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Effects of Histological Chorioamnionitis and Fetal Inflammatory Response Syndrome on the Distribution of Pathogens in Neonatal umbilical Cord Blood Cultures

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Research Article

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Abstract

Objective

To investigate the association between different stages of histological chorioamnionitis (HCA), complicated fetal inflammatory response syndrome (FIRS), and the distribution characteristics of pathogens in neonatal umbilical cord blood cultures(UCBC), so as to provide a basis for the early clinical identification of intrauterine infection.

Methods

A retrospective case-control study was conducted, enrolling 243 newborns delivered at Women's Hospital School of Medicine Zhejiang University from January 2021 to December 2024. According to the results of UCBC, the newborns were divided into a positive group (81 cases) and a negative group (162 cases, matched at a ratio of 1:2). Baseline data such as HCA and FIRS were compared between the two groups, and the high-risk factors affecting positive UCBC results were analyzed. Multivariate logistic regression was used to screen independent risk factors for positive UCBC. Furthermore, the distribution characteristics of pathogens in UCBC under different stages of HCA and FIRS were analyzed.

Results

The incidence rates of moderate to severe HCA, FIRS, placental abruption, acute chorioamnionitis, maternal prenatal temperature ≥ 38.0℃, maternal prenatal pathogenic infection, and maternal pregnancy complicated by cervical incompetence in the UCBC positive group were significantly higher than in the UCBC negative group ($P < 0.05$), and the maternal age was older ($P < 0.05$). Multivariate logistic regression analysis identified that FIRS (OR = 6.38, 95%CI: 2.793–14.573, $P < 0.001$) and prenatal pathogenic infection (OR = 6.049, 95%CI: 2.946–12.418, $P < 0.001$) were independent risk factors for positive UCBC. A total of 86 pathogenic strains were isolated from 81 positive UCBC specimens (5 cases of mixed infection), including 51 Gram-positive strains (59.3%), mainly Enterococcus faecalis (14.0%), group B Streptococcus (7.0%) and Streptococcus mitis (7.0%); 35 Gram-negative strains (40.7%), mainly Escherichia coli (29.1%). Among children with positive UCBC results, the rate of moderate or severe HCA was higher than that in the normal or mild HCA group (55.6% vs 44.4%), but the difference was not statistically significant ($P > 0.05$). The difference between the group with FIRS and the group without FIRS was statistically significant (61.7% vs 38.3%, $P < 0.05$). Escherichia coli(E. coli) detection was significantly higher in HCA complicated with FIRS than in those without FIRS (68.0% vs 32.0%, $P < 0.05$). All Listeria monocytogenes infections were accompanied by moderate or severe HCA and FIRS.

Conclusion

FIRS and maternal prenatal infectious pathogens are independent predictors of positive UCBC.For HCA complicated with FIRS, Gram-negative pathogens such as E. coli should be prioritized in empirical antibiotic coverage.

Introduction

Chorioamnionitis, also termed intra-amniotic infection, refers to infection and inflammation involving the amniotic fluid, placenta, fetus, fetal membranes, or decidua[1].As a common perinatal infectious disorder, it triggers systemic inflammatory responses that increase risks of preterm birth, neonatal lung injury, brain damage, and other adverse outcomes[2].Histological chorioamnionitis (HCA) is defined pathologically by neutrophil infiltration in the chorion, amnion, or decidua, representing a maternal inflammatory reaction[3–4].HCA is often clinically silent and approximately two to three times more prevalent than acute clinical chorioamnionitis[4]. It shows higher diagnostic value for intra-amniotic infection than amniotic fluid culture or clinical chorioamnionitis[5–6].

With progression of inflammation, chorionic vasculitis and funisitis indicate extension to the fetus, a condition defined as fetal inflammatory response syndrome (FIRS)[7].FIRS is strongly associated with increased risks of early-onset sepsis (EOS), bronchopulmonary dysplasia (BPD), intraventricular hemorrhage (IVH), retinopathy of prematurity (ROP), and other severe neonatal complications[8].Most acute inflammatory events arise from ascending bacterial invasion after membrane rupture; a minority are aseptic inflammatory reactions related to uterine distension, hypoxia, or oxidative stress [9].

