A SONOGRAPHIC SHORT CERVIX AS THE ONLY CLINICAL MANIFESTATION OF INTRA-AMNIOTIC INFECTION

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Abstract

OBJECTIVE— A sonographically short cervix is a powerful predictor of spontaneous preterm delivery. However, the etiology and optimal management of a patient with a short cervix in the mid-trimester of pregnancy remain uncertain. Microbial invasion of the amniotic cavity (MIAC) and intra-amniotic inflammation are frequently present in patients with spontaneous preterm labor or acute cervical insufficiency. This study was conducted to determine the rate of MIAC and intra-amniotic inflammation in patients with a cervical length <25 mm in the mid-trimester.

STUDY DESIGN— A retrospective cohort study was conducted of patients referred to our high risk clinic because of a sonographic short cervix or a history of a previous preterm birth. Amniocenteses were performed for the evaluation of MIAC and for karyotype analysis in patients with a short cervix. Fluid was cultured for aerobic and anaerobic bacteria, as well as genital mycoplasmas. Patients with MIAC were treated with antibiotics selected by their physician.

RESULTS— Of 152 patients with a short cervix at 14–24 weeks, 57 had amniotic fluid analysis. The prevalence of MIAC was 9% (5/57). Among these patients, the rate of preterm delivery (<32 weeks) was 40% (2/5). Microorganisms isolated from amniotic fluid included *Ureaplasma urealyticum* (n=4) and *Fusobacterium nucleatum* (n=1). Patients with a positive culture for *Ureaplasma urealyticum* received intravenous Azithromycin. Three patients with *Ureaplasma urealyticum* had a sterile amniotic fluid culture after treatment, and subsequently delivered at term. The patient with *Fusobacterium nucleatum* developed clinical chorioamnionitis and was induced.

CONCLUSION— 1) Sub-clinical MIAC was detected in 9% of patients with a sonographically short cervix (<25 mm); and 2) maternal parenteral treatment with antibiotics can eradicate MIAC caused by *Ureaplasma urealyticum.* This was associated with delivery at term in the three patients whose successful treatment was documented by microbiologic studies.

Keywords

Ultrasound; cervical length; preterm delivery; intra-amniotic infection; chorioamnionitis; cervical insufficiency; short cervix; microbial invasion of the amniotic cavity

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Introduction

A sonographic short cervix is a powerful predictor of spontaneous preterm delivery [2,3,14, 15,17,43]. However, neither the etiology nor the optimal management of patients with a sonographic short cervix has been determined.

Intrauterine infection is a major cause of premature labor and delivery[8]. Previous studies have demonstrated that patients with a dilated cervix before 24 weeks of gestation have a very high incidence of sub-clinical microbial invasion of the amniotic cavity (MIAC) [23,29]. Moreover, the lower the gestational age at which preterm delivery occurs, the higher the rate of MIAC [35,48], intra-amniotic inflammation [55], and histologic chorioamnionitis [24,37, 52].

This study was conducted to determine the rate of intra-amniotic infection in asymptomatic patients with a short cervix in the mid-trimester of pregnancy, and the effect of treatment with antibiotics in patients with a clinically silent intra-amniotic infection and a short cervix.

Population and Methods

A retrospective cohort study was conducted searching our perinatal database of patients referred to our high risk clinic at Hutzel Women's Hospital (Detroit, MI) with the diagnosis of a sonographic short cervix who met the following criteria: (1) singleton gestation; (2) sonographic cervical length < 25 mm; (3) gestational age between 14 and 24 weeks; (4) asymptomatic; (5) cervical dilatation < 2 cm by digital examination; and (6) amniocentesis for microbiological studies of amniotic fluid. The study period was August 2002 to August 2004. Patients with preterm labor, preterm premature rupture of membranes (PROM), placenta previa, bleeding, or a cerclage *in situ* were excluded.

Amniocentesis was performed for clinical indications (e.g., to determine the microbial status of the amniotic cavity and karyotype analysis). Results of the amniocentesis were used in patient management (e.g., antibiotic administration). Amniocentesis in patients with a short cervix was undertaken as part of the standard obstetrical practice, in light of previous observations suggesting an association between a short cervix and histologic chorioamnionitis [11]. Patients were counseled by clinicians, and those who agreed to undergo an amniocentesis for clinical management were asked to donate amniotic fluid and allow collection of clinical information for research purposes, according to a protocol approved by the Wayne State University Institutional Review Board.

