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The Diagnostic Performance of the beta-Glucan Assay in the Detection of Intra-amniotic Infection with *Candida* species

Percy Pacora, MD^{1,2}, Roberto Romero, MD, D.Med.Sci^{1,3,4,5}, Offer Erez, MD^{1,2}, Eli Maymon, MD^{1,2}, Bogdan Panaitescu, MD, PhD^{1,2}, Juan Pedro Kusanovic, MD^{6,7}, Adi L. Tarca, PhD^{1,2}, Chaur-Dong Hsu, MD, MPH², and Sonia S. Hassan, MD^{1,2}

¹Perinatology Research Branch, Division of Obstetrics and Maternal-Fetal Medicine, Division of Intramural Research, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, U.S. Department of Health and Human Services, Bethesda, Maryland, and Detroit, Michigan, USA

²Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, Michigan, USA

³Department of Obstetrics and Gynecology, University of Michigan, Ann Arbor, Michigan, USA

⁴Department of Epidemiology and Biostatistics, Michigan State University, East Lansing, Michigan, USA

⁵Center for Molecular Medicine and Genetics, Wayne State University, Detroit, Michigan, USA

⁶Center for Research and Innovation in Maternal-Fetal Medicine (CIMAF), Department of Obstetrics and Gynecology, Sótero del Río Hospital, Santiago, Chile

⁷Division of Obstetrics and Gynecology, Faculty of Medicine, Pontificia Universidad Católica de Chile, Santiago, Chile

Abstract

INTRODUCTION—A bioassay based on the detection of beta-glucan, a constituent of the cell wall of fungi, has been successfully used to diagnose fungal infections in a variety of biological fluids but not yet in the amniotic fluid.

OBJECTIVE—To determine the diagnostic performance of a beta-glucan bioassay in the detection of *Candida* species in the amniotic fluid.

METHODS—The study population comprised women who had a singleton pregnancy without congenital or chromosomal abnormalities, who experienced preterm labor or preterm prelabor rupture of the fetal membranes, and who underwent transabdominal amniocentesis for clinical indications. Samples of amniotic fluid were cultured for aerobic, anaerobic, genital mycoplasma, *Candida* species and assayed for beta-glucan using the (1→3)-beta-D-glucan-specific *Limulus* amoebocyte lysate test (beta-glucan assay) in all cases. Amniotic fluid interleukin (IL)-6 assay results were also available for all cases. The beta-glucan assay takes about one hour to run: a

concentration > 80 pg/mL was considered positive for fungi. Sterile intra-amniotic inflammation of the amniotic cavity was defined by the presence of an amniotic fluid IL-6 concentration ≥ 2.6 ng/mL and a negative amniotic fluid culture.

RESULTS—1) One hundred and ninety-seven (197) women met the study criteria, of whom 58 (29.4%) had an intrauterine contraceptive device (IUD) in place; 2) twenty (10.2%) women had a culture of proven intra-amniotic *Candida* species-related infection, 19 of whom had a positive beta-glucan assay [sensitivity, 95% (19/20; 95% confidence interval (CI): 75.1%–99.9%]); and 3) the specificity of the beta-glucan assay was 75.1% [133/177; 95% CI: 68.1%–99.9%]. It was affected by the presence of non-fungal intra-amniotic infections and an IUD, but not by the presence of sterile intra-amniotic inflammation, and there was a significant interaction between the presence of an IUD and non-fungal intra-amniotic infections (estimated for the interaction effect = 2.1923, p value = 0.026). The assay's specificity was reduced when non-fungal intra-amniotic infections were diagnosed but only in women who did not have an IUD.

Among women without IUD, the assay's specificity was 91.4% (117/128); it was 93% (106/114) for those without intra-amniotic infection, and 78.6% (11/14) for those with a non-fungal intra-amniotic infection; the difference was not significant (p = 0.09). Among women with IUD, the assay's specificity was 32.7% (16/49); 42.9% (9/21) for those with a non-fungal intra-amniotic infection, and 25% (7/28) for those without intra-amniotic infection, and the difference was significant (p = 0.03).

CONCLUSIONS—The beta-glucan assay is a sensitive, rapid, point-of-care test used to diagnose intra-amniotic *Candida* species-related infection, and it has a high specificity in pregnant women who did not have an IUD in place.

Keywords

Amniotic fluid; *Candida albicans*; fetus; newborn; pregnancy

Introduction

Intra-amniotic fungal infection is often associated with early preterm birth (1–5), fetal death (6–11), and adverse neonatal outcome (12–20). The most common fungus isolated from the amniotic fluid of women with spontaneous preterm parturition [preterm labor with intact membranes or preterm prelabor rupture of the fetal membranes (preterm PROM)] is *Candida albicans* (3, 5, 21–26); additionally, *Candida parapsilosis* (27), *Candida tropicalis* (7, 28–30), and *Candida glabrata* (11, 31, 32) have been isolated from the amniotic fluid and associated with fetal infection. In a meta-analysis (21), the overall frequency of *Candida* species-related infection found in the amniotic fluid of patients with preterm labor (22, 23, 32–42) and preterm PROM (24, 25, 28, 30, 43–46) was 0.6%.

