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The Alabama Preterm Birth Study: Umbilical Cord Blood *Ureaplasma urealyticum* and *Mycoplasma hominis* Cultures in Very Preterm Newborns

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Abstract

Objective—To evaluate the frequency of umbilical cord blood infections with *Ureaplasma urealyticum* and *Mycoplasma hominis* in preterm 23 to 32 week births and to determine their association with various obstetric conditions, markers of placental inflammation, and newborn outcomes.

Study Design—351 mother/infant dyads with deliveries between 23 and 32 weeks gestational age (GA) who had cord blood cultures for *Ureaplasma urealyticum* and *Mycoplasma hominis* had their medical records abstracted, other placental cultures performed, cord IL-6 levels determined, placentas evaluated histologically, and infant outcomes determined.

Results—*Ureaplasma urealyticum* and/or *Mycoplasma hominis* were present in 23% of cord blood cultures. Positive cultures were more common in infants of nonwhite women (27.9 vs 16.8%, p = 0.016), in women less than 20 years of age, in those undergoing a spontaneous compared to an indicated preterm delivery (34.7 vs 3.2%, p = 0.0001), and in those delivering at earlier gestational ages. Intrauterine infection and inflammation were more common among infants with a positive *Ureaplasma urealyticum* and *Mycoplasma hominis* culture as evidenced by placental cultures for these and other bacteria, elevated cord blood IL-6 levels, and placental histology. Infants with positive cord blood *Ureaplasma urealyticum* and *Mycoplasma hominis* cultures were more likely to have neonatal SIRS (41.3 vs 25.7%, p = 0.007, AOR 1.86, 1.08 – 3.21) and probably BPD (26.8 vs 10.1%, p = 0.0001, AOR 1.99, 0.91 – 4.37), but were not significantly different for other neonatal outcomes including RDS, IVH or death.

Conclusion—*Ureaplasma urealyticum* and *Mycoplasma hominis* cord blood infections are far more common in spontaneous versus indicated preterm deliveries and are strongly associated with

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markers of acute placental inflammation. Positive cultures are associated with neonatal systemic inflammatory response syndrome and probably bronchopulmonary dysplasia.

Keywords

Ureaplasma urealyticum; *Mycoplasma hominis*; preterm birth; umbilical cord blood; placental histology; neonatal outcome; neonatal systemic inflammatory response syndrome; bronchopulmonary dysplasia

INTRODUCTION

Ureaplasma urealyticum and *Mycoplasma hominis* are among the organisms most frequently isolated from both placental membranes and amniotic fluid in women with histologic and clinical chorioamnionitis. [1-4] These organisms are commonly found within the uterus in association with spontaneous preterm labor and with preterm premature rupture of the fetal membranes (PPROM).[2,5,6] The earlier the gestational age, the more likely these organisms are to be present in the amniotic fluid, the placenta or in the free membranes.[5] In addition, the intrauterine presence of either organism has been associated with an increased production of a wide variety of cytokines, matrix metalloproteinases and prostaglandins, all believed to be in the causal pathway and/or precursors for spontaneous preterm labor and PPROM.[7-9]

In individual cases, fetal or neonatal infections with these mycoplasmas have been associated with a number of adverse outcomes including chronic lung disease, pneumonias, cerebral white matter lesions, cerebral palsy and death. [4,10,11] However, the proportion of preterm infants that have positive cord blood *Ureaplasma urealyticum* and *Mycoplasma hominis* cultures at birth is unknown, as are the associated risk factors or obstetric conditions, the placental histologic patterns, and the neonatal outcomes. In this study, we evaluated umbilical cord blood cultures for *Ureaplasma urealyticum* and *Mycoplasma hominis* in 351 infants delivered at 23-32 weeks gestational age (GA) and specifically compared the results with various maternal characteristics, obstetric diagnoses, placental histologic findings, cord blood IL-6 levels, and various newborn outcomes.

