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# HIGH TISSUE FACTOR ACTIVITY AND LOW TISSUE FACTOR PATHWAY INHIBITOR CONCENTRATIONS IN PATIENTS WITH PRETERM LABOR

**Offer Erez**[1,2], **Roberto Romero**[1,3], **Edi Vaisbuch**[1,2], **Juan Pedro Kusanovic**[1,2], **Shali Mazaki-Tovi**[1,2], **Tinnakorn Chaiworapongsa**[1,2], **Francesca Gotsch**[1], **Jawed Fareed**[4], **Debra Hoppensteadt**[4], **Nandor Gabor Than**[1], **Bo Hyun Yoon**[5], **Sam Edwin**[1], **Zhong Dong**[1], **Jimmy Espinoza**[1,2], **Moshe Mazor**[6], and **Sonia S. Hassan**[1,2]

[1]Perinatology Research Branch, NICHD/NIH/DHHS, Bethesda, MD, Detroit, MI, United States

[2]Department of Obstetrics and Gynecology, Wayne State University, Detroit, MI, United States

[3]Center for Molecular Medicine and Genetics, Wayne State University, Detroit, MI, United States

[4]Department of Pathology, Loyola University Medical Center, Maywood, IL, United States

[5]Department of Obstetrics and Gynecology, Seoul National University, Seoul, Korea

[6]Department of Obstetrics and Gynecology, Soroka University Medical Center, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel

## Abstract

**Objective—**Preterm labor (PTL) has been associated with an increased thrombin generation in the maternal circulation and amniotic fluid. Tissue factor (TF) is a potent initiator of the coagulation cascade, which can trigger the hemostatic system to generate thrombin. The aims of this study were to determine whether spontaneous PTL with intact membranes is associated with changes in the maternal plasma concentrations and activity of TF and tissue factor pathway inhibitor (TFPI).

**Methods—**This cross-sectional study included women in the following groups: 1) normal pregnancies (n=86); 2) term pregnancies in spontaneous labor (TIL) (n=67) and not in labor (TNL) (n=88); and 3) patients with spontaneous PTL and intact membranes (n=136) that were classified into three sub-groups: a) PTL without intra-amniotic infection and/or inflammation (IAI) who delivered at term (n=49), b) PTL without IAI who delivered preterm (n=54), and c) PTL with IAI who delivered preterm (n=33). Plasma concentrations of TF and TFPI were measured by ELISA, and their activity was measured by chromogenic assays. Non-parametric statistics were used for analysis.

**Results—**1) Among women at term, those with spontaneous labor had a higher median maternal plasma TF and a lower median TFPI concentrations than that of those without labor. 2) Patients with PTL had a significantly lower median maternal plasma TFPI concentration than that of normal pregnant women, regardless of the presence of IAI. 3) There was no significant differences in the median maternal plasma TF concentrations between patients with a normal pregnancy and those with PTL. 4) In contrast, the median TF activity was higher among patients with PTL than in women with normal pregnancies, regardless of the presence of IAI or preterm delivery. 5) However, maternal plasma TFPI activity did not differ among the study groups.

Address correspondence to: Offer Erez, M.D. and 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.

**Conclusion—**Women with preterm parturition, in contrast to those in labor at term, have a higher TF activity and a lower TFPI concentration, without a significant change in the median maternal plasma TF concentration. These observations suggest that the increased thrombin generation reported in patients with PTL may be the result of activation of the extrinsic pathway of the coagulation cascade. In addition, the increased thrombin generation reported in patients with PTL could be due to insufficient anti-coagulation, as reflected by the low maternal plasma TFPI concentration.

### Keywords

Thrombin generation; intra-amniotic inflammation; preterm delivery; parturition; TFPI/TF ratio

## INTRODUCTION

Preterm labor (PTL) with intact membranes is one of the great obstetrical syndromes and is the clinical manifestation of different underlying mechanisms[1], including the following: intrauterine infection[2–5], uteroplacental ischemia[6–9], uterine over-distention[10–12], cervical disease[13–17], abnormal allograft reaction[7], allergic phenomena[18–20], and endocrine disorders[21,22]. In addition, PTL is associated with an increased thrombin generation. Indeed, patients with PTL have a higher median maternal plasma concentration of thrombin-antithrombin (TAT) III complexes than women with a normal pregnancy[23,24] that is not associated with a history of vaginal bleeding during gestation or the presence of intra-amniotic infection/inflammation (IAI)[23]. Thus, the activation of the maternal coagulation cascade may be associated with an episode of PTL regardless of its underlying mechanism.

Systemic maternal inflammation, which has been reported in patients with PTL[25–28], is associated with increased thrombin generation. Indeed: 1) The administration of tumor necrosis factor (TNF)-α to healthy volunteers induces thrombin generation and the activation of coagulation[29]; 2) Proinflammatory cytokines, such as IL-1β and TNF-α, increase mRNA and protein expression of TF by monocytes[30–37] and macrophages[38] and 3) The blocking of interleukin (IL)-6 by specific antibodies attenuates the activation of coagulation in a chimpanzee model of endotoxemia[39]. This activation of the coagulation cascade by systemic inflammation is mediated through the tissue factor (TF) pathway of the coagulation cascade[38,40–44].

