Circulating Fibronectin and Plasminogen Activator Inhibitor-2 Levels as Possible Predictors of Recurrent Placental Syndrome: An Exploratory Study

Carmen A.H. Severens-Rijvers^a Salwan Al-Nasiry^b Chahinda Ghossein-Doha^b
Sara Marzano^f Hugo Ten Cate^c Bjorn Winkens^d Marc A.E. Spaanderman^b
Louis L.H. Peeters^e

Departments of ^aPathology, ^bObstetrics and Gynecology, ^cInternal Medicine and ^dMethodology and Statistics, Maastricht University Medical Centre, CAPHRI Research School, The Division 'Vrouw & Baby', and ^eDepartment of Obstetrics, The Division 'Vrouw & Baby' UMC Utrecht, Utrecht, The Netherlands; ^fDepartment of Obstetrics and Gynecology, Università La Sapienza, Rome, Italy

Key Words

Placental syndrome · Preeclampsia · Gestational hypertension · PAI-2 · Fibronectin

Abstract

Background/Aim: Placental syndromes (PS) are characterized by endothelial dysfunction complicating placental dysfunction. Possible markers for endothelial dysfunction and amount of trophoblast are fibronectin and plasminogen activator inhibitor-2 (PAI-2), respectively. We aimed (1) to determine whether in women with recurrent PS (rPS), this complication is preceded by deviating fibronectin- and PAI-2-levels, and (2) whether this is dependent on pre-pregnant plasma volume (PV). **Methods:** In 36 former patients, we determined fibronectin- and PAI-2-levels in blood-samples collected pre-conceptionally and at 12–16 weeks in their next pregnancy. Differences were analyzed between pregnancies with rPS (n = 12) and without rPS (non-rPS, n = 24) using linear mixed models, with subanalyses based on pre-pregnant normal or subnormal PV. **Results:** We observed higher fibronectin-levels at 12–16 weeks (p < 0.05 and p < 0.01, respectively) and

lower PAI-2-levels at 16 weeks (p < 0.01) in the rPS subgroup, the intergroup differences being larger in women with subnormal PV. **Conclusion:** We showed that former PS patients who developed rPS have raised fibronectin- and reduced PAI-2-levels already in early/mid pregnancy. These deviations are even more prominent in women with subnormal pre-pregnant PV, supporting development of a 2-step screening program for former patients to identify the high-risk subgroup of women who may benefit from closer surveillance.

© 2016 The Author(s)
Published by S. Karger AG, Basel

Introduction

Preeclampsia (PE) is an epitome of the placental syndrome (PS) constituting a major cause of maternal and perinatal morbidity and mortality worldwide. PE has an overall incidence of 4.6% [1], with a higher chance of recurrence in about 25% of former preeclamptic patients [2].

Reprints not available from the authors.

Other clinical disorders within the spectrum of PS are gestational hypertension (GH), fetal growth restriction and/or fetal demise due to placental dysfunction. PS tends to recur in the next pregnancy in any of these different clinical entities [3]. Although the onset of PS is usually in the late second and third trimester, it is strongly associated with defects of placentation in early pregnancy [4], characterized by defective remodeling of uterine spiral arteries [5] leading to placental dysfunction as indicated by both inadequate fetal supply of oxygen and nutrients and excessive release of placental endothelial-toxic microparticles [6–8].

Fibronectin is an extracellular matrix protein released by fibroblasts in the vessel wall in response to mechanical stimuli. Fibronectin promotes fibrin deposition in the extracellular matrix [9]. Therefore, this protein is regarded as a marker for endothelial stress [10]. On the other hand, plasminogen activator inhibitor-2 (PAI-2) is a placenta-specific protease inhibitor [11] released by trophoblast cells. The circulating PAI-2 level typically varies as a function of the total amount of trophoblast [10]. PAI-2 accelerates the conversion of plasminogen into plasmin, which in turn degrades a blood clot [11]. Increased fibronectin and decreased PAI-2 levels precede PE onset as early as at 20 weeks pregnancy [12–15]. Whether these abnormal peripheral levels also prevail earlier in pregnancy in women who later develop PE is unclear.

Previously, we identified a subnormal pre-pregnant plasma volume (PV) as an independent risk factor for recurrent PE in former patients [17]. We also found a higher cardiovascular sympathetic activity in women with a subnormal PV, which indicates a higher level of mechanical stress exerted upon their endothelium [18].

