Circulating Platelet-derived and Placenta-derived Microparticles Expose Flt-1 in Preeclampsia

Christine A. R. Lok, MD, PhD, Anita N. Böing, Ing, Ian L. Sargent, PhD, Suren R. Sooranna, PhD, Joris A. M. van der Post, MD, PhD, Rienk Nieuwland, PhD, and Augueste Sturk, PhD

Background. *Flt-1 is secreted by various cells and elevated concentrations are present in preeclampsia affecting vascular function. Microparticles from these cells may expose Flt-1. We evaluated whether Flt-1 is microparticle-associated in preeclampsia, and established the origin of Flt-1-exposing microparticles.* ***Methods.*** *The concentration of Flt-1 was measured in samples from preeclamptic patients, pregnant and nonpregnant women by enzyme-linked immunosorbent assay. Microparticles were analyzed by flow cytometry. Western blot determined the different forms of Flt-1.* ***Results.*** *Noncell bound Flt-1 was elevated in preeclampsia compared to controls. A fraction (5%) was associated with microparticles in preeclampsia. Flt-1-exposing microparticles were increased in preeclampsia compared to normotensive pregnancy ($p = 0.02$). Full-length Flt-1, was identified in microparticles of platelet and placental origin.* ***Conclusion.*** *Full-length Flt-1 is associated with platelet and placenta-derived microparticles. Possibly, the presentation of Flt-1 on the membrane of a microparticle might alter its function, particularly if it acts in synergism with other exposed vasoactive molecules.*

Key words: Flt-1, microparticles, preeclampsia, sFlt-1, VEGF.

INTRODUCTION

To accommodate the vascular challenges of pregnancy, many growth factors are produced, including vascular endothelial growth factor (VEGF). VEGF is produced by several organs including the placenta, and plays an important role in angiogenesis, regulation of vascular tone and blood pressure. To exert these functions, VEGF binds to one of its transmembrane receptors, VEGFR-1 [fms-like tyrosine kinase 1 (Flt-1)] or VEGFR-2 [kinase-insert domain region (KDR)] which are exposed by endothelial cells and other cells. The biological activity of VEGF is—at least in part—regulated by binding to a circulating, noncell bound (soluble) form of the Flt-1 receptor (sVEGFR-1 or sFlt-1).[1] sFlt-1 is an alternatively spliced product of the *Flt-1* gene, which is released by various cell types into the blood and elevated concentrations have been reported in placental tissue and in maternal serum in preeclampsia.[2] Furthermore, this noncell bound Flt-1 has been reported to be elevated in plasma already before the onset of preeclampsia.[3]

Trophoblast cells expose Flt-1 and release noncell bound Flt-1. These cells also release small vesicles, so-called (placenta-derived) microparticles (MPs) from their outer membrane into the circulation. Concurrently, MPs are also released from maternal leukocytes, endothelial cells, and platelets. MPs have been suggested to play a role in the development of preeclampsia because they impair endothelial

From the Department of Obstetrics and Gynaecology, Academic Medical Center, Amsterdam, Netherlands (CARL, JAMV); Laboratory of Experimental Clinical Chemistry, Department of Clinical Chemistry, Academic Medical Center, Amsterdam, Netherlands (ANB, RN, AS); Nuffield Department of Obstetrics and Gynaecology, University of Oxford, The Women's Centre, John Radcliffe Hospital, Oxford, United Kingdom (ILS); and Department of Maternal Fetal Medicine, Imperial College School of Medicine, Chelsea and Westminster Hospital, London, United Kingdom (SRS).

Address correspondence to: Christine A. R. Lok, MD, PhD, Department of Obstetrics and Gynaecology, H4-Zuid, Academic Medical Center, Meibergdreef 9, Postbus 22660, 1100 DD Amsterdam, Netherlands. E-mail: c.a.lok@amc.uva.nl.

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function in vitro.[4,5] In preeclampsia, both noncell bound Flt-1 as well as the numbers of placenta-derived MPs are increased compared to normal pregnancy.[2,6] Because the concentrations of noncell bound Flt-1 are usually measured in cell-free plasma which contains MP, it is unclear whether the Flt-1 that is detected is (partly) associated with and/or exposed on these circulating MPs.

The aims of this study were to (1) determine whether noncell bound Flt-1 is associated with MP in plasma of patients with preeclampsia, (2) evaluate whether the numbers of Flt-1-exposing MPs are increased in preeclampsia compared to normal pregnancy, (3) establish the cellular origin of Flt-1-exposing MPs, and (4) determine which forms of noncell bound Flt-1 (full-length or truncated Flt-1) are associated with circulating MPs.

