OBSTETRICS

Amniotic fluid infection, inflammation, and colonization in preterm labor with intact membranes

C. Andrew Combs, MD, PhD; Michael Gravett, MD; Thomas J. Garite, MD; Durlin E. Hickok, MD, MPH; Jodi Lapidus, PhD; Richard Porreco, MD; Julie Rael, RN; Thomas Grove, PhD; Terry K. Morgan, MD, PhD; William Clewell, MD; Hugh Miller, MD; David Luthy, MD; Leonardo Pereira, MD; Michael Nageotte, MD; Peter A. Robilio, MD; Stephen Fortunato, MD; Hyagriv Simhan, MD; Jason K. Baxter, MD; Erol Amon, MD; Albert Franco, MD; Kenneth Trofatter, MD; Kent Heyborne, MD; for the ProteoGenix/Obstetrix Collaborative Research Network

OBJECTIVE: The purpose of this study was to compare intraamniotic inflammation vs microbial invasion of the amniotic cavity (MIAC) as predictors of adverse outcome in preterm labor with intact membranes.

STUDY DESIGN: Interleukin-6 (IL-6) was measured in prospectively collected amniotic fluid from 305 women with preterm labor. MIAC was defined by amniotic fluid culture and/or detection of microbial 16S ribosomal DNA. Cases were categorized into 5 groups: infection (MIAC; IL-6, $\geq$11.3 ng/mL); severe inflammation (no MIAC; IL-6, $\geq$11.3 ng/mL); mild inflammation (no MIAC; IL-6, 2.6-11.2 ng/mL); colonization (MIAC; IL-6, $<$2.6 ng/mL); negative (no MIAC; IL-6, $<$2.6 ng/mL).

RESULTS: The infection (n = 27) and severe inflammation (n = 36) groups had similar latency (median, $<$1 day and 2 days, respectively) and similar rates of composite perinatal morbidity and mortality (81% and 72%, respectively). The colonization (n = 4) and negative (n = 195) groups had similar outcomes (median latency, 23.5 and 25 days; composite morbidity and mortality rates, 21% and 25%, respectively). The mild inflammation (n = 47) groups had outcomes that were intermediate to the severe inflammation and negative groups (median latency, 7 days; composite morbidity and mortality rates, 53%). In logistic regression adjusting for gestational age at enrollment, IL-6 $\geq$11.3 and 2.6-11.2 ng/mL, but not MIAC, were associated significantly with composite morbidity and mortality rates (odds ratio [OR], 4.9; 95% confidence interval [CI], 2.2—11.2, OR, 3.1; 95% CI, 1.5—6.4, and OR, 1.8; 95% CI, 0.6—5.5, respectively).

CONCLUSION: We confirmed previous reports that intraamniotic inflammation is associated with adverse perinatal outcomes whether or not intraamniotic microbes are detected. Colonization without inflammation appears relatively benign. Intraamniotic inflammation is not simply present or absent but also has degrees of severity that correlate with adverse outcomes. We propose the designation amniotic inflammatory response syndrome to denote the adverse outcomes that are associated with intraamniotic inflammation.

Key words: chorioamnionitis, intraamniotic infection, intraamniotic inflammation, microbial invasion of the amniotic cavity, morbidity, preterm birth, preterm labor

Cite this article as: Combs CA, Gravett M, Garite TJ, et al. Amniotic fluid infection, inflammation, and colonization in preterm labor with intact membranes. Am J Obstet Gynecol 2014;210:125.e1-15.

From the Center for Research, Education, and Quality, Mednax, Inc., Sunrise, FL (Drs Combs and Garite and Ms Rael); Obstetrix Medical Group, San Jose, CA (Dr Combs); University of Washington Medical Center, Seattle, WA (Drs Gravett and Robilio); ProteoGenix, Inc., Costa Mesa, CA (Drs Hickok and Grove); Oregon Health and Science University, Portland, OR (Drs Lapidus, Morgan, and Pereira); Obstetrix Medical Group of Colorado, Denver, CO (Drs Porreco and Heyborne and Ms Rael); Obstetrix Medical Group, Phoenix Perinatal Associates, Phoenix, AZ (Dr Clewell); Obstetrix Medical Group of Arizona, Tucson, AZ (Dr Miller); Obstetrix Medical Group of Washington, Seattle, WA (Dr Luthy); Obstetrix Medical Group, Southern California, Long Beach, CA (Dr Nageotte); Perinatal Research Group, Nashville, TN (Dr Fortunato); University of Pittsburgh, Pittsburgh, PA (Dr Simhan); Thomas Jefferson University, Philadelphia, PA (Dr Baxter); St. Louis University School of Medicine, St. Louis, MO (Dr Amon); Carolinas Medical Center, Charlotte, NC (Dr Franco); and University Medical Center, Greenville, SC (Dr Trofatter).

Received July 29, 2013; revised Oct. 20, 2013; accepted Nov. 20, 2013.

Supported by a research grant from ProteoGenix, Inc; statistical analyses supported by Hologic, Inc.

Each author had grant support, consulting arrangements, employment, or equity interests with the sponsor, ProteoGenix, Inc. C.A.C. and T.J.G. have served on advisory boards; J.L. has a consulting arrangement, and J.K.B. has an unrelated research grant with Hologic, Inc, which acquired ProteoGenix in 2010.

