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Prediction of short-term newborn infectious morbidity based on maternal characteristics in patients with PPROM and *Ureaplasma* species infection

Predykcja infekcji u noworodków w oparciu o analizę wyników danych pacjentek z przedwczesnym pęknięciem błon płodowych i infekcją *Ureaplasma*

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Abstract

Objectives: *Preterm premature rupture of membranes (PPROM) complicates about 5% of pregnancies. Ureaplasma species is the most common pathogen found in the amniotic fluid in pregnancieneonatal outcome. The aim of the following study was to evaluate the impact of colonization with the Ureaplasma spp. on pregnant women with PPROM, coinfection with different microorganisms, and antimicrobial treatment on neonatal outcome.*

Material and methods: *The study included 30 women with PPROM hospitalized in Division of Reproduction in s complicated by PPROM. It is speculated that it requires a coinfection to produce unfavorable Poznan's K. Marcinkowski University of Medical Sciences. Swabs from cervical canal were obtained for the identification of bacterial and ureaplasmatic infections by culture and PCR.*

Results: *The presence of any infection during the pregnancy after PPROM was confirmed in 22 patients (Ureaplasma spp. in 12 patients, coinfection in 10 women). The cure rate for Ureaplasma species and other infections was 17% (2/12 patients) and 23% (5/22 patients), respectively. There was no correlation between Ureaplasma species infection, coinfection, and cure status with the infection in the newborn. The PPROM to delivery duration also did not affect the newborn infection status. A negative relationship with leukocyte level was detected in patient with newborn infection.*

Conclusions: *The presence of colonization with Ureaplasma species is not attributable to neonatal short-term morbidity. The evaluation of maternal biochemical and microbiological data, regardless of the duration of the pregnancy after PPROM or the cure status, does not add any insight into the newborn infection status.*

Key words: **PPROM** / **short-term newborn infectious** / ***Ureaplasma* spp.** /

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Streszczenie

Cel pracy: *Przedwczesne pęknięcie błon płodowych wikła około 5% wszystkich ciąż i wiąże się z ryzykiem zapalenia błon płodowych oraz zagrożenia porodem przedwczesnym. Ureaplasma species jest patogenem najczęściej występującym w płynie owodniowym ciąż powikłanych PPROM. Wyjaśnienie wpływu koinfekcji na niekorzystne wyniki neonatologiczne wymaga dalszych badań. Celem pracy była ocena wpływu kolonizacji Ureaplasma species, koinfekcji innymi drobnoustrojami i leczenia przeciwbakteryjnego w ciążach powikłanych PPROM na wyniki neonatologiczne.*

Materiał i metody: *Badaniem objęto 30 pacjentek hospitalizowanych w Klinice Rozrodczości Uniwersytetu Medycznego im. Karola Marcinkowskiego w Poznaniu. Pobierano wymazy z szyjki macicy celem identyfikacji infekcji bakteryjnej i ureaplazmatycznej metodą hodowlaną oraz PCR.*

Wyniki: *Infekcję potwierdzono u 22 pacjentek z PPROM (Ureaplasma spp. rozpoznano u 12 pacjentek, natomiast koinfekcję u 10). Odsetek wyleczenia zakażenia Ureaplasma i innymi drobnoustrojami wyniósł odpowiednio 17% (2/12) i 23% (5/22). Nie stwierdzono korelacji między zakażeniem Ureaplasma, koinfekcją, a występowaniem zakażeń u noworodków, niezależnie od statusu wyleczenia. Nie wykazano istotnych różnic między długością czasu od PPROM do porodu, a obecnością infekcji u noworodka. Udowodniono odwrotnie proporcjonalną zależność między poziomem leukocytów u pacjentek, a występowaniem zakażenia u noworodka.*

Wnioski: *Brak zależności między kolonizacją Ureaplasma u pacjentek z PPROM, a krótkoterminową zachorowalnością noworodków. Ocena czynników biologicznych i mikrobiologicznych nie pozwala na przewidzenie stanu noworodka.*

