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RETINOL BINDING PROTEIN 4: AN ADIPOKINE ASSOCIATED WITH INTRA-AMNIOTIC INFECTION / INFLAMMATION

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Abstract

Objective—Retinol binding protein 4 (RBP4), a specific carrier for retinol in the blood, is a novel adipokine that has been implicated in the pathophysiology of insulin resistance, and its gene expression has been associated with adipose tissue inflammation. Recently, proteomic profiling of amniotic fluid (AF) from women with preterm labor (PTL) revealed over-expression of RBP4 in those who delivered preterm. The aim of this study was to determine whether RBP4 is present in AF, and if its concentrations change with gestational age, in the presence of labor, and intra-amniotic infection/inflammation (IAI) in patients with spontaneous PTL

Study design—This cross-sectional study included pregnant women in the following groups: 1) mid-trimester (n=30); 2) term not in labor (n=31); 3) term in labor (n=30); 4) spontaneous PTL without IAI who delivered at term (n=60); 5) PTL without IAI who delivered preterm (n=64); and 6) PTL with IAI (n=56). RBP4 concentrations in AF were determined by ELISA. Non-parametric statistics were used for analyses.

Results—1) RBP4 was detected in all AF samples; 2) among patients with PTL, women with IAI had a higher median AF RBP4 concentration than those without IAI who delivered preterm (1268.9 ng/mL, interquartile range (IQR) 900.3-1970.1 vs. 815.8 ng/mL, IQR 592.4-1098.1; $p<0.001$) and at term (828.7 ng/mL, IQR 499.7-1119.6; $p<0.001$); 3) the median AF RBP4 concentration was higher in women in the midtrimester than in those at term not in labor (1861.1 ng/mL, IQR 1486.2-2034.3 vs. 766.1 ng/mL, IQR 608.5-1154.1; $p<0.0001$; 4) the median AF RBP4 concentration did not differ significantly between patients with PTL without IAI who delivered preterm and those who delivered at term (p=0.7); and 5) among women at term, the median AF RBP4 concentrations was not significantly different between those in labor and those not in labor (p=0.4).

Conclusions—Retinol binding protein 4 is a physiologic constituent of the amniotic fluid. Among patients with PTL, the median AF concentration of immunoreactive RBP4 is elevated in

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pregnancies complicated by IAI. These results suggest that RBP4 may participate in the host response against intra-amniotic infection/inflammation.

Keywords

pregnancy; preterm delivery; preterm labor; microbial invasion of the amniotic cavity; RBP4; chorioamnionitis; adipokines

INTRODUCTION

Adipokines are adipocyte-derived cytokines that provide a link between obesity, insulin resistance and obesity-related inflammatory disorders.[1] This family of biologically active molecules includes peptides and proteins such as leptin (the first adipokine to be discovered[2]), adiponectin, visfatin and resistin, as well as other cytokines and chemokines (i.e., tumor necrosis factor (TNF), interleukin (IL)-6, IL-1 and monocyte chemotactic protein (MCP)-1) which were described to be secreted by adipose tissue.[1] Retinol binding protein 4 (RBP4),[3] has recently been added to the rapidly expanding list of adipokines.[4-6]

Retinol-binding protein 4 belongs to the lipocalin family and is a specific carrier for retinol (vitamin A) in the blood. Until recently, its only function was thought to be the delivery of retinol to peripheral tissues. However, in 2005 Yang et al[3] rekindled interest in this molecule by describing RBP4 as a novel adipokine. Using global gene expression analysis in an adipocye-specific *Glut4* knockout mouse model, RBP4 was found to be over-expressed in adipose tissue, and implicated in the pathophysiology of insulin resistance.[3] Indeed, increased circulating RBP4 concentrations have been reported in patients with diabetes.[3] Moreover, high plasma concentrations of RBP4 precede the development of overt diabetes.[7,8] In contrast, other investigators, in contrast, have not established an association between plasma RBP4 concentrations and insulin resistance.[9,10] Furthermore, emerging evidence suggests that RBP4 plays a role in the inflammatory response. Indeed, in non-diabetic human subjects, *RBP4* gene expression was found to be strongly associated with inflammatory markers (i.e., CD68 and MCP-1) of adipose tissue.[10]

