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Clinical chorioamnionitis at term VI: acute chorioamnionitis and funisitis according to the presence or absence of microorganisms and inflammation in the amniotic cavity

Abstract

Objective: Neonates born to mothers with clinical chorioamnionitis at term are at an increased risk of infection. Acute subchorionitis, chorioamnionitis, and funisitis are considered placental histologic features consistent with acute inflammation according to the Society for Pediatric Pathology. The objectives of this study were to examine the performance of placental histologic features in the identification of: 1) microbial-associated intra-amniotic inflammation (intra-amniotic infection); and 2) fetal inflammatory response syndrome (FIRS).

Methods: This retrospective cohort study included women with the diagnosis of clinical chorioamnionitis at term (n=45), who underwent an amniocentesis to determine: 1) the presence of microorganisms using both cultivation and molecular biologic techniques [polymerase chain reaction (PCR) with broad range primers]; and 2) interleukin (IL)-6 concentrations by enzyme-linked immunosorbent assay (ELISA). The diagnostic performance (sensitivity, specificity, accuracy, and likelihood ratios) of placental histologic features consistent with acute inflammation was determined for the identification of microbial-associated intra-amniotic inflammation and FIRS.

Results: 1) The presence of acute histologic chorioamnionitis and funisitis was associated with the presence of proven intra-amniotic infection assessed by amniotic

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fluid analysis; 2) funisitis was also associated with the presence of FIRS; 3) the negative predictive value of acute funisitis ≥stage 2 for the identification of neonates born to mothers with intra-amniotic infection was <50%, and therefore, suboptimal to exclude fetal exposure to bacteria in the amniotic cavity; and 4) acute funisitis ≥stage 2 had a negative predictive value of 86.8% for the identification of FIRS in a population with a prevalence of 20%.

Conclusion: Acute histologic chorioamnionitis and funisitis are associated with intra-amniotic infection and the presence of FIRS. However, current pathologic methods have limitations in the identification of the fetus exposed to microorganisms present in the amniotic cavity. Further studies are thus required to determine whether molecular markers can enhance the performance of placental pathology in the identification of neonates at risk for neonatal sepsis.

Introduction

Clinical chorioamnionitis is a heterogeneous syndrome [1] characterized by maternal fever accompanied by at least two of the following signs: maternal or fetal tachycardia, maternal leukocytosis, uterine tenderness, or foul-smelling amniotic fluid [2–14]. This syndrome is associated with proven intra-amniotic infection in almost 60% of cases, and is a major risk factor for neonatal sepsis [12, 15–23]. The rapid and accurate diagnosis of maternal intra-amniotic infection is important to guide the management of neonates exposed to intrapartum fever [24–34]. The gold standard for the diagnosis of intra-amniotic infection is amniotic fluid analysis with the use of cultivation and molecular microbiologic techniques to identify the presence of bacteria [35–52] and to assess the inflammatory response [13, 35–37, 53–59].

When information about the amniotic fluid microbiology and inflammatory state is not available, pediatricians must rely on clinical risk factors (e.g., maternal fever, rupture of membranes, etc.) [26–28, 60], signs of neonatal sepsis and laboratory tests, such as neonatal white blood cell and differential counts [61–80], immature to mature ratio of neutrophils [77, 80–83], interleukin (IL)-6 [84–118], IL-8 [81, 91, 100, 101, 104, 106, 109, 113, 119], C-reactive protein (CRP) [68–70, 72, 78, 103, 104, 113, 118, 120–125], procalcitonin [118, 123, 124, 126–128], blood [26, 30, 82,

129–134], and cerebro-spinal fluid cultures [135–144], as well as other biomarkers. Despite intensive investigation, the accurate identification of early-onset sepsis in neonatology remains a conundrum [26–28, 32, 34, 145].

Examination of the placenta, the largest human biopsy, can provide information about fetal exposure to intra-amniotic inflammation or infection [146–169]. Chorionitis and acute histologic chorioamnionitis are histologic lesions that represent a maternal response to inflammatory stimuli in the amniotic cavity, such as bacteria, viruses and fungi, or non-infectious insults, such as danger signals (alarmins resulting from cell stress, apoptosis, and pyroptosis) [156, 158, 163, 170–173]. Inflammation of the umbilical cord or chorionic vessels represents a fetal response and is indicative of a fetus's exposure to inflammatory stimuli before birth [154].

