

***Ureaplasma urealyticum* colonization, prematurity and bronchopulmonary dysplasia**

W.M. van Waarde*, F. Brus*, A. Okken**, J.L.L. Kimpen*

Ureaplasma urealyticum colonization, prematurity and bronchopulmonary dysplasia. W.M. van Waarde, F. Brus, A. Okken, J.L.L. Kimpen. ©ERS Journals Ltd 1997.

ABSTRACT: The aim of the present study was to determine the association between the presence of *Ureaplasma urealyticum* in endotracheal aspirates and bronchopulmonary dysplasia (BPD). In addition, a review of similar studies from the English literature is presented.

During the period February 1990 until March 1991, 108 mechanically-ventilated infants were included in a prospective study. Endotracheal aspirates were cultured for *U. urealyticum*. Birth weight, gestational age and development of BPD was recorded.

Cultures were positive in 23 infants, resulting in a 21% colonization. The infants with positive cultures had a significantly lower gestational age (mean 28.9 vs 31.5 weeks; range 25–40 vs 25–42 weeks; $p=0.0014$). A positive *U. urealyticum* culture was not associated with a low birth weight (mean 1,390 vs 1,690 g; range 675–4,090 vs 700–3,600 g; $p=0.0712$). A positive *U. urealyticum* culture was significantly associated with BPD ($p=0.0373$). However, after correction for gestational age by logistic regression analysis, BPD failed to correlate with the presence of positive *U. urealyticum* cultures.

A MEDLINE search of the English language literature was performed to identify all studies having the association of *U. urealyticum* colonization and BPD. Fourteen controlled studies were found. Five studies found no significant association between *U. urealyticum* colonization and BPD. In two studies, after correction for gestational age, the association between *U. urealyticum* colonization and BPD did not remain significant. In five studies with a significant association between *U. urealyticum* colonization and BPD, no correction for gestational age had taken place.

In conclusion, *U. urealyticum* colonization is not associated with the development of bronchopulmonary dysplasia. *U. urealyticum* is often associated with gestational age and/or low birth weight; to investigate the association between *U. urealyticum* and bronchopulmonary dysplasia correction for both parameters should be made. *Eur Respir J* 1997; 10: 886–890.

*Beatrix Children's Hospital, University Hospital Groningen, Groningen, The Netherlands. **Wilhelmina Children's Hospital, University Hospital for Children and Youth, Utrecht, The Netherlands.

Correspondence: J.L.L. Kimpen
Beatrix Children's Hospital
University Hospital Groningen
P.O. Box 30.001
9700 RB Groningen
The Netherlands

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Ureaplasma urealyticum can be found in the genital tract of 40–80% of women of child-bearing age [1]. *U. urealyticum* colonization has been reported as the cause of prematurity, low birth weight, congenital pneumonia and bronchopulmonary dysplasia (BPD) [2–5]. However, the association with BPD is controversial, because in several studies this association was not significant; and both BPD and *Ureaplasma* are associated with prematurity and low birth weight [2, 6–8]. The aim of the present study was to determine the association between the presence of *U. urealyticum* in endotracheal aspirates and BPD after correction for gestational age. In addition, a review of similar studies from the English literature is presented.

Patients and methods

Patients

A total of 108 mechanically-ventilated infants, admitted to the neonatal intensive care unit (NICU) at the

University Hospital of Groningen during the period February 1990 to March 1991, were included in this prospective study. During this period, 205 infants were treated with mechanical ventilation. The 97 infants for whom no cultures could be obtained were excluded from the study. Most of these infants were ventilated for a very short period (45 for less than 2 days). Twenty nine of the 97 infants died within a few days after birth because of perinatal infection, diaphragmatic hernia, serious respiratory distress syndrome, etc. Only 9 of the 97 infants developed bronchopulmonary dysplasia (fig. 1).

In the study protocol, in principle all ventilated infants were cultured on the day of admission. When children were admitted at weekends, the culture was obtained in the next week. In some children, the culture was later repeated because of errors in obtaining the first culture, or symptoms that are associated with *Ureaplasma*. Eighty six percent of samples were obtained during the infant's first week of life (52% on the first day).

