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The prevalence and clinical significance of amniotic fluid 'sludge' in patients with preterm labor and intact membranes

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KEYWORDS: amniotic fluid 'sludge'; chorioamnionitis; intrauterine inflammation; microbial invasion of the amniotic cavity; preterm delivery; preterm labor; ultrasound

ABSTRACT

Objective *To determine the prevalence and clinical significance of amniotic fluid (AF) 'sludge' observed during transvaginal ultrasound examination of the cervix in patients with preterm labor and intact membranes, and in those with uncomplicated pregnancies.*

Methods *This retrospective study included patients with preterm labor and intact membranes (*n = *84) and those with uncomplicated term pregnancies (*n = *298). The outcome variables included the occurrence of documented microbial invasion of the amniotic cavity (MIAC), histological chorioamnionitis, examination-to-delivery interval, admission to the neonatal intensive care unit (NICU), a composite neonatal morbidity, perinatal death, and delivery within 48 h, 7 days, and < 35 weeks and < 32 weeks. Statistical analysis included Chi-square test, stepwise logistic regression analysis and survival analysis.*

Results *The prevalence of AF 'sludge' was 1% (3/298) in patients with uncomplicated term pregnancies and 22.6% (19/84) in those with preterm labor and intact membranes. Among patients with preterm labor and intact membranes: (1) cervical length ⩽ 15 mm was present in 58.3% (49/84) of the patients; (2) the prevalence of MIAC and histological chorioamnionitis was 12.1% (7/58) and 32.9% (25/76), respectively; (3) the rate of spontaneous preterm delivery within 48 h, 7 days, and < 32 weeks and < 35 weeks of gestation was 13.6% (8/59), 28.8% (17/59), 39.5% (17/43) and 50.8% (30/59), respectively; (4) patients with AF 'sludge' had a higher frequency of positive AF cultures [33.3% (6/18) vs. 2.5% (1/40),* P = *0.003] and histological chorioamnionitis [77.8% (14/18) vs. 19% (11/58),* P < *0.001] than those without AF 'sludge'; (5) a higher proportion of neonates born to patients with AF 'sludge' was admitted to the NICU [64.3% (9/14) vs. 12.9% (8/62),* P < *0.01], had a composite neonatal morbidity [36.8% (7/19) vs. 13.8% (9/65),* P = *0.04] and died in the perinatal period [36.8% (7/19) vs. 4.6% (3/65),* P = *0.001] than those born to women without 'sludge'; (6) a higher proportion of patients with AF 'sludge' had spontaneous delivery within 48 h [42.9% (6/14) vs. 4.4% (2/45),* P = *0.001], within 7 days [71.4% (10/14) vs. 15.6% (7/45),* P < *0.001], < 32 weeks [75% (9/12) vs. 25.8% (8/31),* P = *0.005] and < 35 weeks [92.9% (13/14) vs. 37.8% (17/45),* P < *0.001] than those without AF 'sludge'; and (7) patients with AF 'sludge' had a shorter examination-to-delivery interval than those without AF 'sludge' [AF 'sludge' median, 1 (IQR, 1–5) days vs. no AF 'sludge' median, 33 (IQR, 18–58) days;* P < *0.001].*

Conclusion *The presence of AF 'sludge' in patients with preterm labor and intact membranes is a risk factor for MIAC, histological chorioamnionitis and impending preterm delivery. Copyright © 2005 ISUOG. Published by John Wiley & Sons, Ltd.*

INTRODUCTION

Particulate matter in the amniotic fluid (AF) is present in about 4% of pregnancies during transvaginal ultrasound in the first and early second trimester[1]. The prevalence of this sonographic finding increases with gestational age,

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reaching 88% by 35 weeks[2]. Particulate matter in the first two trimesters of pregnancy has been associated with intra-amniotic bleeding[3,4] and the acrania–anencephaly sequence[5,6], and has been observed in women with high concentrations of maternal serum alpha-fetoprotein[7]. In contrast, in the last trimester of pregnancy, particulate matter and 'echogenic amniotic fluid' have been attributed to the presence of vernix caseosa and/or meconium[8–11], and with a lecithin : sphingomyelin ratio indicative of lung maturity[12,13]. Congenital anomalies associated with particulate matter in the AF include harlequin ichthyosis[14] and epidermolysis bullosa letalis[15].

