Transabdominal amniocentesis in expectant management of preterm premature rupture of membranes: A single center prospective study

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Aims. The aim of this study was to evaluate the role of IL-6 point-of-care test in amniotic fluid obtained from serial amniocentesis in expectantly managed women with PPROM between 24 and 34 weeks of gestation.
Methods. We conducted a prospective observational cohort study which included 62 pregnant women with PPROM in gestational weeks between 22+0 and 34+0. Women aged >18 years were eligible if they presented with PPROM and a singleton pregnancy. Only women who delivered at >24.0 weeks were included in the study. In all women, the maternal blood sampling and a transabdominal amniocentesis were performed at the time of admission prior to the administration of corticosteroids, antibiotics, or tocolytics, to rule out signs of chorioamnionitis. Maternal temperature, maternal serum C-reactive protein (CRP) and white blood cell (WBC) counts were assayed every subsequent day until delivery. Amniotic fluid was used for the clinical assessment (IL-6 point-of-care test, identification of microorganisms in the amniotic fluid. After one week of expectant management of PPROM, second amniocentesis with amniotic fluid sampling was performed in patients who did not deliver. For all newborns, medical records regarding neonatal morbidity and mortality were reviewed.
Results. In total, 62 women aged 19 to 41 years were recruited in the study. The mean gestational age at the time of PPROM was 31+0, the mean gestational age at labor was 32+1, and the median time from PPROM to childbirth was 112 h. IL-6 point-of-care test values above 1,000 pg/mL (positive Il-6 AMC) were found in 12 women (19.4%) with median interval from PPROM to childbirth 56 h (min-max: 6.4-288). IL-6 point-of-care test values below 1,000 pg/mL (negative Il-6 AMC) were found in 51 women (81.0%). The neonatal mortality rate was 1.9% and was associated with prematurity.
Conclusion. The major clinical finding of our study is that serial transabdominal amniocentesis with Il-6 point-of-care test helps to identify a high inflammatory status in amniotic fluid in women with PPROM. Subsequent expectant management of women with PPROM does not lead to worsening of short-term neonatal outcomes.

Key words: PPROM, Il-6, poin-of-care test, amniocentesis

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INTRODUCTION

Early leakage of amniotic fluid in a low week of pregnancy (PPROM) is regarded as an aberration of the integrity of the fetal envelope with efflux of the amniotic fluid that precedes the onset of uterine activity. It complicates about 2-4% (ref.[1]) of all births. It is related to the lower gestational age of the fetuses and in consequence results in higher perinatal and maternal morbidity. PPROM can be subdivided according to gestational age into early PPROM (between 24+0 and 33+6 weeks) and late PPROM (between 34+0 to 36+6 weeks) (ref.[2,3]).

Gestational age is the decisive factor in the management of PPROM cases. An active approach is generally applied above 34+7 gestational week[4]. Although there are data indicating that this limit should be taken as the 35th gestational week to increase neonatal benefit, there is no consensus on this matter[5]. The expectant approach is recommended between 24+0 and 33+7 weeks[3]. Extension of the latency period nonetheless exposes the fetus to complications such as chorioamnionitis, retroplacental hematoma and fetal distress. Recent data suggest a relationship between placental inflammation and important clinical outcomes such as neurologic impairment and chronic lung disease. This has coincided with a more active clinical approach to the management of chorioamnionitis and preterm labor[6].

A key role in PPROM etiology is played by an infection in the choriodecidual space and inflammation[7]. Biochemical processes in women with PROM are accompanied by an increase in matrix metalloproteinases (MMP) (ref.[8]) in amniotic fluid and a decrease in their inhibitors[2,3]. During premature labor, MMPs stimulate primarily proinflammatory cytokines and prostaglandins.

These processes lead to violation of fetal membrane integrity, local inflammation, and ascending bacterial colonization with the development of intra-amniotic inflammatory invasion (MIAC) (ref.[9]). MIAC complicates PPROM in 20-50% of cases and depends on gestational age[10]. In amniotic fluid, the most common bacteria are genital mycoplasmas (*Ureaplasma urealyticum*, *Ureaplasma parvum* and *Mycoplasma hominis*). They are found in 70-80% patients with PPROM (ref.[10]). The presence of bacteria in the amniotic fluid is postulated to activate an intraamniotic innate immune response through the system of pattern recognition receptors, resulting in microbial-associated intraamniotic infection (IAI) (ref.[11]). On the other hand, some endogenous mediators called alarmins (e.g., high mobility group box-1 protein) are released into the amniotic fluid and can trigger development of sterile IAI (the presence of IAI without any proven microorganism in the amniotic fluid) (ref.[12]).

U. urealyticum, *U. parvum* and *Mycoplasma hominis* are most frequently isolated from the amniotic fluid and placenta in cases of histologic and clinical chorioamnionitis and in association with spontaneous preterm labor (PTL) and PPROM (ref.[13]). In addition, HCA is associated with a high concentration of inflammatory mediators in amniotic fluid, including proinflammatory cytokines (TNF-α, IL-1β, IL-6 and IL-8) (ref.[5]) and natural cytokine inhibitors (soluble TNF receptors p55 and p75) (ref.[5]). Kacerovsky et al. have shown that the presence of both MIAC and HCA is associated with strong intraamniotic and fetal inflammatory response[14]. Neonates from this subgroup PPROM may be threatened by high risk of infections, such as early onset sepsis, and thus might benefit from an active management including induction of labor. To identify these infection-related and inflammatory intra-amniotic conditions, evaluation of an amniotic fluid sample is considered a gold standard approach[15]. A positive amniotic interleukin (IL)-6 by point-of-care testing was used as the definition of IAI due to its proven prognostic and clinical utility[16,17]. The objective of the current study was to evaluate the role of IL-6 point-of-care test in amniotic fluid obtained from serial amniocentesis in expectantly managed women with PPROM between 24 and 34 weeks of gestation.

