Preterm prelabor rupture of membranes (PPROM) is not associated with presence of viral genomes in the amniotic fluid


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Abstract
Background: The role of viral infections in preterm prelabor rupture of the membranes (PPROM) is not established. Studies on the presence of viral genomes in the amniotic fluid (AF) collected in pregnancies complicated by PPROM show contradictory outcomes.

Objectives: To investigate AF samples of PPROM pregnancies for the presence of viral genomes.

Study design: AF samples from patients with PPROM were collected during a 4-year (2008–2012) observational study. 174 women were included with selection criteria of singleton pregnancy, PPROM, and maternal age of 18 years and above. PCR was used for detection of human cytomegalovirus (HCMV), herpes simplex virus (HSV), parvovirus B19, human adenoviruses (HAdV), enteroviruses (EV) and human parechovirus (HPeV). The selection of these viral targets was based on literature regarding screening of AF for presence of viral genomes.

Results: Only a single sample was positive out of the 174 tested AFs, HCMV DNA was detected.

Conclusions: PPROM is not associated with active viral infections.
established fetal and/or perinatal viral pathogens parvovirus B19, human cytomegalovirus (HCMV) and herpes simplex virus (HSV), also viruses for which a relationship with the pregnancy outcome is uncertain like human papilloma viruses (HPV), respiratory syncytial virus (RSV), influenza virus, Epstein–Barr virus (EBV), human herpes virus-6 (HHV-6), HADV, AAV and enteroviruses (EV). The significance of detecting genomes of the latter viruses in AF is unclear and remains a subject of debate warranting further investigation [15–22].

A potential role of viruses in PPROM pregnancies has not extensively been investigated. Their presence could provide an explanation for cases where so far no MIAC was detected. The aim of the present investigation was to study well-defined AF samples from PPROM pregnancies for the presence of viral genomes of HCMV, HSV, parvovirus B19, HADV, EV and HPeV. The selection of most targets was based on existing literature regarding screening of AF for viral genomes. HPeV was added because of its relatedness to EV.

2. Study design

2.1. Patients and samples

Two hundred twenty two pregnant women at gestational ages between 24+0 and 36+6 weeks with PPROM admitted to the Department of Obstetrics and Gynecology, University Hospital Hradec Kralove, Czech Republic between May 2008 and May 2012 have been considered for this study. Selection criteria were singleton pregnancy, PPROM, and maternal age ≥ 18 years. PPROM was defined as the leakage of AF prior to the onset of labor, which was diagnosed as described before [7,8,12].

Exclusion criteria were clinical chorioamnionitis, diabetes mellitus, hypertension, pre eclampsia, signs of fetal growth restriction, the presence of either congenital or chromosomal fetal abnormalities, signs of fetal hypoxia, or significant vaginal bleeding. Moreover, women with ultrasound markers of subclinical infections (intraamniotic and/or fetal inflammatory response) were excluded but not women with potential signs of infection such as small fetal thymus or pulsatile flow pattern in fetal splenic vein. In all pregnancies, the gestational age was established based on first trimester ultrasound evaluation.

Forty-eight women had incomplete data or inadequate samples for histopathology and/or PCR analysis: the remaining 174 women were included into the study.

In the Czech Republic, women with PPROM at less than 34 weeks of gestation are treated with corticosteroids for the induction of lung maturation, tocolytics for 48 h, and antibiotics, whereas no treatment except antibiotics is initiated to delay delivery after 34 weeks. Management of PPROM women in the Czech Republic differs substantially from most clinical guidelines. Details have been described previously and can be found in a National Guideline [7,8,12].