As early as 1959, Benirschke proposed that the examination of the umbilical cord using a frozen section could be a rapid and accurate method to identify newborns exposed to infection *in utero* [146]. Decades later, this idea has been revisited [174–176]. However, a major limitation of all clinical studies performed to date in patients with clinical chorioamnionitis at term is that the presence of bacteria in the amniotic cavity remains unknown, and therefore, accurate ascertainment of intra-amniotic infection is not feasible. The objective of the current study was to examine the diagnostic performance of placental histologic features of acute inflammation in the identification of: 1) microbial-associated intra-amniotic inflammation (also termed intra-amniotic infection), and 2) fetal inflammatory response syndrome (FIRS). The latter outcome was selected because there is evidence that both FIRS [98, 177–191] and neonatal systemic inflammation [192–209] are associated with adverse neonatal and infant outcomes.

Materials and methods

This retrospective cohort study included women with the diagnosis of clinical chorioamnionitis at term, who underwent an amniocentesis to identify microorganisms in the amniotic cavity. Patients were identified by searching the clinical database and Bank of Biological Samples of Wayne State University, the Detroit Medical Center, and the Perinatology Research Branch (NICHD/NIH/DHHS). The criteria for entry were: 1) singleton gestation, 2) gestational age of ≥37 weeks, 3) sufficient amniotic fluid obtained by transabdominal amniocenteses for molecular microbiologic studies, and 4) absence of fetal malformations. These patients were included in prior studies, which provide a detailed description of sample collection, microbiological studies, and determination of amniotic fluid IL-6 concentrations and other cytokines [1, 3, 9–11].

All patients provided written informed consent. The use of biological specimens as well as clinical and ultrasound data for research purposes was approved by the Institutional Review Boards of NICHD, Wayne State University, and Sótero del Río Hospital, Santiago, Chile.

Clinical definitions

Microbial invasion of the amniotic cavity was defined according to the results of amniotic fluid culture and PCR/ESI-MS (broad-range PCR coupled with electrospray ionization mass spectrometry) (Ibis® Technology, Athogen, Carlsbad, CA, USA) [1, 45–47, 210]. Microbial-associated intra-amniotic inflammation (also termed intra-amniotic infection) was diagnosed when microorganisms were identified in the amniotic fluid using cultivation or molecular microbiologic techniques and elevated amniotic fluid IL-6 concentrations ($\geq$2.6 ng/mL), as described in detail elsewhere [1, 13, 35–37, 39, 50, 53, 54, 56–59, 211–213].

Clinical chorioamnionitis was diagnosed by the presence of maternal fever (temperature >37.8°C) accompanied by two or more of the following criteria: 1) maternal tachycardia (heart rate >100 beats/min), 2) uterine tenderness, 3) foul-smelling amniotic fluid, 4) fetal tachycardia (heart rate >160 beats/min), and 5) maternal leukocytosis (leukocyte count >15,000 cells/mm^3) [2, 4–8, 12, 214]. FIRS was diagnosed when umbilical cord plasma IL-6 concentrations were >11 pg/mL [178, 187–189, 215–217].

Placental pathology

Tissue samples were obtained from each placenta – one roll from the chorioamniotic membranes and one from the umbilical cord. Two sections were taken from both the chorionic and basal plates. Tissues were formalin-fixed and embedded in paraffin. Five-micrometer-thick sections of tissue blocks were stained with hematoxylin and eosin, and the slides were examined by perinatal pathologists masked to clinical outcomes.

Histopathological changes of the placenta were defined according to the diagnostic criteria proposed by the Perinatal Section of the Society for Pediatric Pathology [156]. Histologic features associated with acute inflammation are acute subchorionitis, acute histologic chorioamnionitis (maternal response), and funisitis/acute chorionic vasculitis (fetal response). The definition and staging of acute histologic chorioamnionitis and acute funisitis are described below.

1. Placental histologic features consistent with acute inflammation (maternal response) were diagnosed based on the presence of inflammatory cells in the chorionic plate and/or in the chorioamniotic membranes [156].
   - Stage 1 (acute subchorionitis/acute chorionitis): accumulation of neutrophils in the subchorionic zone and/or in the chorionic trophoblast layer of the extraplacental membranes.
   - Stage 2 (acute chorioamnionitis): more than a few scattered neutrophils in the chorionic plate and membranous connective tissues and/or in the amnion.
   - Stage 3 (necrotizing chorioamnionitis): marked neutrophilic infiltrate with degenerating neutrophils (karyorrhexis), thickened eosinophilic amniotic basement membrane, and focal amniotic epithelial necrosis.