Tissue factor pathway inhibitor (TFPI), the main inhibitor of the TF pathway of coagulation, is a three Kunitz domain glycoprotein which inhibits thrombin generation through the inhibition of activated factor (F) X and the FVIIa/TF complex[45,46]. The mean maternal plasma concentrations of total TFPI remains increase during the first half of pregnancy, remain relatively constant in the second half[47] and to decrease during labor[48]. The changes in plasma TFPI concentrations during inflammation are not well defined. Patients with chronic inflammation such as inflammatory bowel disease[49], chronic liver disease[50] and atherosclerosis[51], have increased plasma concentrations of TFPI; however, low TFPI concentrations have been reported in acute inflammatory processes such as pneumonia[52].

The profile of the maternal plasma TF and TFPI concentrations have different characteristics among several obstetrical syndromes[53–55]. In comparison to patients with a normal pregnancy, the median maternal plasma TF concentration is higher in patients with preeclampsia[53] or preterm prelabor rupture of membranes (PROM)[54], and lower in women with an SGA (small for gestational age) neonate[53]. Of interest, the median maternal plasma TFPI concentration increases during preeclampsia[53,55], which is

associated with an exaggerated maternal systemic inflammatory response, decreases in patients with preterm prelabor rupture of membranes[54] and does not change in mothers with SGA fetuses[53]. Currently, there is no report regarding the changes in the maternal plasma TF and TFPI concentrations in patients with PTL. Therefore, the aims of this study were to determine the changes in TF, as well as TFPI maternal plasma concentration and activity in preterm and term labor.

## MATERIAL AND METHODS

### Study design and population

This cross-sectional study included women in the following groups: 1) normal pregnancies (n=86); 2) term pregnancies in spontaneous labor (TIL) (n=67) and not in labor (TNL) (n=88); and 3) patients with spontaneous PTL and intact membranes (n=136) that were classified into three sub-groups: a) PTL without intra-amniotic infection and/or inflammation (IAI) who delivered at term (n=49), b) PTL without IAI who delivered preterm (n=54), and c) PTL with IAI who delivered preterm (n=33). Patients with multiple gestations or fetuses with congenital and/or chromosomal anomalies were excluded.

Samples and data were retrieved from our bank of biological samples and clinical databases. Many of these samples have been previously employed to study the biology of inflammation, hemostasis, angiogenesis regulation, and growth factor concentrations in normal pregnant women and in those with pregnancy complications.

All women provided written informed consent prior to the collection of maternal blood. The Institutional Review Boards of Wayne State University and the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD), NIH, DHHS approved the collection and utilization of samples for research purposes.

### Clinical definitions

Women with normal pregnancies met the following criteria: 1) no medical, obstetrical, or surgical complications at the time of the study; 2) gestational ages ranging from 20 to 41 weeks; and 3) delivery of a term infant, appropriate for gestational age, without complications. Preterm labor with intact membranes was diagnosed by the presence of at least two regular uterine contractions every 10 minutes associated with cervical change that required hospitalization before 37 completed weeks of gestation. Intra-amniotic infection was defined by the presence of a positive amniotic fluid culture for microorganisms. Intra-amniotic inflammation was defined as an amniotic fluid WBC count ≥ 100 cells/ml. The results of the amniotic fluid analyses were used for clinical management. An SGA neonate was defined as a birth weight below the $10^{th}$ percentile[56].

### Blood sample collection

All blood samples were collected with a vacutainer into 0.109M trisodium citrate anticoagulant solution (BD; San Jose, CA, USA). The samples were centrifuged at 1300*g* for 10 minutes at 4°C and stored at −70°C until assay.

### Immuno and chromogenic assays

**Human tissue factor immunoassay—**TF concentrations were determined by sensitive and specific immunoassays (American Diagnostica, Greenwich, CT, USA), which recognizes TF-apo, TF, and TF-FVII complexes. The assays were conducted according to the manufacturer's recommendations. The calculated coefficient of variation (CV) in our laboratory was 5.3%, and the sensitivity was 10 pg/ml.

**Human tissue factor activity assay—**TF activity was determined by a chromogenic assay (American Diagnostica, Greenwich, CT, USA). The assays were conducted according to the manufacturer's recommendations. The calculated intra-assay CV was 3.77%, while the inter-assay CV was 6.25%, and the sensitivity of this assay was 0.53 pM.

**Human tissue factor pathway inhibitor immunoassay—**The concentrations of TFPI in maternal plasma were determined by sensitive and specific immunoassays (American Diagnostica, Greenwich, CT, USA). The TFPI ELISA employs, as the capture antibody, a murine anti-TFPI monoclonal that is directed against the Kunitz-1 domain of the TFPI molecule; therefore, it detects both TFPI-1 and TFPI-2, while measuring the total TFPI plasma concentration. The assay was conducted according to the manufacturer's recommendations. The calculated CV in our laboratory was 6.6%, and the sensitivity was approximately 10 ng/ml.

**Human tissue factor pathway inhibitor activity assay—**TFPI activity was determined by a chromogenic assay (American Diagnostica, Greenwich, CT, USA). The assays were conducted according to the manufacturer's recommendations. The calculated intra-assay CV was 5.51%, while the inter-assay CV was 8.74% and the sensitivity was 0.017 unit/ml.