Umbilical cord blood is the residual blood in the placenta and umbilical cord after delivery and cord clamping [10].Umbilical cord blood culture (UCBC) serves as a reliable marker for intrauterine infection with advantages including sufficient volume, non-invasive collection, and reduced risk of iatrogenic anemia in preterm infants[11].Studies indicate UCBC has higher sensitivity and comparable specificity for EOS diagnosis compared with peripheral blood culture (PBC) [12]. Meanwhile, the study also found that pathogens can be detected in the amniotic fluid of most full-term acute chorioamnionitis patients, with a positive blood culture rate of 4.9% in their neonates [13].In contrast, the distribution characteristics of pathogens in UCBC associated with HCA or FIRS, which has a higher incidence than acute chorioamnionitis, require further exploration. This study aims to investigate the effects of varying severities of HCA and FIRS on the pathogen spectrum of UCBC, so as to provide a basis for the early clinical identification of high-risk infants.

Methods

Study design and participants

This retrospective case–control study was approved by the Ethics Committee of Women's Hospital School of Medicine Zhejiang University (approval No. IRB-20230184-R). Subjects were neonates delivered between January 2021 and December 2024. Infants with positive UCBC were assigned to the

case group (n = 81). Controls (n = 162) were UCBC-negative infants without clinical infection, matched 1:2 by gestational age (± 7 days), birth weight (± 250 g), delivery mode, and maternal gestational week (± 1 week) using propensity score matching.

Inclusion and exclusion criteria

Inclusion criteria: (1) Gestational age ≥ 28 weeks; (2)UCBC collected immediately at birth; (3) The mother's placenta was submitted for pathological examination to determine the presence of HCA; (4) Complete clinical data.

Exclusion criteria: (1) Gestational age < 28 weeks; (2) non-standard UCBC collection (suspected contamination); (3) Unqualified pathological section quality of the placenta, making a definite diagnosis impossible; (3) Neonates complicated with severe congenital malformations, immunodeficiency diseases, etc.

Placental pathological examination

Immediately after the delivery of the placenta, it was sent to the pathology department. After fixation in 4% formaldehyde, paraffin embedding, serial sectioning, and HE staining, two senior pathologists reviewed the slides in a double-blind manner. Histological chorioamnionitis was diagnosed in accordance with the consensus criteria of the 2016 Amsterdam International Placenta Workshop: positive results were defined as inflammatory cell infiltration in the chorion, amnion, or decidua. Maternal and fetal inflammatory responses were staged and graded based on infiltration extent and severity:(1) Staging of maternal inflammatory response:Stage 1: acute subchorionitis or chorionitis;Stage 2: acute chorioamnionitis: polymorphonuclear leukocytes extend into fibrous chorion and/or amnion;Stage 3: necrotizing chorioamnionitis: karyorrhexis of polymorphonuclear leukocytes, amniocyte necrosis, and/or amnion basement membrane hypereosinophilia.(2) Grading of maternal inflammatory response:Grade 1: not severe as defined;Grade 2: severe: confluent polymorphonuclear leukocytes or with subchorionic microabscesses.(3) Staging of fetal inflammatory response:Stage 1: chorionic vasculitis or umbilical phlebitis;Stage 2: involvement of the umbilical vein and one or more umbilical arteries;Stage 3: necrotizing funisitis.(4) Grading of fetal inflammatory response:Grade 1: not severe as defined;Grade 2: severe: near-confluent intramural polymorphonuclear leukocytes with attenuation of vascular smooth muscle[14].

Umbilical cord blood culture and pathogen identification

1. Standardized collection of umbilical cord blood: After the delivery of the fetus, the umbilical cord is ligated with hemostatic forceps and transected with surgical scissors. A sterile syringe is used to extract blood remaining in the placenta and umbilical cord. The entire process of umbilical cord blood collection is carried out in strict accordance with aseptic techniques. Collect 1 to 3 ml of umbilical cord blood using the above method and inject it into special neonatal culture bottles for aerobic and facultative anaerobic microorganisms, mix well and send for examination immediately.
2. Culture, identification and antimicrobial susceptibility testing (in accordance with CLSI standards): The submitted cord blood is placed in an automated blood culture analyzer for incubation at a set temperature of 35.5℃. If the incubation period exceeds 5 consecutive days, no pathogenic bacterial growth is deemed present. Following an alarm for positive results, smear staining and microscopic examination are performed to provide a preliminary report to clinicians. The sample is then subcultured onto agar plates such as blood agar for cultivation and purification. A formal report is issued after species identification using an automated microbial identification.