2. Acute funisitis was diagnosed by the presence of neutrophils in the wall of the umbilical vessels and/or the Wharton's jelly, also using previously reported criteria [98, 154, 156, 157, 218–220].
   - Stage 1 (acute chorionic vasculitis/umbilical phlebitis/chorionic vasculitis): neutrophils are identified in the wall of any chorionic plate vessel or in the umbilical vein (few neutrophils are usually present in the Wharton's jelly, but without aggregates or concentric bands of inflammatory cells).
   - Stage 2 (umbilical arteritis): neutrophils are seen in one or both of the umbilical arteries and/or in the umbilical vein.
   - Stage 3 (necrotizing funisitis): presence of neutrophils, cellular debris, and/or mineralization in a concentric band, ring, or halo around one or more umbilical vessel(s).

Statistical analysis

The Kolmogorov-Smirnov test was used to test whether data were normally distributed. Chi-square and Fisher's exact tests were used for comparisons of proportions. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), accuracy, and likelihood ratios (+/−) were all calculated for the identification of microbial-associated intra-amniotic inflammation and FIRS. Statistical analysis was performed using SPSS 19 (IBM Corp, Armonk, NY, USA). A P-value <0.05 was considered statistically significant.

Results

Clinical characteristics of the study population

The descriptive characteristics of the study population stratified by the presence or absence of microbial-associated intra-amniotic inflammation (intra-amniotic infection) were previously reported [3]. Microorganisms most frequently identified in the amniotic cavity were *Ureaplasma* spp. and *Gardnerella vaginalis* [1]. Briefly, patients with clinical chorioamnionitis at term with microbial-associated intra-amniotic inflammation had a significantly higher median amniotic fluid white blood cell count, amniotic fluid IL-6, and umbilical cord plasma IL-6 concentration than those without microbial-associated intra-amniotic inflammation (P<0.001, P<0.001, and P=0.03, respectively) [3]. The frequencies of FIRS and acute inflammatory lesions of the placenta were also significantly greater in patients with clinical chorioamnionitis at term with microbial-associated intra-amniotic inflammation than in those without microbial-associated intra-amniotic inflammation [FIRS: 36% (9/25) vs. 5% (1/20), P=0.03; and acute inflammatory lesions of the placenta: 70.8% (17/24) vs. 25% (5/20), P=0.02] [3].

The frequency of neonates with FIRS was 22.2% (10/45). Among them, 90% (9/10) and 66.7% (6/9) were born to mothers with intra-amniotic infection and with placental histologic lesions consistent with acute amniotic fluid infection respectively, [acute subchorionitis/histologic chorioamnionitis (n=5) or inflammation of the umbilical cord (n=5)] (Table 1). Among neonates with FIRS, whose mothers had placental histologic features consistent with acute inflammation, 83% (5/6) had stage 2 acute histologic chorioamnionitis/funisitis (Table 1). The descriptions of clinical characteristics, bacteria isolated from the amniotic cavity, amniotic fluid and umbilical cord plasma IL-6 concentrations, as well as placental histologic lesions in mothers whose neonates had FIRS, are shown in Table 1.

Overall, 33% (15/45) of neonates born to mothers with clinical chorioamnionitis had suspected neonatal sepsis. Among these neonates, 53.3% (8/15) and 20% (3/15) were exposed to intra-amniotic infection and intra-amniotic inflammation without detectable bacteria, respectively. The remaining 26.7% (4/15) were born to mothers who had no intra-amniotic inflammation. All neonates with suspected neonatal sepsis (diagnosis based on clinical signs and laboratory findings) had negative blood cultures for microorganisms.

Diagnostic performance

The diagnostic performance of placental histologic features consistent with acute inflammation for the identification of microbial-associated intra-amniotic inflammation (also termed intra-amniotic infection) is shown in Table 2. The sensitivity of placental histologic features of acute inflammation ranged from 16.7% to 70.8%. Specifically, acute histologic chorioamnionitis and funisitis ≥stage 2 had sensitivities of 25% (6/24) and 16.7% (4/24), respectively (Table 2). The PPV or NPV of placental histologic features associated with acute inflammation was fair (PPV: 60%–73.9%; NPV: 47.1%–66.7%) (Table 2). Overall, the accuracy of placental histologic features of acute inflammation for the identification of microbial-associated intra-amniotic inflammation only ranged from 50% to 70% (Table 2).

The diagnostic performance of placental histologic lesions for the identification of FIRS is displayed in Table 3. The sensitivity and specificity ranged from 44.4% to 66.7% and 51.4% to 94.3%, respectively. Acute funisitis ≥stage 2 had the highest specificity (94.3%) followed by acute histologic chorioamnionitis ≥stage 2 (82.9%) when compared to other placental histologic lesions (Table 3).

All placental histologic features had an NPV above 80%; however, the PPV ranged from 22.7% to 66.7% (Table 3). Acute funisitis ≥stage 2 had a positive likelihood ratio of 7.8 for the identification of FIRS.