MATERIALS AND METHODS

Patients

The study was approved by the medical ethical committee of the Academic Medical Center. After obtaining written informed consent, blood samples were obtained from preeclamptic patients (n = 20), normotensive pregnant women (n = 20), and nonpregnant controls (n = 20). The women were matched for maternal age (± 5 years) and parity. The preeclamptic patients and normotensive pregnant women were also matched for gestational age (± 2 weeks) at the time of blood sampling. Preeclampsia was defined as: (1) diastolic blood pressure of 110 mm Hg or more on any occasion or 90 mm Hg or more on 2 separate occasions at least 2 hours apart, (2) proteinuria of ≥0.3 g protein per 24 hours, and (3) signs developing after 20 weeks gestational age and values returning to normal within 3 months after delivery. The control groups consisted of healthy women not using any medication.

Collection of Blood Samples

A total of 2 blood samples (9 mL) were taken from the antecubital vein without a tourniquet through a 20-gauge needle using a vacutainer system. The samples were collected into two 4.5-mL tubes containing 0.105 M buffered sodium citrate (Becton Dickinson, San Jose, Calif). Within 30 minutes after collection, cells were removed by centrifugation for 20 minutes at 1560*g* and 20°C. Plasma samples were then divided in 250 μL aliquots, immediately snap frozen in liquid nitrogen to preserve MP structure and then stored at −80°C until further analysis.

Enzyme-linked Immunosorbent Assay

The concentration of both $VEGF_{165}$ and (noncell bound) sFlt-1 in MP-containing plasma and in MP-free plasma (supernatant obtained after centrifugation of the MP-containing plasma for 30 minutes at 18 890*g*) were determined by enzyme-linked immunosorbent assay (ELISA) according to the protocol of the manufacturer (Quantikine, R&D Systems; Abingdon, UK) in 10 patients from each group. The intraassay variation of the VEGF assay was 4.5% to 6.7%, the interassay variation 6.2% to 8.8%, and the minimal detectable level 5.0 pg/mL. The intraassay variation coefficient of the sFlt-1 assay was 2.6% to 3.8% and the interassay variation 7.0% to 8.1%. The sensitivity of the assay was 1.63 to 14.4 pg/mL.

Cell-specific Antibodies

Fluorescein isothiocyanate (FITC)-labeled IgG_1 and phycoerythrin (PE)-labeled IgG_1 and anti-CD8-PE were obtained from Becton Dickinson (San Jose, Calif). Allophycocyanin (APC)-conjugated annexin V was purchased from Caltag (Burlingame, Calif). Monoclonal antibodies directed against endothelial cells (CD62e-FITC) and (s)Flt-1 (Flt-1-PE) were obtained from R&D (Minneapolis, Minn) and CD62e-PE from Ancell (Bayport, Minn). Anti-CD61-FITC (anti-GP-IIIa) and antiglycophorin A-FITC were obtained from DakoCytomation (Glostrup, Denmark). Anti-CD4-FITC was purchased from Sanquin (Amsterdam, the Netherlands). Anti-CD66b was obtained from Immunotech (Beckman Coulter; Mijdrecht, the Netherlands). ED822, a mouse monoclonal antibody to an unknown antigen expressed on the apical surface of the syncytiotrophoblast was used to detect trophoblastic cells.[7,8] The second antibody for the indirect labeling with ED822, GAM(Fab)2-FITC was obtained from Dako. The following final dilutions of antibodies were used: IgG1-FITC (1:10), IgG1-PE (1:10), annexin V-APC (1:20), anti-CD4-FITC (1:2.5), anti-CD8-PE (1:5), anti-CD61-FITC (1:30), anti-CD62e-PE (1:20), anti-CD62e-FITC (1:5), anti-CD66b (1:20), anti-Flt-1-PE (1:15), anti-GlycoA-FITC (1:5), ED822 (1:2.5), and GAM(Fab)2-FITC (1:20).

Isolation of MPs

A sample of 250 μL frozen plasma was thawed on ice and centrifuged for 30 minutes at 18 890*g* and 20°C to pellet the MP. After centrifugation, 225 μL of the supernatant was removed. The MP pellet and remaining supernatant was resuspended in 225 μL phosphate-buffered saline (PBS) with citrate (154 mmol/L NaCl, 1.4 mmol/L phosphate, 10.9 mmol/L trisodium citrate, pH 7.4). After centrifugation for 30 minutes at 18 890*g* and 20°C, 225 μL of the supernatant was removed again. The MP pellet was then resuspended in 75 μL PBS-citrate.

