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The diagnostic performance of the Mass Restricted (MR) score in the identification of microbial invasion of the amniotic cavity or intra-amniotic inflammation is not superior to amniotic fluid interleukin-6

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Abstract

Objective—Intra-amniotic infection/inflammation are major causes of spontaneous preterm labor and delivery. However, diagnosis of intra-amniotic infection is challenging because most are subclinical and amniotic fluid (AF) cultures take several days before results are available. Several tests have been proposed for the rapid diagnosis of microbial invasion of the amniotic cavity (MIAC) or intra-amniotic inflammation. The aim of this study was to examine the diagnostic performance of the AF Mass Restricted (MR) score in comparison with interleukin-6 (IL-6) and matrix metalloproteinase-8 (MMP-8) for the identification of MIAC or inflammation.

Methods—AF samples were collected from patients with singleton gestations and symptoms of preterm labor ($n = 100$). Intra-amniotic inflammation was defined as >100 white blood cells/mm³ (WBCs) in AF; MIAC was defined as a positive AF culture. AF IL-6 and MMP-8 were determined using ELISA. The MR score was obtained using the Surface-Enhanced Laser Desorption Ionization Time of Flight (SELDI-TOF) mass spectrometry. Sensitivity and specificity were

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calculated and logistic regression models were fit to construct receiver-operating characteristic (ROC) curves for the identification of each outcome. The McNemar's test and paired sample non-parametric statistical techniques were used to test for differences in diagnostic performance metrics.

Results—(1) The prevalence of MIAC and intra-amniotic inflammation was 34% (34/100) and 40% (40/100), respectively; (2) there were no significant differences in sensitivity of the three tests under study (MR score, IL-6 or MMP-8) in the identification of either MIAC or intra-amniotic inflammation (using the following cutoffs: MR score >2, IL-6 >11.4 ng/mL, and MMP-8 >23 ng/mL); (3) there was no significant difference in the sensitivity among the three tests for the same outcomes when the false positive rate was fixed at 15%; (4) the specificity for IL-6 was not significantly different from that of the MR score in identifying either MIAC or intra-amniotic inflammation when using previously reported thresholds; and (5) there were no significant differences in the area under the ROC curve when comparing the MR score, IL-6 or MMP-8 in the identification of these outcomes.

Conclusions—IL-6 and the MR score have equivalent diagnostic performance in the identification of MIAC or intra-amniotic inflammation. Selection from among these three tests (MR score, IL-6 and MMP-8) for diagnostic purposes should be based on factors such as availability, reproducibility, and cost. The MR score requires a protein chip and a SELDI-TOF instrument which are not widely available or considered “state of the art”. In contrast, immunoassays for IL-6 can be performed in the majority of clinical laboratories.

Keywords

Biomarker; chorioamnionitis; IL-6; MMP-8; pregnancy; preterm birth; preterm labor; proteomics; reproducibility

Introduction

Preterm birth is the leading cause of perinatal morbidity and mortality worldwide [1–4]. Two-thirds of all preterm births occur after the spontaneous onset of labor with either intact or ruptured membranes [2,5]. Microbial invasion of the amniotic cavity (MIAC) is frequently observed in patients with preterm labor and intact membranes [6–22] and, rarely, in patients with indicated preterm delivery (such as preeclampsia [23] and small-for-gestational-age [24]). Moreover, there is considerable evidence that MIAC is causally linked with spontaneous preterm labor and delivery [25–28].

Under normal circumstances, bacteria are not present in the amniotic cavity [29–32]. Microorganisms can gain access to the amniotic cavity in patients with preterm labor (PTL) and intact membranes [6–22], preterm prelabor rupture of membranes (PROM) [33–41], a short cervix [42–44], cervical insufficiency [45–49], PTL in twin gestations [50–52], vaginal bleeding in the third trimester [53], placenta previa [54,55] or in selected cases of fetal death [56–60].