METHODS

We conducted a prospective observational cohort study which included 62 pregnant women with PPROM in gestational week between 22+0 and 34+0 who were admitted to the Department of Obstetrics and Gynecology, University Hospital Olomouc, Czechia between May 2015 and September 2018. Women aged >18 years were eligible if they presented with PPROM and a singleton pregnancy. Only women who delivered at>24.0 weeks were included in the study. Patients were excluded from the study if they had signs of clinical chorioamnionitis, signs of fetal hypoxia, the presence of either congenital or chromosomal fetal abnormalities, multiple gestation, and cases in which amniocentesis was not possible. Clinical chorioamnionitis was diagnosed in the presence of a maternal temperature of ≥37.8 °C and ≥2 of the following criteria: (1) uterine tenderness; (2) malodorous vaginal discharge; (3) maternal leukocytosis (WBC count of >15,000 cells/mm^3); (4) maternal tachycardia (>100 beats/ min); and (5) fetal tachycardia (>160 beats/min) (ref.[18]).

Enrolled subjects signed informed consent and underwent routine institutional antenatal care for PPROM. This included hospitalization, administration of a course of antenatal steroids (betamethasone acetate/phosphate 12 mg intramuscularly in two doses 24 h apart) and a standardized course of latency antibiotics (parenteral penicillin). Tocolysis was administered for 48 h in the absence of clinical chorioamnionitis, abruptio placentae and fetal compromise. Delivery was undertaken for spontaneous preterm labor, nonreassuring maternal or fetal status, clinical concern for infection, or when a gestational age of 34 weeks was reached.

Clinical diagnosis of membrane rupture was based on a history of amniotic fluid leakage, ultrasound assessment of amniotic fluid volume, a sterile speculum examination confirming amniotic fluid drainage from the cervical os, and biochemical tests when in doubt (AmniSure) (ref.[19,20]). Clinical chorioamnionitis, placental abruption, umbilical cord prolapse, development of fetal distress and uterine surgery history were taken as the indications for cesarean section.

Upon admission, cervical swabs were obtained for the identification of bacteria. Women with a positive cervical culture were correctly treated parenteraly according to the microorganism isolated. In case of negative finding the antibiotics were withdrawn.

In all women, the maternal blood sampling and a transabdominal amniocentesis were performed at the time of admission prior to the administration of corticosteroids, antibiotics, or tocolytics, to rule out signs of chorioamnionitis. Maternal blood sample was obtained by venipuncture of the cubital vein, and was sent to the laboratory immediately following sampling to assess the levels of inflammatory markers (C-reactive protein and white blood cell count) (ref.[21]).

Routine amniocentesis for patients with PPROM was the preferred management strategy at our perinatal service. Amniotic fluid was collected by sterile transabdominal amniocentesis under ultrasound control and used for the clinical assessment (IL-6 point-of-care test, identification of microorganisms in the amniotic fluid). The remaining fluid was subsequently centrifuged and the supernatant stored at -80 °C until analysis. Microbial invasion of amniotic fluid (MIAC) was defined as a positive PCR for genital mycoplasmas and/or a positive amniotic fluid culture[22]. The procedure was deferred in 5 patients with marked oligohydramnios or if the only accessible fluid was found under the central portion of an anterior placenta (Fig. 5). The decision for completing an amniocentesis was at the discretion of the attending perinatologist. In women with amniotic fluid IL-6 point-of-care test>1000 pg/mL active management of labor was a standard approach. Patients who did not progress into labor spontaneously, who had no indications for cesarean

Table 1. Basic characteristics of women with PPROM. The data in the table are presented as the number (%) or mean / median with (minimum – maximum) values.

	TOTAL	IL-6 AMC positive	IL-6 AMC negative	*P*
Number of patients	62	12	50	-
Age of patients (yr)	30.9 ± 5.4	29.9 ± 5.4	31.1 ± 5.5	0.406
mean ± SD (min–max)	(19–41)	(22–39)	(19–41)	
Smoker	12 (19.4%)	4 (33.3%)	8 (16.0%)	0.223
Parity				0.213
0	4 (6.5%)	1 (8.3%)	3 (6.0%)	
1	34 (54.8%)	4 (33.3%)	30 (60.0%)	
2	16 (25.8%)	4 (33.3%)	12 (24.0%)	
3	5 (8.1%)	2 (16.7%)	3 (6.0%)	
4	0 (0%)	0 (0%)	0 (0%)	
5	1 (1.6%)	0 (0%)	1 (2.0%)	
6	1 (1.6%)	0 (0%)	1 (2.0%)	
7	1 (1.6%)	1 (0%)	0 (0%)	
Gestational age at time of PPROM (wk+d)	31+0	29+4	31+3	0.009
mean (min–max)	(22+2 – 34+0)	(25+1 –32+4)	(22+2 – 34+0)	
Gestational age at time of labor (wk+d)	32+1	30+1	32+4	0.001
mean (min–max)	(26+1 – 35+0)	(26+1 – 33+1)	(27+2 – 35+2)	
Latency from PPROM to labor (h)				
median (min–max)	112 (4 –606)	55.9 (6 – 288)	118.5 (4 – 606)	0.042
Type of labor				0.773
vaginal	41 (66.1%)	9 (75.0%)	31 (64.6%)	
Cesarean section	20 (32.3%)	3 (25.0%)	17 (35.4%)	
CRP median (min–max)	6.2 (0.6 – 84.8)	9.5 (0.6 – 84.8)	5.8 (0.6 – 23.5)	0.012
WBC median (min–max)	11.9 (6.9 – 24.0)	12.4 (7.0 – 24.0)	11.9. (6.9 – 21.5)	0.514

Table 2. Microbes identified in the cervical swabs in women with PPROM.