Data sources

Baseline data were collected by reviewing the inpatient electronic medical record system, including neonatal gestational age at birth, birth weight, mode of delivery, Apgar score, maternal age, maternal prenatal body temperature, history of acute chorioamnionitis, prenatal pathogenic infection, etc.

Disease Diagnostic Criteria

Acute chorioamnionitis refers to a syndrome characterized by the presence of a maternal fever (temperature ≥ 37.8°C or ≥ 38.0°C) and 2 or more of these 5 clinical signs: (1) maternal tachycardia (> 100 beats/min); (2) fetal tachycardia (> 160 beats/min); (3) uterine tenderness; (4) purulent or malodorous amniotic fluid or vaginal discharge; and (5) maternal leukocytosis (white blood cell count > 15,000/mm3)[15]

Sample size calculation

Sample size was calculated using G*Power 3.1.9. Assuming a 20% HCA prevalence in controls, OR = 2.5, two-sided α = 0.05, power = 0.80, the minimum required sample was 47 cases and 94 controls. The final sample (81 cases, 162 controls) exceeded this requirement.

Statistical analysis

Data were analyzed using SPSS 26.0. Normally distributed continuous data were presented as mean ± standard deviation (SD) and compared by t-test; non-normally distributed data as median (interquartile range, IQR) and compared by Wilcoxon rank-sum test. Categorical data were presented as n (%) and

compared by χ^2 or Fisher's exact test. Variables with $P < 0.05$ in univariate analysis were entered into multivariate logistic regression. $P < 0.05$ was considered statistically significant.

Results

Study population and baseline characteristics

A total of 13,725 neonatal cord blood specimens were received during the study period. According to the inclusion and exclusion criteria, 81 neonates with positive UCBC were finally selected as the positive group, and 162 neonates in the same period were selected as the negative group at a 1:2 ratio and based on matching principles. There were no statistically significant differences between the two groups in terms of gestational age at birth, body weight, gender, mode of delivery, Apgar score and other indicators (P>0.05).

In the UCBC positive group, the proportions of moderate or severe HCA (55.6% vs 23.5%, P<0.001) and FIRS (61.7% vs 16.0%, P<0.05) were significantly higher than those in the negative group. Maternal age was older, and the proportions of maternal antenatal body temperature ≥38.0℃, acute chorioamnionitis, maternal prenatal pathogenic infection and pregnancy complicated by cervical incompetence were higher in the positive group than in the negative group, with statistically significant differences (P<0.05). There were no statistically significant differences between the two groups in terms of pregnancy mode, premature rupture of membranes, intrauterine distress, meconium-stained amniotic fluid, and maternal education ≥12 years (P>0.05)(Table 1).

Table 1 Baseline characteristics of the two groups

Variable	UCBC positive group (n=81)	UCBC negative group (n=162)	P-value*
Placental pathology			
Moderate or severe HCA, n (%)	45（55.6%）	38（23.5%）	<0.001
FIRS,n (%)	50（61.7%）	26（16.0%）	<0.001
Neonatal characteristics			
Gestational age at birth, weeks, median (IQR)	38.9（32.4-40.1）	38.4（34.0-40.0）	0.90
Birth weight, g, median (IQR)	2990（1935-3560）	3115（1975-3493）	0.91
Male, n (%)	41（50.6%）	82（50.6%）	1.00
Neonatal birth information			
Cesarean section, n (%)	52（64.2%）	94（58.0%）	0.35
Premature rupture of membranes, n (%)	27（33.3%）	54（33.3%）	0.10
Placental abruption, n (%)	1（1.2%）	14（8.6%）	0.02
Intrauterine distress,n（%）	23（28.4%）	43（26.5%）	0.76
Meconium-stained amniotic fluid, n (%)	29（35.8%）	40（24.7%）	0.07
1-min Apgar score, median (IQR)	10（8.5-10）	10（9-10）	0.17
5-min Apgar score, median (IQR)	10（10-10）	10（10-10）	0.05
Maternal characteristics			
Maternal age, years, (Mean ± SD)	32.0±4.7	30.6±3.9	0.04
Maternal education ≥12 years, n (%)	70（86.4%）	129（79.6%）	0.20
History of abortion, n (%)	29（35.8%）	59（36.4%）	0.93
In vitro fertilization, n (%)	12（14.8%）	13（8.0%）	0.10
Multiple pregnancy, n (%)	8（9.9%）	11（6.8%）	0.40
Perinatal factors			
Acute chorioamnionitis, n (%)	67（82.7%）	109（67.3%）	0.01
Maternal temperature ≥38.0°C,n (%)	55（67.9%）	88（54.3%）	0.04
Prenatal pathogenic infection, n (%)	50（61.7%）	27（16.7%）	<0.001
Prenatal antibiotic therapy, n (%)	31（38.3%）	76（46.9%）	0.20