### Statistical analysis

To test whether our data was normally distributed, we used the Kolmogorov-Smirnov and the Shapiro-Wilk tests. Tissue factor and TFPI plasma concentrations were not normally distributed; thus, Kruskal Wallis and Mann–Whitney U tests were used for comparisons of continuous variables. The Chi-square and Fisher's exact tests were used to compare categorical variables. The Spearman's rho test was used to detect a correlation between the concentrations and activity of TF, TFPI, and their ratio to the gestational age at sample collection in women with a normal pregnancy. Multiple logistic regression analysis was performed to study which of the analytes was independently associated with PTL after correction for gestational age at sample collection. A p value $< 0.05$ was considered statistically significant. Analysis was performed with SPSS package, version 12 (SPSS Inc., Chicago, IL, USA).

## RESULTS

The demographic and clinical characteristics of women with normal pregnancies and those with PTL are presented in Table I. Patients with PTL who delivered at term had a lower median maternal age than women with a normal pregnancy. Among patients with PTL, those who delivered preterm (regardless of the presence of IAI) had a lower median gestational age at blood collection than patients with normal pregnancies. Amniocentesis was performed in 83.8% (114/136) of the patients presenting with PTL, of which 14% (16/114) had positive intra-amniotic culture for microorganisms; the frequency of the specific microorganisms is presented in Table II.

Among patients with normal pregnancies, gestational age at sample collection was positively correlated with maternal plasma TFPI activity (r=0.241, p=0.03), but not with TF activity (r=0.17, p=0.15). There was no correlation between TF and TFPI activity in the maternal plasma (r=0.45, p0.72).

### Changes in maternal plasma TF and TFPI concentrations and activity in patients with and without spontaneous labor at term

Patients with spontaneous labor at term had a significantly higher median maternal TF concentration than those women at term not in labor ($p<0.001$) (Figure 1a). In contrast, there was no significant difference in the median maternal plasma TF activity of patients with and without labor at term (TIL: median: 16.7 pM, range 7.0–31.2, vs. TNL: median 15.3 pM, range 2.9–168.3, $p=0.17$).

The median maternal plasma TFPI concentration at term was significantly lower in women in labor, than in those women not in labor ($p<0.001$) (Figure 1b). The median maternal plasma TFPI activity was not significantly different between patients with, and without labor at term (TNL: median 1.2 unit/ml, range 0.2–1.8 vs. TIL: median 1.2 unit/ml, range 0.5–2.7, $p=0.86$). The median maternal TFPI/TF ratio was higher in women at term without labor, than in those with active labor ($p<0.001$) (Figure 1c).

### Changes in the maternal plasma TF concentrations and activity in patients with preterm labor

The median maternal plasma TF concentration did not differ significantly among patients with PTL and those with normal pregnancies (Kruskal Wallis, $p=0.25$) (Figure 2a), nor was it significantly different among the PTL sub-groups (Kruskal Wallis, $p=0.51$).

The median maternal plasma TF activity was significantly higher among the PTL sub-groups than the normal pregnancy group (Kruskal Wallis, $p<0.001$). In the post-hoc analysis, all three PTL sub-groups had a higher median maternal plasma TF activity than that of the normal pregnancy group: 1) PTL who delivered at term: median 14.1 pM, range 6.6–65.2 vs. normal pregnancy: median 9.9 pM, range 0.7–37.6, $p<0.001$; 2) PTL who delivered preterm without IAI: median 12.6 pM, range 7.0–98.7, $p<0.001$; and 3) PTL who delivered preterm with IAI: median 13.1 pM, range 8.1–51.3, $p<0.001$. However, the median maternal plasma TF activity did not differ among the PTL sub-groups (Kruskal Wallis, $p=0.32$).

### Changes in the maternal plasma TFPI concentrations and activity in patients with preterm labor

The median maternal plasma TFPI concentration differed significantly among patients with preterm labor, and those with a normal pregnancy (Kruskal Wallis, $p=0.001$). In the post-hoc analysis, all three PTL sub-groups had a significantly lower median maternal plasma TFPI than that of the normal pregnancy group: 1) PTL who delivered at term: median 54.7 ng/ml, range 28.6–96 vs. normal pregnancy- median 66.1 ng/ml; range 14.3–86.5, $p=0.002$; 2) PTL who delivered preterm without IAI: median 56.7 ng/ml, range 30.3–78.5, $p<0.001$; and 3) PTL who delivered preterm with IAI: median 55.2 ng/ml, range 34.7–96.7, $p=0.023$ (Figure 3). However, the maternal plasma TFPI activity did not differ among patients with PTL and those with normal pregnancy (Kruskal Wallis, $p=0.89$).

### Changes in the maternal plasma TFPI/TF ratio in patients with preterm labor

Patients with PTL had a significantly lower TFPI/TF ratio than in those with a normal pregnancy (Kruskal Wallis, $p=0.039$). However, none of the comparisons between the groups was significant, after correction for multiple comparisons (PTL without IAI- median 174.4, range 26.8–1665.7 vs. normal pregnancy- median 221.5; range: 21.4–3355.3, $p=0.08$; PTL with IAI- median 168.6, range 36.9–621.3 vs. normal pregnancy- median 221.5; range 21.4–3355.3, $p=0.06$; and PTL who delivered at term- median 180.3, range 26.6–890.2 vs. normal pregnancy- median 221.5; range: 21.4–3355.3, $p=0.16$).