The diagnostic indices of acute inflammatory histologic features of the placenta for the identification of intra-amniotic inflammation are shown in Table 4. The sensitivity and NPV for such placental histologic features ranged from 14.7% to 61.7%, and 23.7%–38.1%, respectively. The overall accuracy was <70% in the identification of intra-amniotic inflammation (Table 4).

Discussion

Principal findings of the study

1) The presence of acute histologic chorioamnionitis and funisitis was associated with the presence of proven intra-amniotic infection assessed by amniotic fluid analysis; 2) funisitis was also associated with the presence of FIRS; 3) the NPV of funisitis ≥stage 2 for the identification of neonates born to mothers with intra-amniotic infection was <50%, and therefore suboptimal to exclude fetal exposure to bacteria in the amniotic cavity; and 4) funisitis ≥stage 2 had an NPV of 86.8% for the identification of FIRS in a population with a prevalence of 20%. Further studies are required to determine whether molecular markers can enhance the performance of placental pathology in the identification of neonates at risk for neonatal sepsis.

Acute histologic chorioamnionitis and funisitis: two different types of inflammatory responses and their relationship with microbial invasion of the amniotic cavity

Acute histologic chorioamnionitis or chorionitis consists of the infiltration of the chorion or chorioamniotic membranes by maternal neutrophils and, therefore, is a maternal host response to chemotactic products [156, 158, 163, 170–173]. The mechanism underlying neutrophil infiltration is the presence of high concentrations of neutrophil chemokines in the amniotic fluid, which would generate a gradient promoting the migration of the neutrophils from the decidua to the chorion, and subsequently, the amnion. The following chemotactic factors have been identified in the amniotic cavity of patients in preterm labor: IL-8 [43,

Table 1: Clinical characteristics, amniotic fluid interleukin-6 concentrations, microorganisms identified in the amniotic cavity, and placental histologic features consistent with acute inflammation in mothers whose neonates have fetal inflammatory response syndrome (FIRS) (n=10/45; 22.2%).

No.	Organisms identified by cultivation	Organisms identified by PCR/ESI-MS	Microbial-associated intra-amniotic inflammation (intra-amniotic infection)	GA at delivery (weeks)	Amniotic fluid IL-6 concentration (ng/mL)	Umbilical cord plasma IL-6 concentration (pg/mL)	Maternal response to acute inflammation	Fetal response to acute inflammation
1.	Peptostreptococcus spp.	Gardnerella vaginalis Abiotrophia defectiva	Yes	41^{+1}	97.1	170.85	No	Umbilical arteritis
2.	Fusobacterium spp.	Fusobacterium nucleatum/ periodonticum	Yes	37^{+4}	118.35	103.01	Acute chorioamnionitis	Umbilical arteritis
3.	Ureaplasma urealyticum Streptococcus agalactiae	Ureaplasma urealyticum Gardnerella vaginalis	Yes	40^{+5}	83.19	14.13	Acute chorioamnionitis	Umbilical arteritis
4.	No	No	No	40^{+3}	109.19	12.14	Acute chorioamnionitis	Umbilical arteritis
5.	Ureaplasma urealyticum	Firmicute (Lactobacillus)	Yes	41^{+4}	14.12	101.09	No	No
6.	Ureaplasma urealyticum Enterococcus spp. Gardnerella vaginalis	Peptostreptococcus anaerobius Ureaplasma urealyticum Gardnerella vaginalis	Yes	41^{+1}	19.94	52.84	No	No
7.	Staphylococcus agalactiae Staphylococcus aureus	Staphylococcus agalactiae	Yes	40^{+4}	32.82	31.67	Acute chorioamnionitis	Umbilical phlebitis/ chorionic vasculitis
8.	Staphylococcus agalactiae	Staphylococcus agalactiae	Yes	39^{+4}	10.45	14.78	No	No
9.	No	Acinetobacter spp. Pseudomonas aerunginosa	Yes	40^{+4}	18.41	12.38	Acute subchorionitis/ chorionitis	No
10.	Mycoplasma hominis	Propionibacterium acnes	Yes	38^{+4}	74.08	32.99	N/A[a]	N/A[a]

[a]N/A=A placental pathology report was not available for 1 patient, PCR ESI/MS=broad-range PCR coupled with electrospray ionization mass spectrometry, GA=gestational age, IL=interleukin. Microbial-associated intra-amniotic inflammation defined by the presence of bacteria in the amniotic cavity and intra-amniotic inflammation (IL-6 ≥2.6 ng/mL); fetal inflammatory response is defined by a concentration of umbilical cord plasma IL-6 >11 pg/mL.