The presence of microorganisms in amniotic fluid (AF) can be detected using cultivation and/or molecular techniques [15,16,21,22,29,61–66]. When bacteria are present in the amniotic cavity in the absence of an inflammatory response, the condition is referred to as

MIAC. Once MIAC elicits a localized inflammatory response, the condition is known as intra-amniotic infection [8,14,20,31,38,61,67–73]. Intraamniotic infection and MIAC are largely subclinical, and only a small fraction of patients have evidence of clinical chorioamnionitis [1,12,16,17,43,68,74–76]. Despite being clinically silent, microorganisms may gain access to the fetus and generate a fetal inflammatory response, which is characterized by an elevation of fetal circulating cytokines [77–79], multi-systemic involvement [80–120] and the impending onset of labor [121]. Some patients have intraamniotic inflammation in the absence of demonstrable MIAC for bacteria or viruses – these cases of “sterile” intraamniotic inflammation appear to be associated with adverse pregnancy outcome, yet the cause of the inflammatory process remains to be determined, and may be attributed to “danger signals” or damage-associated molecular patterns (DAMPs) [122–124].

The diagnosis of MIAC, intra-amniotic inflammation, and intra-amniotic infection (which requires a combination of MIAC and intra-amniotic inflammation) is challenging because most infections are subclinical [1,12,16,17,43,68, 74–76]. The “gold standard” for diagnosis of MIAC is the demonstration of microbial growth in AF, which is normally sterile [29–32]; however, AF culture results may take several days to be informative. Therefore, diagnosis has relied *per force* on tests aimed at detecting an intra-amniotic inflammatory process.

We advocated tests used in other body fluids (e.g. cerebrospinal fluid), such as the Gram stain [125–130], AF glucose [126–137] and AF white blood cell (WBC) count [126–128,130,138,139] for the rapid assessment of the presence of bacteria and/or inflammation. Subsequently, other methods were used, including the detection of microbial products, such as endotoxin [25,140–142], the acridine orange stain [143], leukocyte esterase [138,139,144] and gas liquid chromatography of microbial metabolites [145,146]. Immunoassays have also been used for the detection of proteins produced in response to microorganisms or during the course of inflammation, such as interleukin 6 (IL-6) [32,127,137,147–162], other cytokines and chemokines [26,27,77,78,163–188], and rapid tests for matrix metalloproteinase-8 (MMP-8) [160,162,189–194].

In 2005, a method to diagnose intra-amniotic inflammation based on mass spectrometry was reported and referred to as the Mass Restricted (MR) score [195,196]. The MR score was based on the detection of four peaks on mass spectrometry at 3378.2, 3449.7, 10 471.7 and 10 874.4 Daltons. These peaks correspond to four proteins, respectively: neutrophil defensins-1 and -2, and calgranulins A and C [195,196]. The MR score equals the number of these four peaks observed on the mass spectrometry tracing of a particular AF sample, and ranges from 0 to 4; 0 when none of the peaks are present, and 4 when all peaks are present. An MR score of 3 or higher has been proposed as evidence of intra-amniotic inflammation [195,196].

The MR score was formulated and tested in 101 stored AF samples collected in our unit, as previously described [195,196]. These results were subsequently confirmed using fresh, rather than stored, AF samples in a follow-up study of 169 consecutive women with singleton pregnancies and PTL or preterm PROM [197]. The authors compared the performance of the MR score with IL-6 and MMP-8 as well as other biomarkers for the

detection of intra-amniotic inflammation, and claimed that their study “clearly demonstrates the superiority. . . of the MR score in comparison with any other clinical test. . .” in identifying intra-amniotic inflammation, defined as an AF WBC >100 cells/mm³, and MIAC (a positive AF culture for microorganisms) [197]. The sensitivity and specificity of the MR score, IL-6, and MMP-8 for the identification of MIAC and intra-amniotic inflammation reported by the authors are shown in Table 1.

