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ORIGINAL ARTICLE

Tissue factor activity in women with preeclampsia or SGA: a potential explanation for the excessive thrombin generation in these syndromes

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ABSTRACT

Objective: The aim of this study was to determine whether the activity of tissue factor (TF) and tissue factor pathway inhibitor (TFPI) in the plasma of women with preeclampsia (PE) and small for gestational age (SGA) neonate differ from that of normal pregnant women and whether they are related to specific placental lesions.

Methods: This cross-sectional study included the following groups: (1) normal pregnancy ($n = 68$); (2) PE ($n = 128$); and (3) SGA ($n = 56$). Maternal plasma TF and TFPI activity was determined with chromogenic assays.

Results: (1) The median maternal plasma TF activity, but not TFPI activity, differed among the study groups ($p < .0001$ and $p = .4$, respectively); (2) patients with PE had a higher median maternal plasma TF activity than women with normal pregnancies ($p < .0001$) and mothers with SGA fetuses ($p = .002$); (3) among patients with PE, those with distal villous hypoplasia had a higher median maternal TF activity than those without these placental lesions ($p = .018$); and (4) following adjustment for confounding variables, maternal plasma TF and TFPI activity were not associated with an SGA neonate.

Conclusions: Plasma TF activity is higher in women with PE than in those with SGA or normal pregnancies. We propose that these changes may be responsible, at least in part, for the increased *in-vivo* thrombin generation observed in this obstetrical syndrome.

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Introduction

The extrinsic pathway of the coagulation cascade is modulated by two major proteins; it is activated by tissue factor (TF) [1]. Upon its exposure on the endothelium or activated monocytes, tissue factor activates factor VII (FVII), that in turn transforms factor X to its activated form; the latter leads to the generation of thrombin from pro-thrombin [1]. The major inhibitor of TF is tissue factor pathway inhibitor (TFPI) that exerts its action on TF/FVIIa and Factor X through its three Kunitz domains [2,3]. TF has a role in systemic inflammation [4–8], placental implantation [9,10], and angiogenesis [11–16]. During normal pregnancy, TF is abundant in the uterine decidua, fetal membranes, and amniotic fluid [9–11], resulting in an efficient hemostatic mechanism activated during implantation and after delivery [17]. TF also has a soluble form – blood-borne TF – that can be detected in the maternal plasma. The plasma concentration of TF during normal pregnancy is comparable to the non-pregnant state

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[17,18] and increases during labor [19,20]. There is a positive correlation between TF concentration and activity in women with a normal pregnancy [21], and TF activity is higher than the non-pregnant state [22].

Further support for the pro-coagulant state reported in preeclampsia and preterm prelabor rupture of the membranes (PROM) [23,24] is the higher median maternal plasma TF concentration observed in these patients in comparison to women with normal pregnancies [25–28], as well as the low plasma protein *Z* concentrations observed in women with preeclampsia [29] and those with idiopathic intrauterine bleeding and preterm labor [30]. In contrast, in spite of their increased thrombin generation, patients who delivered a small-for-gestational-age (SGA) neonate had a lower median maternal plasma TF concentration than that of women with a normal pregnancy [19]. Patients with both preeclampsia [16,17,25–29] and SGA neonates [16] have increased thrombin generation, as evident by elevated maternal plasma thrombin anti-thrombin (TAT) complexes concentrations [24,31–33]. However, patients with these obstetrical syndromes differ in their maternal TF concentrations. Therefore, we conducted a study that was aimed to test (1) whether TF and TFPI activity can explain the increased thrombin generation in these syndromes and (2) whether the TF and TFPI activity are related to specific placental lesions in each study group.

Material and methods

Study groups and inclusion criteria

This cross-sectional study included patients with singleton gestations in the following groups: (1) women with normal pregnancies ($n = 68$); (2) patients with preeclampsia ($n = 128$); and (3) women who delivered SGA neonates without preeclampsia ($n = 56$). Patients with fetuses who had congenital and/or chromosomal anomalies were excluded.