Of all 108 infants, the following parameters were recorded: sex; gestational age; birth weight; small for

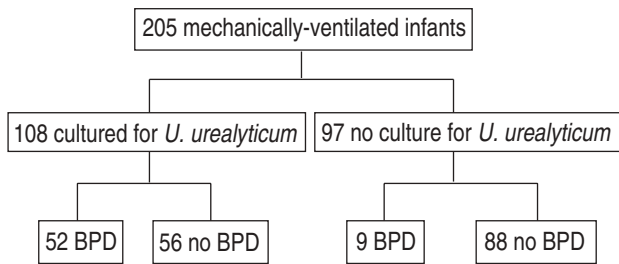


Fig. 1. – The number of infants cultured, or not cultured for *Ureaplasma urealyticum* and the number of infants who developed bronchopulmonary dysplasia (BPD) in both groups.

gestational age (SGA) (birth weight below the 10th percentile for gestational age); premature rupture of membranes (PROM) (>24 h before delivery); mode of delivery (vaginally versus a cesarean section); neonatal respiratory distress syndrome; severity score according to GIEDION *et al.* [9]; development of BPD (defined as a persisting oxygen requirement at 28 days after birth, confirmed by pulse oximetry (arterial oxygen saturation (S_aO_2) >90%)).

Sampling procedure and cultures

Endotracheal aspirates were obtained, from all 108 mechanically-ventilated infants and were transported to the laboratory in mycoplasma transport medium (Sanofi-Pasteur, Marnes la Coquette, France). Maximum time of storage before preparation was 12 h. After filtration, the aspirates were inoculated on mycoplasma duo test-kits (Sanofi-Pasteur), which allow differential titration of *Mycoplasma hominis* and *U. urealyticum*, and on specifically enriched agar plates (Sanofi-Pasteur). The agar plates and test-kits were incubated in 5% CO₂ at 37°C. The plates were examined microscopically for colonies with characteristic morphology [10].

Statistics

Statistical analysis was performed using the Chi-squared test. Logistic regression analysis was performed with BPD as the dependent variable. Gestational age and presence of *U. urealyticum* were chosen as independent variables, because both parameters are associated with BPD and *U. urealyticum* is also associated with prematurity. A p-value less than 0.05 was considered significant.

MEDLINE search of the literature

A MEDLINE search of the English language literature was performed, with use of the medical subject headings "Ureaplasma", "infant", "prematurity", "birth weight" and "bronchopulmonary dysplasia", for the years 1988–1996, to identify all relevant studies. Further studies identified in reference lists of publications noted above were also reviewed. References in review articles and editorials discussing the association between *U. urealyticum* and BPD were examined, to identify any further primary studies. Studies were included if colonization with *U. urealyticum* was determined before BPD development. BPD was defined as in the present study: a requirement for supplemental oxygen at 28 days of age. Several studies also required radiographic changes for the diagnosis of BPD. Studies without a control group were excluded.

Not all *U. urealyticum* cultures were taken from endotracheal aspirates. Some studies had taken nasopharyngeal aspirates or surface specimen. These are mentioned separately. Only full articles in the English language were included.

Results

U. urealyticum was recovered from 23 of the 108 (21%) patients. None of the patients was colonized with *M. hominis*. Six infants with a positive *U. urealyticum* culture also had other microorganisms in their endotracheal aspirate (*Klebsiella*, *Escherichia coli*, Streptococci, Neisseria). In four infants, the first culture was negative for *U. urealyticum* but after 14–26 days a second culture was positive. A positive *U. urealyticum* culture was significantly associated with a lower gestational age (p<0.05) (fig. 2). There was no significant difference in mean