The treatment of fungal intra-amniotic infections improves neonatal outcome (47–49), and it has been proposed that its efficacy can be expected to increase the earlier the infection is diagnosed and treatment begins (47–49). However, the results of traditional cultivation techniques may take from 48 hours up to 14 days to allow the recovery of slowly growing fungi (50, 51). Nevertheless, fungal infection can be diagnosed more rapidly with a bioassay (herein referred to as the beta-glucan assay) based on the identification of (1 $\rightarrow$ 3)-beta-D-

glucan, a component of the cell walls in many fungi. This bioassay has been shown to be effective in diagnosing fungal infections caused by *Candida* (52–54), *Aspergillus* (52, 53), *Cryptococcus* (52), *Trichosporon* (52, 53), *Pneumocystis carinii*/*Pneumocystis jirovecii* (55, 56), *Fusarium* (53, 54), *Trichosporon beigeli* (57), *Saccharomyces cerevisiae* (57), and *Acremonium* (57). The overall sensitivity and specificity of the beta-glucan assay for the detection of systemic fungal infection reported in a meta-analysis of 16 studies (2,979 subjects) were 76.8% and 85.3%, respectively (58).

The beta-glucan assay has been used to evaluate maternal serum (53, 54, 58), urine (59), cerebro-spinal (60–64) and peritoneal (65, 66) fluids, yet it has not been used to evaluate the amniotic fluid for the rapid diagnosis of fungal intrauterine infections. Therefore, the objective of this study was to determine the diagnostic performance of a beta-glucan bioassay for the detection of intra-amniotic fungal infection of women who had or did not have an intrauterine contraceptive device (IUD) in place during an episode of spontaneous preterm parturition.

Materials and Methods

Study population

The study population comprised pregnant women who met the following criteria: a singleton pregnancy without congenital or karyotypic abnormalities; preterm labor and/or preterm PROM evaluated with transabdominal amniocentesis; and a sufficient quantity of stored amniotic fluid (at least 0.5 mL) available to run a beta-glucan assay. Many of the samples had previously been used to study the biology of inflammation, hemostasis, angiogenesis, and growth-factor concentrations.

The study population comprised two separate groups culled from pregnant women with or without an IUD who had preterm parturition and who were enrolled between October 1990 and October 2000 at Hutzel Women's Hospital, Detroit, Michigan, USA, or at Sótero del Río Hospital, Puente Alto, Chile, in a prospective study protocol approved by the Institutional Review Boards of Wayne State University; the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, National Institutes of Health, U.S. Department of Health and Human Services (NICHD/NIH/DHHS); and Sótero del Río Hospital. The patients provided written informed consent.

Clinical definitions

Gestational age was determined by the last menstrual period and confirmed by ultrasound examination or by ultrasound examination alone if the sonographic determination of gestational age was inconsistent with menstrual dating by more than one week.

Preterm parturition included spontaneous preterm labor and preterm PROM. Spontaneous preterm labor was defined as the presence of at least two regular uterine contractions occurring every 10 minutes, associated cervical effacement and shortening, and intact fetal membranes in patients with a gestational age between 20 and 36+ 6/7 weeks (67–69). Preterm prelabor rupture of the fetal membranes was defined as follows: rupture < 37 weeks of gestation occurring at least one hour before the presence of labor (uterine contractions

and cervical effacement and dilatation), diagnosed by the combination of a speculum examination confirming the pooling of amniotic fluid in the vagina, a positive nitrazine test, and a positive ferning test (68, 70–72).

Microbial invasion of the amniotic cavity (MIAC) or intra-amniotic infection was defined as a positive amniotic fluid culture for microorganisms using traditional cultivation techniques (22, 25, 73–81). *Candida* species-related intra-amniotic infection was defined as a positive amniotic fluid culture for *Candida* species using traditional cultivation techniques. Non-fungal intra-amniotic infection was defined as a positive amniotic fluid culture for microorganisms other than *Candida* species or other fungi. Intra-amniotic inflammation was defined as an amniotic fluid interleukin (IL)-6 concentration ≥ 2.6 ng/mL (74, 75, 82–91). Sterile intra-amniotic inflammation was defined as a negative amniotic fluid culture and an amniotic fluid IL-6 concentration ≥ 2.6 ng/mL (74, 75, 82, 83, 85–92).

Amniotic fluid collection and storage—Amniotic fluid specimens were collected by transabdominal amniocentesis for medical indications, such as karyotyping studies and fetal lung maturity, or to rule out intra-amniotic infection. These specimens were collected transabdominally under ultrasound guidance with a sterile aseptic technique using a 22-gauge needle. The first 1–2 mL of amniotic fluid were discarded to avoid maternal cell contamination. Amniotic fluid specimens were transported in a capped sterile syringe to the clinical laboratory at the respective hospitals.

Amniotic fluid specimens were cultured for aerobic and anaerobic bacteria, including *Mycoplasma* and *Ureaplasma*. Specifically, 1–2 drops of amniotic fluid were used to inoculate chocolate agar, trypticase soy agar with 5% sheep blood, and MacConkey agar culture media. Aerobic plates were incubated at 35° C in an 8% CO₂ chamber. Anaerobic plates were incubated at 35° C in an anaerobic chamber containing an atmosphere of 5% CO₂, 10% hydrogen, and 85% nitrogen. All plates were incubated for a total of four days.