Samples and data were retrieved from the Bank of Biological Materials and the clinical databases of the Perinatology Research Branch, *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD), Wayne State University, and the Detroit Medical Center. Many of these samples have been employed to study the biology of inflammation, hemostasis, angiogenesis regulation, and growth factor concentrations in non-pregnant women, women who had normal pregnancies, and 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 NICHD 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 age ranging from 20 to 41 weeks; and (3) delivery of a term infant, appropriate for gestational age, without complications. Preeclampsia was defined as the presence of hypertension (systolic blood pressure $\geq$140 mmHg and/or diastolic blood pressure $\geq$90 mmHg on at least two occasions, 4 h to 1 week apart) and proteinuria ($\geq$300 milligrams in a 24 h urine collection or one dipstick measurement $\geq$2+) [34]. Preeclampsia diagnosed $<$34 weeks of gestation was defined as early onset. A small for gestational age neonate was defined as birthweight $<10^{th}$ percentile [35]. Placental histologic findings were classified according to a diagnostic schema proposed by Redline et al. [36].

Sample collection and immunoassays

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

Human tissue factor immunoassay

TF concentrations were determined by sensitive and specific immunoassays obtained from American Diagnostica (Greenwich, CT), which recognize TF-apo, TF, and TF-FVII complexes. The assays were conducted according to the manufacturer's recommendations. The coefficient of variation (CV) calculated 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 obtained from American Diagnostica (Greenwich, CT). The assays were conducted according to the manufacturer's recommendations. The calculated intra-assay CV was 1.83%, while the inter-assay CV was 3.58%, and the sensitivity of this assay was 0.596 pM.

Human tissue factor pathway inhibitor immunoassay

The concentrations of TFPI in maternal plasma were determined by sensitive and specific immunoassays

obtained from American Diagnostica (Greenwich, CT). The TFPI ELISA employs, as the capture antibody, a murine anti-TFPI monoclonal antibody 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 concentrations. The assay was conducted according to the manufacturer's recommendations. The CV calculated in our laboratory was 6.6%, and the sensitivity was ~10 ng/ml.

Human tissue factor pathway inhibitor activity assay

TFPI activity was determined by a chromogenic assay obtained from American Diagnostica (Greenwich, CT). 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

Maternal plasma concentrations and activity of TF and TFPI were not normally distributed. Thus, the Kruskal–Wallis test with *post-hoc* analysis by the Mann–Whitney *U* test was used for the comparison of continuous variables. Comparisons of proportions were performed by the Chi-square and Fisher's exact tests. The Spearman's rho test was used to detect a correlation between the concentrations and activity of TF, TFPI, and TFPI/TF ratio to the gestational age at sample collection. The ratio of TFPI/TF concentration was previously calculated [26] and included in the logistic regression models. A p value $<.05$ was considered statistically significant. Analysis was performed with SPSS, version 12 (SPSS Inc., Chicago, IL).

Results

Demographic and clinical characteristics

In comparison to women with normal pregnancies, patients with preeclampsia and those who delivered an SGA neonate had a higher median gestational age at sample collection, lower median gestational age at delivery, and lower median birthweight. Women with preeclampsia had lower gestational age at delivery than women who delivered an SGA neonate (Table 1).

Among women with a normal pregnancy (1) gestational age at sample collection positively correlated with maternal plasma TFPI activity ($r = 0.3$, $p = .01$); (2) the correlation between maternal plasma TFPI concentration and activity did not reach statistical significance ($r = 0.3$, $p = .06$); and (3) maternal plasma TF activity was negatively correlated with the TFPI/TF ratio ($r = -0.3$, $p = .006$).

Changes in the median plasma activity of tissue factor and tissue factor pathway inhibitor in the different study groups

The median maternal plasma TF activity, but not TFPI activity, differed among the study groups (Kruskal–Wallis, $p < .0001$, $p = .4$, respectively). Patients with preeclampsia had higher median maternal plasma TF activity than women with normal pregnancies (16.1 pM, range 2.9–91.2 vs. 9.9 pM, range 0.7–37.6,

Table 1. Demographic and clinical characteristics of the study population.