We postulate that in former PS patients, abnormal circulating levels of fibronectin and PAI-2 at 12–16 weeks precede the development of recurrent PS (rPS) in their next pregnancy. To this end, we compared circulating fibronectin and PAI-2 levels before and at 12–16 weeks of the next pregnancy in a cohort of former PS patients who eventually did ($n = 12$) and did not ($n = 24$) develop rPS later on during their pregnancy. In addition, we explored whether the presence of a subnormal pre-pregnant PV enables to more accurately identify former patients who actually develop rPS in their next pregnancy.

Methods

This retrospective, longitudinal, observational cohort study was performed at the Maastricht University Medical Center, Maastricht, the Netherlands, between January 2002 and December 2010. Former ‘severe’ PS patients underwent extensive serial

monitoring in a specialized obstetrical unit as part of their individual risk assessment. They underwent various examinations before and during the first half of their next pregnancy. PS was considered ‘severe’ when its clinical onset was early (<34 weeks) and/or the infant’s birth weight was low ($<2.3\%$) due to placental dysfunction as diagnosed on the basis of abnormal fetal biometry (asymmetric growth) and/or Doppler tracings of the uterine arteries. The study protocol was approved by the institutional Medical Ethics reviewing Committee (MEC 10-4-049). During the study period, we followed women prospectively. Deep-frozen serum samples from 50 women were available for further analyses. Since pre-existing hypertension is also associated with endothelial dysfunction [20], we only enrolled normotensive women (excluding 14 women), all being primiparous, and subdivided them into 2 subgroups depending on whether they did (rPS, $n = 12$) or did not develop recurrent maternal PS (non-rPS, $n = 24$) later on during their pregnancy.

To determine the impact of the pre-existent endothelial reserve capacity, we stratified these 2 groups into subgroups based on their pre-pregnant PV as follows: (1) normal PV (NPV) and non-rPS in the next pregnancy ($n = 12$); (2) NPV and rPS in the next pregnancy ($n = 5$); (3) low PV (LPV) and non-rPS in the next pregnancy ($n = 12$); and (4) LPV and rPS in the next pregnancy ($n = 7$). All participants were Caucasian.

We defined PE as GH, as described in detail previously [21]. A newborn was considered small-for-gestational-age (SGA) when the birth weight was below the 10th centile, based on the most recent Dutch birth weight reference curves [22]. A PV below 48 ml/kg-lean body mass (kg-lbm) was defined as ‘subnormal’, as opposed to a ‘normal’ PV, which was ≥ 48 ml/kg-lbm [23].

We estimated pre-pregnant PV by the I^{125} -labeled albumin indicator dilution method [23]. We collected a venous blood sample after an overnight fast before pregnancy and again at 12–16 weeks in the next pregnancy. These samples were immediately centrifuged and the supernatant plasma stored at -80°C for the later measurement of fibronectin and PAI-2 levels. Fibronectin was determined nephelometrically (Image Beckman Coulter L02.2626. SE1, Beckman Coulter, Fullerton, USA). PAI-2 was determined by ELISA (IMUBIND PAI-2 ELISA, American Diagnostica Inc., Stamford, USA). PAI-2 measurement was only measured in the 2 samples obtained in pregnancy.

We performed statistical analyses using IBM SPSS Statistics for Windows version 20.0 (SPSS, Armonk, N.Y., USA). Data are presented as medians with interquartile range (IQR) for numerical variables and as numbers (with %) for categorical variables. IQRs are presented as 25th and 75th percentiles. Differences between the 2 subgroups were tested using the Mann–Whitney U test for numeric variables and the Fisher’s exact test for categorical variables. The differences in longitudinal trends in outcome parameters between subgroups were assessed using linear mixed models with time (categorical: pre-pregnant, 12–16 weeks of pregnancy), group and group*time as fixed factors and an unstructured covariance structure for the repeated measurements. The interaction of time with the subgroups was assessed to evaluate whether the time course of a studied parameter differed significantly between the groups. Pregnancy outcome and serum levels of fibronectin and PAI-2 were also compared between subgroups based on either the development of rPS (hypertensive vs. normotensive pregnancy) or the presence of a normal or subnormal pre-pregnant PV (LPV vs. NPV). The latter included the following comparisons: NPV-non-

Table 1. Baseline demographic characteristics of the groups

	Non-rPS	rPS
Number	24	12
Pre-pregnant BMI	26.9 (24.3–31.2)	26.3 (21.9–28.2)
Pre-pregnant PV, ml/kg-lbm	48.5 (44.0–52.8)	45.0 (43.0–49.8)
Age	34.8 (31.4–37.9)	32.5 (29.5–35.3)
Obstetric history		
GH/PE, n (%)	17 (70.8)	12 (100)
SGA/stillbirth, n (%)	7 (29.2)	0
Gestational age, weeks	31.9 (30.1–34.2)	30.4 (28.6–33.8)
Birth weight	1,280.0 (885.0–1,817.0)	1,252.5 (735.0–1,643.8)

Data are presented as median (IQR), unless noted otherwise.

