JUKKA OLLIKAINEN

Clinical Relevance of Perinatally Acquired Ureaplasma Urealyticum Involvement in Preterm Infants

Doctoral dissertation

To be presented by permission of the Faculty of Medicine of the University of Kuopio for public examination in Auditorium L3, Canthia, University of Kuopio, on Friday 26th April 2002, at 12 noon

Department of Paediatrics
University of Kuopio
ABSTRACT

To study the clinical consequences of perinatal acquisition of \textit{U. urealyticum} in preterm infants, 176 infants were enrolled in two cohorts, comprising 98 (cohort A) and 78 infants (cohort B). The subjects were born prematurely before 34 weeks of gestation at Kuopio University Hospital. \textit{U. urealyticum} culture samples were collected within the first 12 hours after birth. A total of 163 nasopharyngeal, 116 endotracheal and 127 blood samples were cultured. In the cultures of these samples both A7 agar and a commercial Mycofast kit were used.

\textit{U. urealyticum} involvement was associated with acute respiratory failure. As an indicator of this, the infants with \textit{U. urealyticum} infection in cohort A needed more intensive treatment to achieve adequate ventilation. A higher proportion of infants with \textit{U. urealyticum} infection needed mechanical ventilation than did infants without infection (94% vs. 78%, \(p=0.032\)) and respiratory decompensation was more commonly severe in them (79% vs. 55%, \(p=0.013\)). Furthermore, the clinical diagnosis of respiratory distress syndrome was more common in infected infants (51% vs. 20%, \(p=0.011\)). The development of chronic lung disease was not associated with the presence of \textit{U. urealyticum} in these two cohorts. The presence of \textit{U. urealyticum} infection in infants in cohort A was associated with higher mortality (26% vs. 6%, \(p=0.0070\)). Peripheral blood white cell counts were higher in infants with \textit{U. urealyticum} colonization in cohort B both on the first (median 18.6 vs. 12.4, \(p=0.012\)) and on the second (median 29.0 vs. 15.4, \(P=0.013\)) days of life. The increased need for hospital treatment during the first year of life was associated with \textit{U. urealyticum} colonization in infants of cohort A.

These results suggest that \textit{U. urealyticum} is involved with acute respiratory failure of newborn infants. Because those infants who were colonized with \textit{U. urealyticum} had elevated white cell counts in peripheral blood, \textit{U. urealyticum} is suggested to have the ability to cause inflammatory response in the premature infant. However, the role of \textit{U. urealyticum} in the development of chronic lung disease remains unclear. The increased need for later hospital treatment indicates that perinatally acquired \textit{U. urealyticum} infection influences infants’ health beyond the perinatal era.

National Library of Medicine Classification: WS 410, QW 131, WC 246

Medical Subject Headings: ureaplasma urealyticum; ureaplasma; gram-negative bacteria; mollicutes; mycoplasma; respiratory; infant, premature, diseases
To Tommi and Ville
ACKNOWLEDGEMENTS

This study was carried out at the Department of Pediatrics, Kuopio University Hospital. I wish to express my gratitude and respect to professors Kari Launiala, MD, and Raimo Voutilainen, MD, for providing me the opportunity to do this work.

I wish to express my deepest gratitude to my supervisors Docent Kirsti Heinonen, MD, and Professor Matti Korppi, MD, for their continuous encouragement, ideas and advice. Their inspiring and creative attitude has been significant at all stages of this work. Special thanks for patience.

My sincere thanks are due to reviewers of this thesis Docent Maija Pohjavuori, MD, and Docent Harri Saxén, MD, for their constructive criticism and expert advice.

I am deeply grateful to Docent Marja-Leena Katila, MD, Tarja Heiskanen-Kosma, MD, Heikki Hiekkanen, MD, and Docent Hannu Sarkkinen, MD, for sharing with me their special knowledge concerning microbiological methods and for their supportive and fundamental help throughout the project.

I wish to thank Pirjo Halonen, MSc, for her skillful assistance in statistical problems and Mrs. Liisa Korkalainen, office clerk, for computer assistance and secretarial help.

I thank Vivian Paganuzzi for his assistance in correcting the language of the manuscript.

Kuopio University, Kuopio University Hospital, Kerttu and Kalle Viik Foundation as well as Maud Kuistila Foundation are acknowledged for financial support.

Finally, my warmest thanks belong to my family, Marja, Tommi and Ville.

My dear boys: now the work is done, and now it is the time to switch the computer off!
LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following articles, which are referred to in the text by their Roman numerals:


ABBREVIATIONS

CI Confidence interval
CLD Chronic lung disease
FiO2 Fraction of inspired oxygen
HFOV High-frequency oscillatory ventilation
RDS Respiratory distress syndrome
RR Relative risk
CONTENTS

1. INTRODUCTION 17

2. REVIEW OF THE LITERATURE 17

2.1. History of U. urealyticum 17

2.2. Microbiology of U. urealyticum 18
   2.2.1. Taxonomy 18
   2.2.2. Diagnosis of the presence of U. urealyticum 19

2.3. Epidemiology of U. urealyticum 20
   2.3.1. Epidemiology of U. urealyticum during pregnancy 21
   2.3.2. Neonatal colonization with U. urealyticum 21

2.4. Influence of U. urealyticum on pregnancy 24
   2.4.1. Miscarriage 24
   2.4.2. Intrauterine growth retardation 25
   2.4.3. Preterm birth 25
      2.4.3.1. Prematurity and lower genital tract colonization 25
      2.4.3.2. Prematurity and the presence of U. urealyticum in placental samples 26
      2.4.3.3. Prematurity and U. urealyticum in amniotic fluid 26
   2.4.4. Efficacy of treating maternal U. urealyticum colonization 27

2.5. U. urealyticum and neonatal disease 28
   2.5.1. U. urealyticum and acute respiratory disease 28
   2.5.2. U. urealyticum and chronic lung disease 28
   2.5.3. U. urealyticum and chronic meningitis in premature infants 31
   2.5.4. Efficacy of treating neonatal U. urealyticum colonization 32

2.6. U. urealyticum and laboratory markers of inflammation 32

2.7. Influence of U. urealyticum on the health of infants after perinatal era 33
3. AIMS OF THE STUDY

4. MATERIAL AND METHODS

4.1. Study subjects
4.2. Outcome measures
4.3. Microbiological determinations
   4.3.1. Collection of culture samples
   4.3.2. Microbiological methods
4.4. Data collection
4.5. Definitions
4.6. Ethics
4.7. Statistical analysis

5. RESULTS

5.1. Patient characteristics
5.2. Results of cultures for *U. Urealyticum*
5.3. *U. urealyticum* infection and acute respiratory insufficiency
5.4. *U. urealyticum* infection and mortality
5.5. *U. urealyticum* infection and chronic lung disease of preterm infants
5.6. *U. urealyticum* infection and hospital admissions
during the first year of life
5.7. *U. urealyticum* colonization and blood leukocyte counts
6. DISCUSSION

6.1. Prevalence of *U. urealyticum* colonization and infection 52

6.2. The role of *U. urealyticum* in acute respiratory failure 53
   6.2.1 *U. urealyticum* and the prevalence of respiratory distress syndrome 53
   6.2.2 *U. urealyticum* and the severity of acute respiratory failure 54

6.3. *U. urealyticum* and chronic lung disease 55

6.4. *U. urealyticum* and non-specific markers of inflammation 58

6.5. The influence of *U. urealyticum* on later health 58

6.6. Methodological aspects 59
   6.6.1. Microbiological methods 60
   6.6.2. Definition of infection and colonization 61
   6.6.3. Study population 62
   6.6.4. Statistical analyses 64

7. CONCLUSIONS 65

8. REFERENCES 66
1. INTRODUCTION

Recent research has shown that many newborn infants are colonized by *U. urealyticum* (Hannaford et al. 1999). This colonization seems more common in premature than full term infants (Alfa et al. 1995). Although in full term infants *U. urealyticum* is considered to be harmless (Syrogiannopoulos et al. 1990), in premature infants this microbe has the ability to infect both the respiratory tract (Cassell et al. 1988) and central nervous system (Waltes et al. 1988). Because genital colonization with *U. urealyticum* is frequent in pregnant women, from 47% (Grattard et al. 1995b) to 90% (Hardy et al. 1984), and because *U. urealyticum* is easily transferred to the premature newborn during pregnancy or delivery (Chua et al. 1998), the clinical consequences of *U. urealyticum* colonization have raised concern among pediatricians taking care of premature infants. The fact that routine microbiological methods fail to detect *U. urealyticum* and that routine antimicrobial agents have no effect against it give further cause for concern.

The main aim of this thesis was to establish the clinical picture of perinatally acquired *U. urealyticum* infection among premature infants. Particular attention was paid to the effect of *U. urealyticum* on the development of acute and chronic lung disease. Two unselected cohorts of premature infants, who were all born in the same center, were enrolled.  

2. REVIEW OF THE LITERATURE

2.1. History of *U. urealyticum*

The first Mycoplasma species was discovered in the late 1800s, initially in cattle. As a human pathogen *Mycoplasma pneumoniae* was discovered in the 1930s. The species *U. urealyticum* was discovered in 1954 in patients with non-gonococcal urethritis (Shepard 1954) and was originally named *T-mycoplasmas* (Tiny-mycoplasmas) owing to its small size. Nowadays, *U. urealyticum* is accepted to be a cause of non-gonococcal urethritis, although it causes only about 10% of cases. It is also involved in bacterial vaginosis, together with
several other bacteria (Taylor-Robinson 1997). The pathogenicity of *U. urealyticum* is low and its role in other clinical conditions is controversial.

In the 1970’s *U. urealyticum* colonization of the newborn was shown to be associated with histological signs of chorioamnionitis in placentas (Shurin et al. 1975). This finding led to studies in which the role of *U. urealyticum* was investigated as a cause of chorioamnionitis and preterm delivery (Embree et al. 1980). The suggested association between *U. urealyticum* and chorioamnionitis led to the investigation of the connection between *U. urealyticum* and the diseases of premature infants. Research in both these fields is still active (Yoon et al. 1998a, Hannaford et al. 1999).