	Normal pregnancy ($n = 68$)	Preeclampsia ($n = 128$)	SGA ($n = 56$)
Maternal age (years)	24 (21–27)	25 (20–31)	25 (20–30)
Gravidity[a]			
1	14 (21.2)	43 (33.9)	11 (20.4)
2–5	42 (63.6)	70 (55.1)	36 (66.7)
≥6	10 (15.2)	14 (11.0)	7 (13.0)
Parity[b]			
1	37 (55.2)	93 (73.2)	34 (61.8)
2–5	29 (43.3)	30 (23.6)	20 (36.4)
≥6	1 (1.5)	4 (3.1)	1 (1.8)
Ethnic origin[c]			
African-American	50 (76.9)	106 (84.1)	46 (86.8)
Caucasian	11 (16.9)	13 (10.3)	4 (7.5)
Hispanic	2 (3.1)	5 (4.0)	1 (1.9)
Asian	2 (3.1)	1 (0.8)	1 (1.9)
Other	0	1 (0.8)	1 (1.9)
Gestational age at blood collection (weeks)	31.8 (27.7–34.8)	34.3* (30.2–37.6)	37.2* (31.7–38.4)
Gestational age at delivery (weeks)	39.6 (38.5–40.5)	34.6*‡ (31.0–37.9)	37.6* (34.4–39.0)
Neonatal birthweight (grams)	3335 (3014–3695)	1920* (1215–2702)	2110* (1490–2510)

Data are presented as median (minimum, maximum) or numbers (%). SGA: small for gestational age.
[a]Normal pregnancy ($n = 66$); preeclampsia ($n = 127$); SGA ($n = 54$).
[b]Normal pregnancy ($n = 67$); preeclampsia ($n = 127$); SGA ($n = 55$).
[c]Normal pregnancy ($n = 65$); preeclampsia ($n = 126$); SGA ($n = 53$).
*$p < 0.05$ in comparison to normal pregnancy.
‡$p < 0.05$ in comparison to SGA.

respectively; $p < .0001$), and patients with SGA fetuses (12.9 pM, range 2.5–74.4, $p = .002$). The latter group had higher median maternal plasma TF activity than women with normal pregnancies ($p = .0001$) (Figure 1).

Early-onset preeclampsia was associated with higher median maternal plasma TF activity than term preeclampsia (18.7 pM, range 8.4–65.9 vs. 15.3 pM, range 2.9–91.2, respectively; $p = .016$) (Figure 2).

To determine the association between maternal plasma TF and TFPI activity and preeclampsia or SGA, we constructed three multivariate logistic regression models and demonstrated that (1) maternal plasma TF activity and gestational age at sample collection, but not TFPI activity, were independently associated with the development of preeclampsia (Table 2); (2) the independent association between TF activity and the development of preeclampsia remained significant even after the introduction of the TFPI/TF ratio into the model (Table 3); and (3) by contrast, only gestational age at sample collection, but not TF or TFPI activity, was associated with the delivery of an SGA neonate (Table 4).

Placental histological findings in patients with preeclampsia and SGA and their association with the changes in tissue factor and tissue factor pathway inhibitor concentrations and their ratio (TFPI/TF)

Placental histology was available from 88.3% (113/128) of patients in the preeclampsia group and 78.6% (44/56) of patients from the SGA group, and the specific findings are presented in Table 5. Increased syncytial knots were more frequent in the placentae of patients with preeclampsia than in those of patients with SGA neonates [54% (61/113) vs. 27.3% (12/44), respectively; $p = .004$]. Among patients with preeclampsia, those with distal villous hypoplasia had a higher median maternal TF activity than those without