Flow Cytometry

A total of 5 μL of the MP suspension was diluted in 35 μL CaCl2 (2.5 mmol/L)-containing PBS. Then, 5 μL APC-labeled annexin V was added to all tubes plus 5 μL of the cell-specific monoclonal antibody or isotype-matched control antibodies. The samples were then incubated in the dark for 15 minutes at room temperature. After incubation, 900 μL of calcium-containing PBS was added to all tubes (except to the annexin V control, to which 900 μL citrate-containing PBS was added). Samples were analyzed for 1 minute in a flow cytometer (FACS Calibur) with CellQuest software (Becton Dickinson). Both forward scatter (FSC) and sideward scatter (SSC) were set on logarithmic gain. MPs were identified on the basis of their size and density and on their capacity to bind annexin V. Annexin V measurements were corrected for autofluorescence. Labeling with cell-specific monoclonal antibodies was corrected for identical concentrations of isotype-matched control antibodies. Double labeling of anti-CD4-FITC, anti-CD8-PE, anti-CD61-FITC, anti-CD62e-FITC, anti-CD66b and anti-GlycoA-FITC with anti-Flt-1-PE were performed to investigate the origin of the Flt-1-exposing MPs. Calculation of the number of MPs per liter plasma was based upon the particle count per unit time, the flow rate of the flow cytometer, and the net dilution during sample preparation of the analyzed MP suspension.

Double Labeling of ED822 and Flt-1

Samples (n = 13) with high numbers of Flt-1-exposing MPs were selected to investigate whether they originated from trophoblast cells, using an indirect staining procedure. Samples with low numbers of Flt-1-exposing MPs were not investigated, because the losses due to the 2 washing steps, which were necessary to enable indirect staining of MPs, result in very low MP numbers for analysis. MPs (5 μL aliquots) were incubated for 15 minutes at room temperature in a final volume of 50 μL of PBS containing 2.5 mmol/L CaCl2 (PBS/Ca, pH 7.4) and unlabeled ED822, anti-Flt-1-PE and annexin V-APC. After incubation with the antibody, the MPs were washed with 200 μL of PBS/Ca. Then, 5 μL GAM(Fab)2-FITC was added, and the mixtures were again incubated for 15 minutes at room temperature. Subsequently, 300 μL of buffer was added and the MPs were analyzed by flow cytometry.

Western Blotting

Because the ELISA does not discriminate between full-length Flt-1 and alternatively spliced Flt-1, the elevated plasma levels of noncell bound Flt-1 in preeclampsia may be either ''truly soluble'' Flt-1 or transmembrane Flt-1 associated with MPs. These forms can be discriminated by Western blot analysis.

For Western blotting, the total MP population was isolated from 750 μL plasma by centrifugation for 1 hour at 18 890*g*. Because of the higher volume of this plasma sample, 1 hour of centrifugation was necessary to pellet all MP. Then, 725 μL MP-free plasma (supernatant) was removed and stored. The remaining 25 μL plasma containing the MP was resuspended in 725 μL PBS and then centrifuged again for 1 hour at 18 890*g* and 20°C. Then, 740 μL of the supernatant was removed. The MP pellet was dissolved in reducing sample buffer (final volume 25 μL) and 6 μL MP-free plasma was diluted with 210 μL PBS. The diluted MP-free plasma was further diluted 2-fold with reducing sample buffer (final dilution 72 times, which is the same concentration as the residual plasma in the MP samples). The samples (10 μL) were transferred to gradient gels (4%-15%) and blotted to polyvinylidene fluoride (PVDF; both from BioRad; Hercules, Calif). Membranes were blocked with 5% bovine serum albumin (BSA) (Sigma Aldrich; Zwijndrecht, the Netherlands) for 1 hour. Western blots were labeled with an antibody recognizing full-length Flt-1 as well as its truncated, noncell bound form, in 2.5 % BSA (Abcam; Cambridge, United Kingdom). For staining, goat antirabbit peroxidase-labeled was used (Dako). For detection, a Lumi-light plus conjugate was used (Roche; Indianapolis, Ind). A lysate from interleukin-1alpha (IL-1α) stimulated human umbilical vein endothelial cells (HUVECs, 1 hour, 5 ng/mL) was used as positive control.

Table 1. Patient Characteristics

	Preeclampsia (n = 20)	Normotensive Pregnancy (n = 20)	Nonpregnant Controls (n = 20)	P^a	P^b
Age (years)	30.0 ± 4.9	29.2 ± 3.6	29.9 ± 3.6	NS	NS
Gestational age					
At study (weeks)	29.4 ± 2.2	30.3 ± 3.0	–	NS	–
At delivery (weeks)	31.9 ± 3.1	39.1 ± 1.8	–	.0001	–
Blood pressure					
Systolic (mm Hg)	159 ± 15	114 ± 13	113 ± 15	.0001	.0001
Diastolic (mm Hg)	99 ± 9	67 ± 9	70 ± 9	.0001	.0001
BMI (kg/m^2)	27.1 ± 6.1	23.7 ± 4.9	21.9 ± 2.1	NS	NS
Proteinuria (g/L)	6.1 ± 4.7	–	–	–	–
Parity					
Primiparous	18	18	–	–	–
Multiparous	2	2	–	–	–
Birth weight (grams)	1324 ± 566	3351 ± 585	–	.0001	–

Abbreviations: BMI, body mass index; NS, nonsignificant.
[a] Difference between preeclampsia and normotensive pregnant controls.
[b] Difference between preeclampsia and nonpregnant controls.