In light of the association between gestational age at sample collection and TFPI activity, we constructed a logistic regression model including an episode of preterm labor as the dependent variable. Maternal age, gestational age at blood draw, maternal plasma TF and TFPI concentration and activity, as well as the interaction between the concentration and activity of each analyte were included as covariates. Maternal plasma TFPI concentration (OR 0.87, 95%CI 0.77–0.98), TF activity (OR 1.14, 95%CI 1.04–1.26), gestational age at blood draw (OR 0.72, 95%CI 0.67–0.79) and maternal age (OR 0.9, 95%CI 0.84–0.97) were all independently associated with an episode of PTL (Table 3).

## DISCUSSION

### Principle findings of the study

1) Patients with PTL had a significantly higher median maternal plasma TF activity than that of women with a normal pregnancy, regardless of the presence of IAI or gestational age at delivery.2) Patients with PTL had a significantly lower median maternal plasma TFPI concentration than women with a normal pregnancy, regardless of the presence of IAI or gestational age at delivery. 3) There were no significant differences in the median maternal plasma TF concentrations and TFPI activity between patients in the PTL group with or without IAI, and women with a normal pregnancy. 4) The TFPI/TF ratio was lower in patients with PTL. 5) Maternal plasma TFPI concentration and TF activity were independently associated with the occurrence of an episode of PTL.

### The association between inflammation and coagulation

Preterm labor is a syndrome that results from different underlying mechanisms, such as intrauterine infection/inflammation, increased thrombin generation in the maternal circulation, and an increased maternal systemic inflammatory response[1,5]. The coagulation cascade and the inflammatory system can activate and augment the action of each other[38,40,41]. This interaction is particularly important during pregnancy because the placentas of patients with PTL or preterm PROM often have inflammatory lesions as well as thrombotic lesions[6,57].

### Maternal inflammation and circulating tissue factor concentration and activity

During acute systemic inflammation (i.e. endotoxemia), there is activation of the coagulation cascade through the TF pathway[38,40,41]. Evidence in support of this view includes the following: 1) Proinflammatory cytokines, such as IL-1β and TNF-α, increase mRNA and protein expression of TF by monocytes[30–37] and macrophages[38]. 2) The administration of low dose endotoxin to healthy volunteers is associated with a 125-fold increase in TF mRNA monocyte concentration[58]. 3) Activated monocytes that express TF on their membrane secrete TF into the plasma in its free form[59–63], or in micro-particles[60].

In the current study, labor at term was associated with a higher median maternal plasma TF concentration, without a significant change in its activity. Preterm labor was associated with an increase in TF activity in the maternal plasma without a significant change in its concentration, in comparison to women with a normal pregnancy, while patients with preterm PROM had an increased maternal plasma TF concentration[54] and activity (unpublished data). A possible explanation for the differences between PTL and preterm PROM may derive from the specific component of the common pathway of parturition, which is activated in each obstetrical syndrome[64]. While preterm PROM is associated with the activation of the decidua and the membranes, myometrial activation is the major component of preterm labor with intact membranes[64]. This is relevant because the decidua and the membranes have a high TF concentration[65–67]. We propose that during labor at

term or in preterm PROM, activation of the decidua and membranes may lead to TF shedding into the maternal circulation during preterm PROM and normal labor at term. Conversely, activation of the myometrium may be associated with lower shedding of TF into the maternal circulation. Nevertheless, both PTL and preterm PROM are pathologic processes which are associated with an increased maternal systemic inflammatory response[25,68], that contributes to tissue factor activation in the maternal circulation. However, the physiologic process of labor at term, although defined as inflammatory in nature, is not associated with a similar increase in TF activity.

### The effect of the activation of the hemostatic system on maternal inflammation

The activation of the coagulation cascade induces the secretion of proinflammatory cytokines.[38,40,41,44]. Indeed, the expression of TF in sites of injury or on platelets, leads to the generation of the TF/FVIIa/FXa complex, which in turn leads to thrombin generation and activation of the protease-activated receptor (PAR)-2. The latter enhances the production of IL-6[44,69–71]. Evidence in support of this concept includes the observation that the administration of antibodies against the binding site of FXa to TF/FVIIa complexes attenuates tissue damage and thrombosis in baboons with *E. coli*-induced sepsis[72]. The proposed mechanism of action is that the TF/FVIIa complex cannot activate PAR-2 without the contribution of FXa[72]. Thus, during systemic inflammation, FXa plays a dual role: 1) activation of the prothrombinase complex leading to the generation of thrombin[73–80]; and 2) amplification of the inflammatory process by inducing IL-6 secretion from endothelial cells[81,82] and monocytes[70–72]. The latter may contribute to the systemic maternal inflammation previously reported among patients with PTL[25].

### The effect of TF activity on thrombin generation in patients with preterm labor

In this study, we could not demonstrate an increase in the median maternal plasma TF concentration; however, there was an increase in TF activity in the maternal plasma of patients with PTL. Since the assay we used in this study measures TF activity through the generation of FXa in a given sample, increased TF activity reflects elevated FXa generation that leads to the increased thrombin generation previously reported among patients with PTL[23,24,83]. Similarly to the increased thrombin generation, the median maternal plasma TF activity was increased in all sub-groups of PTL, regardless of the presence of intra-amniotic infection/inflammation, or a subsequent preterm delivery.

