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ORIGINAL ARTICLE

Histologic chorioamnionitis in extremely preterm births, microbiological findings and infant outcome

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ABSTRACT

Background: Histologic chorioamnionitis (HCA) is most often caused by ascending bacterial infection originating from the cervicovaginal tract.

Objectives: To investigate whether HCA with a fetal inflammatory response (FIR) has a worse clinical outcome than HCA alone. Further, if FIR or a positive maternal microbiologic culture obtained prior to birth were related to adverse neonatal outcomes in a cohort of extremely preterm (EP) neonates.

Methods: Prospective observational cohort study recruiting EP singleton pregnancies (gestational age at birth $\leq$28 weeks) with confirmed HCA. FIR was defined by fetal neutrophils in the chorionic vessels and/or umbilical vessels. Positive culture was defined as growth of potentially pathogenic bacteria in a sample from the cervicovaginal tract prior to birth, or if a cervicovaginal culture was lacking, a culture result from the placenta was used. Logistic regression was used to estimate odds ratios and 95% confidence intervals for the associations between FIR, a positive culture result and adverse outcomes, defined as bronchopulmonary dysplasia (BPD), brain pathology assessed by magnetic resonance imaging, retinopathy of prematurity, necrotizing enterocolitis, early-onset neonatal sepsis, and perinatal death. A composite outcome variable included one or more adverse outcomes.

Results: We included 71 cases with HCA, of which 51 (72%) had FIR. Maternal age, rate of clinical chorioamnionitis (CCA), preterm pre-labor rupture of membranes (PPROM), the number of women receiving antenatal steroids and antibiotics, and the rate of positive maternal cultures of potentially pathogenic bacteria were all significantly higher in the HCA with FIR. Neonates in the FIR group had significantly higher levels of blood leukocytes compared to those without. FIR was associated with a longer interval from PPROM to delivery (log-rank test: $p = .022$). Microbiological sampling had been performed in 63 (89%) cases, of which 60 (95%) were cervicovaginal samples. No associations were found between a positive culture and adverse neonatal outcomes, in contrast to FIR, that was significantly associated to BPD and brain pathology.

Conclusions: In a cohort of EP pregnancies with confirmed HCA, the presence of FIR was associated with advanced maternal age, CCA, PPROM, antenatal steroids and antibiotics, and a positive maternal culture of potentially pathogenic bacteria. However, the presence of FIR, and not a positive culture, was associated with adverse neonatal outcomes.

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1. Introduction

The cause of preterm birth is multifactorial, but ascending maternal infection with development of chorioamnionitis is associated with preterm pre-labor rupture of membranes (PPROM) and low gestational age (GA) at birth [1,2]. In addition to prematurity itself and the risk of short-term complications, chorioamnionitis is associated with adverse long-term outcomes such as cerebral palsy, white matter injuries and neurodevelopmental impairment [3]. However,

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chorioamnionitis is a heterogeneous condition and is often clinically silent. Leviton et al. found an increased risk of cerebral white matter injury and subsequent cerebral palsy in a group of extremely preterm infants where the placentas contained low-virulent microorganisms [4]. Other studies have shown that there is a spectrum of vaginal microbiome states linked to risk of preterm birth, and that these vary across populations [5].

Histologic chorioamnionitis (HCA) with a fetal inflammatory response (FIR) is characterized by the presence of fetal neutrophils in the chorionic vessels and/or umbilical vessels. FIR is considered the histological counterpart of the clinical fetal inflammatory response syndrome (FIRS), defined as elevated interleukin-6 (IL-6) levels in umbilical cord blood [6]. Elevated IL-6 levels are regarded as a key factor for identification of intrauterine inflammation. Although amniotic fluid analysis for IL-6 is a well-documented method of diagnosing intrauterine inflammation [7], it requires amniocentesis, which is an invasive procedure that carries risk of complications [8]. The use of noninvasive bedside tests to detect elevated cytokine levels in cervicovaginal fluid in order to predict preterm birth has also been studied [9]. Still there are no biochemical markers for FIRS in clinical practice.

Based on this prior knowledge and the strong need for clinical and biochemical markers that can predict FIR and adverse outcomes following preterm birth, the aim of this study was to explore whether HCA with and without FIR differed regarding clinical characteristics and microbiologic culture result obtained prior to birth. Further, if FIR or positive cultures were related to adverse neonatal outcomes in a cohort of neonates born extremely preterm (EP).