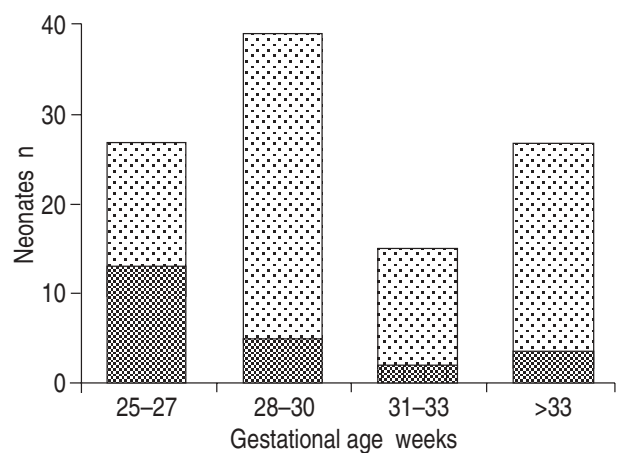


Fig. 2. – The number of infants with positive (■) or negative (▨) *Ureaplasma urealyticum* culture, related to gestational age.

Table 1. – Clinical parameters of newborn infants, stratified by the presence or absence of *Ureaplasma urealyticum* in endotracheal aspirates

Clinical parameter	Ureaplasma present (n=23)	Ureaplasma absent (n=85)	p-value
Sex M/F	12/11 (21)/(22)	46/39 (79)/(78)	NS
Gestational age weeks	28.9±4.0 (25–40)	31.5±4.3 (25–42)	0.0014
Birth weight g	1390±862 (675–4090)	1690±792 (700–3600)	NS
SGA	2 (9)	17 (20)	NS
PROM	7 (35)	14 (17)	NS
Delivery			
Vaginal	14 (26)	40 (74)	NS
Section	9 (17)	45 (83)	
Severity of IRDS			
Grade 0	4 (17)	29 (34)	NS
Grade 1	11 (48)	21 (25)	NS
Grade 2	7 (30)	26 (31)	NS
Grade 3	1 (4)	8 (9)	NS
Grade 4	0 (0)	1 (1)	NS
BPD	16 (70)	36 (42)	0.0373

Data are presented as absolute number and percentage in parenthesis, or as mean±SD and range in parenthesis, as appropriate. M: male; F: female; SGA: small for gestational age; PROM: premature rupture of membranes; IRDS: infant respiratory distress syndrome; BPD: bronchopulmonary dysplasia; NS: nonsignificant.

birth weight between the infants positive and negative for *U. urealyticum* (table 1 and fig. 3). Colonized infants did not differ with respect to sex, SGA, PROM, method of delivery and severity of respiratory distress syndrome (table 1). BPD occurred in 16 of 23 *U. urealyticum* positive infants, and in 36 of 85 *U. urealyticum* negative infants ($p=0.0373$). However, logistic regression analysis showed that BPD was significantly related to decreasing gestational age but not to the presence of a positive *U. urealyticum* culture (table 2 and fig. 4).

Fourteen controlled studies were found in the MEDLINE search (table 3). In five studies, endotracheal aspirates were taken [5, 7, 8, 11, 12]. Seven studies had obtained nasopharyngeal cultures [13–19]. In six of these seven studies endotracheal cultures were obtained in a

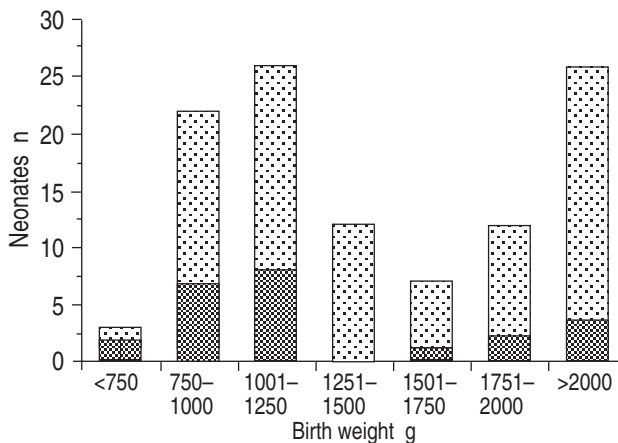


Fig. 3. – The number of infants with positive (■) or negative (□) *Ureaplasma urealyticum* culture, related to birth weight.