This study was conducted to examine the diagnostic performance of the MR score in comparison with IL-6, and MMP-8 in the identification of MIAC and intra-amniotic inflammation using the original AF samples examined in developing the MR score [195,196]. This was possible because the original study of the MR score was conducted in collaboration with our unit, and we measured IL-6 and MMP-8 concentrations in 100 of the 101 stored AF samples. Comparing the diagnostic performance of IL-6 and MMP-8 with that of the MR score using these samples has two unique advantages. First, it eliminates potential confounding factors related to technique - that is, potential differences in expertise, subjective evaluation of peaks, and the technique used for mass spectrometry. Second, because the MR score was defined based on these samples, this data set should maximize the diagnostic performance of the MR score. Therefore, the aim of this study was to examine the diagnostic performance of the AF MR score in comparison with that of IL-6 and MMP-8 for the diagnosis of MIAC and inflammation.

Methods

Study population

This study included a total of 100 AF samples remaining out of the total 101 samples used for the study originally reported by Buhimschi et al. [195,196] that were selected from the 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). Samples were collected by ultrasound guided transabdominal amniocentesis in patients with symptoms of PTL or preterm PROM. PTL was diagnosed in the presence of regular uterine contractions (at least three in 30 minutes) and documented cervical changes that required admission to the hospital before 37 weeks of gestation. The collection of these samples and the method of storage have been previously described in detail [195,196].

Originally, 77 samples were used to define the MR score [195,196]. These AF samples were selected on the basis of known outcomes (spontaneous PTL or symptoms of PTL but who delivered at term). Subsequently, an additional 24 AF samples, bringing the total to 101 samples, were selected to examine the diagnostic performance of the MR score for the identification of MIAC and inflammation. Mass spectrometry tracings were obtained for these samples using SELDITOF (Ciphergen, Fremont, CA) between May 2001 and April 2002.

The current study was conducted to compare the diagnostic performance of the MR score to that of IL-6 and MMP-8 for the identification of MIAC and intra-amniotic inflammation, and for the diagnosis of delivery before 34 weeks of gestation. The rationale for the selection

of such outcome was based on the severity and frequency of adverse outcomes from spontaneous preterm birth in preterm infants (<34 weeks of gestation) [198,199]. The IL-6 and MMP-8 assays of the 100 AF samples were conducted in October 2002 in preparation for the presentation of the results in abstract form at the annual meeting of the Society for Maternal Fetal Medicine in 2003 [195]. Intra-amniotic inflammation was defined as an AF WBC count >100 cells/mm³; MIAC was defined as a positive AF culture. Demographic and clinical information about the mothers and neonates was extracted from medical records.

Immunoassays for IL-6 and MMP-8

Determination of IL-6 concentrations was performed using a commercially available enzyme-linked immunosorbent assay (ELISA) (R & D Systems, Minneapolis, MN) with a sensitivity of 2.3 pg/mL. Inter- and intra-assay coefficients of variations were 9.02% and 7.24%, respectively. MMP-8 concentrations were determined with the use of a commercially available ELISA (Amersham Pharmacia Biotech, Inc. Arlington Heights, IL). The sensitivity of the assay in our laboratory was 0.06 ng/mL; inter- and intra-assay coefficients of variation were 4.6% and 3.7%, respectively.

Statistical analysis

Sensitivity and specificity were calculated and logistic regression models were fit to construct ROC curves portraying the respective diagnostic performance of each of the three markers (MR score, IL-6 and MMP-8) in identifying each of the three selected obstetrical complications (MIAC, intra-amniotic inflammation and preterm delivery before 34 weeks of gestation). Sensitivity and specificity were calculated using thresholds previously reported [195,196] and, separately, sensitivity using cutoffs determined at a fixed false-positive rate of 15%. An additional threshold (IL-6 >2.6 ng/mL) based on previous publications was used to calculate sensitivity and specificity for IL-6 in identifying preterm delivery before 34 weeks of gestation [194,200]. The McNemar's test and paired sample non-parametric statistical techniques were used to examine differences in diagnostic performance comparing the MR score to IL-6 and MMP-8 for the identification of selected outcomes. A 5% threshold for type I error was used to determine statistical significance. Statistical analyses were performed using SAS version 9.3 (SAS, Cary, NC).