Microbes	TOTAL (n=62)	IL-6 AMC positive (n=12)	IL-6 AMC negative (n=50)	*P*
Negative finding	21 (33.9%)	3 (25.0%)	18 (36.0%)	0.735
Ureaplasma spp.	20 (32.3%)	5 (41.7%)	15 (30.0%)	0.500
Escherichia coli	10 (16.4%)	2 (16.7%)	10 (20.0%)	1.000
Group B Streptococcus spp.	7 (11.3%)	1 (8.3%)	6 (12.0%)	1.000
Mycoplasma spp.	7 (11.5%)	2 (16.7%)	5 (10.0%)	0.612
Enterococcus spp.	7 (11.3%)	1 (8.3%)	6 (12.0%)	1.000
Klebsiella spp.	2 (3.3%)	2 (16.7%)	0 (0%)	**0.035**
Pseudomonas	1 (1.6%)	0 (0%)	1 (2.0%)	1.000
Yeasts	1 (1.6%)	0 (0%)	1 (2.0%)	1.000

Table 3. Microbes identified in the amniotic fluid from amniocentesis in women with PPROM.

Microbes	TOTAL (n=62)	IL-6 AMC positive (n=12)	IL-6 AMC negative (n=50)	*P*
Negative finding	48 (78.7%)	5 (41.7%)	43 (87.8%)	0.002
Ureaplasma spp.	10 (16.1%)	6 (50.0%)	4 (8.2%)	0.002
Group B Streptococcus spp.	1 (1.6%)	1 (8.3%)	0 (0%)	0.197
Peptostreptococcus	1 (1.6%)	0 (0%)	1 (2.0%)	1.000
Lactobacillus	1 (1.6%)	0 (0%)	1 (2.0%)	1.000
Klebsiella spp.	1 (1.6%)	0 (0%)	1 (2.0%)	1.000
Citrobacter	1 (1.6%)	0 (0%)	1 (2.0%)	1.000
Mycoplasma	1 (1.6%)	1 (8.3%)	0 (0%)	0.197
Haemophilus	1 (1.6%)	0 (0%)	1 (2.0%)	1.000

section, with no signs of clinical chorioamnionitis, with amniotic fluid IL-6 point-of-care test <1000 pg/mL and without increase in maternal CRP and white blood cell count, were followed up. In a subgroup of patients with gestational age below 26 weeks a conservative clinical management prevailed even in cases with positive Il-6 point-of-care test[1].

Maternal temperature, maternal serum C-reactive protein (CRP) and white blood cell (WBC) counts were assayed every subsequent day until delivery. After one week of expectant management of PPROM, second amniocentesis with amniotic fluid sampling was performed in 10 patients who did not deliver. In patients with amniotic fluid IL-6 point-of-care test>1000 pg/mL an active management of labor was adopted. Subgroup of patients with IL-6 point-of-care test <1000 pg/mL continued with expectant management of PPROM.

Analysis of amniotic fluid samples for IL-6 concentrations

Amniotic fluid was used for the clinical assessment (IL-6 point-of-care test, identification of microorganisms in the amniotic fluid. For the automated electrochemiluminiscence immunoassay method, the amniotic fluid IL-6 concentrations (pg/mL) were measured using the immuno-analyzer Milenia POCScan (Milenia Biotec, Giesen, Germany) (ref.[23]). For each patient, 0.1 ml of amniotic fluid was pipetted to the sampling well of the Picoscan cassette. The IL-6 cassette is a lateral flow immunoassay that requires 20 min incubation at room temperature. The Picoscan analyzer measures the color intensity of the test band and calculates the IL-6 concentration (pg/mL) according to a stored standard curve.

Detection of *Ureaplasma species* and *Mycoplasma hominis* in the amniotic fluid

DNA was isolated from the amniotic fluid using the QIAamp DNA mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions (using the protocol for isolating bacterial DNA from biological fluids). RT-PCR was performed using the Rotor-Gene 6000 instrument (Qiagen, Hilden, Germany) employing the commercial AmpliSens®C. Ureaplasma/M. hominis-FRT kit (Federal State Institution of Science, Central Research Institute of Epidemiology, Moscow, Russia) to detect DNA from *Ureaplasma species* and *Mycoplasma species* in a common PCR tube. As a control, we included a PCR run for beta-actin, a housekeeping gene, to exclude the presence of PCR inhibitors[24].

For all newborns, medical records regarding neonatal morbidity and mortality were reviewed. Short-term neonatal outcome included the presence of the following outcomes: the incidence of early-onset sepsis (EOS) and late-onset sepsis (LOS). Definitive cases of sepsis were those infants with positive blood cultures. The sepsis cases in the first 7 days were defined as early sepsis, and sepsis cases after the seventh day were accepted as late sepsis[25].

In 56 cases, the placenta was sent for histological examination after delivery. Acute chorioamnionitis was diagnosed based on the degree of polymorphonuclear leukocyte infiltration that was inspected separately in the free membranes (amnion and choriondecidua), chorionic plate and umbilical cord, according to criteria proposed by Salafia et al.[26,27]. Chronic choriomnionitis was defined by the infiltration of the organ by lymphocytes, plasma cells and/or macrophages[28]. This study was approved by the Institutional Review Board Committee. Statistical analysis of the results was carried out using the IBM SPSS Statistics version 22. The normality of data was tested using Shapiro-Wilk test. To analyse correlation between maternal CRP and IL-6 point-of-care test Spearman's correlation analysis was used. The Fisher´sexact test was used to compare Il-6 AMC positive and negative groups in categorical parameters. The Student´s test or Mann-Whitney U-test were used to compare the differences between groups for quantitative variables. Tests were performed at a 5% level of significance.