Table 2. – Logistic regression analysis with respect to BPD, with p-values, odds ratio (OR) and 95% confidence intervals (95% CI)

Factor	p-value	OR	95% CI
Gestational age	0.00013	0.79	0.69–0.89
Positive culture	0.20430	2.0	0.68–6.2

BPD: bronchopulmonary dysplasia.

Table 3. – Literature data on the correlation between a positive *Ureaplasma urealyticum* culture and prematurity, low birth weight and bronchopulmonary dysplasia

First author [Ref.]	Infants n	Specimen type	Prematurity	Low birth weight	BPD	Correction for prematurity
CASSELL [5]	200	ETA	-	-	S	No
SANCHEZ [20]	111	Surface	NS	NS	S	No
IZRAELI [14]	99	NPA/ETA	S	S	S	No
HOROWITZ [15]	214	NPA/ETA	NS	NS	S	No
DYKE [6]	224	Surface	S	S	NS	No
VALENCIA [13]	69	NPA	NS	NS	NS	No
SAXEN [11]	49	ETA	NS	NS	NS	No
ALFA [16]	240	NPA/ETA/ Surface	-	-	S	No
WANG [17]	107	NPA/ETA	-	-	S	Yes
SMYTH [7]	138	ETA	S	NS	NS	Yes
PAYNE [18]	93	NPA/ETA	NS	NS	S	Yes
HEGGIE [8]	224	ETA	S	S	NS	Yes
JONSSON [19]	93	NPA/ETA	S	S	S	Yes
ILES [12]	40	ETA	NS	NS	S	Yes

ETA: endotracheal aspirate; NPA: nasopharyngeal aspirate; s: significant; ns: nonsignificant; -: not investigated.

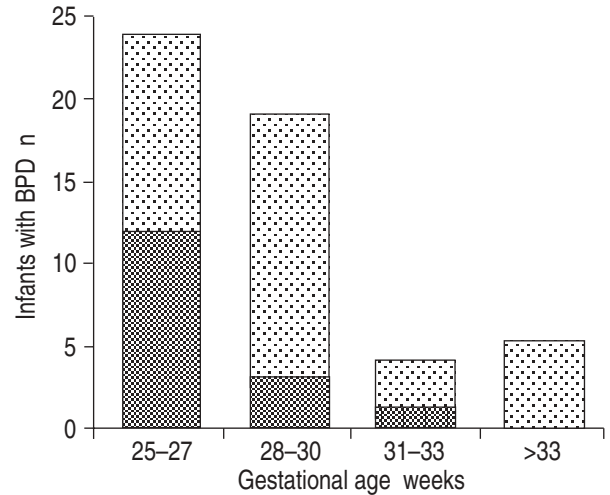


Fig. 4. – The number of infants with bronchopulmonary dysplasia (BPD) and positive (■) or negative (□) *Ureaplasma urealyticum* culture, related to gestational age.

subpopulation [14–19]. In two studies, surface cultures were taken (eye, throat, vagina, gastric aspirate) [6, 20].

Nine studies showed a significant correlation between positive *U. urealyticum* culture and BPD. In five studies, no correction had been performed for gestational age [5, 14–16, 20]. Two studies described a significant association between *U. urealyticum* colonization and BPD, that disappeared after multivariate analysis [18, 19]. Only two studies showed a significantly higher risk for developing BPD in *U. urealyticum* colonized infants after correction for gestational age [12, 17]. Five studies showed no significant correlation between *U. urealyticum* colonization and BPD [6–8, 11, 13].

Discussion

A positive *U. urealyticum* culture has been related to prematurity [2, 6–8, 14, 19]. This was confirmed in the present study. Colonization has also been associated with BPD [5, 12, 14–20]. We also found a significant

association between *U. urealyticum* and BPD. However, after correction for gestational age, *U. urealyticum* colonization did not contribute to the development of BPD. The most significant factor in the development of BPD was decreasing gestational age. This is in agreement with previous studies [7, 18, 19]. WANG *et al.* [21] recently reported a meta-analysis on studies on *U. urealyticum* colonization and BPD. The pooled estimate of relative risks for BPD in infants colonized with *U. urealyticum* in comparison with those not colonized was 1.75 (95% confidence interval (95% CI) 1.53–1.99) [21]. Confounding variables closely linked to *U. urealyticum* and BPD (such as gestational age and birth weight) were not excluded.