AF sampling, offered to women with PPROM as a part of our local standard protocol was carried out as described previously [7,8,12]. Ultrasound-guided trans-abdominal amniocentesis was performed on admission prior to the administration of corticosteroids, antibiotics, or tocolytics, and approximately 5 ml of AF was aspirated. Upon collection, AF samples were immediately processed on admission prior to the administration of corticosteroids, antibiotics, or tocolytics, and approximately 5 ml of AF was aspirated. Upon collection, AF samples were immediately processed on admission prior to the administration of corticosteroids, antibiotics, or tocolytics, and approximately 5 ml of AF was aspirated. Upon collection, AF samples were immediately pro-
In spite of the risk pregnancies they detected HHV6, HCMV, parvovirus B19 and HSV are well-established causes of miscarriage, preterm birth, inflammation and postnatal morbidity, but the reasons for these associations remain unclear. In total, 44% of samples were positive in the order of EBV in frequency order. Overall, 2% of AF samples were positive and there was no association with pregnancy outcome [22]. HAdV genomes were not detected, which contradicts the previously mentioned studies wherein HAdV genomes were the most commonly detected viral genomes in AF [16–21]. A recently published study detected no viral genomes in AF from 13 women with PPROM [26]. In line with the negative studies, we did not find viral genomes in a well-defined cohort of 174 PPROM cases, except for a single AF sample that was positive for HCMV DNA at a low concentration of 5 copies/ml of which interpretation is uncertain. The AF sample was furthermore positive for Ureaplasma urealyticum. On follow-up the child was healthy. In retrospect, cord blood was tested and showed a fetal inflammatory response with an IL-6 level of 1810 pg/ml and a HCMV DNA load of 100 copies/ml was found which is still of uncertain significance.

Published literature and our own results show a heterogeneous picture suggesting different categories: (i) low-risk pregnancies without viral genomes detected [14], (ii) low-risk (at the time of AF-sampling) pregnancies with positivity ranging between 2 and 41%, a variation that partly depended on the viruses selected for study [15–22], (iii) high-risk pregnancies (PPROM) without detectable viruses or a single positive sample, so far in one study [26] and our present study, and (iv) pregnancies with high suspicion of a viral infection and a correspondingly high percentage (41%) of AF samples that were virus PCR positive [27]. Variations in the viruses investigated and patient selection hampers a serious comparison. A majority of AF samples were drawn in the second trimester and without suspicion of an infection or fetal abnormalities. Particularly for HAdV the results were contradictory. Furthermore, our and some of the other studies observed a poor correlation between amniotic inflammation and detection of virus genomes [16,22].

How can these inconsistencies be explained? Infection of the fetus with HCMV and parvovirus B19 can undisputedly have serious adverse effects: upon vertical transmission, HCMV can cause fetal damage, intra-amniotic inflammation, premature birth and sequelae that develop post-partum [28]. Parvovirus B19 is a cause of adverse pregnancy outcome with, e.g., miscarriage and fetal hydrops [29]. Both viruses can, however, cause asymptomatic infection of the fetus and both can cause latency in the mother from which viral DNA can leak into the circulation [30,31]. Thus, a positive PCR in AF or cord blood may not be sufficient evidence of fetal infection. A formal proof of congenital infection requires probably additional postpartum investigations.
### Table 3
An overview of virus studies in the amniotic fluids.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Source of AF</th>
<th>Gestational age of sampling (weeks)</th>
<th>Relationship of virus in AF to pregnancy/fetal pathology by respective authors</th>
<th>Total samples/patients</th>
<th>DNA viruses</th>
<th>RNA viruses</th>
</tr>
</thead>
<tbody>
<tr>
<td>McLean et al. [14]</td>
<td>gAMC with low risk for fetal infection.</td>
<td>12–32</td>
<td>Related to possibility of fetal infection</td>
<td>AF 0/243</td>
<td>0</td>
<td>ND</td>
</tr>
<tr>
<td>Van den Veyver et al. [27]</td>
<td>Women with fetuses at risk for intrauterine viral infection.</td>
<td>15–35</td>
<td>Related to fetal pathology</td>
<td>303 (^{9}) AF-95/253</td>
<td>9 (3%)</td>
<td>ND</td>
</tr>
<tr>
<td>Wenstrom et al. [16]</td>
<td>Women with unexplained abortion within 30 days after gAMC.</td>
<td>14–22</td>
<td>Unrelated to pregnancy loss</td>
<td>154</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Burguete et al. [15]</td>
<td>gAMC.</td>
<td>14–25</td>
<td>No conclusive pathological deduction</td>
<td>60</td>
<td>0</td>
<td>ND</td>
</tr>
<tr>
<td>Baschat et al. [17]</td>
<td>gAMC without structural and chromosomal abnormalities.</td>
<td>18 ± 2 (mean ± SD)</td>
<td>Viral genomes maybe present in normal sonography</td>
<td>686</td>
<td>0</td>
<td>ND</td>
</tr>
<tr>
<td>Baschat et al. [18]</td>
<td>gAMC with low risk for fetal infection yielding normal karyotype.</td>
<td>15–27</td>
<td>Related to fetal abnormalities</td>
<td>1090</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Reddy et al. [19]</td>
<td>gAMC yielding normal karyotype.</td>
<td>19 ± 3 and 20 ± 5 (mean ± SD)</td>
<td>Related to fetal abnormalities</td>
<td>423(^{9})</td>
<td>0</td>
<td>ND</td>
</tr>
<tr>
<td>Miller et al. [20]</td>
<td>gAMC with low risk for fetal infection and normal fetal anatomy and karyotype.</td>
<td>15–23</td>
<td>Viral infection not related to pregnancy outcome</td>
<td>686(^{9})</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Adams et al. [21]</td>
<td>AMC for karyotyping and viral PCR testing for history or ultrasound based indication.</td>
<td>16–28</td>
<td>Viral infections related to few fetal abnormalities</td>
<td>1191</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Gervasi et al. [22]</td>
<td>Second trimester AMC for clinical indications. PPROM.</td>
<td>16–20</td>
<td>Not associated with pregnancy outcome</td>
<td>0</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Naresh and Simhan [26]</td>
<td>24–34</td>
<td>13</td>
<td>Unrelated to PPROM</td>
<td>0</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

