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Clinical chorioamnionitis at term III: how well do clinical criteria perform in the identification of proven intra-amniotic infection?

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Abstract

Objective: The diagnosis of clinical chorioamnionitis is based on a combination of signs [fever, maternal or fetal tachycardia, foul-smelling amniotic fluid (AF), uterine tenderness and maternal leukocytosis]. Bacterial infections within the amniotic cavity are considered the most frequent cause of clinical chorioamnionitis and an indication for antibiotic administration to reduce maternal and neonatal morbidity. Recent studies show that only 54% of patients with the diagnosis of clinical chorioamnionitis at term have bacteria in the AF and evidence of intra-amniotic inflammation. The objective of this study was to examine the performance of the clinical criteria for the diagnosis of chorioamnionitis to identify patients with microbial-associated intra-amniotic inflammation (also termed intra-amniotic infection).

Materials and methods: This retrospective cross-sectional study included 45 patients with the diagnosis of clinical chorioamnionitis at term, whose AF underwent analysis for: 1) the presence of microorganisms using both cultivation and molecular biologic techniques [polymerase chain reaction (PCR) with broad primers], and 2) interleukin (IL)-6 concentrations by enzyme-linked immunosorbent assay. The diagnostic performance (sensitivity, specificity, accuracy, and likelihood ratios) of each clinical sign and their combination to identify clinical chorioamnionitis were determined using microbial-associated intra-amniotic inflammation [presence of microorganisms in the AF using cultivation or molecular techniques and elevated AF IL-6 concentrations (≥ 2.6 ng/mL)] as the gold standard.

Results: The accuracy of each clinical sign for the identification of microbial-associated intra-amniotic inflammation (intra-amniotic infection) ranged between 46.7% and 57.8%. The combination of fever with three or more clinical criteria did not substantially improve diagnostic accuracy.

Conclusion: In the presence of a fever during labor at term, signs used to diagnose clinical chorioamnionitis do not accurately identify the patient with proven intra-amniotic infection (i.e., those with microorganisms detected

by culture or molecular microbiologic techniques and an associated intra-amniotic inflammatory response).

Keywords: Amniocentesis; fever; foul-smelling amniotic fluid; funisitis; histological chorioamnionitis; intra-amniotic inflammation; maternal leukocytosis; preterm birth; sterile inflammation; tachycardia; uterine tenderness.

Introduction

The term “clinical chorioamnionitis” refers to an entity diagnosed by the presence of fever ($>37.8^{\circ}\text{C}$) and at least two of the following criteria: maternal tachycardia (>100 beats/min), maternal leukocytosis [white blood cell (WBC) count $>15,000$ cells/ mm^3], uterine tenderness, fetal tachycardia (>160 beats/min), and foul-smelling amniotic fluid (AF) [1–10]. More than 35 years ago, Gibbs recognized the challenges in the diagnosis of intra-amniotic infection (microbial-associated intra-amniotic inflammation) as he indicated that the clinical criteria were neither sensitive nor specific [1].

The clinical diagnosis of chorioamnionitis is an indication for antimicrobial administration, given that a randomized clinical trial of patients with this condition near term found that the frequency of neonatal bacteremia was significantly greater in patients who were not given antibiotics before delivery than in those who were treated in the neonatal period. This classic trial by Gibbs et al. is the basis for clinical practice today [6].

Epidural anesthesia and analgesia for labor and delivery have gained wide acceptance, and are used in more than 80% of cases in maternity hospitals [11, 12]. About 10% to 30% of patients who receive epidural analgesia develop a fever [11–29], and the differential diagnosis between clinical chorioamnionitis and an epidural-induced fever is challenging. This has resulted in the increased administration of antibiotics to mothers in labor [17, 18, 21, 30, 31] and their newborns [18, 21, 32, 33], and in the implementation of septic workups in newborns [11, 12, 15, 18, 21, 25, 32, 34, 35]. Recent evidence suggests that the administration of antibiotics has important effects on the microbiome in adults [36–46] and in the neonatal period [37, 40, 43–45, 47–68].