2. Materials and methods

2.1. Participants and study design

This study was part of a prospective, population-based project, BabyPEP, described in detail previously [10]. Briefly, women with threatening spontaneous or medically indicated preterm delivery at Haukeland University Hospital, a tertiary hospital in Bergen, Norway, were invited to participate after informed consent if the neonates were expected to be born before GA $\leq$28 weeks. Threatening preterm birth is a clinical term including cervical ripening, regular and painful uterine contractions, and discharge of mixed blood and mucus. In the present study, we included singleton pregnancies and neonates with a postnatal verified HCA. No neonates had mothers with preeclampsia. The enrollment period was from 25 October 2010 to 24 September 2018 (Figure 1).

2.2. Exposures

GA was estimated based on ultrasound biometry in 1st trimester or at 17–19 weeks of gestation (84.5%) or according to *in vitro* fertilization data (15.5%). PPROM was defined as a rupture of the membranes that took place more than one hour before regular contractions (start of labor). According to the national guidelines, a cervicovaginal swab for culturing prior to antibiotic therapy is indicated in women with PPROM or premature contractions. Aerobic cultures were performed routinely, whereas mycoplasma detection by Polymerase Chain Reaction (PCR) analysis was not part of the clinical routine. A positive culture was defined as growth of potentially pathogenic bacteria (i.e. group B streptococci (GBS), *Escherichia coli* (*E. coli*) or other *Enterobacterales*) in a sample from the cervicovaginal tract prior to birth. In three cases where a cervicovaginal sample prior to birth was missing, we used culture results from the placenta as a proxy for cervicovaginal colonization.

If PPROM was confirmed, antibiotic therapy (Benzylpenicillin intravenously) was routinely given to the mother until the culture result was available. In cases of a positive GBS culture, penicillin therapy was continued up to a total of 7 days. If the cultures were negative, penicillin treatment was discontinued. In cases of known colonization of other bacteria than GBS, antibiotic treatment according to the antibiogram was given during labor. If an antibiogram was not available, empirical treatment with gentamicin (6 mg/kg body weight/day intravenously) was given. The type of antibiotics given during labor were later compared with the culture results and evaluated by one of the authors (JK). If a woman with a positive GBS culture received Penicillin during labor, it was defined as *adequate empiric antibiotic treatment*. However, if there was a positive *E. coli* culture and the woman only received Penicillin and not gentamicin, it was defined as *not adequate empiric antibiotic treatment*. The results from the cervicovaginal and placental samples were obtained from the electronic patient records. We coded treatment with antenatal antibiotics Yes/No. Antenatal corticosteroids (betamethasone) were administered as intramuscular injections of 12 mg and repeated after 24 h, if possible. In this study, any dose of antenatal corticosteroids was coded Yes/No. Clinical chorioamnionitis (CCA) was defined as maternal fever with temperature at least 38.0 °C