Recently, our group has demonstrated that increased amniotic fluid concentrations of adipokines, such as visfatin[11] and resistin,[12] are associated with intra-amniotic infection/inflammation (IAI). Furthermore, proteomic profiling of the amniotic fluid of patients with spontaneous preterm labor (PTL) revealed that RBP4 is over-expressed in patients who delivered preterm, compared to those with an episode of PTL who delivered at term.[13] While this increased expression was noted regardless of the presence or absence of IAI, it was more profound in microbial invasion of the amniotic cavity.[13]

Thus, the aim of this study was to determine whether the amniotic fluid concentrations of RBP4 change with advancing gestational age, spontaneous labor at term, and in the presence of intra-amniotic infection/inflammation in patients with spontaneous preterm labor and intact membranes.

MATERIALS AND METHODS

Study design and population

A cross-sectional study was conducted by searching our clinical database and bank of biological specimens, and included pregnant women in the following groups: 1) women in the mid-trimester of pregnancy (14-18 weeks) who underwent amniocentesis for genetic indications and delivered a normal neonate at term (n=30); 2) normal pregnant women at

term with (n=30) and without spontaneous labor (n=31); 3) patients with an episode of spontaneous PTL and intact membranes who were classified into: a) PTL without IAI who delivered at term (n=60); b) PTL without IAI who delivered preterm (n=64); and c) PTL with IAI (n=56).

All participants provided written informed consent prior to the collection of amniotic fluid. The collection of amniotic fluid and its utilization 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 (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 at term (≥37 weeks) a healthy neonate with appropriate birthweight for gestational age [14,15]. Spontaneous preterm labor was defined by 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. Preterm delivery was defined as delivery before 37 completed weeks of gestation. Intra-amniotic infection was defined as a positive amniotic fluid culture for micro-organisms. Intra-amniotic inflammation was diagnosed by an amniotic fluid IL-6 concentration ≥2.6 ng/mL.[16] Histologic chorioamnionitis was diagnosed based on the presence of inflammatory cells in the chorionic plate and/or chorioamniotic membranes, and acute funisitis was diagnosed by the presence of neutrophils in the wall of the umbilical vessels and/or Wharton's jelly, using the criteria previously described.[17,18]

Amniotic fluid sample collection

Amniotic fluid samples were obtained by transabdominal amniocenteses performed for genetic indication, evaluation of microbial status of the amniotic cavity, and/or assessment of fetal lung maturity in patients approaching term. Women at term not in labor underwent amniocentesis for the assessment of fetal lung maturity prior to 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 of the amniotic cavity and fetal lung maturity. These patients were retrospectively considered to be in labor at term if all the following criteria were met: (1) spontaneous labor; (2) delivery within 24 hours of amniocentesis; (3) analysis of amniotic fluid consistent with maturity; (4) birthweight >2500 grams; and (5) absence of complications of prematurity and physical examination of the newborn by pediatricians consistent with a term neonate. 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 (WBC) count, glucose concentration and Gram-stain were also performed shortly after collection as previously described.[19-21] The results of these tests were used for subsequent clinical management. Amniotic fluid IL-6 concentrations were employed only for research purposes. 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 markers of infection at the time of retrieval, however, all samples have subsequently been found to have an amniotic fluid IL-6 concentration <2.6 ng/mL.

Placenta, umbilical cord and chorioamniotic membranes were collected in patients with spontaneous PTL and intact membranes who delivered within 72 hours of amniocentesis, and the presence or absence of histologic chorioamnionitis and/or funisitis was assessed.

The 72 hours time period was chosen in order to preserve a meaningful temporal relationship between amniotic fluid RBP4 concentrations and placental histopathologic findings.

Determination of retinol binding protein 4 concentration in amniotic fluid

Amniotic fluid concentration of retinol binding protein 4 was determined by sensitive enzyme-linked immunoassays (Millipore Corporation, St. Charles, MO, USA). The RBP4 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 the manufacturer's recommendations. Amniotic fluid samples were incubated in duplicate wells of the micro titer plates, which had been pre-coated with antibodies specific for the analyte (RBP4). 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, with color developing 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 RBP4 in amniotic fluid samples were determined by interpolation from individual standard curves. The calculated inter- and intra-assay coefficients of variation for RBP4 immunoassays in our laboratory were 6.98% and 6.05%, respectively. The sensitivity was calculated to be 0.09 ng/mL.