MATERIALS AND METHODS

The overall Alabama Preterm Birth Study, which included 457 consecutive singleton deliveries of infants born between 23 and 32 weeks from 1996 to 2001, has been described previously. [12-17] Findings from this data set related to IL-6 levels,[12] inflammatory placental lesions,[13] male/female differences in placental inflammatory markers, [14] the use of corticosteroids in the face of placental inflammation,[15] placental histolologic findings in recurrent preterm births,[16] and the importance of the placental lesion - diffuse decidual leukocytoplastic necrosis[17] - have all been evaluated. The study reported here specifically focuses on the subset of 351 women/infant pairs in this population who had umbilical cord blood cultures for *Ureaplasma urealyticum* and *Mycoplasma hominis*.

A maternal chart review was performed by a trained research nurse to gather demographic and obstetric characteristics such as maternal race, education, age and parity, smoking status, diabetes and preeclampsia, and whether the delivery was spontaneous or indicated. The diagnosis of preclampsia required a blood pressure of greater than 140/90 on at least one occasion and the presence of proteinuria (1+ or greater). Spontaneous preterm birth was defined as delivery after either spontaneous preterm labor or spontaneous preterm premature rupture of membranes (PPROM). Indicated preterm birth was defined as delivery effected for maternal or fetal indications.

Umbilical cord blood was collected from 351 of the preterm infants using an aseptic technique including cleansing of the needle puncture site with alcohol. The cord blood was cultured for *Ureaplasma urealyticum* and *Mycoplasma hominis* as previously described,[18] and it is these infants that are the focus of this study. Failure to collect cord blood cultures was generally due to insufficient blood available after the routine clinical cord blood studies were obtained. Il-6 was assayed as previously reported.[12] Values greater than 34.5 pg/mL, the 95th percentile of women who had an indicated preterm birth in this population, were considered elevated. The chorioamnionic space was cultured for *Ureaplasma urealyticum* and *Mycoplasma hominis* and other aerobic and anaerobic organisms as previously described.[19] Membrane cultures for *Ureaplasma urealyticum* and *Mycoplasma hominis* as well as other organisms were available for all 351 of the placentas where cord blood was available.

Placental histology for each of the 351 preterm neonates with cord blood cultures was available for study. In each case, a minimum of two membrane rolls, two complete sections of umbilical cord (one from the placental and one from the fetal end of the cord), and two to four transmural parenchymal sections were submitted for routine histology. All cases were evaluated histologically by a single pathologist (OF-P), using a placental evaluation protocol adapted from Bendon et al.[20] For this study, the free membranes, chorionic plate, and umbilical cord were evaluated qualitatively for the presence of polymorphonuclear (PMN) infiltration. A PMN infiltration was considered present with PMNs in an essentially linear distribution. Specifically, scattered, entrapped, single or sparsely distributed cells were not scored as an infiltration. The number of PMNs in an infiltration generally exceeded 20/ high power field (HPF 400x) but 5-10 or more/HPF were also scored as a PMN infiltration. The membranes, chorionic plate, and decidua basalis were evaluated for the presence of chronic lymphohistiocytic and plasmacytic inflammation, and the chorionic plate and cord for thrombosis.[21-24] The umbilical cord was evaluated for funisitis, defined as neutrophilic infiltrate in cord vessel walls and/or Wharton's jelly. The decidua basalis was also evaluated for the presence of plasma cells, acute microfocal hemorrhages, and hemosiderin. In addition, the decidua basalis was evaluated for the presence of diffuse decidual leukocytoclastic necrosis, a band-like distribution of coagulative necrosis variably admixed with a deeper region containing karyorrhectic debris.[17]

Neonatal outcome data through hospital discharge or death was also recorded. Neonatal systemic inflammatory response syndrome (SIRS) was defined as the presence of negative cerebrospinal fluid and blood cultures plus clinically suspected sepsis or a band: band + polymorphonuclear cell ratio of 0.15 or greater. The diagnoses of grade 3 or 4 intraventricular hemorrhage (IVH) or cystic PVL were made using ultrasound criteria.[25] Necrotizing enterocolitis (NEC) stage 2 or greater was considered present if diagnosed clinically by the neonatologist. Respiratory distress syndrome (RDS) was defined as the documentation of any of these 3 criteria: (1) infant oxygen requirement at 6 hours through 24 hours of life, (2) an abnormal chest radiograph consistent with RDS within the first 24 hours of life, and (3) need for surfactant. Bronchopulmonary dysplasia (BPD) was defined as infant oxygen requirement at 28 days and chronic lung disease as oxygen requirement at 36 weeks of life.