2.2. Microbiology of *U. urealyticum*

2.2.1. Taxonomy

Currently the mycoplasmas comprise six genera. They are the smallest free-living organisms in nature. Three out of a total of approximately 70 species of mycoplasmas are considered to be human pathogens: *Mycoplasma pneumoniae*, *Mycoplasma hominis* and *Ureaplasma urealyticum*.

All mycoplasmas share some typical features. For example, they lack a cell wall. As a result, they are pleomorphic and resistant to antimicrobial agents that inhibit cell wall synthesis. They do not stain well with Gram staining and their size is comparable to that of myxoviruses; these characteristics make them invisible on routine microscopy. With the exception of aerobic *M. pneumoniae*, they are facultative anaerobes. Mycoplasmas are stringent in growth media, susceptible to drying and requiring urea in their metabolism. Consequently, they are not readily detected by standard culture methods.
The species *U. urealyticum* is currently classified into two biovars and 14 serovars (Kong et al. 1999a). The denomination of the two biovars varies; the terms biovars 1 and 2 are used (Kong et al. 1999a) together with biovar parvum and biovar T960 (Abele-Horn et al. 1997a, Grattard et al. 1995a). Recent investigations suggest that these two biovars actually represent two distinct species of mycoplasmas, namely *Ureaplasma parvum* (former biovar 1) and *Ureaplasma urealyticum* (former biovar 2; Kong 1999b). Both of these biovars (or species) have been detected in clinical samples from humans, biovar 1 more often (Kong et al. 1999a, Abele-Horn et al. 1997a, Grattard et al. 1995b). Serotyping of these two biovars (or species) has been difficult, which causes problems when studying associations between individual serovars and diseases (Kong et al. 1999a). The 14 serovars of *U. urealyticum* are classified in the two biovars as follows: biovar 1 contains serovars 1, 3, 6, and 14, while biovar 2 contains the other ten of the currently known biovars (Grattard et al. 1995a).

### 2.2.2. Diagnosis of the presence of *U. urealyticum*

In laboratory cultures of *U. urealyticum*, liquid in-house media such as A7 broth are commonly used. These media commonly contain horse serum with yeast extract together with penicillin G, urea and manganese sulfate (Hargrave et al. 1995). In addition, commercial kits for the cultivation of *U. urealyticum* have been developed.

Diagnostic tests based on polymerase chain reaction (PCR) for *U. urealyticum* were developed in the early 1990s. They seem to have higher sensitivity (Blanchard et al. 1993, Teng et al. 1994, Nelson et al. 1998) than conventional cultural methods in the detection of *U. urealyticum* in clinical samples. However, PCR based tests may show false-positive results due to contamination of the reagents with target DNA (Razin 1994).

Serological diagnostic methods for *U. urealyticum* have been used in only a few clinical studies (Gallo et al. 1983, Quinn et al. 1983, Horowitz et al. 1995a, Cunningham et al. 1996). They have not attained wide use in clinical work. The antigens expressed by *U. urealyticum*
show variability (Zheng et al. 1992, Cheng et al. 1994), and multiple serovars are often present in a single clinical sample (Kong et al. 1999a, Nelson et al. 1998, Heggie et al. 2001). Furthermore, newborn infants show only weak or no serological responses (Dinsmoor et al. 1989).

Like other Mycoplasmas, *U. urealyticum* is usually sensitive to erythromycin and its derivatives (Matlow et al. 1998). Erythromycin resistance of *U. urealyticum* is described (Hentschel et al. 1993), but it seems to be uncommon: Martinez et al. (2001) found no erythromycin resistance in their study. Tetracycline resistance is more common: 16% of *U. Urealyticum* strains were resistant (Martinez et al. 2001).

2.3. Epidemiology of *U. urealyticum*

It has been suggested that *U. urealyticum* is spread primarily by sexual contact. Sexual activity increases the prevalence of *U. urealyticum* colonization (Russo et al. 1981, Shafer et al. 1985, Bump et al. 1986), and *U. urealyticum* colonization has even been considered to be an indicator of sexual activity (Chambers et al. 1987). However, also non-sexual transmission routes are common: 8% of prepubertal girls (Hammerschlag et al. 1987) and 41% of newborn infants (Iwasaka et al. 1986) had vaginal colonization with *U. urealyticum*, and the respective figure in virginal adolescent was 33% (Bump et al. 1986). These findings suggest either vertical transmission from the antecedent generation or transmission via non-sexual contact. Gender seems to influence *U. urealyticum* colonization; adolescent girls have a colonization rate eightfold that of boys of the same age (Foy et al. 1975). Socioeconomic status, racial-ethnic factors and age have been associated with *U. urealyticum* colonization (Harrison et al. 1983, Carey et al. 1991).
2.3.1. Epidemiology of *U. urealyticum* during pregnancy

Colonization of the genital tract with *U. urealyticum* is common during pregnancy; frequencies of from 47 % (Grattard et al. 1995b) to 90 % (Hardy et al. 1984) have been detected. *U. urealyticum* is easily transmitted during vaginal delivery from the colonized mother to her newborn, but *U. urealyticum* has been detected in newborns even after section delivery with intact membranes (Bowman et al. 1998). In term deliveries, transmission rates have varied from 38% (Abele-Horn et al. 1997b) to 88% (Chua et al. 1998). The transmission rate has been considered to be higher in preterm than term infants (Alfa et al. 1995), and up to 95% transmission rates have been detected in preterm deliveries (Abele-Horn et al. 1997b). The transmission of *U. urealyticum* is suggested to be inversely related to birth weight in premature deliveries (Sanchez et al. 1990, Alfa et al. 1995). Some studies have shown that neonatal colonization is higher in the context of premature rupture of membranes (Sanchez et al. 1987, Saxen et al. 1993) but this has not been found consistently (Sanchez et al. 1990).

2.3.2. Neonatal colonization with *U. urealyticum*

The rate of neonatal *U. urealyticum* colonization has varied widely in previous reports. Horowitz et al. (1992) found no positive samples for *U. urealyticum* in a cohort of 100 full term infants; on the other hand, Sanchez et al. (1987) detected a colonization rate of 45% when the mother was colonized. In preterm infants the colonization rate with *U. urealyticum* seems to be higher; rates of 37% (Illes et al. 1996) to 56% (Heggie et al. 1994) have been detected in tracheal samples, and up to 80% rates have been detected in very low birth weight infants when both tracheal and nasopharyngeal samples have been cultured (Izraeli et al. 1991). Table 1 shows the *U. urealyticum* colonization rates in 17 studies and Table 2 shows the *U. urealyticum* infection rates in five studies. In these tables the results are presented with no regard to maternal status of colonization. Overall, positive cultures have been detected in from 0% (Horowitz et al. 1992) to 43% (Pacifico et al. 1997) of cases in
nasopharyngeal samples, in from 9% (Groneck et al. 1996) to 55% (Panero et al. 1995) in tracheal samples, and in from 0% (Valencia et al. 1993) to 28% (Cassell et al. 1988) in blood samples. Seventeen studies have evaluated the effect of gestational age or birth weight on the colonization rate with *U. urealyticum*. Ten studies have shown that colonization rate increases with decreasing gestational age (Izraeli et al. 1991, Horowitz et al. 1992, Smyth et al. 1993, Crouse et al. 1993, Luton et al. 1994, Heggie et al. 1994, Jonsson et al. 1994, Alfa et al. 1995, Abele-Horn et al. 1997b, Pacifico et al. 1997), but such an association was not found in seven studies (Cassell et al. 1988, Sanchez et al. 1988, Payne et al. 1993, Saxen et al. 1993, Ills et al. 1996, Lyon et al. 1998, Bowman et al. 1998). (The studies in Tables 1 and 2 were obtained by MEDLINE search by using the keywords throat, nasopharynx, (endo)trachea(l), blood and ureaplasma.)
Table 1.

Rates of *U. urealyticum* colonization reported in previous clinical studies

<table>
<thead>
<tr>
<th>Authors, year</th>
<th>Sample site</th>
<th>Percentage</th>
<th>Subjects of colonization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horowitz et al. 1992</td>
<td>nasopharynx</td>
<td>0 %</td>
<td>full-term infants</td>
</tr>
<tr>
<td></td>
<td>nasopharynx, trachea</td>
<td>24%</td>
<td>preterm infants</td>
</tr>
<tr>
<td>Groneck et al. 1996</td>
<td>trachea</td>
<td>9%</td>
<td>preterm infants</td>
</tr>
<tr>
<td>Patterson et al. 1998</td>
<td>trachea, nasopharynx</td>
<td>16%</td>
<td>&lt; 1251g</td>
</tr>
<tr>
<td>Heggie et al. 1994</td>
<td>trachea</td>
<td>17%</td>
<td>full- and preterm infants</td>
</tr>
<tr>
<td>Bowman et al. 1998</td>
<td>trachea</td>
<td>18%</td>
<td>&lt; 1000g</td>
</tr>
<tr>
<td>Payne et al. 1993</td>
<td>trachea, nasopharynx</td>
<td>18%</td>
<td>&lt; 1251g</td>
</tr>
<tr>
<td>Jonsson et al. 1998</td>
<td>trachea, nasopharynx</td>
<td>19%</td>
<td>&lt; 30 weeks</td>
</tr>
<tr>
<td>Jonsson et al. 1994</td>
<td>trachea, nasopharynx</td>
<td>19%</td>
<td>full- and preterm infants</td>
</tr>
<tr>
<td>van Waarde et al. 1997</td>
<td>trachea</td>
<td>21%</td>
<td>full- and preterm infants</td>
</tr>
<tr>
<td>Hannaford et al. 1999</td>
<td>trachea</td>
<td>27%</td>
<td>&lt; 28 weeks</td>
</tr>
<tr>
<td>Saxen et al. 1993</td>
<td>trachea</td>
<td>29%</td>
<td>&lt; 30 weeks</td>
</tr>
<tr>
<td>Wang et al. 1988</td>
<td>gastric aspirate, nasopharynx and trachea</td>
<td>33%</td>
<td>&lt; 1250g</td>
</tr>
<tr>
<td>Abele-Horn et al. 1998</td>
<td>trachea</td>
<td>36%</td>
<td>&lt; 1500g</td>
</tr>
<tr>
<td>Iles et al. 1996</td>
<td>trachea</td>
<td>37%</td>
<td>&lt; 31 weeks</td>
</tr>
<tr>
<td>Da Silva et al. 1997</td>
<td>trachea, nasopharynx</td>
<td>37% a</td>
<td>&lt; 1501g</td>
</tr>
<tr>
<td></td>
<td></td>
<td>45% b</td>
<td>&lt; 1501g</td>
</tr>
<tr>
<td>Sanchez et al. 1988</td>
<td>eye, throat, vagina, rectum</td>
<td>41%</td>
<td>&lt; 2000g</td>
</tr>
<tr>
<td>Abele-Horn et al. 1997b</td>
<td>nasopharynx, throat, trachea</td>
<td>56%</td>
<td>full- and preterm infants</td>
</tr>
<tr>
<td>Izraeli et al. 1991</td>
<td>throat, trachea</td>
<td>18%</td>
<td>28 – 36 weeks</td>
</tr>
<tr>
<td></td>
<td></td>
<td>80%</td>
<td>&lt; 28 weeks</td>
</tr>
</tbody>
</table>

a: based on culture methods

b: based on PCR methods
Table 2.