Results

Demographic and clinical characteristics of the study population are presented in Table 2. Forty percent (40/100) of the AF samples showed intra-amniotic inflammation based on AF WBC count >100 cells/mm³. A positive AF culture for microorganisms was present in 34% (34/100) of the AF samples. Sixty-two (62%) mothers delivered prior to 34 weeks of gestation: 40 delivered spontaneously, and 22 were delivered for maternal and/or fetal indications.

Diagnostic performance in identifying intra-amniotic inflammation

Table 3 shows the sensitivity and specificity for each biomarker in identifying intra-amniotic inflammation using analyte cut-off values previously published (MR score >2, IL-6 >11.4 ng/mL and MMP-8 >23 ng/mL) [197]. There was no difference in sensitivity when

comparing the MR score to IL-6 or MMP-8, both using previously identified cut-off values and when using thresholds selected based on a fixed false-positive rate of 15% (both $p = 1.0$). There was also no difference in specificity comparing the MR score to IL-6 for the identification of intra-amniotic inflammation when using the previously identified thresholds, whereas MMP-8 had a lower specificity than either of these two markers (both $p < 0.001$). ROC curves characterizing each marker's performance in identifying intra-amniotic inflammation are shown in Figure 1. The areas under the ROC curves (AUCs) for IL-6 (0.98) and MMP-8 (0.97) were not statistically significantly different from that of the MR score (0.97) (each $p = 0.7$).

Diagnostic performance in identifying MIAC

Table 4 shows the sensitivity and specificity for each marker in identifying MIAC using the previously identified thresholds (MR score >2 , IL-6 >11.4 ng/mL and MMP-8 >23 ng/mL). There was no difference in sensitivity when comparing the MR score to IL-6 or MMP-8 for the identification of MIAC, either when using previously identified cut-off values ($p > 0.3$) or when using separate thresholds selected based on a fixed false-positive rate of 15% ($p > 0.3$). There was also no difference in specificity comparing the MR score to IL-6 for the identification of MIAC when using the previously identified thresholds, whereas MMP-8 had a lower specificity than either of these two markers (both $p < 0.01$). ROC curves characterizing the performance of the MR score, IL-6, and MMP-8 in identifying MIAC are shown in Figure 2. The AUCs for IL-6 (0.83) and MMP-8 (0.85) were not statistically significantly different from that of the MR score (0.86; $p = 0.3$ and $p = 0.8$, respectively).

Diagnostic performance in identifying preterm delivery before 34 weeks of gestation

Tables 5 and 6 show the sensitivity and specificity for the MR score, IL-6 and MMP-8 in identifying all deliveries before 34 weeks of gestation and spontaneous preterm delivery before 34 weeks of gestation, respectively. Using previously identified thresholds, there was no significant difference in sensitivity or specificity when comparing the MR score to IL-6 for the identification of all ($p = 0.7$ and $p = 1.0$, respectively) and, separately, for spontaneous deliveries, before 34 weeks (both $p = 1.0$). In contrast, the sensitivity for the MR score was significantly lower than that of MMP-8 ($p = 0.02$), whereas the specificity of MMP-8 was significantly lower than that of both the MR score and IL-6 (both $p < 0.05$) for the identification of spontaneous, but not overall, deliveries before 34 weeks of gestation.

There was also no significant difference in the sensitivity when comparing the MR score to either IL-6 or MMP-8 using cut-offs selected by fixing the false-positive rate at 15%, either for the identification of all or, separately, for spontaneous preterm delivery before 34 weeks of gestation ($p > 0.6$). In addition, there was no difference in sensitivity comparing the MR score to IL-6 when using a separate threshold (IL-6 >2.6 ng/mL) for the identification of these complications. ROC curves characterizing the performance of the MR score, IL-6, and MMP-8 in identifying patients who delivered spontaneously prior to 34 weeks of gestation are shown in Figure 3. The AUC for the MR score (0.76) was not statistically different from those of IL-6 (0.80) or MMP-8 (0.79) in identifying this outcome (each $p = 0.2$); the same was true for the identification of all preterm deliveries before 34 weeks of gestation (each $p > 0.3$).