Statistical analysis

Shapiro-Wilk and Kolmogorov-Smirnov tests were used to test for normal distribution of the data. Since amniotic fluid RBP4 concentrations were not normally distributed, non parametric tests were used for analyses. Correlations between continuous variables were 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. Among patients with PTL and intact membranes, a receiver-operating characteristic (ROC) curve analysis was performed to determine amniotic fluid RBP4 concentration cutoff for identification of patients who had IAI. A multivariable logistic regression model (stepwise) was utilized to examine the association between the RBP4 concentrations, maternal age, pre-pregnancy body mass index (BMI), smoking status, gestational age at amniocentesis, and the presence of IAI. A p-value of <0.05 was considered statistically significant. Statistical analysis was performed with SPSS package version 12 (SPSS Inc, Chicago, IL, USA).

RESULTS

Demographic and clinical characteristics of the study population

Table I displays the demographic and clinical characteristics of patients in the midtrimester, term not in labor and term in labor groups. Table II presents the demographic and clinical characteristics of patients with spontaneous PTL and intact membranes. Among patients with PTL, those with IAI had a significantly lower median gestational age at amniocentesis than both patients without IAI who delivered preterm and those who delivered at term (Table II).

Amniotic fluid RBP4 in normal pregnancies

Retinol-binding protein 4 was detected in all amniotic fluid samples (n=271). The median amniotic fluid RBP4 concentration in the mid-trimester was higher than that of women at term not in labor (1861.1 ng/mL, interquartile range (IQR) 1486.2-2034.3 vs. 766.1 ng/mL, IQR 608.5-1154.1; p<0.0001, Figure 1). Among women at term, the median amniotic fluid RBP4 concentration did not differ significantly between women with spontaneous labor and those not in labor (term in labor: 836.8 ng/mL, IQR 589.5-1112.8; p=0.4, Figure 1).

Amniotic fluid RBP4 in spontaneous PTL and intact membranes

Among patients with PTL, the median amniotic fluid RBP4 concentration differed significantly between the three study groups (Kruskal-Wallis, p<0.001). Women with IAI had a higher median amniotic fluid RBP4 concentration than those with PTL without IAI who delivered preterm (1268.9 ng/mL, IQR 900.3-1970.1 vs. 815.8 ng/mL, IQR 592.4-1098.1; p<0.001) and those with PTL without IAI who delivered at term (828.7 ng/mL, IQR 499.7-1119.6; p<0.001, Figure 2). The median amniotic fluid RBP4 concentration did not differ significantly between patients with PTL without IAI who delivered preterm and those without IAI who delivered at term (p=0.7, Figure 2).

The ROC curve of amniotic fluid RBP4 concentration for the identification of IAI among patients with PTL with intact membranes is displayed in Figure 3 (area under the curve (AUC) 0.71, p<0.001). A cutoff value for amniotic fluid RBP4 concentration of ≥1149.8 ng/mL had a sensitivity of 58.9% and a specificity of 79.8% for the identification of IAI in patients with PTL and intact membranes. In a backward stepwise multivariable logistic regression model including IAI as the dependent variable and maternal age, pre-pregnancy BMI, smoking status, gestational age at amniocentesis and amniotic fluid RBP4 concentration as covariates, only an amniotic fluid RBP4 concentration ≥1149.8 ng/mL and pre-pregnancy BMI were independently associated with the presence of IAI (Table 3).

Among patients with PTL and intact membranes, the amniotic fluid RBP4 concentrations correlated significantly with amniotic fluid WBC count (r=0.20, p=0.007) and IL-6 concentrations (r=0.37, p<0.001), but not with amniotic fluid glucose concentrations (r=-0.36, p=0.6).

Amniotic fluid concentration of RBP4 and placental inflammation

Among patients with spontaneous PTL and intact membranes with IAI, 75% (42/56) delivered within 72 hours of amniocentesis, and placental histopathologic results were available from 74% (31/42) of those patients. The median amniotic fluid RBP4 concentration was significantly higher in patients with IAI who had histologic chorioamnionitis and/or funisitis than in those without these placental lesions (1901.0 ng/mL, IQR 1107.3-2492.0 vs. 968.2 ng/mL, IQR 634.7-1396.7; p=0.01, Figure 4).