Pregnancy comorbidities			
Gestational diabetes mellitus, n (%)	20（24.7%）	35（21.6%）	0.60
Hypothyroidism in pregnancy, n (%)	7（8.6%）	17（10.5%）	0.65
Hypertensive disorders in pregnancy, n (%)	12（14.8%）	22（13.6%）	0.79
Cervical insufficiency, n (%)	12（14.8%）	9（5.6%）	0.02
Autoimmune disease, n (%)	3（3.7%）	7（4.3%）	0.82

HCA:histological chorioamnionitis ;FIRS: fetal inflammatory response syndrome;IQR: interquartile range; SD: standard deviation.

Independent risk factors for positive UCBC

Variables with P < 0.05 identified in univariate analysis were subsequently entered into the multivariate logistic regression model. The results demonstrated that FIRS (OR = 6.38, 95% CI: 2.79–14.57, P < 0.001) and maternal prenatal pathogenic infection (OR = 6.05, 95% CI: 2.95–12.42, P < 0.001) were independent risk factors for positive neonatal UCBC.(Table 2)

Table 2 Multivariate logistic regression analysis of risk factors for positive UCBC

Variable	**β**	**SE**	**Wald value**	**P-value**	**Odds ratio**	**95% CI**
Moderate or severe HCA	-0.09	0.423	0.046	0.830	0.913	0.399~2.092
FIRS	1.853	0.421	19.333	<0.001	6.380	2.793~14.573
Maternal age	0.083	0.043	3.748	0.053	1.086	0.999~1.181
Placental abruption	0.586	0.282	2.97	0.085	1.626	0.935~2.823
Acute chorioamnionitis	-0.207	0.571	0.131	0.717	0.813	0.266~2.491
Maternal temperature ≥38.0°C	0.786	0.511	2.366	0.124	2.195	0.806~5.975
Prenatal pathogenic	1.800	0.367	24.054	<0.001	6.049	2.946~12.418
Cervical insufficiency	0.095	0.611	0.024	0.877	1.100	0.322~3.644

HCA: histological chorioamnionitis; FIRS: fetal inflammatory response syndrome; OR: odds ratio; CI: confidence interval.

Pathogen distribution in UCBC

A total of 86 pathogenic strains were isolated from 81 UCBC positive specimens (including 5 cases of mixed infection). Among the 86 isolated pathogenic strains, 51 were Gram-positive bacteria (59.3%) and 35 were Gram-negative bacteria (40.7%), with no fungi detected.Distribution of Gram-positive bacteria: Enterococcus faecalis 12 strains (14.0%), Streptococcus mitis 6 strains (7.0%), Streptococcus gallolyticus 6 strains (7.0%), Group B Streptococcus(GBS) 6 strains (7.0%), Listeria monocytogenes 5 strains (5.8%), Staphylococcus capitis 2 strains (2.3%), Streptococcus anginosus 2 strains (2.3%), Gardnerella vaginalis 2 strains (2.3%), and 10 other Gram-positive pathogenic strains (11.6%), including Staphylococcus epidermidis, Peptostreptococcus anaerobius, Staphylococcus aureus, etc.Distribution of Gram-negative bacteria: Escherichia coli (E. coli) 25 strains (29.1%), Bacteroides spp. 5 strains (14.3%), and 5 other strains (5.8%), including Pseudomonas aeruginosa, Prevotella spp, etc.Escherichia coli combined with Enterococcus faecalis was the most common in mixed infections.(Table 3).