Discussion

Principal findings of this study

(1) There were no significant differences in sensitivity at a fixed false-positive rate of 15%, or AUC, in identifying either MIAC or intra-amniotic inflammation, or preterm delivery before 34 weeks of gestation, when comparing the MR score to AF concentrations of IL-6 or MMP-8; and (2) there were no significant differences in specificity in identifying either MIAC or intra-amniotic inflammation when comparing the MR score to AF concentrations of IL-6 using previously identified thresholds [197]. These findings contradict the claim that the MR score is “clearly superior” to any other clinical test for the diagnosis of MIAC and inflammation [197].

The frequency and clinical significance of microbial invasion of the amniotic cavity and intra-amniotic inflammation

A positive AF culture for bacteria has been reported in approximately 10% of patients with spontaneous PTL and intact membranes [14,15,21,130], 30–40% of patients with preterm PROM [37,41,127,201,202], 9% of patients with a short cervix [42–44], 51% of patients with acute cervical insufficiency [45–49], 10% of patients with PTL and twin gestations [50–52], and 14% of patients with idiopathic vaginal bleeding [53]. With the use of molecular microbiologic techniques, the frequency with which bacteria have been found in AF is even higher [19,21,22,66,203–214]. Moreover, the presence of microbial footprints detected with polymerase chain reaction, even in the absence of microbial growth in the laboratory, is associated with adverse pregnancy outcome [200].

Microbial invasion of the amniotic cavity and intra-amniotic inflammation are risk factors for impending preterm delivery and perinatal mortality and morbidity (e.g. otitis media [215], congenital pneumonia [216,217], admission to the neonatal intensive care unit [202,218], respiratory distress syndrome or chronic lung disease [218–220], congenital sepsis [19,218,219,221], cerebral palsy [112–114,117,118, 222–230] and necrotizing enterocolitis [147,163,231]). Moreover, intra-amniotic infection is associated with clinical chorioamnionitis [217,231–233] and puerperal endometritis [234], and may be complicated by maternal sepsis [235,236] and disseminated intravascular coagulation [237,238].

The early identification of MIAC has implications for the clinical management of the patient with PTL and preterm PROM. For example, in patients with PTL, intact membranes, and the presence of MIAC, tocolytics should not be administered, because they are ineffective and increase the risk of pulmonary edema [239]. After a positive diagnosis, antibiotic treatment can be initiated immediately, rather than waiting for delivery. A randomized clinical trial in which antimicrobial therapy began before delivery, rather than after, showed a decreased rate of neonatal sepsis with early treatment [240]. This trial was conducted in pregnancies near term and was discontinued on the recommendation of the Data and Safety Monitoring Board after the observation of an increased rate of adverse events (sepsis) when treatment was delayed [240]. A similar trial has not been conducted in cases of subclinical MIAC in preterm gestation. However, such a trial may not be ethically possible – it would be difficult to argue that the preterm fetus, generally considered as immunocompromised in comparison

with the term fetus/neonate, would not be harmed by delayed treatment (see a detailed discussion of this issue in reference [42]).

Reproducibility in science and medicine

Replication is a cornerstone of scientific validity [241–244]. Many claims by prestigious laboratories and journals have not been subsequently replicated, despite several attempts [245,246]. The lack of replication and its implications are particularly worrisome when dealing with diagnostic tests or therapies intended for clinical applications in humans [247–249]. In the case of pregnancy, false-positive or false-negative results may lead to very serious consequences.

We were not able to replicate the claim that the MR score is superior to other tests (e.g. IL-6) for the detection of MIAC or intra-amniotic inflammation. The findings in this study suggest that IL-6 and the MR score had a higher specificity (although not sensitivity) than MMP-8 for detecting intra-amniotic inflammation (Table 3).

There was no difference in sensitivity when comparing the MR score to IL-6 in identifying the patient who will deliver before 34 weeks of gestation, regardless of the thresholds employed (e.g. those proposed by Buhimschi et al. [MR score >2, IL-6 >11.3 ng/mL], cut-offs selected based on a fixed false-positive rate of 15% or an IL-6 threshold proposed in prior studies [2.6 ng/mL]) [200].