During the study period, our institution's database recorded 152 patients with a sonographic cervical length < 25 mm and a gestational age between 14 and 24 weeks. Of these, 57 underwent an amniocentesis.

Retrieval of amniotic fluid

Amniotic fluid was retrieved by transabdominal amniocentesis under ultrasonographic guidance. The fluid was then transported to the laboratory in a capped plastic syringe and cultured for aerobic and anaerobic bacteria, as well as mycoplasmas. White blood cell count, glucose concentration and Gram stain for microorganisms were performed in amniotic fluid shortly after collection, using methods previously described [28,31,33].

Sonographic examination of the cervix

Sonographic evaluation of cervical length was conducted by transvaginal ultrasound. Sonographic examinations were performed with standard equipment (Advanced Technology Laboratories, Bothell, Washington; Seimens SI450, Issaquah, WA; Acuson XP128, Mountain

View, CA). All examinations were performed by Registered Diagnostic Medical Sonographers and reviewed by a perinatologist. Transvaginal cervical length measurements were obtained using the technique described by Iams et al [17,18].

Gestational age was determined by last menstrual period or by ultrasound, in the case where the discrepancy between the ultrasound and the menstrual dating was greater than two weeks. Patient clinical and demographic data, past obstetrical history, and pregnancy outcome were obtained by chart review.

Statistical Analysis

Comparisons were performed with Chi square, Fisher's exact tests and Mann-Whitney U tests.

Results

One hundred fifty-two patients had a transvaginal cervical length < 25 mm at 14–24 weeks during the study period. Fifty-seven patients had amniotic fluid analyses. No patients had evidence of labor. Table 1 details the patient demographic data. The prevalence of positive amniotic fluid cultures was 9% (5/57). Among these patients with a positive amniotic fluid culture, the rate of preterm delivery at less than 32 weeks was 40% (2/5). Microorganisms isolated from amniotic fluid were *Ureaplasma urealyticum* (n=4) and *Fusobacterium nucleatum* (n=1). One patient had a positive culture for *Staphylococcus aureus,* but a negative Gram stain of amniotic fluid, no white blood cells, and a normal glucose determination. This patient was considered to have a contaminant and, therefore, was not included among individuals with a positive culture. No antibiotics were administered, and the patient delivered at 35 weeks with severe preeclampsia. The placenta showed no histologic evidence of chorioamnionitis.

Four patients with a positive culture for *Ureaplasma urealyticum* received intravenous Azithromycin for seven days, and had a second amniocentesis after treatment. All patients had a second amniotic fluid culture which was negative for microorganisms. Three of the four women subsequently delivered at term, and one showed histologic evidence of chorioamnionitis. There was one case with a first positive culture for *Ureaplasma urealyticum* who had sterile amniotic fluid at the time of the second amniocentesis, but delivered preterm (7 weeks after the second amniocentesis). This patient had histologic evidence of chorioamnionitis.

One patient had a positive amniotic fluid culture for *Fusobacterium nucleatum,* developed clinical chorioamnionitis shortly after the amniocentesis, and labor was induced. The clinical and laboratory information for all cases with MIAC are displayed in Table 2. Table 3 displays the pregnancy outcome of the study population.

Comment

Principal findings of this study

1) Nine percent of women with a sonographic short cervix (defined as a cervical length < of 25 mm) had MIAC; 2) MIAC was sub-clinical in nature, as none of the patients showed evidence of clinical chorioamnionitis at the time of presentation; 3) the most frequent organism isolated from the amniotic fluid was *Ureaplasma urealyticum*; and 4) treatment with antibiotics administered to the mother was associated with eradication of the *Ureaplasma urealyticum* in three of four cases, and these patients delivered at term. One patient with a positive *Ureaplasma urealyticum* culture who received treatment and subsequently had a negative amniotic fluid culture delivered preterm, indicating that treatment was not successful in all cases. Our findings suggest that a short cervix, detected with ultrasound, can be the only clinical manifestation of

MIAC, and that intra-amniotic infection in the mid-trimester of pregnancy can be treated with antibiotics.