This observation is relevant given the following findings: 1) patients with preterm labor who subsequently delivered at term had a higher rate of SGA neonates and a higher proportion of placental vascular lesions[84]; and 2) a subset of patients with PTL had vascular lesions in their placenta, [6,85] and 7–20% of inflammatory lesions in the placenta were accompanied by vascular lesions in preterm parturition[6,57]. Thus, the increased TF activity among patients with PTL contributes to a higher generation of FXa that, along with the physiologic increase in the maternal plasma concentrations of FVII and FX during gestation[86–89], may be the underlying mechanism leading to the increased thrombin generation reported in patients with PTL.

### Changes in TFPI concentration incomplicated pregnancies

The finding that patients with PTL have a lower median maternal plasma TFPI concentration than in women with normal pregnancies is novel. Moreover, this observation was independently associated with an episode of PTL, Similarly, maternal plasma TFPI concentrations were lower among patients with preterm PROM[54] or a fetal death[90]. In contrast, patients with preeclampsia had a higher median maternal plasma TFPI concentration[53,55].

The process leading to the lower median maternal TFPI plasma concentration observed in patients with PTL is not clear. In contrast to PPROM[54], the lower median plasma TFPI concentration in patients with PTL is not associated with a higher median TF plasma concentration, further supporting a different profile of the activation of the coagulation cascade in these pregnancy complications.

Of interest, thrombin has an inhibitory effect on the production of TFPI by endothelial cells[91], and the increased thrombin generation observed in patients with PTL may be associated with a concomitant reduction in TFPI production by the maternal vascular endothelium. The placenta is an additional source for TFPI (mainly TFPI-2[92–101]), and preterm parturition may be associated with a lower production of TFPI by the placenta, contributing to the low maternal plasma concentrations detected in patients with PTL. Indeed, patients with vascular complications of pregnancy (preeclampsia, eclampsia, placental abruption, fetal growth restriction, and fetal demise) have a lower placental concentration of total TFPI and TFPI mRNA expression than in women with normal pregnancies[102],. However, currently there is no report regarding the placental production of TFPI in patients with PTL or preterm PROM.

## Insufficient anti-coagulation as a mechanism for thrombin generation among patients with PTL

The lower median maternal plasma TFPI concentration in patients with PTL, regardless of the presence of infection or gestational age at delivery, suggests that the increased thrombin generation observed among these patients may derive not only from an increased activation of the hemostatic system, but also from insufficient anti-coagulation. The latter can be due to either low concentrations of the anticoagulant proteins, or as a result of an abnormal balance between coagulation factors and their inhibitors.

### Low concentration of anti-coagulant proteins in patients with PTL

The activation of factor X is an important step in the coagulation cascade leading to the conversion of prothrombin (factor II) into thrombin. Therefore, the inhibition of FXa by TFPI, protein Z/protein Z dependant protease complex, and antithrombin III[103], leads to a reduction in the generation of thrombin. The findings of the current study regarding the low median maternal plasma TFPI concentration among patients with PTL, and the findings previously reported by our group regarding low concentrations of protein Z/protein Z dependent protease complex in patients with PTL without IAI[104], suggest that the insufficient inhibition of factor Xa may contribute to the increased thrombin generation reported in patients with PTL.

### Changes in the balance between coagulation and anti-coagulation in patients with PTL

The overall balance between the concentration and activity of the coagulation factors and the anti-coagulation proteins is one of the determining factors of thrombin generation. In the normal state, the immunoreactive concentrations of TFPI in the plasma are 500 to 1000 times higher than that of TF[105], suggesting that an excess of anti-coagulant proteins closely controls the coagulation cascade activity. In the current study, although PTL was not associated with a significant change in the median maternal plasma TF concentration, the TFPI/TF ratio was lower than that of normal pregnant women, mainly due to decreased TFPI concentrations. Our group previously reported that patients with preterm PROM[54], as well as those with preeclampsia[53], have a lower median maternal plasma TFPI/TF ratio than that of normal pregnant women. The lower TFPI/TF ratio in patients with preeclampsia occurs despite the increase in the median maternal plasma TFPI concentration observed in these patients. This suggests that the balance between TF and its natural inhibitor may better reflect the overall activity of the TF pathway of coagulation, than the individual

concentrations of TF or TFPI. We propose that a subset of patients with PTL have a low anti-coagulation proteins concentration, or a lower ratio between the coagulation factors and their inhibitors, which contributes to the increased thrombin generation observed in these patients.

In conclusion, women with preterm parturition, in contrast to labor at term, have a higher TF activity and a lower TFPI concentration without a significant change in the median maternal plasma TF concentration. These observations suggest that the increased thrombin generation reported in patients with PTL may be the consequence of the activation of the TF pathway of the coagulation cascade. Moreover, the low maternal plasma TFPI concentration suggests that deficient anti-coagulation may be an additional mechanism leading to the increased thrombin generation reported in patients with PTL.