Statistical Analysis

Data were analyzed with the Statistical Package of the Social Science software for Windows, release 11.5 (SPSS Benelux BV; Gorinchem, the Netherlands). The demographic characteristics of patients were normally distributed and therefore presented as means ± SD and compared with analysis of variance (ANOVA) and Bonferroni post hoc tests. The data from the flow cytometric analysis and ELISA were not normally distributed and therefore presented as medians and ranges and analyzed with Kruskal-Wallis Tests for differences among the 3 study groups and Mann-Whitney *U* tests for comparisons between the preeclampsia and normotensive pregnancy group or the nonpregnant controls. If the concentration of a sample was below the detection limit of the ELISA assay, calculations were performed with the lowest measurable concentration. A probability value of less than .05 was considered statistically significant. Correlations were calculated with a 2-sided bivariate Pearson correlation test.

RESULTS

Patient Characteristics

Patient characteristics are summarized in Table 1. Age and body mass index (BMI) were comparable between groups. As expected, birth weight and gestational age at delivery were significantly lower in the preeclamptic women compared to normotensive pregnant women. Both systolic and diastolic blood pressures were significantly higher in preeclampsia compared to the control groups. The majority of women were primiparous in both the preeclamptic and normotensive pregnant groups. In the preeclamptic group, 12 patients used antihypertensive medication (60%) and 5 patients (25%) suffered from hemolysis, elevated liver enzymes, low platelets (HELLP) syndrome besides preeclampsia.

Plasma Concentrations of VEGF and sFlt-1

The plasma concentrations of VEGF were below the detection limit in all preeclamptic patients (<7.8 pg/mL; Figure 1A). The concentration of VEGF was 8.1 pg/mL (range 7.8-44.5 pg/mL) in normotensive pregnant patients and 16.7 pg/mL (range 11.1-24.4 pg/mL) in nonpregnant women. In contrast to VEGF, the concentration of noncell bound Flt-1 was significantly elevated in preeclampsia (Figure 1B; 2288 pg/mL, range 1106-4763 pg/mL) compared to normotensive pregnant women (227 pg/mL, range 153-369 pg/mL) and nonpregnant women (31.2 pg/mL, range 31.2-65.0 pg/mL).

When the plasma was centrifuged to remove MP, about 95% of noncell bound Flt-1 remained in the MP-free supernatant from preeclamptic patients. Thus, about 5% (range 0%-13%) of noncell bound Flt-1 was associated with MPs in this group.

Circulating MPs Expose Noncell Bound Flt-1

Total numbers of MPs were decreased in preeclampsia compared to both normotensive pregnant women and

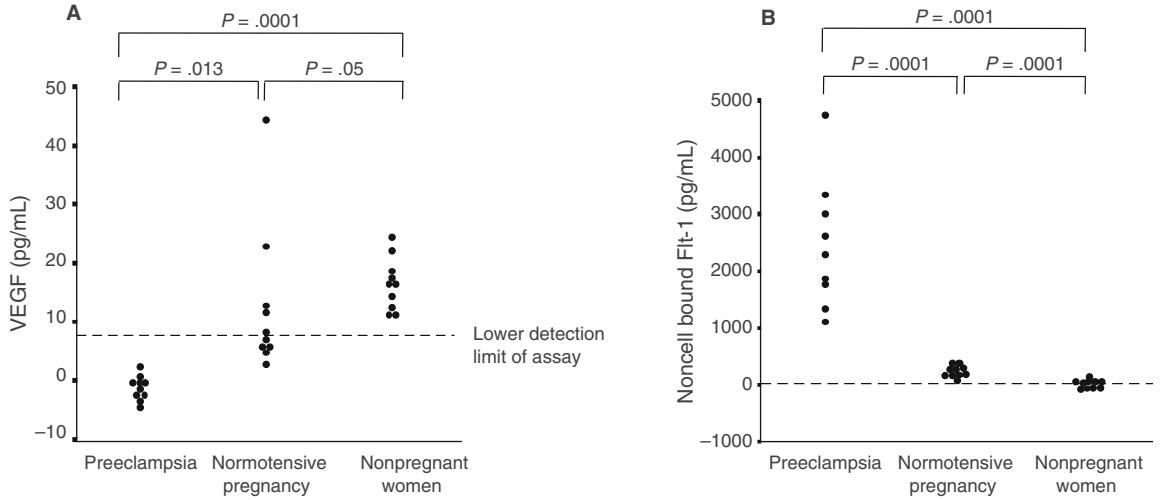

Figure 1. Concentration of VEGF and noncell bound Flt-1 in plasma. The concentrations of (A) VEGF and (B) noncell bound Flt-1 are shown. Each dot represents 1 patient. The dotted lines represent the lower detection limit of these assays. Flt-1 = fms-like tyrosine kinase 1; VEGF = vascular endothelial growth factor.