Słowa kluczowe: **PPROM** / **zakażenia u wcześniaków** / ***Ureaplasma* spp.** /

Preterm premature rupture of membranes (PPROM) complicates about 5% of pregnancies and is considered a cause of premature labor in 40–60% of cases [1]. Infection is the most commonly cited cause for PPROM [2]. In turn, chorioamnionitis is the most common and serious complication of PPROM. According to different reports, it occurs in 10–60% of preterm membrane rupture cases [3, 4]. The longer the interval from PPROM to delivery, more likely the chorioamnionitis will occur. Therefore, PPROM poses clinician with a therapeutic dilemma—should labor occur early after rupture of membranes, often producing a premature newborn with inherent problems of prematurity, or should the labor be postponed, with possible development of intraamniotic infection—and its own set of problems. Apart from standard infections with aerobic and anaerobic bacteria, there is a threat of infection with *Ureaplasma* species. This pathogen is said to be a part of urogenital flora in up to 25% of patients with premature rupture of membranes [5]. The distinguishing features of this mycoplasma are as follows: smallest size, lack of cell wall, and resistance to commonly prescribed antibiotics, including beta-lactams [6, 7]. The incidence of vertical transmission of *Ureaplasma* infection in term newborns without respiratory disorders was assessed at 38% [8]. The contribution of *Ureaplasma* spp. to neonatal disease (including pneumonia and respiratory distress syndrome) is conflicting. It has been confirmed that there is a presence of *Ureaplasma* spp. in cases of PPROM, but it has been suggested that evidence of the mere presence is not enough to be causative of a disease [9]. It is speculated that it might require a coinfection to produce unfavorable neonatal outcome.

The objective of our study was to determine whether colonization of the vagina with *Ureaplasma* spp. of pregnant women with PPROM was associated with adverse neonatal outcome. Furthermore, we aimed at determining if successful antimicrobial treatment had any effect on decreasing the incidence of infection in newborns, and if a coinfection with different microorganism induced different problems during the neonatal period.

Material and method

The study was conducted in Division of Reproduction and Neonatal Infection Ward in Poznan's K. Marcinkowski University of Medical Sciences. The study included 30 women with PPROM, which is defined as rupture occurring between 24–34th week of gestation. Exclusion criteria were as follows: fetus with congenital anomalies, marked hypotrophy of the fetus, amniocentesis in current pregnancy. The mean age of the patients was 33 years (24–44 years old). There were 5 primiparous and 25 multiparous patients, respectively. The mean time of the membrane rupture was 31 weeks' gestation (18–34 weeks' gestation). The rupture-to-delivery interval was 10 days (3–79 days) and the mean week of delivery was 33rd week of gestation (28–36 weeks' gestation). The protocol involved placing a sterile speculum in the vagina at the time of admission and collection of leaking amniotic fluid form cervical canal to a sterile swab (two standard dry ones and one with culture media). The swabs were sent to Microbiology Unit in our hospital for the identification of bacterial and ureaplasmatic infections. In addition, the DNA was extracted and PCR was carried out to test for the presence of *Ureaplasma* spp. in amniotic fluid.

Collection the amniotic fluid from the cervical canal.

Using sterile swabs, we collected samples from the cervical canal . Two swabs were sent to the microbiological unit for analysis. Another swab, intended to identify bacteria by qPCR, was frozen (-200°C) until assay.

Identification of the Ureaplasma spp. in PCR assay.

Swab was suspended in 1.5 ml of sterile saline (0.9% NaCl). DNA isolation was conducted with the obtained suspension of 200 µl, using QIAamp MiniElute Virus Spin Kit Qiagen (Hilden, Germany). The identification of *Ureaplasma parvum* or/and *Ureaplasma urealitycum* DNA was conducted using FTD urethritis plus detection kit (Fast-track Diagnostics, Luxembourg), containing specific primers and fluorescent probes and RotorGene

Table I. The incidence of infection, results of treatment, and markers of inflammation in infected and healthy newborns.