Acute subchorionitis/chorionitis=maternal response to acute inflammation stage 1, Acute chorioamnionitis=maternal response to acute inflammation stage 2, Necrotizing chorioamnionitis and subacute chorioamnionitis=maternal response to acute inflammation stage 3, Umbilical phlebitis/chorionic vasculitis=acute funisitis stage 1, Umbilical arteritis=acute funisitis stage 2, Necrotizing funisitis=acute funisitis stage 3.

Table 2: The diagnostic performance of placental histologic features consistent with acute inflammation for the identification of intra-amniotic infection (microbial-associated intra-amniotic inflammation) in patients with clinical chorioamnionitis at term (n=25/45; 55.56%).[a]

	Sensitivity (%)		Specificity (%)		Positive likelihood ratio (95% CI)	Negative likelihood ratio (95% CI)	Positive predictive value (%)		Negative predictive value (%)		Accuracy
	% (n)	95% CI	% (n)	95% CI			% (n)	95% CI	% (n)	95% CI	
Acute subchorionitis, chorioamnionitis (n=22/44)	66.7% (16/24)	(44.7–84.3)	70.0% (14/20)	(45.7–88.0)	2.2 (1.0–4.6)	0.5 (0.3–0.9)	72.7% (16/22)	(49.8–89.2)	63.6% (14/22)	(40.7–82.8)	68.2% (30/44)
Acute funisitis (n=13/44)	33.3% (8/24)	(15.7–55.3)	70.0% (14/20)	(45.7–91.3)	1.3 (0.5–3.4)	0.9 (0.6–1.3)	61.5% (8/13)	(31.6–86.0)	48.4% (15/31)	(30.2–66.9)	52.3% (23/44)
Acute inflammatory histologic features of the placenta (n=23/44)	70.8% (17/24)	(48.9–87.3)	71.4% (15/21)	(47.8–88.0)	2.4 (1.2–4.8)	0.4 (0.2–0.8)	73.9% (17/23)	(51.6–89.7)	66.7% (17/21)	(43.0–85.4)	70.5% (31/44)
Acute histologic chorioamnionitis ≥stage 2 (n=10/44)	25.0% (6/24)	(9.8–46.7)	80.0% (16/20)	(56.3–94.1)	1.3 (0.4–3.8)	0.9 (0.7–1.3)	60.0% (6/10)	(26.4–87.6)	47.1% (16/34)	(29.8–64.9)	50% (22/44)
Acute funisitis ≥stage 2 (n=6/44)	16.7% (4/24)	(4.8–37.4)	90.0% (18/20)	(68.3–98.5)	1.7 (0.3–8.2)	0.9 (0.7–1.2)	66.7% (4/6)	(22.7–94.7)	47.4% (18/38)	(30.9–64.2)	50% (22/44)

[a]A placental pathology report was not available for 1 patient.

CI=confidence interval, intra-amniotic infection (microbial-associated intra-amniotic inflammation) is defined by the presence of bacteria in the amniotic cavity and intra-amniotic inflammation (IL-6≥2.6 ng/mL).

Acute subchorionitis/chorionitis=maternal response to acute inflammation stage 1, Acute chorioamnionitis=maternal response to acute inflammation stage 2, Necrotizing chorioamnionitis and subacute chorioamnionitis=maternal response to acute inflammation stage 3, Umbilical phlebitis/chorionic vasculitis=acute funisitis stage 1, Umbilical arteritis=acute funisitis stage 2, Necrotizing funisitis=acute funisitis stage 3.

Acute inflammatory histologic features of the placenta: acute subchorionitis, chorioamnionitis and/or acute funisitis.

Table 3: The diagnostic performance of placental histologic features consistent with acute inflammation for the identification of fetal inflammatory response syndrome (FIRS) in patients with clinical chorioamnionitis at term (n=9/45; 20%).[a]