nonpregnant controls (Figure 2A). There were no correlations between the numbers of MPs and either blood pressure, proteinuria, or birth weight. The majority of the MPs (94%-98%) originated from platelets in all groups (data not shown). The number of endothelial cell–derived MPs did not differ between groups (Figure 2B). In contrast, the numbers of MPs exposing Flt-1 were elevated in preeclamptic patients compared to normotensive pregnant women (Figure 2C; $P = .02$). Moreover, the fraction of Flt-1-exposing MP (Figure 2D) was elevated in preeclampsia (1.36%, range 0%-16%) compared to normotensive pregnancy (0.23%, range 0%-40%; $P = .001$) and nonpregnant controls (median 0.30%, range 0%-4%; $P = .0001$). There was no difference in the number of MP between preeclamptic patients with or without HELLP syndrome or between patients using antihypertensive medication and patients not using medication.

Further Characterization of MP-associated (Noncell Bound) Flt-1

Figure 3A shows placenta-derived MPs exposing Flt-1. In Figure 3B, a representative Western blot of MP fractions and MP-free plasma is presented. A total of 2 different concentrations of HUVEC lysates were used as positive controls, showing a single 150 kd (full-length) form of Flt-1. Lysates from isolated MP fractions and the corresponding MP-free plasma samples of 2 preeclamptic patients and 2 pregnant controls are presented. All MP lysates contained detectable amounts of 150-kd Flt-1, indicating that MPs expose the transmembrane form of Flt-1. Because the corresponding (MP-free) plasma samples did not contain a full-length form, this 150-kd Flt-1 is exclusively associated with circulating MPs. These plasma samples contained several proteins with lower molecular weights staining for Flt-1, including a protein of 110 kd, which theoretically could be noncell bound Flt-1. Because proteins of similar molecular weight also stained in plasma samples of nonpregnant women, however, aspecific binding could not be excluded.

Cellular Origin of MPs Exposing Noncell Bound Flt-1

A clearly detectable population of circulating Flt-1-exposing MPs was present in plasma samples from 13 women (9 patients with preeclampsia and 4 women with a normotensive pregnancy). To establish the cellular origin of these circulating Flt-1-exposing MPs, double labeling experiments were performed using a panel of various cell-specific antibodies. Microparticles originating from erythrocytes, T-helper cells, T-suppressor cells, granulocytes, or endothelial cells did not stain for Flt-1. The majority of the Flt-1-exposing MPs (77%-86%) originated from platelets (Figure 4A). The other Flt-1 exposing MPs (approximately 19% in the preeclamptic patients) double stained with ED822, confirming their placental origin (Figure 4B). The median number of the ED822-exposing MP in preeclamptic patients was

