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Pentraxin 3 in Amniotic Fluid: A Novel Association with Intra-amniotic Infection and Inflammation

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Abstract

Objective—Pentraxin 3 (PTX3) is a soluble pattern recognition receptor that has an important role in immunoregulation and vascular integrity. The aim of this study was to determine if PTX3 is present in amniotic fluid (AF) and if its concentration changes with gestational age, in the presence of labor, and in cases of intra-amniotic infection/inflammation (IAI) associated with spontaneous preterm labor (PTL) or preterm prelabor rupture of membranes (PPROM).

Study design—This was a cross-sectional study which included the following groups: 1) mid-trimester (n=45); 2) uncomplicated pregnancies at term with (n=48) and without (n=40) spontaneous labor; 3) women with PTL and intact membranes: a) who delivered at term (n=44); b) who delivered preterm without IAI (n=40); and c) who delivered preterm with IAI (n=62); 4) women with PPROM with (n=63) and without (n=36) IAI. Pentraxin-3 concentration in AF was determined by ELISA. Non-parametric statistics were used for analyses.

Results—1) Among women in preterm labor with intact membranes, the median AF PTX3 concentration was significantly higher in women with IAI than in those without IAI (7.95 ng/mL vs. 0.38 ng/mL; $p<0.001$) and than in those who delivered at term (0.55 ng/mL; $p<0.001$); 2) women with PPROM and IAI had a higher median amniotic fluid PTX3 concentration than those without IAI (9.12 ng/mL vs. 0.76 ng/mL; $p<0.001$); 3) the median AF PTX3 concentration did not change with gestational age (mid-trimester: 0.79 ng/mL vs. term not in labor: 0.58 ng/mL; $p=0.09$); and 4) among women at term, no significant differences were observed in the median AF PTX3 concentration between women with spontaneous labor and those not in labor (0.54 ng/mL vs. 0.58 ng/mL, respectively; $p=0.9$).

Conclusions—PTX3 is a physiologic constituent of the AF, and its concentration is elevated in the presence of IAI, suggesting that PTX3 may play a role in the innate immune response against intra-amniotic infection.

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Keywords

preterm labor; preterm delivery; preterm prelabor rupture of membranes; PPROM; pregnancy; amniocentesis; microbial invasion of the amniotic cavity; MIAC; cytokines; pattern recognition receptors

Introduction

Preterm labor (PTL) is a syndrome [119], and one of the most important mechanism of disease is intrauterine infection, the only pathological process for which a causal link with prematurity has been established [18,36,46,47,[49],[61],[75],[77],[88],[91],[128],129,131,134,135]. Intra-amniotic infection and/or inflammation (IAI) is present in about one third of women with spontaneous PTL with intact membranes [133,168] and is associated with the development of the fetal inflammatory response syndrome (FIRS) [48,126], and severe neonatal morbidity [7,24,25, 45,[57],[80],101,164-167,169].

Several investigators [5,40,87,152,155-157] have reported on the antimicrobial activity of components of the amniotic fluid (AF), which are involved in the innate and adaptative immune response against microorganisms. The innate component of the immune system represents the first line of defense against infection and includes a wide range of non-specific mechanisms [32,35,55,59,[60],73,125,150,153]. One of the mechanisms by which the innate immune system recognizes microorganisms is mediated through pattern recognition receptors (PRRs) [66], which bind to surface markers on microorganisms [58,100,124].

Pentraxins are essential components of the humoral arm of the innate immune response and act as soluble PRRs [12,41] in response to pro-inflammatory signals and Toll-like receptors (TLRs) activation [4,8,103,171]. Pentraxin 3 (PTX3) is produced and released by a variety of cell types such as mononuclear cells, phagocytes, dendritic cells, fibroblasts, and endothelial cells [1,3,17,30,[51],64,74,79,106]. PTX3 recognizes microbial products, opsonizes fungi, selected Gram-positive and Gram-negative bacteria, viruses, and activates complement [12], and it is considered an acute phase response protein, because its concentrations increase considerably and rapidly in plasma of patients with systemic inflammatory response syndrome, sepsis, or septic shock [89]. Thus, the objective of this study was to determine if PTX3 is present in AF, if its concentration changes with gestational age, spontaneous labor at term, and in the presence of IAI in women with spontaneous PTL with intact membranes and in those with preterm prelabor rupture of the membranes (PPROM).

Materials and Methods

Study design and population

A cross-sectional study was carried out by searching our clinical database and bank of biological samples, and included 378 pregnant women in the following groups: 1) Women at 14-18 weeks gestation whose amniocentesis was conducted for genetic indications (n=45) and who subsequently had an uncomplicated pregnancy; 2) Uncomplicated term pregnancies with (n=48) and without (n=40) spontaneous labor; 3) Women with PTL and intact membranes without IAI who delivered at term (n=44); without IAI who delivered preterm (n=40); and with IAI (n=62); and 4) Women with PPROM with (n=63) and without IAI (n=36).

