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ORIGINAL ARTICLE

A rapid interleukin-6 bedside test for the identification of intra-amniotic inflammation in preterm labor with intact membranes

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Abstract

Objective: Preterm birth is associated with 5–18% of pregnancies and is the leading cause of neonatal morbidity and mortality. Amniotic fluid (AF) interleukin-6 (IL-6) is a key cytokine for the identification of intra-amniotic inflammation, and patients with an elevated AF IL-6 are at risk for impending preterm delivery. However, results of the conventional method of measurement (enzyme-linked immunosorbent assay; ELISA) are usually not available in time to inform care. The objective of this study was to determine whether a point of care (POC) test or lateral-flow-based immunoassay for measurement of AF IL-6 concentrations can identify patients with intra-amniotic inflammation and/or infection and those destined to deliver spontaneously before term among women with preterm labor and intact membranes.

Methods: One-hundred thirty-six women with singleton pregnancies who presented with symptoms of preterm labor and underwent amniocentesis were included in this study. Amniocentesis was performed at the time of diagnosis of preterm labor. AF Gram stain and AF white blood cell counts were determined. Microbial invasion of the amniotic cavity (MIAC) was defined according to the results of AF culture (aerobic and anaerobic as well as genital mycoplasmas). AF IL-6 concentrations were determined by both lateral flow-based immunoassay and ELISA. The primary outcome was intra-amniotic inflammation, defined as AF ELISA IL-6 $\geq$ 2600 pg/ml.

Results: (1) AF IL-6 concentrations determined by a POC test have high sensitivity (93%), specificity (91%) and a positive likelihood ratio of 10 for the identification of intra-amniotic inflammation by using a threshold of 745 pg/ml; (2) the POC test and ELISA for IL-6 perform similarly in the identification of MIAC, acute inflammatory lesions of placenta and patients at risk of impending spontaneous preterm delivery.

Conclusion: A POC AF IL-6 test can identify intra-amniotic inflammation in women who present with preterm labor and intact membranes and those who will subsequently deliver spontaneously before 34 weeks of gestation. Results can be available within 20 min – this has important clinical implications and opens avenues for early diagnosis as well as treatment of intra-amniotic inflammation/infection.

Keywords

Acute chorioamnionitis, acute funisitis, biomarkers, ELISA, microbial invasion of the amniotic cavity, prematurity, preterm birth, point of care test

History

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Introduction

Preterm birth affects 5–18% of pregnancies [1–7] and is the leading cause of neonatal morbidity and mortality [8–15]. One of every four women who deliver preterm has an intra-amniotic infection that is largely subclinical [16–48]. Microbial-associated preterm labor is mediated by inflammatory processes that involve the production of cytokines such as interleukin (IL)-1 [49–56], IL-6 [32,53,54,56–74], IL-10 [75,76], tumor necrosis factor-alpha (TNF-α) [53,74,77–80], chemokines [53,54,66,67,81–95], matrix-degrading enzymes [96–106] and other inflammatory-related proteins [56,107–124], which activate the common pathway of parturition [1,3,125–127]. Multiple studies have shown that

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amniotic fluid (AF) IL-6 concentrations are superior to AF white blood cell (WBC) counts, glucose, Gram stain or equivalent to proteomic markers in identifying intra-amniotic infection and microbial invasion of the amniotic cavity (MIAC) [58,60,71,128–132]. Moreover, even in the absence of demonstrable microorganisms in the amniotic cavity, an elevated AF IL-6 concentrations is associated with an increased risk of adverse pregnancy and neonatal outcomes in the context of preterm labor [46,72,133–136], preterm prelabor rupture of the membranes (preterm PROM) [137,138] and a short cervix [139]. Thus, AF IL-6 concentrations have both diagnostic and prognostic value.

Currently, it usually takes hours to determine AF IL-6 concentrations, and the results are often unavailable in time to inform clinical decisions. A point of care (POC) test (lateral flow-based immunoassay) has been widely used in the settings of adult [140] and neonatal sepsis [141,142], as well as for other inflammation-related conditions [143]. It was not until recently that such tests were used in obstetrics. In a pilot study, our group showed that AF IL-6 concentrations determined using a POC test were strongly correlated with those measured by conventional enzyme-linked immunosorbent assay (ELISA) (Spearman's $\rho = 0.92$) [144]. Moreover, POC IL-6 test results can identify patients with preterm PROM who are destined to deliver preterm and/or have acute histological chorioamnionitis [145–147].