RESULTS

In total, 62 women aged 19 to 41 years were recruited in the study. The mean age of women was 30.9 years. The mean gestational age at the time of PPROM was 31+0, median 31+6, minimum 22+2 and maximum 34+0 (Table 1, Fig. 1). The mean gestational age at labor was 32+1, median 32+4, minimum 26+1 and maximum 35+0. The median time from PPROM to childbirth was 112 h (4.7 days), minimum latency length was 4 h and maximum 606 h (25.3 days). Maternal CRP values ranged from 0.6 to 84.8 mg/L with a median of 6.2 mg/L. In 31 women (49.2%) the maternal CRP value was above 6 mg/L. To evaluate correlation between maternal CRP, and IL-6 point-of-care test Spearman's correlation analysis was applied. This showed a significant positive correlation between maternal CRP and IL-6 point-of-care test (r=0.364; *P*=0.006) (Fig. 4).

The majority of patients 41 (66.1%) delivered vaginally, caesarean section was performed in 20 (32.3%) women (Table 1). Negative microbial finding was found 21 (33.9%) and 48 (77.4%) of cervical swabs and amniotic fluid samples respectively. *Ureaplasma spp.* was the most common microbial finding present in 20 (32.3%) cervical swabs and in 10 (16.1%) amniotic fluid samples (Table 2 and 3). The median of antibiotic treatment interval was 3 days (min-max: 1-9). In all women, antibiotic therapy has been initiated parenteraly with Penicillin G (PNC) as latency treatment which was subsequently modified according to the results of microbial cultures. In 18 cases (28.6%) macrolide antibiotics were administered. PNC alone or in combination with Clarithromycin was administered in 44 (71.0%), and 11 (17.7%) patients respectively (Table 4). Signs of acute or chronic chorioamnionitis were found in 16 (25.8%) and 3 (4.8%) patients respectively (Table 5).

The median IL-6 point-of-care (Il-6 AMC) test values from amniocentesis was 264 pg/mL (min-max: 50-10,000) (Fig. 3). IL-6 point-of-care test values above 1,000 pg/mL (positive Il-6 AMC) were found in 12 women (19.4%). The mean age of women was 29.9 years (min-max: 22-39). The mean gestational age at PPROM was 29+4, median

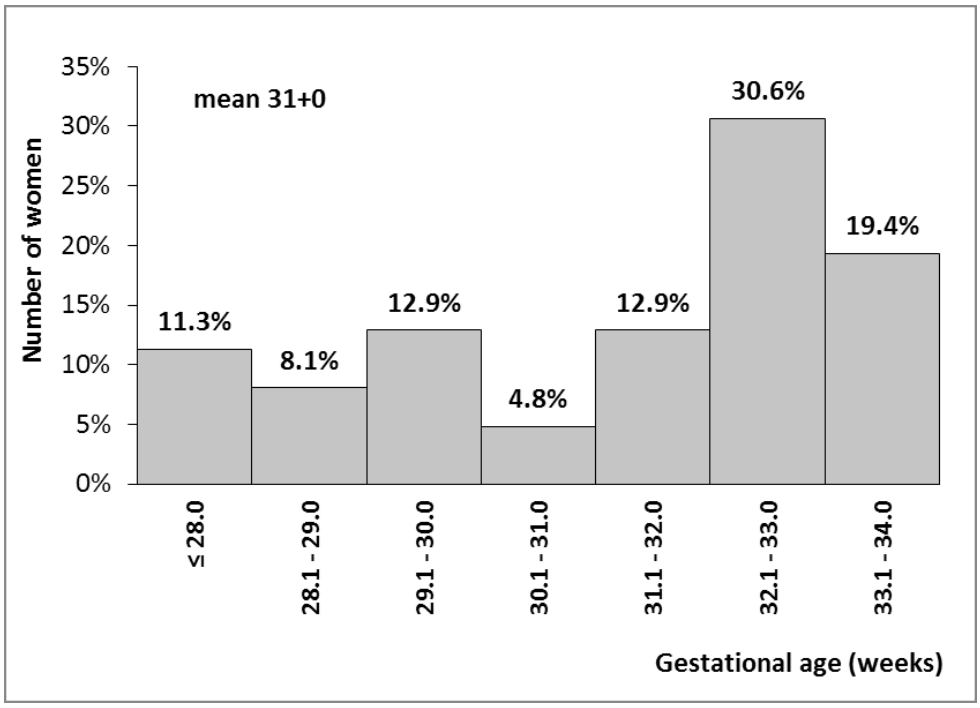

Fig. 1. Distribution of women according to gestational age at PPROM.