Dense aggregates of particulate matter giving the ultrasound appearance of AF 'sludge' are frequently seen, in the proximity of the internal cervical os, in patients with preterm labor and intact membranes. However, the prevalence and clinical significance of this ultrasound finding have not been determined.

METHODS

Study design

A retrospective study was conducted by searching our clinical database and digital library of ultrasound images collected from August 1999 to December 2002. The inclusion criteria were singleton pregnancies and gestational ages between 20 and 35 weeks. Uncomplicated pregnancies included only patients that delivered at term neonates whose birth weights were adequate for gestational age. All patients signed informed consent to use the clinical and ultrasound data for research purposes, and the protocols were approved by the Institutional Review Boards of the Sotero del Rio Hospital, Wayne State University and the National Institute of Child Health and Human Development (NICHD).

Preterm labor was defined by the presence of regular uterine contractions with cervical changes before 37 completed weeks of gestation. Microbial invasion of the amniotic cavity (MIAC) was defined as a positive AF culture for micro-organisms in fluid obtained by amniocentesis. Neonatal morbidity was considered to be present if the neonate had necrotizing enterocolitis, suspected sepsis, intraventricular hemorrhage, or if the neonate required assisted ventilation due to respiratory distress syndrome. Suspected neonatal sepsis was diagnosed in the absence of a positive neonatal blood culture if two or more of the following criteria were found: (1) white blood cell (WBC) count < 5000 cells/mm^3; (2) polymorphonuclear leukocyte count of < 1800 cells/mm^3; and (3) ratio of bands to total neutrophils > 0.2. Clinical chorioamnionitis was diagnosed in the presence of maternal temperature of $\geqslant 38.7$°C and two or more of the following criteria: (1) uterine tenderness; (2) malodorous vaginal discharge; (3) maternal leukocytosis (WBC $> 15\,000$ cells/mm^3; and (4) fetal tachycardia (> 160 bpm).

Sonographic assessment of the cervix

Transvaginal ultrasound was performed with commercially available ultrasound systems (Acuson Sequoia, Siemens, Mountain View, CA and Voluson 730, General Electric Medical Systems, Kretztechnik, Zipf, Austria) equipped with endovaginal transducers with frequency ranges of 5–7.5 MHz and 5–9 MHz, respectively. Sonographic examination of the cervix was performed as previously described[16,17]. Amniotic fluid 'sludge' was defined as the presence of dense aggregates of particulate matter in the proximity of the internal cervical os (Figure 1). An experienced sonographer, blinded to clinical outcome, reviewed the two-dimensional images and three-dimensional (3D) volume datasets of the cervix to determine the presence or absence of AF 'sludge'. The AF 'sludge' volume was measured using the 3D volume dataset with the Virtual Organ Computer-aided AnaLysis (VOCAL) ultrasound technique and the values correlated with the WBC count in the AF (Figure 2).

Analysis

Outcome variables included MIAC, histological chorioamnionitis, admission to the neonatal intensive care unit (NICU), composite neonatal morbidity and spontaneous delivery within 48 h, 7 days, and < 32 weeks and < 35 weeks, as well as the examination-to-delivery interval. Proportions were compared using Chi-square or Fisher's exact tests. Stepwise logistic regression analysis was performed to determine the relationship between the presence of 'sludge' and several potential explanatory variables (cervical length < 15 mm, cervical funneling, cervical dilatation by digital examination, gestational age at the time of ultrasound examination, and vaginal bleeding in the index pregnancy). Survival analysis was performed to assess the examination-to-delivery interval according to the presence or absence of AF 'sludge'.

