Lethal neonatal respiratory failure by perinatal transmission of *Ureaplasma parvum* after maternal PPROM

Tödliches neonatales respiratorisches Versagen durch perinatale Übertragung von *Ureaplasma parvum* nach mütterlichem PPROM

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Abbreviations:

Ampl. HF	high-frequency oscillatory ventilation amplitude
APGAR	Appearance, Pulse, Grimace, Activity, Respiration (scoring systeme)
BPD	bronchopulmonary dysplasia
CRP	c-reactive protein
CTG	cardiotocography
EONS	early onset neonatal sepsis
HF-frequ.	high-frequency oscillatory ventilation frequency
HFO	high-frequency oscillatory ventilation
IL-6	interleukin 6
MAP	mean arterial pressure
NICU	neonatal intensive care unit
NO	nitric oxide
PC-SIMV	pressure controlled synchronized intermittent mechanical ventilation
PCT	procalcitonin
PEEP	positive end-expiratory pressure
PEONS	PEONS-trial
	Prediction of Early Onset Neonatal Sepsis after maternal PPROM by microbiome analysis
PIP	peak inspiratory pressure
PPROM	preterm premature rupture of membranes
rDNA	ribosomal deoxyribonucleic acid
WBC	White blood cell count
wga	Weeks of gestational age

Abstract

A primiparous pregnant woman was admitted due to preterm premature rupture of membranes (PPROM) in 27+0 week of gestational age (wga). Conventional vaginal microbiological analysis had no pathological finding. Management decisions based on national guidelines included antenatal corticoids, tocolytics and antibiotics. Unstoppable efforts of preterm labor in 28+0 wga and supposed amniotic infection syndrome necessitated emergency cesarean section. The preterm infant underwent NICU therapy, developed an early-onset neonatal sepsis and therapy refractory pulmonary insufficiency with consecutive right heart failure, resulting in death on the 36th day of life. Microbiota analyses by 16Sr DNA sequencing was performed from maternal vaginal swabs and from neonatal pharyngeal swabs. Maternal antibiotic treatment resulted in depletion of physiological vaginal colonisation with *Lactobacillus crispatus*. *Ureaplasma parvum* became the dominant vaginal microorganism at delivery and was detected in high relative abundance in neonatal specimen. Progressive radiological air-space changes and interstitial pathologies associated with *Ureaplasma* infection (bronchopulmonary dysplasia type III) were seen early at 3rd and distinctly from 14th day of life. This clearly demonstrates the need of vaginal colonization diagnostics in PPROM patients and awareness of the consecutive risks in the preterm. Vaginal microbiome analysis may allow individualized and targeted maternal and fetal diagnostic, prophylactic and therapeutic strategies to identify, protect and treat the high-risk neonates after PPROM.

Zusammenfassung

Die Aufnahme erfolgte in der 27+0 Schwangerschaftswoche (SSW) aufgrund eines frühen vorzeitigen Blasensprunges (PPROM). Die konventionelle vaginale mikrobiologische Diagnostik verblieb unauffällig. Das Management umfasste leitliniengerecht die Applikation antenataler Steroide, Tokolytika und Antibiotika. Unaufhaltsame Frühgeburtsbestrebungen in der 28+0 SSW unter Verdacht eines Amnioninfektionssyndroms erforderten eine Notsectio. Das Frühgeborene erhielt eine sofortige NICU-Versorgung, entwickelte eine early-onset neonatale Sepsis und eine therapierefraktäre Lungen- mit konsekutiver Rechtsherzinsuffizienz, die zum Tod am 36. Lebenstag führte. Es wurden Mikrobiomanalysen mittels 16Sr DNA-Sequenzierung aus mütterlichen Vaginalabstrichen und aus neonatalen Rachenabstrichen durchgeführt. Die Antibiotikatherapie depletierte die physiologische

vaginalen Kolonisation mit *Lactobacillus crispatus*. *Ureaplasma parvum* wurde als die dominierende vaginale mikrobielle Spezies und in den neonatalen Proben mit hoher relativer Abundanz nachgewiesen. Radiologisch wurden progressive Lungenveränderungen im Zusammenhang mit Ureaplasmen (bronchopulmonale Dysplasie Typ III) nachgewiesen. Dies zeigt deutlich die Notwendigkeit einer exakten vaginalen Kolonisationsdiagnostik bei PPROM-Patientinnen mit Relevanz für konsekutive Risiken bei Frühgeborenen. Die vaginale Mikrobiomanalyse kann dabei individualisierte mütterliche und neonatale diagnostische, prophylaktische und therapeutische Strategien zur Identifizierung, zum Schutz und zur Behandlung der Hochrisiko-Neugeborenen nach PPROM verbessern.