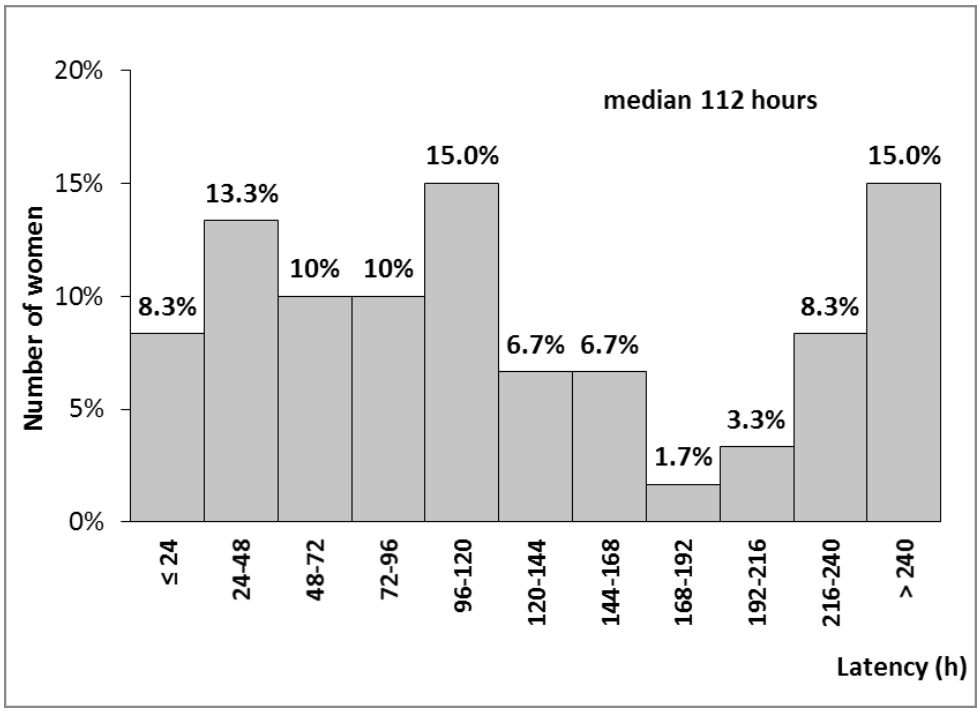

Fig. 2. Distribution of latency time from PPROM to labor.

29+5, minimum 25+1 and maximum 32+4. The mean gestational age at birth was 30+1, median 29+6, minimum 26+1 and maximum 33+1. Median interval from PPROM to childbirth was 56 h (min-max: 6.4-288) (Table 1). IL-6 point-of-care test values below 1,000 pg/mL (negative Il-6 AMC) were found in 50 women (80.6%). Lower gestational age at time of PPROM (P=0.009), lower gestational age at time of labor (P=0.001) and shorter latency period from PPROM to labor (P=0.042) were shown in women with positive Il-6 AMC. Maternal CRP values in women with IL-6 point-of-care test value > 1,000 pg/mL ranged from 0.6 to 85 mg/L with a median of 9.5 mg/L. In 10 women (83.3%), the maternal CRP was above 6 mg/L. Levels of maternal CRP were lower (median 5.8; range 0.6 – 23.5) in Il-6 AMC negative subgroup (P=0.012), while white blood cell counts were not different between both groups (Table 1). In cervical culture, the presence of *Klebsiella spp.* was found to be high in women with positive IL-6

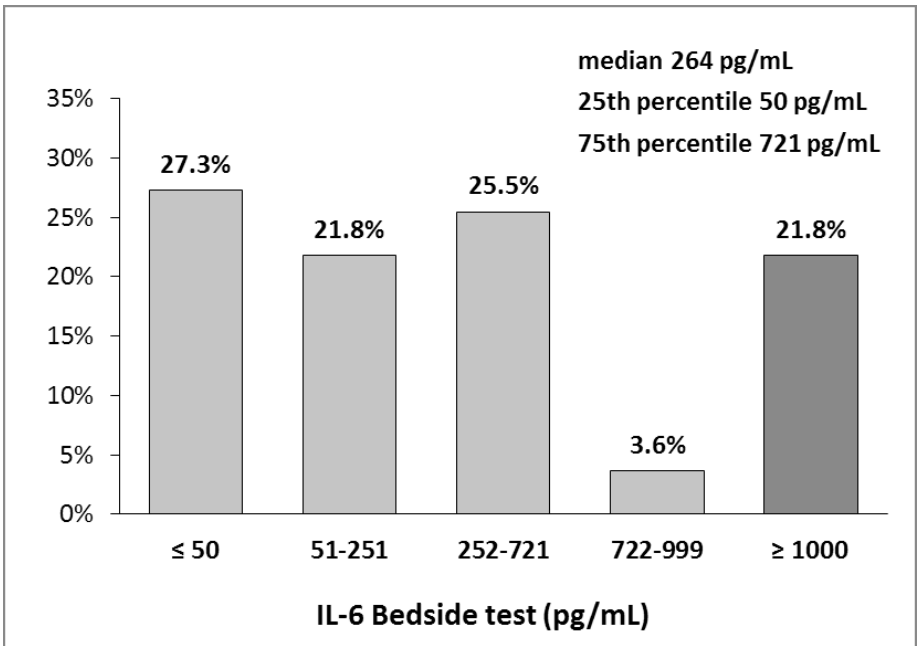

Fig. 3. Distribution of IL-6 point-of-care test values in women with PPROM.