RESULTS

Clinical characteristics of the study population

Eighty-four patients met the entry criteria for preterm labor. The prevalence of AF 'sludge' was 1% (3/298) among uncomplicated pregnancies and 22.6% (19/84)

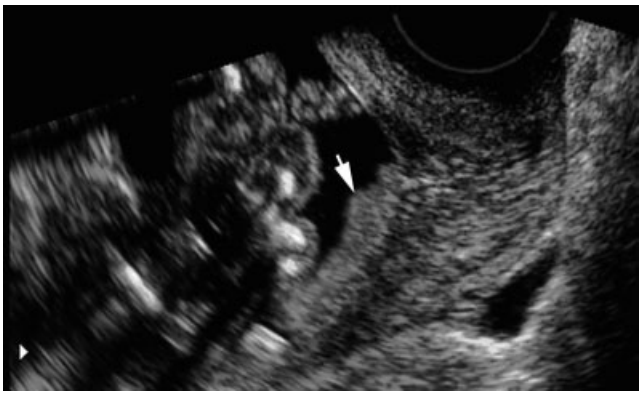

Figure 1 Aggregates of particulate matter (amniotic fluid 'sludge') (arrow) are seen in the proximity of the internal cervical os in a patient with preterm labor and intact membranes.

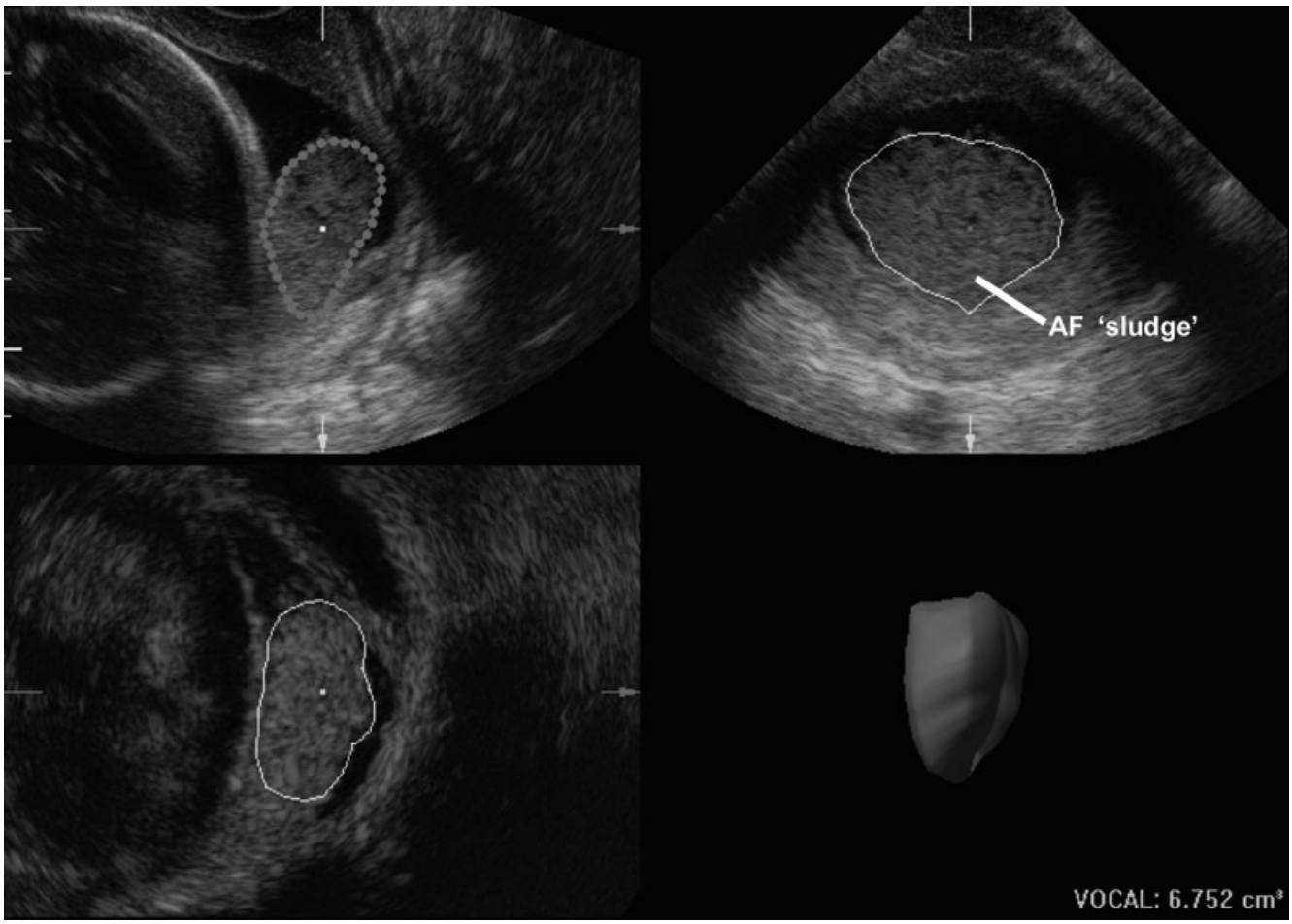

Figure 2 Three-dimensional multiplanar display and volume measurement with the VOCAL technique of amniotic fluid 'sludge' in a patient with preterm labor and intact membranes with positive amniotic fluid culture for *Fusobacterium nucleatum*.

among patients with preterm labor and intact membranes. Table 1 describes the demographic characteristics of the patients with preterm labor and intact membranes according to the presence or absence of AF 'sludge'. There were no significant differences in the ethnicity, parity, previous preterm delivery, cervical cerclage in the index pregnancy or use of tocolysis between patients with and without 'sludge'. Patients with 'sludge' had significantly lower gestational age at examination, gestational age at delivery and lower birth weight compared to those without 'sludge' (Table 2). A higher proportion of women with 'sludge' had a history of vaginal bleeding during the index pregnancy compared to those without AF 'sludge', but the frequency of vaginal spotting at presentation or discolored AF at amniocentesis was not different between the two groups in question (Table 2). There was no significant correlation between the AF 'sludge' volume and the WBC count in the AF (Figure 2).