Presented at the 33rd annual meeting of the Society for Maternal-Fetal Medicine, San Francisco, CA, Feb. 11-16, 2013.

Reprints not available from the authors.

0002-9378/$36.00 • • http://dx.doi.org/10.1016/j.ajog.2013.11.032

Intrauterine infection and inflammation are well-documented causes of preterm labor with intact fetal membranes, especially at very early gestational ages.[1] Cultures for microorganisms in amniotic fluid demonstrate microbial invasion of the amniotic cavity (MIAC) in 20-60% of women with preterm labor at <28 weeks of gestation and 10-25% at 28-32 weeks of gestation.[2-4] Culture-proven MIAC is associated with short latency to delivery and high rates of perinatal morbidity and mortality.[2-5]

Even with culture-negative amniotic fluid, however, women in preterm labor often have intraamniotic inflammation, which is evidenced by elevated amniotic fluid levels of inflammatory markers such as interleukin-6 (IL-6),[3,5-11] other proinflammatory cytokines and chemokines,[6,10-13] tumor necrosis factor alpha,[4,6,10,14] or matrix metalloproteinase-8 (MMP-8).[15-17] Whether or not the amniotic fluid culture is positive, intraamniotic inflammation is associated with short latency[3-5,7,9,12-18] and high rates of perinatal morbidity and mortality.[3-5,14,17,18]

One explanation for the morbidity that is associated with culture-negative intraamniotic inflammation is that many cases actually have MIAC but that the amniotic fluid cultures are falsely negative. Using polymerase chain reaction (PCR) amplification, several groups have demonstrated prokaryotic 16S subunit ribosomal RNA or the DNA coding for it (rDNA) in amniotic fluid in many culture-negative preterm labor cases.[19-26] The microbes that are identified by 16S PCR techniques are often facultative organisms that are difficult to culture with standard techniques. Preterm labor cases with 16S PCR-proven MIAC have similar outcomes to cases with culture-proven MIAC,[19-21,23-25] which suggests true infection and not simply detection of nonviable microbial degradation products.

In principle, MIAC and the intraamniotic inflammatory response are distinct entities. In the simplest model, each of them can be either present or absent; therefore, states of amniotic fluid are possible: (1) infection (MIAC and inflammatory response both present), (2) inflammation (inflammatory response present, MIAC absent); (3) colonization (MIAC present, inflammatory response absent); (4) negative (both absent).

Moreover, the inflammatory response is not simply present or absent but is a continuum. A recent report suggested that clinical outcomes correlated with gradations in inflammatory response that varied from "no" to "minimal" to "severe," with categories defined by the number of biomarkers that are present in amniotic fluid.[27] To our knowledge, there has been no previous report that has investigated whether the severity of outcomes might be graded similarly based on the concentration of a single inflammatory marker.

The aims of the present investigation were to compare the outcomes of preterm labor in women with intraamniotic infection, inflammation, or colonization and to examine whether the outcomes are related to the severity of the inflammatory response as defined by intraamniotic IL-6 levels.

Materials and Methods

This report involved a subset of subjects from a larger multicenter study, the goal of which was to develop a noninvasive test to screen for intraamniotic infection based on cervicovaginal proteins. The protocol was approved by the local institutional review board at each participating site.

Inclusion/exclusion criteria

We included consenting women who were at least 18 years old with singleton pregnancies at 15.0-36.9 weeks of gestation in spontaneous preterm labor with intact fetal membranes and who underwent amniocentesis to evaluate for intraamniotic infection and to measure amniotic fluid IL-6. *Preterm labor* was defined as regular uterine contractions plus at least 1 of the following: cervical dilation ≥2 cm; cervical length by transvaginal sonography ≤30 mm; or a positive cervicovaginal fetal fibronectin test. The protocol required cervical length by transvaginal sonography or fetal fibronectin test only if cervical dilation was <2 cm. Each of the participating sites was a tertiary perinatal center where amniocentesis was offered routinely to women with preterm labor, although several centers restricted the procedure to <34 weeks of gestation. Exclusion criteria were ruptured membranes, major fetal anomaly, fetal aneuploidy, or a medical indication for preterm birth.

TABLE 1
Number of subjects per site by gestational age

Site no.	Gestational age, wk <30	30-33.9	≥34	Total
1	7	9	4	20
3	9	5	0	14
4	14	13	0	27
5	15	4	0	19
6	3	4	0	7
7	2	2	1	5
8	21	7	3	31
9	1	0	0	1
10	15	40	26	81
11	26	10	9	45
12	11	12	8	31
13	2	2	2	6
14	4	5	1	10
16	0	1	0	1
19	0	1	1	2
20	3	1	1	5
Total	133	116	56	305

Combs. Inflammation, infection, preterm labor. Am J Obstet Gynecol 2014.

Specimens

Amniotic fluid was obtained by transabdominal amniocentesis with the use of sonographic guidance and antiseptic skin preparation. A 5-mL aliquot was sent to the local hospital laboratory for assessment of glucose concentration, white blood cell count (WBC), Gram stain, and aerobic and anaerobic culture, which included genital mycoplasmas at some laboratories. A 10-mL aliquot of amniotic fluid was frozen at −80°C and