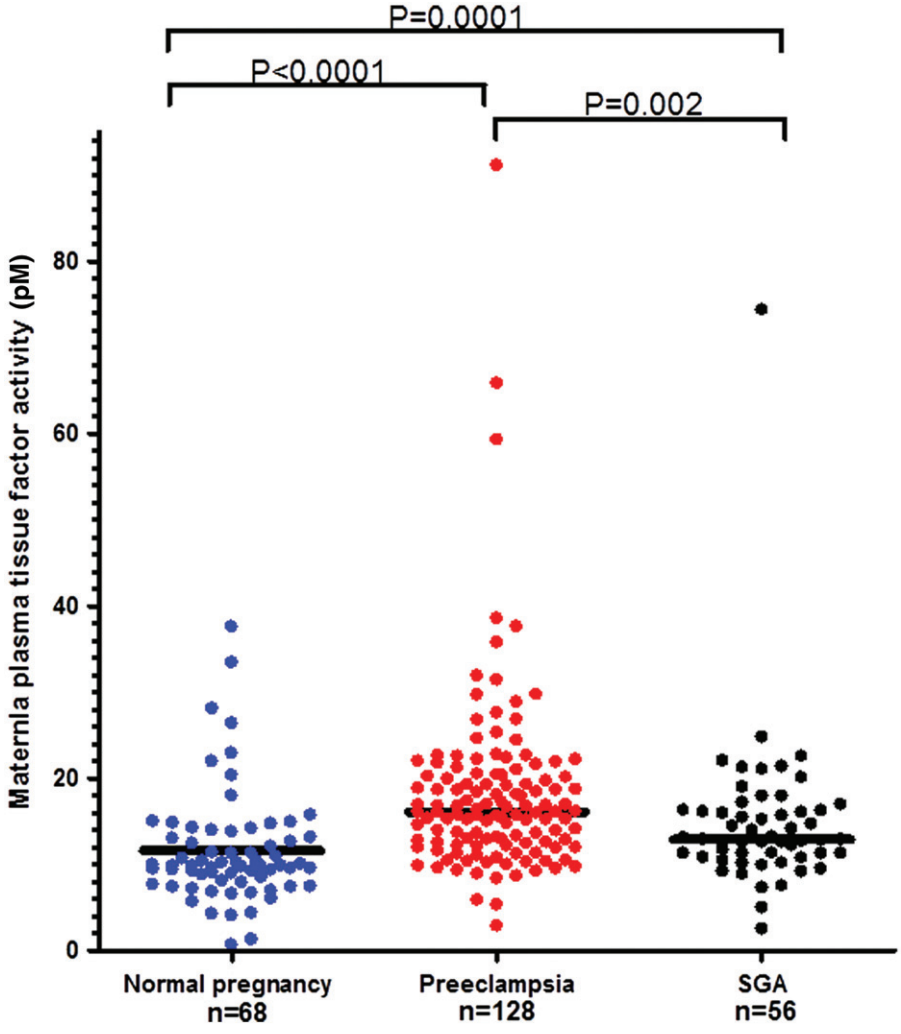

Figure 1. Maternal plasma tissue factor activity in women with normal pregnancies, preeclampsia or SGA neonates.

this lesion (20.1 pM, range 8.4–91.2 vs. 16.1 pM, range 2.9–59.3, respectively; $p = .018$).

Discussion

Principal findings of the study

(1) Patients with preeclampsia or women who delivered an SGA neonate had higher median maternal plasma TF activity than those with normal pregnancies; (2) by contrast, the maternal plasma TFPI activity did not differ significantly among the study groups; (3) patients who developed early-onset preeclampsia had higher median maternal plasma TF activity than those who developed this syndrome at term; (4) maternal plasma TF activity was independently associated with the development of preeclampsia even after adjustment for gestational age at sample collection and with the maternal TFPI/TF ratio; and (5) there was no association between the delivery of an SGA neonate with maternal plasma TF and TFPI activity after adjustment for gestational age at sample collection.