All women provided written informed consent prior to the collection of AF. The collection and utilization of AF for research purposes was approved by the Institutional Review Boards of the participating institutions and the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, NIH, DHHS. Many of these samples have been used previously to

study the biology of inflammation, hemostasis, and growth factor concentrations in uncomplicated pregnancies and those with adverse pregnancy outcomes.

Definitions

Women were considered to have an uncomplicated pregnancy if they did not have any medical, obstetrical, or surgical complication, and delivered a normal neonate at term, which was appropriately grown for gestational age [2,50]. Spontaneous PTL was defined as the presence of regular uterine contractions occurring at a frequency of at least two every 10 minutes associated with cervical change that required hospitalization before 37 completed weeks of gestation. PPROM was diagnosed by sterile speculum examination which confirmed pooling of AF in the vagina in association with nitrazine and ferning tests when necessary, before 37 weeks of gestation and prior to labor. Intra-amniotic infection was defined as a positive AF culture for micro-organisms. Intra-amniotic inflammation was diagnosed by an AF interleukin (IL)-6 concentration $\geq$2.6 ng/mL[168]. Histologic chorioamnionitis was diagnosed on the basis of inflammatory cells in the chorionic plate and/or chorioamniotic membranes [110]. Acute funisitis was diagnosed by the presence of neutrophils in the wall of the umbilical vessels and/or Wharton's jelly using criteria previously described [99].

Sample collection

The AF samples were obtained by transabdominal amniocentesis for genetic indications, for evaluation of microbial status of the amniotic cavity and/or for assessment of fetal lung maturity in women approaching term in whom the dates were uncertain. For these women to be considered as term, the following criteria had to be fulfilled: 1) analysis of AF consistent with maturity; 2) birthweight >2500g; 3) absence of respiratory distress syndrome or other complications of prematurity; and 4) pediatric neonatal examination consistent with a term neonate. Samples of AF were transported to the laboratory in a sterile capped syringe and cultured for aerobic/anaerobic bacteria and genital mycoplasmas. White blood cell (WBC) count, glucose concentration and Gram-stain were also performed shortly after collection as previously described [122,127,132]. The results of these tests were used for clinical management. AF 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. The AF IL-6 concentrations were used only for research purposes. Among women with spontaneous preterm labor with intact membranes who delivered within 72 hours of amniocentesis, placenta, umbilical cord, and chorioamniotic membranes were collected, and the presence or absence of histologic chorioamnionitis and/or funisitis was assessed. The 72 hour interval was chosen to preserve a meaningful temporal relationship between AF PTX 3 concentration and placental histopathologic findings.

Determination of human PTX3 concentration in amniotic fluid

Specific and sensitive enzyme-linked immunoassays (Linco Research, St. Charles, MO, USA) were used to determine concentrations of PTX3 in human AF. The PTX3 assays were validated for use in human AF in our laboratory prior to their use in this study. Validation included spike and recovery experiments which produced parallel curves indicating that AF constituents did not interfere with antigen-antibody binding in this assay. Immunoassays were carried out according to the manufacturer's recommendations. The AF samples were incubated in duplicate wells of the micro titer plates, which had been pre-coated with antibodies specific for PTX3. During this incubation, the PTX3 present in the standards or AF 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 PTX3 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, with color

developing in proportion to the amount of the PTX3 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 PTX3 in AF samples were determined by interpolation from individual standard curves. The calculated inter-assay and intra-assay coefficients of variation for PTX3 in our laboratory were 2.7% and 3.9% respectively. The sensitivity was 0.126 ng/ml.

The concentrations of PTX3 in AF samples were determined by extrapolation from individual standard curves. The calculated inter-assay and intra-assay coefficients of variation for PTX3 in our laboratory were 2.7% and 3.9% respectively. The sensitivity was 0.126 ng/ml.

Statistical analysis

The normality of the data was tested using the Shapiro-Wilk and Kolmogorov-Smirnov tests. Since AF PTX3 concentrations were not normally distributed, non-parametric tests were used for analyses. Comparisons between proportions were performed with the Chi-square test. Kruskal-Wallis with post-hoc analysis and Mann-Whitney U tests were used for continuous variables. Adjustment for multiple comparisons was performed using the Bonferroni method [11]. Analysis of covariance (ANCOVA) was used to examine the difference of AF PTX3 concentration between the PTL and PPROM subgroup while adjust for storage time. Spearman rank correlation was utilized to assess correlations between AF concentrations of PTX3, IL-6, glucose and WBC count. A p-value of <0.05 was considered statistically significant. The statistical package used was SPSS v.15.0 (SPSS Inc., Chicago, IL, USA).