We recently reported, when the AF of patients with clinical chorioamnionitis at term is examined using cultivation and molecular microbiologic techniques, that only 54% of patients have microbial-associated intra-amniotic inflammation in the amniotic cavity [69]. Moreover, 24% of patients have intra-amniotic inflammation without detectable bacteria, and 22% do not have any evidence

of intra-amniotic inflammation [69]. Traditionally, one expects that only patients with bacterial infections may benefit from antibiotic administration; however, our observations suggest that relying on the conventional criteria for the diagnosis of clinical chorioamnionitis may result in over-treatment with antimicrobial agents. Therefore, it is timely that the diagnostic performance and accuracy of clinical signs for the diagnosis of clinical chorioamnionitis be revisited using a gold standard for the identification of microbial-associated intra-amniotic inflammation, which represents intra-amniotic infection.

Materials and methods

This retrospective cross-sectional study included women with the diagnosis of clinical chorioamnionitis at term who underwent transabdominal amniocentesis to identify microorganisms in the amniotic cavity. Patients were identified by searching the clinical database and the Bank of Biological Samples of Wayne State University, the Detroit Medical Center, and the Perinatology Research Branch (NICHD/NIH). The criteria for entry were: 1) singleton gestation; 2) gestational age ≥ 37 weeks; 3) sufficient AF obtained by transabdominal amniocenteses for molecular microbiologic studies; and 4) absence of fetal malformations. A subset of these patients was included in prior studies, which provides a detailed description of sample collection, microbiological studies, and determination of AF IL-6 concentrations [69, 70].

All patients provided written informed consent and the use of biological specimens as well as clinical and ultrasound data for research purposes were approved by the Institutional Review Boards of the NICHD, Wayne State University, and the S tero del R o Hospital, Santiago, Chile.

Clinical definitions

Microbial invasion of the amniotic cavity was defined according to the results of AF culture and PCR/ESI-MS (Ibis[®] Technology – Athogen, Carlsbad, CA, USA) [71–74]. Microbial-associated intra-amniotic inflammation (intra-amniotic infection) was diagnosed when microorganisms were identified in AF using cultivation or molecular techniques and elevated AF IL-6 concentrations (≥ 2.6 ng/mL) were found, as described in detail elsewhere [69, 75–92].

Clinical chorioamnionitis was diagnosed by the presence of maternal fever (temperature $>37.8^{\circ}\text{C}$) accompanied by two more of the following criteria: 1) maternal tachycardia (heart rate >100 beats/min); 2) uterine tenderness; 3) foul-smelling AF; 4) fetal tachycardia (heart rate >160 beats/min); and 5) maternal leukocytosis (leukocyte count $>15,000$ cells/ mm^3) [1–9, 69, 70, 80]. Acute histologic chorioamnionitis was diagnosed based on the presence of inflammatory cells in the chorionic plate and/or in the chorioamniotic membranes [77, 83, 93–102], and acute funisitis was diagnosed by the presence of neutrophils in the wall of the umbilical vessels and/or in the Wharton’s jelly, also using previously reported criteria [93, 103–108]. Fetal inflammatory response syndrome (FIRS) was diagnosed when umbilical cord blood IL-6 concentrations were ≥ 11 pg/mL [72, 105, 109–119].

Statistical analysis

The Kolmogorov-Smirnov test was used to test whether data were normally distributed. A Chi-square or Fisher's exact test was used for comparisons of proportions. Kruskal-Wallis and the Mann-Whitney *U*-tests were used to compare median concentrations of analytes between and among groups. Sensitivity, specificity, accuracy, and likelihood ratios (+/−) were calculated for the identification of microbial-associated intra-amniotic inflammation. Statistical analysis was performed using SPSS 19 (IBM Corp, Armonk, NY, USA). A *P* value <0.05 was considered statistically significant.

Results

Descriptive characteristics

The descriptive characteristics of the study population stratified by the presence or absence of microbial-associated intra-amniotic inflammation or intra-amniotic infection are displayed in Table 1. Fever was a requirement for

the diagnosis of clinical chorioamnionitis. The most frequent clinical signs of chorioamnionitis were maternal tachycardia (91.1%; 41/45), followed by fetal tachycardia (75.6%; 34/45) and maternal leukocytosis (WBCs >15,000 cell/mm³) (73.3%; 33/45). Uterine tenderness and foul-smelling AF were found in <10% of the study population (uterine tenderness: 8.9%, 4/45; foul-smelling AF: 6.7%, 3/45) (Table 1). There were no significant differences in the frequency of each clinical sign between clinical chorioamnionitis with and without microbial-associated intra-amniotic inflammation (*P*>0.05). All patients had an epidural. Amniocenteses were performed before the administration of epidural analgesia in 78% (35/45) of the study participants. All but two of these women received antibiotics, which were administered in most of the cases (88.4%; 38/43) after amniocentesis (Table 1). In three patients, the amniocentesis was performed approximately 5 min after the administration of antibiotics, and in two women, the amniocentesis was performed 45 min after treatment. The information about the microorganisms identified in AF has been published previously [69]. The

Table 1: Characteristics of the study population.