Data analyses were performed with SAS version 8 software (SAS Institute, Inc, Cary, NC). Frequencies and means between groups were compared with the use of Chi-square tests for discrete variables and t tests and analysis of variance for continuous variables. Logistic regression analyses, adjusting for gestational age, race and infant sex, were used to determine the odds ratio (OR) for a positive *Ureaplasma urealyticum* and/or *Mycoplasma hominis* culture to be associated with spontaneous preterm birth, various placental findings, and with the newborn outcomes SIRS and BPD. A P-value ≤ 0.05 was chosen to define statistical significance. The study was approved by the UAB Institutional Review Board.

RESULTS

A positive culture for *Ureaplasma urealyticum* or *Mycoplasma hominis* or both was present in 82 of the 351 (23.4%) umbilical cord bloods of infants delivering at 23-32 weeks. Of the women with a positive cord blood culture for *Ureaplasma urealyticum* and/or *Mycoplasma hominis*, 43 (52%) had *Ureaplasma urealyticum* only, 21 (26%) had *Mycoplasma hominis* only, and 18 (22%) had both *Ureaplasma urealyticum* and *Mycoplasma hominis.* Since the results were generally similar for women with *Ureaplasma urealyticum* or *Mycoplasma hominis*, for the analyses described below, we elected to dichotomize our analyses so that those with *Ureaplasma urealyticum*, *Mycoplasma hominis*, or both were considered positive, while those without *Ureaplasma urealyticum* or *Mycoplasma hominis* were considered negative.

Infants with positive cord blood cultures for *Ureaplasma urealyticum* and/or *Mycoplasma hominis* weighed less (1122 ± 350g vs 1204 ± 402g, p = 0.0003) and were born one week earlier (28.0 ±2.4 wks vs 29.0 ± 2.1 wks, p = 0.098). Table 1 presents the relationship of positive *Ureaplasma urealyticum* and/or *Mycoplasma hominis* cultures to maternal and obstetric characteristics. Non-white women and women <20 years of age were significantly more likely to be culture positive as were those who had a spontaneous preterm birth. The earlier the gestational age at delivery, the greater the likelihood of being culture positive. On the other hand, women with preeclampsia and an indicated preterm birth were significantly less likely to have a positive cord blood culture for *Ureaplasma urealyticum* and/or *Mycoplasma hominis*. Adjusting for maternal race, GA and infant sex, the OR and 95% CI for women with an indicated preterm birth to have a positive *Ureaplasma urealyticum* and/or *Mycoplasma hominis* culture was 0.07, 0.02 – 0.19, (p <0.0001) and for preeclampsia, 0.07, 0.03 – 0.20 (p <0.0001).

Placentas from infants with a positive cord blood culture for *Ureaplasma urealyticum* and/or *Mycoplasma hominis* were significantly more likely to have positive placental cultures for any organism and for *Ureaplasma urealyticum* and/or *Mycoplasma hominis* specifically. (Table 2) In addition, positive cord blood cultures for *Ureaplasma urealyticum* and/or *Mycoplasma hominis* were significantly more common in the presence of elevated IL-6 levels. Also, placentas with acute inflammation in the free membranes, chorionic plate and umbilical cord were significantly more likely to have positive *Ureaplasma urealyticum* and/or *Mycoplasma hominis* cultures. None of the other placental histologic characteristics, including the presence of decidual plasma cells and diffuse decidual leukocytoclastic necrosis, decidual membrane necrosis, and hemosiderin and microfocal hemorrhage in the decidua basalis were associated with positive *Ureaplasma urealyticum*/*Mycoplasma hominis* cultures. The OR and 95% CI for an elevated IL-6 to be associated with positive *Ureaplasma urealyticum* and/or *Mycoplasma hominis* cultures was 5.82, 3.15 – 10.78 (p <0.0001). For acute inflammation of the free membranes and funisitis, the ORs and 95% CIs for having positive *Ureaplasma urealyticum* and/or *Mycoplasma hominis* cultures were 5.18, 2.86 – 9.39 (p <0.0001) and 3.33, 1.94 – 5.70, respectively (p <0.0001).