Amniotic fluid not required for clinical purposes was centrifuged to remove cellular and particulate matter. Aliquots of amniotic fluid were stored at –70° C until analysis.

The beta-glucan assay test

The (1→3)-beta-D-glucan-specific *Limulus* amoebocyte lysate (LAL) assay (beta-glucan assay; Fungitell®, Associates of Cape Cod, East Falmouth, MA, USA) is an aqueous extract, derived from the amoebocytes (blood cells) of the horseshoe crab (*Limulus polyphemus*), the most widely used method to measure (1→3)-beta-D-glucan in human serum (50, 53). The assay is based upon modification of the LAL pathway. This reagent, modified to eliminate Factor C, bypasses the activated factor B, and reacts only to (1→3)-beta-D-glucan; it does not react to other polysaccharides, including beta-glucans with different glycosidic linkages. When (1→3)-beta-D-glucan is present in a sample, it activates factor G, a serine protease zymogen. The activated Factor G converts the inactive pro-clotting enzyme to the active clotting enzyme (93) that, in turn, cleaves para-nitroaniline (pNA) from the chromogenic peptide substrate Boc-Leu-Gly-Arg-pNA, creating a chromophore that absorbs at 405 nm. The reagent is used in the beta-glucan kinetic assay to detect the rate of optical density increase produced by a sample. This rate is interpreted

against a standard curve to produce an estimate of (1→3)-beta-D-glucan concentration in the sample (93). Beta-glucan activity was calibrated with pure pachyman, a linear beta-glucan. All assays were performed in triplicate using a microtiter plate.

Amniotic fluid samples (5 µL) were pretreated for 10 minutes at 37° C with an alkaline reagent (20 µL; 0.125 M KOH/0.6 M KCl) to inactivate serine proteases as well as inhibitors in human serum and to enhance the reactivity to activated factor G (94, 95). After adding the beta-glucan assay reagent, the microtiter plate was inserted into a ThermoMax plate reader (Molecular Devices LLC, Sunnyvale, CA, USA), pre-incubated to 37° C, and a kinetic assay was run using SoftMax Pro software (Molecular Devices). An amniotic fluid beta-glucan concentration ≥ 80 pg/mL was interpreted as a positive result; an amniotic fluid beta-glucan concentration < 79 pg/mL was considered a negative test.

Statistical Analysis

The Kruskal-Wallis test was used to compare continuous variables (birthweight and gestational age at amniocentesis) as these were not normally distributed by the Shapiro-Wilk test. The Chi-square and Fisher's exact tests were used for comparison between categorical variables.

A log linear model was used to fit a multilayer contingency table of the data to determine the factors that affected assay specificity and the interactions between those factors. The statistical package used was SPSS Version 19 (IBM Corporation, Armonk, New York, USA). A p-value of <0.05 was considered statistically significant.

Results

Characteristics of the study population

One hundred and ninety-seven (197) women met the study criteria: the demographic characteristics of these women are shown in Table 1. Fifty-eight (29.4%) nulliparous women had an IUD in place: 57 (98.3%) were from Chile and one (1.7%) was from Detroit. All women who did not have an IUD in place were recruited from the Detroit area: 90.6% (126/139) of women were of African-American descent and 41% (57/139) were nulliparous (Table 1). Women with an IUD were more frequently Hispanic ($p<0.001$), and women without IUD were more frequently African-American ($p<0.001$). Women who had an IUD in place also underwent amniocentesis at an earlier gestational age and had a lower median birthweight than women who did not have an IUD in place (Table 1).

The frequency of intra-amniotic infection and fungal intra-amniotic infection

Fifty-five of 197 (27.9%) women had MIAC (Figure 1): 35 (17.8%) had a non-fungal intra-amniotic infection, 15 (7.6%) had *Candida* species alone -related intra-amniotic infections, and 5 (2.5%) had mixed *Candida species* and non-fungal intra-amniotic infections. Forty of 197 (20.3%) women had inflammation of the amniotic cavity alone without infection (sterile intra-amniotic inflammation). *Candida* species were the only fungi isolated.

Microbial invasion of the amniotic cavity was significantly more frequent in women with an IUD than in those without an IUD [51.7% (30/58) versus 18.0% (25/139); $p < 0.001$]. Non-

fungal intra-amniotic infections and intra-amniotic infection by *Candida* species only were significantly more frequent in women who had an IUD in place than in those who did not (Non-fungal intra-amniotic infection : with IUD, 36.2 % (21/58) versus without IUD, 10.1 % (14/139); $p < 0.001$); intra-amniotic-infection by *Candida* species only : with IUD, 13.8% (8/58) versus without IUD, 5.0% (7/139); $p=0.04$) (Figure 2).

Candida intra-amniotic infections and sterile inflammation of the amniotic cavity were also more frequent among women who had an IUD in place, but the differences were not statistically significant [Candida intra-amniotic infection: with IUD, 15.5% (9/58) versus without IUD, 7.9% (11/139), $p = 0.12$; Sterile intra-amniotic inflammation: with IUD, 27.6% (16/58) versus without IUD, 17.3% (24/139), $p = 0.12$] (Figure 2). Only one in five women with an IUD in place had a sterile intra-amniotic cavity without inflammation, whereas the intra-amniotic cavity was sterile without inflammation in almost two-thirds of the women without an IUD in place ([20.7% (12/58) versus 64.7% (90/139), $p < 0.001$] (Table 1).

