Published in final edited form as:

Am J Obstet Gynecol. 2008 October ; 199(4): 426.e1–426.e7. doi:10.1016/j.ajog.2008.06.075.

TOTAL HEMOGLOBIN CONCENTRATION IN AMNIOTIC FLUID IS INCREASED IN INTRA-AMNIOTIC INFECTION/INFLAMMATION

Edi VAISBUCH, MD, Roberto ROMERO, MD, Offer EREZ, MD, Juan Pedro KUSANOVIC, MD, Francesca GOTSCH, MD, Nandor G. THAN, MD, PhD, Shali MAZAKI-TOVI, MD, Pooja MITTAL, MD, Sam EDWIN, PhD, and Sonia S. HASSAN, MD

¹Perinatology Research Branch, NICHD/NIH/DHHS, Bethesda, Maryland and Detroit, Michigan, USA

²Center for Molecular Medicine and Genetics, Wayne State University, Detroit, Michigan, USA

³Wayne State University School of Medicine, Department of Obstetrics and Gynecology, Detroit, Michigan, USA

Abstract

Objective—Discolored amniotic fluid (AF) has been associated with intra-amniotic infection/inflammation (IAI) in patients with preterm labor (PTL). The presence of hemoglobin and its catabolic products have been implicated as a cause for AF discoloration. The aim of this study was to determine whether there is an association between total hemoglobin concentration in AF and gestational age, spontaneous labor (term and preterm) and the presence or absence of IAI.

Study design—This cross-sectional study included patients in the following groups: 1) mid-trimester ($n=65$); 2) term not in labor ($n=22$); 3) term in labor ($n=47$); 4) spontaneous PTL who delivered at term ($n=92$); 5) PTL without IAI who delivered preterm ($n=76$); 6) PTL with IAI ($n=81$); 7) preterm prelabor rupture of the membranes (PPROM) with IAI ($n=48$); and 8) PPROM without IAI ($n=49$). Total hemoglobin concentrations in amniotic fluid were determined by an enzyme-linked immunoassay. Non-parametric statistics were used for analysis.

Results—1) Hemoglobin was detected in all AF samples ($n=480$); 2) The median AF total hemoglobin concentration at term was significantly higher than in mid-trimester [520.6 ng/mL, interquartile range (IQR) 271.2–1549.2 vs. 58.5 ng/mL, IQR 26.1–200.8; $p<0.001$]; 3) Among patients with PTL, the median AF total hemoglobin concentration was significantly higher in patients with IAI than in those without IAI (4671.8 ng/mL, IQR 1294.2–8620.7 vs. 2013.6 ng/mL, IQR 629.2–5420.4; $p=0.01$) or women who delivered at term (1143.4 ng/mL, IQR 451.8–4037.9; $p=0.001$); 4) Similarly, among patients with PPROM, the median AF total hemoglobin concentration was significantly higher in patients with IAI than in those without IAI (10753.7 ng/mL, IQR 2053.9–56026.6 vs. 2281 ng/mL, IQR 938.2–9191.7; $p=0.02$); 5) Women at term in labor had a higher median hemoglobin concentration than those not in labor (1952.6 ng/mL, IQR 709.6–6289.2 vs. 520.6 ng/mL, IQR 271.1–1549.2; $p=0.003$).

Address correspondence to: Roberto Romero, M.D., Perinatology Research Branch, NICHD, NIH, DHHS, Wayne State University/Hutzel Women's Hospital, 3990 John R, Box 4, Detroit, MI 48201, USA, Telephone (313) 993-2700, Fax: (313) 993-2694, prbchiefstaff@med.wayne.edu, Edi Vaisbuch, MD, Perinatology Research Branch, NICHD, NIH, DHHS, Wayne State University/Hutzel Women's Hospital, 3990 John R, Box 4, Detroit, MI 48201, USA, Telephone (313) 993-2700, Fax: (313) 993-2694, evaisbuch@med.wayne.edu.

Presented at the 28th Annual Meeting of the Society for Maternal-Fetal Medicine, Dallas, TX, Jan. 28-Feb. 2, 2008.

Condensation

Hemoglobin can be detected in all amniotic fluid samples and the total hemoglobin concentration in amniotic fluid increases in pregnancies complicated with intra-amniotic infection/inflammation.

Conclusions—1) The AF concentration of immunoreactive total hemoglobin increases with advancing gestational age, and is elevated in pregnancies that are complicated with IAI. Spontaneous labor at term is associated with higher AF concentrations of total hemoglobin.