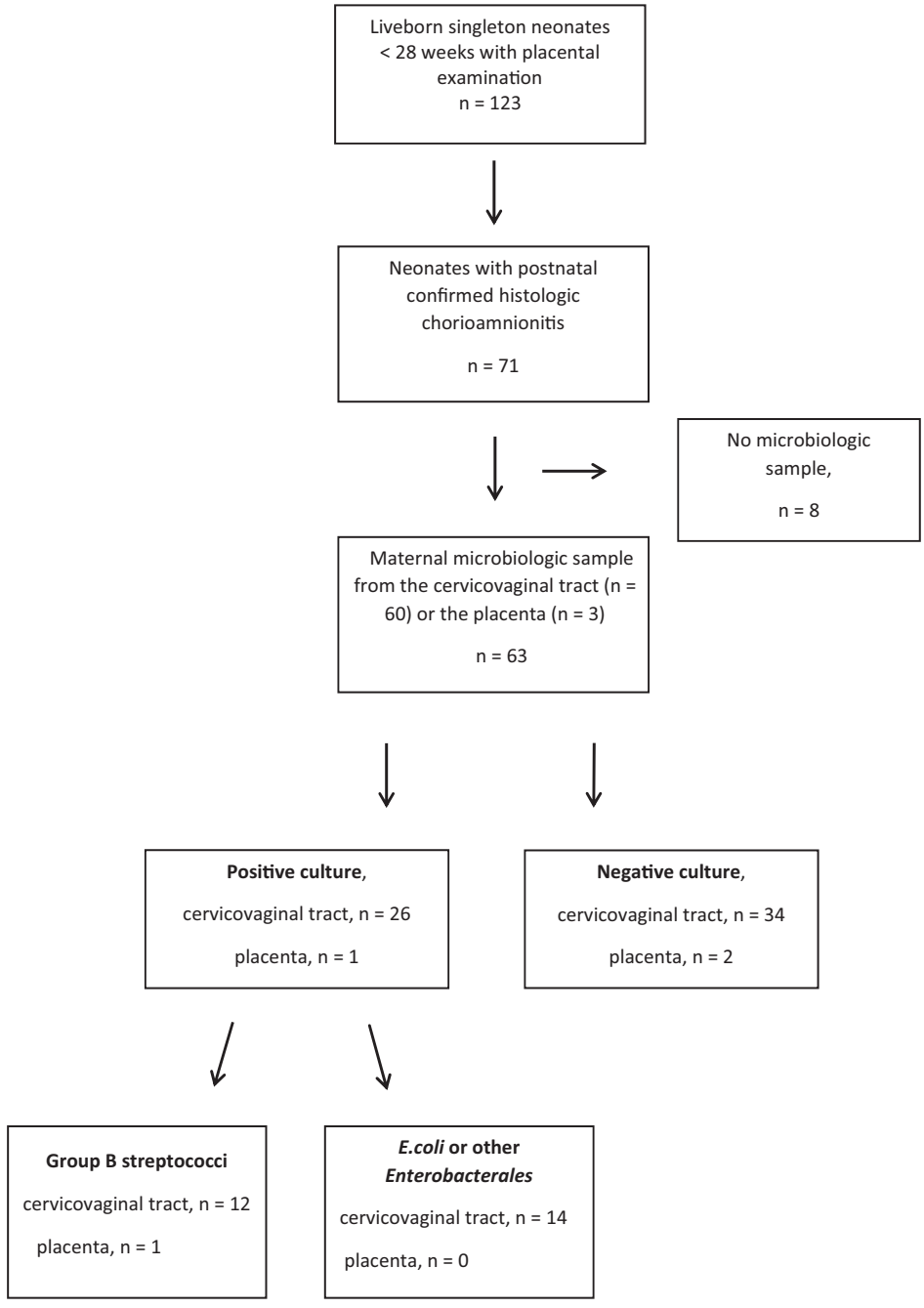

Figure 1. Flowchart of the study-population for the prospective observational study on singleton pregnancies and infants with a postnatal verified histologic chorioamnionitis, enrolled at Haukeland University Hospital (Norway) from 25 October 2010 to 24 September 2018.

(rectal), and at least two of the following symptoms: maternal tachycardia (>100 beats per minute), fetal tachycardia (>160 beats per minute), maternal leukocytosis ($>15 \times 10^9$/L), uterine tenderness, or malodorous amniotic fluid/discharge [11].

2.3. Outcomes

Bronchopulmonary dysplasia (BPD) was defined by need of oxygen supplementation or ventilatory support at 36 weeks GA [12]. Brain pathology was evaluated by magnetic resonance imaging (MRI) performed at term-equivalent age and was dichotomized into *No pathology* versus *Any pathology*. Perinatal death was defined as death within the first 28 days of life. Necrotizing enterocolitis included cases verified by pre-operative abdominal x-ray and ultrasound examination, during surgery, or by autopsy. Retinopathy of prematurity was graded as defined by the Committee for Classification of Prematurity [13] and dichotomized

into no/mild retinopathy (grade 1–2) and severe retinopathy (grade 3–5). Early-onset neonatal sepsis (EONS) was clinical symptoms of infection within 72 h of life and a positive blood culture and/or an increasing level of C-reactive protein (CRP) >30 during the course of the infection. A composite outcome variable of .any adverse outcomes. was constructed for each neonate provided that at least one of the above mentioned conditions were diagnosed. The z-scores for birthweights were calculated with reference to the 2013 Fenton growth charts [14].

2.4. Classification of placental histological findings

A routine histopathologic sampling in agreement with the Amsterdam Consensus was carried out in all the included placentas. Adequate samplings were performed with at least two sections from the umbilical cord, two sections from a membrane roll, a section from the cord insertion site and two full-thickness sections from normal appearing parenchyma in the inner 2/3. Additional samples were taken from macroscopically abnormal parenchyma.

HCA was defined as infiltration of neutrophils into the placenta, extraplacental membranes and the umbilical cord. FIR was defined by the presence of neutrophils in the vessel wall of the chorionic plate or in the umbilical vein, and/or arteries [15].