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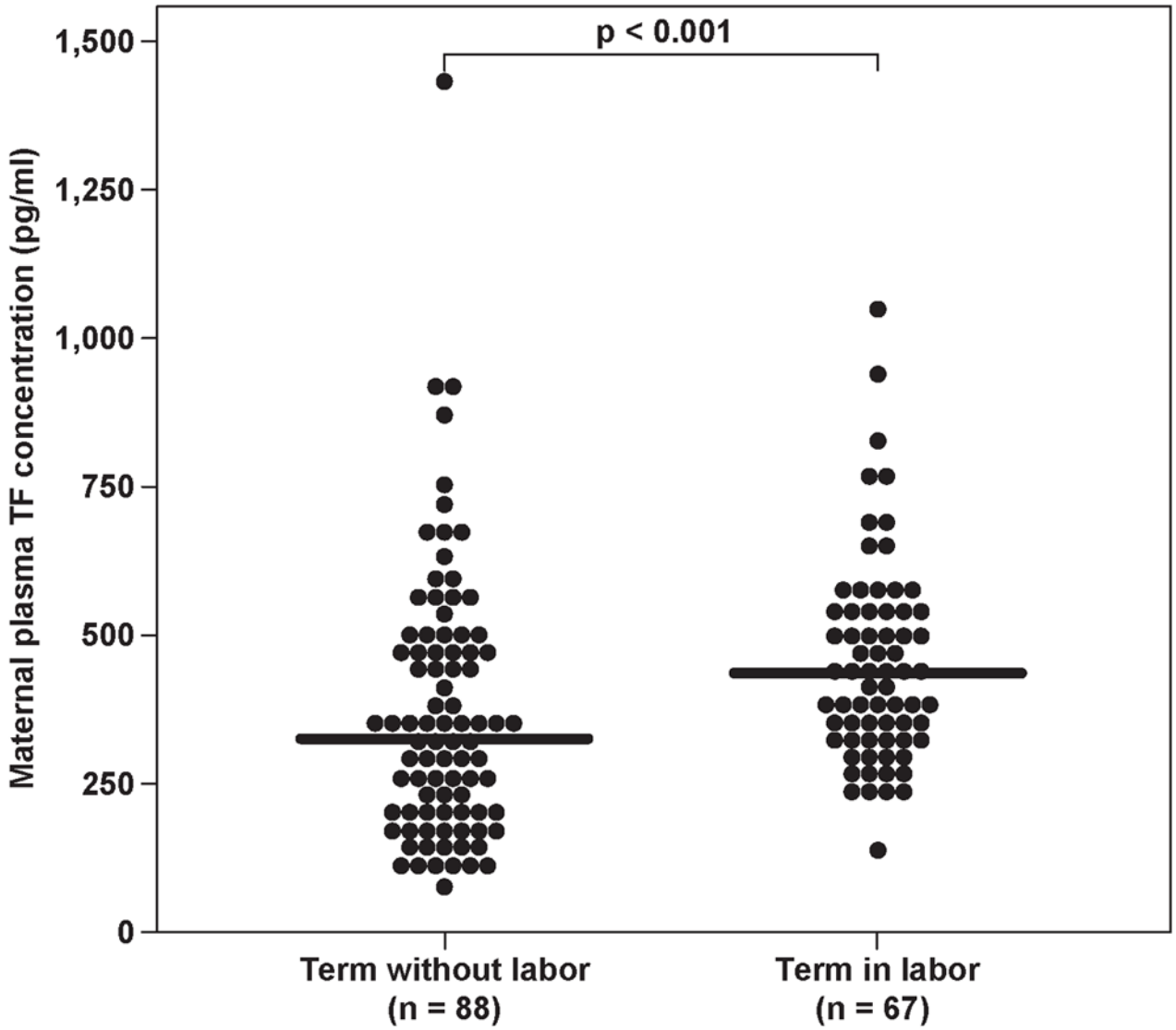