Rates of *U. urealyticum* infection reported in previous clinical studies

<table>
<thead>
<tr>
<th>Authors, year</th>
<th>Sample site</th>
<th>Percentage of infection</th>
<th>Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lyon et al. 1998</td>
<td>trachea</td>
<td>13%</td>
<td>&lt; 30 weeks</td>
</tr>
<tr>
<td>Cassell et al. 1988</td>
<td>trachea</td>
<td>23%</td>
<td>&lt; 1000 g</td>
</tr>
<tr>
<td>Ilies et al. 1996</td>
<td>trachea</td>
<td>37%</td>
<td>&lt; 31 weeks</td>
</tr>
<tr>
<td>Pacifico et al. 1996</td>
<td>trachea</td>
<td>50%</td>
<td>&lt; 1501 g</td>
</tr>
<tr>
<td>Panero et al. 1995</td>
<td>trachea or blood</td>
<td>55%</td>
<td>&lt; 1301 g</td>
</tr>
</tbody>
</table>

2.4. Influence of *U. urealyticum* on pregnancy

2.4.1. Miscarriage

In 1983 Quinn et al. showed that *U. urealyticum* infection was associated with spontaneous abortions. Naessens et al. (1987) showed that cervical and endometrial colonization with *U. urealyticum* was associated with recurrent spontaneous abortion and that *U. urealyticum* was often isolated from placentae with histological signs of infection in the context of fetal loss. In 1994 Joste et al. showed by comparing first trimester spontaneous and elective abortions that *U. urealyticum* was present in chorion in 26% of cases with spontaneous abortions compared with 0% of cases with elective abortion. However, some studies have not found any association between *U. urealyticum* and miscarriage (Harrison 1986, Harrison et al. 1983).
2.4.2. Intrauterine growth retardation

Embree et al. (1980) showed that placental infection with *U. urealyticum* was associated with prematurity, low birth weight and intrauterine growth retardation. In 1987 McCormack et al. showed that erythromycin treatment of pregnant women who had vaginal cultures positive for *U. urealyticum* led to higher birth weight of the child when compared with untreated controls. However, this effect was detected only when treatment was given during the third trimester; treatment in midtrimester having no effect. Germain et al. (1994) correlated the results of vaginal and cervical cultures to birth weight of infants in a large population of 13,000 pregnant women. They showed that the relative risk for intrauterine growth retardation caused by *U. urealyticum* colonization was statistically significant but low, 1.20. In contrast, several studies have not detected any association between maternal *U. urealyticum* colonization and low birth weight (Ross et al. 1981, Harrison et al. 1983, Iwasaka et al. 1986, Zlatnik et al.1986, Naessens et al. 1989).

2.4.3. Preterm birth

2.4.3.1. Prematurity and lower genital tract colonization

Minkoff et al. showed in 1984 that vaginal colonization of *U. urealyticum* in early pregnancy was associated with preterm labor. Similar results were reported in studies by Lamont et al. (1986 and 1987) and McDonald et al. (1992). In contrast, studies by McGregor et al. (1990), Carey et al. (1991) and Paul et al. (1998) have shown that cervicovaginal colonization of *U. urealyticum* does not increase the risk for preterm delivery or premature rupture of membranes. Of special interest is the finding that vaginal and cervical swabs have poor correlation with intrauterine infection (Carroll et al. 1996).
2.4.3.2. Prematurity and the presence of *U. urealyticum* in placental samples

In 1988 Hillier et al. showed that *U. urealyticum* was isolated more often from the chorioamnion surface of the placenta in preterm deliveries than in term deliveries. Similar results were reported by Kundsün et al. in 1996; *U. urealyticum* infection of the placenta was detected more often in preterm spontaneous deliveries than in preterm deliveries induced for maternal complications.

2.4.3.3. Prematurity and *U. urealyticum* in amniotic fluid

Watts et al. (1992), Yoon et al. (2000) and Romero et al. (1990) studied cases with preterm labor and intact membranes, and observed that positive amniotic fluid cultures were associated with earlier delivery than was the case with negative cultures for *U. urealyticum*. Furthermore, Romero et al. (1989) showed by using a similar study design that the presence of *U. urealyticum* in the amniotic fluid was associated with low gestation and high incidence of RDS and infectious diseases of the newborn.

Gray et al. (1992) and Bashiri et al. (1999) showed by cultures of midtrimester genetic amniocentesis that cases, which were positive for *U. urealyticum*, had unfavorable outcome (either preterm delivery or fetal loss). Bashiri et al. (1999) also showed that those cases with amniotic fluid samples positive for *U. urealyticum* had high levels of interleukine-6. Likewise, Horowitz et al. (1995b) detected an association between the presence of *U. urealyticum* in the amniotic fluid and premature delivery.

The potential ability of *U. urealyticum* to induce premature labor is suggested by at least two findings. Firstly, *U. urealyticum* has phospholipase activity (De Silva et al. 1986, De Silva et al. 1991), which is speculated to trigger the onset of labor by degradation of placental phospholipids. Secondly, *U. urealyticum* has the ability to cause an inflammatory reaction when invading the chorioamnion (Yoon et al. 1998a, Bashiri et al. 1999, Yoon et al. 2000), leading to
poor pregnancy outcome. However, the true relationship between *U. urealyticum* colonization of the genital tract and the onset of preterm labor is thus far unresolved. The high frequency of *U. urealyticum* colonization in the lower genital tract during uncomplicated pregnancy, in up to 90% of cases (Hardy et al. 1984), makes the evaluation of its role difficult. Furthermore, *U. urealyticum* is commonly accepted to be one of the several microbes in bacterial vaginosis; other microbes in this context also have associations with pregnancy outcome (Germain et al. 1994).

At present, *U. urealyticum* colonization of the genital tract is not commonly accepted to have an effect on pregnancy outcome. A detrimental effect has been suggested if *U. urealyticum* invades the chorioamnion prematurely (Gray et al. 1992, Yoon et al. 1998a, Yoon et al. 2000, Horowitz et al. 1995a, Horowitz et al. 1995b). Such microbial invasion seems infrequent in symptomless pregnancies, in only from 0.33% (Gray et al. 1992) to 2.8% (Horowitz et al. 1995) of cases, but may be common in cases with preterm labor and intact membranes (10% (Romero et al. 1990) to 19% (Watts et al. 1992)) and cases with preterm rupture of membranes (21% (Yoon et al. 1998b) to 34% (Romero et al. 1992)).

2.4.4. Efficacy of treating maternal *U. urealyticum* colonization

Winkler et al. (1989) and Antsaklis et al. (1997) showed that in patients with threatened preterm labor treatment with erythromycin delayed delivery, compared with placebo or standard treatment. Similar results were found by Ogasawara et al. (1997). Interestingly, in this study erythromycin treatment did not affect the rate of neonatal colonization with *U. urealyticum*. In 1999 Berg et al. showed that erythromycin treatment of *U. urealyticum* colonization, detected by genetic amniocentesis, led to a reduced rate of pregnancy loss. In contrast, Eschenbach et al. (1991) showed that long-term treatment with erythromycin did not affect pregnancy outcome in symptomless pregnant women with *U. urealyticum* colonization. Thus, these results suggest that in normal pregnancies *U. urealyticum* colonization requires no treatment, but there may be subgroups of patients in which treatment may prove beneficial.
2.5. *U. urealyticum* and neonatal disease

2.5.1. *U. urealyticum* and acute respiratory disease

Previous research throws little light on the association between acute respiratory disease and *U. urealyticum* colonization. In 1984 Taylor-Robinson et al. were able to isolate *U. urealyticum* in 19-33% of the newborn infants in a special care unit. They found no association between *U. urealyticum* isolation and respiratory distress. In 1985 Quinn et al. described a case report of fatal neonatal pneumonia caused by *U. urealyticum*. Romero et al. (1989) showed that more infants were born prematurely and had higher incidence of RDS if the amniotic fluid was infected by *U. urealyticum*. Abele-Horn et al. (1997b) followed pregnant women with and without *U. urealyticum* colonization and found that *U. urealyticum* colonization was associated with prematurity and RDS. However, the development of RDS was not controlled for prematurity in either of these studies. The studies by Cassell et al. (1988), Saxen et al. (1994) and Sanchez et al. (1988) found no association between *U. urealyticum* and RDS. Hannaford et al. (1999) suggested that *U. urealyticum* colonization actually was a negative predictor of RDS.

2.5.2. *U. urealyticum* and chronic lung disease

In 1988 Cassell et al. studied a cohort of 200 intubated preterm infants. They showed that in a subgroup of 48 infants with birth weight less than 1000 gm *U. urealyticum* infection, detected by endotracheal culture samples, was associated with increased mortality and CLD. Saxen et al. published in 1993 the first study in which no association was found between *U. urealyticum* and CLD.