Diagnostic accuracy of the beta-glucan assay

The beta-glucan assay was positive in 19 of the 20 cases for which *Candida* species were cultured from the amniotic fluid specimens (sensitivity, 95%) (Tables 2 and 3). The overall specificity of the beta-glucan assay was 75.1% (133/177) (Tables 2 and 4).

The specificity of the assay was affected by the presence of an IUD and non-fungal intra-amniotic infection but not by the presence of sterile inflammation; there was an interaction between the presence of an IUD and non-fungal infection (estimated for the interaction effect = 2.1923, $p = 0.026$) (Tables 4 and 5).

Non-fungal intra-amniotic infection lowered the assay's specificity but only for women who did not have an IUD in place. Although non-fungal intra-amniotic infections were more common in women who had an IUD in place, non-fungal intra-amniotic infections did not lower the assay's specificity when compared to women with an IUD in place but who did not have a non-fungal intra-amniotic infection.

The specificity of the beta-glucan assay for women who did not have an IUD in place was 91.4% (117/128); it was 93% (106/114) for those who did not and 78.6% (11/14) for those who did have a non-fungal intra-amniotic infection; the difference was not statistically significant ($p = 0.09$) (Table 5). The assay's specificity for women who had an IUD in place was 32.7% (16/49); it was 42.9% (9/21) for those who did have and 25% (7/28) for those who did not have a non-fungal intra-amniotic infection; the difference was statistically significant ($p = 0.03$) (Table 5). The probability of culture-positive intra-amniotic *Candida* species-related infection in a woman without an IUD in place who had a positive beta-glucan assay was 47.6% (10/21) and 21.4% (9/42) in a woman with an IUD in place (Table 3).

Discussion

This is the first study to report the use of a beta-glucan assay to diagnose fungal intra-amniotic infection. The principal findings of the study are as follows: 1) the beta-glucan assay detected *Candida* species-related intra-amniotic infection with 95% sensitivity (95% CI, 75.1–99.9); 2) the assay's specificity was 75.1% (95% CI, 68.1–81.3%) for the entire study population, 91.4% (95% CI, 85.1–95.6%) for women without an IUD, and 32.7% (95% CI, 20.0–47.5%) for those with an IUD; and 3) the assay's specificity was affected by the presence of non-fungal intra-amniotic infection and an IUD but not by sterile inflammation of the amniotic cavity; there was a significant interaction between the presence of an IUD and non-fungal intra-amniotic infection on the assay's specificity. Non-fungal infection of the amniotic cavity decreased the assay's specificity but only in women who did not have an IUD. The presence of an IUD significantly decreased the specificity of the assay.

Intra-amniotic *Candida* infection

Candida species colonize the vagina in at least 20% of all women (96), and the frequency rises to 30% in pregnant women (97). Candidiasis during pregnancy may be associated with an increased risk of pregnancy complications such as preterm PROM and poor pregnancy outcome (47, 98–100). Moreover, there is evidence that the eradication of *Candida* species in pregnancy may reduce the risk of preterm birth (101). Despite the clinical frequency of *Candida* species in the lower genital tract, they appear to be infrequent invaders of the amniotic cavity based on their low prevalence in the amniotic fluid (21).

In the current study, the rate of intra-amniotic *Candida* species-related infection was 7.9% (11/139) for pregnant women without an IUD and 15.5% (9/58) for pregnant women with an IUD in place. The association between the presence of an IUD and intra-amniotic infection with *Candida* species has been previously reported (1, 4, 102–106). Roque et al. (5) reported in a meta-analysis of 54 cases that 50% of patients who had an intra-amniotic infection with *Candida* species also had an IUD, and these factors are associated with severe infectious morbidity.

The IUD has the lowest failure rate (1%) of all contraception methods (107), and its use is recommended as a first-line contraceptive method for nearly all women, including adolescents and nulliparous women (108–110). Approximately 10% of North American women use an IUD as a contraceptive, and more than 60% of this group use the device for more than 48 months (111). However, pregnancy occurring with the presence of an IUD is associated with an increased rate of complications, e.g., intrauterine infection often due to fungi (1, 4, 102–105, 112), late spontaneous abortion (106, 113, 114), spontaneous preterm labor/birth (103, 113–120), histologic chorioamnionitis/funisitis (121), abruptio placentae (103), adverse neonatal outcome (5, 7, 8, 29, 104, 105, 112, 113, 115–120, 122–132), maternal sepsis (113, 116, 133), and even maternal death (134). In a large cohort study of 12,297 patients (103), 1.6% (196/12,297) conceived while using an IUD and 56.1% (110/196) of these pregnant women with an IUD delivered preterm. The prevalence of positive amniotic fluid cultures from patients who had an IUD in place was 45.9% (45/98), and *Candida* species-related intra-amniotic infection was present in 31% (14/45) of

pregnancies that occurred with an IUD, a rate five times higher compared to patients who conceived without an IUD (6.3%; 11/174) (103).