IL-6 was reported to have a high sensitivity for the identification of MIAC in patients with PTL and intact membranes, with values ranging from 80% to 100% [151]. The cut-off of 11.3 ng/mL, established by our group two decades ago, was derived from an analysis of AF samples from 120 patients with spontaneous PTL and/or intact membranes, in which the prevalence of positive AF cultures was 9.2% (11/120) [151]. The analysis showed that, using a cut-off of 11.3 ng/mL, IL-6 was the most sensitive test (100%) for the detection of MIAC compared to glucose (81%), WBC count (63.6%), and Gram stain (63.6%); ($p < 0.01$ for all) [151]. The diagnostic performance of 11.3 ng/mL as a cutoff of IL-6 to identify a positive AF culture was confirmed by our group in another set of patients [150], and subsequently, by other investigators [161,250,251]. The threshold of 2.6 ng/mL was also proposed by our unit to detect intra-amniotic inflammation after we observed that patients with concentrations of IL-6 above 2.6 ng/mL were at risk for preterm delivery, and frequently had evidence of acute chorioamnionitis and/or funisitis on placental examination [200]. Moreover, even with negative cultures, an elevated IL-6 concentration was associated with a short amniocentesis-to-delivery interval and a significantly higher rate of neonatal adverse outcomes [200]. Subsequently, the value of this cut-off as a marker of intra-amniotic inflammation has been confirmed by others [71,162,252].

Strengths and limitations of the study

The diagnostic performance of selected markers was compared using the same AF samples originally used to define the MR score, and, if anything, should have biased the diagnostic performance in favor of the MR score. Yet, the diagnostic performance of the MR score was similar to that of IL-6. In contrast to the findings of the MR score (which have not been independently reproduced), the claims about the sensitivity and specificity of IL-6 in the

diagnosis of MIAC [147,151,200,253] have been independently confirmed [161,250,251,254].

Proteomics is used to identify candidate biomarkers in biological fluids and tissues [255–258]; yet, proteomics is largely a discovery (rather than diagnostic) tool. In general, a proteomics approach is used to discover differentially expressed proteins/peptides between diseased and non-diseased states, and targeted assays are designed to identify and quantify differentially-expressed biomarkers which can distinguish health from disease. The targeted assays implemented after discovery are generally immunoassays, because of the wide availability of these assay platforms (e.g. ELISAs). However, chemical assays using mass spectrometry to identify specific biomarkers are also possible. This was one of the hopes of using SELDI-TOF and the MR score for the rapid identification of intra-amniotic inflammation. Unfortunately, the diagnostic performance of the MR score is not superior to that of a single ELISA for IL-6, and the instrumentation to perform SELDI-TOF has not gained popularity in clinical laboratories. It has accordingly been largely abandoned in research laboratories in favor of more sensitive and accurate mass spectrometry techniques. Proteomic platforms have been used successfully to identify biomarkers for the adequate identification of spontaneous preterm birth and adverse pregnancy outcome using cervical/vaginal fluid [259–264], AF [265–269] and maternal serum [265,270,271].

Conclusions

Immunoassays for IL-6 or MMP-8 can be used to identify intra-amniotic inflammation and MIAC (which is frequently associated with intra-amniotic inflammation) with equivalent diagnostic performance to the MR score. There is no justification to use the MR score, which requires SELDITOF-technology which is not available in most clinical units. Proteomics may be used to discover new biomarkers of intra-amniotic inflammation, infection, or other disease states associated with preterm labor. Advances in mass spectrometry may render these approaches feasible (and even chemical assays of biomarkers possible) in the clinical setting, and this possibility requires future studies.

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Figure 1.

Receiver operating characteristic curve analysis for the use of IL-6, MMP-8 and the Mass Restricted (MR) score for the detection of intra-amniotic inflammation. The AUCs for using IL-6 or MMP-8 were not statistically significantly different from that of the MR score (each $p = 0.7$).

ROC Curve of Detection of a Positive Amniotic Fluid Culture (n = 100)

Figure 2.