Table 1 Demographic characteristics of patients with preterm labor and intact membranes according to the presence of amniotic fluid 'sludge'

Demographic characteristic	*No 'sludge'* (n = *65*)	*'Sludge' present* (n = *19*)	P
Maternal age (years)	24.2 ± 6	23.1 ± 6.8	NS
Race			
African–American	93.8 (61/65)	84.2 (16/19)	NS
Caucasian	4.6 (3/65)	15.8 (3/19)	NS
Other	1.5 (1/65)	0.0	NS
Parity			
Nulliparous	37.5 (24/64)	42.1 (8/19)	NS
Parous	62.5 (40/64)	57.9 (11/19)	NS
Prior preterm delivery	32.3 (21/65)	15.8 (3/19)	NS

Data are expressed as percentage (number) or mean ± SD. NS, not significant.

Prevalence of outcome variables among patients with preterm labor and intact membranes

Cervical length ⩽ 15 mm was present in 58.3% (49/84) of the patients. The prevalence of MIAC and histological chorioamnionitis was 12.1% (7/58) and 32.9% (25/76), respectively, and the rate of spontaneous preterm delivery within 48 h, 7 days, and < 32 weeks and < 35 weeks was 13.6% (8/59), 28.8% (17/59), 39.5% (17/43), and 50.8% (30/59), respectively.

Relationship between AF 'sludge' and a positive AF culture and histological chorioamnionitis

Patients with AF 'sludge' had a higher frequency of positive AF cultures [(33.3% (6/18) vs. 2.5%

Table 2 Clinical characteristics of patients with preterm labor and intact membranes according to the presence of amniotic fluid 'sludge'