Key Words

PPROM, EONS, Ureaplasma parvum, bronchopulmonary dysplasia, microbiome analysis

Schlüsselworte

PPROM, EONS, Ureaplasma parvum, Bronchopulmonale Dysplasie, Mikrobiomanalyse

Introduction

Preterm birth is associated with a high risk for short- and long-term neonatal complications and lifelong deficits, disabilities and disorders for the child. Around 30 to 40 % of all spontaneous preterm births [1, 2] are caused by preterm premature rupture of membranes (PPROM). PPROM is defined as a spontaneous rupture of the fetal membranes before the onset of labour at less than 37 weeks of gestational age (wga). The rupture of membranes is predominantly induced by vaginal infections with pathogenic bacteria (vaginal dysbiosis), subsequently leading to maternal and neonatal morbidities by ascending infections from the vagina to the uterine cavity, placenta and fetus [3]. The clinical management of PPROM < 34 wga is challenging and has to balance the prolongation of the pregnancy to prevent preterm

birth and the risk of progressing inflammation like chorioamnionitis associated with subsequent poor neonatal outcome. Standard clinical guidelines include a prophylactic antibiotic therapy, antenatal corticoid steroids for fetal lung maturation and tocolytic therapy if necessary. Close monitoring of the fetal heart rate as well as of the maternal inflammatory markers white blood cell count (WBC), interleukin 6 (IL-6), C-reactive protein (CRP) and procalcitonin (PCT) is also recommended [4].

Until now, neonatal sepsis remains a leading cause of neonatal mortality and morbidity, especially among very-low-birth-weight neonates (< 1500 g) [5-7]. Unfortunately, the signs and symptoms of neonatal sepsis are nonspecific and diverse [5]. The risk of EONS development after PPROM is no less than 14-22 % [1, 3]. Specific pathogens like *Ureaplasma* spp., especially *Ureaplasma parvum* [8-10], are frequently observed in preterm and very low birth weight neonates [8, 9, 11, 12]. Related pulmonal problems in these neonates, like acute respiratory distress syndrome, bronchopulmonary dysplasia [13-19] or chronic lung disease lead to sequelae and increased mortality [20-23].

Established diagnostic tools are currently unable to provide a rapid and accurate prediction of the estimated risk for poor neonatal outcomes. Hence, there is an unmet and indispensable need to identify this high-risk group of neonates as early as possible and to detect potentially harmful pathogens, especially Ureaplasma and Mycoplasm in these patients.

Case Report

A 29-year old primiparous woman was admitted to the university hospital in the 27+0 week of gestation due to preterm premature rupture of membranes (PPROM). Maternal history remained unremarkable: no specific risk factors for PPROM or preterm birth could be

identified. The initial ultrasonographic examination determined an inconspicuous vital female singleton in breech presentation with an oligohydramnion. Blood test revealed slightly elevated CRP 8.4 mg/l (normal range < 7.5 mg/l) and WBC 12.5 gpt/l (normal range 4.4-11.3 gpt/l), while maternal vital signs and cardiotocography (CTG) remained normal.

Daily routine controls of WBC, CRP, IL-6, as well as daily CTG recordings and evaluation of maternal vital signs including temperature, heart rate and blood pressure were performed. The patient gave informed consent to take part in the observational PEONS-trial, which aims to improve the prediction of an early onset neonatal sepsis (EONS) in neonates after maternal PPROM. Within this trial microbiota analyses by 16S rDNA sequencing was performed from maternal vaginal swabs before antibiotic treatment (day 0, hospital admission), during antibiotic treatment (day 5) and directly prior to caesarean section (day 7), as well as from neonatal pharyngeal swabs and meconium additionally to conventional microbiological culture.