2.5. Statistics

The chi-square or Fisher's exact test was used for comparison of categorical variables, presented as proportions. For continuous variables Student's *T*-test or Mann–Whitney's *U*-test was used, as appropriate, and presented as mean with standard deviations. Kaplan–Meier survival curves for the PPROM-to-delivery interval were compared between the FIR groups with the log-rank test. We used binary logistic regression analyses to explore the association between FIR and possible confounders. FIR served as the dependent variable and the confounders were included in the multivariable regression analysis if significant in the unadjusted analyses. Similarly, the adverse outcome variables served as the dependent variable, FIR and a positive culture served as independent variables. The analysis was also adjusted for the use of antenatal steroids and antenatal antibiotics in the multivariable regression analysis. The results are presented as odds ratios (ORs) with 95% confidence intervals (CIs). The data were analyzed using SPSS Statistics for Windows, version 26.0 (IBM Corp, New York, USA).

2.6. Ethical approval

The Regional Committees for Medical and Health Research Ethics approved the study (REK ID 2010/496), and the mothers gave written informed consent.

3. Results

From a cohort of 123 EP live born singletons, 71 cases had HCA and were enrolled in this study (Figure 1). FIR was present in 51 (72%) cases. Table 1 shows the characteristics and neonatal outcomes according to the presence or absence of FIR. Cases with FIR were characterized by advanced maternal age, CCA, PPROM, antenatal corticosteroid administration, antenatal antibiotics, and a positive maternal culture of potentially pathogenic bacteria. In the FIR group, neonates had significantly higher levels of blood leukocytes compared to the group without. There were no significant differences between the two groups (with and without FIR) regarding adequate empiric antibiotic treatment. Further, no differences were found regarding neonatal outcomes, including the composite adverse outcome. Of 71 cases with HCA, 63 (89%) cases had a microbiologic sample result. A cervicovaginal sample prior to birth had been performed in 60 cases (95%), and in three cases lacking a cervicovaginal sample, a placenta sample was available. Of the samples from the cervicovaginal tract, 26 (41.3%) had a positive culture with potentially pathogenic bacteria, of which GBS was found in 12 cases (46.2%). Among the three placenta samples, one case was positive for GBS (Figure 1). The time from cervicovaginal sampling to birth varied from 0 to 8 days, with the majority of samples taken within 2 days before birth (76%) (data not shown). A significantly longer interval from PPROM to delivery was seen in pregnancies complicated by FIR (Figure 2; log-rank test $p = .022$). In those without FIR, 80% (16/20) of the deliveries occurred within 24 h compared to 41% (21/51) in the FIR group.

Maternal age, antenatal antibiotics, antenatal steroids and PPROM were all associated with FIR in the unadjusted binary logistic regression analysis. Although a positive culture of potentially pathogenic bacteria was not significantly associated with FIR ($p = .050$), it was included in the multivariable analysis. Only maternal age and antenatal steroids were significantly associated to FIR in the adjusted analysis (Table 2). Excluding the three cases with cultures from the

Table 1. Maternal and neonatal characteristics of 71 cases with histologic chorioamnionitis (HCA), with and without a fetal inflammatory response (FIR) enrolled at Haukeland University Hospital (Norway) from 25 October 2010 to 24 September 2018.