Results

Demographic and clinical characteristics of the study population

Table I presents the demographic and clinical characteristics of women in the mid-trimester, term not in labor and term in labor groups. Predictably, women in the genetic amniocentesis group had a significantly higher median maternal age and significantly lower median gestational age at amniocentesis than women at term not in labor. Table II and III display the demographic and clinical characteristics of women with spontaneous PTL and intact membranes and those with PPROM, respectively. In women with PTL and intact membranes, those with IAI had a significantly lower median gestational age at amniocentesis than those without IAI who delivered preterm. Women with IAI had also a lower gestational age at delivery compared to women without IAI who delivered preterm and at term. In women with PPROM, the birth weight and gestational age were significantly lower in women with IAI than in those without IAI.

Amniotic fluid PTX3 concentration did not change with advancing gestational age or in the presence of labor at term

PTX3 was detected in 95.2% (360/378) of all AF samples. There were no significant differences in the median AF PTX3 concentration between women in the mid-trimester and those with an uncomplicated term pregnancy who were not in labor (0.79 ng/mL vs. 0.58 ng/mL, respectively; p=0.09) (Figure 1). Similarly, no significant differences were observed in the median AF PTX3 concentration between women at term in labor and those not in labor (0.54 ng/m vs. 0.58 ng/mL, respectively; p=0.9) (Figure 1).

Amniotic fluid PTX3 concentrations are increased in the presence of intra-amniotic infection/inflammation in women with spontaneous preterm labor and intact membranes

Among women with PTL, those with IAI had a significantly higher median AF concentration of PTX3 compared to those without IAI who delivered preterm (7.95 ng/mL vs. 0.38 ng/mL, respectively; $p<0.001$) and than those without IAI who delivered at term (0.55 ng/mL; $p<0.001$) (Figure 2). There were no significant differences in the median AF PTX3 concentration between women with PTL without IAI who delivered preterm and those who delivered at term ($p=0.6$) (Figure 2). These results did not change after adjusting for gestational age at amniocentesis, and storage time (ANCOVA).

Amniotic fluid PTX3 concentrations are increased in the presence of intra-amniotic infection/inflammation in women with PPROM

Women with PPROM and IAI had a significantly higher median AF PTX3 concentration than women with PPROM without IAI (9.12 ng/mL, vs. 0.76 ng/mL, respectively; $p<0.001$) (Figure 3). These results did not change after adjusting for gestational age at amniocentesis, and storage time (ANCOVA).

Correlation of amniotic fluid PTX3 concentration and other indirect markers of intra-amniotic infection/inflammation

A significant correlation was observed between AF PTX3 concentrations and IL-6, WBC count and glucose concentration in women with spontaneous PTL and those with PPROM (Spearman rho coefficient: IL-6 0.74, $p<0.001$; WBC count 0.49; $p<0.001$; and glucose -0.3, $p<0.001$).

Amniotic fluid PTX3 concentrations and histological chorioamnionitis

Fifty-two women with spontaneous PTL delivered within 72 hours, and histologic chorioamnionitis was present in 62% (23/37) of the cases with available placental pathologic examination. The median AF PTX3 concentration was significantly higher in women with histologic chorioamnionitis compared to those without placental inflammation (28.5 ng/mL, vs. 1.32 ng/mL, respectively; $p=0.002$) (Figure 4).

Discussion

Principal findings of the study

1) Pentraxin 3 is a physiologic constituent of the AF; 2) in women with spontaneous PTL with intact membranes, as well as in those with PPROM, the median AF PTX3 concentration was significantly elevated in the presence of IAI; 3) amniotic fluid PTX3 concentrations correlated significantly with indirect AF markers of IAI, such as IL-6, as well as with histologic chorioamnionitis; and 4) advancing gestational age and spontaneous labor at term were not associated with significant changes in the median AF PTX3 concentrations.

What is Pentraxin 3?

Pentraxins are a group of evolutionarily conserved soluble PRRs and essential components of the humoral arm of the innate immune response, together with other soluble PRRs such as mannose-binding lectin, ficolins and the complement cascade [41]. Pentraxins are characterized by a distinctive cyclic pentameric structure [41,44] and can be divided into short and long pentraxins, since they share a C-terminal pentraxin-like domain but the long pentraxins hold a unique and unrelated long N-terminal domain [9,13,17,21,[34],[41],[44],[79],85, 102,103,151]. C-reactive protein (CRP) and serum amyloid P-component (SAP) are short pentraxins. CRP was the first described fluid-phase pattern recognition molecule and named after its ability to bind in a calcium-dependent manner the C-polysaccharide of *Streptococcus*

pneumoniae [12]. CRP and SAP are acute-phase proteins that regulate innate resistence to microbes and scavenging of cellular debris [103].