	Clinical chorioamnionitis at term without microbial- associated intra-amniotic inflammation (n=20)	Clinical chorioamnionitis at term with microbial- associated intra- amniotic inflammation (n=25)	P-value
Maternal age (years)	21.5 (18.25–25.75)	19 (18–24)	0.26
Nulliparity	50% (10/20)	76% (19/25)	0.07
Body mass index (kg/m ²)	23.75 (21.68–26.53)	23.5 (21.65–24.55)	0.59
Maternal tachycardia (heart rate >100 beats/min)	95% (19/20)	88% (22/25)	0.41
Fetal tachycardia (heart rate >160 beats/min)	70% (14/20)	80% (20/25)	0.43
Leukocytosis [white blood cell (WBC) count >15,000 cell/mm ³]	70% (14/20)	76% (19/25)	0.65
Foul-smelling AF	5% (1/20)	8% (2/25)	0.69
Uterine tenderness	5% (1/20)	12% (3/25)	0.41
≥3 criteria	45% (9/20)	56% (14/25)	0.46
≥4 criteria	0% (0/20)	8% (2/25)	0.20
AF WBC count (cell/mm ³)	5 (0–37.75)	300 (39–900)	<0.001
AF glucose (mg/dL)	9 (9–11.5)	9 (7–9)	0.01
AF IL-6 (ng/mL)	2.53 (0.91–4.40)	14.12 (5.73–36.85)	<0.001
AF IL-6 ≥2.6 ng/mL	50% (10/20)	100% (25/25)	<0.001
Maternal blood IL-6 (ng/mL)	2.54 (0.91–4.40)	0.009 (0.005–0.046)	0.92
Umbilical cord blood IL-6 (pg/mL)	2.70 (2.09–4.95)	6.52 (2.53–23.23)	0.03
Gestational age at delivery (weeks)	39.25 (38.38–39.85)	41.1 (39.6–41.1)	0.002
Gestational age at amniocentesis (weeks)	39.25 (38.19–39.83)	40.6 (39.6–41.1)	0.002
Birthweight (g)	3385 (3035–3755)	3570 (3375–3800)	0.18
Neonatal sepsis	10% (2/20)	16% (4/25)	0.56
Fetal inflammatory response syndrome	5% (1/20)	36% (9/25)	0.03
Acute inflammatory lesions of the placenta ^a	25% (5/20)	70.8% (17/24) ^b	0.02

Data presented as median (interquartile) or % (n); AF=Amniotic fluid, IL=Interleukin.

^aAcute inflammatory lesions of placenta: acute histologic chorioamnionitis and/or acute funisitis.

^bPlacental pathology report was not available for one patient.

most frequent microorganisms were *Ureaplasma* spp. and *Gardnerella vaginalis* [69].

Patients with clinical chorioamnionitis at term with microbial-associated intra-amniotic inflammation had a significantly higher median AF WBC count, AF IL-6, and umbilical cord blood IL-6 concentration than those without microbial-associated intra-amniotic inflammation ($P < 0.001$, $P < 0.001$, and $P = 0.03$, respectively). 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% vs. 5%; $P = 0.03$, and acute inflammatory lesions of placenta: 70.8% vs. 25%; $P = 0.02$) (Table 1).

Diagnostic performance

The performance of criteria for the diagnosis of clinical chorioamnionitis in the identification of microbial-associated intra-amniotic inflammation is shown in Table 2. The sensitivity of maternal and fetal tachycardia and maternal leukocytosis ranged from 75% to 90%; however, the specificity was poor for these criteria, ranging from 0% to 30%. In contrast, foul-smelling AF and uterine tenderness had a high specificity (95%) but a low sensitivity (8% and 12%, respectively) for the identification of microbial-associated intra-amniotic inflammation. Altogether, the diagnostic accuracy for each clinical criterion ranged between 46.7% and 57.8%. The combination of fever with three or more clinical criteria did not further improve the diagnostic accuracy for the identification of microbial-associated intra-amniotic inflammation (Table 2).