Keywords

chorioamnionitis; microbial invasion of the amniotic cavity; pregnancy; preterm labor; preterm prelabor rupture of membranes (PPROM); vaginal bleeding

INTRODUCTION

The presence of hemoglobin and its degradation products in the amniotic cavity following an episode of intra-amniotic bleeding may lead to discoloration of the amniotic fluid.^{1,2} In patients with preterm labor (PTL) and intact membranes, discoloration of the amniotic fluid is associated with microbial invasion of the amniotic cavity.³ Because bacteria require nutrients for growth,^{4,5} it has been postulated that even small amounts of intra-amniotic bleeding can potentially serve as abundant media for microbial growth, which leads to increased risk for microbial invasion of the amniotic cavity and subsequent preterm parturition.

Recently, microbial invasion of the amniotic cavity was implicated as a common etiology of vaginal bleeding as it has been detected in 14% of pregnant women with “idiopathic” vaginal bleeding.⁶ Furthermore, microbial invasion of the amniotic cavity and vaginal bleeding were associated with subsequent preterm prelabor rupture of membranes (preterm PROM) and early preterm delivery.⁶ It has been proposed that subclinical intrauterine infection can cause deciduitis and decidual bleeding that leads to vaginal bleeding and subsequent adverse pregnancy outcomes.⁶

This study was conducted to determine whether the amniotic fluid concentration of total hemoglobin changes with advancing gestational age, with spontaneous labor at term, and in the presence of intra-amniotic infection/inflammation (IAI) in patients with spontaneous PTL and intact membranes and in women with preterm PROM.

MATERIALS AND METHODS

Study design and population

A cross-sectional study was conducted by searching our clinical database and bank of biological specimens. This study consisted of pregnant women in the following groups: 1) women in the mid-trimester of pregnancy (14–18 weeks of gestation) who underwent amniocentesis for genetic indications and delivered a normal neonate at term ($n=65$); 2) normal pregnant women at term with spontaneous labor ($n=47$) and 3) normal pregnant women at term not in labor ($n=22$); 4) women with an episode of PTL and intact membranes who were classified into: a) PTL who delivered at term ($n=92$); b) PTL who delivered preterm (<37 weeks gestation) without IAI ($n=76$); and c) PTL with IAI ($n=81$); and 5) women with preterm PROM with ($n=48$) and without IAI ($n=49$).

All women provided written informed consent prior to the collection of amniotic fluid. The use of amniotic fluid for research purposes was approved by the Institutional Review Boards of participating institutions and the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD/NIH/DHHS). Many of these samples have been used previously to study the biology of inflammation, hemostasis, angiogenesis regulation, and growth factor concentrations in normal pregnant women and those with pregnancy complications.

Definitions

Patients were considered to have a normal pregnancy outcome if they did not have obstetrical complications, and delivered a term neonate (≥ 37 weeks) of appropriate birthweight for gestational age^{7,8} without complications. Spontaneous PTL was defined by the presence of regular uterine contractions occurring at a frequency of at least two every 10 minutes associated with cervical change before 37 completed weeks of gestation that required hospitalization. Preterm PROM was diagnosed by sterile speculum examination confirming pooling of amniotic fluid in the vagina in association with nitrazine and ferning tests when necessary, before 37 weeks of gestation and in the absence of labor. Women at term not in labor underwent amniocentesis for the assessment of fetal lung maturity before cesarean section. Women at term in labor consisted of women who were suspected to have PTL because of uncertain dates and had an amniocentesis for the assessment of microbial invasion and fetal lung maturity. However, if they delivered a baby who weighed >2500 grams without complications of prematurity, they were considered to represent patients in spontaneous labor at term. Intra-amniotic infection was defined as a positive amniotic fluid culture for microorganisms. Intra-amniotic inflammation was diagnosed by an amniotic fluid interleukin (IL)-6 concentration >2.6 ng/mL.⁹

Amniotic fluid collection

Amniotic fluid samples were obtained from transabdominal amniocenteses that were performed for evaluation of microbial status of the amniotic cavity and/or assessment of fetal lung maturity. A sample of amniotic fluid was transported to the laboratory in a sterile capped syringe and cultured for aerobic/anaerobic bacteria and genital *Mycoplasmas*. White blood cell count, glucose concentration and Gram-stain were also performed shortly after collection. The results of these tests were used for subsequent clinical management. Amniotic fluid not required for clinical assessment was centrifuged for 10 minutes at 4°C , and the supernatant was aliquoted and stored at -70°C until analysis. Mid-trimester samples were not evaluated for infection. However, all samples had an amniotic fluid IL-6 concentration ≤ 2.6 ng/mL.