Infants whose cord blood cultures were positive for *Ureaplasma urealyticum* and/or *Mycoplasma hominis* were significantly more likely to have neonatal systemic inflammatory response syndrome (41.3 vs 25.7%, p=0.007) and bronchopulmonary displasia (26.8 vs 10.1%, p = 0.0001) but were not significantly different for other neonatal outcomes including RDS, IVH or death. (Table 3) Adjusting for maternal race, GA and infant sex, the OR and 95% CI for a positive *Ureaplasma urealyticum* and/or *Mycoplasma hominis* culture to be associated with SIRS was 1.86, 1.08 – 3.21, (p = 0.026) and for BPD 1.99, 0.91 – 4.37. (p = 0.0872)

COMMENT

Ureaplasma urealyticum and *Mycoplasma hominis* are commonly found in vaginal and cervical fluids with rates reported as high as 80%.[4,26] Most studies find little relationship between these lower genital tract colonizations and various adverse pregnancy outcomes, although they are more common in women with risk factors for preterm birth such as young maternal age and black race.[4,10,26]

In contrast to lower genital tract cultures, positive *Ureaplasma urealyticum* and/or *Mycoplasma hominis* cultures (and/or DNA PCR) of the placental membranes and amniotic fluid have been consistently associated with histologic chorioamnionitis, preterm birth and adverse perinatal outcomes. [1-6,10,11] Positive results are more commonly found in those delivering at the lowest birthweights and earliest gestational ages. There are isolated reports of adverse neonatal outcomes in infants with sepsis associated with positive *Ureaplasma urealyticum* and *Mycoplasma hominis* cultures.[4] However, we are not aware of studies in which a large number of infant umbilical cord bloods were prospectively cultured for *Ureaplasma urealyticum* and *Mycoplasma hominis* and these results compared to maternal and obstetric characteristics, other markers of placental inflammation, and newborn outcomes.

In this large population of 23 to 32 week preterm infants, *Ureaplasma urealyticum* and/or *Mycoplasma hominis* were present in 23% of cord blood cultures. Positive cultures were more commonly found in nonwhite (predominantly black) women, in women less than 20 years of age, in those undergoing a spontaneous compared to an indicated delivery, and in those delivering at earlier gestational ages. Intrauterine infection and inflammation were more common in those infants with a positive *Ureaplasma urealyticum* and/or *Mycoplasma hominis* culture as evidenced by placental cultures for *Ureaplasma urealyticum* or *Mycoplasma hominis* and other bacteria, elevated cord blood IL-6 levels, and placental membrane and umbilical cord histology. Infants with positive cord blood *Ureaplasma urealyticum* and/or *Mycoplasma hominis* cultures were more likely to have neonatal SIRS and probably BPD.

About two-thirds of ≤32 week infants are born preterm either because they undergo spontaneous PROM or labor.[16] The underlying etiology of most of these early preterm births appears to be bacterial chorioamnionitis for which histologic chorioamnionitis is a marker.[13] In this study, the cord bloods of 35% of infants born following spontaneous labor or membrane rupture at 23 to 32 weeks gestational age were positive for *Ureaplasma urealyticum* and/or *Mycoplasma hominis* as were the umbilical cord bloods of 44% of all infants born at 23 and 24 weeks gestational age and 27% of all infants born at 25 to 28 weeks. Given the frequency of these infections and their association with SIRS and likely with BPD,[10] it seems reasonable to determine if infants in these categories would benefit from routine culture for *Ureaplasma urealyticum* and/or *Mycoplasma hominis* and subsequent treatment with an antibiotic effective against these organisms. Similarly, we question whether treatment of women likely to deliver an early gestational age infant with an antibiotic effective against these organisms might reduce subsequent neonatal morbidity and mortality. Studies that answer these questions should make an important contribution to the long-term outcome of early gestational age infants, especially those born following spontaneous preterm labor and PPROM.