In this study, we examined whether AF IL-6 concentrations, determined by a POC test, can identify patients with preterm labor with intact membranes who have intra-amniotic inflammation and/or infection, and/or deliver spontaneously before term, relative to the performance of concentrations determined by conventional ELISA.

Material and methods

Study population

A retrospective cohort study was conducted by searching the clinical database and bank of biological samples of Wayne State University, the Detroit Medical Center and the Perinatology Research Branch of the *Eunice Kennedy Shriver* National Institutes of Child Health and Human Development (NICHD; Detroit, MI) to identify patients with a diagnosis of spontaneous preterm labor with intact membranes. Patients were included if they met the following criteria: (1) singleton gestation; (2) trans-abdominal amniocentesis performed between 20 and 35 weeks of gestation with microbiological studies; (3) available AF for the performance of microbiologic studies; and (4) neonatal outcomes were known. Patients were excluded from this study if they had placenta previa or if their fetus had a chromosomal or structural anomaly.

Patients with the diagnosis of preterm labor with intact membranes were counseled by their treating physicians about the potential value of identifying microorganisms in AF. Women who agreed to undergo an amniocentesis were asked to donate additional AF other than that required for clinical studies, and to allow collection of clinical information for research purposes. Further management of these patients was at the discretion of the attending physician. All patients provided written informed consent, and the use of biological specimens and clinical data for research purposes were approved by the Institutional Review Boards of NICHD and Wayne State University.

Biological samples and analysis

AF was transported in a capped sterile syringe to the clinical laboratory where it was cultured for aerobic and anaerobic bacteria, including genital mycoplasmas. AF not required for clinical assessment was centrifuged for 10 min at 4 °C and stored at −70 °C until analysis. Evaluation of WBC count, glucose concentration and Gram stain of AF were also performed shortly after collection. The presence of intra-amniotic infection/inflammation was assessed by determination of AF IL-6 concentration by ELISA.

Clinical definitions

Preterm labor was diagnosed by the presence of at least two regular uterine contractions every 10 min associated with cervical changes in patients with a gestational age between 20 and 36 6/7 weeks. Acute histologic chorioamnionitis was diagnosed according to previously described criteria [148,149]. Funisitis was diagnosed when neutrophil infiltration was detected into the umbilical vessel walls or Wharton's jelly using previously reported criteria [150–152]. Intra-amniotic inflammation was diagnosed when the AF IL-6 concentration was $\geq$2600 pg/ml ($\geq$2.6 ng/ml), as determined by ELISA [46,87,117,153]. MIAC was defined according to the results of AF culture. Intra-amniotic infection was defined as a combination of MIAC with intra-amniotic inflammation.

Analysis of AF samples for IL-6 concentrations

AF IL-6 concentrations (pg/ml) were determined both by ELISA and the lateral flow-based immunoassay POC test. For ELISA, AF IL-6 concentrations were determined by immunoassays obtained from R&D Systems (Minneapolis, MN). The POC determination of AF IL-6 concentrations (pg/ml) was performed using a lateral flow-based immunoassay POC test (Milenia QuickLine® IL-6; Milenia Biotec, Bad Nauheim, Germany). The details and performance of ELISA [37,46,53,57,60,153–157] and POC immunoassays have been previously described [144]. The IL-6 POC test inter- and intra-assay coefficients of variations are 15.5% and 12.1%, respectively.

Study outcomes

The primary outcomes in this study are intra-amniotic inflammation and positive AF culture. Secondary outcomes include the occurrence of spontaneous preterm delivery (within 24 h, 48 d and 7 d of admission), spontaneous preterm delivery (<28 and <34 weeks of gestation) and the presence of placental lesions consistent with acute inflammation (acute histologic chorioamnionitis and/or acute funisitis). The relationships between acute histologic chorioamnionitis and AF IL-6 concentrations were examined in 55 patients who delivered within three days of amniocentesis. This interval was chosen to preserve a meaningful temporal relationship between the results of amniocentesis and placental pathology.

Table 1. Clinical characteristics of the study population.