The clinical significance of a short cervix detected with ultrasound

The shorter the sonographic cervical length in the mid-trimester of pregnancy, the higher the likelihood of spontaneous preterm delivery [1–3,14,15,17,19,43,45,46]. However, there is no agreement as to what is a sonographic short cervix. For example, Iams et al. [17] reported the results of a prospective study of low-risk women for preterm delivery who underwent sonographic examination at 24 and 26–28 weeks of gestation. The authors concluded that a cervix of 26 mm or shorter at 24 weeks increased the risk for spontaneous preterm delivery (Relative risk:6.19; 95% CI: 3.84–9.97) [17]. However, the prevalence of spontaneous preterm delivery (defined as < 35 weeks) in this study was 4.3%, while the positive predictive value was 17.8% for a cervical length ≤ 25 mm at 24 weeks gestation [17]. Other investigators, including members of our group, have proposed a cut-off of 15 mm, because a cervical length of 15 mm or less is associated with nearly a 50% risk of spontaneous preterm delivery at 32 weeks of gestation or less when neonatal morbidity is substantial [14,15].

It is clear, however, that sonographic cervical length is not a screening test for spontaneous preterm delivery, as only a fraction of all patients who have a spontaneous preterm birth have a short cervix in the mid-trimester. Previous studies conducted at our institution indicated that only 8% of all patients with a preterm delivery at less than 32 weeks of gestation have a cervical length of 15 mm or less [14]. For this reason, we advocate that a sonographic cervical length is only a method for risk assessment for spontaneous preterm delivery and not a screening test. Cervical length can modify the *a priori* risk for preterm delivery. For example, a woman with a history of preterm delivery or one with a twin or triplet gestation will have a higher risk for preterm delivery than a patient without such history and the same cervical length [7,12,13, 22,27,40,41,44,47,49].

Why do patients have a short cervix?

The causes of a sonographic short cervix are largely unknown. A congenital short cervix has been reported in patients exposed to diethylstilbestrol (DES)[38], though this is an extremely rare occurrence nowadays. Previous studies suggest that maternal age, body mass index, ethnicity, and obstetrical history are independent explanatory variables of sonographically determined cervical length [16]. One interpretation of these observations is that the combination of genetic and environmental factors may play a role in determining cervical length. For instance, pregnant adolescents, undernourished women (low body mass index), individuals of African-Caribbean or African-American origin, and those with a previous preterm delivery have a higher risk of spontaneous preterm birth [4,16]. It is possible that these factors confer risk by altering, among other biological determinants of preterm birth, cervical length.

Since the uterine cervix is a connective tissue structure[20], lack of appropriate nutrition during development (in utero or during critical periods of reproductive development, such as adolescence) conceivably may lead to inadequate extracellular matrix deposition in the cervix, and this may predispose to a short cervix. For example, a low body mass index (below 19.8) is an independent risk factor for preterm delivery (OR: 3.6, 95% CI: 1.6–8.0) [39]. However, this association may operate through a short cervix. If the low body mass index is due to environmental factors, the short cervix could be an acquired phenomenon. The possibility that maternal under-nutrition or fetal growth restriction may predispose to a short cervix needs to be considered.

Several authors have documented a relationship between a previous obstetrical history of preterm birth and cervical length after pregnancy [10,18]. Iams et al. reported a study of cervical length in patients with: 1) a previous history on cervical insufficiency; 2) a previous preterm delivery ≤ 26 weeks; 3) a previous preterm delivery at 27–32 weeks; 4) a previous preterm delivery at 33–35 weeks; and 5) a control group of women with previous term delivery. A strong relationship was established between cervical length in the index pregnancy and previous obstetric history. This relationship appears linear and patients considered to have a typical history of an incompetent cervix did not constitute a unique group[18]. Similar results have been reported by Guzman et al. who described a strong relationship between previous obstetric history and cervical length in the subsequent pregnancy [10]. Specifically, they observed that the frequency of a short cervix (cervical length < 2cm) or progressive shortening of the cervix to a length of < 2cm was associated with the gestational age at delivery in the previous pregnancy [10]. Collectively, these studies suggest a relationship between a history of preterm delivery and the cervical length in a subsequent pregnancy. A short cervical length in the subsequent pregnancy may reflect the result of genetic and environmental factors predisposing to preterm delivery, which can express themselves in changes in cervical structure.

Another cause for a short cervix is premature cervical ripening in response to a pathologic stimulus such as infection. Previous studies have indicated that up to 50% of patients presenting with painless cervical dilatation before 24 weeks of gestation have a positive amniotic fluid culture for microorganisms [23,29]. In these cases, infection can be a cause of cervical insufficiency, or may be secondary to prolonged exposure of chorioamniotic membranes to the microbial flora present in the lower genital tract [29].