Clinical characteristic	*No 'sludge'* (n = *65*)	*'Sludge' present* (n = *19*)	P
Gestational age at examination (weeks)	29.9 ± 3.5	26 ± 3.8	< 0.001*
Gestational age at delivery (weeks)	35.6 ± 3.9	27.4 ± 4.8	< 0.001*
Birth weight (g)	2446 ± 747	1081 ± 700	< 0.001*
Cerclage in the index pregnancy	6.2 (4/65)	21.1 (4/19)	NS
Vaginal spotting in the index pregnancy	23.1 (15/65)	15.8 (3/19)	NS
Vaginal bleeding in the index pregnancy	4.6 (3/65)	31.6 (6/19)	0.004*
Discolored amniotic fluid	37.9 (11/29)	35.7 (5/14)	NS
Cervical length			
< 15 mm	47.7 (31/65)	94.7 (18/19)	< 0.001*
< 25 mm	75.4 (49/65)	100.0 (19/19)	0.03*
Tocolysis (magnesium sulfate)	73.0 (46/63)	73.3 (11/15)	NS
Subsequent preterm PROM in the index pregnancy	16.9 (11/65)	26.3 (5/19)	NS

Data are expressed as percentage (number) or mean ± SD. *$P < 0.05$. NS, not significant; PROM, premature rupture of membranes.

Table 3 Outcome variables according to the presence of amniotic fluid 'sludge'

Outcome variable	*No 'sludge'* (n = *65*)	*'Sludge' present* (n = *19*)	P
Clinical chorioamnionitis	1.5 (1/65)	10.5 (2/19)	NS
Positive amniotic fluid cultures	2.5 (1/40)	33.3 (6/18)	0.003*
Histological chorioamnionitis	19.0 (11/58)	77.8 (14/18)	< 0.001*
Composite of neonatal morbidity	13.8 (9/65)	36.8 (7/19)	0.04*
Admission to NICU	12.9 (8/62)	64.3 (9/14)	< 0.001*
Perinatal death	4.6 (3/65)	36.8 (7/19)	0.001*
Spontaneous delivery			
Within 48 h	4.4 (2/45)	42.9 (6/14)	0.001*
Within 7 days	15.6 (7/45)	71.4 (10/14)	< 0.001*
< 32 weeks	25.8 (8/31)	75.0 (9/12)	0.005*
< 35 weeks	37.8 (17/45)	92.9 (13/14)	< 0.001*

Data are expressed as percentage (number). *$P < 0.05$. NICU, neonatal intensive care unit; NS, not significant.

(1/40), $P = 0.003$] and histological chorioamnionitis [77.8% (14/18) vs. 19% (11/58), $P < 0.001$] than those without AF 'sludge' (Table 3). Stepwise logistic regression analysis indicated that the presence of AF 'sludge' was an independent factor for the occurrence of positive AF culture and histological chorioamnionitis (Table 4). Microorganisms isolated from the AF of patients with 'sludge' included *Ureaplasma urealyticum* ($n = 1$), *Fusobacterium nucleatum* ($n = 1$), *Candida albicans* ($n = 1$), *Peptostreptococcus* spp. ($n = 1$), Group B streptococci ($n = 1$) and *Gardnerella vaginalis* ($n = 1$). One patient without 'sludge' had a positive AF culture for *Acinetobacter* spp. and *Ureaplasma urealyticum*.

Table 4 Stepwise logistic regression analysis of the presence of amniotic fluid 'sludge' after controlling for covariates as an explanatory variable for the occurrence of outcome variables

Outcome variable	*OR*	*95% CI*	P
Positive amniotic fluid cultures	19.2	1.14–332	0.04*
Histological chorioamnionitis	8.3	1.3–50.9	0.02*
Admission to NICU	2.9	0.5–16.8	NS
Composite neonatal morbidity	0.85	0.16–4.6	NS
Perinatal death	3.3	0.3–40.5	NS
Spontaneous delivery			
Within 48 h	19.6	1.5–257.4	0.02*
Within 7 days	11.7	1.7–81.6	0.01*

*$P < 0.05$. NICU, neonatal intensive care unit; NS, not significant; OR, odds ratio.

Relationship between AF 'sludge' and neonatal outcome

A higher proportion of neonates born to patients with AF 'sludge' was admitted to the NICU [64.3% (9/14) vs. 12.9% (8/62), $P < 0.001$], had a composite neonatal morbidity [36.8% (7/19) vs. 13.8% (9/65), $P = 0.04$] and died in the perinatal period [36.8% (7/19) vs. 4.6% (3/65), $P = 0.001$] than those born to patients without 'sludge'. Stepwise logistic regression analysis indicated that the presence of AF 'sludge' was not an independent factor for the occurrence of these outcome variables (Table 4). As expected, the regression analysis indicated that gestational age at examination was the most important determinant for perinatal mortality.