Characteristic	Median (interquartile range) or percent ($n = 136$)
Maternal age (years)	24 (20–29)
Nulliparity	33.8% (46/136)
Prior preterm delivery	37.5% (51/136)
Gestational age at amniocentesis (weeks)	30.9 (27–32.4)
Amniotic fluid glucose (mg/dl)	24 (17–30.8)
Amniotic fluid white blood cell (cell/m^3)	1.5 (0–13)
Microbial invasion of the amniotic cavity (%)	16.2% (22/136)
Intra-amniotic inflammation (ELISA IL-6 $\geq$ 2600 pg/ml) (%)	44.1% (60/136)
Gestational age at delivery (weeks)	33.3 (28.2–36.9)
Interval from amniocentesis to delivery (d)	8 (1–36.8)
Spontaneous deliver within one day after amniocentesis (%)	33.8% (46/136)
Spontaneous deliver within two days after amniocentesis (%)	43.4% (59/136)
Spontaneous deliver within seven days after amniocentesis (%)	47.8% (65/136)
Spontaneous delivery at <28 weeks of gestation (%)	22.8% (31/136)
Spontaneous delivery at <34 weeks of gestation (%)	54.4% (74/136)
Spontaneous delivery at <37 weeks of gestation (%)	64% (87/136)
Acute histologic chorioamnionitis (%)*	57.4% (31/54)
Acute funisitis (%)*	38.9% (21/54)
Acute inflammatory lesions of placenta (%)*	57.4% (31/54)

Data presented as median (interquartile range) or % (*n*).
Acute inflammatory lesions of placenta include acute histologic chorioamnionitis and acute funisitis.
*Included only patients who had interval from amniocentesis to delivery <3 d ($n = 59$). Among these patients, placenta histology reports were not available in four patients, and placental histopathology reports were not available in 13/136 patients.

Statistical analysis

The Kolmogorov–Smirnov test was used to assess normality of arithmetic data distributions. The Kruskal–Wallis and Mann–Whitney *U* tests were used to make comparisons among and between groups for arithmetic variables. Chi-square or Fisher's exact test were used for comparisons of categorical variables. Statistical analysis was performed using SPSS 19 (IBM Corp, Armonk, NY) and SAS 9.4 (Cary, NC). A p value <0.05 was considered statistically significant.

Results

Characteristics of the study population

A total of 136 women with preterm labor with intact membranes were included in this study. Their clinical characteristics are listed in Table 1. The prevalence of MIAC and intra-amniotic inflammation was 16.2% (22/136) and 44.1% (60/136), respectively. Most of the participants had spontaneous preterm deliveries, specifically 22.8% (31/136) at <28 weeks, 54% (74/136) at <34 weeks and 64% (87/136) at <37 weeks. The rates of spontaneous preterm delivery within 24 h, 48 h and 7 d were 33.8% (46/136), 43.4% (59/136) and 47.8% (65/136), respectively. The median (interquartile range) gestational age at amniocentesis was 30.9 (27–32.4) weeks. Of the 54 women who delivered within three days of the amniocentesis, and had placenta pathologic reports, 57.4% (31/54) had acute histologic chorioamnionitis, and most of their offspring were diagnosed with funisitis [67.7% (21/31)].

Table 2 lists the microorganisms identified by AF culture, gestational age at delivery, concentrations of IL-6 (by ELISA and POC test), AF inflammatory response and the type or absence of placental lesions consistent with acute inflammation in women with MIAC. The most frequent microorganism identified was *Ureaplasma urealyticum*, which was identified in 18% (4/22) of these women.

The diagnostic performance of an AF IL-6 POC test for the identification of intra-amniotic inflammation

Upon inspecting a receiver operating characteristic curve for the identification of intra-amniotic inflammation [area under curve = 0.94 (0.90–0.99)], a threshold of $\geq$745 pg/ml was selected for the POC test (Figure 1). Table 3 lists, at this cut-off, the POC test had a sensitivity of 93% and a specificity of 91%.