Table 3

Pathogen	No. of strains	Proportion (%)
Gram-positive bacteria	51	59.3
Enterococcus faecalis	12	14.0
Group B Streptococcus	6	7.0
Streptococcus mitis	6	7.0
Streptococcus gallolyticus	6	7.0
Listeria monocytogenes	5	5.8
Staphylococcus capitis	2	2.3
Streptococcus anginosus	2	2.3
Gardnerella vaginalis	2	2.3
Others	10	11.6
Gram-negative bacteria	35	40.7
Escherichia coli	25	29.1
Bacteroides species	5	14.3
Others	5	14.3
Total	86	100

HCA and pathogen distribution

Among the 81 children with positive UCBC, 36 cases (44.4%) had normal or mild HCA, and 45 cases (55.6%) had moderate or severe HCA, with no statistically significant difference between the two groups (P>0.05). E. coli was detected in 14 cases (56.0%) of those with moderate or severe HCA and 11 cases (44.0%) of those with normal or mild HCA, showing no statistically significant intergroup difference (P>0.05). Group B streptococcus was detected in 3 cases each (50.0% respectively) in the moderate or severe HCA group and the normal or mild HCA group.All 5 cases of Listeria monocytogenes infection were complicated with moderate to severe HCA and FIRS.

FIRS and pathogen distribution

Grouped by whether FIRS was combined: 31 cases (38.3%) in the non-FIRS group and 50 cases (61.7%) in the FIRS group, with a statistically significant difference between the two groups (P<0.05). Escherichia coli() was detected in 17 cases (68.0%) of those with FIRS and 8 cases (32.0%) of those without FIRS, showing a statistically significant difference (P<0.05). Group B streptococcus was found in 4 cases (66.7%) of those with FIRS and 2 cases (33.3%) of those without FIRS.Among them, 13 children with Escherichia coli infection had moderate or severe HCA on placental pathology combined with FIRS, accounting for 52.0% of the total number of Escherichia coli infections; all 5 children with mixed infection and 5 children with Listeria monocytogenes infection had moderate or severe HCA combined with FIRS.

Discussion

Neonatal sepsis remains the leading cause of morbidity and mortality among newborns worldwide[16].Chorioamnionitis is currently used as a primary risk factor for identifying infants at risk of EOS[17],which may be due to the fact that fetuses exposed to chorioamnionitis show structural damage to the fetal immune system and epithelial barrier, making it easier for microorganisms to invade the body And it is caused by the decline in the newborn's ability to resist pathogens after birth[18].Chorioamnionitis is mainly classified into a clinical or a histopathologic identity or from a microbiologic point of view: (1) the clinical diagnosis of chorioamnionitis is based on clinical signs, eg, maternal fever, uterine tenderness, malodorous discharge, and maternal and fetal tachycardia, and on laboratory abnormalities, ie, leukocytosis; (2) HCA is diagnosed by histopathologic examination of the placenta; the characteristic morphologic feature of acute HCA is diffuse infiltration of neutrophils into the chorioamniotic membranes; and (3) microbiologic chorioamnionitis, or intraamniotic infection, refers to the presence of microorganisms in the amniotic fluid, retrieved by amniocentesi[19].HCA represents a prevalent obstetric complication, with an incidence of 3–5% in term placentas and as high as 90% in placentas delivered at 21–24 weeks of gestation [4]. Characterized by an insidious clinical onset, HCA is typically asymptomatic in most cases. Consequently, a considerable proportion of hidden chorioamnionitis remains undiagnosed and untreated in a timely manner, thereby imposing potential adverse impacts on both maternal and fetal well-being [20].Existing evidence indicates that HCA is associated with a spectrum of adverse neonatal outcomes, including preterm birth, sepsis, BPD, IVH, necrotizing enterocolitis(NEC), and cerebral palsy (CP)[21-22]. Furthermore, long-term sequelae of HCA

have been linked to various neurodevelopmental disorders, such as autism spectrum disorder, and epilepsy [23]. Accumulating research has also revealed a significant association between HCA and elevated risks of neonatal mortality and severe perinatal complications, underscoring the clinical importance of early identification and diagnosis of this condition[24].Meanwhile, the incidence of HCA among preterm infants exhibits a significant negative correlation with gestational age [25]. Epidemiologically, HCA occurs in approximately 50% of preterm infants delivered before 28 weeks of gestation [26].Upon invasion of the amniotic cavity by pathogenic microorganisms, both maternal and fetal immune systems are activated, triggering the release of pro-inflammatory cytokines including interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) [27].