Management decision was based on national guidelines, including antenatal corticoids (2 x 12 mg betamethasone intramuscular at interval of 24 hours), accompanying tocolytic treatment with the NO donor nitroglycerin 10 mg transdermal as well as antibiotic treatment. The antibiotic treatment started with azithromycin 1 g orally as single dose and ampicillin (3 x 2 g per day) intravenously at the day of admission, but was changed to a calculated therapy with cephalexin (3 x 500 mg per day) on day 4 (27+3 weeks of gestational age (wga) because of an increase of IL-6, while WBC and CRP remained normal. Conventional vaginal microbiological culture at admission reported no conspicuous colonization. Due to again rising inflammatory blood parameters a second escalation (meropenem; 3 x 500 mg

per day) became necessary on day 5 (27+5 wga) whereas no clinical sign of maternal infection as fever or tachycardia was observed. On the 8th day after PPROM (28+0 wga) contractions with unstoppable efforts of preterm labor appeared. The resulting cervical ripening with footling presentation and suspicious diagnosis of amniotic infection syndrome necessitated delivery by emergency cesarean section. Histological examination of the placenta, amniotic membranes and umbilical cord according to Redline criteria [24] showed signs of inflammation on the maternal site with low to moderate acute chorioamnionitis (stadium 2, grade 1) and on the fetal site with acute umbilical panvasculitis (stadium 2, grade 2).

The preterm infant (weight 1200 g, length 41 cm, head circumference 26.5 cm) underwent immediate neonatal intensive care unit (NICU) maximal therapy. A severe respiratory adaptation disorder was observed (APGAR-score 3/6/9) with no spontaneous respiration. Intubation and ventilation were necessary directly after birth. Surfactant was applied in the 10th minute of life. Ventilation had to be intensified in the first hours of life (PC-SIMV: PIP 25, PEEP 10, freq. 75/min).

Laboratory values on the first day of life were IL-6 261.4 pg/ml (normal range < 7.5 pg/ml) CRP 115.5 mg/l (normal range < 7.0 mg/l), WBC 25.5 gpt/l (normal range 7.8 -15.9 gpt/l) and respiratory acidosis with pH level 6.97 and pCO_2 10.4 kPa. The patient showed clinical signs of early-onset neonatal sepsis with circulatory problems, hypotension and catecholamine requirement. The capillary refilling time was more than 3 sec. Antibiotic therapy with meropenem 80 mg/kg/d and vancomycin 15 mg/kg/d was initiated. On the 2nd day of life, the antibiotic therapy was intensified and clarithromycin 20 mg/kg/d was additionally administered.

The first x-ray examination showed a severe respiratory distress syndrome (level 4). Recurrent bilaterally pneumothoraxes occurred and the installation of a thoracic drainage was necessary. Echocardiography showed signs of severe pulmonary hypertension. In the absence of lung compliance, escalated HFO ventilation (MAP 16, Ampl. HF 45, HF-freq. 9) was initiated in combination with NO ventilation (20 ppm). Under HFO, improved pCO_2 elimination was observed, but insufficient oxygenation could be achieved. A switch to conventional ventilation was necessary. Finally, the intermittent changes in both ventilation modes were required. The thoracic drainage could not be removed despite several attempts because of the repeated occurrence of re-pneumothoraces (4 times). The recurrent pneumothoraces and the radiographically detectable changes in the sense of bullous and atelectatic lesions in the lungs induced a genetic examination to exclude a congenital surfactant deficiency (SF-associated protein deficiency). The findings were normal. The thoracic drains were finally removed on the 14^{th} day of life. In persistent high oxygen demand anti-inflammatory treatment with hydrocortisone 3 mg/kg/d started on the 10^{th} day of life. There was only a slight improvement. Even a second treatment cycle did not fundamentally improve oxygen demand.

The blood culture results were negative and conventional microbiological examinations of the throat, rectal swab and tracheal aspirates showed no harmful conspicuities. Despite successful EONS therapy, the infant developed a therapy refractory pulmonary insufficiency with consecutive right heart failure, resulting in death on the 36^{th} day of life.