Of seven women with negative ELISA AF IL-6 tests who had positive POC test results, six (85%) delivered within two days of amniocentesis at <34 weeks of gestation. Two of these women also had positive AF cultures: one had *Streptococcus* spp./*Gemella morbillorum* in addition to placental lesions consistent with acute inflammation; the other had Gram-negative bacilli, but placental histopathology was not available. On the other hand, four patients had positive ELISA AF IL-6 test results, yet the results of the POC test were negative. Two of these four patients delivered at term, none had a positive AF culture, and one had an AF IL-6 concentration above the cutoff used to define intra-amniotic inflammation (2609 pg/ml; determined by ELISA). This patient did not have placental lesions consistent with acute inflammation.

The diagnostic performance of an AF IL-6 POC test for the identification of MIAC and acute inflammatory lesions of placenta

Table 3 lists the performance of the POC test in identifying patients with MIAC and those with placental lesions consistent with acute inflammation was equivalent to that of conventional ELISA. Among patients with MIAC, 18.2% (4/22) had negative ELISA IL-6 results (Table 2). However, POC AF IL-6 was elevated in two of these four patients. One

Table 2. Clinical characteristics, amniotic fluid inflammatory response and acute inflammatory placental lesions in patients with microbial invasion of the amniotic cavity using cultivation techniques.

No.	Organisms	GA at delivery (weeks)	AF glucose (mg/dl)	AF WBC (cell/mm^3)	ELISA IL-6 (pg/ml)	Point of care IL-6 (pg/ml)	Acute histological chorioamnionitis	Acute funisitis
1.	*Prevotella* spp., *Enterococcus faecalis*	25^{+1}	10	1	52 637	3208	No	No
2.	*Mycoplasma hominis*	33	1	420	172 301	4613	N/A	N/A
3.	*Ureaplasma urealyticum*	28^{+6}	13	180	9433	10 000	Acute chorioamnionitis	No
4.	*Candida albicans*	26^{+3}	10	2160	201 261	4448	Necrotizing chorioamnionitis	No
5.	*Streptococcus agalactiae*	25	10	4	93 638	3575	Necrotizing chorioamnionitis	No
6.	*Candida albicans*, *Lactobacillus* spp.	33^{+1}	10	43	200 626	3554	Acute subchorionitis	No
7.	*Haemophilus influenza*	30^{+6}	10	40	92 063	6467	Necrotizing chorioamnionitis	Necrotizing funisitis
8.	*Fusobacterium* spp., Gram-negative bacilli	21^{+6}	19	1564	317 655	6228	Subchorionic microabscesses	Necrotizing funisitis
9.	Gram-negative bacilli	21^{+1}	20	66	242 699	5934	Subacute chorioamnionitis	Umbilical arteritis
10.	*Streptococcus agalactiae*	25^{+2}	19	5	248 889	8208	Acute chorioamnionitis	Umbilical arteritis
11.	Gram-positive cocci	22^{+5}	10	125	470 626	5540	Necrotizing chorioamnionitis	Umbilical arteritis
12.	*Fusobacterium* spp., Gram-negative bacilli	28^{+1}	1	22	301 426	3996	Acute chorioamnionitis	Umbilical arteritis
13.	*Ureaplasma urealyticum*	33	10	500	85 962	4628	Acute chorioamnionitis	Umbilical phlebitis/chorionic vasculitis
14.	*Streptococcus anginosus*, *Streptococcus mitis*	22^{+6}	10	10	73 254	7246	Acute chorioamnionitis	Umbilical phlebitis/chorionic vasculitis
15.	*Bacteroides* spp., *Mobiluncus* spp., *Clostridium sporogenes*	22^{+4}	10	295	517 846	4748	Necrotizing chorioamnionitis	Umbilical phlebitis/chorionic vasculitis
16.	*Ureaplasma urealyticum*	39^{+2}	19	2	1274	155	No	No
17.	Gram-negative bacilli	26^{+5}	N/A	610	1779	10 000	N/A	N/A
18.	*Streptococcus* spp., *Gemella morbillorum*	31^{+6}	10	1920	741	6796	Necrotizing chorioamnionitis	Umbilical arteritis
19.	*Candida albicans*	32^{+4}	10	1292	96 334	4252	Acute chorioamnionitis	Necrotizing funisitis
20.	*Staphylococcus capitis*	28^{+6}	20	24	360 503	4374	Subacute chorioamnionitis	Umbilical arteritis
21.	*Mobiluncus* spp.	32	10	570	76 888	8144	Acute chorioamnionitis	Umbilical phlebitis/chorionic vasculitis
22.	*Ureaplasma urealyticum*	34^{+3}	24	0	244	60	Acute subchorionitis	No