Relationship between AF 'sludge' and impending preterm delivery

A higher proportion of patients with AF 'sludge' had spontaneous delivery within 48 h [42.9% (6/14) vs.

Table 5 Diagnostic indices of amniotic fluid 'sludge'

Outcome variable	*Prevalence (% (number))*	*Sensitivity (%)*	*Specificity (%)*	*PPV (%)*	*NPV (%)*	*LR (+)*	*LR (−)*
Positive amniotic fluid cultures	12.1 (7/58)	86	76	33	98	3.6	0.19
Histological chorioamnionitis	32.9 (25/76)	56	92	78	81	7.1	0.48
Spontaneous delivery							
Within 48 h	13.6 (8/59)	75	84	43	96	4.8	0.30
Within 7 days	28.8 (17/59)	59	90	71	84	6.2	0.46

LR, likelihood ratio; NPV, negative predictive value; PPV, positive predictive value.

4.4% (2/45), $P = 0.001$], within 7 days [71.4% (10/14) vs. 15.6% (7/45), $P < 0.001$], < 32 weeks [75% (9/12) vs. 25.8% (8/31), $P = 0.005$] and < 35 weeks [92.9% (13/14) vs. 37.8% (17/45), $P < 0.001$] than those without AF 'sludge'. Stepwise logistic regression analysis indicated that the presence of 'sludge' was an independent factor associated with the likelihood of spontaneous delivery within 48 h and 7 days, but not < 32 weeks or < 35 weeks (Table 4). The diagnostic indices of the AF 'sludge' are displayed in Table 5. Survival analysis demonstrated that patients with AF 'sludge' had a shorter examination-to-delivery interval compared to those without AF 'sludge' [AF 'sludge' median, 1 (IQR, 1–5) days vs. no AF 'sludge' median, 33 (IQR, 18–58) days, $P < 0.001$] (Figure 3). Cox regression analysis showed similar results after controlling for cervical length < 15 mm, gestational age at examination, and positive amniotic fluid culture (Hazard ratio : 0.19, CI 95% 0.08–0.46).

DISCUSSION

Our results indicate that AF 'sludge' during transvaginal examination of the cervix is present in 22.6% of patients with preterm labor and intact membranes, and is a risk factor for MIAC, histological chorioamnionitis and impending preterm delivery.

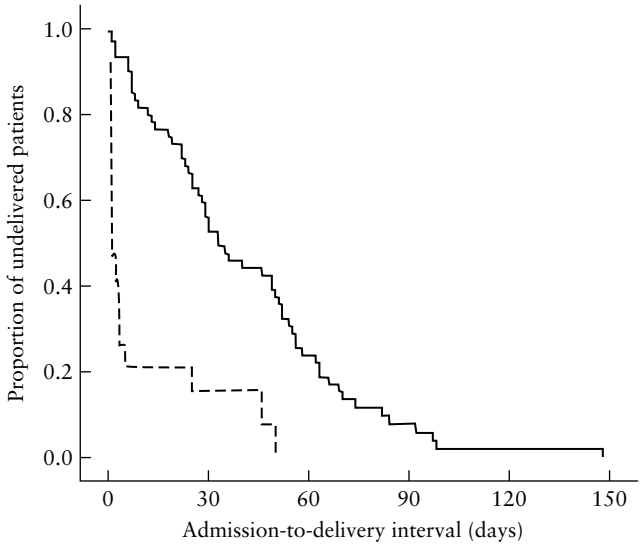

Figure 3 Survival curve of the admission-to-delivery interval (days) according to the presence of 'sludge' (Kaplan–Meier with log rank test, $P < 0.001$). Patients with amniotic fluid 'sludge' (dashed line) had a shorter examination-to-delivery interval compared to those without 'sludge' (solid line). [AF 'sludge' median 1 (IQR, 1–5) days vs. no AF 'sludge' median, 33 (IQR, 18–58) days, $P < 0.001$].