DISCUSSION

Principal findings of this study

1) Retinol binding protein 4 was detected in all amniotic fluid samples; 2) Intra-amniotic infection/inflammation in patients with PTL was associated with a higher median amniotic fluid RBP4 than those without IAI; 3) The median amniotic fluid RBP4 concentration was higher in the mid-trimester (14-18 weeks) than at term; 4) Spontaneous labor at term was not associated with changes in the median amniotic fluid RBP4 concentration.

Retinol binding protein 4: an old carrier of retinol and a novel adipokine

Retinol binding protein, first described by Kanai, Raz and Goodman in 1968,[22] is the specific carrier of retinol in the blood, belongs to the lipocalin (transporters for small hydrophobic molecules) family, and has a molecular weight of approximately 21-22 kDa.[22] Retinol binding protein 4 is a plasma protein,[23] while RBP1, RBP2,[24] RBP5 and RBP7 are cellular proteins, and RBP3 is the interstitial form.[25]

Recently, RBP4, previously thought to play a role only in the delivery of retinol from the liver to peripheral tissues, has been classified as a novel adipokine implicated in the pathophysiology of insulin resistance.[3] Several lines of evidence support this view: 1) the expression of the gene encoding RBP4 is increased in the adipose tissue of mice with adipocyte-specific ablation of *GLUT4*;[3] 2) circulating RBP4 concentrations are significantly increased in humans with diabetes;[3] 3) patients who are destined to develop type 2 diabetes mellitus have higher plasma/serum RBP4 concentrations before the development of overt disease;[7,8] and 4) a high concentration of circulating RBP4 is an independent factor contributing to acute insulin response in obese subjects.[26] Of note, other studies have not found an association between RBP4 and insulin resistance.[9,10] In addition, among non-diabetic men, the serum concentrations of RBP4 are not significantly different between lean, overweight and obese subjects, and circulating RBP4 concentrations are not associated with age, BMI, waist-to-hip ratio, or metabolic parameters, including insulin sensitivity.[26] In summary, despite the fact that the role of RBP4 in diabetes and insulin resistance is controversial,[6] there is a growing body of evidence that supports the association between RBP4, metabolic complications and obesity-related disorders.

In addition to their suggested role in insulin resistance, adipocytokines have also been implicated in the pathophysiology of inflammatory disorders such as asthma,[27-29] inflammatory bowel disease,[30-32] rheumatoid arthritis,[33,34] as well as obesity.[3,6,11,35-44] Moreover, some adipocytokines, such as resistin[12,38,45,46] and visfatin,[11,47,48] have an immunoregulatory effect on the innate immune responses, while others (i.e. leptin) have been shown to regulate both the innate[49-52] and the adaptive limbs of the immune system.[53,54] Likewise, RBP4[1,6,12] has also been linked to inflammation. Indeed, *RBP4* gene expression has been found to be strongly associated with inflammatory markers (i.e., CD68 and MCP-1) of adipose tissue in non-diabetic human subjects.[10]

Amniotic fluid RBP4 concentrations in normal pregnancies

Retinol binding protein 4 was detectable in the amniotic fluid of all patients included in this study, suggesting that RBP4 is a physiologic component of human amniotic fluid. Moreover, RBP4 concentrations were higher in the mid-trimester than at term. These findings raise a question regarding the source of this protein in the amniotic fluid. RBP in amniotic fluid can be of maternal or fetal origin. In the rat fetal circulation, RBP and retinol concentrations increase in parallel from 11 to 14 days of gestation, and this has been suggested to result from the transplacental transport of retinol bound to RBP.[55] Between 16 and 20 days of gestation, rat fetal concentrations of RBP increase significantly and this has been attributed to fetal synthesis of RBP.[55] Based on these results, it has been suggested that maternal RBP-retinol can cross the placenta.[55,56] However, Quadro et al,[57] using retinol binding protein-deficient (RBP -/-) mice and mice that express human RBP on the RBP-/- background, demonstrated that neither maternal nor fetal RBP crosses the placenta. Thus, amniotic fluid RBP can be derived from either the fetus or membranes. Indeed, animal studies have demonstrated that RBP is synthesized by the amnion, chorion and allantoic sac in the bovine[58] as well as by the yolk sac and fetal liver in the rat.[59] Furthermore, the latter report suggested that retinol transport to the fetus may involve RBP synthesized in the yolk sac and fetal tissues.[59]

