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ARTICLE

Analysis of the Incidence and Influencing Factors of *Ureaplasma urealyticum* Infection in Premature Neonates

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Abstract

Aims/Background Premature newborns face a higher risk of *Ureaplasma urealyticum* (UU) infection, which is closely associated with many diseases in the neonatal period. Therefore, this study retrospectively analysed the occurrence of UU infection in premature newborns and further explored its influencing factors, which are instrumental for enabling early diagnosis of UU infection in premature infants.

Methods In this retrospective study, 870 preterm infants born in the Affiliated Yangming Hospital of Ningbo University from June 2021 to December 2024 were selected as the study subjects. Ninety-three premature infants who tested positive for UU in nasopharyngeal aspirates were included in the UU group (n = 93) as cases, whereas 777 premature infants who tested negative for UU were included in the non-UU group (n = 777). Medical records and laboratory test results of the two groups of premature infants were collected. Univariate analysis and multiple logistic regression analysis were performed to identify factors influencing UU infection in premature newborns. Predictive efficacy of indicators showing differences in predicting UU infection in premature newborns was evaluated by means of receiver operating characteristic (ROC) curve analysis.

Results The incidence rate of UU infection in preterm neonates was 10.69% (93/870). Univariate analysis identified maternal age ($p < 0.001$), gestational hypertension ($p = 0.016$), delivery method ($p < 0.001$), premature rupture of membranes ($p = 0.006$), gestational age ($p = 0.001$), white blood cell count ($p < 0.001$), neutrophil count ($p < 0.001$), serum C-reactive protein ($p < 0.001$) and procalcitonin levels ($p = 0.003$) as factors associated with UU infection in premature newborns. The multiple logistic regression analysis identified natural birth/forceps delivery ($p < 0.001$), low gestational age ($p = 0.004$), and serum C-reactive protein >0.5 mg/L ($p = 0.001$) as significant risk factors for UU infection in premature newborns. Concerning the combined use of delivery method (natural birth/forceps delivery), gestational age and serum C-reactive protein (>0.5 mg/L), the area under the curve (AUC) was found to be 0.800 (0.758–0.843, $p < 0.001$), with a sensitivity of 74.2%, specificity of 75.3%, and Youden index of 0.495.

Conclusion The incidence of UU infection in preterm infants stands at about 10%, with delivery method, gestational age and C-reactive protein level being the independent influencing factors. The combination of these three factors holds certain clinical potential in predicting neonatal UU infection.

Key words: premature infants; newborn; *Ureaplasma urealyticum*; infection; influencing factor

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Introduction

Ureaplasma urealyticum (UU) belongs to the family Mycoplasma and the genus *Ureaplasma*. It is the smallest prokaryotic organism, mainly colonising the surface

of the urinary and reproductive tract and infant respiratory mucosa (Jonduo et al, 2022; Stavart et al, 2023; Xianchun et al, 2023). Some research has found that UU infection is closely related to the occurrence of genital infection-related diseases such as cervicitis (Bender and Gundogdu, 2022), postpartum endometritis (Chaim et al, 2003), and persistent non-gonococcal urethritis (Zinsli et al, 2023). In addition, other studies have found that during pregnancy, UU infection can promote the expression of inflammatory cytokines, exacerbate inflammatory responses, interfere with inflammation clearance, and is also associated with adverse pregnancy outcomes such as premature birth and low birth weight. For example, Miyoshi et al (2022) found that vaginal UU positivity is an independent predictor of preterm birth in women with adverse symptoms from preterm birth and/or cervical shortening. According to a study by Huang et al (2024) on UU infection among women in Southwest China, UU positivity is associated with an increased risk of premature birth and low birth weight in newborns.