**Abbreviations viruses:** AAV-2: adeno-associated virus-2; EV: enteroviruses; HAdV: human adenoviruses; HCMV: human cytomegalovirus; HHV: human herpes virus; HPV: human papilloma viruses; HSV: herpes simplex virus; Parvo-B19: parvovirus-B19; RSV: respiratory syncytial virus; VZV: varicella zoster. Other abbreviations: AF: amniotic fluid; AMC: amniocentesis; gAMC: genetic amniocentesis; NA: not available; ND: not done; %: percentage.

- All presented percentages are rounded to nearest decimal.
- Van den Veyver et al. [26] show % PCR positives calculated as positives (shown in table) of 303 patients samples. Total 253 AF of which 95 were positive.
- Reddy et al. [19] did not give actual numbers only percentages.
- Miller et al. [20] have calculated the % of positive amniotic fluids for only 1 virus (n = 37). 

\(^{9}\) Miller et al. [20] have calculated the % of positive amniotic fluids for only 1 virus (n = 37).
Further consideration that a positive PCR can be the result of viral latency in cells, either in the form of episomes or after integration into the host genome, has explicitly been discussed for HHV-6 [22], but it holds also for most of the other viruses studied in AF: for AAV, as was already suggested by Bruguer et al. [15], for EBV which is latently present as episomes in B-lymphocytes [32], and for HADV for which latency has been reported in T-lymphocytes [33]. Latency has even been reported for EV in peripheral blood mononuclear cells [34]. Consequently, the presence of viral genomes in AF may be the by-product of a physiological cell turn over and of pathological conditions as placental insufficiency and/or inflammation [31,35]. Even more trivial explanations as method of sample collection and handling of material or the primers that are selected for virus detection may explain incongruent outcomes [32]. Of course, the presence of viral genomes in AF may also point to an active infection but that has to be proven by post-natal investigation.

We conclude that in most cases PPROM is not associated with presence of viral genomes and that HCA without detectable MIAC is not explained by an active viral infection. Our hypothesis that detection of viral genomes in AF can reflect latency without any clinical consequence for the fetus requires additional study. This is highly warranted, because it may fundamentally change the interpretation of future AF studies.

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Competing interest
None.

Ethical approval
This study was approved by the Institutional Review Board committee (March 19, 2008; No. 200804 S01P), and informed consent was received from all participants.

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