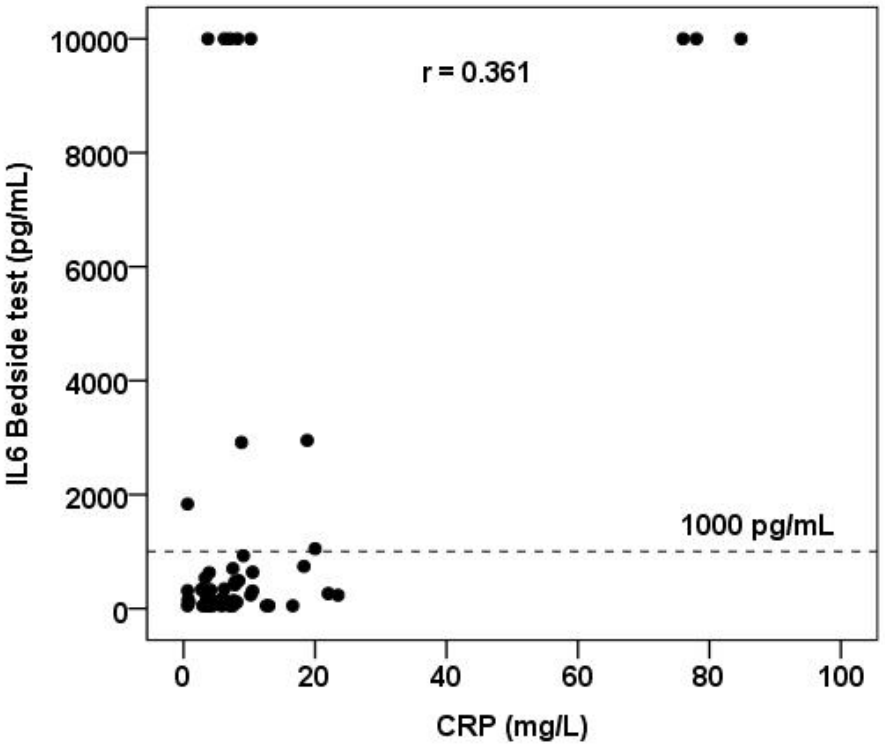

Fig. 4. Correlation between maternal CRP and IL-6 point-of-care test in women with PPROM.

AMC (16.7%) while negative (0%) in patients with IL-6 point-of-care test below 1,000 pg/mL, *P*=0.035. In amniotic fluid, the presence of *Ureplasma spp.* was higher in patients with positive Il-6 AMC (50.0%) when compared to women with negative Il-6 AMC (8.2%), *P*=0.002. The presence of other microbes was not significantly different between groups (Table 3). Age, smoking, parity and type of labor were no different between groups of women with negative or positive Il-6 AMC (Table 1, Fig. 2). Acute chorioamnionitis was found more often in women with positive Il-6 AMC as compared with subgroup of women with negative Il-6 AMC (66.8% vs. 16.0%), *P*=0.011. Chronic chorioamnionitis or no signs of placental inflammation were found more frequently in women with negative IL-6 AMC (*P*=0.011) (Table 5).

Early-onset neonatal sepsis was diagnosed in 8 neonates (12.7%) (Fig. 5). In 4 out of 8 neonates placental signs of acute chorioamnionitis were shown, in 3 cases

Table 4. Antibiotic treatment in women with PPROM.

Antibiotics		IL-6 AMC positive (n=12)	IL-6 AMC negative (n=50)	*P*
PNC	44 (71.0%)	7 (58.3%)	37 (74.0%)	0.305
PNC + Clarithromycin	11 (17.7%)	2 (16.7%)	9 (18.0%)	1.000
PNC + Azithromycin	2 (3.2%)	0 (0%)	2 (4.0%)	1.000
Clindamycin	2 (3.2%)	2 (16.7%)	0 (0%)	0.035
Clarithromycin	1 (1.6%)	0 (0%)	1 (2.0%)	1.000
Ampicillin and Sulbactam + Azithromycin	1 (1.6%)	0 (0%)	1 (2.0%)	1.000
Amoxicillin + Clavulanic Acid + Azithromycin	1 (1.6%)	1 (8.3%)	0 (0%)	0.194

Table 5. Placental histopathology in women with PPROM.

Histological finding	TOTAL (n=62)	IL-6 AMC positive (n=12)	IL-6 AMC negative (n=50)	*P*
Acute inflammation	16 (25.8%)	8 (66.8%)	8 (16.0%)	**0.011**
purulent inflammation	7 (11.3%)	2 (16.6%)	5 (10.0%)	0.614
Chronic inflammation	3 (4.8%)	1 (8.3%)	2 (4.0%)	1.000
Without inflammation	26 (41.9%)	1 (8.3%)	35 (70.0%)	**0.011**

placental histopathology was negative. In 1 case, placental histology was not performed. This patient was shown as Il-6 AMC negative, with negative microbial finding from amniotic fluid and maternal CRP at the level 8.1 mg/L. She delivered at 34+3 weeks with positive microbial finding in the cervical culture (*E.colli, Pseudomonas aeruginosae*) In one out of 8 cases (from the Il-6 AMC positive subgroup) the newborn after six days of latency died (7 days after delivery; 26+1, 700 g) due to prematurity (respiratory distress syndrome). In this case the placenta had signs of purulent acute chorioamnionitis. Late neonatal sepsis was observed in 2 newborns (3.2%) (Fig. 5). In one case a woman in the Il-6 AMC positive subgroup delivered at 26+1 (850 g) weeks, in the second case a woman in the Il-6 AMC negative subgroup delivered at 27+2 (1025 g) weeks. In both cases, the placental histology was negative.

DISCUSSION

To reduce maternal and neonatal infections and gestational-age-dependent morbidity in expectant management, a 7-day course of therapy with a combination of intravenous ampicillin and erythromycin followed by oral amoxicillin and erythromycin is recommended for women with preterm PROM who are less than 34+0 weeks of gestation[4,1]. however, there are no clear recommendations on how often and using which parameters expectant management should be performed. In this prospective study we incorporated repeated transabdominal amniocentesis into expectant management of women with PPROM to be able more precisely evaluate the risk of MIAC and subsequent HCA. The assessment of Il-6 from amniotic fluid is one of the most common approaches in evaluating the systemic inflammatory response and its intensity in women with PPROM.