PTX3, also called TNF stimulated gene 14 (TSG14) [78,79], is a 381 amino acids protein with a molecular weight of 40 kDa, and its gene is located on chromosome 3 [14,17,85,148]. PTX3 was the first long pentraxin identified [17,78,79] and other members of this family subsequently discovered are neuronal pentraxin 1, [95,143] neuronal pentraxin 2, [63,159] neuronal pentraxin receptor [28,72], and guinea pig apexin [94,113]. Similarly to CRP, PTX3 performs as an acute phase response protein in plasma: its physiologic concentration is low (≤2 ng/ml) but increases rapidly (peak at 6-8 hours) and dramatically (200-800 ng/ml) during inflammatory conditions such as autoimmune disease, endotoxic shock, infections, degenerative disorders and sepsis [86,89,104].

PTX3 is expressed by human peripheral blood monocytes in response to IL-1β and Tumor Necrosis Factor-α (TNF-α) or after stimulation with microbial components such as lypopolysaccharide (LPS) [13,41,67], while IL-6, monocyte chemotactic protein 1 (MCP-1/CCL2), macrophage colony-stimulating factor (M-CSF), granulocyte–macrophage colony-stimulating factor (GM-CSF), or interferon-γ (IFN-γ), are not strong inducers of PTX3 [3, 12]. Interestingly, IL-10 as a mild inducer of PTX3 in monocytes and dendritic cells [105], and it can amplify PTX3 production induced by LPS [12,41].

PTX3 is also present in neuthrophil granules [65], acting as a reservoir for a rapid release after microbial recognition [12]. Dendritic cells [29,30] produce high concentrations of PTX3 in response to LPS or TLR agonists such as peptidoglycan (TLR2), double-stranded DNA (TLR3), *Candida* (TLR4), and flagellin (TLR5) [30]. In contrast to neutrophils, dendritic cells and macrophages produce PTX3 *de novo* in response to inflammatory signals [12]. Other cell types that produce PTX3 *in vitro* are endothelial cells [17,79,108], smooth muscle cells [74], epithelial cells [93], adipocytes [1], fibroblasts [144,145], synovial cells [82] and chondrocytes [163].

Pentraxin 3 and normal pregnancy

Only few studies have investigated PTX3 during pregnancy. It has been demonstrated that the maternal blood PTX3 concentration is significantly higher during normal pregnancy compared to non-pregnant women [19,137], supporting the view that normal pregnancy is a pro-inflammatory state [23,31,62,83,[84],[90],114,139,140,149]. However, conflicting results have been reported regarding the changes in maternal circulating PTX3 concentration throughout gestation. While Rovere-Querini et al. [137] reported an increase in the maternal serum PTX3 concentrations with advancing gestational age and the highest concentration during labor, Cetin et al. [19] found no change in maternal plasma PTX3 concentrations during pregnancy.

Pentraxin 3 in pregnancy complications

Women with preeclampsia have a significantly higher (6 to 10-fold) median serum/plasma PTX3 concentration than women with uncomplicated pregnancies [19,137]. Moreover, it has been reported that serum PTX3 concentrations correlate with the severity of preeclampsia [137]. Since PTX3 is expressed in endothelial cells [17,108], it was proposed [19] that elevated circulating concentrations of PTX3 in women with preeclampsia may represent a state of endothelial dysfunction that characterizes this obstetrical syndrome [16,20,43,68,[107],[111],[112], 115-117,138,154]. Indeed, PTX3 has been recently considered to be a marker of vascular bed injury in conditions such as myocardial infarction [76,104,142] and disorders associated with autoimmunity such as small-vessel vasculitis [39,162], rheumatoid arthritis [82], psoriasis [10], and Wegener granulomatosis [161]. Vascular endothelial cells and smooth muscle cells produce high concentrations of PTX3 in response to inflammatory signals, suggesting a role

as a regulator of endothelium during thrombogenesis and ischemic vasculature disease [17, 74,108,118].

A single study reported on maternal circulating PTX3 concentrations in women with preterm delivery. Assi et al. [6] reported that, regardless of the clinical presentation (PTL or PPROM), women with a preterm delivery (<34 weeks) had a significantly higher maternal plasma PTX3 concentration (but not in vaginal fluid) than normal pregnant women. Moreover, women with placental vasculopathy had significantly higher plasma PTX3 concentrations than those without these placental lesions. In contrast, no differences were found in the peak plasma or peak vaginal concentration of PTX3 between women with clinical and/or histologic chorioamnionitis and those without these conditions. The authors suggested that elevated PTX3 concentrations in maternal plasma of women with PTL or PPROM may be associated to mechanisms other than intra-uterine infection, such as insults related to placental underperfusion [6].

Pentraxin 3 in amniotic fluid in normal pregnancy and term parturition

There is a paucity of information regarding PTX3 concentration in AF. In this study, PTX3 was detected in 95% of AF samples suggesting that this molecule is a physiologic constituent of the AF. In addition, we observed that PTX3 concentrations did not change significantly with advancing gestational age. This finding is in agreement with a report by Greco et al. [54] who compared PTX3 concentrations in AF obtained in mid-trimester amniocenteses and during elective cesarean sections from uncomplicated pregnancies [54].