N/A: results were not available; WBC, white blood cell count; AF, amniotic fluid; acute subchorionitis/chorionitis = acute histologic chorioamnionitis stage 1; acute chorioamnionitis = acute histologic chorioamnionitis stage 2; necrotizing chorioamnionitis and subacute chorioamnionitis = acute histologic chorioamnionitis stage 3; subchorionic microabscesses = severe acute histologic chorioamnionitis; umbilical phlebitis/chorionic vasculitis = acute funisitis stage 1; umbilical arteritis = acute funisitis stage 2; necrotizing funisitis = acute funisitis stage 3.

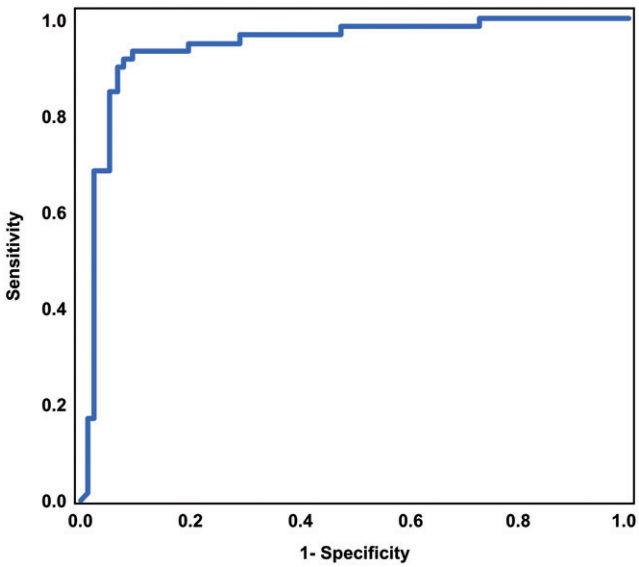

Figure 1. Receiver operating characteristic curve that describes the performance of point of care test amniotic fluid interleukin-6 in the identification of intra-amniotic inflammation (determined by ELISA IL-6 $\geq$ 2600 pg/mL) (area under the curve for amniotic fluid IL-6 point of care test = 0.94; 95% confidence interval: 0.90–0.99, $p < 0.001$).

of these patients had acute histologic chorioamnionitis and funisitis; therefore, this implied true intra-amniotic infection. The placental pathology report was not available for the other patient.

Of note, one of the two patients with MIAC who had negative AF IL-6 test results for both assays (POC and ELISA) delivered at term and did not have placental lesions consistent with acute inflammation, suggesting the possibility of contamination [i.e. false-positive AF culture (*U. urealyticum*)].

The diagnostic performance of an AF IL-6 POC test for the identification of impending preterm delivery

Table 4 lists the performance of the POC and ELISA AF IL-6 tests in identifying women who had spontaneous preterm deliveries. Both tests had equivalent positive likelihood ratios in identifying patients who had a spontaneous preterm delivery within one day of amniocentesis, or those who would subsequently deliver spontaneously at <28 weeks of gestation. Sensitivity and specificity were each marginally higher for the POC test than for the ELISA in identifying women who would deliver spontaneously within either two or seven days of amniocentesis. In contrast, sensitivity was slightly higher, whereas specificity was slightly lower, when comparing the performance of the POC test to that of the ELISA for the identification of spontaneous preterm delivery at <34 weeks of gestation. Yet, confidence intervals for estimates characterizing the diagnostic performance of the POC test overlapped with those of the ELISA test, indicating statistically equivalent performance in assessing the risk of spontaneous preterm delivery.

Discussion

Principal findings of the study: (1) AF IL-6 concentrations determined by a POC test have high sensitivity (93%) and specificity (91%) for the identification of intra-amniotic inflammation, by using a threshold of 745 pg/ml and (2) the POC test and ELISA for IL-6 determination perform similarly in identifying patients with MIAC, acute inflammatory lesions of placenta and risk of impending spontaneous preterm delivery in patients with preterm labor with intact membranes.