Discussion

The additional microbiota analyses by 16S rDNA sequencing within the PEONS-trial (*ClinicalTrials.gov Identifier: NCT03819192)* had been performed subsequently after clinical treatment of the patients from maternal vaginal swabs, as well as from neonatal pharyngeal

swabs and meconium. Amplicon libraries of the V1–V2 region of the 16S rDNA were sequenced on a MiSeq system (2x250 bp; Illumina, Hayward, CA), and merged and aligned reads were clustered allowing differences in two nucleotides [25, 26]. The most relevant species identified are indicated in figure 1B. Others, include members of different non-relevant genera such as *Prevotella spp., Ralstonia spp*., *Burkholderia spp*., *Stenotrophomonas spp*., *Propionibacterium spp*. as well as *Corynebacterium spp*. The microbiota analysis detected initial a high relative abundance of *Ureaplasma parvum* besides the dominating *Lactobacillus crispatus* in the maternal specimen. Maternal antibiotic treatment resulted in depletion of *L. crispatus,* but not of *U. parvum. U. parvum* became the dominant microorganism immediately before delivery and was also detectable in high relative abundance in neonatal specimens.

An ascending amnion infection of *Ureaplasma* spp. with the possibility of vertical transmission in utero or perinatally is possible [27-29]. *Ureaplasma* spp., especially *Ureaplasma parvum* [8, 9], is frequently observed in preterm and very low birth weight neonates [8, 11]. *Ureaplasma*-induced inflammation, either directly cytokine-associated, or due to higher host vulnerability to secondary impact factors like the immature immunological processes of an preterm infant is discussed to be the mutual underlying mechanism in prenatal, perinatal and neonatal morbidities [12, 23, 27]. Especially *Ureaplasma parvum* serovars seam to increase the risk of spontaneous preterm birth [30].
Inflammation and mechanical ventilation as main risk factors for the development of bronchopulmonary dysplasia (BPD) in prematurely born infants [18, 31, 32] as well as an imbalance to proinflammation has been widely acknowledged as key feature in the BPD pathogenesis [18]. The BPD risk therefore is tripled in infants colonized with *Ureaplasma*

spp. [33], likely due to emerging inflammatory processes prenatally. Related pulmonal problems in these neonates, like respiratory distress syndrome, bronchopulmonary dysplasia [13-17] or chronic lung disease lead to sequelae and increased mortality [20-22]. Accordingly, radiological manifestation of bronchopulmonary dysplasia (type III) was observed early at 3rd day and more distinctly from 14th day of life onwards (figure 1A). The progressive air-space changes described here are known to be associated with *Ureaplasma* infections [34-38].

A special feature in the problematic constellation of detection on one side and the differentiation between colonization and infection on the otherside in the perinatological context is that the bacteria *Ureaplasma* spp. do not survive in routine culture media and are not detected by Gram staining, but require complex growth media or molecular-based techniques [27]. Thus, *Ureaplasma spp.* are not covered by routine diagnostics. In the case described, the routine diagnostics also showed inconspicuous results.

Furthermore, the optimal indications for treatment as well as treatment regimens itself of both maternal and neonatal *Ureaplasma* colonization or infection is poorly defined. Eradication approaches have not proven successful, so far, and standardized regimens are missing [12, 27, 39].

In summary this case demonstrates impressively:

1) the limitations of the current conventional tools and routine diagnostic procedures in PPROM patients and their preterm infants

2) the indispensable necessity of an interdisciplinary obstetrical and neonatal awareness in the diagnostic and therapeutic management of PPROM patients and their preterm infants

3) the need of an early and effective detection of a colonization with potentially harmful pathogens, especially *Ureaplasma* and *Mycoplasma [21, 40],* in PPROM patients and their preterm infants. Even if it is unclear if and to what extend maternal antimicrobial therapy is able to treat intrauterine infections or to eradicate those pathogens [40], the knowledge on their presence may fasten targeted neonatal antimicrobial therapy. And furthermore

4) that microbiome analysis may give detailed insights into the presence and relative abundance of potentially pathogenic microorganisms, which may result in optimized individual and targeted maternal and fetal diagnostic, prognostic, prophylactic and therapeutic procedures [1, 3].

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Figure 1. (A) Chest x-ray at the 14th day of life: Early radiographic manifestation similar to bronchopulmonary dysplasia (type III) in association with *Ureaplasma* infection. (B) 16S rDNA-analysis of maternal vaginal swabs from PPROM diagnosis (Day 0) to caesarean section (Day 7), neonatal pharyngeal swabs (Ph) and meconium (Me). Others include different non-relevant species, such as *Prevotella spp., Ralstonia spp*., *Burkholderia spp*., *Stenotrophomonas spp*., *Propionibacterium spp*., *Corynebacterium spp*..