Characteristics	HCA with FIR ($n = 51$)	HCA without FIR ($n = 20$)	p-Value[a]
Maternal and pregnancy			
Age (years), mean (SD)	32.1 (5.2)	26.2 (4.8)	<.001
BMI (kg/m^2), mean (SD)	26.4 (6.6)	23.3 (4.1)	.089
Norwegian origin, *n/N* (%)	43/51 (84.3)	18/20 (90.0)	.850
Smoking, *n/N* (%)	4/51 (7.8)	5/20 (25.0)	.105
Primiparity, *n/N* (%)	27/51 (52.9)	14/20 (70.0)	.191
Preeclampsia, *n/N* (%)	0/51 (0.0)	0/20 (0.0)	
Clinical chorioamnionitis, *n/N* (%)	14/51 (27.5)	0/20 (0.0)	.007
PPROM, *n/N* (%)	27/51 (52.9)	3/20 (15.0)	.004
Antenatal steroids, *n/N* (%)	49/51 (96.1)	14/20 (70.0)	.005
Antenatal antibiotics, *n/N* (%)	36/51 (70.6)	5/20 (25.0)	<.001
Positive culture of potentially pathogenic bacteria[b], *n/N* (%)	24/48 (50.0)	3/15 (20.0)	.040
Adequate empiric antibiotic treatment, *n/N* (%)	14/26 (53.8)	0/4 (0.0)	.103
Onset of labor, *n/N* (%)			.690
Spontaneous	43/51 (84.3)	19/20 (95.0)	
Induced	2/51 (3.9)	0/20 (0.0)	
Cesarean section	6/51 (11.8)	1/20 (5.0)	
Mode of delivery, *n/N* (%)			.057
Spontaneous vaginal	36/51 (70.6)	15/20 (85.0)	
Forceps	0/51 (0.0)	2/20 (10.0)	
Cesarean section	15/51 (29.4)	3/20 (15.0)	
Maternal vascular malperfusion, *n/N* (%)	10/51 (19.6)	3/20 (15.0)	.746
Placental weight (grams)[c], mean (SD)	252.6 (76.8)	244.1 (88.1)	.533
Placental weight <10th percentile, *n/N* (%)	2/50 (4.0)	0/20 (0.0)	1.000
Neonatal			
Gestational age (weeks^{days}), mean (range)	25^5 (23^0–27^6)	25^6 (23^1–27^6)	.582
Birth weight (grams), mean (SD)	860.6 (214)	883.8 (229)	.769
Birth weight z-score, mean (SD)	0.31 (0.6)	0.31 (0.9)	.490
Small for gestational age, *n/N* (%)	1/51 (2.0)	0/20 (0.0)	1.000
Male sex, *n/N* (%)	27/51 (52.9)	6/20 (30.0)	.191
Apgar score <4 at 5 min, *n/N* (%)	10/51 (19.6)	3/20 (15.0)	.746
pH <7.0[d], *n/N* (%)	5/51 (9.8)	2/20 (10.0)	1.000
CRP[e], mean (SD)	15.1 (18.9)	7.8 (3.5)	.815
Leukocyte level (LPK)[e], mean (SD)	26.6 (15.9)	12.3 (6.2)	<.001
Ventilator treatment first 24h after birth, *n/N* (%)	39/49 (79.6)	16/19 (84.2)	1.000
Perinatal death, *n/N* (%)	4/51 (7.8)	4/20 (20.0)	.209
Bronchopulmonary dysplasia, *n/N* (%)	32/47 (68.1)	6/15 (40.0)	.052
Brain pathology by MRI, *n/N* (%)	25/41 (61.0)	4/13 (30.8)	.057
Retinopathy (≥grade 3), *n/N* (%)	15/45 (33.3)	5/16 (31.3)	.836
Early onset neonatal sepsis, *n/N* (%)	4/51 (7.8)	0/20 (0.0)	.571
Necrotizing enterocolitis, *n/N* (%)	7/51 (13.7)	4/20 (20.0)	.491
Composite adverse outcome[f], *n/N* (%)	39/48 (81.3)	14/19 (73.7)	.517

[a]Chi-square, Fisher's Exact test or Mann–Whitney *U* test as appropriate; [b]dominant growth of group *B streptococci*, *E.coli* or other *Enterobacterales* in cervicovaginal ($n = 60$) or placental culture ($n = 3$); [c]trimmed weight without cord and membranes; [d]lowest arterial value measured within first 24 h; [e]highest level measured within 48 h; [f]including any of the following: perinatal death, bronchopulmonary dysplasia, brain pathology by MRI, retinopathy, early-onset neonatal sepsis, necrotizing enterocolitis.

Abbreviations: SD: standard deviation; BMI: body mass index; PPROM: preterm pre-labor rupture of membranes; CRP: C-reactive protein.

placenta did not change the results (data not shown). We explored if FIR and a positive microbiologic culture affected neonatal outcome (Table 3). FIR was significantly associated with BPD and brain pathology, while a positive bacterial culture was not predictive of any adverse neonatal outcome. Excluding the three placenta samples from the analyses did not affect the overall result (data not shown).

4. Discussion

In this study of EP births with confirmed HCA, we found that maternal age, CCA, PPROM, administration of antenatal steroids, antibiotics, a positive maternal culture of potentially pathogenic bacteria, and higher levels of blood leukocytes in the neonates were associated with FIR. Our results also indicate that FIR, but not the cervicovaginal bacterial colonization per se, plays the major role regarding adverse outcome in EP neonates.