At present, 21 reports can be identified by MEDLINE search using medical subject headings "*U. urealyticum*" and "chronic lung disease" or "bronchopulmonary dysplasia", in which the clinical association between CLD and *U. urealyticum* has been the main interest of the study. In these studies there are great variation in patient enrollment, microbiological diagnostic procedures
and in the definition of CLD. If we exclude these differences an association between CLD and *U. urealyticum* was suggested in 13 and no association was suggested in 8 of the 21 reports. The results of these 21 studies are presented in Table 3, including data on the association between the presence of *U. urealyticum* and gestational age and/or birth weight of the children. In 11 out of these 21 studies the results were presented using risk ratios of *U. urealyticum* for the development of CLD. Figure 1 summarizes these results graphically. The relative risks were less than two in six of the 11 studies. The shape of the plot is asymmetrical, suggesting a publication bias for not publishing studies without a detected effect.
Table 3.

Data on the association between *U. urealyticum* and chronic lung disease in 21 reports.

<table>
<thead>
<tr>
<th>Authors, year</th>
<th>Association with CLD</th>
<th>Association with prematurity</th>
<th>Correction for prematurity</th>
<th>Number of infants in the analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heggie et al. 2001</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>137</td>
</tr>
<tr>
<td>Harnaaford et al. 1999</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>99</td>
</tr>
<tr>
<td>Abele-Horn et al. 1998</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>97</td>
</tr>
<tr>
<td>Perzigan et al. 1998</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>105</td>
</tr>
<tr>
<td>Pacifcico et al. 1997</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>32</td>
</tr>
<tr>
<td>Abele-Horn et al. 1997b</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>170</td>
</tr>
<tr>
<td>VanWaarde et al. 1997</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>108</td>
</tr>
<tr>
<td>Da Silva et al. 1997</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>108</td>
</tr>
<tr>
<td>Iles et al. 1996</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>40</td>
</tr>
<tr>
<td>Garland et al. 1996</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>44</td>
</tr>
<tr>
<td>Alfa et al. 1995</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>26</td>
</tr>
<tr>
<td>Jonsson et al. 1994</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>89</td>
</tr>
<tr>
<td>Dyke et al. 1993</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>112</td>
</tr>
<tr>
<td>Smyth et al. 1993</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>56</td>
</tr>
<tr>
<td>Payne et al. 1993</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>93</td>
</tr>
<tr>
<td>Saxen et al. 1993</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>45</td>
</tr>
<tr>
<td>Horowitz et al. 1992</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>114</td>
</tr>
<tr>
<td>Izraeli et al. 1990</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>99</td>
</tr>
<tr>
<td>Sanchez et al. 1988</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>111</td>
</tr>
<tr>
<td>Wang et al. 1988</td>
<td>+</td>
<td>NS</td>
<td>+</td>
<td>95</td>
</tr>
<tr>
<td>Cassell et al. 1988</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>48</td>
</tr>
</tbody>
</table>

CLD = chronic lung disease

NS = not studied
Figure 1.
Funnel plot presentation of results of 11 studies in which *U. urealyticum*-related relative risks for the development of chronic lung disease were reported.

The *U. urealyticum* related relative risk for the development of chronic lung disease is presented on the horizontal axis. The numbers of infants in the analysis are presented on the vertical axis. △ indicates studies with no control for prematurity and X indicates studies controlled for prematurity.

2.5.3. *U. urealyticum* and chronic meningitis in premature infants

In 1986 Likitnukul et al. were unable to isolate *U. urealyticum* from 199 CSF samples of infants less than 3 months of age with suspected septic infection. In 1987 Garland et al. described a case report of neonatal meningitis caused by *U. urealyticum*, and Neal et al. (1994) described the case of a preterm infant with intraventricular haemorrhage who had *U.
urealyticum} isolated from the CSF. In 1988 Waites et al. noted that \textit{U. urealyticum} was the commonest bacteria (8%) detected in CSF samples of high-risk premature infants. In 1990 Waites et al. found similar findings in CSF samples from newborn community hospital patients, the rate of positive CSF cultures for \textit{U. urealyticum} being 1.6% in that study. Heggie et al. (1994) found a lower incidence of CSF infection with \textit{U. urealyticum}, 0.2%.

2.5.4. The efficacy of treating neonatal \textit{U. urealyticum} colonization

In 1998 Lyon et al. randomly assigned 75 infants with less than 30 weeks of gestation to receive erythromycin intravenously at birth for seven days or to no treatment. In that cohort, the incidence of \textit{U. urealyticum} infection - detected by cultures of endotracheal samples - was low, 9%, and finally only 3 infants with \textit{U. urealyticum} infection were treated with erythromycin. The treatment with erythromycin did not affect the development of CLD. Similar results were detected by Jonsson et al. in 1998 using randomized treatment in a cohort of 155 infants born before 30 weeks of gestational age. Bowman et al. (1998) studied 124 infants with birth weight less than 1000g and those 22 (18%) with \textit{U. urealyticum} growth in endotracheal samples were treated with erythromycin. There was no difference in the development of CLD between those infants with treated \textit{U. urealyticum} colonization and those with any \textit{U. urealyticum} colonization; the authors claim that the result indicates the efficacy of the treatment.

2.6. \textit{U. urealyticum} and laboratory markers of inflammation

The association between \textit{U. urealyticum} and increased leukocyte counts was originally reported in the case report of Ohlsson et al. (1993). Panero et al. (1995) detected that those premature infants who had \textit{U. urealyticum} growth in endotracheal or blood samples had higher leukocyte counts. The other studies relating \textit{U. urealyticum} with neonatal disease do not report leukocyte counts; this is also the case in studies focusing on \textit{U. urealyticum} and cytokines in endotracheal samples.
In vitro studies of Stancombe et al. (1993) and Crouse et al. (1998) have suggested the ability of *U. urealyticum* to cause an inflammatory reaction. These studies showed that cultivated cell lines released inflammatory cytokines when co-incubated with *U. urealyticum*. Similar findings were detected in vivo by Groneck et al. (1996) and Patterson et al. (1998). However, Lyon et al. (1998) did not find any association between *U. urealyticum* and concentrations of inflammatory cytokines in bronchoalveolar lavage samples.

### 2.7. The influence of *U. urealyticum* on the health of infants after the perinatal era

In 1981 Stagno et al. detected that 21% of the infants who were hospitalized with pneumonitis between 1-3 months of age were infected with *U. urealyticum*. Control infants had a colonization rate of 4%. In contrast, Syrogiannopoulos et al. (1990) prospectively followed full-term infants with and without *U. urealyticum* colonization up to three months of age and did not find any difference in the lower respiratory tract infections between these groups. Apart from these two reports there are no data concerning the health-related effects of perinatal *U. urealyticum* colonization beyond the perinatal era. This is true especially in premature infants.
3. AIMS OF THE STUDY

The main purposes of the present study were to investigate whether the presence of *U. urealyticum* in preterm infants, detected immediately after birth, associates with specific immediate clinical symptoms or signs; whether the presence of *U. urealyticum* is related to the development of CLD; and whether the presence of *U. urealyticum* has an influence on later health of premature infants.

The specific aims were:

1. To evaluate whether the presence of *U. urealyticum* worsens the acute respiratory insufficiency of preterm infants.

2. To determine whether the duration of assisted ventilation or supplemental oxygen are dependent on the presence of *U. urealyticum*.

3. To investigate whether the presence of *U. urealyticum* affects the nonspecific laboratory markers of inflammation.

4. To assess whether the presence of *U. urealyticum* affects the need for later hospital treatment during the first year of life.
4. MATERIAL AND METHODS

4.1. Study subjects

The criterion for enrollment to this study was premature birth before 34 weeks gestation at Kuopio University Hospital. Children born in other hospitals and admitted to the neonatal intensive care unit of Kuopio University Hospital were not enrolled. The clinical presentation of the newborn did not affect the enrollment: infants with congenital malformations or overt other diseases were also included. The children were enrolled in two periods: June 1990 to December 1991 (Cohort A, 115 infants) and February 1993 to July 1994 (Cohort B, 92 infants). Of these 207 children, 17 were excluded because of parental refusal and 14 because of insufficient data or sample collection. Thus, 176 infants constituted the final study cohort; there were 98 infants in cohort A and 78 infants in cohort B. Gestational age was determined by an early gestational ultrasound examination in 82% of cases and after birth by Dubowitz score in all cases. The mothers of the infants had received comparable antenatal care.

4.2. Outcome measures

To assess the presence and severity of respiratory insufficiency, the need for oxygen or assisted ventilation was recorded daily in all study infants. In addition, the highest peak inspiratory pressure, the highest fraction of inspired oxygen and the mode of mechanical ventilation when needed (conventional or high-frequency oscillatory ventilation) were recorded daily. To determine the presence and severity of CLD, the need for supplemental oxygen or assisted ventilation together with chest radiographic findings were evaluated at the age of 28 days and 36 weeks of post-conceptional age in all children.

To measure inflammatory response, serum C-reactive protein concentration and white blood cell counts were recorded for two days in the infants of cohort B. White blood cell counts
were determined by an automated cell counter (Coulter Counter S+). The concentration of C-reactive protein was measured by the immunonephelometric method (Vallance et al. 1991, Montagne et al. 1992).

To assess the need for hospital treatment during the first year of life, hospital admissions and the lengths of hospital stays were recorded retrospectively in infants of cohort A.

4.3. Microbiological determinations

4.3.1. Collection of culture samples

Nasopharyngeal culture samples (163 samples) were obtained as a part of routine care of the respiratory tract. Endotracheal samples (116 samples) were collected if the child required endotracheal intubation within 12 hours after birth. Both nasopharyngeal and endotracheal samples were taken by mechanical suction: the suction catheters were flushed with sterile saline, if necessary, and two drops of the samples were transferred into the transport medium. Blood cultures (127 samples) were taken if septic infection was suspected; after sterile peripheral venipuncture, two drops of blood were inoculated into the transport medium. Culture sampling was restricted to 12 hours after delivery to avoid nosocomial contamination. Tissue samples for *U. urealyticum* cultures were used for cohort A; at autopsy, tissue samples were taken from peripheral lung tissue and from the cortical area of the parietal lobe of the brain. The samples were immediately transported to the laboratory in the transport medium. The transport medium of samples for *U. urealyticum* cultures was 10B broth (Shepard 1983) for cohort A and MS UMM (International Mycoplasma, Signes, France) for cohort B.
4.3.2. Microbiological methods

In all infants, *U. urealyticum* was cultured using A7 agar (Shepard et al 1976). In addition to A7 agar, a Mycofast® All-In cultivation and identification kit (International Mycoplasma, Toulon, France) was used for cohort B. Agar plates and Mycofast cultures were incubated under 5% carbon dioxide for seven and two days, respectively. Bacterial growth was observed daily. *U. urealyticum* was detected on agar by the typical morphology of colonies and by the production of urease, and in the Mycofast kit by their susceptibility pattern to lincomycin, trimethoprim-sulfamethoxazole and erythromycin.