Spontaneous labor at term is regarded as an inflammatory process [22,37,38,56,[69]-[71],[81], [96]-[98],[120],[121],[123],130,141,146,158]. In the study reported herein, labor at term was not associated with a significant change in the AF concentration of PTX3, whereas Rovere-Querini et al. [137] reported that the maternal serum PTX3 concentrations peaked during labor. These results suggest that PTX3 in AF may have a limited role in the physiologic process of parturition at term.

Pentraxin 3 in amniotic fluid in intra-amniotic infection/inflammation

The findings that IAI is associated with an elevated median AF concentration of PTX3 in women with PTL and in those with PPROM, as well as in women with histologic chorioamnionitis, are novel. Among women with PTL or PPROM, the presence of IAI was associated with a 16-fold and a 12-fold increase in AF PTX3 concentrations, respectively. Similarly, women with histologic chorioamnionitis had a dramatically higher AF PTX3 concentration (22-fold) than those without placental inflammation. Furthermore, a significant correlation was observed between AF PTX3 concentrations and indirect markers of intra-amniotic infection, such as IL-6. Recently, Greco et al. [54] reported an increased concentration of PTX3 in amniotic fluid collected from the vaginal fornix from women with PPROM, and the AF concentration of PTX3 correlated with the presence of histologic chorioamnionitis.

Compelling evidence supports the notion that PTX3 plays an important role against bacterial infection caused by *Staphylococcus aureus, Pseudomonas aeroginosa, Salmonella typhimurium, Klebsiella pneumoniae, Streptococcus pneumoniae, Neisseria meningitides* [42,67], fungal infection caused by *Aspergillus fumigatus and Paracoccidioides brasiliensis* [27,42], and viral infections, such as cytomegalovirus (CMV) and H3N2 influenza virus [15, 109]. Indeed, PTX3 confers resistance to viral infections by binding both human and murine CMV, limiting viral entry and infectivity in dendritic cells [15], and also by inhibiting influenza viruses [109] through different mechanisms such as inhibition of hemagglutination, neutralization of virus infectivity, and inhibition of viral neuraminidase [12]. In addition, the binding of PTX3 with C1q, which was the first described ligand for PTX3, activates the

classical pathway of the complement system and facilitates pathogen recognition by phagocytes [92,136]. PTX3 also modulates factor H, which is considered the main soluble regulator of the alternative pathway, preventing an exaggerated activation of the complement system [26]. Thus, it has been proposed that PTX3 participates in the crosstalk between the cellular and humoral arms of the innate immunity in response to microbial invasion by facilitating the activity of the cellular arm of the innate immune response and modulating complement activation [12]. This support the concept of activation of the innate immune system and the complement pathway as part of the inflammatory response to microbial invasion of the AF [33,147,160].

What is the origin of Pentraxin 3 in amniotic fluid?

The origin of PTX3 in the AF and the main compartment contributing to the higher concentrations in cases with IAI is still unknown. Several potential sources can be suggested: 1) PTX3 was shown to be physiologically expressed in fetal membranes (amniotic epithelium, chorionic mesoderm) from uncomplicated pregnancies [53,137]. Furthermore, its expression increased in membranes from pregnancies complicated by PPROM and/or with histologic chorioamnionitis [53]. This suggests that the fetal membranes may contribute to the higher AF concentration of PTX3 observed in cases with IAI and histologic chorioamnionitis; 2) the fetus is capable of mounting an inflammatory response to the presence of microbial invasion of the amniotic cavity [48,52,126] characterized by systemic activation of the innate immune system. Indeed, it has been reported that CRP, one of the short pentraxins, is significantly higher in preterm neonates from mothers with a positive AF culture than in those with negative culture as well as in neonates with funisitis than in those without funisitis [170]. Although there are no data regarding PTX3 in cord blood, it is possible that AF PTX3 may represent, in part, a fetal inflammatory response to acute intra-amniotic infection; and 3) in maternal circulation, PTX3 concentrations were shown to increase with advancing gestation and to peak during term labor [137]. However, the lack of significant change in AF PTX3 concentrations throughout gestation and during term parturition suggests that maternal blood and amniotic fluid are two independent compartments.

In conclusion, this study demonstrates that PTX3 is a physiologic constituent of the amniotic fluid, and its concentration is significantly elevated in the presence of intra-amniotic infection/inflammation, suggesting that PTX3 may play a role in the innate immune response against intra-amniotic infection.