BMI = Body mass index.

No significant differences were found.

Table 2. Outcome of current pregnancy per group

	Non-rPS	rPS
Number	24	12
MAP at 12 weeks GA	81.0 (75.5–86.8)	88.0 (80.0–91.0)
GH, n (%)	–	7 (58.3)
GA at onset, weeks		36.4 (29.6–38.3)
PE, n (%)	–	5 (41.7)
GA at onset, weeks		36.0 (33.4–37.8)
GA at birth, weeks	38.7 (37.7–40.3)	38.1 (37.2–40.1)
Birth weight	3,280.0 (2,986.3–3,615.0)	3,007.5 (2,525.0–3,310.0)
SGA, n (%)	4 (16.7)	3 (25)

Data are presented as median (IQR), unless noted otherwise.

MAP = Mean arterial pressure; GA = gestational age.

No significant differences were found.

rPS vs. NPV-rPS, LPV-non-rPS vs. LPV-rPS, NPV-rPS vs. LPV-rPS, and NPV-non-rPS vs. LPV-non-rPS. A p value <0.05 was considered to indicate statistical significance.

Results

Table 1 lists baseline demography and table 2 lists pregnancy outcomes of the study population. Both former PS patient groups were comparable, although the variation in most variables was large.

Table 3 shows that the former patients in the rPS group differed from those in the non-rPS group by higher fibronectin levels at 12–16 weeks ($p < 0.05$ and $p < 0.01$, respectively) and by $\approx 30\%$ lower PAI-2 levels at 16 weeks ($p < 0.01$). These differences were similar when only the more

Table 3. (Pre-)pregnant circulating concentrations of fibronectin and PAI-2

	Pre	12 weeks GA	16 weeks GA
Fibronectin, mg/l			
Non-rPS	254.3 (19.1)	263.8 (15.7)	254.9 (17.2)
rPS	317.8 (34.0)	336.4 (22.7) ^a	346.0 (24.7) ^a
PAI-2, ng/ml			
Non-rPS	–	34.1 (4.0)	63.0 (4.1) ^b
rPS	–	23.4 (5.9)	43.5 (5.8) ^{a, b}

Data are presented as estimated means (SE), using linear mixed model.

GA = Gestational age.

^a Significant difference ($p < 0.05$) compared to rPS.

^b Significant within-group change ($p < 0.05$) compared to 12 weeks GA.

Table 4. Pregnancy outcome in each subgroup based on pre-pregnancy plasma volume

	NPV-non-rPS	NPV-rPS	LPV-non-rPS	LPV-rPS
Number	12	5	12	7
Pre-pregnant PV*	52.5 (52.0–56.8)	50.0 (48.5–52.5)	44.0 (42.3–46.5)	43.0 (41.0–45.0)
MAP at 12 weeks GA	79.5 (74.5–89.3)	80.0 (77.5–90.5)	82.0 (74.0–86.8)	88.0 (83.3–92.8)
GH, n (%)	–	3 (60)	–	4 (57.1)
GA at onset, weeks	–	35.3 (34.1–36.4)	–	38.0 (25.0–38.6)
PE, n (%)	–	2 (40)	–	3 (42.9)
GA at onset, weeks	–	36.8 (36.0–37.6)	–	34.0 (32.9–38.0)
GA at birth, weeks	38.9 (37.8–42.0)	37.9 (37.1–39.4)	38.3 (37.7–40.4)	39.1 (37.3–40.3)
Birth weight, g	3,225.0 (2,597.5–3,425.0)	3,055.0 (2,730.0–3,290.0)	3,300.0 (3,097.5–3,685.5)	2,760.0 (2,230.0–3,400.0)
SGA, n (%)	2 (16.7)	0	2 (16.7)	3 (42.9)

Data are presented as median (IQR), unless noted otherwise.

MAP = Mean arterial pressure; GA = gestational age.

* Per definition significant difference ($p < 0.05$) between groups, no significant differences in other values found.