Determination of total hemoglobin concentration in amniotic fluid

Amniotic fluid concentration of human total hemoglobin was determined by sensitive enzyme-linked immunoassays (ALPCO Diagnostics; Salem, NH, USA). Total hemoglobin immunoassay was validated for human amniotic fluid in our laboratory prior to the conduction of this study. Validation included spike and recovery experiments which produced parallel curves indicating that amniotic fluid constituents did not interfere with antigen-antibody binding in this assay. Immunoassays were carried out according to manufacturer recommendations. Amniotic fluid samples were incubated in duplicate wells of the micro titer plates, which have been pre-coated with antibodies specific for the analyte (total hemoglobin). During this incubation, the analyte present in the standards or amniotic fluid samples was bound by the immobilized antibodies in the respective assay plates. After repeated washing and aspiration to remove all unbound substances, an enzyme-linked polyclonal antibody specific for the analyte was added to the wells of the assay plates. Unbound enzyme conjugate was removed by repeated washing and a substrate solution was added to the wells of the assay plates and color developed in proportion to the amount of the analyte bound in the initial step. Color development was stopped with the addition of an acid solution and the intensity of color was read using a programmable spectrophotometer (SpectraMax M2, Molecular Devices, Sunnyvale, CA, USA). The concentrations of total hemoglobin in amniotic fluid samples were determined by interpolation from individual standard curves. The calculated inter- and intra-assay coefficients of variation for total hemoglobin immunoassay in our laboratory were 5.8% and 3.6%, respectively. The lower limit of detection (sensitivity) for total hemoglobin assay was calculated to be 0.29 ng/mL.

Statistical analysis

Shapiro-Wilk and Kolmogorov-Smirnov tests were used to test for normal distribution of the data. Since amniotic fluid total hemoglobin concentrations were not normally distributed, non-parametric tests were used for analyses. Correlation between continuous variables was assessed by the Spearman's rank correlation test. Comparisons between proportions were performed with Chi-square test. Kruskal-Wallis with post-hoc test (Mann-Whitney U tests) was used for continuous variables. Multiple logistic regression analysis and analysis of covariance (ANCOVA) were used to investigate the association between the subgroups of PTL, preterm PROM, term in labor and not in labor, and the total hemoglobin amniotic fluid concentration, gestational age at amniocentesis, and storage time. A p -value of <0.05 was considered statistically significant. Statistical analysis was performed with SPSS package version 14 (SPSS Inc, Chicago, IL, USA).

RESULTS

Hemoglobin was detected in all amniotic fluid samples ($n=480$) that were tested. Tables 1 and 2 display the demographic and clinical characteristics of patients with spontaneous PTL and intact membranes and women with preterm PROM, respectively. The median gestational age at amniocentesis was significantly lower among patients with PTL with IAI than in the other two subgroups of PTL (Table 1), and among patients with preterm PROM with IAI, when compared to patients with preterm PROM without IAI (Table 2). Information on the gross appearance of the collected amniotic fluid was available for 86.7% of the study population (416/480), of whom 15.1% (63/416) had a discolored amniotic fluid sample [red or brown discoloration 7% (29/416), turbid 5% (21/416) and meconium-like 3.1% (13/416)]. Among the PTL groups, patients who delivered preterm with or without IAI were more likely to have a discolored amniotic fluid than those who delivered at term [PTL without IAI who delivered at term 1.1% (1/89) vs. PTL with IAI 8.9% (7/79); $p=0.02$ and vs. PTL without IAI who delivered preterm 11% (8/73); $p=0.007$]. Similarly, patients with preterm PROM and IAI were more likely to have discolored amniotic fluid than those without IAI [43.8% (21/49) vs. 22.5% (11/48); $p=0.03$]. The median storage time did not differ significantly within the PTL groups (Table 1), as well as within the preterm PROM groups (Table 2) or at term (not in labor and in labor) groups.