The prevalence of MIAC in patients with a short cervix compared to other complications of pregnancy

The frequency of MIAC in patients with an asymptomatic short cervix is quite similar to that of patients presenting with preterm labor and intact membranes [35], but lower than those with preterm PROM (30%) [36] and acute cervical insufficiency (50%) [29].

Ureaplasma urealyticum: the most common microorganism found in the amniotic cavity in women with a sonographic short cervix

The observations reported herein that *Ureaplasma urealyticum* is the most common microorganism found in the amniotic cavity is consistent with those made in cases of patients in preterm labor with intact membranes, preterm PROM, acute cervical insufficiency, term PROM, and vaginal bleeding [8,32,51,53,54,56]. The reasons why *Ureaplasma urealyticum* is a frequent isolate from the amniotic cavity are unknown. This organism, found in the lower genital tract of up to 80% of normal pregnant women, has been detected in endometrial cultures [5,21,42]. It is possible that *Ureaplasma urealyticum* gains access to the endometrial cavity with sperm in the non-pregnant state, to cause endometritis and, subsequently, an intra-amniotic infection during pregnancy. The other alternative is that *Ureaplasma urealyticum* is prone to gain access from the lower genital tract to the amniotic cavity in some women, while not in others. If this is the case, the factors responsible for this susceptibility need to be understood.

The clinical significance of microbial invasion in patients with a short cervix

Gray et al. reported that all women with a positive amniotic fluid culture for *Ureaplasma urealyticum* at the time of genetic amniocentesis delivered a preterm neonate with histologic evidence of chorioamnionitis [9]. This study, however, did not contain information about cervical length.

We were not able to examine the natural history of MIAC in the context of this study, as the results were used for patient management. Patients with MIAC were treated with antibiotics. The current ethical framework governing research in human subjects indicates that the fetus exposed to an invasive procedure must have the possibility of benefiting from the information acquired from the procedure. Therefore, we find it difficult to envision the circumstances in which MIAC can be identified and then an intervention withheld to determine the natural history of this condition. The second limitation of this study is that we have used microbial culture rather than molecular microbiological techniques. We have previously demonstrated that standard culture techniques underestimate the rate of microbial footprints in the amniotic cavity, and that patients with bacterial footprints but negative amniotic fluid cultures have a similar outcome to those with a positive amniotic fluid culture for bacteria [53,54]. Thus, the current study provides a minimum estimate of the frequency of MIAC in women with a short cervix. It is, however, possible that the rate of microbial invasion may increase with the use of molecular microbiologic techniques for the detection of bacteria and viruses [6,25,26,50,53].

Treatment with antibiotics can eradicate MIAC in women with a short cervix

An important observation of our study is that three patients with positive amniotic fluid cultures for *Ureaplasma urealyticum* underwent treatment with intravenous Azithromycin, and a repeat amniocentesis demonstrated that the amniotic cavity had become sterile and the patient delivered at term. This information is consistent with two previous reports that parenteral antibiotic therapy can eradicate MIAC [30,34]. However, the significance of this report is that, in the past, maternal treatment has accomplished eradication in patients who had preterm PROM, and this time it was accomplished in women with intact membranes. This result suggests that maternal antibiotic administration can successfully eradicate MIAC in patients in the mid-trimester of pregnancy. The implications of this observation are substantial, since Gray et al reported a 0.37% (9/2461) prevalence of positive cultures for *Ureaplasma urealyticum* in amniotic fluid samples obtained during second trimester genetic amniocenteses. After exclusion of a therapeutic abortion case, all women (8/8) with positive amniotic fluid cultures had either a fetal loss within four weeks of amniocentesis (n=6) or preterm delivery (n=2). All had histologic evidence of chorioamnionitis [9]. Our findings suggest that treatment with antibiotics may be successful. A limitation of our study is that the natural history of intra-amniotic infection in women with intact membranes is not known. The traditional assumption has been that once MIAC has occurred, it has a progressive course. Still, it is possible that in certain patients the host defense mechanisms suffice to control some cases of MIAC, and in such cases intra-amniotic inflammation/infection could be transient and not lead inevitably to preterm delivery. It is important to note that one patient with a positive amniotic fluid culture with *Ureaplasma urealyticum* was treated with antibiotics and had a second amniocentesis, suggesting eradication of the infection within the amniotic cavity, and still had a preterm delivery. Noteworthy is the fact that while the number of white blood cells in the first amniocentesis was 3 per mm^3, the second amniocentesis performed after treatment had 90 white blood cells per mm^3, but a negative culture. These results suggest that treatment will not be successful in all cases. It is conceivable that the microorganisms were eradicated or that the inoculum size was decreased to such a degree that a positive culture could not be obtained. Another possibility is that the antibiotic used for the treatment remained in the amniotic fluid and interfered with the growth of the microorganism in culture. The net result may have been a negative culture. However, progressive intra-amniotic inflammation occurred and resulted in a preterm delivery. Indeed, the white blood cell count in the second amniocentesis increased dramatically, and the placenta of the patient in question showed histologic evidence of chorioamnionitis. The clinical implications of these observations are that not all patients will be successfully treated, and that care needs to be exercised in monitoring the inflammatory response and optimizing the methods for the identification of microorganisms in the amniotic