Consistent with other studies [16,17], we have previously shown that FIR is a strong marker of BPD and brain pathology in EP births [10]. The challenge is therefore to identify maternal and neonatal characteristics, which are associated with FIR, with the aim to prevent it. As HCA with FIR is diagnosed after birth, knowledge of such factors is essential for timely and adequate surveillance and treatment of EP neonates at risk.

Advanced maternal age is associated with pregnancy complications and adverse outcomes, which may partly be explained by changes in the placenta. A

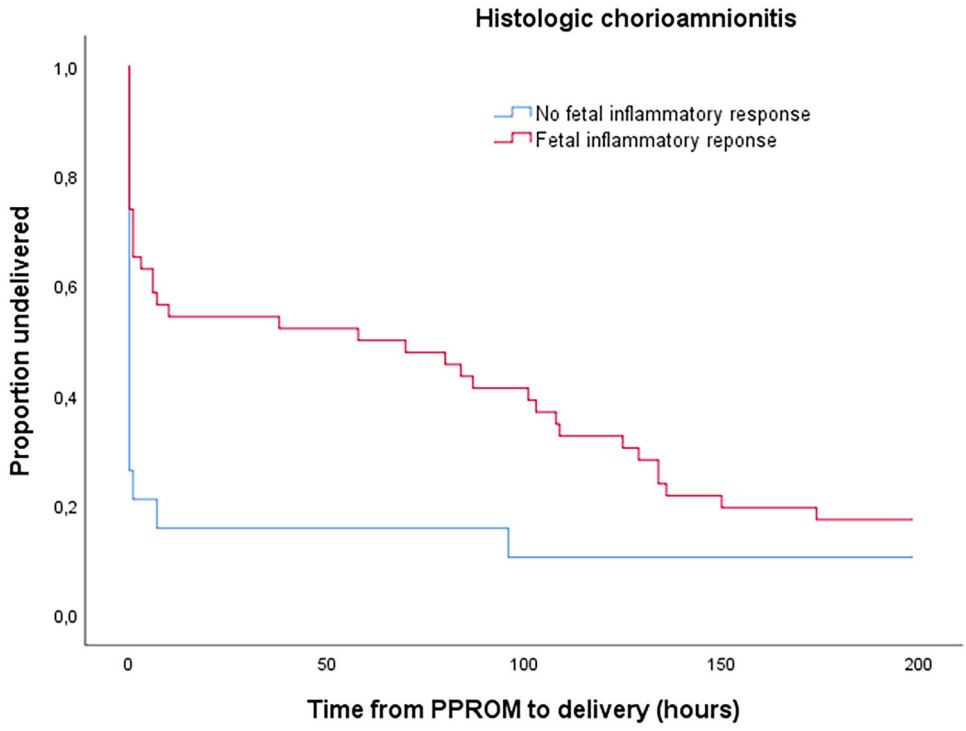

Figure 2. Kaplan–Meier curves for time from preterm pre-labor rupture of membranes to delivery according to fetal inflammatory response in 71 extremely preterm births at Haukeland University Hospital (Norway) from 25 October 2010 to 24 September 2018 (log-rank test: $p = .022$).

Table 2. Results from logistic regression of fetal inflammatory response according to maternal and pregnancy characteristics in extremely preterm births at Haukeland University Hospital (Norway) from 25 October 2010 to 24 September 2018.

	Fetal inflammatory response			
Variables	Unadjusted models OR 95% CI	*p*-Value	Adjusted models OR 95% CI	*p*-Value
Maternal age, years[a]	1.28 (1.12, 1.47)	<.001	1.28 (1.05, 1.55)	.008
BMI ≥30 kg/m^2	3.34 (0.68, 16.45)	.101		
Smoking	0.26 (0.06, 1.08)	.063		
Parity ≥1	2.07 (0.69, 6.25)	.185		
Antenatal antibiotics	8.75 (2.52, 30.39)	<.001	3.17 (0.59, 17.16)	.170
Antenatal steroids	10.50 (1.91, 57.88)	.003	15.41 (0.79, 302.6)	.038
Vaginal bleeding	1.25 (0.39, 4.06)	.708		
PPROM[b]	6.38 (1.66, 24.47)	.002	5.47 (0.73, 40.96)	.079
Cesarean section	2.36 (0.60, 9.27)	.193		
Positive culture of potentially pathogenic bacteria[c]	4.00 (1.00, 15.99)	.050	1.42 (0.27, 7.38)	.676

[a]As continuous variable; [b]Preterm pre-labor rupture of membranes; [c]Cervicovaginal ($n = 60$) or placental ($n = 3$) culture, including group *B streptococci*, *E.coli,* or other *Enterobacterales*.