FIRS refers to the systemic inflammatory reaction mounted by the fetus in response to intrauterine insults, characterized by increased concentrations of inflammatory cytokines within the fetal circulation. A cord blood IL-6 level of ≥ 11 pg/mL is widely accepted as a diagnostic criterion for FIRS [28–29]. Notably, FIRS has been strongly associated with an elevated risk of neonatal mortality and multiple adverse neonatal complications [27].A meta-analysis demonstrated that approximately 50%–70% of preterm infants with HCA are complicated by concurrent FIRS. In terms of perinatal outcomes, preterm infants with HCA accompanied by FIRS exhibit a significantly poorer prognosis than those with HCA alone. Furthermore, this meta-analysis also revealed that among preterm infants with a gestational age of less than 28 weeks, the occurrence of EOS is significantly higher in those with HCA combined with FIRS compared with those with HCA alone[8].However, another systematic review and meta-analysis investigating the association between HCA and clinical chorioamnionitis (CA) with EOS in preterm infants delivered before 37 weeks of gestation yielded distinct findings. Pooled analysis based on unadjusted data demonstrated a significant association between CA and EOS. Furthermore, in contrast to isolated CA exposure (i.e., chorioamnionitis without funisitis, representing umbilical inflammation associated with chorioamnionitis), concurrent funisitis did not confer an additional increased risk of neonatal sepsis.In our study, although moderate or severe HCA was associated with positive UCBC in univariate analysis, it did not demonstrate independent predictive value in multivariate models. This discrepancy may be attributable to the fact that histologic staging of HCA reflects the temporal progression of inflammation rather than the actual pathogen load. In contrast, FIRS (i.e., funisitis or chorionic vasculitis) were identified as an independent risk factor for positive UCBC, indicating that pathogenic microorganisms have breached the placental barrier and invaded the fetal circulation, thereby establishing a closer association with positive UCBC results. This further suggests that, compared with isolated chorioamnionitis, FIRS serves as a more reliable indicator of fetal infection and adverse neonatal outcomes [30].

Microbial invasion of the amniotic cavity occurs mainly via three routes:(1) ascending from the vagina and cervix;(2) hematogenous dissemination through the placenta (transplacental infection);and (3) accidental introduction during invasive obstetric procedures, including amniocentesis, percutaneous fetal blood sampling, chorionic villus sampling, or shunting procedures [19].Among these pathways, ascending infection represents the predominant route underlying intrauterine infection [31].The presence of HCA and FIRS indicates that the fetus may have been exposed to pathogenic microorganisms.

Notably, findings from the present study identified FIRS and antenatal pathogen infection as independent risk factors for positive UCBC, suggesting that the fetal systemic inflammatory response to intrauterine infection is closely associated with hematogenous transmission of pathogens.

GBS is a β-hemolytic Gram-positive bacterium that commonly colonizes the rectum and vagina of healthy women, with an average colonization rate of 17.9%. It is recognized as one of the most prevalent pathogens associated with chorioamnionitis. When infection occurs during labor, GBS is vertically transmitted to neonates in approximately 36% of cases [32–34].In developed countries, GBS remains the leading etiological agent of neonatal sepsis and meningitis [35]. For instance, in the United Kingdom, EOS attributable to GBS infection occurs in approximately 1 in 2000 live births [36]. Nevertheless, the majority of neonates with GBS infection are clinically asymptomatic [37].Recent studies conducted in the Chinese population have reported that the prevalence of chorioamnionitis is 16.93% in GBS-positive women and 8.86% in GBS-negative women [38].Conversely, the rates of GBS positivity were 13.56% in patients with HCA and 3.53% in those without HCA [20], indicating that the occurrence of GBS in HCA-positive cases was approximately 3.8-fold higher than in HCA-negative cases.In this study, a total of 6 GBS strains were identified among pathogenic isolates from UCBC, accounting for 7% (6/86) of all positive cultures.Of these 6 neonates with GBS infection, 5 (83.3%) had concurrent HCA, while only 1 (16.7%) was HCA-negative.Notably, 4 cases (66.7%) of GBS infection were accompanied by FIRS, whereas 2 cases (33.3%) occurred in the absence of FIRS.The infection status of GBS in HCA-merging cases and non-HCA GBS cases is basically consistent with previous studies.Of note, prophylactic antibiotic administration in GBS-positive pregnant women not only reduces the risk of infection by other opportunistic pathogens but also mitigates the likelihood of HCA [20].