Table 5. (Pre-)pregnant circulating concentrations of fibronectin and PAI-2 for plasma volume-based subgroups

	Pre	12 weeks GA	16 weeks GA
Fibronectin, mg/l*			
NPV-non-rPS	247.3 (22.3)	262.2 (19.7)	237.7 (20.3)
NPV-rPS	218.9 (35.6)	266.4 (30.6)	262.6 (31.4)
LPV-non-rPS	262.5 (20.6)	265.3 (19.7)	273.3 (20.6)
LPV-rPS	552.0 (70.0) ^a	370.7 (27.9) ^{a, b}	385.5 (28.7) ^{a, b}
PAI-2, ng/ml**			
NPV-non-rPS	–	40.3 (5.7)	65.5 (5.7)
NPV-rPS	–	27.1 (9.2)	56.3 (8.6)
LPV-non-rPS	–	28.4 (5.5)	60.4 (5.7)
LPV-rPS*	–	20.8 (7.6)	33.0 (7.6) ^{a, b}

Data are presented as estimated means (SE), using linear mixed model. GA = Gestational age.

^a Significant difference ($p < 0.05$) between LPV-non-rPS and LPV-rPS.

^b Significant difference ($p < 0.05$) between NPV-rPS and LPV-rPS.

* Within-group changes: LPV-rPS showed a significant decline in fibronectin levels at 12 and 16 weeks GA, compared to the pre-pregnant value.

** Within-group changes: except for LPV-rPS, all groups showed a significant rise in PAI-2 levels at 16 weeks GA.

homogeneous subgroup of former patients with a history of GH was included (data not shown). While fibronectin levels did not change over time, the PAI-2 levels increased consistently between 12 and 16 weeks ($p < 0.001$ in both groups).

Table 4 shows that stratification of pregnancy outcome based on pre-pregnant PV did not lead to clustering of baseline demographic features in either of the former PS patient group.

Table 5 presents the results from the subgroup analysis after subdividing both the rPS and non-rPS groups into normal and subnormal PV subgroups. Despite comparable incidences of rPS in both PV subgroups (5 of 17 (29%) cases in the NPV subgroup and 7 of 19 (37%) cases in the LPV subgroup), this subdivision led to clear inter-group differences in fibronectin and PAI-2 levels. Circulating fibronectin levels in LPV-rPS subgroup were higher than in LPV-non-rPS subgroup ($p = 0.001$). Even though pregnancy induced a decline in the fibronectin levels in LPV-rPS subgroup, these levels were still higher than in the 3 other PV subgroups at mid-pregnancy.

Meanwhile, in all subgroups except LPV-rPS, circulating PAI-2 levels increased between 12 and 16 weeks ($p < 0.001$). As a consequence, PAI-2 levels at 16 weeks were lower in LPV-rPS than in both LPV-non-rPS and NPV-rPS ($p < 0.01$ and $p < 0.05$, respectively).

Discussion

In this exploratory study, we found that in the subgroup of former PS patients with a subnormal PV, those who developed rPS in their next pregnancy differed from their counterparts who did not develop rPS by higher fibronectin and lower PAI-2 levels, mainly at 16 weeks of gestation. Our results are in line with our previous observations [19, 24] that (1) both elevated fibronectin levels and subnormal PV prior to a next pregnancy predispose former PS patients to rPS in their next pregnancy and (2) rPS is preceded by indirect signs of placental insufficiency already at 12 weeks. Interestingly, in a previous study,

we found that in the non-pregnant state endothelial activation seems to indicate a lower endothelial functional reserve capacity, and thus probably also a lower cardiovascular reserve capacity [25].

Obviously, a major limitation of this study was the modest size of our study population which limits its statistical power. Therefore, we were unable to determine robust cutoff values for fibronectin and PAI-2, which are needed to estimate the positive and negative predictive values. In conjunction with the exploratory character of this study, we also decided not to correct for multiple testing or confounders but to perform subgroup analysis instead. The observed differences between non-rPS and rPS, and the results of the subgroup analysis support the view that former PS patients developing rPS in their next pregnancy can be identified among their counterparts who do not develop rPS, on the basis of 3 parameters: (1) pre-pregnant PV, (2) circulating fibronectin and (3) PAI-2 levels at 16 weeks. In this context, it is relevant to mention that fibronectin is an easy, fairly accurate and non-invasive method to estimate endothelial function [10]. This also applies to PAI-2 levels, which vary as a function of total amount of trophoblast. In most cases, low peripheral PAI-2 levels in mid-pregnancy are consistent with the presence of a relatively small placenta, without providing information on placental function [18]. PAI-2 inhibits urokinase-type plasminogen activator and both are produced by trophoblast cells at the fetal-maternal interface. Reduced circulating PAI-2 levels at 12–16 weeks are often an early sign of imminent placental dysfunction possibly due to defective fibrinolytic activity in the intervillous space because of enhanced fibrin deposition, a common feature in pregnancies complicated by a PS. Lastly, our study groups show a heterogenous obstetrical history, which could be a source of bias or confounding factor. Yet, PE, GH, SGA and IUFD all predispose to each other in a subsequent pregnancy [3] and share a common placental etiology [7, 8]. Nevertheless, despite the aforementioned limitations, this study is still unique because of the pre-conceptual and early- to mid-pregnant measurements.