AF IL-6 POC test for the identification of intra-amniotic inflammation and impending preterm delivery

Compelling evidence indicates that patients with intra-amniotic inflammation are at greater risk for impending preterm delivery and other adverse outcomes, even without identifiable microorganisms [46,72,133–139,158]. We have previously demonstrated that sterile intra-amniotic inflammation (inflammatory process in which microorganisms are neither detected by cultivation nor molecular methods) is more common than microbial-associated intra-amniotic inflammation in patients with preterm labor and intact membranes [72,134], asymptomatic sonographic short cervix [139] and preterm PROM [138]. Moreover, we have shown that sterile intra-amniotic inflammation is associated with adverse pregnancy outcomes; hence the importance of identifying patients with intra-amniotic inflammation [72,134,139].

In this study, we have demonstrated that a POC AF IL-6 test has high sensitivity and specificity in the identification of intra-amniotic inflammation and spontaneous preterm delivery. While its performance in identifying infection/inflammation-related obstetrical outcomes was comparable to that of AF IL-6 concentrations determined by ELISA, the POC assay can yield results within 20 min. Hence, unlike conventional ELISA, POC AF IL-6 results can be available in time to inform clinical decisions, similar to a rapid matrix metalloproteinase-8 (MMP-8) test, which has been shown to identify intra-amniotic infection/inflammation in patients with preterm labor and intact membranes with >80% sensitivity and >90% specificity [103]. Furthermore, the MMP-8 test was found to be useful in the identification of intra-amniotic inflammation in patients with preterm PROM [104], MIAC in patients at risk for preterm delivery [159] and funisitis in patients with preterm delivery [160].

It is interesting that six of seven patients with positive POC test and negative ELISA results had early spontaneous preterm deliveries (<34 weeks) within two days of amniocentesis. This suggests that the POC test contributes additional risk information beyond that provided by conventional ELISA. It is further noteworthy that two of the four patients with negative POC and positive ELISA test results delivered at term, one of them did not have placental lesions consistent with acute inflammation, and this patient had a positive ELISA result (2609 pg/ml).

In a prior study, we showed that AF IL-6 concentrations determined by a POC test were on an average 30% lower than that determined by conventional ELISA. Thus, it is not surprising that a lower AF IL-6 cut-off was selected for the POC to identify patients with intra-amniotic inflammation in this study ($\geq$745 pg/ml). Kacerovsky et al. proposed a higher cut-off (1000 pg/ml) in a study using the same assay to

Table 3. Diagnostic performance of point of care AF IL-6 concentrations and ELISA AF IL-6 for identification of intra-amniotic infection and/or inflammation and placental lesions consistent with acute inflammation.