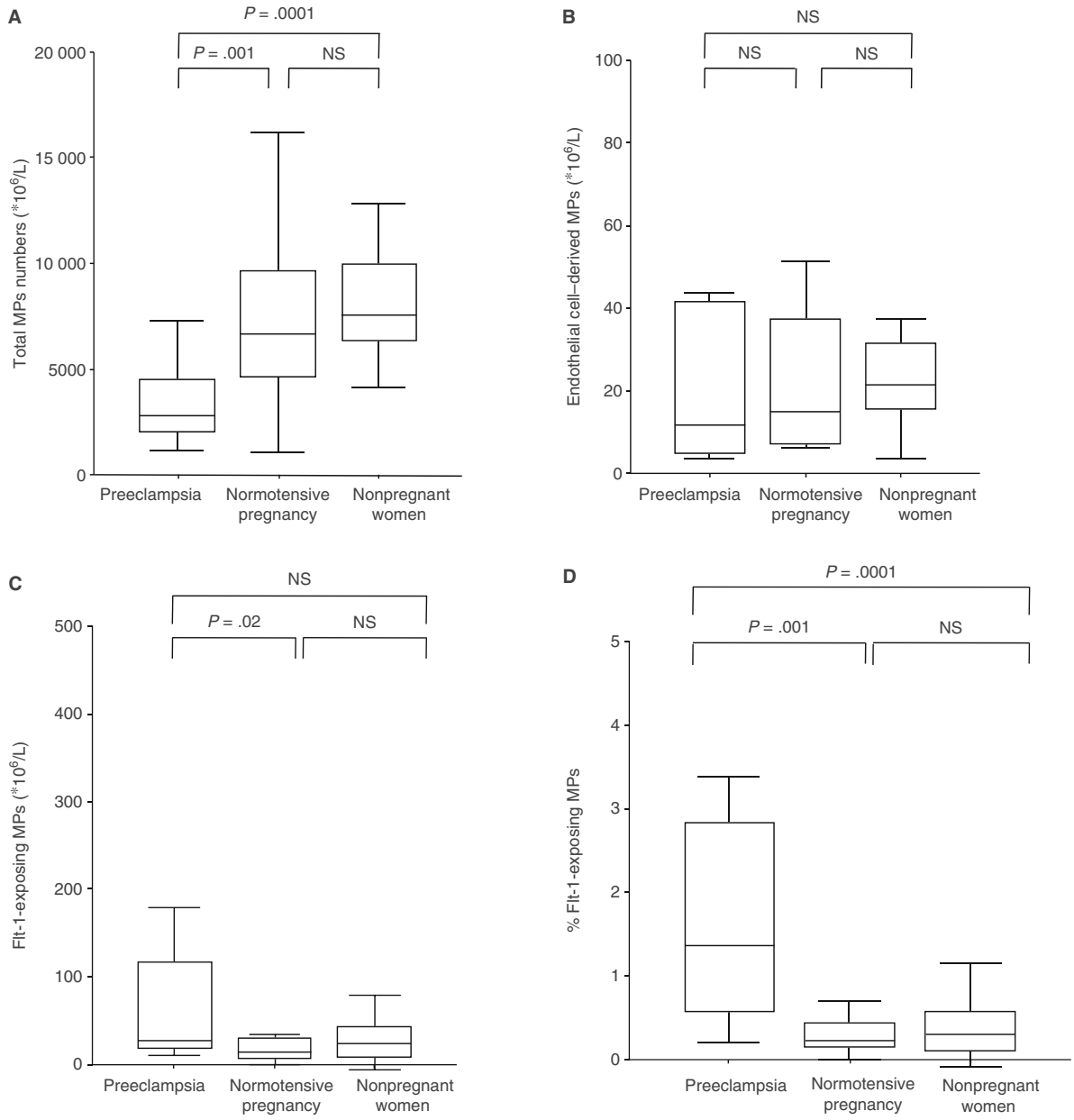

Figure 2. Circulating MPs in preeclampsia. This figure shows box plots of the number of (A) total MPs, (B) endothelial cell–derived MPs, (C) Flt-1-exposing MPs, and (D) the proportion of Flt-1-exposing MPs. Flt-1 = fms-like tyrosine kinase 1; MP = microparticles.

49×10^3 (range 17×10^3-243×10^3) which was about 4.8% of the total number of MPs.

Although the majority of the Flt-1-exposing MPs was derived from platelets, this was only a minor fraction of all platelet-derived MPs (PMP). Nevertheless, this fraction was elevated in preeclampsia (0.7%, range 0.20%-1.60%) compared to normotensive pregnant controls (0.3%, range 0.1%-0.6%; $P = .01$) but not to nonpregnant controls (0.6%, range 0.1%-3.4%; $P > .05$). There was no correlation between the number of PMP and the number of Flt-1-exposing MP. In contrast, a strong correlation was present between the total number of ED822-exposing MP and Flt-1-exposing MP ($r = .744$, $P = .009$).