Changes in maternal plasma tissue factor during normal and complicated pregnancies

During normal pregnancy, TF is abundant in the uterine decidua [37,38], fetal membranes, and amniotic fluid [9,10,20,21], resulting in an efficient hemostatic mechanism activated during implantation and after delivery [17]. The maternal plasma concentration of TF during normal pregnancy is similar to the non-pregnant state and increase during labor [11,15].

Table 2. Multiple logistic regression analysis of the association of maternal plasma tissue factor pathway inhibitor and tissue factor concentrations — activity as well as the interactions between each analyte concentration and activity and preeclampsia.

Factor	OR (95% CI)
Gestational age at sample collection (weeks)	1.09 (1.03–1.14)
TF activity (pM)	1.07 (1.04–1.11)
TFPI activity (unit/ml)	1.6 (0.8–3.2)

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

Table 3. Multiple logistic regression analysis of the association of maternal plasma tissue factor pathway inhibitor/tissue factor ratio and preeclampsia.

Factor	OR (95% CI)
Gestational age at sample collection (weeks)	1.13 (1.06–1.22)
TF activity (pM)	1.08 (1.02–1.13)
TFPI/TF ratio	0.97 (0.96–0.98)
TFPI activity (unit/ml)	2.8 (0.94–8.35)

TFPI/TF: Tissue factor pathway inhibitor/Tissue factor; OR: odds ratio; CI: confidence interval.

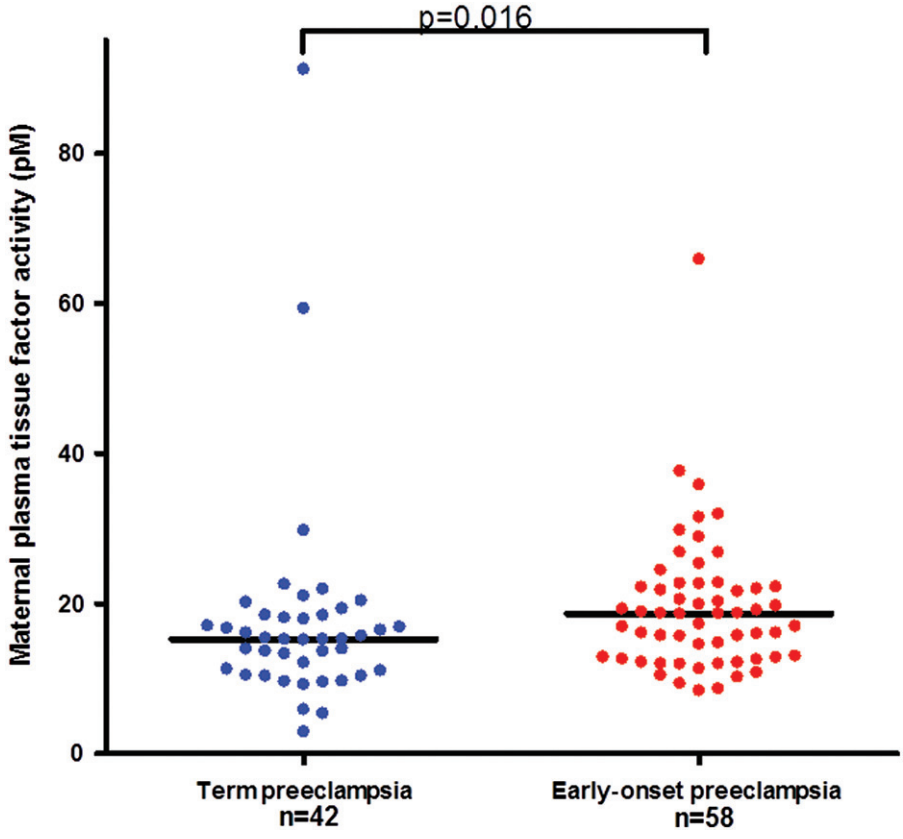

Figure 2. Maternal plasma tissue factor activity in women with early-onset or term preeclampsia.