E. coli and GBS are the main pathogens causing EOS. With the increasing application of prenatal screening and intrapartum antibiotic prophylaxis (IAP), the incidence of EOS caused by GBS has gradually decreased, while the infection rate of E. coli has been on the rise, especially among preterm infants. Meanwhile, E. coli is also a major bacterium responsible for neonatal intracranial infection and death [39-40]. In addition, Stoll et al. found that the proportion of mothers with HCA was significantly higher in neonates with E. coli infection than in those with GBS infection (81% vs. 52%; adjusted P = 0.03) [39]. Regarding the mechanism by which E. coli induces intrauterine infection, a metabolomic analysis of serum samples from preterm infants with HCA revealed that the pathogenic E. coli infection pathway is involved in HCA-related metabolic changes, indicating that persistent intrauterine infection caused by Gram-negative bacteria is the most common cause of HCA [41]. Furthermore, animal experiments have confirmed that lipopolysaccharide (LPS) from Gram-negative bacteria can strongly activate the fetal immune system and induce FIRS[42]. In our study, E. coli was detected in 14 cases (56.0%) of infants with moderate or severe HCA and 11 cases (44.0%) of infants with normal or mild HCA, with no statistically significant difference between the two groups ($P > 0.05$). In contrast, E. coli was detected in 17 cases (68.0%) of infants with concurrent FIRS and 8 cases (32.0%) of infants without FIRS, and the difference between the two groups was statistically significant ($P < 0.05$). These findings suggest that HCA caused by E. coli is more severe and is more likely to be accompanied by FIRS.

Listeria monocytogenes (LM) is a severe foodborne infection, and pregnant women represent a high-risk population. Maternal LM infection may lead to adverse pregnancy outcomes including miscarriage, stillbirth, preterm birth, and congenital neonatal infection [43].LM exhibits distinct placental tropism, enabling it to invade trophoblast cells, breach the placental barrier, and infect the placenta, thereby inducing chorioamnionitis and/or chorionic vasculitis. Involvement of the fetus and amniotic cavity subsequently results in mother-to-child vertical transmission, which may trigger intrauterine fetal demise or preterm delivery [44-45].In the present study, all neonates with culture-confirmed LMs in umbilical cord blood were complicated by moderate or severe HCA and FIRS. This observation may be attributed to the ability of LM, upon entering the amniotic cavity, to stimulate both maternal and fetal immune systems, resulting in abundant release of proinflammatory cytokines and subsequent development of HCA and FIRS. However, given the limited existing evidence, high-quality clinical studies are warranted to further elucidate the underlying relationship between listeriosis and these inflammatory conditions.

Conclusion

FIRS and antenatal pathogen infection are independent predictors of positive UCBC. For HCA complicated by FIRS, high vigilance should be paid to infections caused by Gram-negative bacteria such as E. coli, while special attention should also be given to specific pathogens including LM.

Several limitations of this study should be acknowledged. First, HCA can only be definitively diagnosed via delayed pathological examination, and not all puerperae undergo this confirmatory testing. This diagnostic limitation may result in underdiagnosis of HCA and subsequent missed opportunities for timely intervention, highlighting the need for further research to identify reliable biomarkers that facilitate early screening of HCA cases. Second, this study was designed as a single-center retrospective analysis with a limited sample size, which may restrict the generalizability of the findings. Additionally, long-term follow-up data were lacking, precluding the assessment of the long-term impact of infection on neonatal prognosis. Therefore, multicenter prospective studies with larger sample sizes and comprehensive long-term follow-up are warranted in the future to validate the current findings and address these limitations.

Abbreviations

HCA
histological chorioamnionitis
FIRS
fetal inflammatory response syndrome
UCBC
umbilical cord blood cultures
EOS
early-onset sepsis
BPD
bronchopulmonary dysplasia

IVH
intraventricular hemorrhage
ROP
retinopathy of prematurity
PBC
peripheral blood culture
NEC
enecrotizing enterocolitis
CP
cerebral palsy
IL-6
interleukin-6
TNF-α
tumor necrosis factor-α
CA
clinical chorioamnionitis
GBS
Group B streptococcus
IAP
intrapartum antibiotic prophylaxis
E. Coli
Escherichia coli
LPS
lipopolysaccharide
LM
Listeriosis

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Women's Hospital School of Medicine Zhejiang University (approval No. IRB20230184R) and conducted in accordance with the Declaration of Helsinki. Written informed consent was waived due to the retrospective nature of the study.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Tong Dong, Lixin Ma,Bingqing Ding, and Rong Tao. The first draft of the manuscript was written by Tong Dong, Lixin Ma,Bingqing Ding and Yingying Bao. All authors commented on previous versions of the manuscript.

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