Women with a normal pregnancy at term not in labor had a significantly higher median total hemoglobin concentration in amniotic fluid than women in the mid-trimester [term not in labor: 520.6 ng/mL, interquartile range (IQR) 271.2–1549.2 vs. mid-trimester: 58.5 ng/mL, IQR 26.1–200.8; $p<0.001$; Figure 1]. Although the median amniotic fluid samples storage time differ significantly between mid-trimester and term not in labor samples, this had no significant contribution ($p=0.9$) to the model (ANCOVA).

Patients at term in labor had a significantly higher median amniotic fluid total hemoglobin concentration than women at term not in labor (term in labor: 1952.6 ng/mL, IQR 709.6–6289.2 vs. term not in labor: 520.6 ng/mL, IQR 271.2–1549.2; $p=0.003$; Figure 1).

Because of the significant changes in total hemoglobin amniotic fluid concentration with advancing gestational age and in the gestational age at the time of amniocentesis among patients with spontaneous PTL and intact membranes and those with preterm PROM, a multiple logistic regression model was set, controlling for gestational age at amniocentesis. Among patients with spontaneous PTL, women with IAI had a significantly higher amniotic fluid total hemoglobin concentration than those who delivered preterm without IAI (PTL with IAI: 4671.8 ng/mL, IQR 1294.2–8620.8 vs. PTL without IAI: 2013.6 ng/mL, IQR 629.3–5420.4; $p=0.01$) and women with PTL who delivered at term (median 1143.4 ng/mL, IQR 451.8–4037.9; $p=0.001$) (Figure 2). The median amniotic fluid median total hemoglobin concentration did

not differ significantly between women with PTL without IAI and those with PTL who delivered at term (Figure 2). Similarly, patients with preterm PROM with IAI had a significantly higher median amniotic fluid total hemoglobin concentration than women with preterm PROM without IAI (preterm PROM and IAI: 10753.7 ng/mL, IQR 2053.9–56026.6 vs. preterm PROM without IAI: 2281 ng/mL, IQR 938.2–9191.7; $p=0.02$) (Figure 3).

Information regarding history of vaginal bleeding during the index pregnancy was available from 345 patients (71.9% of the study population). Fifty-seven women (16.5%) had history of vaginal bleeding during the index pregnancy. The median amniotic fluid total hemoglobin concentration was not significantly different in patients with or without a history of vaginal bleeding ($p=0.2$). Patients with PTL and IAI [18.3% (13/71); $p=0.03$], and women with PTL without IAI who delivered preterm [21.1% (12/57); $p=0.02$] had a higher prevalence of vaginal bleeding history during pregnancy than those with PTL who delivered at term [5.6% (3/54)]; however, among these three groups, the median total hemoglobin concentration in amniotic fluid was not significantly different in patients with or without a history of vaginal bleeding ($p=0.9$, $p=0.5$ and $P=0.5$, respectively).

Information about a previous amniocentesis was available for 83.1% of the patients (345/415) who had amniocentesis after the mid-trimester. Only 23 patients (6.6%) had a previous amniocentesis; of these, only 3 amniotic fluid samples were described as bloody. The amniotic fluid total hemoglobin concentrations did not differ significantly among women who had a previous amniocentesis from that of patients who did not have a prior amniocentesis ($p=0.7$).

COMMENT

Principal findings of this study

1) Total hemoglobin was detectable in all amniotic fluid samples; 2) the concentration of immunoreactive total hemoglobin in amniotic fluid increased with gestational age; 3) women with spontaneous labor at term had a higher median amniotic fluid concentration of total hemoglobin than those not in labor at term; and 4) patients with IAI (regardless of the membrane status) had a higher median amniotic fluid concentration of total hemoglobin than those without IAI.

What is the origin of Hemoglobin in the amniotic fluid?

The presence of hemoglobin in all amniotic fluid samples deserves comment. Sources of hemoglobin in amniotic fluid include blood contamination during amniocentesis (maternal or fetal). However, such a contamination at the time of amniocentesis is rare, with maternal origin ranging from 0.3–10.8%, whereas fetal injury rates range between 0.6–2%, in an old review before the widespread use of ultrasound.¹⁰