Aggregates of hyperechogenic particulate matter in the gallbladder of adult patients have been described as biliary 'sludge'[18,19]. This sonographic finding is characterized by the presence of low-level echoes that layer in the dependent portion of the gallbladder without acoustic shadowing[19], and is associated with ascending microbial invasion of the gallbladder[20]. Indeed, biliary 'sludge' is considered a risk factor for positive bile cultures obtained by percutaneous aspiration of the gallbladder[21]. Moreover, the presence of bacterial biofilms in the gallbladder has been proposed to be a key event in the formation of biliary 'sludge'[22].

Microbial invasion of the amniotic cavity is generally prevented by components of the innate and adaptive immune system, including the cervical epithelium[23] and mucus plug[24–27], chorioamniotic membranes[28,29] and cells of the decidua, amnion and chorion, including neutrophils, macrophages, natural killer cells and throphoblast[30,31]. However, the integrity of the chorioamniotic membranes is not sufficient to prevent MIAC. Indeed, micro-organisms have been isolated from the AF of asymptomatic patients at the time of genetic amniocentesis[32–34] in 12.8% (379/2963)[35] of patients with preterm labor and intact membranes and in 18.8% (17/90)[36] of patients in labor at term. Thus, these micro-organisms can penetrate intact membranes[37].

The observation that patients with preterm labor and intact membranes with 'sludge' are more likely to have microbiological and histological evidence of MIAC and impending preterm delivery is novel. During MIAC, microbial proliferation may be prevented by a local inflammatory response elicited by cytokines[38–40], chemokines[41], matrix degrading enzymes[42–46], antimicrobial peptides[47,48] and cells of the innate immune system[49,50]. Micro-organisms may protect themselves from these host defense mechanisms by producing and embedding themselves in matrices of polymeric compounds, also known as bacterial biofilms[51]. Evidence in favor of this view includes: (1) bacteria can remain viable within a biofilm despite elevated concentrations of proinflammatory cytokines, including IL-1β, IL-12 and interferon-γ[51], and (2) human leukocytes can penetrate bacterial biofilms *in vitro* but are not able to phagocyte the embedded bacteria[51]. Thus, the possibility that AF

'sludge' may represent clusters of bacterial biofilms and inflammatory cells should be considered.

It is possible that aggregates of exfoliated cells from the fetal digestive, respiratory and urinary tracts, amniotic membranes, fetal skin and umbilical cord[52] may also contribute to the presence of AF 'sludge' and participate in the host response during MIAC. Indeed, it has been proposed that exfoliated fetal skin cells account for the antimicrobial properties of the vernix caseosa, where the presence of several antimicrobial peptides, including α-defensins, cathelicidin (LL-37), psoriasin and ubiquitin, has been described[53]. In contrast, our results indicate that debris from intra-amniotic bleeding in the index pregnancy may not contribute to the AF 'sludge'. Indeed, the proportion of discolored AF, an index of earlier intra-amniotic bleeding[54–56], was not different between the study groups. Moreover, vaginal bleeding, also associated with discolored amniotic fluid[57], was included as a confounding variable in the logistic regression analysis, which indicated that AF 'sludge' is an independent explanatory variable for the occurrence of intra-amniotic infection and histological chorioamnionitis.

An inherent limitation of this study is its retrospective nature. However, 68% (57/84) of cases were examined with 3D ultrasound, and thus we were able to examine not only the pictures taken by the sonographer at the time of the examination but also the volume dataset. We were not able to identify cases in which AF 'sludge' could be seen in a parasagittal scan that did not include the endocervical canal. However, this possibility remains and only further studies will clarify this issue.

Collectively, our observations indicate that the sonographic finding of AF 'sludge' is associated with microbiological and histological evidence of intra-amniotic infection and impending preterm delivery. We propose that this sonographic sign may identify patients at risk for MIAC, who in turn are at risk for preterm delivery and short- and long-term complications such as cerebral palsy and chronic lung disease.

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