Antimicrobial properties of the amniotic fluid against fungi

Under normal circumstances, the amniotic fluid is sterile (135–139). Systematic investigations using cultivation and molecular microbiologic techniques have demonstrated that most women with a normal pregnancy outcome do not show evidence of bacteria (140, 141), viruses (142–144), or fungi (138) in the amniotic cavity. The absence of microorganisms is presumably accomplished by the presence of components of the innate immune system and antimicrobial peptides in the amniotic fluid (145).

The amniotic fluid concentration of antimicrobial peptides (e.g., bactericidal/permeability-increasing protein and calprotectin) increases with advancing gestational age, and their median concentration in the amniotic fluid at mid-trimester gestation is significantly reduced as compared to term gestation in normal pregnancies (145). In fact, amniotic fluid has antimicrobial properties against potential pathogenic microorganisms (146–152), and several studies demonstrated that human amniotic fluid from patients in their second and third trimesters of pregnancy can efficiently inhibit the growth of *Candida albicans* *in vitro* (146, 153, 154). Therefore, *Candida* species-related intra-amniotic infection in early pregnancy is more deleterious than during the third trimester of pregnancy (1–10, 100, 155, 156).

The current study reports that 25% (5/20) of the *Candida* species isolated in pregnancies with preterm labor were associated with other microorganisms. Additionally, our group previously reported that 4.8% (2/42) of the amniotic fluid samples from women with clinical chorioamnionitis at term was caused by *Candida* species (one identified as *Candida albicans* and the other as *Candida famata*) (75). Both cases indicated the presence of mixed flora: one was associated with Gram-negative bacilli and the other with *Staphylococcus epidermidis*, *Lactobacillus species*, and *Micrococcus luteus* (75). These two studies suggest that *Candida* species-related intra-amniotic infection is more frequently associated with other microorganisms observed in early preterm gestation. The impaired anti-microbial property of the amniotic fluid found early in pregnancy may be a common pathogenic mechanism for the presence of a concurrent *Candida* species-related intra-amniotic infection. Thus, early pregnancies associated with cervical incompetence (2), cervical cerclage (1, 2, 102, 157), use of an IUD (1, 4, 102, 103), *in vitro* fertilization (9, 31, 158, 159), premature rupture of the fetal membranes (31), and immunocompromised individuals (160) are at increased risk of *Candida* species-related intra-amniotic infection as a result of the combination of an impaired anti-microbial property of the amniotic fluid early in pregnancy and an ascending infection of *Candida* species through the uterine cervical canal and fetal membranes (161, 162).

Congenital candidiasis and fetal response to *Candida* species-related intra-amniotic infection

Although congenital candidiasis has been reported to be 0.8% (4/492), based on *Candida* species-related placental infection in a series of 494 random consecutive singleton deliveries (163), congenital candidiasis is more commonly associated with the systemic spread and

worsened outcome for preterm neonates compared to term neonates (156, 164). Systemic neonatal candidiasis is estimated to occur in 2% to 4% of neonates with very low birthweight (13, 14, 102, 147, 165–167). The prevalence of *Candida* species-related infection in early preterm neonates weighing less than 1500 grams (very low birthweight) was 4.8% (7/146) during the first 24 hours of life (16). Additionally, there is an inverse correlation between the incidence of *Candida* species-related infection and neonatal birthweight (168) and gestational age (156, 169).

Candida species-related intra-amniotic infection is associated with severe fetal inflammatory response and adverse pregnancy outcomes that include early preterm birth (1–5, 20) and fetal death (6, 9, 10, 155, 165, 170). Several experiments utilizing sheep models demonstrated the pathogenicity of *Candida albicans* in the amniotic fluid: 1) *Candida albicans* colonization of the amniotic cavity rapidly resulted in a robust intrauterine inflammation characterized by an increase in IL-1 β , IL-8 in the amniotic and lung fluids, fetal hypercortisolemia, leukopenia, and thrombocytopenia, and an elevated mRNA expression of IL-1 β , IL-8, IL-6, tumor necrosis factor-alpha (TNF- α), and monocyte chemoattractant protein (MCP)-1 in the fetal lung, skin, and membranes (171); 2) a *Candida albicans* intrauterine infection in early pregnancy causes systemic fetal candidiasis, an infection associated with robust systemic inflammatory response and progressive cardiac dysfunction (172, 173); 3) intra-amniotic exposure to *Candida albicans* provokes acute systemic and neuro-inflammatory responses with concomitant white-matter injury (174); and 4) *Candida albicans* colonization of the amniotic cavity causes fetal intestinal infection, mucosal injury, and inflammation (175). In humans, the information regarding the effect of fetal candidiasis is mainly derived from case reports and case series (1–5) of late abortions (6, 114), preterm labor (21, 33), fetal death, and clinical chorioamnionitis (20, 176).

Intra-amniotic infection with candidiasis may be treatable with anti-fungal medication *in utero*. Treatment with fluconazole, administered intra-amniotically or intraperitoneally, in an animal model was associated with a significant reduction in the fetal IL-6 concentration on Day 3 in comparison to the untreated group. Intra-amniotic exposure to *Candida albicans* did not attenuate fetal neuro-inflammation and white-matter injury but rather reduced fetal mortality (174). Maneenil et al. (177) demonstrated that the severe fetal inflammatory response caused by intra-amniotic infection with *Candida albicans* in fetal sheep was transiently decreased with the use of fluconazole and that maternal anti-fungal therapy may prevent fetal injury associated with *Candida* species-related intrauterine infection. These results are in agreement with three clinical reports showing that early diagnosis and maternal as well as intra-amniotic administration of anti-fungal treatment improved the neonatal outcome in cases with intra-amniotic candidiasis (47–49).