Receiver operating characteristic curve analysis for the use of IL-6, MMP-8 and the Mass Restricted (MR) score for the detection of a positive amniotic fluid culture. The AUCs for IL-6 and MMP-8 were not statistically significantly different from that of the MR score ($p = 0.3$ and $p = 0.8$, respectively).

ROC Curve of Spontaneous Delivery Before 34 Weeks of Gestation (n = 100)

Figure 3.

Receiver-operating characteristic curves for the use of IL-6, MMP-8 and the Mass Restricted (MR) score for the detection of intra-amniotic inflammation. The areas under the ROC curves for IL-6 and MMP-8 were not statistically significantly different from that of the MR score (each $p = 0.2$).

Table I

Performance of IL-6, MMP-8, and the MR score for the detection of intra-amniotic inflammation and infection (defined as AF WBC count >100 cells/mm³ and a positive AF culture, respectively) as originally reported in reference [197].

Biomarker	Intra-amniotic inflammation		Intra-amniotic infection	
	Sensitivity (%) [95% CI]	Specificity (%) [95% CI]	Sensitivity (%) [95% CI]	Specificity (%) [95% CI]
MR score 3	93.0 [84.1–97.4]	92.4 [89.2–94.0]	79.5 [68.4–87.8]	85.6 [81.7–88.5]
IL-6 >11.4 ng/mL	45.0 [34.5–52.4]	94.8 [91.2–97.4]	45.2 [36.6–52.9]	61.9 [44.7–77.3]
MMP-8 >23 ng/mL	92.9 [82.5–97.5]	66.7 [62.9–68.3]	90.7 [79.9–96.2]	65.0 [61.3–67.0]

MR score: Mass restricted score; IL-6: Interleukin 6; MMP-8: Matrix metalloproteinase 8. Source reference [197].

Demographic and clinical characteristics of the study population, and results of the amniotic fluid analysis

Table II

Clinical characteristics	Patients who delivered at term (≥ 37 weeks)		Patients who delivered preterm (<37 weeks)			
	WBC (-) AFC (-) (n=29)		WBC (+) AFC (+) (n=27)	WBC (+) AFC (-) (n=13)	WBC (-) AFC (+) (n=7)	WBC (-) AFC (-) (n=24)
Age (years) [†]	25.9 (6.9)		26.7 (6.1)	23.2 (4.4)	28.9 (4.6)	24.0 (5.5)
Ethnicity, n (%), African-American) [∞]	23 (79.3)		24 (88.9)	10 (76.9)	7 (100)	23 (95.8)
Parity [§]	1 [0-5]		1 [0-7]	1 [0-5]	2 [0-6]	1 [0-9]
GA at amniocentesis (weeks) [†]	29.3 (3.3)		26.5 (4.2)	27.0 (2.2)	31.9 (1.5)	30.0 (3.5)
GA at delivery (weeks) [†]	38.6 (1.3)		26.7 (4.2)	27.8 (2.2)	33.0 (1.2)	31.1 (3.7) *
PPROM, n (%) [∞]	0 (0)		12 (44.4%) *	4 (30.8%) *	6 (85.7%) *	10 (41.7%) *
Indicated delivery, n (%) [∞]	3 (10.3)		12 (44.4%) *	1 (7.7%)	4 (57.1%) *	8 (33.3%) *
Spontaneous delivery, n (%) [∞]	26 (89.7)		15 (55.6%) *	12 (92.3%)	3 (42.9%) *	16 (66.7%) *
Birthweight (g) [†]	3248.2 (411.7)		1010 (566.2)	1076.2 (381.3)	1491.9 (325) *	1776.3 (643.0) *
Amniotic fluid analysis						
WBC (cell/mm ³) [§]	5 [0-80]		1000 [260-19200] *	1000 [200-14800] *	10 [3-88] *	3 [0-62] *
Positive Gram stain, n (%) [∞]	0 (0)		17 (63.0) *	1 (7.7)	3 (42.9) *	1 (4.2)
Glucose concentration <10 mg/dL, n (%) [∞]	0 (0)		16 (59.3) *	6 (46.2) *	0 (0)	0 (0)
Storage IL-6 (years) [§]	3.0 [1.5-9.0]		2.5 [1.4-2.5]	2.9 [1.6-3.6]	3.0 [1.9-8.7]	2.4 [1.2-9.7]
Storage MMP8 (years) [§]	3.0 [0.7-10.2]		2.5 [0.7-9.7]	3.0 [1.1-9.0]	3.1 [1.9-8.7]	2.4 [1.3-9.7]
Placental pathology						
Histological chorioamnionitis, n (%) [∞]	1 (6.3)		25 (92.6) *	13 (100) *	2 (33.3)	8 (36.4)