	Infected newborns	Non-infected newborns	*p*
***Ureaplasma* spp. infections during PPROM**			Chi² w. Yates correction
Ureaplasma spp. positive	5	7	0,940
Ureaplasma spp. negative	9	9	
***Ureaplasma* treatment result during PPROM**			Fisher's Exact Test
Ureaplasma spp. cured	2	0	0,470
Ureaplasma spp. not cured	5	5	
Infection with other pathogens than *Ureaplasma* spp.			Fisher's Exact Test
Cured	2	3	0,624
Not cured	10	7	
CRP [mg/L] median (range)	9,56 (1,4-222,9)	4,00 (1,1-17,8)	0,661*
	OR=1,069 95%CI (0,93-1,228)		0,39
WBC [G/L] median (range)	9,87 (5,8-15,3)	15,23 (3,7-32,6)	0,038*
	OR=0,79, 95%CI (0,6250,999)		0,049**
The PPROM to delivery length [days] median (range)	13 (3-79)	9 (2-37)	0,183*
	OR=1,03, 95%CI (0,987-1,087)		0,158**

*Mann-Whitney Rank Sum Test, **Logistic Regression Analysis.

3000 thermocycler (Corbett Research, Australia) according to the protocol and thermal profile of the PCR reaction supplied by the manufacturer.

All the patients received a standard treatment consisting of ampicillin 1 g q 6 h and erythromycin 300 mg three times daily. If the presence of *Ureaplasma* spp. was confirmed, the patients were subject to additional treatment with azithromycin two times 500 g/day for three days. Curative treatment was defined as lack of the presence of pathogens in the last culture immediately preceding the delivery. The cervical canal swab was repeated every 7 day until delivery in each patient. The patients were monitored using CTG(cardiotocography), Doppler studies, and biochemical indices (WBC, CRP) to detect any signs of fetal distress or infection. The decision to deliver was left to the discretion of managing physician. The study group comprised 24 women with preterm PPROM. All newborns after delivery were evaluated for signs of infection.

For the statistical evaluation of the results, SigmaStat 3.5 (Dundas Software Ltd., Germany) was used. To examine the statistical significance of the variable distribution, the chi-square test with Yates correction and Fisher's Exact Test was used. We assumed $p < 0.05$ for statistical significance.

Results

Obtained results are summarized in Table1. The presence of any infection at some point during the pregnancy after PPROM was confirmed in 22 patients. The presence of *Ureaplasma* species was confirmed in 12 patients while coinfection with other pathogens was found in 10 women with PPROM.

The cure rate, defined as no infection in cervical culture before delivery, for *Ureaplasma* species was 17% (2/12 patients), and cure rate for other infections was 23% (5/22 patients).

There was no correlation with *Ureaplasma* spp. infection during the PPROM-to-delivery interval and infections detected in newborns. Furthermore, there was no statistically significant difference in the presence of infections in the newborn and successful treatment of *Ureaplasma* colonization. Also no correlation was found between coinfection with other pathogens and infection in the newborn, regardless of cure status.

Regarding the CRP and leukocyte levels, using logistic regression analysis, we have detected a negative relationship with leukocyte levels in patients with newborn infection. The lower the leukocyte count, the more likely was the infection of the newborn to be detected (OR = 0,79; $p = 0,49$). The PPROM to delivery length also did not affect the newborn infection status (OR = 1,03; $p = 0,158$).