To overcome the delay of a diagnosis of intra-amniotic fungal infection, which usually takes 2 to 14 days (50, 51), the use of a simple test providing a rapid, easy-to-interpret result is recommended; such a point-of-care test requires low maintenance and is cost-effective (69, 70, 178–182). Amniotic fluid matrix metalloproteinase (MMP)-8 (83, 178, 183–191) and IL-6 (82, 137, 180–182, 192–224) have diagnostic and prognostic value in the identification of intra-amniotic inflammation, imminent spontaneous preterm delivery, acute inflammatory lesions of the placenta, and adverse neonatal outcome. Additionally, concentrations of

MMP-8 and IL-6 perform better than the amniotic fluid white blood cell count, glucose concentration, and Gram stain for the identification of intra-amniotic inflammation/infection (83, 178, 184, 200–202, 205).

The rapid-assay kits evaluating IL-6 and MMP-8 in the amniotic fluid are currently being introduced for the timely identification of intra-amniotic infection and inflammation (within 15 to 20 minutes) without the need of sophisticated laboratory equipment (179); both assays show the same sensitivity for the detection of intra-amniotic inflammation (85.7%; 18/210); however, the specificity of the rapid MMP-8 test was higher than that of the rapid IL-6 with a cutoff of 745 pg/mL in the identification of intra-amniotic inflammation (179). Moreover, patients with a positive MMP-8 rapid test who did not have intra-amniotic infection or inflammation detected by either a standard cultivation technique or an amniotic fluid white blood cell count delivered preterm and showed evidence of acute histologic chorioamnionitis (179). Currently, the beta-glucan assay may yield results in less than one hour; and due to its high sensitivity (95%) and specificity (75%), it may identify patients with *Candida* species-related intra-amniotic infection who would benefit from prompt anti-fungal treatment (47–49).

Collectively, this evidence suggests that intra-amniotic infection with *Candida* species carries a high rate of fetal/neonatal morbidity and mortality that may be prevented with early diagnosis and *in utero* treatment. The introduction of point-of-care tests specifically intended for the identification of *Candida* species may improve the early detection and the prompt treatment of *Candida* species-related intra-amniotic infection.

Performance indices of the beta-glucan assay

The finding that the beta-glucan assay utilized in this study has a sensitivity of 95% and a specificity of 75.1% for the detection of *Candida* species-related intra-amniotic infection in women with preterm parturition is novel. The assay's values of sensitivity and specificity are similar to those reported in the cerebro-spinal fluid of children with fungal meningitis (60). The diagnostic indices of the beta-glucan assay for fungal intra-amniotic infection were as good as or better than those reported for other biological fluids (54, 58, 225).

Ostrosky-Zeichner et al. (54) reported that the beta-glucan assay, using a cutoff of 80 pg/mL in the serum of 163 patients with invasive fungal infection, had a sensitivity of 64.4% and a specificity of 92.4% as well as a positive predictive value of 89% and a negative predictive value of 73%. Of the 107 patients with proven candidiasis, 77.6% had a positive beta-glucan assay; among 118 patients with a positive culture for fungi who were treated with anti-fungal medication, 69.5% had a positive beta-glucan test (54). Karageorgopoulos et al. (58) conducted a meta-analysis in which 594 of 2,979 patients had proven fungal infection in their serum. The authors reported a pooled sensitivity and specificity of the beta-glucan assay as 76.8% and 85.3%, respectively, and an average false-positive rate of 1% among 310 beta-glucan assays performed for healthy individuals (58). In another meta-analysis that included 15 studies, Lu et al. (226) found a sensitivity of 76%, a specificity of 85%, a positive likelihood ratio of 5.05, and a negative likelihood ratio of 0.28 for the beta-glucan assay in serum. To date, the best diagnostic performance of a beta-glucan assay, reported by Malani et al. (60), showed a sensitivity of 96% and a specificity of 95% to identify and

diagnose proven fungal meningitis as well as a sensitivity of 84% and a specificity of 95% for the diagnosis of probable or proven spinal or para-spinal fungal infection among 233 patients.

Furthermore, a multi-center prospective study conducted in South Africa included 72 neonates with clinically suspected late-onset sepsis who were also at high risk for fungaemia; Mackay et al. (227), using a beta-glucan assay at a level of 80 pg/ml, found its sensitivity and specificity to be 70.7% and 77.4% as well as positive and negative predictive values of 80.6% and 66.7%, respectively, for the diagnosis of invasive fungal disease in neonates.