	Sensitivity (%)		Specificity (%)		Positive likelihood ratio (95% CI)	Negative likelihood ratio (95% CI)	Positive predictive value (%)		Negative predictive value (%)		Accuracy
	% (n)	95% CI	% (n)	95% CI			% (n)	95% CI	% (n)	95% CI	
Acute subchorionitis, chorioamnionitis (n=22/44)	55.6% (5/9)	(21.4–86.0)	51.4% (18/35)	(34.0–68.6)	1.1 (0.6–2.3)	0.9 (0.4–1.9)	22.7% (5/22)	(7.9–45.4)	81.8% (18/22)	(59.7–94.7)	52.3% (23/44)
Acute funisitis (n=13/44)	55.6% (5/9)	(21.4–86.0)	77.1% (27/35)	(59.9–89.6)	2.4 (1.1–5.7)	0.6 (0.3–1.2)	38.5% (5/13)	(14.0–68.4)	87.1% (27/31)	(70.2–96.3)	72.7% (32/44)
Acute inflammatory histologic features of the placenta (n=23/44)	66.7% (6/9)	(30.1–92.1)	51.4% (18/35)	(34.0–68.6)	1.4 (0.8–2.4)	0.7 (0.2–1.7)	26.1% (6/23)	(10.3–48.4)	85.7% (18/21)	(63.6–96.8)	54.5% (24/44)
Acute histologic chorioamnionitis ≥stage 2 (n=10/44)	44.4% (4/9)	(13.9–78.6)	82.9% (29/35)	(66.3–93.4)	2.6 (0.9–7.3)	0.7 (0.4–1.2)	40.0% (4/10)	(12.4–73.6)	85.3% (29/34)	(68.9–94.9)	75. % (33/44)
Acute funisitis ≥stage 2 (n=6/44)	44.4% (4/9)	(13.9–78.6)	94.3% (33/35)	(80.8–99.1)	7.8 (1.7–35.9)	0.6 (0.3–1.1)	66.7% (4/6)	(22.7–94.7)	86.8% (33/38)	(71.9–95.5)	72.7% (32/44)

[a]A placental pathology report was not available for 1 patient.

CI=confidence interval, FIRS=fetal inflammatory response syndrome is defined by umbilical cord interleukin-6 concentration >11 pg/mL.

Acute subchorionitis/chorionitis=maternal response to acute inflammation stage 1, Acute chorioamnionitis=maternal response to acute inflammation stage 2, Necrotizing chorioamnionitis and subacute chorioamnionitis=maternal response to acute inflammation stage 3, Umbilical phlebitis/chorionic vasculitis=acute funisitis stage 1, Umbilical arteritis=acute funisitis stage 2, Necrotizing funisitis=acute funisitis stage 3.

Acute inflammatory histologic features of the placenta: acute subchorionitis, chorioamnionitis and/or acute funisitis.

Table 4: The diagnostic performance of placental histologic features consistent with acute inflammation for the identification of intra-amniotic inflammation in patients with clinical chorioamnionitis at term (n=35/45; 77.8%).[a]

	Sensitivity (%)		Specificity (%)		Positive likelihood ratio (95% CI)	Negative likelihood ratio (95% CI)	Positive predictive value (%)		Negative predictive value (%)		Accuracy
	% (n)	95% CI	% (n)	95% CI			% (n)	95% CI	% (n)	95% CI	
Acute subchorionitis, chorioamnionitis (n=22/44)	58.8% (20/34)	(40.7–75.3)	80% (8/10)	(44.4–98.9)	2.9 (0.8–10.5)	0.5 (0.3–0.9)	90.9% (20/22)	(70.8–98.6)	36.4% (8/22)	(17.2–59.3)	63.6% (28/44)
Acute funisitis (n=13/44)	32.4% (11/34)	(17.4–50.5)	80% (8/10)	(44.4–96.9)	1.6 (0.4–6.1)	0.9 (0.6–1.3)	84.6% (11/13)	(54.5–97.6)	25.8% (8/31)	(11.9–44.6)	43.2% (19/44)
Acute inflammatory histologic features of the placenta (n=23/44)	61.8% (21/34)	(43.6–77.8)	80% (8/10)	(44.4–96.8)	3.1 (0.9–10.9)	0.5 (0.3–0.8)	91.3% (21/23)	(71.9–98.7)	38.1% (8/21)	(18.2–61.6)	65.9% (29/44)
Acute histologic chorioamnionitis ≥stage 2 (n=10/44)	23.5% (8/34)	(10.8–41.2)	80% (8/10)	(44.4–96.9)	1.2 (0.3–4.7)	0.9 (0.7–1.4)	80.0% (8/10)	(44.4–96.9)	25.5% (8/34)	(10.8–41.2)	36.4% (16/44)
Acute funisitis≥stage 2 (n=6/44)	14.7 % (5/34)	(5.0–31.1)	90% (9/10)	(55.5–98.3)	1.5 (0.2–11.2)	0.95 (0.7–1.2)	88.3% (5/6)	(36.1–97.2)	23.7% (9/38)	(11.5–40.3)	34% (14/44)

[a]A placental pathology report was not available for 1 patient.

CI=confidence interval, IL=interleukin, intra-amniotic inflammation is defined by amniotic fluid IL-6 concentration ≥2.6 ng/mL.

Acute subchorionitis/chorionitis=maternal response to acute inflammation stage 1, Acute chorioamnionitis=maternal response to acute inflammation stage 2, Necrotizing chorioamnionitis and subacute chorioamnionitis=maternal response to acute inflammation stage 3, Umbilical phlebitis/chorionic vasculitis=acute funisitis stage 1, Umbilical arteritis=acute funisitis stage 2, Necrotizing funisitis=acute funisitis stage 3.