Changes in the maternal plasma TF concentration have been reported in several pregnancy complications. Indeed, in comparison to normal pregnancy, the median maternal plasma TF concentration increases in patients with preeclampsia [39] or those with preterm PROM [25], decreases in those who deliver an SGA neonate [39], and does not change significantly in patients with preterm labor [19] or in women with a fetal demise [21]. Along with the increased maternal plasma TAT III complexes concentration reported in these obstetrical syndromes [23,25–27,29,40], the changes in the maternal plasma TF concentration were regarded as additional evidence for the presence of a procoagulant state in preeclampsia and preterm PROM.

The association between maternal plasma tissue factor concentration and procoagulant activity

It is unclear whether the maternal plasma TF concentration reflects a state of activation of the coagulation cascade, systemic maternal inflammation, or both. Indeed, the pro-coagulant activity of plasma immunoreactive TF (blood-borne TF) of non-pregnant patients is a controversial topic. While Butenas et al. [41] demonstrated that blood-borne TF has very little or no pro-coagulant activity, and that only the administration of exogenous, active TF generates a whole blood and plasma clot after the inhibition of the contact factor (factor XIIa) [41], others [42–46] have proposed that blood-borne TF does not initiate the coagulation cascade, but rather propagates clot formation by attaching to activated platelets, further enhancing this process.

The assay used in this study measures TF activity through the generation of factor Xa (FXa) in a given sample. Thus, increased TF activity is reflected in elevated FXa formation that leads to increased thrombin generation. Therefore, the positive correlation between maternal plasma TF concentration and activity in normal pregnant women, previously reported by our group [19] suggests that during gestation circulating TF has pro-coagulant properties that may be part of the hypercoagulable state of normal pregnancy.

Table 4. Multiple logistic regression analysis of the association of maternal plasma tissue factor pathway inhibitor and tissue factor concentrations, activity as well as the interaction between each analyte concentration and activity and SGA.

Factor	OR (95% CI)
Gestational age at sample collection (weeks)	1.12 (1.05–1.2)
TFPI activity (unit/ml)	0.93 (0.38–2.25)
TF activity (Pm)	0.99 (0.96–1.2)

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

Preeclampsia, SGA and tissue factor activity

The finding that maternal plasma TF activity was independently associated with the development of preeclampsia, but not with the delivery of an SGA neonate, is novel.

What are the possible mechanisms leading to increased tissue factor activity among patients with preeclampsia?

The following are possible factors contributing to the higher TF plasma concentration among patients with preeclampsia: (1) the exaggerated systemic maternal inflammation characterizing patients with preeclampsia [47–55]. Indeed, patients with this syndrome have increased monocyte activation when compared to those with a normal pregnancy [49,55–64], as well as a higher monocyte metabolic activity and oxidative

Table 5. A comparison of placental histologic lesions between patients with preeclampsia and patients who delivered an SGA neonate.

Placental histologic findings	Preeclampsia ($n = 113$)	SGA ($n = 44$)	p value
Findings consistent with amniotic fluid infection			
Chorioamnionitis, maternal response	7 (6.2)	4 (9)	.5
Funisitis, fetal response	2 (1.8)	4 (9)	.05
Findings consistent with maternal underperfusion			
Remote villous infarcts	21 (18.6)	5 (11.4)	.3
Recent villous infarcts	6 (5.3)	0	.2
Increased syncytial knots	61 (54)	12 (27.3)	.004
Villous agglutination	14 (12.4)	7 (15.9)	.6
Increased intervillous fibrin	26 (23.0)	7(15.7)	.4
Distal villous hypoplasia	28 (24.8)	6 (13.6)	.19
Persistent muscularization of basal plate arteries	4 (3.5)	2 (4.5)	.6
Mural hypertrophy of decidual arterioles	8 (7.1)	4 (9.1)	.7
Acute atherosis of basal plate arteries/decidual arterioles	16 (14.2)	2 (4.5)	.1
Findings consistent with fetal vascular thrombo-occlusive disease			
Villous changes	9(6.1)	4 (9.1)	.8
Chronic villitis with obliterative fetal vasculopathy	9 (8)	5 (11.4)	.5