Several other research groups reported that abnormal fibronectin [13, 15, 27–29] and PAI-2 levels [14, 30–32] precede the onset of a PS, presumably from 20 weeks pregnancy onward [13–15, 29, 30, 32]. One study measured fibronectin at 13 weeks, but the authors only performed a trend analysis based on 3 consecutive measurements in the first, second and third trimester [16]. Our data indicate that former PS patients – previously identified with a subnormal pre-pregnant PV – may already have raised fibronectin levels at 12 weeks.

Previously, we reported that a subnormal PV prior to pregnancy predisposes former PS patients to rPS in their next pregnancy [17]. Subnormal PV is a suitable surrogate for reduced venous capacitance and is accompanied by raised cardiovascular sympathetic activity [18, 23, 33], both independently predisposing not only to rPS [17], but also to premature cardiovascular morbidity [34].

In conclusion, this study supports the concept that former PS patients with a subnormal PV destined to develop an rPS in their next pregnancy differ from their counterparts who do not develop rPS in their next pregnancy by elevated peripheral fibronectin levels together with reduced circulating PAI-2 levels at 16 weeks pregnancy. It follows that the former PS patients may benefit from a 2-step approach consisting of a pre-pregnancy PV measurement followed by a PAI-2 and fibronectin measurement at 16 weeks to determine the risk of developing rPS. Obviously, our findings require confirmation in a much larger population to determine the predictive power of this model before being implemented in clinical practice. Currently, no suitable predictive tests are available to guide the management of former PS patients in future pregnancies. Thus, exploring novel strategies based on plausible biological molecules involved in the pathogenesis of PS women, could offer new perspectives to guide the management of these high-risk pregnancies.

Acknowledgments

The authors want to acknowledge Timo Ekhart, Departments of Obstetrics and Gynecology, Maastricht University Medical Centre, Maastricht, The Netherlands, for the collection and entry of the data.

Disclosure Statement

The authors report no conflict of interest. There was no external funding of this study.

References

- 1 Abalos E, et al: Global and regional estimates of preeclampsia and eclampsia: a systematic review. *Eur J Obstet Gynecol Reprod Biol* 2013;170:1–7.
- 2 Sep SJ, et al: Simple prepregnant prediction rule for recurrent early-onset hypertensive disease in pregnancy. *Reprod Sci* 2009;16:80–87.
- 3 Lykke JA, Paidas MJ, Langhoff-Roos J: Recurring complications in second pregnancy. *Obstet Gynecol* 2009;113:1217–1224.