4.4. Data collection

The study infants were followed prospectively as long as they stayed in the hospital; in addition, data on hospital admissions were collected retrospectively during the first year of life in Study III. In Study I, peak inspiratory pressure and fraction of inspired oxygen were recorded using structured data collection; in Study II the data were recorded as raw values.

Kuopio University Hospital, where all the infants were born, services a population of approximately one million people in the eastern and central parts of Finland. In addition, there are four central hospitals with pediatric wards in that district. To obtain data on hospital admissions of the children in cohort A, the clinical charts at these five hospitals were reviewed. The lengths and diagnoses of all hospital stays were recorded and the principal indication for each hospital treatment was noted.

4.5. Definitions

A culture was defined as positive when *U. urealyticum* was grown on A7 agar in Study I, and by growth on both A7 agar and Mycofast® in Study II. *U. urealyticum* colonization was defined as the presence of *U. urealyticum* in samples obtained from the nasopharynx or
trachea. *U. urealyticum* infection was defined as the presence of *U. urealyticum* in samples obtained from the trachea, blood or CSF; and additionally, in Study I from postmortem brain and lung tissue biopsies. The term “*U. Urealyticum* involvement” was used for cases where there was either colonization or infection.

RDS was considered to be present if the child needed supplemental oxygen or assisted ventilation and had typical radiographic findings within 24 hours after birth. Serious respiratory failure was considered to be present if HFOV, FiO2 more than 60%, or peak inspiratory pressures more than 18 cmH2O were needed to correct ventilatory insufficiency. CLD was considered to be present if the child had typical radiographic findings and needed supplemental oxygen or assisted ventilation at the age of 28 days (Shennan et al. 1988). CLD was defined as severe if the child met the criterion of CLD and needed supplemental oxygen or mechanical ventilation at 36 weeks of postconceptional age (Lyon et al. 1998).

The indications for later hospital admissions were classified into four categories: 1) indications related to the respiratory tract, including respiratory tract infections, obstructive airway disease and CLD; 2) indications related to the central nervous system, including rehabilitation periods for disturbed development and treatment periods for complications of prematurity; 3) indications related to prematurity, including study periods for growth and development and hospitalizations for feeding difficulty in early infancy; 4) miscellaneous indications not classified into the three above-defined categories.

**4.6. Ethics**

The study was approved by the Research Ethics Committee of Kuopio University Hospital. Parental consent was obtained before enrollment.
4.7. Statistical analysis

Chi-square analysis and Fisher’s exact probability tests were used for discrete data. The Mann-Whitney test was used for nonparametric data and for non-normally distributed continuous data. Multivariate analysis was performed using logistic regression. The association between *U. urealyticum* and CLD was analyzed in two ways. First, only children who survived for 28 days were included, and second, a sensitivity analysis was performed using the combination “death or CLD”.
5. RESULTS

5.1. Patient characteristics (I, II)

The basic characteristics of the study population are presented in Table 4. No significant differences were detected between these two cohorts. The distribution of gestational age in these two cohorts is presented as combined graphically in Figure 2.

Table 4.

The basic characteristics of the study population in cohorts A and B.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Cohort A</th>
<th>Cohort B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No = 98</td>
<td>No = 78</td>
</tr>
<tr>
<td>Gestational age*</td>
<td>29.6 (27.5-32.5)</td>
<td>31.6 (27.9-33.0)</td>
</tr>
<tr>
<td>Birth weight*</td>
<td>1330 (940-1760)</td>
<td>1480 (1010-1990)</td>
</tr>
<tr>
<td>Apgar score at 1 minute of age*</td>
<td>6 (5-8)</td>
<td>7 (5-8)</td>
</tr>
<tr>
<td>Male gender</td>
<td>52 (53%)</td>
<td>43 (55%)</td>
</tr>
<tr>
<td>Caesarean section</td>
<td>60 (61%)</td>
<td>40 (51%)</td>
</tr>
<tr>
<td>Surfactant use</td>
<td>35 (36%)</td>
<td>33 (42%)</td>
</tr>
<tr>
<td>Dexamethasone use</td>
<td>12 (12%)</td>
<td>15 (19%)</td>
</tr>
</tbody>
</table>

* Values are medians, with 25 and 75 percentiles in parentheses.
Figure 2.
The distribution of gestational age in 176 study infants. Cohorts A and B are combined in this graph.

5.2. Results of cultures for *U. urealyticum* (I, II)

In the total of 176 preterm infants, there were 70 (41%) infants with at least one sample positive for *U. urealyticum*. The results of the cultures by sample sites are presented in Table 5. *U. urealyticum* infection was detected in 43 (44%) of the 98 infants in cohort A and *U. urealyticum* colonization in 11 (14%) of the 78 infants in cohort B.
Table 5.

Detection of *U. urealyticum* by culture in cohorts A and B.

<table>
<thead>
<tr>
<th>Site of culture</th>
<th>Cohort A</th>
<th>Cohort B</th>
</tr>
</thead>
<tbody>
<tr>
<td>samples</td>
<td>No = 98</td>
<td>No = 78</td>
</tr>
<tr>
<td>Nasopharynx (No = 163)</td>
<td>42 (47%)</td>
<td>9 (14%)</td>
</tr>
<tr>
<td>Trachea (No = 116)</td>
<td>28 (41%)</td>
<td>4 (8%)</td>
</tr>
<tr>
<td>Blood (No = 127)</td>
<td>23 (34%)</td>
<td>1 (2%)</td>
</tr>
</tbody>
</table>

The presence of *U. urealyticum* was not associated with low gestational age or low birth weight in either cohort (Tables 6 and 7). There were no significant differences in gender, in Apgar scores at one minute, in the mode of delivery, or in the use of surfactant or dexamethasone between infants with and without *U. urealyticum* culture findings (Tables 6 and 7).
Clinical features in 98 preterm infants with and without *U. urealyticum* infection in cohort A

<table>
<thead>
<tr>
<th></th>
<th>Present (n = 47)</th>
<th>Absent (n = 51)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age (wk)*</td>
<td>28 (27-30)</td>
<td>30 (28-33)</td>
<td>0.06</td>
</tr>
<tr>
<td>Birth weight (gm)*</td>
<td>1200 (810-1630)</td>
<td>1390 (1020-1890)</td>
<td>0.11</td>
</tr>
<tr>
<td>Apgar score at 1 min.*</td>
<td>7 (4-8)</td>
<td>6 (5-8)</td>
<td>0.97</td>
</tr>
<tr>
<td>Male gender</td>
<td>27 (57%)</td>
<td>25 (49%)</td>
<td>0.40</td>
</tr>
<tr>
<td>Caesarean section</td>
<td>26 (55%)</td>
<td>34 (67%)</td>
<td>0.25</td>
</tr>
<tr>
<td>Surfactant</td>
<td>18 (38%)</td>
<td>17 (33%)</td>
<td>0.61</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>3 (6%)</td>
<td>9 (18%)</td>
<td>0.089</td>
</tr>
</tbody>
</table>

* Values are medians, with 25 and 75 percentiles in parentheses.
Table 7.

Clinical features in 78 infants with and without *U. urealyticum* colonization in cohort B

<table>
<thead>
<tr>
<th></th>
<th>Present (n = 11)</th>
<th>Absent (n = 67)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age (weeks)*</td>
<td>29 (27-32)</td>
<td>31 (28-33)</td>
<td>0.16</td>
</tr>
<tr>
<td>Birth weight (g)*</td>
<td>1370 (980-1930)</td>
<td>1500 (1070-1990)</td>
<td>0.53</td>
</tr>
<tr>
<td>Apgar score at 1 min.*</td>
<td>6 (3-9)</td>
<td>7 (5-8)</td>
<td>0.77</td>
</tr>
<tr>
<td>Male gender</td>
<td>7 (64%)</td>
<td>36 (54%)</td>
<td>0.75</td>
</tr>
<tr>
<td>Caesarean section</td>
<td>3 (27%)</td>
<td>37 (55%)</td>
<td>0.086</td>
</tr>
<tr>
<td>Surfactant</td>
<td>6 (55%)</td>
<td>27 (40%)</td>
<td>0.51</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>3 (27%)</td>
<td>12 (18%)</td>
<td>0.43</td>
</tr>
</tbody>
</table>

* Values are medians, with 25 and 75 percentiles in parentheses.

5.3. *U. urealyticum* infection and acute respiratory insufficiency (I, II)

More children with *U. urealyticum* infection in cohort A needed mechanical ventilation than infants not infected. The frequency of RDS was higher in infants with (51%) than those without (20%) *U. urealyticum* infection (p=0.0011). Respiratory failure when present was classified as serious in 79% of infants with and in 55% of infants without *U. urealyticum* infection (p=0.013). The length of mechanical ventilation or the need for supplemental oxygen were not associated with *U. urealyticum* infection (Table 8).

In Study II, in which ventilator settings were recorded in more detail, the infants with *U. urealyticum* colonization required higher fraction of inspired oxygen during assisted ventilation than infants without colonization (FiO2= 0.97 vs. 0.70, median; p=0.020). However, this association was detected only within 24 hours after birth. Furthermore, more infants with *U. urealyticum* colonization required HFOV than did infants without colonization.
(5 of 11 vs. 9 of 67, p=0.010). There were seven infants who were colonized with *U. urealyticum* and needed mechanical ventilation, and six (86%) of them were treated with surfactant.