Giorlandino et al¹¹ reported, in repeated amniocenteses performed on 20 women 2 weeks after a first procedure (due to laboratory contamination of the first cell cultures), that blood-stained amniotic fluid could be found in 75% of women. The authors reported a significantly higher red blood cell count and hemoglobin concentration in the amniotic fluid of women who had previously underwent a transplacental amniocentesis when compared to those who did not have a transplacental procedure, and speculated that bleeding may occur after withdrawing the needle, leaving this contamination unrecognized in most cases.¹¹ Nevertheless, it has been observed that, even if contamination with red blood cells occurred during amniocentesis, centrifugation usually prevented hemolysis and hemoglobin release.^{12,13} In the study presented herein, information regarding whether the amniocentesis was trans-placental or not was available in 143 patients, and 23 (16%) had a trans-placental amniocentesis. No significant differences were found in the median total hemoglobin concentration between women with

and without trans-placental amniocentesis ($p=0.3$). Moreover, the detection of total hemoglobin in all amniotic fluid samples (although the gross appearance of the majority the samples was described as clear fluid) and the significant differences in total hemoglobin concentration with advancing gestational age, in the presence of spontaneous labor at term, and between women with and without IAI, strongly suggest other sources.

A source for hemoglobin in amniotic fluid can be intra-amniotic bleeding prior to amniocentesis, although this condition is rare^{14;15} and may derived from the maternal¹⁵⁻¹⁷ or fetal¹⁸ circulation. Maternal intra-amniotic bleeding has been reported in association with placental abruption,^{19;20} circumvallated placenta,¹⁵ as well as PTL^{15;16} with clinical¹⁴ or histological chorioamnionitis.¹⁶ Intra-amniotic bleeding of fetal origin has been associated with a sacrococcygeal teratoma¹⁸ and fetal transfusion.²¹ However, the reported cases were acute events with noticeable amount of intra-amniotic maternal or fetal bleeding, leading to maternal or fetal compromise. It is possible that a more subtle or chronic intra-amniotic maternal or fetal bleeding is subclinical.^{11;21-23} Another possible source for the hemoglobin in the amniotic fluid, however under-investigated, may be transmembranous passage from extra-amniotic bleeding.

The existence of free hemoglobin in amniotic fluid and its association with brownish coloration of the amniotic fluid or clinical outcomes has been previously reported,^{13;24-27} although there are conflicting accounts regarding the presence of hemoglobin in clear amniotic fluid.^{13;25-27} Francoual et al²⁷ measured total hemoglobin concentration (colorimetrically with tetramethylbenzidine) in amniotic fluid obtained by amniocentesis or from the vaginal pool in 78 patients in the late third trimester. A higher mean total hemoglobin concentration was found in “meconium-stained” amniotic fluid than in “clear” amniotic fluid (mean: 288 ± 442 mg/L, range 5–2500 vs. 55 ± 87 mg/L, range 0–434; $p<0.01$). Yet, the authors reported that hemoglobin was present in most samples of “clear” amniotic fluid.²⁷ In contrast, Legge²⁵ examined 208 second trimester amniotic fluid samples for pigmentation (visually and spectrophotometrically), and reported that none of the clear amniotic fluid samples contained abnormal pigmentation, while 14 of the 15 dark brown colored samples had chemically detectable hemoglobin (14 had hemoglobin A and 8 had also hemoglobin F).²⁵ Moreover, Hankins et al²⁶ detected total and fetal hemoglobin in brown, green and clear second trimester amniotic fluid samples, with higher concentrations in brown amniotic fluid.²⁶ These authors reported that fetal hemoglobin was determined to be 20% to 100% of the total hemoglobin. Weiner et al¹³ looked for the presence or absence of hemoglobin peaks of free hemoglobin chains using mass spectrometry in 37 women undergoing amniocentesis before rescue cerclage and 39 control women undergoing genetic mid-trimester amniocentesis. Hemoglobin peaks were found in 27% (12/37) of the cerclage group and in none of the control group (0/39). In the rescue cerclage group, the presence of hemoglobin peaks in the amniotic fluid was associated with a shorter amniocentesis-to-delivery interval than in the absence of hemoglobin peaks (median 6 days, range 0–100 vs. median 38 days, range 0–148; $p<0.04$).¹³ However, no amniotic fluid culture results were reported, hemoglobin measurement was qualitative, discrimination between the beta and gamma (fetal hemoglobin) chains was not possible because of the proximity of peaks of the hemoglobin chains, and the overall detection rate of hemoglobin was low [15.8% (12/76)].