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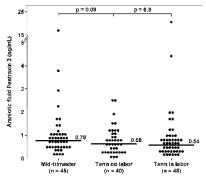

Figure 1. Amniotic fluid concentrations of Pentraxin 3 (PTX3) in normal pregnancies at mid-trimester and in those at term with and without labor

There were no differences in the median amniotic fluid PTX3 concentration between women in the mid-trimester and those with a normal pregnancy at term not in labor [0.79 ng/mL, IQR 0.57-1.08 vs. 0.58 ng/mL, IQR 0.27-1.05, respectively; p=0.09]; no significant differences were observed in the median amniotic fluid PTX3 concentration between women with spontaneous labor at term and those at term not in labor (0.58 ng/mL, IQR 0.27-1.05 vs 0.54 ng/mL, IQR 0.34-0.82, respectively; p=0.9).

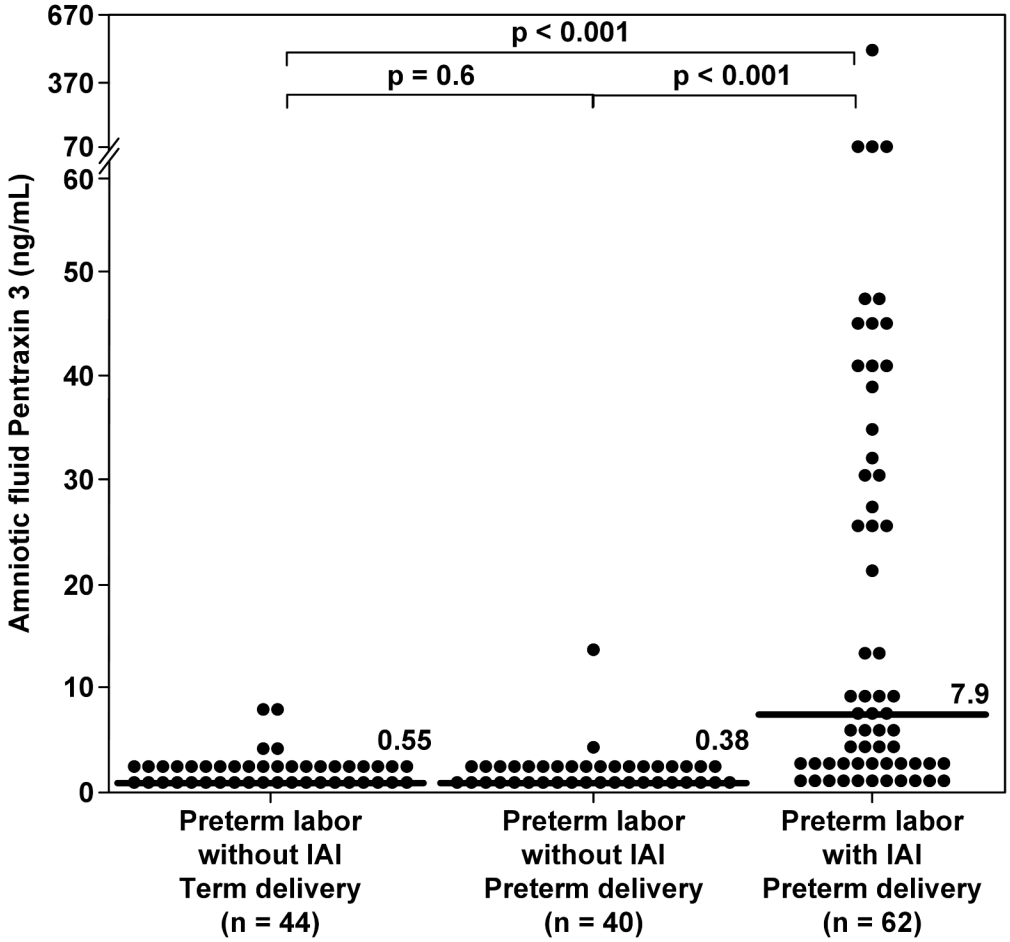

Figure 2. Amniotic fluid concentrations of Pentraxin 3 (PTX3) among women with spontaneous preterm labor (PTL) and intact membranes

The median amniotic fluid concentration of PTX3 was significantly higher in women with intra-amniotic infection/inflammation (IAI) than in women who delivered preterm without IAI (7.9 ng/mL, IQR 1.7–35.3 vs. 0.38 ng/mL, IQR 0.22–0.82; p<0.001) and in those who delivered at term (0.55 ng/mL, IQR 0.24–1.19; p<0.001). Among women without IAI, there was no significant difference in the median amniotic fluid concentration of PTX3 between those who delivered preterm and those who delivered at term. (0.38 ng/mL, IQR 0.22-0.82 vs 0.55 ng/mL, IQR 0.24-1.19; p= 0.6).