Acute inflammatory histologic features of the placenta: acute subchorionitis, chorioamnionitis and/or acute funisitis.

48, 221–228], neutrophil activating peptide [221, 222, 229], CXCL-13 [230], leukotriene B4 [231], epithelial cell-derived neutrophil-activating peptide-78 (ENA-78) [229], Exodus-1 (CCL20) [232], and CXCL-6 [233].

Funisitis represents a fetal inflammatory response, in which circulating fetal neutrophils migrate through the umbilical vein and artery with subsequent infiltration of the Wharton's jelly [154, 156]. The first stage of funisitis is phlebitis, followed by arteritis, and then infiltration of the Wharton's jelly [154, 156]. Chorionic vasculitis is the equivalent of funisitis in the chorionic plate of the placenta [154].

The presence of acute chorioamnionitis diagnosed by histologic examination of the placenta has traditionally been considered as evidence of intra-amniotic infection. Indeed, this lesion is classified by the Society for Pediatric Pathology as consistent with "amniotic fluid infection" [156]. The scientific basis for this infection has been that several studies have shown a strong relationship between the presence of bacteria in the amniotic fluid and acute inflammatory lesions of the placenta in patients who delivered within 48 h of amniocentesis with preterm labor and intact membranes. For example, in a population of patients with preterm labor and intact membranes, where the prevalence of microbial invasion of the amniotic cavity (determined with cultivation techniques) was 38% (35/92), the PPV of acute funisitis was 78.7% and that of acute chorioamnionitis was 71%. The NPV for microbial invasion of the amniotic cavity, defined as a positive amniotic fluid culture, ranged between 82% for funisitis and 86% for chorioamnionitis. Similar observations have been reported by other investigators [234]. In term gestations, acute histologic chorioamnionitis is more common in patients who underwent spontaneous labor than in those who delivered by cesarean section without labor [160, 235]. Moreover, the more advanced cervical dilatation at the time of a cesarean delivery, the greater the risk of acute histologic chorioamnionitis and microbial invasion of the amniotic cavity [236, 237]. Other studies have documented that acute histologic chorioamnionitis is associated with the presence of bacteria in the chorioamniotic space [150, 151, 238], although the strength of this association varies between term and preterm gestations [172, 173]. For example, in preterm birth, the frequency of funisitis ranges from 17% to 44.8% [98, 154, 218, 239–241], while at term, funisitis is present in only 6.1%–7.2% and 1.1%–4% of cases with and without labor, respectively [157, 160, 235, 237], and its frequency is higher in patients who have rupture of the membranes [157].

Placental histologic features associated with amniotic fluid infection for the identification of neonates at risk for infection/sepsis

Acute inflammatory histologic features of the placenta are associated with microbial invasion of the amniotic cavity, intra-amniotic inflammation/infection [46, 53, 98, 147–155, 157, 159–162, 165–169, 237, 238, 242–246], and neonatal infection [147–149, 155, 245, 247–256]. A previous study reported that adding placental inflammation (amnionitis) to clinical factors (gestational age, clinical chorioamnionitis, duration of labor, maternal age, race, and parity), which are used in the identification of neonatal infection, decreased the administration of antibiotics from 35% to 10% [257]. Hoang et al. also proposed to use the presence or absence of acute histologic chorioamnionitis to guide the duration of antimicrobial treatment in neonates at risk for sepsis [258].

We report herein that the acute inflammatory histologic features of the placenta could not accurately identify neonates born to mothers with intra-amniotic infection/inflammation, or neonates with FIRS/suspected sepsis. These findings are consistent with previous reports [164, 259]. Cuna et al. reported that the presence of acute histologic chorioamnionitis did not improve the predictive performance of other biomarkers for the identification of early-onset neonatal sepsis [259]. Moreover, in that study, none of the clinically stable term infants with acute histologic chorioamnionitis had early-onset neonatal sepsis, suggesting that isolated acute subchorionitis/histologic chorioamnionitis is not associated with adverse neonatal outcomes or the requirement of antimicrobial treatment [259]. Other investigators have also reported that 38% (53/139) of patients with "clinical chorioamnionitis" did not have histologic evidence of chorioamnionitis [153], and that only 4% of women at term gestation with acute histologic chorioamnionitis were found to have microorganisms in the placenta, despite using both cultivation and molecular microbiologic techniques [164]. These observations, as well as our findings reported herein, indicate that the conventional methods to examine the human placenta with hematoxylin and eosin have limitations in determining whether intra-amniotic infection was present or whether the inflammatory response in the umbilical cord can be attributed to microorganisms.