2021 review has concluded with poorer placentation and altered placental development related to advanced maternal age [18]. The concept of "inflammaging" was introduced in 2000, claiming that during aging a sterile, low-grade inflammation may develop [19]. Pregnancy-associated inflammaging is therefore of particular interest. The association between maternal age and the development of FIR in the present study is in keeping with a recent report on maternal age as a pro-inflammatory factor in pregnancy [20]. The finding is important as it adds to a growing body of evidence regarding unfavorable consequences of increasing age among childbearing women [21]. Maternal obesity has also been associated with a proinflammatory state [22]. We did not find that maternal BMI significantly influenced the risk of FIR, but this could be due to the relatively small sample size (Table 2). The results from our study emphasize that lifestyle factors may have an impact on FIR [6].

Interestingly, we found significant associations between the administration of antenatal steroids and antibiotics with FIR in the univariate analyses, but in the adjusted multivariate analysis only antenatal steroids reached significance. The association is difficult to explain. Although we did not register the time

Table 3. Adverse neonatal outcomes according to fetal inflammatory response and positive bacterial culture in unadjusted and adjusted logistic regression models for extremely preterm singletons with histologic verified chorioamnionitis in extremely preterm births enrolled at Haukeland University Hospital (Norway) from 25 October 2010 to 24 September 2018.

Outcomes	BPD		Brain pathology		Perinatal death	
Predictors	Unadjusted model OR 95% CI	Adjusted model[a] OR 95% CI	Unadjusted model OR 95% CI	Adjusted model[a] OR 95% CI	Unadjusted model OR 95% CI	Adjusted model[a] OR 95% CI
Fetal inflammatory response	5.54 (1.16, 26.50)	15.99 (1.64, 155.7)	4.99 (0.85, 29.26)	10.74 (1.05, 110.4)	0.41 (0.08, 2.25)	0.35 (0.05, 2.43)
Positive culture of potentially pathogenic bacteria[c]	0.85 (0.25, 2.85)	0.99 (0.28, 3.59)	0.91 (0.26, 3.19)	1.04 (0.28, 3.95)	0.62 (0.10, 3.73)	0.58 (0.09, 3.64)

Outcomes[d]	ROP ≥ grade 3		Composite adverse outcome[b]	
Predictors	Unadjusted model OR 95% CI	Adjusted model[a] OR 95% CI	Unadjusted model OR 95% CI	Adjusted model[a] OR 95% CI
Fetal inflammatory response	0.96 (0.23, 3.97)	1.05 (0.23, 4.86)	2.16 (0.56, 8.34)	4.76 (0.93, 24.38)
Positive culture of potentially pathogenic bacteria[c]	1.13 (0.34, 3.72)	1.17 (0.34, 4.01)	1.12 (0.31, 4.08)	1.53 (0.40, 5.84)

Abbreviations: BPD: bronchopulmonary dysplasia; ROP: retinopathy of prematurity; OR: odds ratio; CI: confidence interval.
[a]Adjusted for antenatal steroids (no/yes) and antenatal antibiotics (no/yes); [b]Including at least one of adverse outcomes; [c]Cervicovaginal ($n = 60$) or placental ($n = 3$) culture, including group *B streptococci, E.coli* or other *Enterobacterales*.
[d]For the outcome variables early-onset neonatal sepsis and necrotizing enterocolitis the numbers were too small for further analyses.

interval from administration of antenatal steroids to delivery in our study, a study from 1992 indicates that administration of antenatal steroids close to the delivery may induce a leukocytosis in the premature infant [23], which may explain FIR. Another possible explanation may be that FIR is established in the placenta before birth in extremely premature neonates, and that antenatal steroids may enhance the inflammation itself. However, the growing dilemma on the use of antenatal corticosteroids and long-term consequences may support the complexity in this field [24].

Molecular-based studies have shown that not only known pathogens such as GBS and *E. coli* [25,26], but also bacteria regarded as part of the normal genital flora may cause chorioamnionitis. The mechanisms are unclear, but microorganisms may interact with host factors released during inflammation [27], and may differ in accordance to other possible confounding factors such as the duration and timing of the inflammatory process, ethnicity, stress and GA [28–30].