In contrast to previous studies,^{13;27} the results reported herein demonstrate that hemoglobin is present in the amniotic fluid of normal and complicated pregnancies. Thus, detection of hemoglobin in amniotic fluid should not be necessarily considered as an abnormal finding. In general, in normal pregnancies, an immunoassay may detect hemoglobin from a “physiologic” subclinical decidual bleeding that can be normal during the implantation phase and early pregnancy.¹ However, the detected hemoglobin can be of fetal origin,²⁶ since the immunoassay used in this study could not discriminate between hemoglobin A and hemoglobin F.

Thus, although intra-amniotic bleeding secondary to amniocentesis may be responsible for the presence of hemoglobin in the amniotic fluid in some cases, it is possible that a hemorrhagic event prior to the amniocentesis is responsible for the detected hemoglobin in amniotic fluid in the majority of cases. Yet, the source of this bleeding remains to be defined.

Total hemoglobin in amniotic fluid of women in spontaneous labor at term

We found a significantly higher median amniotic fluid total hemoglobin concentration in women with spontaneous labor at term than in women not in labor, suggesting that physiologic parturition may be associated with some degree of intra-amniotic bleeding. A possible explanation is that uterine contractions or active labor may cause vessel damage (placental or membrane) and intra-amniotic bleeding.

Why is the presence of hemoglobin in amniotic fluid associated with IAI?

The current view is to consider the amniotic fluid to be sterile, as less than 1% of women not in labor at term will have a positive amniotic fluid culture. Moreover, clear amniotic fluid was found to have antibacterial activity, reaching its peak at 36 to 40 weeks of gestation.²⁸⁻³³

Resolution of focal hemorrhage involves the breakdown of hemoglobin into various heme pigments and iron. Bacteria require nutrients to growth and cause infection, and one of these bacterial growth factors is iron.^{4;5} Most extracellular iron is bound to high-affinity iron-binding proteins such as transferrin (iron transport) and lactoferrin (iron scavenging).⁵ Clear amniotic fluid has antibacterial²⁸⁻³¹ and antifungal activity,³⁴ and a role for transferrin and lactoferrin in inhibition of microbial growth in amniotic fluid by scavenging iron, has been proposed.^{30; 31;34-36} Oka et al.³¹ demonstrated restoration of the antibacterial activity against *Escherichia coli* by adding transferrin into heat-treated amniotic fluid, but simultaneous addition of transferrin and sufficient concentration of iron to form a transferrin-iron complex resulted in the loss of antibacterial activities. Thadepalli et al.³⁰ reported that amniotic fluid inhibited *in vitro* growth of *Escherichia coli* in the presence of low iron concentration and iron-saturation of transferrin, but had a non-inhibitory effect when iron concentration was elevated and iron-saturation of transferrin was more than 50%. Thus, the higher the iron-saturation of transferrin, the easier bacteria can capture iron from transferrin. Similarly, Ahn et al.³⁷ reported that antibacterial activity of amniotic fluid is closely related with low iron-availability.

In a cross-sectional study including 268 pregnant women, Pacora et al.³⁸ reported that lactoferrin was detectable in 85.4% (229/268) of amniotic fluid samples. In patients with spontaneous preterm labor and intact membranes or those with preterm PROM, intra-amniotic infection was associated with a significant increase in amniotic fluid lactoferrin concentrations. In contrast, term parturition was associated with a significant decrease in lactoferrin concentration in amniotic fluid, suggesting that lactoferrin is part of the repertoire of host defense mechanisms against intra-amniotic infection.³⁸

The hemoglobin detected in the amniotic fluid may be due to a primary intra-amniotic infection and/or an inflammatory process that can lead to deciduitis and hemorrhage (maternal, fetal or both), and subsequently to the activation of the common terminal pathway of parturition.³⁹ Indeed, Gomez et al.⁶ suggested that vaginal bleeding may be the only clinical manifestation of a subclinical intra-uterine infection, which can cause deciduitis and hemorrhage. In a retrospective cohort study of women presented with idiopathic vaginal bleeding between 18 and 35 weeks gestation, microbial invasion of the amniotic cavity was detected in 14% of the cases (16/114) and was associated to subsequent early preterm delivery and preterm PROM.⁶

Strength and limitations of the study

The main strength of this study is its subject sample size in terms of numbers and phenotype. In addition, we used a sensitive and specific immunoassay for total hemoglobin. A limitation is that this immunoassay does not discriminate between maternal and fetal hemoglobin.