GA=gestational age; PPRM=preterm prelabor rupture of membranes; WBC=white blood cell count (+WBC: WBC count > 100 cells/mm³); AFC=amniotic fluid cultures.

Missing placental pathology, n=16 (13 in term delivery group).

* p < 0.05 versus "normal" group († Dunnett's, ∞ Fisher's exact, § Dunn's tests).

† Data presented as mean (SD).

§ Data presented as median [range].

Table III

Diagnostic performance of IL-6, MMP-8 and the MR score for the identification of intra-amniotic inflammation

Biomarker	Sensitivity (%)	95% CI	Specificity (%)	95% CI
MR score 3	95.0% (38/40)	83.1–99.4	95.0% (57/60)	86.1–99.0
IL-6 >11.4 ng/mL	95.0% (38/40)	83.1–99.4	91.7% (55/60)	81.6–97.2
MMP-8 >23 ng/mL	97.5% (39/40)	86.8–99.9	70.0% (42/60)	56.8–81.2

MR score: Mass restricted score; IL-6: Interleukin 6; MMP-8: Matrix metalloproteinase 8.

Table IV

Diagnostic performance of IL-6, MMP-8 and the MR score to identify a positive amniotic fluid culture or microbial invasion of the amniotic cavity (MIAC)

Biomarker	Sensitivity (%)	95% CI	Specificity (%)	95% CI
MR score 3	88.2% (30/34)	72.6–96.7	83.3% (55/66)	72.1–91.4
IL-6 >11.4 ng/mL	85.3% (29/34)	68.9–95.1	78.8% (52/66)	67.0–87.9
MMP-8 >23 ng/mL	94.1% (32/34)	80.3–99.3	62.1% (41/66)	49.3–73.8

MR score: Mass Restricted score; IL-6: Interleukin 6; MMP-8: Matrix metalloproteinase 8.

Table V

Diagnostic performance of the MR score, IL-6 and MMP-8 for the identification of patients who delivered before 34 weeks of gestation (spontaneous and indicated)

	Delivery before 34 weeks of gestation			
	Sensitivity	95%CI	Specificity	95%CI
MR score 3	66.1% (41/62)	53.0–77.7	100% (38/38)	90.8–100
IL-6 >2.6 ng/mL	77.4% (48/62)	65.0–87.1	89.5% (34/38)	75.2–97.1
IL-6 >11.4 ng/mL	69.4% (43/62)	56.4–80.4	100% (38/38)	90.8–100
MMP-8 >23 ng/mL	83.9% (52/62)	72.3–92.0	86.8% (33/38)	71.9–95.6

MR score: Mass Restricted score; IL-6: Interleukin 6; MMP-8: Matrix metalloproteinase 8.

Table VI

Diagnostic performance of the MR score, IL-6, and MMP-8 for spontaneous delivery <34 weeks of gestation

	Deliveries < 34 weeks			
	Sensitivity	95%CI	Specificity	95%CI
MR score 3	67.5% (27/40)	50.9–81.4	76.7% (46/60)	64.0–86.6
IL-6 >2.6 ng/mL	80% (32/40)	64.4–91.0	66.7% (40/60)	53.3–78.3
IL-6 >11.4 ng/mL	70% (28/40)	53.5–83.4	75% (45/60)	62.1–85.3
MMP-8 >23 ng/mL	85% (34/40)	70.2–94.3	61.7% (37/60)	48.2–73.9

MR score: Mass restricted score; IL-6: Interleukin 6; MMP-8: Matrix metalloproteinase 8.