Table 3 shows the diagnostic indices for the identification of intra-amniotic inflammation, regardless of the presence or absence of microorganisms detected by cultivation or molecular microbiologic techniques.

Discussion

Criteria for the diagnosis of clinical chorioamnionitis have considerable limitations if the goal is to identify the patient with bacterial-associated intra-amniotic inflammation. The standard diagnostic criteria of clinical chorioamnionitis include fever and two or more of the following: maternal and fetal tachycardia, uterine tenderness, foul-smelling AF, and maternal leukocytosis [1–10]. The rationale for the precise cut-off used to define fever and maternal and fetal tachycardia was discussed in detail by Newton

Table 2: The diagnostic performance of clinical criteria for the identification of microbial-associated intra-amniotic inflammation (intra-amniotic infection) in patients with clinical chorioamnionitis at term.

Clinical signs	Sensitivity (%)		Specificity (%)		Positive likelihood ratio (95% CI)	Negative likelihood ratio (95% CI)	Accuracy (%)
	% (n)	95% CI	% (n)	95% CI			
Maternal tachycardia (heart rate > 100 beats/min)	88.0 (22/25)	(68.75–97.31)	5.0 (1/20)	(0.83–24.95)	0.93 (0.78–1.10)	2.40 (0.27–21.35)	51.1 (23/45)
Fetal tachycardia (heart rate > 160 beats/min)	80.0 (20/25)	(59.29–93.09)	30 (6/20)	(11.97–54.27)	1.14 (0.81–1.62)	0.67 (0.24–1.87)	57.8 (26/45)
WBC count > 15,000	76 (19/25)	(54.87–90.58)	30 (6/20)	(11.97–54.27)	1.09 (0.76–1.56)	0.80 (0.30–2.10)	55.6 (25/45)
Foul-smelling AF	8.00 (2/25)	(1.22–26.07)	95 (19/20)	(75.05–99.17)	1.6 (0.16–16.40)	0.97 (0.83–1.13)	46.7 (21/45)
Uterine tenderness	12.0 (3/25)	(2.69–31.25)	95 (19/20)	(75.05–99.17)	2.40 (0.27–21.35)	0.93 (0.78–1.10)	48.9 (22/45)
≥ 3 criteria	56.00 (14/25)	(34.94–75.57)	55 (11/20)	(31.55–76.90)	1.24 (0.69–2.26)	0.80 (0.44–1.45)	55.6 (25/45)
≥ 4 criteria	8.00 (2/25)	(1.22–26.07)	100 (20/20)	(83.01–100)	0.92 (0.82–1.03)	–	48.9 (22/45)

CI = Confidence interval, AF = amniotic fluid.

Microbial-associated intra-amniotic inflammation: presence of microorganisms in AF and intra-amniotic inflammation [amniotic fluid interleukin-6 ≥ 2.6 mg/dL; $n = 25/45$ (55.56%)].

Table 3: The diagnostic performance of clinical signs for the identification of intra-amniotic inflammation in patients with clinical chorioamnionitis at term.

Clinical signs	Sensitivity (%)		Specificity (%)		Positive likelihood ratio (95% CI)	Negative likelihood ratio (95% CI)	Accuracy (%)
	% (n)	95% CI	% (n)	95% CI			
Maternal tachycardia (heart rate > 100 beats/min)	88.57 (31/35)	(73.24–96.73)	0 (0/10)	(0–31.03)	0.89 (0.79–1.00)	–	68.8 (31/45)
Fetal tachycardia (heart rate > 160 beats/min)	77.14 (27/35)	(59.86–89.55)	30 (3/10)	(7.03–65.16)	1.10 (0.71–1.72)	0.76 (0.25–2.35)	66.67 (30/45)
WBC count > 15,000	74.30 (26/35)	(56.74–87.48)	30 (3/10)	(7.03–65.16)	1.06 (0.68–1.66)	0.86 (0.28–2.58)	64.4 (29/45)
Foul-smelling AF	5.71 (2/35)	(0.87–19.19)	90.0 (9/10)	(55.46–98.34)	0.57 (0.06–5.67)	1.05 (0.84–1.31)	24.44 (11/45)
Uterine tenderness	8.57 (3/35)	(1.90–23.08)	90.0 (9/10)	(55.46–98.34)	0.86 (0.10–7.37)	1.02 (0.81–1.28)	26.67 (12/45)
≥ 3 criteria	48.57 (17/35)	(31.39–66.00)	40.0 (4/10)	(12.40–73.63)	0.81 (0.44–1.49)	1.29 (0.56–2.93)	46.67 (21/45)
≥ 4 criteria	5.71 (2/35)	(0.87–19.19)	100 (10/10)	(68.97–100)	–	0.94 (0.87–1.02)	26.67 (12/45)