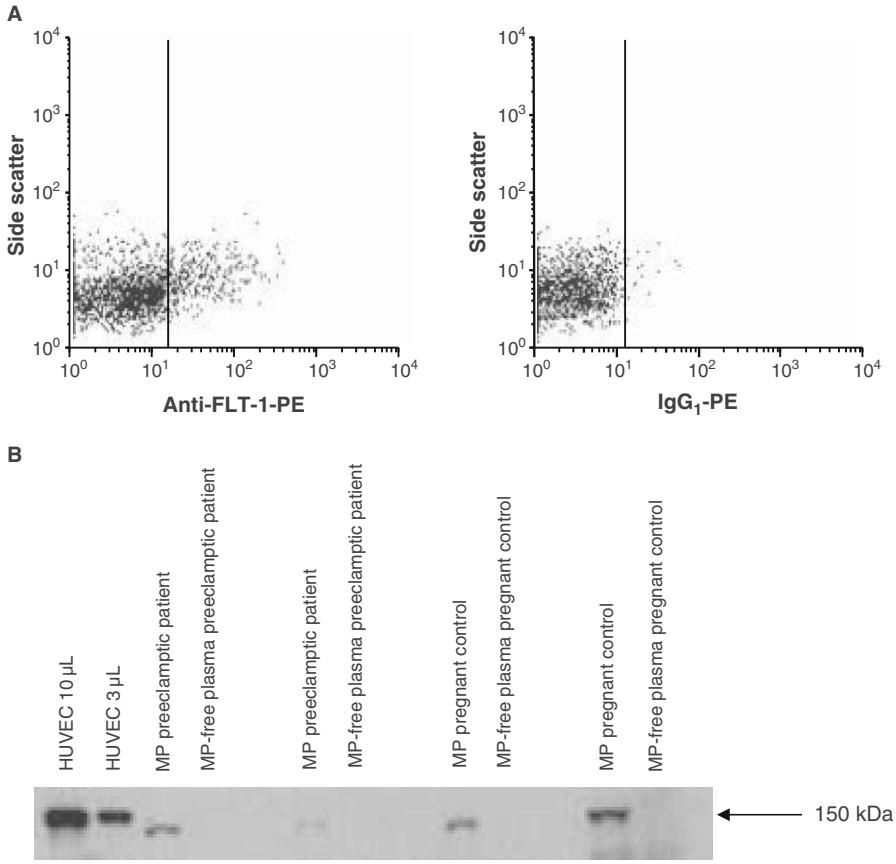

Figure 3. Flt-1 is associated with circulating MP. A, Dot plots of a representative experiment showing the staining of MP isolated from preeclamptic plasma. As outlined in the Materials and Methods section, MPs were identified based on FSC, SSC, and binding of annexin V. MPs were double stained with either anti-Flt-1 (left) or its isotype (IgG1) control antibody (right). Based on control antibody binding, a fluorescence threshold was set on the X axis to be able to identify the Flt-1-exposing MPs, that is those MPs on the right side of the fluorescence threshold. B, Western blot showing lysates of MPs and corresponding MP-free plasma. The MP-free plasma samples had a similar (diluted) quantity of remnant plasma as present in the corresponding MP lysates (see Materials and Methods) of 2 normotensive pregnant and 2 preeclamptic patients. MPs lysates contain a 150-kd band corresponding full-length Flt-1. HUVEC: A culture supernatant of HUVEC used as a positive control for the Flt-1 analyses. Flt-1 = fms-like tyrosine kinase 1; FSC = forward scatter; HUVEC = Human umbilical vein endothelial cell; IgG = immunoglobulin G; MP = microparticle; PE = phycoerythrin; SSC = sideward scatter.

DISCUSSION

In this study, we confirmed that the concentration of noncell bound Flt-1 is elevated in preeclampsia compared to normotensive pregnancies and nonpregnant controls. A fraction of noncell bound Flt-1 is associated with MPs. Thus, at least 2 different forms of noncell bound Flt-1 concurrently occur in plasma of preeclamptic patients. First, a major fraction of (truly) ''soluble'' Flt-1, and second a minor fraction of full-length Flt-1 which is exclusively associated with MPs.

Total numbers of MPs were decreased in preeclampsia probably reflecting the decreased platelet count in preeclampsia.[9] Our present study shows that plasma from preeclamptic patients contains substantial numbers of platelet-derived and placenta-derived MPs exposing Flt-1. It is tempting to speculate that the increased presence of such MPs contributes to the reported effects of MPs on the endothelium. Various in vitro models of angiogenesis have shown that serum of pregnant women induced endothelial cells to form tube-like structures whereas serum from preeclamptic patients inhibited this formation.[1] Moreover, addition of noncell bound Flt-1 to normal serum also inhibited this tube formation. This inhibitory action could be overcome by the addition of VEGF and placental growth factor. In fact, administration of noncell bound Flt-1 to rats mimics several of the clinical symptoms of preeclampsia, including hypertension and glomerular endotheliosis.[1] In an animal model, MPs also induced various features characteristic