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Table 1

The percent of positive cord blood cultures for *Ureaplasma urealyticum* and/or *Mycoplasma hominis* by demographic and obstetric factors

Maternal Characteristics	UU and/or MH Present n = 82/351	P-value
Parity (%)		
0	26.9	0.106
≥1	19.5	
Race (%)		
Non-white	27.9	0.016
White	16.8	
Age (years) (%)		
<20	35.8	0.010
20-30	19.9	
>30	18.5	
Smoker (%)		
Yes	14.6	0.160
No	24.5	.
Diabetes (%)		
Yes	8.3	0.189
No	24.9	
Education (%)		
<12 years	24.2	0.869
≥12 years	23.4	
Type of PTB (%)		
Indicated	3.2	<0.0001
Spontaneous	34.7	
Preeclampsia (%)		
Yes	3.4	<0.0001
No	33.6	
Gestational age (%)		
23-24	44.4	0.018
25-28	27.1	
29-32	18.5	
Infant sex (%)		
Male	27.6	0.064
Female	19.2	

UU = *Ureaplasma urealyticum;* MH = *Mycoplasma hominis* PTB=Preterm birth

Table 2

Placental findings (%) in women with and without positive cord blood cultures for *Ureaplasma urealyticum* and/or *Mycoplasma hominis* (N=351)

	UU and/or MH in cord blood		
Placental Finding	**Present**	**Absent**	**P-value**
Any placental culture positive	90.1	47.6	<0.0001
MH or UU placental culture positive	77.8	21.6	<0.0001
Cord Blood IL-6 > 34.5 pg/ml	57.6	19.7	<0.0001
Acute inflammation			
Free membranes	76.9	37.7	<0.0001
Chorionic plate	74.4	30.4	<0.0001
Umbilical cord	51.3	25.1	<0.0001
Chronic inflammation (lymphohistiocytic)			
Free membranes	5.1	10.3	NS
Chorionic plate	1.3	0.4	NS
Decidua basalis	10.1	8.5	NS
Plasma cells: decidua	2.7	3.1	NS
Necrosis: membrane decidua	7.7	14.5	NS
Hemosiderin: decidua basalis	4.0	3.8	NS
Microfocal hemorrhage: decidua basalis	6.5	10.3	NS
Thrombosis: chorionic plate	15.8	11.2	NS
Diffuse decidual leukocytoclastic necrosis	20.0	28.9	NS

MH = *Mycoplasma hominis*: UU = *Ureaplasma urealyticum*; IL-6 = Interleukin-6

Table 3

Newborn outcomes in infants with positive vs negative cord blood cultures for *Ureaplasma urealyticum* and/or *Mycoplasma hominis*

	UU and MH Cultures		
Newborn Outcome	**Positive**	**Negative**	**P-value**
RDS	65.8	64.9	0.877
CLD	8.6	5.2	0.257
BPD	26.8	10.1	0.0001
IVH (grade 3/ 4)	8.8	6.6	0.517
PVL	3.8	2.3	0.493
SIRS	41.3	25.7	0.007
NEC	18.3	14.2	0.363
Death	8.5	9.3	0.835

RDS = Respiratory distress syndrome; CLD = Chronic lung disease; BPD = Bronchopulminary displasia; IVH = Intraventricular hemorrhage; PVL = Periventricular leukomalacia; SIRS = Systemic inflammatory response syndrome; NEC= Necrotizing enterocolitis; UU = *Ureaplasma urealyticum*; MH = *Mycoplasma hominis*