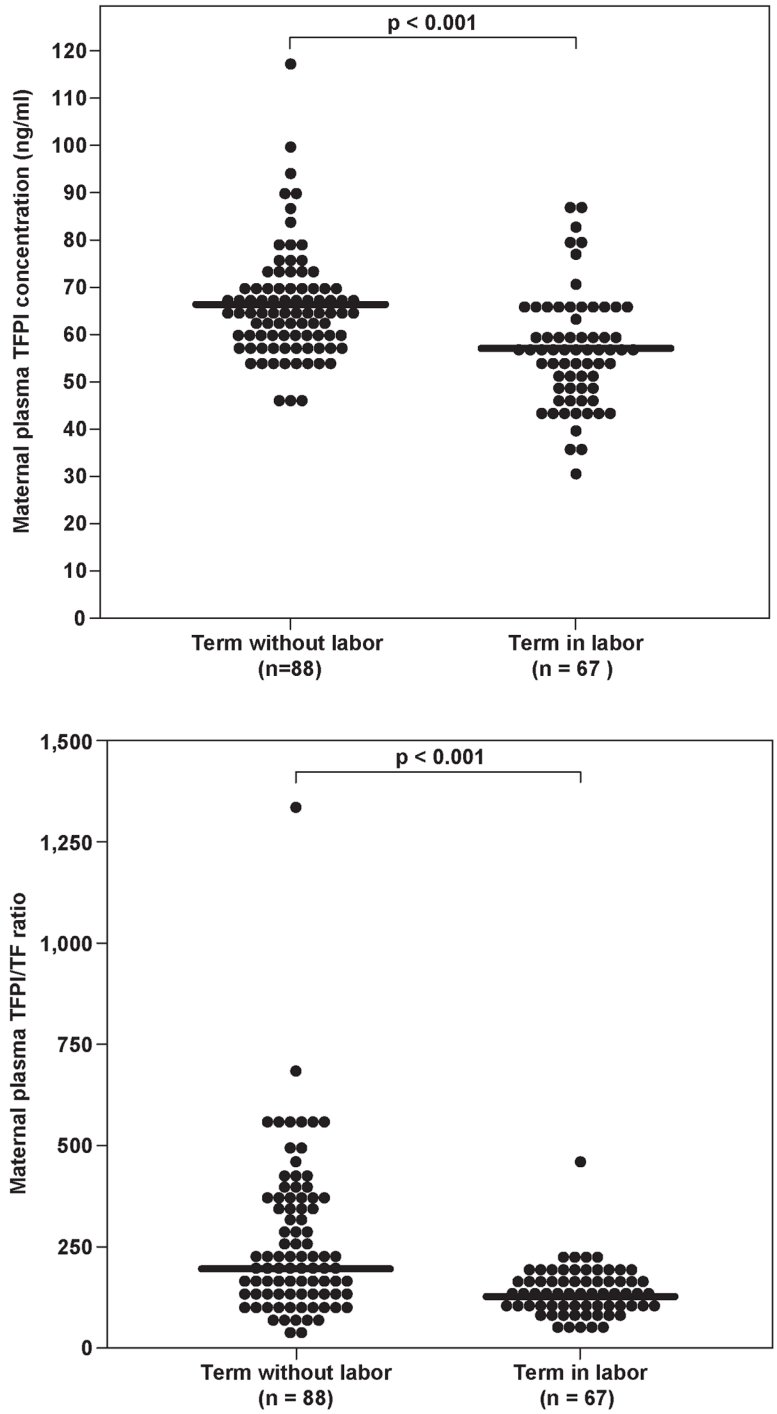


**Figure 1.**

Maternal tissue factor (TF) (TIL: median 432.8 pg/ml, range 130.1–1043.7 vs. TNL: median 320.7 pg/ml, range 70.5–1415.2) (1a) and tissue factor pathway inhibitor (TFPI) (TIL: median 56.6 ng/ml; range 30–87.7, vs. TNL: median 65.3 ng/ml, range 45.5–117.8) (1b) plasma concentrations and the TFPI/ TF ratio (TNL: median 201.4, range 40.1–1326.2 vs. TIL: median 126.2, range 48.3–457.3) (1c) between women with and without spontaneous labor at term.

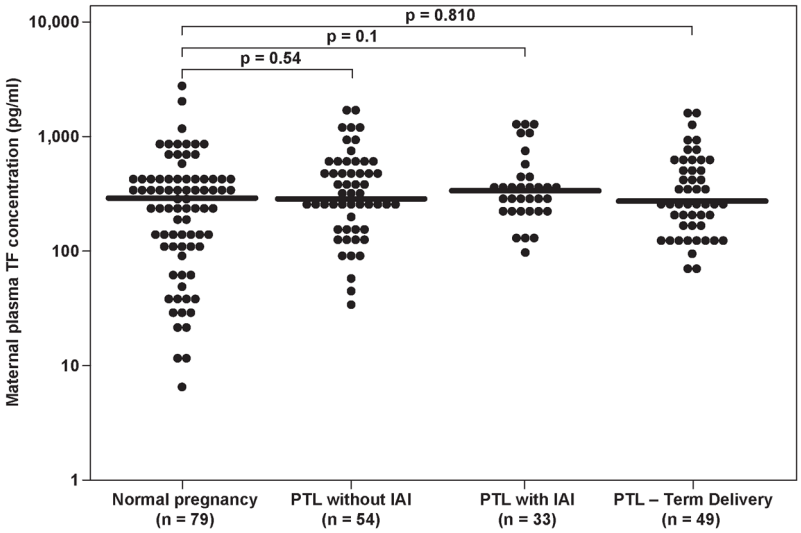


**Figure 2.**

Maternal plasma tissue factor (TF) concentration in women with normal pregnancy and patients with preterm labor according to the presence of intra-amniotic infection/ inflammation and those who delivered at term. (Normal pregnancy- median 291.5 pg/ml; range 6.3–2662.2; PTL who delivered at term: median 258.6 pg/ml, range 65.9–1495.3; PTL who delivered preterm without IAI: median 272.97 pg/ml, range 33.5–1774.6; PTL who delivered preterm with IAI: median 345.7pg/ml, range 98.5–1237.0)