**Table 8.**

Outcome variables of study infants with and without *U. urealyticum* infection in cohort A

<table>
<thead>
<tr>
<th></th>
<th>Present (n = 47)</th>
<th>Absent (n = 51)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assisted ventilation</td>
<td>44 (94%)</td>
<td>40 (78%)</td>
<td>0.032</td>
</tr>
<tr>
<td>HFOV</td>
<td>17 (36%)</td>
<td>11 (22%)</td>
<td>0.11</td>
</tr>
<tr>
<td>Days of assisted ventilation</td>
<td>5 (2-10)</td>
<td>5 (1-28)</td>
<td>0.47</td>
</tr>
<tr>
<td>Respiratory distress syndrome</td>
<td>24 (51%)</td>
<td>10 (20%)</td>
<td>0.0011</td>
</tr>
<tr>
<td>Serious respiratory failure</td>
<td>37 (79%)</td>
<td>28 (55%)</td>
<td>0.013</td>
</tr>
<tr>
<td>Days of supplemental oxygen</td>
<td>11 (4-66)</td>
<td>22 (2-83)</td>
<td>0.87</td>
</tr>
<tr>
<td>Death</td>
<td>12 (26%)</td>
<td>3 (6%)</td>
<td>0.0070</td>
</tr>
</tbody>
</table>

* Only the 84 children with a need for assisted ventilation included

a: Values are medians with 25 and 75 percentiles in parentheses.

b: Either HFOV, FiO2 > 0.6 or peak inspiratory pressure >18 cmH2O needed

HFOV = high-frequency oscillatory ventilation
In several previous studies, low gestation and low birth weight have been associated with the prevalence of *U. urealyticum* infection. In the present study, neither had significant association with the presence of *U. urealyticum* (Table 7). Logistic regression analysis was used to investigate the role of *U. urealyticum* infection in the development of acute respiratory disease. After controlling for gestational age, gender and Apgar score the association between *U. urealyticum* infection and RDS remained significant in cohort A (*p* = 0.0025, relative risk 4.9, 95% confidence interval 1.7 - 14). Similarly, *U. urealyticum* infection was a significant predictor of the need for HFOV independently of gestational age, gender and Apgar score in cohort B (*p*=0.026, RR = 12, 95% CI 1.4 - 110). However, the need for assisted ventilation and the presence of serious respiratory failure lost their significance after controlling for gestational age, gender and Apgar score.

5.4. *U. urealyticum* infection and mortality (I)

Of the 98 study infants in cohort A, 15 (15%) died, all of them before the age of 3 weeks. The mortality of infants was higher in infants with than in those without *U. urealyticum* infection (Table 8). Even after adjustment for prematurity this association was significant and, in fact, *U. urealyticum* infection was a more important predictor of death than was prematurity (Table 9). Respiratory distress syndrome was considered as the cause of death in 10 infants. In cohort B *U. urealyticum* colonization was not associated with mortality.
Table 9.

The results of logistic regression analysis when death of the patient was used as a dependent variable.

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>$p$</th>
<th>RR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>U. urealyticum</em> infection</td>
<td>0.030</td>
<td>4.7</td>
<td>1.16-19.20</td>
</tr>
<tr>
<td>Gestational age</td>
<td>0.16</td>
<td>0.98</td>
<td>0.78-1.23</td>
</tr>
<tr>
<td>Gender</td>
<td>0.70</td>
<td>0.78</td>
<td>0.59-1.02</td>
</tr>
<tr>
<td>Apgar score</td>
<td>0.071</td>
<td>0.78</td>
<td>0.22-2.75</td>
</tr>
</tbody>
</table>

This logistic regression correctly classified 83% of patients.

5.5. *U. urealyticum* infection and chronic lung disease of preterm infants (IV)

The presence of CLD was assessed at 28 days of age; the infants who died before that were excluded from the analysis. CLD developed in 18 (51%) infants with and in 23 (48%) infants without *U. urealyticum* infection among the 83 infants in cohort A ($p=0.75$). The respective values were 4 (40%) and 21 (35%) for the 70 infants with and without *U. urealyticum* colonization in cohort B ($p=0.76$). There were no specific subgroups formed by different gestational ages or birth weights with any association between *U. urealyticum* and CLD. In sensitivity analysis those infants who died before CLD could be assessed (the age of 28 days) were included in the CLD group to form the “death or CLD” group. This analysis showed no association between *U. urealyticum* and CLD. Even if alternative definitions for CLD were used (the 28 day criterion with and without radiographic criteria or the 36 weeks criterion), no association between CLD and *U. urealyticum* was found.
5.6. *U. urealyticum* infection and hospital admissions during the first year of life (III)

All the 40 infants in cohort A who survived and had both tracheal and blood samples collected for the culture of *U. urealyticum* were followed for 12 months to explore the association of perinatal *U. urealyticum* infection with later need for hospital care. These children required a total of 73 hospital admissions resulting in 734 hospital days until age 12 months. Ninety percent of these admissions were classified into the three main disease categories (respiratory tract-related indications, central nervous system-related indications, and prematurity-related indications). The children with perinatal *U. urealyticum* infection required more inpatient care than those not infected: the difference was significant in hospital days but not in hospital treatment periods (Tables 10 and 11). The difference in hospital days between infants with and those without *U. urealyticum* infection was significant for respiratory diseases but not for other disease categories. Within respiratory diseases, CLD and obstructive airway disease caused more hospital days in children with *U. urealyticum* infection than in those without infection (Tables 10 and 11).
Table 10.

Number of hospital admissions during the first year of life in infants with and those without perinatal *U. urealyticum* infections

<table>
<thead>
<tr>
<th>Reason for hospital admission</th>
<th>Present (No = 22)</th>
<th>Absent (No = 18)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory disease</td>
<td>25</td>
<td>9</td>
<td>0.19</td>
</tr>
<tr>
<td><em>Chronic lung disease</em></td>
<td>11</td>
<td>3</td>
<td>0.051</td>
</tr>
<tr>
<td><em>Obstructive airway disease</em></td>
<td>10</td>
<td>0</td>
<td>0.033</td>
</tr>
<tr>
<td>Neurological disease</td>
<td>5</td>
<td>3</td>
<td>0.43</td>
</tr>
<tr>
<td>Prematurity</td>
<td>8</td>
<td>5</td>
<td>0.91</td>
</tr>
<tr>
<td>Other reason</td>
<td>17</td>
<td>1</td>
<td>0.73</td>
</tr>
<tr>
<td>Total admissions</td>
<td>55</td>
<td>18</td>
<td>0.052</td>
</tr>
</tbody>
</table>

a: One patient with asthma who had CLD in the perinatal period
Table 11.

Number of hospital days during the first year of life in infants with and those without perinatal *U. urealyticum* infections

<table>
<thead>
<tr>
<th>Reason for hospital admission</th>
<th>Present</th>
<th>Absent</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory disease</td>
<td>350</td>
<td>129</td>
<td>0.27</td>
</tr>
<tr>
<td>-Chronic lung disease</td>
<td>269</td>
<td>71</td>
<td>0.045</td>
</tr>
<tr>
<td>-Obstructive airway disease (^a)</td>
<td>50</td>
<td>0</td>
<td>0.033</td>
</tr>
<tr>
<td>Central nervous system disease</td>
<td>47</td>
<td>4</td>
<td>0.40</td>
</tr>
<tr>
<td>Prematurity</td>
<td>82</td>
<td>46</td>
<td>0.89</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>67</td>
<td>9</td>
<td>0.081</td>
</tr>
<tr>
<td>Total</td>
<td>546</td>
<td>188</td>
<td>0.047</td>
</tr>
</tbody>
</table>

\(^a\): One patient with asthma who had CLD in the perinatal period

5.7. *U. urealyticum* colonization and blood leukocyte counts (II)

*U. urealyticum* colonization was detected in eleven (14\%) of 78 infants in cohort B. The peripheral blood leukocyte counts on both the first and the second day after birth were significantly higher in children with *U. urealyticum* colonization than in those not colonized (Table 12). This association persisted even if the six children with other bacterial growths in the tracheal samples were excluded from the analysis. Notably, all blood cultures were sterile for conventional bacteria. CRP concentrations were low in both groups, the medians being < 10 mg/ml (N.S.).
Leukocyte counts in peripheral blood in infants with and those without *U. urealyticum* colonization.

<table>
<thead>
<tr>
<th></th>
<th>Present (No = 11)</th>
<th>Absent (n=67)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>18.6 (2.7)</td>
<td>12.4 (1.1)</td>
<td>0.012</td>
</tr>
<tr>
<td>Day 2</td>
<td>29.0 (9.2)</td>
<td>15.4 (2.2)</td>
<td>0.013</td>
</tr>
</tbody>
</table>

Values are means, with standard error of the mean in parentheses.
6. DISCUSSION

In this thesis, U. urealyticum involvement was associated with the presence and severity of acute respiratory failure. As an indicator of this, the infants with U. urealyticum involvement needed more intensive treatment to achieve adequate ventilation. They needed higher fraction of inspiratory oxygen in a ventilator and more often needed high-frequency oscillatory ventilation than infants without U. urealyticum. Furthermore, the clinical diagnosis of respiratory distress syndrome was commoner (51%) in infants with U. urealyticum infection than in children without infection (20%). The development of chronic lung disease was not associated with U. urealyticum in these two cohorts. The presence of U. urealyticum infection was associated with high mortality (26%).

Peripheral blood white cell counts were elevated in infants with U. urealyticum colonization within two days after birth. The need for hospital treatment during the first year of life was associated with U. urealyticum infection. When compared with non-infected infants, the infants with U. urealyticum infection needed more hospital days especially for chronic lung disease.