Premature infants are at a high risk for infection due to barrier dysfunction and congenital and acquired immune response dysfunction (Bhati et al, 2023; Marissen et al, 2023; Tétu et al, 2022). UU can invade amniotic fluid early in pregnancy and is the most commonly isolated microbe in the inflamed placenta, making premature infants at a higher risk of UU infection (Libra et al, 2024; Sprong et al, 2020). UU infection is also closely associated with many diseases during the neonatal period: for example, Cultrera et al (2006) pointed out that UU infection in the lower respiratory tract of preterm newborns can increase the risk of respiratory distress syndrome in preterm infants. Chen et al (2024) found that there was a correlation between UU infection and the occurrence and development of bronchopulmonary dysplasia in premature infants. Based on an investigation on preterm infants with a birth weight $\leq$1250 grams, Ozdemır et al (2012) found that respiratory UU infection was independently correlated with severe retinopathy requiring treatment. A study by Li et al (2022) revealed that among 14 cases of necrotising vasculitis, 9 (64.3%) umbilical cords were UU-positive as detected by polymerase chain reaction (PCR).

The above-mentioned research confirms the correlation between UU infection and adverse pregnancy outcomes as well as neonatal diseases. However, untimely detection of UU infection could result in delayed treatment, leading to long-term adverse effects on newborns, which can be serious or even life-threatening. In light of this, we conducted a retrospective analysis of the occurrence of UU infection in premature infants with gestational age $<$37 weeks, and further explored its influencing factors, which are instrumental for enabling early diagnosis of UU infection in premature infants.

Methods

Study Participants

In this retrospective study, 870 preterm infants born in the Affiliated Yangming Hospital of Ningbo University from June 2021 to December 2024 were selected as the study subjects. Ninety-three premature infants who tested positive for UU in

nasopharyngeal aspirates were included in the UU group ($n = 93$) as cases, whereas 777 premature infants who tested negative for UU were included in the non-UU group ($n = 777$).

Inclusion criteria of this study are as follows: (1) infants with a gestational age >28 weeks and <37 weeks; (2) infants who had been tested for UU in the nasal pharyngeal aspirate within 48 hours of birth; (3) infants whose mothers had not taken any antibiotics in the past month and did not have a history of sexually transmitted diseases; and (4) infants with complete clinical data. Newborns with other types of bacterial or viral infections, severe metabolic or genetic diseases, or major deformities, as well as those whose ceased receiving treatment or were dying, were excluded from the present study.

Procedures

We collected medical records and laboratory test results of the two groups of premature infants, including maternal age, body mass index (BMI), primipara/multipara, gestational hypertension, gestational diabetes mellitus, delivery method, late-onset oligohydramnios, premature rupture of membranes, hormone used during pregnancy, gender of premature infants, gestational age, birth weight, 1-min Apgar score, 5-min Apgar score, white blood cell count, neutrophil count, mean platelet volume, blood platelet count, serum C-reactive protein, and procalcitonin. Apgar score includes five indicators: skin colour, heart rate, breathing, muscle tone, and response to stimulation, used to gain a sense of the health status of newborns and determine whether they suffer from asphyxia or other problems. The score is evaluated by professional doctors with rich clinical experience according to the scoring criteria (Rozycki and Yitayew, 2022). White blood cell count, neutrophil count, mean platelet volume, and blood platelet count were measured using the fully automatic hemocytometer (Model: XN-2800, Manufacturer: SYSMEX, Kobe, Japan). Enzyme-linked immunosorbent assay (ELISA) was employed to measure serum C-reactive protein and procalcitonin levels, with the kits purchased from Shanghai Enzyme-linked Biotechnology Co., Ltd., Shanghai, China. The ELISA kit numbers corresponding to serum C-reactive protein and procalcitonin are ml106583 and ml106700, respectively.

Statistical Analysis

Statistical analysis was performed using SPSS v23.0 (IBM SPSS Corp., Armonk, NY, USA). Categorical variables were analysed using the Chi-squared or Chi-squared correction test, expressed as count and percentage. The Shapiro-Wilk test was used to assess the normality of continuous data. Presented as mean ± standard deviation, normally distributed data were analysed using the independent samples *t*-test. Expressed as median (min, max), non-normally distributed data were analysed using the Mann-Whitney *U* test. Univariate analysis and multiple logistic regression analysis were performed to identify factors influencing UU infection in premature newborns. Predictive efficacy of indicators showing differences in predicting UU infection in premature newborns was evaluated by means of receiver

operating characteristic (ROC) curve analysis. A p-value < 0.05 was considered statistically significant.