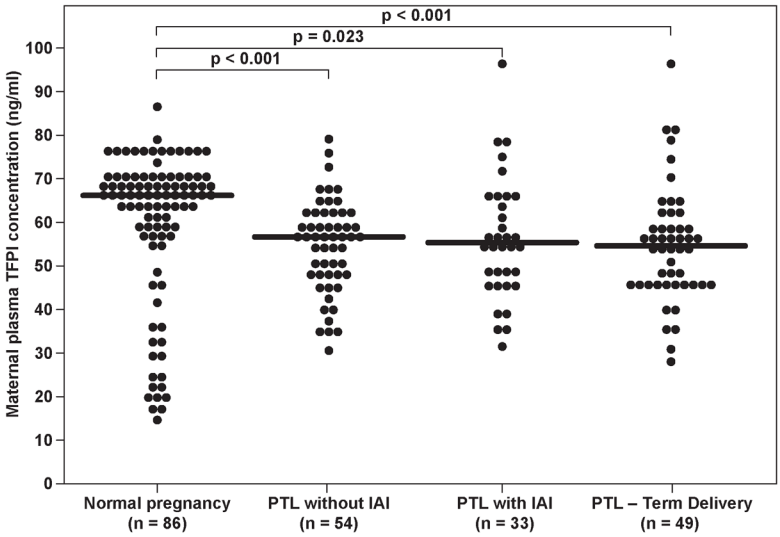


**Figure 3.**

Maternal plasma tissue factor pathway inhibitor (TFPI) concentrations in women with normal pregnancy and patients with preterm labor according to the presence of intra-amniotic infection/inflammation and those who delivered at term. (Normal pregnancy-median 66.1 ng/ml; range 14.3–86.5; PTL who delivered at term: median 54.7 ng/ml, range 28.6–96; PTL who delivered preterm without IAI: median 56.7 ng/ml, range 30.3–78.5; PTL who delivered preterm with IAI: median 55.2 ng/ml, range 34.7–96.7)

## Table I

Demographic and clinical characteristics of the study population

| | Normal pregnancy (n= 86) | PTL without IAI (n=54) | PTL with IAI (n=33) | PTL delivered at term (n=49) |
|---|---|---|---|---|
| Maternal age (years) | 24 (21,27) | 22 (19,29) | 23(19,27) | 20 (18,24) * |
| Gravidity€ | | | | |
| 1 | 18 (21.4) | 8 (14.8) | 12 (36.4) | 11 (22.4) |
| 2–5 | 53 (63.1) | 39 (72.2) | 17 (51.5) | 32 (65.3) |
| ≥6 | 13 (15.5) | 7 (13) | 4 (12.1) | 6 (12.2) |
| Parity§ | | | | |
| 1 | 46 (54.1) | 30 (55.6) | 24 (72.7) | 31(63.3) |
| 2–5 | 38 (44.7) | 21(38.8) | 6 (18.2) | 18(36.7) |
| ≥6 | 1 (1.2) | 3(5.6) | 2 (6.1) | 0 |
| Ethnic origin£ | | | | |
| African-Americans | 67 (80.7) | 42 (79.2) | 27(81.8) | 42 (87.5) |
| Caucasian | 11 (13.3) | 8 (15.1) | 5 (15.2) | 3 (6.2) |
| Hispanic | 2 (2.4) | 3 (5.7) | 0 | 1 (2.1) |
| Asian | 3 (3.6) | 0 | 1 (3) | 2 (4.2) |
| Gestational age at blood collection (weeks) | 31.1 (27.3, 33.6) | 29.5 * (25.1,32.2) | 26.1 * (24.6,31.6) | 31.4 (29.3,32.5) |
| Gestational age at delivery (weeks) | 39.6 (38.4,40.7) | 31.6 * (26.2, 34.7) | 27.9 * (25, 33.5) | 38.2 * (37.3, 38.9) |
| Cesarean delivery * | 24 (33.8) | 20 (37.7) | 6(18.2) | 0 * |
| Neonatal birthweight | 3342.5 (3057.5, 3641.8) | 1690 * (880, 2335) | 1040 * (642.5, 1755) | 2948 * (2710, 3255) |
| SGA | 0 | 5 (9.4) * | 5 (15.1) * | 12 (24.5) * |

Data are presented as median (25–75 percentile) or numbers (%)

* p<0.05 in comparison to the normal pregnancy group

PTL- preterm labor; IAI- intraamniotic infection/inflammation; SGA – small for gestational age

## Table II

Prevalence of intra-amniotic microorganisms in patients with preterm labor that underwent diagnostic amniocentesis (n=16)

| Microorganism | Number (%) |
|---|---|
| Mixed flora | 4 (25) |
| *Fusobacterium nucleatum* | 3 (18.8) |
| *Gardnerella Vaginalis* | 1 (6.2) |
| *Ureaplama Urealiticum* | 1 (6.2) |
| *Mycoplasma Hominis* | 1 (6.2) |
| *Streptococcus Agalactia* | 1 (6.2) |
| Others | 5 (31.3) |

Data are presented as numbers (%)

## Table III

Multiple logistic regression analysis of the association of maternal plasma tissue factor concentrations and activity as well as tissue factor pathway inhibitor and activity and preterm labor

| Factor | OR ( 95% CI) |
|---|---|
| Gestational age at sample collection (wk) | 0.72 (0.67–0.79) |
| Maternal age ( years) | 0.90 (0.84–0.97) |
| TF concentration (pg/ml) | 1.0 (0.99–1.0) |
| TF activity (pM) | 1.14 (1.04–1.25) |
| TF activity by TF concentration | 1.0 (1.0–1.0) |
| TFPI concentration (ng/ml) | 0.87 (0.77–0.98) |
| TFPI activity (unit/ml) | 0.032 (0–8.8) |
| TFPI activity by TFPI concentration | 1.06 (0.97–1.17) |

OR: odds ratio; CI: confidence interval; TF- tissue factor; TFPI- tissue factor pathway inhibitor