The finding of higher mid-trimester concentrations of RBP4 in amniotic fluid compared to term gestation is novel. This decrease of RBP4 concentration with advancing gestational age is similar to the pattern of alpha feto-protein, a fetus specific protein. The concentration of the latter decreases significantly from the second trimester of pregnancy to term gestation.[60,61] Thus, this finding further supports the concept of a fetal origin of amniotic fluid RBP4.

Our finding of a higher mid-trimester concentration of RBP4 in amniotic fluid compared to term is in apparent discrepancy with two previous reports by Sklan et al,[56,59] which describe the amniotic fluid concentrations of RBP4 at different gestational ages. In the rat, the amniotic fluid concentrations of RBP (n=12, radioimmunoassay) did not change significantly along gestation.[59] Similarly, no significant differences were observed in the RBP concentrations determined by radial immunodiffusion in amniotic fluid obtained from a total of 10 patients at 16 to 18 and 37 to 42 weeks of gestation.[56] The small number of subjects and the different assays employed may account for this apparent inconsistency.

Amniotic fluid RBP4 concentration is increased in women with preterm labor and intra-amniotic infection/inflammation

The increased RBP4 concentration in the amniotic fluid of women with PTL and IAI reported herein is novel. This finding confirms a recent report from our group[13] in which, using proteomic profiling of the amniotic fluid of patients with preterm labor, as a discovery tool, over-expression of RBP4 was observed in patients who delivered preterm compared to those with an episode of PTL who delivered at term, regardless of the presence of intra-amniotic infection/inflammation. RBP4 expression, however, was more profound in infected amniotic fluid.[13] Of note, we could not demonstrate a significant difference in the amniotic fluid concentration of RBP4 between patients with PTL without IAI who delivered preterm or at term. Moreover, the lack of difference in the presence of term labor does not support a role for RBP4 in term or preterm parturition but, rather, in infection/inflammation.

The association between amniotic fluid RBP4 concentrations and IAI in patients with spontaneous PTL and intact membranes is consistent with recent reports from our group concerning other adipokines such as visfatin,[11] resistin,[12] and adiponectin,[62] in which IAI was associated with increased amniotic fluid adipokine concentrations. Moreover, the amniotic fluid concentration of RBP4 was independently associated with IAI in patients with PTL and correlated with other indices of intra-amniotic inflammation such as amniotic fluid WBC count ($r=0.20$, $p=0.007$) and IL-6 concentrations ($r=0.37$, $p<0.001$). In addition, the median amniotic fluid RBP4 concentration was higher among patients with IAI and histologic chorioamnionitis and/or funisitis than among those with IAI without these placental lesions.

The cross-sectional nature of this study does not allow us to discern a cause and effect relationship between amniotic fluid RBP4 and infection/inflammation. However, given the newly described role of RBP4 in the pathogenesis of insulin resistance, it is tempting to postulate that the higher RBP4 concentrations in amniotic fluid are related to the increased fetal demand for energy in cases of intra-amniotic infection/inflammation. In humans, insulin maintains normal glucose levels in the blood by binding to its receptor on the cell membrane, stimulating glucose uptake into muscle and fat cells through the *GLUT4* transporter, as well as inhibition of glucose production in the liver. Yang *et al.*[3] demonstrated that RBP4 may be the link between *GLUT4* suppression and insulin resistance. Elevated concentrations of RBP4 impair insulin signaling in the muscle, inhibits glucose uptake, and interfere with insulin-mediated suppression of glucose production in the liver, causing elevation of blood glucose concentrations.[63] Collectively, these findings may point to a role for RBP4 in the fetal inflammatory response to acute infection.