		Point of care IL-6 test (cut-off 745 pg/ml)		ELISA IL-6 (cut-off 2600 pg/ml)	
Outcomes	Diagnostic performance	% (*n*)	95% CI	% (*n*)	95% CI
Intra-amniotic inflammation [44.1% (60/136)]	Sensitivity	93.3 (56/60)	(83.8–98)	100% diagnostic performance. ELISA IL-6 is a gold standard test for the identification of intra-amniotic inflammation	
	Specificity	90.8 (69/76)	(82–96)		
	Positive predictive value	88.9 (56/63)	(78.4–95.4)		
	Negative predictive value	94.5 (69/73)	(86.6–98.5)		
	Positive likelihood ratio	10.1 (93.3/9.2)	(5–21)		
	Negative likelihood ratio	0.07 (6.7/90.8)	(0.03–0.2)		
Microbial invasion of the amniotic cavity (MIAC) identified by culture [16.3% (22/136)]	Sensitivity	90.9 (20/22)	(70.8–98.6)	81.8 (18/22)	(59.7–94.7)
	Specificity	62.3 (70/114)	(52.7–71.2)	63.2 (71/114)	(53.6–72.0)
	Positive predictive value	31.8 (20/63)	(20.6–44.7)	30.0 (18/60)	(18.9–43.2)
	Negative predictive value	97.3 (71/73)	(90.4–99.6)	94.7 (71/76)	(87.1–98.5)
	Positive likelihood ratio	2.41 (90.9/37.7)	(1.8–3.2)	2.2 (81.8/36.8)	(1.6–3.0)
	Negative likelihood ratio	0.15 (9.1/62.3)	(0.04–0.6)	0.3 (18.2/63.2)	(0.1–0.7)
Acute inflammatory lesions of placenta (chorioamnionitis or funisitis) [only patients who delivered within three days after amniocentesis were included [57.4% (31/54)]]	Sensitivity	93.6 (29/31)	(78.5–99)	93.6 (29/31)	(78.5–99.0)
	Specificity	43.5 (10/23)	(23.2–65.5)	52.2 (12/23)	(30.6–73.2)
	Positive predictive value	69.1(29/42)	(52.9–82.3)	72.5 (29/40)	(56.1–85.4)
	Negative predictive value	83.3 (10/12)	(51.6–97.4)	85.7 (12/14)	(57.2–97.8)
	Positive likelihood ratio	1.7 (93.6/56.5)	(1.1–2.4)	2.0 (93.6/47.8)	(1.3–3.03)
	Negative likelihood ratio	0.15 (6.4/43.5)	(0.04–0.6)	0.12 (6.4/52.2)	(0.03–0.5)
Acute funisitis [only patients who delivered within three days after amniocentesis were included [38.9% (21/54)]]	Sensitivity	100 (21/21)	(83.8–100)	95.2 (20/21)	(76.1–99.2)
	Specificity	36.4 (12/33)	(20.4–54.9)	39.4 (13/33)	(22.9–57.9)
	Positive predictive value	50.0 (21/42)	(34.2–65.8)	50.0 (20/40)	(33.8–66.2)
	Negative predictive value	100.0 (12/12)	(73.4–100)	92.9 (13/14)	(66.1–98.8)
	Positive likelihood ratio	1.57 (100/63.6)	(1.2–2)	1.6 (95.2/60.6)	(1.2–2.1)
	Negative likelihood ratio	0	–	0.12 (4.8/39.4)	(0.02–0.8)

CI: confidence interval.

Table 4. Diagnostic performance of point of care AF IL-6 concentrations and ELISA AF IL-6 for identification of patients with spontaneous preterm delivery.

		Point of care IL-6 test (cut-off 745 pg/ml)		ELISA IL-6 (cut-off 2600 pg/ml)	
Outcomes	Diagnostic performance	% (*n*)	95% CI	% (*n*)	95% CI
Spontaneous preterm delivery within one day after amniocentesis [33.8% (46/136)]	Sensitivity	84.8 (39/46)	(71.1–93.6)	78.3 (36/46)	(63.6–89.0)
	Specificity	73.3 (66/90)	(63–82)	73.3 (66/90)	(63–82.1)
	Positive predictive value	62 (39/63)	(48.8–73.9)	60.0 (36/60)	(46.5–72.4)
	Negative predictive value	90.4 (66/73)	(81.2–96.0)	86.8 (66/76)	(77.1–93.5)
	Positive likelihood ratio	3.2 (84.4/26.7)	(2.2–4.6)	2.9 (78.3/26.7)	(2.0–4.3)
	Negative likelihood ratio	0.21 (15.2/73.3)	(0.1–0.4)	0.3 (21.7/73.3)	(0.2–0.5)
Spontaneous preterm delivery within two days after amniocentesis [43.4% (59/136)]	Sensitivity	79.7 (47/59)	(67.2–89)	71.2 (42/59)	(57.9–82.2)
	Specificity	79.2 (61/77)	(68.5–87.6)	76.6 (59/77)	(65.6–85.5)
	Positive predictive value	74.6 (47/63)	(62.1–84.7)	70.0 (42/60)	(56.8–81.2.5)
	Negative predictive value	83.6 (61/73)	(73–91.2)	77.6 (59/76)	(66.6–86.4)
	Positive likelihood ratio	3.8 (79.7/20.8)	(2.4–6)	3.1 (71.2/23.4)	(2–4.7)
	Negative likelihood ratio	0.3 (20.3/79.2)	(0.2–0.4)	0.4 (28.8/76.6)	(0.3–0.6)
Spontaneous preterm delivery within seven days after amniocentesis [47.8% (65/136)]	Sensitivity	78.5 (51/65)	(66.5–87.7)	72.3 (47/65)	(59.8–82.7)
	Specificity	83.1 (59/71)	(72.3–90.9)	81.69 (58/71)	(70.7–89.9)
	Positive predictive value	81 (51/63)	(69.1–89.7)	78.3 (47/60)	(65.8–87.9)
	Negative predictive value	80.8 (59/73)	(69.9–89.1)	76.3 (58/76)	(65.2–85.3)
	Positive likelihood ratio	4.6 (78.5/16.9)	(2.7–7.9)	4.0 (72.3/18.31)	(2.4–6.6)
	Negative likelihood ratio	0.3 (21.5/83.1)	(0.16–0.4)	0.3 (27.7/81.69)	(0.2–0.5)
Spontaneous preterm delivery <28 weeks [22.8% (31/136)]*	Sensitivity	87.1 (27/31)	(70–96.3)	83.9 (26/31)	(66.3–94.5)
	Specificity	75.0 (6/8)	(35.1–96.7)	75.0 (6/8)	(35.1–96.1)
	Positive predictive value	93.1 (27/29)	(77.2–99.0)	92.9 (26/28)	(76.5–98.9)
	Negative predictive value	60.0 (6/10)	(26.4–87.6)	54.6 (6/11)	(23.5–83.1)
	Positive likelihood ratio	3.5 (87.1/25)	(1.04–11.7)	3.4 (83.9/25)	(1.0–11.3)
	Negative likelihood ratio	0.2 (12.9/75)	(0.06–0.5)	0.22 (16.1/75)	(0.09–0.53)
Spontaneous preterm delivery <34 weeks [54.4% (74/136)]†	Sensitivity	77.03 (57/74)	(65.8–86.01)	72.97 (54/74)	(61.4–82.6)
	Specificity	71.43 (5/7)	(29.3–95.5)	85.71 (6/7)	(42.2–97.6)
	Positive predictive value	96.6 (57/59)	(88.3–99.5)	98.2 (54/55)	(90.2–99.7)
	Negative predictive value	22.7 (5/22)	(7.9–45.4)	23.08 (6/26)	(90.3–99.7)
	Positive likelihood ratio	2.7 (77.03/28.57)	(0.8–8.8)	5.1 (72.97/14.29)	(0.19–0.51)
	Negative likelihood ratio	0.32 (22.97/71.43)	(0.17–0.6)	0.32 (27.03/85.71)	(0.19–0.51)