Goudjil et al. (228) found that the beta-glucan concentration was higher in 18 cases with neonatal invasive *Candida* species-related infection as compared to 43 non-infected neonates (364 pg/mL versus 89 pg/mL; $p < 0.001$). The authors concluded that the optimal cut-off for distinguishing between non-infected and infected patients was 125 pg/mL (sensitivity, 84%; specificity, 75%) (228). A cutoff > 523 pg/ml for a beta-glucan assay of serum was reported for two low-birthweight neonates with a culture-proven *Candida parapsilosis* infection (229); another group of investigators utilized a cut-off > 200 pg/mL for a case series report on five preterm neonates with *Candida* species-related infections already proven by their positive blood cultures.(230). A retrospective study of 47 preterm neonates conducted by Cornu et al. (231) found that the median beta-glucan concentration was higher in 26 neonates with probable or proven invasive fungal infection versus 20 neonates without fungal colonization/infection [149 pg/ml (interquartile range (IQR): 85–364) versus 39 pg/ml (IQR: 20–94), $p < 0.001$]. A beta-glucan assay with a cutoff > 106 pg/ml had a sensitivity of 61.5% and a specificity of 81% for the diagnosis of invasive fungal infection; the beta-glucan assay concentration decreased with anti-fungal treatment. Therefore, the authors concluded that a beta-glucan assay may be useful for the early identification of invasive fungal infection and for monitoring the efficacy of anti-fungal therapy (231).

There are two likely explanations for why the assay's specificity was affected by non-fungal infections in the amniotic fluid and by the presence of an IUD. First, the beta-glucan test is very sensitive at detecting endotoxin in Gram-negative bacterial infections (232–235); therefore, a positive assay result for women who had a non-fungal infection was likely caused by the assay's cross-reactivity with other non-fungal microorganisms. The assay's specificity for women who did not have an IUD was 93% (106/114) in the absence of a non-fungal infection; that rate fell to 79% (11/14) if a non-fungal infection was present.

Second, IUDs are frequently contaminated with *Candida albicans* (236), and the longer an IUD has been in place, the more likely it is to be contaminated with *Candida* species (237, 238). *Candida* species, once attached to an IUD in the uterine cavity, become encapsulated within a protective extracellular matrix (biofilm form) that prevents detection of the flora when cultivation and molecular microbiological methods are used (239–246). However, the *Candida* species within the biofilm may secrete beta-glucan that then diffuses into the amniotic cavity and becomes detectable by the beta-glucan assay. This is the most likely explanation for the high “false-positive” rate of the beta-glucan assay for women who had an IUD as well as for the finding that the assay's specificity was lower in women who had a

non-fungal infection than in those who did not. As in women with an IUD, the frequency of occult *Candida* species infections may be higher in women who do not have a non-fungal infection than in those who do.

Candida species-related intra-amniotic infections were five times more common in the absence of a non-fungal infection in women who had an IUD in place [20% (8/36)] than in women who had a non-fungal intra-amniotic infection [4.5% (1/22)], although the difference did not reach statistical significance ($p = 0.13$, Fisher's exact test) (Tables 1 and 3). *Candida* species-related intra-amniotic infections were also significantly more common in the absence of a non-fungal intra-amniotic infection in women who had an IUD in place than in women who did not have an IUD [20% (8/36) versus 5.8% (7/121), $p = 0.007$]. Additional studies will be required to determine whether these associations are spurious or related to the protective biofilm that forms around an IUD, a factor that could decrease the probability of non-fungal intra-amniotic infections while at the same time causing occult *Candida* infections.

Strengths and limitations of the study

The strengths of this study are the large number of amniotic fluid samples that were assayed and the subsequent evaluation of factors affecting the assay's performance. The limitations of the study are the lack of information on the duration of each IUD's placement and the need for a biofilm study on the presence of *Candida* species inside of the patients' IUDs.

Conclusion

The beta-glucan assay, a sensitive, rapid method used to diagnose *Candida* species-related intra-amniotic infections, has high specificity and sensitivity rates for pregnant women who do not have an IUD in place.

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Figure 1.
The frequency of microbial invasion of the amniotic cavity (MIAC) in the study population.

Figure 2.

The frequency of sterile intra-amniotic inflammation and intra-amniotic infection by *Candida* species only, mixed flora (*Candida* species and non-fungal microorganisms), and non-fungal microorganisms in pregnant women with and without an intrauterine contraceptive device (IUD).

Table 1

Demographic and clinical characteristics of the study population.

	Pregnant women with an IUD (n=58)	Pregnant women without an IUD (n=139)	p value
Age (years)	29.0 (24.5–34.0)	23.0 (20.0–27.0)	<0.001
Body Mass Index (kg/m ²)	26.2 (23.7–29.8)	24.7 (21.1–32.4)	0.26
Gravidity	2.0 (1.0–3.0)	3.0 (2.0–4.0)	<0.001
Nulliparity	0 (0%)	57 (41.0%)	<0.001
Ethnicity			
African-American	1 (1.7%)	126 (90.6%)	<0.001
Caucasian	0 (0%)	10 (7.2%)	0.03
Hispanic	57 (98.3%)	1 (0.7%)	<0.001
Other	0 (0%)	2 (1.4%)	1.00
Gestational age at amniocentesis (weeks)	24.9 (21.9–29.5)	29.4 (24.6–32.0)	<0.001
Gestational age at delivery (weeks)	28.9 (24.9–33.2)	34.4 (28.4–37.9)	<0.001
Birthweight (kg)	1.51 (0.827–2.21)	2.29 (1.15–2.85)	0.01
Sterile intra-amniotic inflammation	16 (27.6%)	24 (17.3%)	0.12
Without intra-amniotic infection/inflammation	12 (20.7%)	90 (64.7%)	<0.001

IUD: Intrauterine contraceptive device; IL: interleukin.