Results

Incidence of UU Infection in Premature Newborns

Among the 870 preterm neonates born during the same period, 93 had UU infection. The incidence rate of UU infection in preterm neonates was 10.69% (93/870).

Univariate Analysis of UU Infection in Premature Newborns

There was no statistically significant difference in BMI, primipara/multipara, gestational diabetes mellitus, late-onset oligohydramnios, and hormones used during pregnancy between the two groups of postpartum women ($p > 0.05$). There was no statistically significant difference in gender, birth weight, 1-min Apgar score, 5-min Apgar score, mean platelet volume and blood platelet count between the two groups of premature infants ($p > 0.05$). Univariate analysis identified maternal age ($p < 0.001$), gestational hypertension ($p = 0.016$), delivery method ($p < 0.001$), premature rupture of membranes ($p = 0.006$), gestational age ($p = 0.001$), white blood cell count ($p < 0.001$), neutrophil count ($p < 0.001$), serum C-reactive protein ($p < 0.001$) and procalcitonin levels ($p = 0.003$) as factors associated with UU infection in premature newborns (Table 1).

Multiple Logistic Regression Analysis of UU Infection in Premature Newborns

Multiple logistic regression was performed with UU infection occurrence as the dependent variable. The independent variables include maternal age, gestational hypertension (no = 0, yes = 1), delivery method (cesarean section = 0, natural birth/forceps delivery = 1), premature rupture of membranes (no = 0, yes = 1), gestational age, white blood cell count, neutrophil count, serum C-reactive protein ($\leq$0.5 mg/L = 0, $>$0.5 mg/L = 1) and procalcitonin levels. The analysis identified natural birth/forceps delivery ($p < 0.001$), low gestational age ($p = 0.004$), and serum C-reactive protein $>$0.5 mg/L ($p = 0.001$) as significant risk factors for UU infection in premature newborns (Table 2).

Delivery Method, Gestational Age and Serum C-Reactive Protein in Predicting UU Infection in Premature Newborns

The area under the curve (AUC) for the combined use of delivery method (natural birth/forceps delivery), gestational age and serum C-reactive protein ($>$0.5 mg/L) was found to be 0.800, with a sensitivity of 74.2%, specificity of 75.3%, and Youden index of 0.495 (Table 3, Fig. 1).

Discussion

This study found that the incidence rate of UU infection in premature newborns was 10.69% (93/870). Through univariate analysis and multiple logistic regression analysis, we found that the delivery method (natural birth/forceps delivery), low

Table 1. Univariate analysis of UU infection in premature newborns.

Variable	*n*	UU group (*n* = 93)	Non-UU group (*n* = 777)	$t/\chi^2/Z$	*p*-value
Maternal age (years)		29.01 ± 5.76	30.85 ± 4.92	3.337	<0.001
BMI (kg/m^2)		26.59 ± 4.14	27.43 ± 4.70	1.645	0.100
Primipara/multipara [*n* (%)]				2.709	0.100
Primipara	435	54 (58.06)	381 (49.03)		
Multipara	435	39 (41.94)	396 (50.97)		
Gestational hypertension [*n* (%)]				5.777	0.016
Yes	141	7 (7.53)	134 (17.25)		
No	729	86 (92.47)	643 (82.75)		
Gestational diabetes mellitus [*n* (%)]				0.414	0.520
Yes	230	22 (23.66)	208 (26.77)		
No	640	71 (76.34)	569 (73.23)		
Delivery method [*n* (%)]				28.554	<0.001
Natural birth/forceps delivery	356	62 (66.67)	294 (37.84)		
Cesarean section	514	31 (33.33)	483 (62.16)		
Late-onset oligohydramnios [*n* (%)]				0.000	1.000
Yes	36	4 (4.30)	32 (4.12)		
No	834	89 (95.70)	745 (95.88)		
Premature rupture of membranes [*n* (%)]				7.501	0.006
Yes	362	51 (54.84)	311 (40.03)		
No	508	42 (45.16)	466 (59.97)		
Hormones used during pregnancy [*n* (%)]				0.006	0.938
Yes	203	22 (23.66)	181 (23.29)		
No	667	71 (76.34)	596 (76.71)		
Gender of premature infants [*n* (%)]				0.059	0.809
Male	450	47 (50.54)	403 (51.87)		
Female	420	46 (49.46)	374 (48.13)		
Gestational age (weeks)		34.39 ± 2.16	35.04 ± 1.76	3.262	0.001
Birth weight (g)		2369.09 ± 520.03	2399.72 ± 549.15	0.511	0.609
1-min Apgar score [*n* (%)]				2.231	0.135
Ten points	786	80 (86.02)	706 (90.86)		
<Ten points	84	13 (13.98)	71 (9.14)		
5-min Apgar score [*n* (%)]				2.152	0.142
Ten points	841	87 (93.55)	754 (97.04)		
<Ten points	29	6 (6.45)	23 (2.96)		
White blood cell count ($\times 10^9$/L)		12.50 (9.73, 15.78)	10.50 (8.00, 13.20)	3.983	<0.001
Neutrophil count ($\times 10^9$/L)		7.00 (4.95, 9.50)	5.60 (3.40, 7.60)	4.134	<0.001
Mean platelet volume (fL)		9.80 ± 0.88	9.76 ± 0.70	0.471	0.638
Blood platelet count ($\times 10^9$/L)		264.68 ± 66.93	258.16 ± 57.42	1.013	0.311
Serum C-reactive protein [*n* (%)]				18.757	<0.001
>0.5 mg/L	26	10 (10.75)	16 (2.06)		
≤0.5 mg/L	844	83 (89.25)	761 (97.94)		
Serum procalcitonin (ng/mL)		0.17 (0.10, 0.28)	0.14 (0.10, 1.90)	2.977	0.003