6.1. Prevalence of U. urealyticum colonization and infection

The frequency of U. urealyticum infection was 44% in the earlier cohort and the frequency of U. urealyticum colonization 14% in the later cohort. These rates compare well with those reported in previous studies, in which the rate of U. urealyticum colonization has varied from 0% to 80% and the rate of U. urealyticum infection from 13% to 55% (see Tables 1 and 2). The prevalence of U. urealyticum involvement has been reported to be dependent on socio-economic factors, age and ethnic factors in adult populations (Harrison et al. 1983, Carey et al. 1991). In the present study, the patients were rather homogenous with respect to these factors. The mothers of the study infants were all of Finnish origin, and at that time socio-
economic differences were low in Finnish society. In the perinatal era, the rate of both vertical transmission (Sanchez et al. 1990, Abele-Horn et al. 1997b) and neonatal colonization (Izraeli et al. 1991, Alfa et al. 1995, Horowitz et al. 1992) of *U. urealyticum* have been inversely related with gestational age. Neonatal colonization with *U. urealyticum* may also be dependent on maternal age (Bowman et al. 1998). In the present study, as in some others (Hannaford et al. 1999, Abele-Horn et al. 1998, Perzigian et al. 1998), the rates of *U. urealyticum* involvement were not associated with gestational age or birth weight. To some extent, the variations in *U. urealyticum* involvement between different studies reflect different patient selection and natural variation in maternal status of *U. urealyticum*. Furthermore, diagnostic methods have varied; repeated sample collection (Pacifico et al. 1997, Illes et al. 1996) and new sensitive methods such as PCR (Nelson et al. 1998), for example, increase positive *U. urealyticum* findings. These facts emphasize the role of microbiological approach and patient selection when investigating *U. urealyticum* involvement in infants.

6.2. The role of *U. urealyticum* in acute respiratory failure

An association was detected between *U. urealyticum* involvement and different variables used to measure acute respiratory disease. The need for assisted ventilation and the severity of respiratory failure were such parameters in the first cohort and the need for HFOV and the need for inspiratory oxygen in the second cohort. These findings suggest that the preterm infants with *U. urealyticum* involvement may develop more severe acute respiratory disease than infants without *U. urealyticum*.

6.2.1 *U. urealyticum* and the prevalence of respiratory distress syndrome

A significant association was detected between respiratory distress syndrome and *U. urealyticum* infection. The association between *U. urealyticum* infection and RDS remained significant also after controlling for prematurity in a logistic regression model. The
association between RDS and *U. urealyticum* involvement has been evaluated in only a few previous studies: many studies focusing on CLD and *U. urealyticum* have not reported data on RDS (Horowitz et al. 1992, Smyth et al. 1993, Wang et al. 1988, Payne et al. 1993, Valencia et al. 1993, Jonsson et al. 1994, Panero et al. 1995, Iles et al. 1996, Bowman et al. 1998). Furthermore, in some previous studies all enrolled infants have had RDS (Pacifico et al. 1997, Heggie et al. 1994), thus rendering impossible the evaluation of the role of *U. urealyticum* in that disease. No association was detected between *U. urealyticum* and RDS in the studies of Cassell et al. (1988) and Saxen et al. (1994), both of which used populations with high prevalence of RDS (93% and 96%, respectively), or in the study of Sanchez et al. (1988). Apart from the present investigation, there is only one study (Abele-Horn et al. 1997b) suggesting an association between *U. urealyticum* colonization and the development of RDS.

The ability of *U. urealyticum* to cause acute respiratory disease has been documented in animal studies (Rudd et al. 1985, Crouse et al. 1990, Walsh et al. 1993) in which inoculation of *U. urealyticum* has caused pneumonia in newborn animals. The ability of *U. urealyticum* to cause pneumonia in humans has been suggested in many case reports (Quinn et al. 1985, Waite et al. 1989, Brus et al. 1991), as well as in three prospective clinical studies (Crouse et al. 1993, Panero et al. 95, Pacifico et al. 1997). The association between *U. urealyticum* infection and acute respiratory disease was detected in the present study. It is well-known that bacterial pneumonia in preterm infants may be indistinguishable from RDS (Ablow et al. 1977). If that is also the case for *U. urealyticum* pneumonia, the trials focusing on the association of *U. urealyticum* with RDS or pneumonia may actually evaluate the same process.

6.2.2 *U. urealyticum* and the severity of acute respiratory failure

In previous reports, the severity of acute respiratory decompensation has attracted only minor interest, and the need for HFOV has not been used as an indicator of severity of
respiratory decompensation. However, the length of assisted ventilation is reported in some previous studies, but such studies show conflicting results. Some studies (Saxen et al. 1993, Horowitz et al 1992, Jonsson et al. 1994 and Lyon et al. 1998) show no association between *U. urealyticum* infection and the length of mechanical ventilation. The studies of Panero et al. (1995), Pacifico et al. (1997) and Perzigian et al. (1998) show longer ventilator therapies in infants with than in those without *U. urealyticum* infection. However, these studies did not adjust their results for gestational age. It is notable that in the present study the need for HFOV was significantly associated with *U. urealyticum* infection even after adjustment for gestational age. In the study of Bowman et al. (1998) no difference was detected in the maximal ventilator settings when comparing infants with and those without *U. urealyticum* colonization.

Most previous studies focusing on respiratory disease and *U. urealyticum* have been designed to investigate CLD; thus, acute events have been poorly documented. Often, the study design rendered impossible the evaluation of the association between *U. urealyticum* and the need for assisted ventilation: in nine studies, for example, only intubated infants were included (Crouse et al. 1993, Cassell et al. 1988, Saxen et al. 1994, Jonsson et al. 1994, Panero et al. 1997, Iles et al. 1996, vanWaarde et al. 1997, Lyon et al. 1998 and Bowman et al. 1998). Furthermore, many studies have not reported at all the need for mechanical ventilation (Alfa et al. 1995, Payne et al. 1993, Heggie et al. 1994, Wang et al. 1988, Smyth et al. 1993). In the present study, both the need for and the mode of assisted ventilation were carefully documented and a significant association with *U. urealyticum* involvement was demonstrated.

6.3. *U. urealyticum* and chronic lung disease

In the present study no association was found between *U. urealyticum* and CLD. On the basis of previous data, the role of *U. urealyticum* in the development of CLD had remained unclear. Of the 21 previous studies, 13 have shown and eight have failed to show an
association between *U. urealyticum* and CLD (see Table 2). Two of the three studies in which the effect of antibiotic treatment against *U. urealyticum* has been evaluated have shown no beneficial effect on the development of CLD (Jonsson et al. 1998, Lyon et al. 1998, Bowman et al. 1998). A recently published meta-analysis of previous studies supported an association between *U. urealyticum* colonization and the development of CLD (RR 1.72 CI 1.5 to 1.96; Wang et al. 1995).

Because the frequency of CLD increases with lowering gestational age, as shown also in the present study, gestational age is an important confounding factor. In 1997, van Waarde et al. showed that 56% of the reports suggesting an association between *U. urealyticum* and CLD lacked the control for gestational age. As seen in Table 3, eight studies showed and 13 studies failed to show that low gestational age increases the prevalence of *U. urealyticum*. Figure 1 presents the results of eleven previous studies in which relative risks for the development of CLD were reported. Figure 1 shows that the relative risks are low in many studies. In addition, the shape of the plot is asymmetrical, suggesting a publication bias for not publishing studies without a detected effect.

Many previous studies that have failed to detect an association between CLD and *U. urealyticum* were carried out in Europe (Smyth et al. 1993, Saxen et al. 1994, Jonsson et al. 1994, vanWaarde et al. 1997)), as was the present study. In contrast, the majority of studies that have shown an association are of non-European origin (Wang et al. 1988, Sanchez et al. 1988, Cassell et al. 1988, Izraeli et al. 1990, Horowitz et al. 1992, Alfa et al. 1995, Garland et al. 1996, Perzigian et al. 1998, Hannaford et al. 1999). Differences between centers in the principles of treatment of premature infants may influence the development of CLD and the role of *U. urealyticum* in it. The etiology of CLD is multifactorial being dependent on many aspects of treatment, e.g. nutrition (Hallman et al. 1992) and fluid therapy (Van Marter et al. 1990). The prevalence of *U. urealyticum* may be dependent on ethnic factors (Carey et al. 1991). In the present study, the treatment was standardized and
all infants were of the same ethnic origin, increasing the reliability of the observed negative result.

The frequency of CLD, 37%, detected in this study, compares well with the 17% (Sanchez et al. 1988) to 60% (Iles et al. 1996) frequencies in previous studies. The rates of treatments with dexamethasone and exogenous surfactant were similar in U. urealyticum positive and negative infants. Also other clinical characteristics were rather similar between U. urealyticum positive and negative groups.

In this study the presence of CLD was assessed at 28 days of age. The infants who died before that age may have biased the results. Because of that, sensitivity analysis was performed, by analyzing “death or CLD” group. This analysis did not affect the results.

In many previous studies, evaluation of the association between U. urealyticum and CLD has focused on infants who were more premature than those in this study: the study of Wang et al. (1988) enrolled only infants with birth weight less than 1250g, and in the analysis of Cassell et al. (1988) U. urealyticum was related to CLD only in infants with birth weight less than 1000g. In this study, there was no association between CLD and U. urealyticum involvement even when different gestational ages or birth weights were used for analysis.

The definitions of U. urealyticum colonization and infection has varied in previous studies, as discussed previously, as has the definition of CLD (Sanchez et al. 1988, van Waarde et al. 1997, Lyon et al. 1998). In the present study, no associations were observed between CLD and the presence of U. urealyticum even when alternative criteria for the presence of U. urealyticum and CLD were employed.
6.4. *U. urealyticum* and non-specific markers of inflammation

The infants with *U. urealyticum* colonization had higher blood leukocyte counts than infants without colonization. However, CRP values were identical. Increased leukocyte levels are commonly accepted to represent a sign of inflammation. Blood cultures were negative for conventional bacteria and the association between elevated white cell count and *U. urealyticum* colonization persisted even when those few infants with other bacterial growth in tracheal samples were excluded from the analysis.

Previously, increased leukocyte counts have been associated with *U. urealyticum* infection in the studies of Ohlsson et al. (1993) and Panero et al. (1995). Leukocyte levels have not been reported in most other studies. The ability of *U. urealyticum* to cause an inflammatory response has been shown in studies in which the presence of *U. urealyticum* has been associated with elevated cytokine concentrations (interleukine-6, interleukine-8 and tumor necrosis factor-alpha) in vitro (Stancombe et al. 1993, Crouse et al. 1998). These findings have also been detected in vivo: Patterson et al. (1998) studied 96 preterm infants and showed that *U. urealyticum* colonization was associated with elevated cytokine concentrations (interleukine-1-beta and tumor necrosis factor-alpha) in tracheobronchial aspirates. Comparable findings were detected in the study of Groneck et al. (1996).