cavity. This information is important for both patient care and the improvement of the treatment of intra-amniotic infection/inflammation.

In conclusion, this study demonstratesthat a short cervix may be the only manifestation of sub-clinical MIAC, and that eradication of these infections is possible and may be associated with a normal term delivery.

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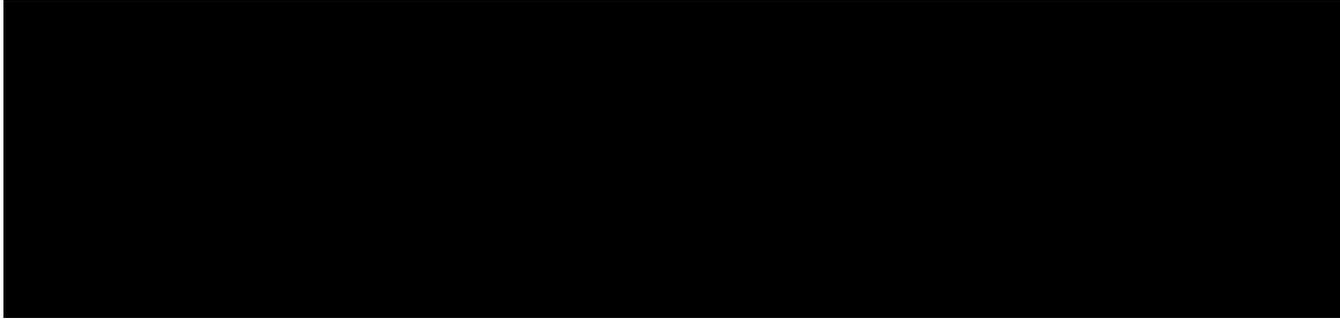
Table 1

Demographic data of the study population

Parameter	n = 57
Maternal age (years, mean ± SD)	27 ± 9
Parity	
0 (%, n)	53% (30)
1 or more (%, n)	47% (27)
History of preterm birth	
Yes	58% (33)
No	42% (24)
Ethnicity	
African American	93% (53)
Caucasian	3.5% (2)
Hispanic	1.8% (1)
Asian	1.8% (1)

Table 2

Clinical characteristics of patients with a short cervix (< 25 mm) and microbial invasion of the amniotic cavity

GA: gestational age; AF: amniotic fluid; WBC: white blood cell; amnio: amniocentesis; PROM: premature rupture of membranes.

*
Dosage = 1 gram intravenous every 24 hours X 7 days.

Table 3

Pregnancy outcome of patients with a short cervix (< 25 mm)

Parameter	All patients (n=57)	MIAC (n=5)	No MIAC (n=52)
Gestational age at amniocentesis [weeks, mean ± sd, median (range)]	20.3 + 1.9	20.3 ± 1.7	20.3 ± 2.0
	20.4 (15.2–23.5)	20.4 (18–22.6)	20.4 (15.2–23.5)
Spontaneous preterm delivery			
≤ 35 weeks	45.6% (26/57)	40% (2/5)	46% (24/52)
≤ 32 weeks	31.6% (18/57)	40% (2/5)	31% (16/52)
≤ 28 weeks	22.8% (13/57)	40% (2/5)	21% (11/52)
Clinical chorioamnionitis	1.8% (1/57)	20% (1/5)	0% (0/52)
Histological chorioamnionitis	54% (26/48)	60% (3/5)	53% (23/43)
Gestational age at delivery [weeks, mean ± sd, median(range)]	33.5 ± 6.2	33.2 ± 8.1	33.5 ± 6.5
	36 (18–42)	36.5 (20–40.1)	36 (18–42)

MIAC: microbial invasion of the amniotic cavity; all comparisons were non-significant (p>0.05).