- 4 Roberts JM, Hubel CA: The two stage model of preeclampsia: variations on the theme. *Placenta* 2009;30(suppl A):S32–S37.
- 5 Formigli L, et al: Aponecrosis: morphological and biochemical exploration of a syncretic process of cell death sharing apoptosis and necrosis. *J Cell Physiol* 2000;182:41–49.
- 6 Gyselaers W, et al: Role of dysfunctional maternal venous hemodynamics in the pathophysiology of pre-eclampsia: a review. *Ultrasound Obstet Gynecol* 2011;38:123–129.
- 7 Avagliano L, et al: Abnormal spiral artery remodelling in the decidual segment during pregnancy: from histology to clinical correlation. *J Clin Pathol* 2011;64:1064–1068.
- 8 Brosens I, et al: The 'great obstetrical syndromes' are associated with disorders of deep placentation. *Am J Obstet Gynecol* 2011;204:193–201.
- 9 Mosesson MW: Fibrinogen and fibrin structure and functions. *J Thromb Haemost* 2005;3:1894–1904.
- 10 Blum A, et al: Endothelial dysfunction in preeclampsia and eclampsia: current etiology and future non-invasive assessment. *Isr Med Assoc J* 2003;5:724–726.
- 11 Medcalf RL, Stasinopoulos SJ: The undecided serpin. The ins and outs of plasminogen activator inhibitor type 2. *FEBS J* 2005;272:4858–4867.
- 12 The Netherlands Perinatal Registry. [Homepage on the internet], March 2008. <http://www.perinatreg.nl/>.
- 13 Bodova KB, et al: Fibronectin, plasminogen activator inhibitor type 1 (PAI-1) and uterine artery Doppler velocimetry as markers of preeclampsia. *Dis Markers* 2011;30:191–196.
- 14 Chappell LC, et al: A longitudinal study of biochemical variables in women at risk of preeclampsia. *Am J Obstet Gynecol* 2002;187:127–136.
- 15 Dane C, et al: Maternal plasma fibronectin and advanced oxidative protein products for the prediction of preeclampsia in high risk pregnancies: a prospective cohort study. *Fetal Diagn Ther* 2009;26:189–194.
- 16 Paarlberg KM, et al: Total plasma fibronectin as a marker of pregnancy-induced hypertensive disorders: a longitudinal study. *Obstet Gynecol* 1998;91:383–388.
- 17 Aardenburg R, et al: Low plasma volume following pregnancy complicated by pre-eclampsia predisposes for hypertensive disease in a next pregnancy. *BJOG* 2003;110:1001–1006.
- 18 Aardenburg R, et al: A subnormal plasma volume in formerly preeclamptic women is associated with a low venous capacitance. *J Soc Gynecol Investig* 2005;12:107–111.
- 19 Ray JG, et al: Cardiovascular health after maternal placental syndromes (CHAMPS): population-based retrospective cohort study. *Lancet* 2005;366:1797–1803.
- 20 Yannoutsos A, et al: Pathophysiology of hypertension: interactions between macro and microvascular alterations through endothelial dysfunction. *J Hypertens* 2014;32:216–224.
- 21 Report of the national high blood pressure education program working group on high blood pressure in pregnancy. *Am J Obstet Gynecol* 2000;183:S1–S22.
- 22 The Netherlands Perinatal Registry. <http://www.perinatreg.nl/> (cited September 2009).
- 23 Spaanderman ME, et al: Latent hemodynamic abnormalities in symptom-free women with a history of preeclampsia. *Am J Obstet Gynecol* 2000;182(1 pt 1):101–107.
- 24 Spaan JJ, et al: Metabolic syndrome as a risk factor for hypertension after preeclampsia. *Obstet Gynecol* 2012;120(2 pt 1):311–317.
- 25 Spaanderman ME, et al: Subclinical signs of vascular damage relate to enhanced platelet responsiveness among nonpregnant formerly preeclamptic women. *Am J Obstet Gynecol* 2006;194:855–860.
- 26 Celermajer DS, et al: Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *Lancet* 1992;340:1111–1115.
- 27 Aydin T, Varol FG, Sayin NC: Third trimester maternal plasma total fibronectin levels in pregnancy-induced hypertension: results of a tertiary center. *Clin Appl Thromb Hemost* 2006;12:33–39.
- 28 Madazli R, et al: Prediction of preeclampsia with maternal mid-trimester placental growth factor, activin A, fibronectin and uterine artery Doppler velocimetry. *Int J Gynaecol Obstet* 2005;89:251–257.
- 29 Rasanen J, et al: Comprehensive maternal serum proteomic profiles of preclinical and clinical preeclampsia. *J Proteome Res* 2010;9:4274–4281.
- 30 Akolekar R, et al: Maternal plasma plasminogen activator inhibitor-2 at 11 to 13 weeks of gestation in hypertensive disorders of pregnancy. *Hypertens Pregnancy* 2011;30:194–202.
- 31 Hunt BJ, et al: Pregnancy outcome and fibrinolytic, endothelial and coagulation markers in women undergoing uterine artery Doppler screening at 23 weeks. *J Thromb Haemost* 2009;7:955–961.
- 32 Parra M, et al: Screening test for preeclampsia through assessment of uteroplacental blood flow and biochemical markers of oxidative stress and endothelial dysfunction. *Am J Obstet Gynecol* 2005;193:1486–1491.
- 33 Spaanderman ME, et al: The effect of pregnancy on the compliance of large arteries and veins in healthy parous control subjects and women with a history of preeclampsia. *Am J Obstet Gynecol* 2000;183:1278–1286.
- 34 Scholten RR, et al: Co-occurrence of cardiovascular and prothrombotic risk factors in women with a history of preeclampsia. *Obstet Gynecol* 2013;121:97–105.