Notes: UU, *Ureaplasma urealyticum*; BMI, body mass index.

Table 2. Multiple logistic regression analysis of UU infection in premature newborns.

Variable	β	Standard error	Wald	*p*-value	Odds ratio	95% CI	
						Lower	Upper
Maternal age	–0.040	0.023	2.955	0.086	0.961	0.918	1.006
Gestational hypertension	–0.420	0.369	1.296	0.255	0.657	0.319	1.354
Delivery method	1.136	0.241	22.235	<0.001	3.113	1.942	4.992
Premature rupture of membranes	0.286	0.235	1.476	0.224	1.331	0.839	2.109
Gestational age	–0.163	0.056	8.496	0.004	0.850	0.761	0.948
White blood cell count	0.024	0.023	1.129	0.288	1.024	0.980	1.071
Neutrophil count	0.042	0.028	2.235	0.135	1.043	0.987	1.102
Serum C-reactive protein	1.625	0.498	10.642	0.001	5.079	1.913	13.486
Serum procalcitonin	0.025	0.024	1.028	0.311	1.025	0.977	1.075
Constant	3.428	2.047	2.805	0.094	30.806	-	-

Notes: UU, *Ureaplasma urealyticum*; CI, confidence interval.

Table 3. Prediction efficacy of delivery mode, gestational age, and serum C-reactive protein for UU infection in premature infants.

Variable	AUC	*p*-value	Cut-off	Sensitivity	Specificity	Youden index	95% CI
Delivery method	0.644	<0.001	-	66.7	62.2	0.288	0.585–0.703
Gestational age	0.607	0.001	35.64 weeks	66.7	52.4	0.201	0.547–0.667
Serum C-reactive protein	0.613	<0.001	-	59.1	66.4	0.255	0.545–0.682
Combined	0.800	<0.001	-	74.2	75.3	0.495	0.758–0.843

Notes: AUC, area under the curve; CI, confidence interval; UU, *Ureaplasma urealyticum*.

gestational age and serum C-reactive protein (>0.5 mg/L) were independent risk factors for UU infection in premature newborns.