In conclusion, this study demonstrates that among patients with PTL, the amniotic fluid concentration of immunoreactive RBP4 is elevated in pregnancies complicated with IAI and, to a greater extent, in cases with histological chorioamnionitis. These results suggest that the newly classified adipokine, RBP4, participates in the host response against intrauterine infection/inflammation.

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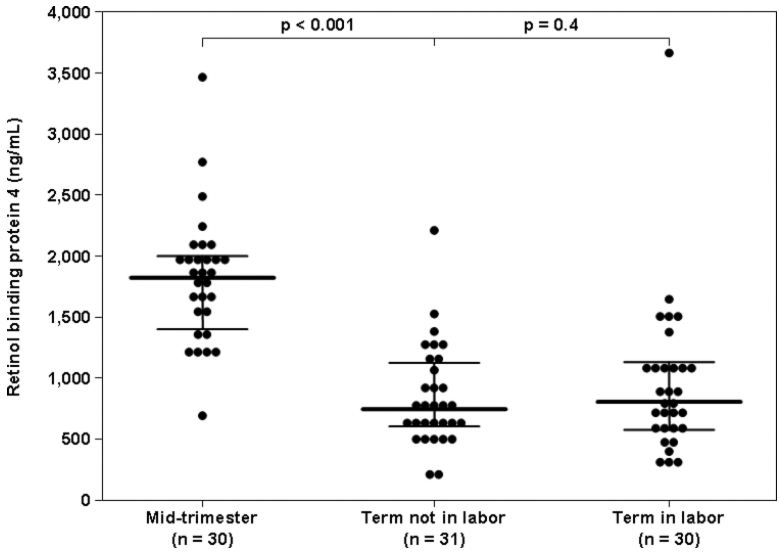

Figure 1.

Amniotic fluid concentrations of retinol-binding protein 4 (RBP4) in normal pregnancies in the mid-trimester and at term, in labor and not in labor. The median amniotic fluid RBP4 concentration was higher in women in the mid-trimester than in those at term not in labor [1861.1 ng/mL, interquartile range (IQR) 1486.2-2034.3 vs. 766.1 ng/mL, IQR 608.5-1154.1; p<0.0001]. Among women at term, the median amniotic fluid RBP4 concentration did not differ significantly between women in labor and those not in labor (Term not in labor: 766.1 ng/mL, IQR 608.5-1154.1 vs. term in labor: 836.8 ng/mL, IQR 589.5-1112.8; p=0.4). The black lines represent the median and IQR.

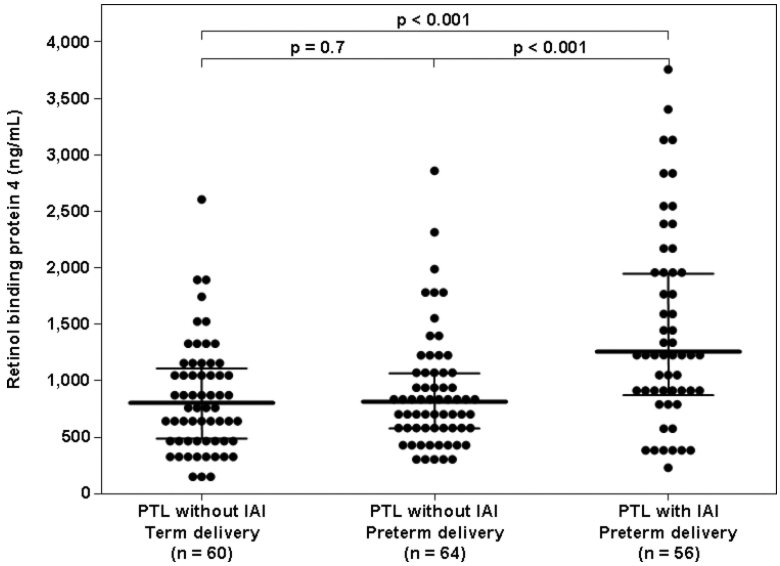

Figure 2.