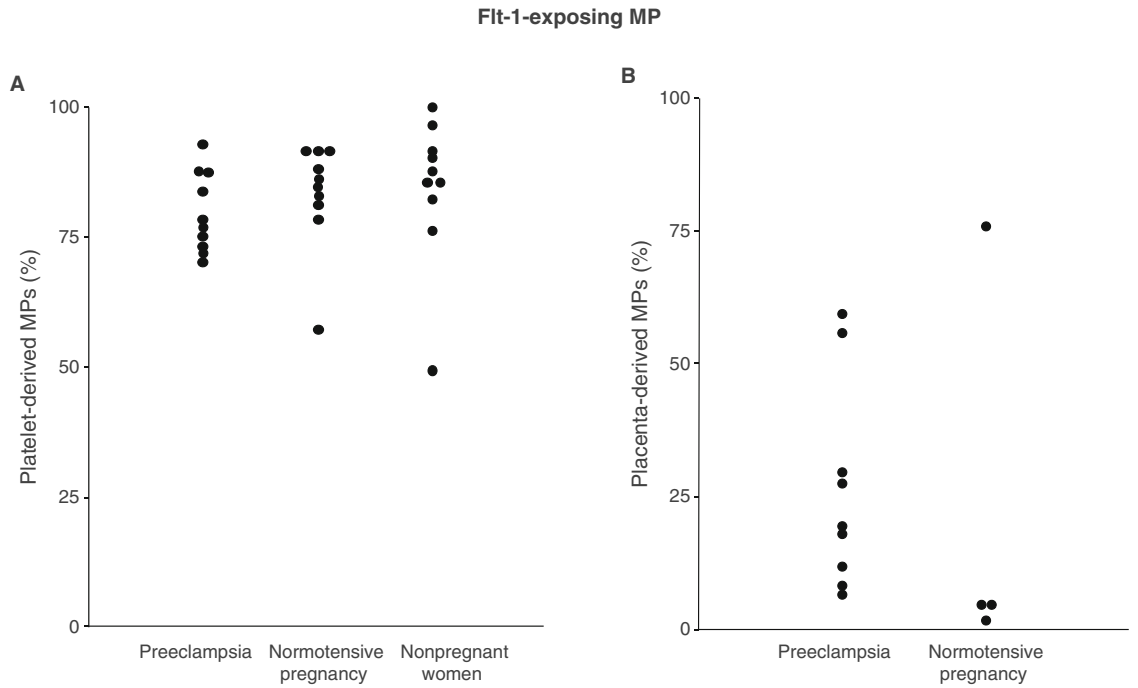

Figure 4. The cellular origin of Flt-1-exposing MPs. To establish the cellular origin of Flt-exposing MPs, we double stained Flt-1-exposing MP with antibodies directed against either platelet (A) or trophoblast cell (B) antigens. Each dot represents the percentage of Flt-1-exposing MP from platelets (A) or placenta (B) from each individual patient. These results include one preeclamptic patient with HELLP syndrome. Flt-1 = fms-like tyrosine kinase 1; HELLP = hemolysis, elevated liver enzymes, low platelets; MP = microparticles.

of preeclampsia,[10] and incubation of myometrial arteries with PMPs from preeclamptic patients impaired bradykinin-mediated relaxation.[11,12]

We found that the majority of Flt-1-exposing PMPs originated from platelets. This is not surprising, because PMPs compose the largest subgroup of MPs in both pregnant women and preeclamptic patients. Moreover, Flt-1 has been shown to be exposed on activated platelets enabling a feed forward of VEGF on thrombin-activated platelets.[13] The remaining Flt-1-exposing MPs originated from placenta. Despite the fact that these MPs constitute only a relatively small fraction of the total number of circulating MPs in the maternal blood, still significantly elevated numbers are present in plasma from preeclamptic women compared to normal pregnancy.[6,13] One may argue that these differences are the result of intrauterine growth retardation accompanying the preeclampsia. However, we did not find a correlation between birth weight and Flt-1-exposing MPs in any of the groups. Some of our patients developed HELLP syndrome. Future research might aim at looking for differences in patients with only preeclampsia and not HELLP as suggestions are made that this is a separate disease entity. However, preeclampsia is a heterogeneous disease and HELLP syndrome is part of the total spectrum in preeclamptic patients.

Plasma of some patients contained substantial numbers of Flt-1-exposing MPs. This variation between patients can be caused by the time of sample collection. In our measurements, we assume that MPs are continuously shed into the circulation. However, it has been postulated that for example placenta-derived MPs are episodically shed into the circulation, possibly after placental incidents like ischemia.[6] This may also apply to other subpopulations of MPs. It also should be mentioned that MPs isolated from plasma do not reflect the entire in vivo situation, because particular subsets of MPs may already have been removed from the circulation by either clearance or adherence to the (damaged) endothelium or to other cells. Indeed, it has been shown that placenta-derived MPs can be taken up by monocytes.[8] Thus, the true proportion of Flt-1-exposing MPs originating from the placenta may be higher in vivo.

Perfusion of subcutaneous fat arteries with in vitro prepared placenta-derived MPs impaired the relaxation response, suggesting that such MPs are indeed capable of modulating endothelial ''dysfunction.''[5] Taken together, these data may point to a possible effect of MPs

containing Flt-1. This study is the first step to elucidate the role of differences in particular subsets of MPs in preeclampsia. Additional studies in small resistance arteries are essential to demonstrate the functional consequences of these differences.

Nonglycosylated full-length Flt-1 has a molecular weight of 150 kd.[14] The noncell bound (and non-MP associated) Flt-1 molecule lacks the transmembrane and cytoplasmic regions, and has a lower molecular weight. Different molecular weights of the truly soluble Flt-1 have been reported in the literature, depending on the origin and the glycosylation status of the molecule. Our finding of full-length Flt-1 with a molecular weight of 150 kd in the MP fractions is in line with earlier reports.[14] To which extent the biological activity of MP-bound Flt-1 in our study contributes to the pathophysiological development of preeclampsia, however, remains to be determined.

In conclusion, the concentration of noncell bound Flt-1 is significantly elevated in plasma from preeclamptic patients. Different forms of noncell bound Flt-1 coexist in such plasma samples, that is a minor fraction of a full-length (transmembrane) form (150 kd) that is associated with MPs, and a major fraction of truncated Flt-1, which is truly ''soluble.'' The biological activities of the coexisting forms of (s)Flt-1 in preeclampsia are of great interest and remain to be established. It is possible that the presentation of Flt-1 on the membrane of an MP might alter its function, particularly if it acts in synergism with other vasoactive molecules expressed alongside it on the MP.

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