CI: confidence interval.
*The analysis was performed only in patients who had amniocentesis at <28 weeks ($n = 39$).
†The analysis was performed only in patients who had amniocentesis at <34 weeks ($n = 81$).

determine AF IL-6 concentrations among women with preterm PROM for the identification of MIAC (or the combination of MIAC with acute histological chorioamnionitis) [146]. Other investigators who used a POC test to determine IL-6 concentrations in vaginal fluid from women with preterm PROM have reported high negative predictive value for the detection of intra-amniotic inflammation, comparable to that observed in our study (97.4% versus 94.5%) [147]. Yet, the positive predictive value in our study was higher than that of vaginal fluid IL-6 concentration POC test (88.9% versus 50%). Vousden et al. reported the use of POC IL-6 in vaginal fluid to determine pregnancy outcomes in asymptomatic high-risk patients of preterm birth [145]. Using a cut-off of 56 pg/ml, vaginal fluid IL-6 concentrations had 81% sensitivity and 65% specificity to identify patients who delivered <28 weeks of gestation [145]. This diagnostic performance is slightly lower than that of POC AF IL-6 in this study. The optimal cutoff value is to be determined in accordance with regard to risk/benefit ratios for specific interventions.

Strengths and limitations

The strengths of this study include: (1) we included a homogenous group of patients with preterm labor with intact membranes rather than including patients with preterm prelabor rupture of membranes who have a higher prevalence of intra-amniotic infection/inflammation and (2) the POC test was not used to inform treatment. A limitation is that we used cultivation technique to identify microorganisms in the amniotic cavity. Thus, non-culturable bacteria, which could have been identified by molecular microbiologic techniques, may be not able to be detected.

Conclusion

A POC AF IL-6 test can identify intra-amniotic inflammation as determined by ELISA in women with preterm labor and intact membranes, and it also performs equivalently in identifying those who subsequently deliver spontaneously before term. Further studies are warranted to determine whether POC AF IL-6 results can inform treatment decisions sufficient to improve pregnancy outcomes in such patients.

Declaration of interest

Authors declare no conflict of interest.

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