Amniotic fluid concentration of retinol-binding protein 4 (RBP4) among women with spontaneous preterm labor and intact membranes (PTL). The median amniotic fluid RBP4 concentration was higher in patients with intra-amniotic infection/inflammation (IAI) than in women with PTL without IAI who delivered preterm [1268.9 ng/mL, interquartile range (IQR) 900.3-1970.1 vs. 815.8 ng/mL, IQR 592.4-1098.1; $p<0.001$], and than those with PTL without IAI who delivered at term (828.7 ng/mL, IQR 499.7-1119.6; $p<0.001$)). Among women with PTL without IAI, there was no difference in the median amniotic fluid concentration of RBP4 between those who delivered preterm and those who delivered at term (p=0.7). The black lines represent the median and IQR.

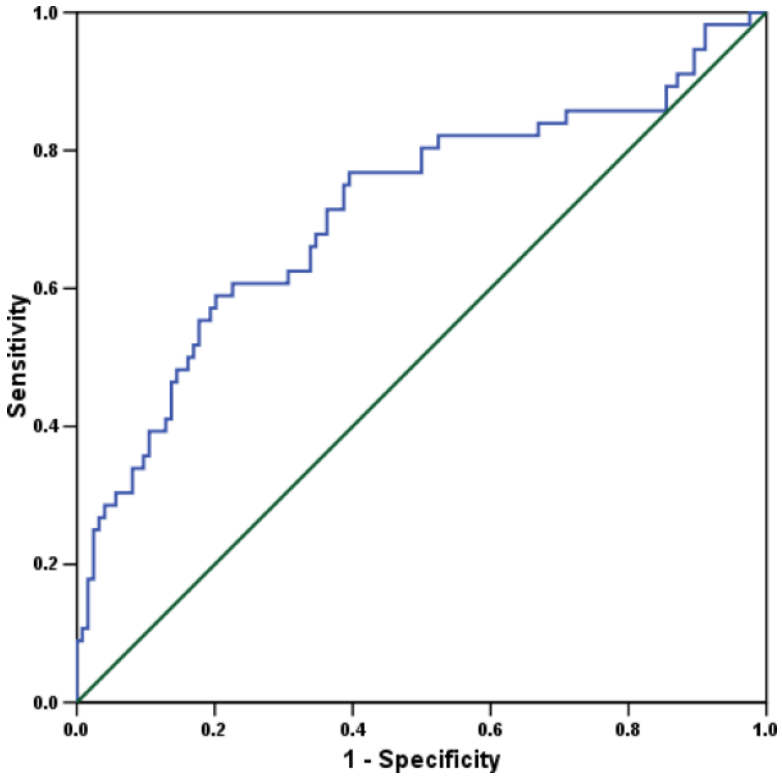

Figure 3.

The receiver-operating characteristic (ROC) curve of amniotic fluid RBP4 concentration for the identification of IAI among patients with PTL with intact membranes (area under the curve (AUC) 0.71, $p<0.001$). Derived from this ROC curve, a cutoff value for amniotic fluid RBP4 concentration of ≥1149.8 ng/mL had a sensitivity of 58.9% and a specificity of 79.8% for the identification of IAI in patients with PTL and intact membranes.

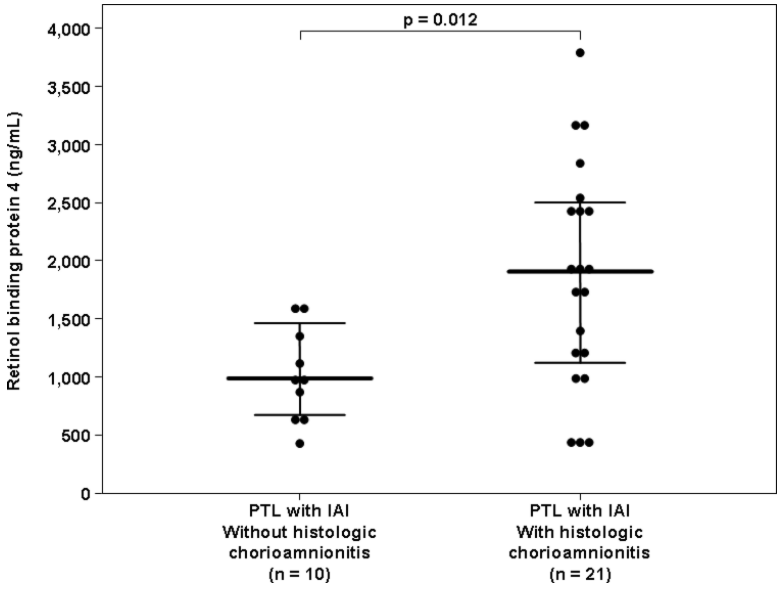

Figure 4.