6.5. The influence of *U. urealyticum* on later health

The effects of *U. urealyticum* involvement on the health of preterm infants beyond the perinatal era has not previously been studied. The results of this study showed that those infants who had *U. urealyticum* infection at birth require more hospital care than infants without infection. The increased need for hospital care resulted mainly from hospital admissions for respiratory diseases. Interestingly, the difference between infants with and without *U. Ureaplasma* infection was significant only in relation to CLD.
As discussed above, CLD was not associated with the presence of *U. urealyticum* but acute respiratory insufficiency was. The fact that the infants who had *U. urealyticum* infection at birth still needed more hospital care for CLD until age 12 months indicates that infants with infection recover from CLD slowly. Because no culture samples were collected after 12 hours after birth, the possibility of continuous *U. urealyticum* infection in the respiratory tract can not be ruled out. However, *U. urealyticum* is not considered a pathogen beyond the perinatal era (Syrogiannopoulos et al. 1990). This increased need for hospital care for CLD during the first year of life is caused by more serious respiratory disease these infants had initially.

The results of the study suggest that perinatal *U. urealyticum* infection affects infants' health beyond the perinatal era. No previous studies have evaluated the effect of prenatal *U. urealyticum* involvement on later health of preterm infants. In 1981, Stagno et al. showed an increased rate of *U. urealyticum* infection in full-term infants aged 1 to 3 months with hospital admission for pneumonitis, when compared with control infants. However, Syrogiannopoulos et al. (1990) studied the presence of *U. urealyticum* colonization at birth and later respiratory tract infections in full-term infants, and found no association.

### 6.6. Methodological aspects

All the preterm infants included in this study were born in the study center, and all had less than 34 weeks of gestational age. This study did not focus particularly on very or extremely low birth weight infants. However, gestational age was included as a confounding factor and in some analyses specific subgroups, formed by different gestational ages and birth weights, were studied.
6.6.1. Microbiological methods

The study population consisted of two separate cohorts. Different microbiological methods were used for these cohorts. For the cultures for *U. urealyticum*, A7 agar (Shepard et al. 1976) was used for both cohorts. In addition, a commercial Mycofast® cultivation and identification kit was used for the later cohort. To avoid false-positive culture results, growth on both culture methods was required before a sample was regarded as positive. This led to a significant difference in positive culture results between the two cohorts, as was expected. PCR or other sensitive non-cultural methods were not used. The sensitivity of cultural methods, when compared with PCR methods, is from 77% (Nelson et al. 1998) to 94% (Luki et al. 1998). The selection of strict criteria for the presence of *U. urealyticum* in clinical samples reduced the risk of false positive cases, but some true positive cases were probably missed, leading rather to underestimation than overestimation of the role of *U. urealyticum*.

The difference in the frequency of positive cultures may also reflect natural variation in the prevalence of *U. urealyticum* infection: a decrease in the incidence of *U. urealyticum* infection over time has been reported (Lyon et al. 1998).

Culture sampling was restricted to 12 hours after birth to avoid nosocomial contamination (Sanchez 1993). That restriction may have led to underestimation of the true prevalence of *U. urealyticum* in study infants: repeated culture sampling within seven days after birth has been shown to find more cases than were found immediately after birth (Iles et al. 1996, Pacifico et al. 1997). However, the repeated culture sampling may, at least potentially, detect cases with horizontal transmission of *U. urealyticum*; the frequency of horizontal spread is not known (Sanchez 1993).

Serologic tests share some problems in the diagnostics of *U. urealyticum*: multiple serovars of *U. urealyticum* are often found in a single clinical specimen (Kong et al. 1999a); antigen variability of *U. urealyticum* has been reported (Cheng et al. 1994, Zheng et al.1992); and
newborns show weak serologic responses (Dinismoor et al. 1989). Serologic tests were not used to detect *U. urealyticum* in this study.

### 6.6.2. Definition of infection and colonization

The definitions of *U. urealyticum* colonization and infection have varied in previous reports. Colonization is often defined as the presence of *U. urealyticum* in the samples obtained from the nasopharynx or trachea (Jonsson et al. 1998, Patterson et al. 1998, DaSilva et al. 1997, Jonsson et al. 1994, Payne et al. 1993, Horowitz et al. 1992). Another common definition for *U. urealyticum* colonization is to regard colonization as positive endotracheal samples (Abele-Horn et al. 1998, Cordero et al. 1997, Abele-Horn et al. 1997b, vanWaarde et al. 1997, Smyth et al. 1993). Some studies have used culture sampling from the nasopharynx, trachea or gastric aspirate (Wang et al. 1988); likewise, eye, throat, vagina and rectum (Sancez et al. 1987, 1988 and 1990), throat, eyes and vagina (Syrogiannopoulos et al. 1990), or ear and gastric aspirates (Luton et al. 1994) have been used to define *U. urealyticum* colonization.

In some studies, the presence of *U. urealyticum* in endotracheal samples is taken as a sign of *U. urealyticum* infection (Lyon et al. 1998, Iles et al. 1996). A wider definition of *U. urealyticum* infection was used by Pacifico et al. (1997), who included all infants with a positive culture in samples obtained from the nasopharynx, trachea or blood in the infection group. *U. urealyticum* infection has also been defined as positive findings in the cultures of endotracheal or blood samples (Cassell et al. 1988), and *U. urealyticum* infection of the central nervous system as positive CSF samples (Waites et al. 1988 and Waites et al. 1990).

In this thesis, *U. urealyticum* colonization was defined as the presence of *U. urealyticum* in samples obtained from the nasopharynx or trachea. *U. urealyticum* infection was defined as the presence of *U. urealyticum* in samples obtained from the trachea, blood or CSF, and,
additionally, from postmortem lung and brain tissue samples in the first cohort. The presence of *U. urealyticum* in any sample is termed "*U. urealyticum* involvement". These definitions compare well with the definitions most commonly used in previous studies. Invasive samples were not obtained in infants without clinical disease; consequently, endotracheal and blood samples were not obtained in all infants.

### 6.6.3. Study population

All 176 study infants in the two cohorts were born at the Kuopio University Hospital: infants born in other centers and admitted to this hospital were not enrolled. All preterm deliveries with lower than 34 weeks gestational age in the County of Kuopio are cared for in the Kuopio University Hospital. However, the Kuopio University Hospital serves as referral center for three other counties with four central hospitals. In the present study, the mothers in three central hospitals with impending preterm delivery at 28 weeks or less of gestational age were referred to the study center prior to labor. Thus, the study infants represent a sample of newborn premature infants from a distinct geographic area.

The number of preterm deliveries decreases with decreasing gestation. However, because three central hospitals referred mothers with preterm delivery before 28 weeks of gestational age, the distribution of gestational age was two-peaked. The highest frequencies were at 28 and 33 weeks (Figure 4).

When the results of the present study are included, the majority of European reports do not suggest an association between *U. urealyticum* and CLD, whereas most non-European studies do. The organisation of hospital care in Finland differs from that in North America, where mothers with impending preterm delivery are not centralized in referral centres, although infants are after birth. In Finland, mothers with impending preterm delivery are admitted prior to delivery to centres with neonatal intensive care units. This difference in treatment policy is important when comparing the results of the present study to those of

The study infants of the present thesis represented unselected cohorts of infants who were all born in a study centre with a clear geographical cover. This makes the results of the evaluation applicable to clinical practice.
6.6.4. Statistical analyses

This thesis evaluated the possible associations of *U. urealyticum* involvement with diseases and outcome of premature infants. Many diseases of premature infants, such as CLD, are more severe and more common in more premature infants. Consequently, the main results were controlled for low gestational age by using a logistic regression model. Many previous studies have evaluated the role of *U. urealyticum* in specific groups of preterm infants, such as very low birth weight infants. Therefore, repeated analyzes were done using different birth weight and gestational age cut-off points. Furthermore, sensitivity analysis was performed in the analysis of CLD to avoid bias caused by patients dying before CLD was defined. The two cohorts were rather large, 98 and 87 infants, allowing a reliable statistical analysis of the data.
7. CONCLUSIONS

1. The perinatally acquired *U. urealyticum* involvement associates with acute respiratory disease in preterm infants. That is indicated by the increased needs for different treatment modalities in infants with the presence of *U. urealyticum*. To achieve adequate ventilation, more infants with the presence of *U. urealyticum* needed assisted ventilation, high frequency oscillatory ventilation and a higher fraction of inspired oxygen in the ventilator.

2. The difference in the fraction of inspired oxygen was only detected on the first day of life. This is an important finding to keep in mind when designing future intervention studies. If the influence of *U. urealyticum* on the respiratory disease is strongest immediately after birth, antimicrobial therapy may have too little time to show clinical effect. Complementing the treatment of neonatal disease with antenatal antibiotic treatment should be evaluated.

3. As the presence of *U. urealyticum* in premature infants associates with both clinical respiratory disease and inflammatory response, the role of *U. urealyticum* as a commensal microbe should be re-evaluated, at least in preterm infants.

4. The effect of *U. urealyticum* on the respiratory morbidity of preterm infants is seen up to twelve months’ age, so is not limited to the perinatal era.

5. The role of *U. urealyticum* in the development of chronic lung disease remains unclear.
8. REFERENCES


Chua KB, Ngeow YF, Ng KB, Chye JK, Lim CT. Ureaplasma urealyticum and Mycoplasma hominis isolation from cervical secretions of pregnant women and nasopharyngeal secretions of their babies at delivery. Singapore Med J 1998;39:300-302.


Martinez MA, Ovalle A, Santa-Cruz A, Barrera B, Vidal R, Aguirre R. Occurrence and antimicrobial susceptibility of Ureaplasma parvum (Ureaplasma urealyticum biovar 1) and Ureaplasma urealyticum (Ureaplasma urealyticum biovar 2) from patients with adverse pregnancy outcomes and normal pregnant women. Scand J Infect Dis 2001;33:604-10.