Acute funisitis is more specific but less sensitive than acute histologic chorioamnionitis for the identification of intra-amniotic infection [154]. This is easy to understand because acute chorioamnionitis merely reflects the presence of inflammatory stimuli in the amniotic cavity, such as microorganisms or danger signals [156, 158, 163,

170–173]. However, if the microorganisms in the amniotic cavity do not invade the human fetus, funisitis should not be present.

Funisitis was reported in 70% of preterm neonates with sepsis [260]. In contrast, in our previous study, which included 832 consecutive patients who delivered within 72 h of amniocentesis, funisitis was present in 17% of patients at term with positive amniotic fluid culture for bacteria [157], and none of these neonates had neonatal sepsis. Therefore, the clinical significance of funisitis in term and preterm gestations is substantially different [157, 239]. We have proposed that microbial invasion of the amniotic cavity during the course of spontaneous labor at term is short-lived, resulting in a more modest intra-amniotic inflammatory response, as well as a lower risk of FIRS, than in preterm gestation [41]. Intra-amniotic infection in patients presenting with an episode of preterm labor is likely to have occurred days or weeks before the initiation of labor and to have eventually led to the initiation of parturition with intact or ruptured membranes [261, 262]. Evidence in support of this is that patients with positive amniotic cultures for microorganisms at the time of genetic amniocentesis in the midtrimester eventually have preterm labor or preterm prelabor rupture of the membranes, which occurs frequently after a period of weeks [261–263]. Prolonged exposure to microorganisms in preterm gestations with subclinical microbial invasion of the amniotic cavity is more likely to result in fetal invasion. This would explain why congenital neonatal sepsis is more frequent in preterm than in term neonates [264, 265]. This view is consistent with our report about the differences in fetal IL-6 umbilical cord concentrations in response to microbial invasion of the amniotic cavity between term and preterm gestations [41].

The most likely explanation for the limitations of placental pathology to identify intra-amniotic infection is that the inflammation of the chorioamniotic membranes is a non-specific mechanism of host defense against danger signals of both microbial and non-microbial origin. We have recently reported that sterile intra-amniotic inflammation is common in patients with clinical chorioamnionitis at term [1], preterm labor with intact membranes [36, 39], preterm prelabor rupture of the membranes [35], and a short cervix [37]. These patients often exhibit evidence of acute chorioamnionitis in the absence of microorganisms in the amniotic fluid. The mechanisms responsible for the acute inflammatory response in the absence of microorganisms remain to be discovered, although we have previously proposed that alarmins could also play a role [39, 54]. The possibility that patients with sterile inflammation may have had an intra-amniotic infection controlled by the host, which leads to eradication of the microorganisms but an unresolved intra-amniotic inflammatory response, cannot be excluded.

In the current study, we focused on examining the relationship between placental histology and FIRS, because it is now evident that many of the complications of infection are mediated by the effects of systemic inflammation rather than the presence of organisms [177–209]. Indeed, patients could have asymptomatic bacteremia, which is transient and does not have adverse outcomes; yet, the combination of bacteria and systemic inflammation can lead to sepsis or septic shock [26–28, 32, 133]. In addition, the presence of FIRS or neonatal systemic inflammation is associated with adverse outcomes, even in the absence of detectable bacteria by cultivation and molecular microbiologic techniques, suggesting that an exaggerated inflammatory response plays a major role in neonatal outcomes [82, 98, 177–209, 266]. Previous studies have also shown a correlation between the concentrations of TNF-α in neonatal blood and the progression of neonatal sepsis to septic shock [267, 268].

Strengths and limitations

The major strength of this study is that both cultivation and molecular microbiologic techniques were used to identify microorganisms in the amniotic cavity; therefore, the diagnosis of microbial invasion was based on state-of-the-art methodologies. However, a larger sample size and replication are desirable. The use of molecular markers to identify the presence of microorganisms in the extrachorionic membranes, chorionic plate, and umbilical cord could be an important approach in providing morphologic evidence of the location of microorganisms in different sites. This could be accomplished using fluorescent *in situ* hybridization with probes aimed at identifying the conserved regions of the prokaryote genome, or alternatively, using stains for bacteria in tissues [161, 269]. Similarly, markers for neutrophil activation can improve the performance of conventional examination of the placenta [270].

Conclusion

Acute histologic chorioamnionitis and funisitis are associated with intra-amniotic infection and the presence of FIRS. Current pathologic methods have limitations in

the identification of the fetus exposed to microorganisms present in the amniotic cavity. The use of molecular methods to identify bacteria in tissues and neutrophil activation may improve the performance of histologic examination of the placenta in the prediction of neonates at risk for sepsis.

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