Intraamniotic Il-6

In our study we identified 12 (19.4%) (Table 1) cases of PPROM as Il-6 positive, using cut-off value of >1,000 pg/mL. The cut-off values of the amniotic fluid IL-6 concentration to identify IAI in women with PPROM have been developed for the past years. In 1993, Romero et al. established the amniotic fluid IL-6 concentration cut-off value of 7.9 ng/mL in women with PPROM with a sensitivity of 81%, specificity of 75%, positive predictive value of 67%, and negative predictive value of 86% (ref.[29]). In 2014 Kacerovsky et al. established the amniotic fluid IL-6 poin-of-care test cut-off value of 1000 pg/mL in women with PPROM with a sensitivity of 60%, specificity of 94%, positive predictive value of 75%, and negative predictive value of 88% to be optimal for prediction of both MIAC and HCA (ref.[21]). Musilova et al. using the same point-of-care detection system as our group observed presence of microbial-associated IAI (both IAI and MIAC) in 21% (34/166) of women. If the diagnosis of IAI was expanded to include both a positive IL-6 and neutrophil infiltration of the amnion (histological amnionitis), then the proportion of women with IAI was 23% (ref.[30]). These data correlate with our results, although a slightly higher proportion of IAI in the study group of Musilova et al. could be explained by their lower cut-off level for Il-6 (745 pg/mL).

Maternal CRP

In a subgroup of patients with positive Il-6 AMC test we have found higher levels of maternal CRP as compared to Il-6 AMC test negative woment. Maternal serum CRP has been proposed as a marker of infection and inflammation in several diseases[31]. With MIAC and HCA, CRP exhibited a weak and contradictory association. A systematic review by Martinez et al. concluded that there was no evidence to support the use of CRP as a tool for the

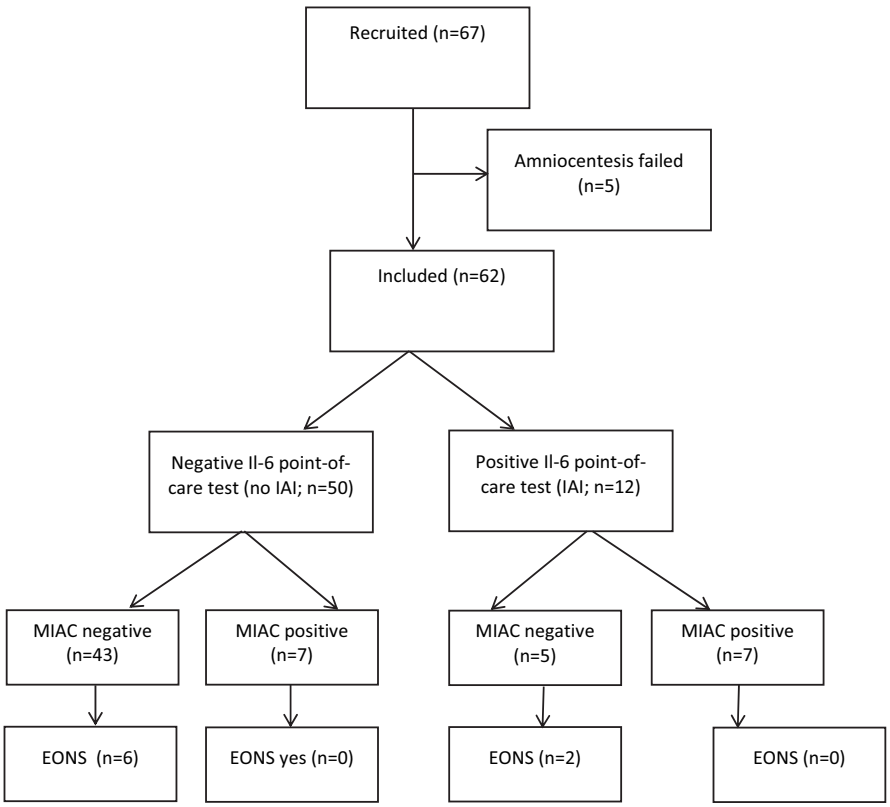

Fig. 5. Flowchart of the study.

prediction of HCA and significant differences were reported when CRP levels were compared between PPROM women with or without MIAC (ref.[32,33]). Stepan et al. in their large study found out that only the extreme cut-off levels of CRP, represented by the 95th percentile, predicted the presence of MIAC and HCA below 32 weeks of gestation. However, the low sensitivity limits the clinical utility of this detection method[34]. Also, in our data we found very low correlation between Il-6 poin-of-care test and levels of maternal CRP (Fig. 4).

White blood cell counts

There was no difference in white blood cell counts between the Il-6 AMC positive and negative subgroups in our study. Different maternal WBC count cutoff values have been proposed to predict MIAC, histological or clinical chorioamnionitis, and neonatal infection[35,36]. However, none of these values have good predictive indices. Musilova et al. showed a maternal WBC count cutoff value 14.0×109/L to have a good negative predictive value, but its positive predictive value and likelihood ratio were poor. Our data are consistent with their conclusion that this prevents WBC count cutoff value from being used in the clinical setting[20].

Microbial findings in cervical cultures

Ureaplasma spp. was detected in cervical cultures of 20 (32.3%) and in amniotic fluid samples of 10 (16.1%) patients and was the most common microbial pathogen in our study group (Table 2). *Ureaplasma spp.* are considered a part of normal genital flora and have an average colonisation rate of 40-80% (ref.[37]). In their study, Gupta et al. determined the presence of

Ureaplasma urealyticum in the lower genital tract of 32% of infertile women[38] However, according to recently published data, *U. urealyticum*, *U. parvum* and *Mycoplasma hominis* are most frequently isolated from the amniotic fluid and placenta in cases of histologic and clinical chorioamnionitis and in association with spontaneous PTL and PROM (ref.[39,40]). Kacerovsky et al. found *Ureaplasma spp*. in 21% of women with PPROM (ref.[21]). More recently, Pavlidis et al. used a mouse model to show ascending infection of Ureaplasma parvum is associated with preterm birth. The study reports an increase in preterm birth from 13% to 28% following vaginal colonisation with Ureaplasma and upregulation of pro-inflammatory cytokines, aligning with the human clinical response[41]. Thus, our data are in accordance with recent literature showing *Ureaplasma spp.* as one of the most common microbes in female genital tract, and associated with adverse pregnancy outcomes such as preterm birth, stillbirth, histologic chorioamnionitis, and neonatal morbidities.