The UU infection has an exclusively high incidence rate in women of childbearing age, translating to even higher risk in pregnant women due to the influence of estrogen in the body, the change of vaginal pH value and other factors during pregnancy, which result in the disruption of the balance of the ecological environment in the vagina, a condition conducive to the growth and survival of bacteria and pathogens in the vagina and thereby their infectability (Oh et al, 2024; Przybylski et al, 2024; Zhang et al, 2023). However, there are relatively few studies on neonatal UU infection, and their research findings present discordances: Ma et al (2024) investigated 7257 hospitalised newborns in China and found that the incidence of UU infection was 7.73% (561/7257). Abe et al (2024) tested gastric fluid samples from 47 newborns in the intensive care unit and found that 9 (19%) of them were positive for UU. Viscardi et al (2020) found that among 121 premature infants in the intensive care unit, 36% (44 cases) were UU-positive. In their other report (Viscardi et al, 2008), DNA testing was performed on blood and cerebrospinal fluid samples from 313 infants with extremely low birth weight, revealing a UU-positive rate of 23.6% (74 cases). A study of 214 pregnant women in Israel (Peretz et al, 2020) found that the incidence of UU infection in their delivered newborns was

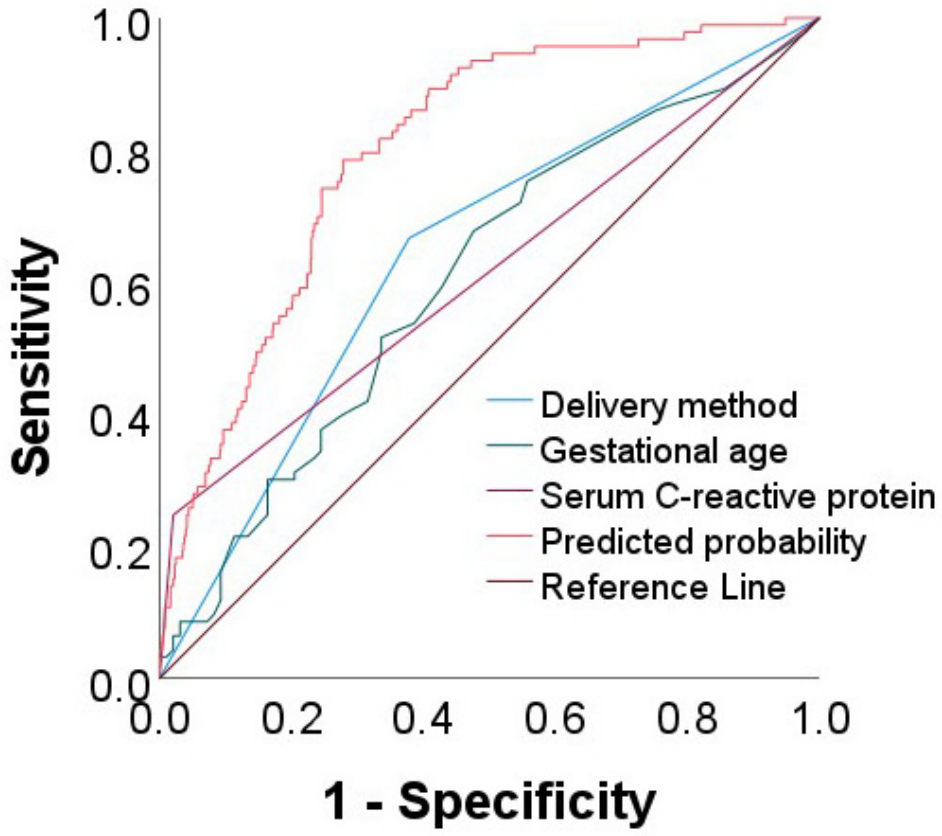

Fig. 1. ROC curve of the combination of delivery method, gestational age, and serum C-reactive protein for predicting UU infection in premature newborns. Notes: ROC, receiver operating characteristics; UU, *Ureaplasma urealyticum*.

28.5%. The incidence of UU infection in this study was 10.69%, accounting for about one-tenth of preterm infants, which once again confirms that UU infection is closely related to preterm birth. Using R software for statistical analysis and Microbiome Analyst and Calypso software for microbiome analysis, Park et al (2022) found that UU infection was highly correlated with preterm birth. Through analysis using next-generation sequencing (NGS) technology, Tirone et al (2022) found that vaginal colonization of UU was associated with spontaneous preterm birth.