Data are presented as numbers (%).

burst than those delivering an SGA neonate [65]. Peripheral blood monocytes from the uterine vein of patients with preeclampsia showed a higher degree of activation in comparison to those obtained from the cubital vein [60], and this has been proposed to be the consequence of maternal monocyte activation during their passage through the placental bed of patients with preeclampsia [60]. (2) A second mechanism to consider is the production of microparticles containing TF and their release into the maternal circulation. Activated monocytes are a possible source of such microparticles that express TF on their membranes [5,41,66–72] and shed microparticles containing TF [41,46,73–76] into the plasma during systemic inflammation. The placenta may be an additional source for microparticles. Indeed, patients with preeclampsia have increased syncytiotrophoblast and endothelial macrovesicle production [77–82]. Additionally, a higher apoptosis rate was observed in cultured cytotrophoblasts of patients with preeclampsia and fetal growth restriction, and the primary placental tissues of these patients had 1.8- and 1.9-fold increases in the apoptosis rate compared to the controls [83]. Placentas of patients with preeclampsia have higher degrees of ischemia [84] and apoptosis [82] associated with microparticles generation. Placental microparticles that would carry the placental isoform of TF, which is different than recombinant TF and has a higher affinity for FVIIa [85], and syncytiotrophoblast microvesicles released from the placentas of patients with preeclampsia, exhibit increased TF activity than that of women who had normal pregnancies; a recent study suggested that macrovesicles released from the placentas of patients with preeclampsia exhibit increased TF activity, and when they were added to platelet-poor plasma, they led to increased thrombin generation [86]. (3) Patients with preeclampsia had a significant reduction in their microparticle-induced thrombin generation after treatment with anti-FVII antibodies [87]. Thus, the authors [87] concluded that a higher proportion of thrombin generation is derived from the extrinsic pathway of coagulation in patients with preeclampsia. This is in accord with our own findings, since TF is the activator of the extrinsic pathway of coagulation, and the increased maternal plasma activity of this coagulation factor among patients with preeclampsia reported herein may contribute to the observation made by VanWijk et al. [87]. Moreover, maternal plasma TF activity is increased in patients who developed early-onset preeclampsia, suggesting an association between TF activity and the severity of this syndrome.

Tissue factor and maternal thrombin generation in women who delivered an SGA neonate

The finding that women who delivered an SGA neonate have higher median maternal plasma TF activity than women who had normal pregnancies is novel. TF activity, however, was not independently associated with the delivery of an SGA neonate. The association between the higher TAT III complexes concentration reported in these patients [24] and the activation of TF in the maternal plasma is not clear. Our group reported that although women who delivered SGA neonates have faster thrombin generation kinetics, they do not generate significantly more thrombin than women who had normal pregnancies [88]. In addition, the median maternal plasma TF concentration was lower in the SGA group than in the preeclampsia as well as the normal pregnancy groups, while TFPI concentration and activity did not differ from that of women with a normal pregnancy [39]. Collectively, these findings suggest that patients with an SGA neonate have higher TF activity compared to women who had normal pregnancies, and this may contribute to the faster thrombin generation kinetics in these patients. However, the low maternal plasma TF concentration and the normal TFPI concentration result in a higher TFPI/TF ratio that may lead to less activation of the extrinsic pathway of coagulation.

In conclusion, maternal plasma TF activity, although high in patients who developed preeclampsia and those who delivered an SGA neonate, is independently associated only with the development of preeclampsia, and is higher in patients with the early-onset form than in those who developed preeclampsia later during gestation. This finding supports the procoagulant activity of blood-borne TF in complicated pregnancies, especially among patients with preeclampsia, and further emphasizes the role of the extrinsic pathway of coagulation in this obstetrical syndrome.

Disclosure statement

No potential conflict of interest was reported by the authors.

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