Values are expressed as median (interquartile range) or number (percentage).

Candida intra-amniotic infection: Identification of *Candida* species in the amniotic fluid by culture.

Mixed infection intra-amniotic infection: Identification of *Candida* species and non-fungal microorganism(s) by culture.

Sterile inflammation: Amniotic fluid IL-6 2.6 ng/mL and negative amniotic fluid culture result.

Sterile without inflammation: Amniotic fluid IL-6 <2.6 ng/mL and negative amniotic fluid culture result.

The Mann Whitney *U* test was used for continuous variables; the chi-square and Fisher's exact tests for categorical variables.

Table 2

The diagnostic performance of a beta-glucan test for identifying intra-amniotic *Candida* infection

	Study population (n=197)		Women with IUD (n=58)		Women without IUD (n=139)	
	% (n)	95% CI	% (n)	95% CI	% (n)	95% CI
Frequency of <i>Candida</i> intra-amniotic infection						
Sensitivity	10.2% (20/197)	6.3%–15.2%	15.5% (9/58)	7.4%–27.4%	7.9% (11/139)	4.0%–13.7%
Specificity	95.0% (19/20)	75.1%–99.9%	100.0% (9/9)	63.4%–100.0%	90.9% (10/11)	58.7%–99.7%
Positive likelihood ratio	75.1% (133/177)	68.1%–81.3%	32.7% (16/49)	20.0%–47.5%	91.4% (117/128)	85.1%–95.6%
Negative likelihood ratio	3.8	2.9–5.0	1.5	1.2–1.8	10.6	5.8–19.2
Positive predictive value	0.1	0.01–0.5	0.0		0.1	0.02–0.7
Negative predictive value	30.2% (19/63)	24.7%–36.3%	21.4% (9/42)	18.3%–24.9%	47.6% (10/21)	33.4%–62.2%
	99.3% (133/134)	95.2%–99.9%	100.0% (16/16)		99.2% (117/118)	94.7%–99.9%

LEGEND. CI: confidence interval; IUD: intrauterine contraceptive device.

Beta-glucan assay results stratified by infection-inflammation status of the amniotic cavity, intra-amniotic infection, and the presence of an intrauterine contraceptive device (IUD).

Table 3

		Women with an IUD		Women without an IUD		Overall		Total
		Beta-glucan assay		Beta-glucan assay		Beta-glucan assay		
		Positive	Negative	Positive	Negative	Positive	Negative	
Infection-inflammation status of the amniotic cavity	-Intra-amniotic infection with inflammation	20	9	10	7	30	16	46
	-Intra-amniotic infection without inflammation	1	0	3	5	4	5	9
	-Absence of intra-amniotic infection and inflammation	8	4	5	85	13	89	102
	-Sterile inflammation	13	3	3	21	16	24	40
	Total	42	16	21	118	63	134	197
Intra-amniotic infection	Candida spp.	9	0	10	1	19	1	20
	-Candida species only	8	0	7	0	15	0	15
	-Candida spp. and non-fungal microorganism(s)	1	0	3	1	4	1	5
	Non-fungal microorganism(s)	12	9	3	11	15	20	35
	Absence of intra-amniotic infection	21	7	8	106	29	113	142
	Total	42	16	21	118	63	134	197

Beta-glucan assay results for 177 women without intra-amniotic *Candida* infection stratified by infection-inflammatory status of the amniotic cavity and the presence of an intrauterine contraceptive device (IUD).

Table 4

Infection and inflammation status of the amniotic cavity	Women with an IUD		Women without an IUD		Overall	
	Beta-glucan assay		Beta-glucan assay		Beta-glucan assay	
	Positive	Negative	Positive	Negative	Positive	Negative
Intra-amniotic inflammation	Present	Intra-amniotic infection	12	9	2	6
	Absent	Absence of intra-amniotic infection	13	3	3	21
Total		Intra-amniotic infection	0	0	1	5
		Absence of intra-amniotic infection	8	4	5	85
			33	16	11	117
					44	133
						177

Table 5

Effects of non-fungal intra-amniotic infection, intra-amniotic inflammation, and an intrauterine contraceptive device (IUD) on the specificity of the beta- glucan assay.

Patients	Total (n)	Negative assay (n)	Specificity
Overall	177	133	75.1%
Women with an IUD	49	16	32.7%
Women without an IUD	128	117	91.4%
No IUD, no intra-amniotic infection	114	106	93.0%
No IUD, non-fungal intra-amniotic infection	14	11	78.6%
No IUD, no intra-amniotic infection, no intra-amniotic inflammation	90	85	94.4%
No IUD, no inflammation, non-fungal intra-amniotic infection	6	5	83.3%
No IUD, no intra-amniotic infection, no intra-amniotic inflammation	24	21	87.5%
No IUD, non-fungal-intra-amniotic infection, intra-amniotic inflammation	8	6	75.0%
IUD, no intra-amniotic infection	28	7	25.0%
IUD, non-fungal intra-amniotic infection	21	9	42.9%
IUD, no intra-amniotic infection, no intra-amniotic inflammation	12	4	33.3%
IUD, no intra-amniotic infection, intra-amniotic inflammation	16	3	18.8%
IUD, non-fungal intra-amniotic infection, intra-amniotic inflammation	21	9	42.9%

LEGEND. n: number.