Conclusion and future research

Hemoglobin can be detected in the amniotic fluid of all pregnant women; however, the total hemoglobin concentration was significantly higher in women in spontaneous term labor than in women at term not in labor and significantly higher in women with IAI than in those without IAI. Collectively, our findings suggest an association between the presence of hemoglobin and microbial invasion of the amniotic cavity. However, preterm delivery caused by conditions that are unrelated to IAI may also be associated with high amniotic fluid hemoglobin concentration. Further studies are needed to determine the source of hemoglobin in amniotic fluid and its significance.

Acknowledgments

This research was supported by the Intramural Research Program of the National Institute of Child Health and Human Development, NIH, DHHS.

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Figure 1. Amniotic fluid concentration of total hemoglobin in normal pregnancies in the mid-trimester and at term in labor and not in labor

(Legend) The median amniotic fluid concentration of total hemoglobin was significantly higher in women at term who were not in labor than in women in the mid-trimester [520.6 ng/mL, interquartile range (IQR) 271.2–1549.2 vs. 58.5 ng/mL, IQR 26.1–200.8; $p < 0.001$]. Women at term in labor had a higher median amniotic fluid total hemoglobin concentration than those not in labor (1952.6 ng/mL, IQR 709.6–6289.2 vs. 520.6 ng/mL, IQR 271.2–1549.2; $p = 0.003$).

Figure 2. Amniotic fluid concentration of total hemoglobin among women with spontaneous preterm labor and intact membranes

(Legend) The median amniotic fluid concentration of total hemoglobin was significantly higher in patients with intra-amniotic infection/inflammation (IAI) than in women without IAI (4671.8 ng/mL, interquartile range (IQR) 1294.2–8620.7 vs. 2013.6 ng/mL, IQR 629.2–5420.4; $p=0.01$) and than in those who delivered at term (1143.4 ng/mL, IQR 451.8–4037.9; $p=0.001$). Among women with PTL without IAI, there was no significant difference in the median amniotic fluid concentration of total hemoglobin between those who delivered preterm and those who delivered at term.

Figure 3. Amniotic fluid concentration of total hemoglobin among women with preterm prelabor rupture of the membranes

(Legend) The median amniotic fluid concentration of total hemoglobin was significantly higher in patients with IAI than in women without IAI (10753.7 ng/mL, interquartile range (IQR) 2053.9–56026.6 vs. 2281 ng/mL, IQR 938.2–9191.7; $p=0.02$).

Table 1

Demographic and clinical characteristics of women presenting with spontaneous preterm labor

Variable	Spontaneous PTL and intact membranes			<i>p</i> [*]
	delivered at term (<i>n</i> =92)	without IAI (<i>n</i> =76)	with IAI (<i>n</i> =81)	
Maternal age (years)	22 (20–27)	21 (19–29)	24 (20–28)	NS
GA at amniocentesis (weeks)	30.5 (26.5–32.6)	31.1 (27.4–32.1)	26.3 (24.1–32)	0.002
Amniotic fluid sample storage time (years)	5.6 (4.9–7.6)	5.8 (5.0–7.2)	5.9 (5.5–6.8)	NS
GA at delivery (weeks)	38.6 (37.9–39.6)	34.5 (32.6–35.3)	28 (24.8–32.9)	<0.001
Birthweight (grams)	3050 (2789–3370)	2225 (1760–2507)	1060 (646–1887)	<0.001

Values expressed as median (interquartile range)

GA: gestational age; PTL: preterm labor; IAI: intra-amniotic infection/inflammation; NS: not significant.

* Kruskal-Wallis test with Bonferroni correction.

Table 2

Demographic and clinical characteristics of women presenting with preterm PROM

Variable	Preterm PROM		<i>p</i> [*]
	without IAI (<i>n</i> =49)	with IAI (<i>n</i> =48)	
Maternal age (years)	25 (23.7–33.1)	28.5 (22–35.5)	<i>NS</i>
GA at amniocentesis (weeks)	32.6 (28.8–33.1)	29.2 (25.4–31.3)	0.001
Amniotic fluid sample storage time (years)	4.9 (3.6–5.8)	5.0 (4.4–5.8)	<i>NS</i>
GA at delivery (weeks)	33.3 (31.2–34.3)	30 (27.8–31.9)	<0.001
Birthweight (grams)	2020 (1685–2285)	1520 (1060–1820)	<0.001

Values expressed as median (interquartile range)

GA: gestational age; IAI: intra-amniotic infection/inflammation; PROM: prelabor rupture of the membranes; NS: not significant.

*
Mann-Whitney U-test