CI=Confidence interval, AF=amniotic fluid.

Intra-amniotic inflammation (includes both microbial-associated intra-amniotic inflammation and intra-amniotic inflammation without demonstrable microorganisms): amniotic fluid interleukin-6 ≥ 2.6 ng/mL; n = 35/45 (77.78%).

[8], who articulated that the thresholds for maternal and fetal tachycardia are the 90th percentile [8], and for the WBC count, the 80th percentile [120].

Our findings indicate that clinical signs of chorioamnionitis do not accurately identify patients with microbial-associated intra-amniotic inflammation or intra-amniotic infection. Maternal and fetal tachycardia, as well as maternal leukocytosis, had low specificity (5%–30%), whereas foul-smelling AF and uterine tenderness had poor sensitivity (<15%) for the diagnosis of microbial-associated intra-amniotic inflammation. Our observations are consistent with those of a prior study which demonstrated that fever and maternal and fetal tachycardia were not reliable for the identification of acute histologic chorioamnionitis [121]. Several investigators have shown that the majority of women with acute inflammatory placental lesions do not have microorganisms detectable using either cultivation or molecular microbiologic techniques in the chorioamniotic membranes [122–129]. Histologic chorioamnionitis is more sensitive than clinical chorioamnionitis in the identification of patients with a positive AF culture for microorganisms [130].

The diagnosis of intra-amniotic infection in this study was based on the combination of the presence of microorganisms identified by cultivation or molecular microbiologic methods and intra-amniotic inflammation as a gold standard [71–74, 83–86, 88, 89, 131]. We previously reported that intra-amniotic inflammation with detectable microorganisms was associated with acute histologic chorioamnionitis and funisitis [69, 83–86] and elevated inflammation-related protein concentrations in the AF [70, 83–86].

Efforts to improve the identification of patients with proven intra-amniotic infection are worthwhile because a high rate of false-positive diagnoses has clinical and financial implications. Often, mothers with a fever during labor are given antibiotics [17, 18, 21, 30, 31] – such intervention renders the results of neonatal cultures less reliable, and this frequently results in antibiotic administration in the neonatal period [18, 21, 32, 33], increased septic workups [11, 12, 15, 18, 21, 25, 32, 34, 35], and separation of the neonates from the parents while antibiotic treatment takes place in the nursery [132–135].

We propose that analysis of AF obtained using a transcervical AF collector may facilitate the rapid diagnosis of patients with intra-amniotic inflammation from those who do not have an inflammatory process [136]. Further studies are required to determine if such an approach may reduce the utilization of antibiotics in both patients in labor and in the neonatal period.

The major strength of this study is that both cultivation and molecular microbiologic techniques were used

to identify microorganisms in the amniotic cavity collected by transabdominal amniocentesis – therefore, the diagnosis of microbial invasion is based on a gold standard. Most work in clinical chorioamnionitis has been based on a case definition which relies heavily on clinical signs. The shortcomings of such an approach have become clear, now that sterile inflammation has emerged as an important entity in patients at term [137] as well as those in preterm labor [83, 86], those with preterm pre-labor rupture of the membranes [84], or those with an asymptomatic sonographic short cervix [85]. Non-microbial-associated inflammation appears to account for a sizable segment of patients with clinical chorioamnionitis at term [69, 80].

Conclusion

Criteria used for the diagnosis of clinical chorioamnionitis at term do not accurately identify the subset of patients with intra-amniotic infection or bacterial-associated intra-amniotic inflammation. Further work is required to explore whether such diagnosis is possible by using AF obtained with a transcervical AF collector [136].

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