Antibiotic treatment

The most common antibiotic regimen used in our study was parenteral PNC alone administered in 71.0% of patients, followed by combination of PNC with Clarithromycin which was administered in 17.7% of cases (Table 4). The practice of administering antibiotics to patients with preterm PROM is grounded in the results of multiple randomized clinical trials and a large systematic review and meta-analysis[42-44]. Antimicrobial

agents have been shown to prolong the latency period, decrease neonatal infection, and reduce respiratory morbidity[44,45]. Several antibiotic regimens have been used in patients with preterm PROM, including ampicillin, amoxicillin-clavulanate, penicillin, erythromycin, mezlocillin, pipericillin, cefexin, cefizox, gentamicin, clindamycin, azithromycin and combinations of different agents[44]. The current recommendation in Czechia includes intravenous PNC G (ref.[22]). However, penicillin is not effective in the treatment of PPROM (ref.[44]). Therefore we adopted parenteral antibiotic treatment according to the microorganism isolated from the amniotic fluid. Gomez et al. followed 46 patients with preterm PROM for whom amniocenteses were performed between 18–32 weeks. In their study the prevalence of intra-amniotic inflammation for the initial amniocentesis was 39%, seven patients had a positive amniotic fluid culture for bacteria (ref.[46]). Nevertheless, at the time of the follow-up amniocentesis, six of these seven patients still had a positive amniotic fluid culture despite having received antibiotics[46]. The authors hypothesized that although the overall effect of antibiotic administration in patients with preterm PROM is beneficial in the short term, there is a subset of fetuses who will be exposed to a chronic intrauterine inflammatory process.

Serial intraamniotic inflammation

In our study we observed no patients with positive amniotic fluid microbial cultivation undergoing serial amniocentesis. However, in three out of these ten cases the Il-6 AMC test was positive. Intensity of intra-amniotic inflammatory response increases gradually when innate imunity defense is activated by the presence of bacteria in either the amniotic fluid or the fetal membranes. However, it has been suggested that intra-amniotic inflammation is elicited not only by the activation of pattern recognition receptors recognizing specific

motifs on the surface of bacteria called pathogen-associated molecular pattern (PAMS) but also by endogenous molecules that signal tissue and cellular damage called "alarmins"[47,48].

Activated immunocompetent cells produce a broad spectrum of inflammatory mediators and chemokines responsible for attraction and migration of neutrophils, macrophages, and other imine cells to the placenta and fetal membranes[49]. This could possibly explain elevated Il-6 AMC levels in our patients with negative microbial results in amniotic fluid samples from serial amniocentesis.

Chorioamnionitis and early-onset neonatal sepsis

Histological chorioamnionitis complicates almost half of all PPROM cases that occur prior to 34 weeks' gestation[50]. In our study, 26 (41.9%) (Table 5) out of 62 women were diagnosed with histopathological sign sof chorioamnionitis which correlates with published data. Maternal chorio-amnionitis after PPROM is associated with higher risks of early-onset neonatal sepsis (EONS) (10.0% vs. 2.8%; aOR 3.102; 95% CI 2.306–4.173; $P<0.001$) (ref.[51]). We found placental signs of acute chorioamnionitis in 4 (6.3%) out of 8 cases of neonates diagnosed with early-onset neonatal sepsis (Table 5). Walker et al. found that only 33% of infants remained undelivered after one week following a PPROM history (ref.[52]). In this report, infants with a longer latency were more likely to die compared with age-matched controls. However, Test et al. found no correlation between prolonged latency periods (more than 72 h) and increased neonatal mortality rate[53]. In our study, latency period ranged from 4 to 606 hours and the neonatal mortality was found to be 1.6% due to prematurity (Fig. 2). Fetal losses tend to occur at earlier gestational ages following PPROM. In one series of PPROM cases, all deaths occurred at less than 30 weeks. The intrauterine fetal death rate (FDIU) was reported as 0.9% while the neonatal mortality rate was reported as 5% (ref.[54]). Baser et al., found the FDIU rate to be 1.5%. All cases were at less than 29 weeks. The neonatal mortality rate was 1.9% and was associated with prematurity[55]. Some studies have found that chorioamnionitis was associated with neonatal sepsis, however, the present study did not found any association[56,57].

CONCLUSION

In conclusion, the major clinical finding of our study is that serial transabdominal amniocentesis with Il-6 point-of-care test helps to identify a high inflammatory status in amniotic fluid in women with PPROM. This seems to be a decisive factor for determination between active and expectant management of women with PPROM. Prolonged latency period with expectant management did not lead to increased neonatal mortality rate. Further studies are required to determine whether long-term neonatal outcomes differ according to the specific inflammatory response.

ABBREVIATIONS

GBS Group B streptococcus; HCA, histological chorioamnionitis; IL-1β, IL-6, IL-8 Interleukin 1β, 6, 8; MIAC, microbial invasion into the amniotic cavity; PPROM, preterm premature rupture of membranes; PNC, penicillin; sRAGE, soluble receptor for advanced glycation end products; TLR Toll-like receptor; TNF-α, tumour necrosis factor α.

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