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

Mateusz Mikołajczyk¹, Przemysław Krzysztof Wirstlein¹, Magdalena Wróbel², Jan Mazela³, Karolina Chojnacka³, Jana Skrzypczak¹

1 Department of Gynecology and Obstetrics, Division of Reproduction, Poznan University of Medical Sciences, Poland
2 Gynecological and Obstetric Clinical Hospital in Poznan, Poland
3 Department of Neonatology, Poznań University of Medical Sciences, Poland

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 pregnancies and neonatal 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 Poznani’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. /
Streszczenie

Cel pracy: Przedwczesne pęknięcie błon płodowych wiąże się z ryzykiem zapalenia błon płodowych oraz zagrożenia porodem przedwczesnym. Ureaplasma species jest patogenem najczęściej występującym w płynie owodniowym ciąży powikłanych PROM. 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 ciągach powikłanych PROM na wyniki neonatologiczne.

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

 Wyniki: Infekcje potwierdzono u 22 pacjentek z PROM (Ureaplasma spp. rozpoznano w 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 PROM do porodu, a obecnością infekcji u noworodka. Udowodniono odwrotną proporcjonalną zależność między poziomem leukocytów u pacjentek a występowaniem zakażenia u noworodka.

Wnioski: Brak zależności między kolonizacją Ureaplasma u pacjentek z PROM, a krótkoterminowym zachorowaniem noworodków. Ocena czynników biologicznych i mikrobiologicznych nie pozwala na przewidzenie stanu noworodka.

Słowa kluczowe: PPROM / zakażenia u goedeń / Ureaplasma spp. /

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 PROM, 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, amniosentesis 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 ureaplastic 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 urealyticum 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.

<table>
<thead>
<tr>
<th></th>
<th>Infected newborns</th>
<th>Non-infected newborns</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ureaplasma spp. infections during PPROM</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ureaplasma spp. positive</td>
<td>5</td>
<td>7</td>
<td>0.940</td>
</tr>
<tr>
<td>Ureaplasma spp. negative</td>
<td>9</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td><strong>Ureaplasma treatment result during PPROM</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ureaplasma spp. cured</td>
<td>2</td>
<td>0</td>
<td>0.470</td>
</tr>
<tr>
<td>Ureaplasma spp. not cured</td>
<td>5</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td><strong>Infection with other pathogens than Ureaplasma spp.</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cured</td>
<td>2</td>
<td>3</td>
<td>0.624</td>
</tr>
<tr>
<td>Not cured</td>
<td>10</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>CRP [mg/L] median (range)</td>
<td>9.56 (1.4-222.9)</td>
<td>4.00 (1.1-17.8)</td>
<td>0.661*</td>
</tr>
<tr>
<td></td>
<td>OR=1.069 95% CI</td>
<td>(0.93-1.228)</td>
<td>0.39</td>
</tr>
<tr>
<td>WBC [G/L] median (range)</td>
<td>9.87 (5.8-15.3)</td>
<td>15.23 (3.7-32.6)</td>
<td>0.038*</td>
</tr>
<tr>
<td></td>
<td>OR=0.79, 95% CI</td>
<td>(0.6250.999)</td>
<td>0.049**</td>
</tr>
<tr>
<td>The PPROM to delivery length [days] median (range)</td>
<td>13 (3-79)</td>
<td>9 (2-37)</td>
<td>0.183*</td>
</tr>
<tr>
<td></td>
<td>OR=1.03, 95% CI</td>
<td>(0.987-1.087)</td>
<td>0.158**</td>
</tr>
</tbody>
</table>

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

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, 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

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 the presence of pathogens in the last culture immediately preceding the delivery. The cervical canal swab was repeated every 7 days until delivery in each patient. The patients were monitored using CTG(cariontography), 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).
the cervical canal as it is the “passage” between the intraterine 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 Kacervsky 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 Kacervsky 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 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 the guidelines. 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 fetuses, 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 eradication of 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


Źródło finansowania: Praca była finansowana z Badania statutowych Kliniki Rozwojowo-rodzniczego nr 592-01-01/11/042-03014.

Konflikt interesów: Autorzy nie zgłaszają konfliktu interesów oraz nie otrzymali żadnego wynagrodzenia związanego z powstawaniem pracy.

References