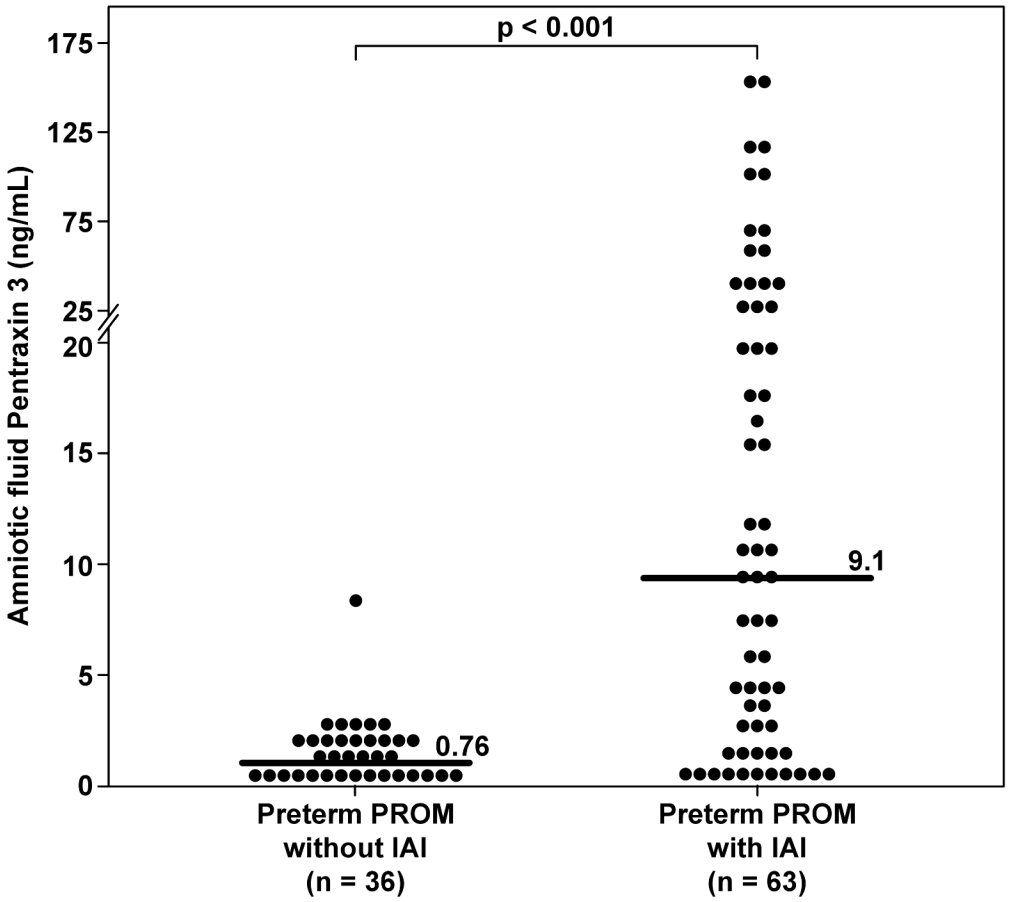

Figure 3. Amniotic fluid concentrations of Pentraxin 3 (PTX3) in women with preterm prelabor rupture of the membranes (PPROM)

The median amniotic fluid concentration of PTX3 was significantly higher in women with intra-amniotic infection/inflammation (IAI) than in those without IAI (9.1 ng/mL, IQR 1.85-29.6 vs. 0.76 ng/mL, IQR 0.34–1.53; $p<0.001$).