Amniotic fluid concentrations of retinol-binding protein 4 (RBP4) among women with spontaneous preterm labor (PTL) and intra-amniotic infection/inflammation (IAI) who delivered within 72 h of amniocentesis and have placental histopathologic diagnosis: The median amniotic fluid RBP4 concentration was significantly higher in patients with IAI and histologic chorioamnionitis than in women with IAI without histologic chorioamnionitis [1901.0 ng/mL, interquartile range (IQR) 1107.3-2492.0 vs. 968.2 ng/mL, IQR 634.7-1396.7; p=0.01]. The black lines represent the median and IQR.

Table 1

Demographic and clinical characteristics of women with normal pregnancies in the mid-trimester and at term, with and without spontaneous labor.

	Mid-trimester (*n*=30)	***p*[a]**	**Term not in labor (*n* = 31)**	***p*[b]**	**Term in labor (*n* = 30)**	***p*[c]**
Maternal age (years)[†]	36 (35-38)	<0.001	28 (21.5-33)	<0.001	22 (19-26.2)	<0.001
GA at amniocentesis (weeks)[†]	16.2 (16-17)	<0.001	38.5 (37.9-39.6)	*NS*	38.4 (38-39.2)	<0.001
GA at delivery (weeks)	39 (38-40)	*NS*	38.5 (37.9-39.6)	*NS*	38.4 (38-39.2)	*NS*
Birth weight (grams)	3346 (3218-3671)	*NS*	3200 (3015-3634)	*NS*	3295 (3092-3510)	*NS*

Values expressed as median (interquartile range)

GA: gestational age; NS: not significant.

[a] *p* between mid-trimester and term not in labor

[b] *p* between term not in labor and term in labor

[c] *p* mid-trimester and term in labor

[†] $p<0.001$, Kruskal-Wallis test

Table 2

Demographic and clinical characteristics among women presenting with spontaneous preterm labor (PTL) and intact membranes.

	Spontaneous PTL and intact membranes					
	Without IAI, term delivery (*n*=60)	***p*[a]**	**without IAI, preterm delivery (*n* = 64)**	***p*[b]**	**with IAI, preterm delivery (*n* = 56)**	***p*[c]**
Maternal age (years)	23 (18.5-30)	*NS*	23 (20-30)	*NS*	23 (20-28.2)	*NS*
GA at amniocentesis (weeks)[†]	32 (30.4-34)	*NS*	32.2 (30.1-33.4)	<0.001	28.4 (25.1-33.2)	0.001
GA at delivery (weeks)[††]	39 (38.1-39.9)	<0.001	34.8 (33.7-35.8)	<0.001	29.5 (25.4-33.4)	<0.001
Birth weight (grams)[††]	3240 (2972-3622)	<0.001	2480 (2025-2780)	<0.001	1425 (765-2185)	<0.001

Values expressed as median (interquartile range)

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

[a] *p* between PTL without IAI who delivered at term and PTL without IAI who delivered preterm

[b] *p* between PTL without IAI who delivered preterm and PTL with IAI who delivered preterm

[c] *p* between PTL without IAI who delivered at term and PTL with IAI who delivered preterm

[†] $p=0.001$

[††] $p<0.001$; Kruskal-Wallis test

Table 3

A backward stepwise multivariable logistic regression model including IAI as the dependent variable and maternal age, pre-pregnancy BMI, smoking status and gestational age at amniocentesis and amniotic fluid RBP4 concentration ≥1149.8 ng/mL as covariates.

	Odds ratio	95% CI	p
Maternal age	0.94	0.88-1.01	0.08
Pre-pregnancy BMI	1.17	1.05-1.30	0.004
Smoking status	1.79	0.63-5.09	0.3
GA at amniocentesis	0.91	0.77-1.09	0.3
Amniotic fluid RBP4 concentration ≥1149.8 ng/mL	4.6	1.91-11.05	0.001

CI: confidence interval; BMI: body mass index; GA: gestational age; RBP4: retinol binding protein 4