Understanding the pathogenic and host factors is important for the screening and treatment of UU infection in premature infants. In our study, we found that natural birth/forceps delivery, low gestational age, and abnormal increase of serum C-reactive protein level were independent influencing factors of UU infection in premature newborns. For these three factors, we have the following explanations: (1) First of all, different delivery methods may lead to differences in the microflora acquired by newborns at birth. Newborns delivered vaginally are more susceptible to the influence of vaginal microflora. UU has a higher colonisation rate in the female reproductive tract and can be vertically transmitted to newborns through the mother's birth canal, so cesarean section can reduce the risk of neonatal UU infection. Two studies conducted by Sánchez and Regan (1987) found that the rate of UU-positive detection in newborns of mothers with UU-positive cervix was significantly higher than that of mothers with UU-negative cervix, providing evidence for

vertical transmission of UU. Sun and Fu (2021) found that the delivery mode and premature rupture of membranes were independent risk factors for UU infection in premature infants. In a study of 5893 women with urinary tract infection, Xu et al (2018) found that natural birth could lead to increased UU infection rate. (2) Secondly, a lower gestational age is synonymous with a less developed immune system, which is marked by worse barrier function, conducive to increased risk of infection (Peterson et al, 2021). In addition, a lower gestational age along with a longer duration of premature membrane rupture raises the possibility of vertical transmission (Chatzakis et al, 2020). From an investigation of 49 patients with premature rupture of membranes or who had preterm delivery, Kirchner et al (2007) found that the presence of UU in maternal amniotic fluid culture was associated with low gestational age and low birth weight. (3) Finally, the inflammatory response mediated by UU infection will lead to an increase in the blood level of inflammatory factors in newborns. As a sensitive inflammatory marker, the increase of C-reactive protein level indicates the existence of an inflammatory response. A study by Kwak et al (2015) discovered for the first time that positive culture of UU vaginal secretions is correlated with elevated maternal serum C-reactive protein, concurring with our observations. In addition, consistent with the univariate analysis results of this study, Sun and Fu (2021) pointed out that pregnancy-induced hypertension is a protective factor for UU infection, but the mechanism behind said protection remains unclear.

This study has several limitations: the study was a single-centre trial with a relatively small sample size. In addition, although all pertinent data we could gather from medical records and laboratory test results of the mothers and their preterm infants had been considered and analysed, we still could not rule out that there may be other factors influencing the occurrence of UU infection in preterm newborns. The diagnostic process of neonatal UU infection in this study entailed leveraging positive tracheal secretions as the standard; however, without expanding the analysis to using gastric fluid samples, we believe several cases ended up as missed diagnoses. In addition, when univariate and multivariate logistic regression analyses are used, multicollinearity problems may result when there is a correlation between the independent variables, which may affect the predictive performance of the model. Therefore, in the follow-up study, we will conduct a multi-centre study, expand the sample size, and include more possible influencing factors, including those that have been tested in this analysis.

Conclusion

In summary, with an approximately 10% incidence rate of UU infection in preterm newborns, early screening emerges as a prevention strategy of great significance. Delivery method, low gestational age, and elevated C-reactive protein are independent risk factors of UU infection in premature newborns, and can be combined to assist in predicting the occurrence of UU infection in newborns.

Key Points

- The incidence rate of UU infection in preterm neonates was 10.69% (93/870).
- According to univariate analysis, maternal age, gestational hypertension, delivery method, premature rupture of membranes, gestational age, white blood cell count, neutrophil count, serum C-reactive protein, and procalcitonin levels are factors associated with UU infection in premature newborns.
- The multiple logistic regression analysis identified delivery method, low gestational age, and serum C-reactive protein >0.5 mg/L as significant risk factors of UU infection in premature newborns.
- According to ROC analysis, the combination of delivery mode, gestational age, and C-reactive protein has certain predictive value for UU infection in premature newborns.

Availability of Data and Materials

All data included in this study are available from the corresponding author upon reasonable request.

Author Contributions

JHL and FWC designed the research study and wrote the first draft. JHL and CQZ performed the research. JHL and CQZ analyzed the data. All authors contributed to the important editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

This study was approved by the Institutional Ethical Committee of the Affiliated Yangming Hospital of Ningbo University (approval number: 2025-01-009). The study complied with the Declaration of Helsinki (2013) and all postpartum women signed informed consent.

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Conflict of Interest

The authors declare no conflict of interest.

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