In the present study, only endotracheal cultures were performed. Endotracheal colonization has been considered to be the most representative of lower airway colonization, and is the method of choice to evaluate colonization of the lower airway tract in preterm infants [5, 18]. The difference in culture sites for *U. urealyticum* between studies could be one of the reasons for disagreement in the results concerning the association of *U. urealyticum* with BPD.

Because tracheal aspirates were not obtained from all artificially-ventilated infants in the present study, it is possible that the results were confounded by selection of the more critically ill infants for collection of specimens. The 48% frequency of BPD in the total study population is suggestive. The frequency of BPD in the population who were not cultured was only 9%. However, there are several reasons for this low percentage of BPD. Firstly, many infants were ventilated for very short periods of time (45 for less than 2 days), because of wet lung disease, perinatal asphyxia, *etc.* Secondly, several infants died some days after birth (30%). Thirdly, the percentage of premature infants was much lower (35 versus 61% <30 weeks in the cultured group).

Twenty one percent of the cultured patients had a positive culture for *U. urealyticum*. This is in agreement with the percentage colonization reported in the literature [18, 19].

Four infants in the present study initially had a negative *U. urealyticum* culture but a positive culture after a period of 14–26 days. The difference between the first and second culture could have been a consequence of an error in processing the first cultures. Nosocomial transmission could also have occurred, as has been suggested by SANCHEZ and REGAN [22] and ILES *et al.* [12]. However, confirmation of nosocomial infection requires improvement of *U. urealyticum* characterization, for instance by using serotyping of isolates. When *Ureaplasma* was acquired at or just before delivery; concentrations of *Ureaplasma* could be too low to identify it in culture. Meanwhile, in infants with symptoms which could be attributed to *U. urealyticum* infection, culture should be repeated.

One potential mechanism by which *U. urealyticum* colonization could contribute to BPD is by causing chronic pulmonary infection, resulting in prolonged oxygen requirement. Both the infection and the oxygen supplementation could lead to BPD [5, 23]. However, it is difficult to make a distinction between *U. urealyticum* colonization and infection. PAYNE *et al.* [18] found a greater incidence of >2+ polymorphonuclear leucocytes

in the tracheal aspirate at 2±1 days of age in infants colonized with *U. urealyticum*. *U. urealyticum* infected fibroblasts produced a significant increase in interleukin-6 and -8 (IL-6 and IL-8), independent of hyperoxic exposure [24]. OHLSSON *et al.* [25] described an elevated white blood cell (WBC) count in very low birth weight (VLBW) infants, who were colonized with *U. urealyticum* in their nasopharynx or trachea. The increase in WBC count was due to an increase in the number of mature and immature neutrophils [25]. Serological studies showed a serovar-specific antibody response to *U. urealyticum* in neonates, including serovar-specific immunoglobulin M (IgM) antibody [26].

A randomized, placebo-controlled trial of erythromycin treatment of *U. urealyticum* colonized neonates, to determine whether eradication of *U. urealyticum* would decrease the incidence of BPD, could possibly provide further information. This effort would require a large number of subjects. In such a study, a confounding variable could be the presence of other infectious agents susceptible to erythromycin, or a *U. urealyticum* strain resistant to erythromycin. Moreover, further prospective studies to distinguish between *U. urealyticum* colonization and infection are indicated.

In conclusion, the present study shows that *U. urealyticum* colonization is not associated with bronchopulmonary dysplasia, after correction for gestational age. In many similar studies, no correction for gestational age has been performed [5, 14–16, 20]. The present study confirms data from two recent studies rejecting the association between *U. urealyticum* colonization and bronchopulmonary dysplasia after correction for gestational age [18, 19]. Correction for confounding variables, closely linked to *U. urealyticum* colonization (such as gestational age and birth weight) is essential in studying the association between *U. urealyticum* colonization and bronchopulmonary dysplasia.

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