Discussion

PPROM and associated preterm labor remains one of the most challenging aspects of modern perinatal medicine. One of the most common complications of preterm PPROM is ascending infection. The role of *Ureaplasma* spp. as a causative factor for PPROM and pathogen responsible for neonatal adverse effects is still debatable. In our study, the rate of confirmed *Ureaplasma* spp. infection in patients with PPROM was 33,6% (12 patients). This is in line with the lowest estimates of *Ureaplasma* colonization during pregnancy [10]. We have utilized both culture and PCR to detect *Ureaplasma* in cervical swabs. There is some controversy regarding the presence of *Ureaplasma* in different compartments. The rate of colonization might be different for vaginal swabs, cervical samples, direct amniotic fluid samples, and, finally, the cord blood taken during cordocentesis. Our aim was to provide an easy and affordable method to estimate the bacterial burden in patients with PPROM. Most studies focus on

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the cervical canal as it is the "passage" between the intrauterine and vaginal compartments. The vaginal flora is often responsible for the ascending infection, while the draining amniotic fluid gives us clues as to what is the situation within the amniotic sac and, therefore, in the fetus. This was recently confirmed by Kacerovsky et al. as they confirmed that 80% of women with *Ureaplasma* infection in the cord blood had also exhibited the infection in the amniotic fluid [11]. However, the range of *Ureaplasma* colonization in the setting of PPROM is very wide – from 15% to 68% [12, 13]. This wide variation might reflect the differences in socioeconomic and geographic variations in the prevalence in the *Ureaplasma* infection.

Contrary to some authors, we have found no correlation between *Ureaplasma* colonization and neonatal infection [14, 15]. This was also true for patients with and without eradication of the pathogen at the time of delivery. Also no correlation was found between the week of preterm delivery and infection status in the newborn. Our results strengthen the recent study by Kacerovsky et al. They have failed to detect any impact on short-term neonatal morbidity with regard to cord blood presence of *Ureaplasma* spp. This might be explained by the fact that according to some studies, the *Ureaplasma* does not induce an inflammatory reaction [16]. Therefore, the fetal inflammatory reaction might not be observed in the mere presence of *Ureaplasma* species. However, we have also confirmed that 10 women with PPROM were also infected with other pathogens, which were treated according to antibiogram. After the correction for this fact, we have also failed to see a correlation between the infection in the neonate and the eradication rate for different bacterial species detected with the cervical swab. The cure rate in our current study for *Ureaplasma* was 17% and for other bacteria 23%. The relatively low cure rate might be partially explained by the fact that the mean rupture to delivery time was only 10 days (shortest time being 3 days). Also, the extreme long times to delivery achieved in some patients (79 days) were conductive to ascending infection. The fetus, having only IgG antibodies crossing from the mother to defend itself from infection, constitutes a perfect feeding ground for any bacteria. Most of the studies look at this aspect not with repeat PCR as a proof of cure, but rather with observation of decline in adverse effects (preterm birth, infection in the newborn). This approach has a serious flaw as demonstrated by elegant study by Ogasawara et al. [17]. It proved that the cure rate as estimated by the vertical transmission rates was not different; however, a prolongation of pregnancy was achieved. By using antibiotics we are not only affecting the presence of *Ureaplasma*. Antibiotics exert their action on a wide range of bacteria; therefore, the observed improvements in adverse effects might be attributable to eradicating different bacterial species [18]. Also the biological effects of antibiotics reach far above the antimicrobial actions in the human organism.

Conclusions

In summary, in this paper we present supporting evidence to a thesis that mere presence of colonization with *Ureaplasma* species is not attributable to neonatal short-term morbidity. Also the currently prevailing believe, that achieving improvement in neonatal outcomes with antibiotic treatment is attributable to eradication of *Ureaplasma* is questionable, as we have achieved very low cure rates, proven by repeated sampling of the cervical environment. Currently, we do not have any insight into the newborn infection status, as judged by the maternal biochemical and microbiological data, regardless of the duration of the pregnancy after PPROM or the cure status. Therefore, optimal course of action in preterm PPROM, which is early versus delayed delivery, still remains unknown.

Oświadczenie autorów

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