Although the etiology of PPROM is multifactorial, vaginal microbiota composition is an established risk factor [31]. Interestingly, studies using cultivation or molecular techniques have shown that the outcome in neonates with PPROM and intra-amniotic inflammation without detectable microorganisms (sterile intra-amniotic inflammation) is similar to outcomes in neonates who were exposed for microorganisms in the amniotic cavity [32,33].

We also found a significant association between FIR and PPROM and demonstrated that most births without FIR occur before 24 h, compared with a longer PPROM-to-delivery interval in the FIR group. An explanation may be that prolonged exposure to PPROM increases the time the fetus is exposed to pro-inflammatory factors. Alternatively, a prolonged exposure to PPROM increases the risk of ascending bacterial infection, which in turn increases the risk of development of FIR. Since FIR is a strong marker of neonatal adverse outcome, one may argue to shorten the pregnancy after PPROM. However, an intervention (such as induction or cesarean delivery) must be balanced against the major impact of gestational age at birth on both long- and short term neonatal morbidity and mortality. In addition, the time necessary to achieve full effect of betamethasone for lung maturation is 24 h between the doses. Further, the multifactorial etiology of PPROM and preterm birth complicates the identification of individuals that will benefit from early delivery. The increasing risk of FIR during ongoing pregnancy after PPROM should initiate careful monitoring in order to identify signs of chorioamnionitis. Risk factors such as advanced maternal age and probably maternal obesity should also be taken into account [34].

The use of culture-based methods for detecting bacteria has its limitations. Not all bacteria are cultivable, and time lag from sampling to plating of the bacteria on culture media may lead to bacterial death. Usually, the time from confirmed PPROM to start of antibiotic prophylaxis is very short (a few hours). In our population, penicillin was given routinely in cases of PPROM, with a bactericidal effect on GBS, but a weaker or absent effect on bacteria like *E.coli* and

other *Enterobacterales*. However, we did not register the time interval between PPROM and prophylactic administration of antibiotics, therefore we cannot rule out that the antibiotics administered to mothers with PPROM were given too late or for a too short time to slow down or reverse the development of FIR.

In cases with a positive maternal culture of potentially pathogenic bacteria, we evaluated the antibiotic treatment given during labor, and found that FIR developed in 53.8% of the cases despite adequate empiric antibiotic treatment during labor (Table 1). These results indicate that the development of FIR probably preceded labor and could not be prevented by antibiotic treatment given during labor. In line with that, three out of four cases with EONS occurred despite adequate antibiotic treatment during labor.

Most of the samples were from the cervicovaginal tract, but in a few cases the cervicovaginal samples were missing and we used an available placental sample instead. One of these three samples showed growth of GBS. Although the sample compartment was different, we argue that a positive placental sample with GBS could serve as a surrogate, reflecting an ascending infection from the cervicovaginal tract. Whereas the opposite situation, a positive cervicovaginal culture does not always result in a positive placental culture.

The low number of adverse events in our study is a limitation, and is reflected in the wide confidence intervals (Tables 2 and 3). The group of EP neonates constitutes less than 0.5% of all births, and by focusing on the group with HCA, the number of cases was even more restricted. However, this is a group of special interest since these neonates carry a high risk of lifelong morbidity.

The strength of the study is the prospective design with detailed information on fetal and maternal characteristics, the use of antenatal antibiotics and steroids, neonatal outcomes, adequate placental sampling and bacterial cultures.

Our results are in line with prior studies that acknowledge the complexity between extremely preterm birth, vaginal microbiomes, inflammation, infection and neonatal outcome [35–37]. HCA with FIR may play a role in risk assessment and prognostication in EP neonates. We agree with the conclusion by Salas et al. that detailed placental pathology for early detection of infants with FIR may help guide individualized postnatal care [38]. Adding placental findings to known demographic and clinical risk factors and clinical presentation may improve our ability to more accurately predict neonatal outcomes, counsel parents and make personalized targeted follow-up of these children with the goal to improve long-term outcome.

5. Conclusion

In this cohort of EP pregnancies with confirmed HCA, the presence of FIR was associated with advanced maternal age, CCA, PPROM, antenatal use of steroids and antibiotics, and positive maternal cultures of potentially pathogenic bacteria. However, only maternal age and antenatal steroids remained significantly associated to FIR in a multivariable analysis. The presence of FIR was associated with adverse neonatal outcomes, but a positive maternal culture was not.

Disclosure statement

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