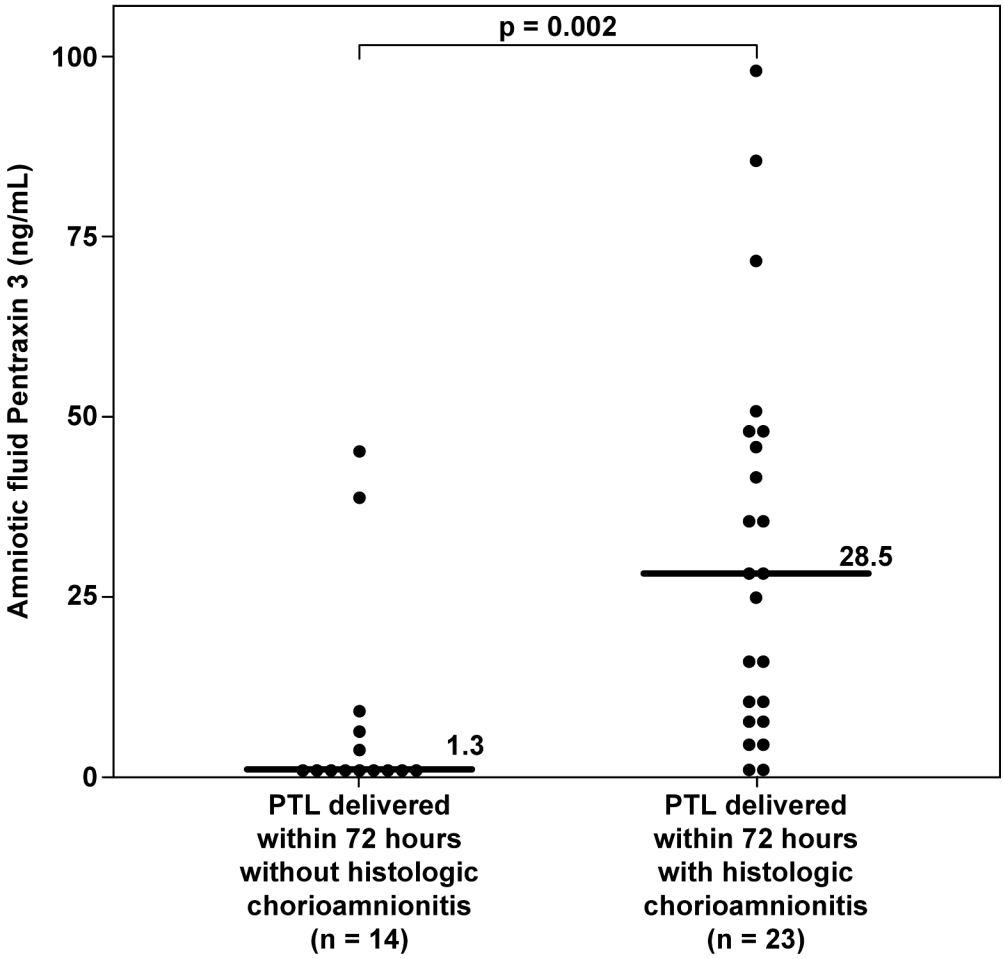

Figure 4. Amniotic fluid concentrations of Pentraxin 3 (PTX3) in women with spontaneous preterm labor with and without histologic chorioamnionitis who delivered within 72 hours from amniocentesis

Women with histologic chorioamnionitis and/or funisitis had a significantly higher median PTX3 concentration in amniotic fluid than those without histologic inflammation (28.5 ng/mL, IQR 9.25-48.68 vs 1.32 ng/mL, IQR 0.63-7.36; p=0.002).

Table I

Demographic and clinical characteristics of women in the mid-trimester and those at term with and without spontaneous labor

	Mid-trimester (n=61)	p[a]	Term No labor (n=50)	Term In labor (n=49)	p[b]
Maternal age (years)	36 (35-38)	<0.001	27 (21-32)	23 (19-30)	NS
GA at amniocentesis (weeks)	16 (16-17)	<0.001	39 (38-40)	39 (37.8-40)	NS
GA at delivery (weeks)	40 (38-40)	NS	39 (38-40)	39 (37.8-40)	NS
Birthweight (grams)	3,320 (3,064-3,570)	NS	3,260 (3,055-3,595)	3,250 (3,060-3,620)	NS

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

GA: gestational age; **NS:** not significant.

p[a]: comparison between women in the mid-trimester and those at term not in labor

p[b]: comparison between women at term not in labor and those at term in labor

Table II

Demographic and clinical characteristics of women presenting with spontaneous preterm labor with intact membranes

	PTL without IAI Term delivery (n=44)	p	PTL without IAI Preterm delivery (n=40)	p[a]	PTL with IAI Preterm delivery (n=62)	p[b]
Maternal age (years)	23 (20-27)	NS	21.5 (20-28.8)	NS	22 (20-26.8)	NS
Smoking	28.6 (6/21)	<0.05	3.6 (1/28)	<0.05	24.2 (8/33)	NS
GA at amniocentesis (weeks)	30.5 (27.7-33.2)	NS	31.9 (28.1-33.3)	<0.05	28.9 (26.5-32.7)	NS
GA at delivery (weeks)	39.1 (38.1-40)	<0.001	34.6 (33.2-35.8)	<0.001	30.6 (27.0-32.9)	<0.001
Birthweight (grams)	3,154 (2,910-3,505)	<0.001	2,455 (1,973-2,693)	<0.001	1,515 (930-2,112)	<0.001

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

p: comparison between PTL who delivered at term and PTL without IAI

p[a]: comparison between PTL who delivered preterm without IAI and PTL with IAI

p[b]: comparison between PTL who delivered at term and PTL with IAI

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

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Table III

Demographic and clinical characteristics of women presenting with preterm prelabor rupture of membranes

	PPROM without IAI (n=44)	PPROM with IAI (n=47)	p
Maternal age (years)	24.5 (20-31)	26 (22-32)	NS
Smoking	28.6 (4/14)	29 (9/31)	NS
GA at amniocentesis (weeks)	31.5 (28.1-32.6)	30 (27-32)	NS
GA at delivery (weeks)	32.7 (30.9-33.8)	30.7 (28.4-32.6)	<0.05
Birthweight (grams)	1,837 (1,455-2190)	1,660 (1,304-1,895)	<0.05

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

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