

Circulation

JOURNAL OF THE AMERICAN HEART ASSOCIATION



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Circulation published online August 1, 2011

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75214

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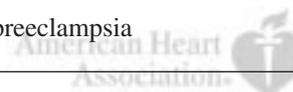
Ravi Thadhani, MD, MPH; Tuelay Kisner, MD*; Henning Hagmann, MD*; Verena Bossung, MD; Stefanie Noack, RN; Wiebke Schaarschmidt, MD; Alexander Jank, MD; Angela Kribs, MD; Oliver A. Cornely, MD; Claudia Kreyssig, MD; Linda Hemphill, MD; Alan C. Rigby, PhD; Santosh Khedkar, PhD; Tom H. Lindner, MD; Peter Mallmann, MD; Holger Stepan, MD; S. Ananth Karumanchi, MD; Thomas Benzing, MD

Background—Targeted therapies to stabilize the clinical manifestations and prolong pregnancy in preeclampsia do not exist. Soluble fms-like tyrosine kinase 1 (sFlt-1), an alternatively spliced variant of the vascular endothelial growth factor receptor 1, induces a preeclampsia-like phenotype in experimental models and circulates at elevated levels in human preeclampsia. Removing sFlt-1 may benefit women with very preterm (<32 weeks) preeclampsia.

Methods and Results—We first show that negatively charged dextran sulfate cellulose columns adsorb sFlt-1 in vitro. In 5 women with very preterm preeclampsia and elevated circulating sFlt-1 levels, we next demonstrate that a single dextran sulfate cellulose apheresis treatment reduces circulating sFlt-1 levels in a dose-dependent fashion. Finally, we performed multiple apheresis treatments in 3 additional women with very preterm (gestational age at admission 28, 30, and 27+4 weeks) preeclampsia and elevated circulating sFlt-1 levels. Dextran sulfate apheresis lowered circulating sFlt-1, reduced proteinuria, and stabilized blood pressure without apparent adverse events to mother and fetus. Pregnancy lasted for 15 and 19 days in women treated twice and 23 days in a woman treated 4 times. In each, there was evidence of fetal growth.

Conclusions—This pilot study supports the hypothesis that extracorporeal apheresis can lower circulating sFlt-1 in very preterm preeclampsia. Further studies are warranted to determine whether this intervention safely and effectively prolongs pregnancy and improves maternal and fetal outcomes in this setting. (*Circulation*. 2011;124:00-00.)

Key Words: angiogenic factor ■ apheresis ■ preeclampsia



Preeclampsia is a devastating medical complication of pregnancy associated with significant maternal and fetal morbidity and mortality.¹ The risk is highest in very preterm (<32 weeks) preeclampsia when the infant mortality rate is 70 times higher than at term.^{2,3} Delivery of the placenta remains the only effective means to treat preeclampsia. Randomized trials have tested nonspecific interventions including antihypertensive agents; however, the ability of these and other interventions to prevent or stabilize the clinical manifestations and prolong pregnancy in preterm preeclampsia is limited.^{4–6} The underlying pathogenesis of preeclampsia has remained elusive, hampering successful development of targeted interventions for the condition.

Clinical Perspective on p ●●●

The prevailing hypothesis suggests that preeclampsia involves a placental factor that circulates to distal organs and causes damage to the vasculature.⁷ We and others have suggested that excess placental derived soluble fms-like tyrosine kinase 1 (sFlt-1) or the soluble vascular endothelial growth factor (VEGF) receptor 1, an alternatively spliced variant of VEGF receptor 1, mediates the signs and symptoms of preeclampsia, and elevated circulating levels are associated with clinical preeclampsia.^{8–11} Circulating sFlt-1 levels in very preterm preeclampsia are among the highest observed.^{12,13} Soluble fms-like tyrosine kinase 1

Received March 29, 2011; accepted June 27, 2011.

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The online-only Data Supplement is available with this article at <http://circ.ahajournals.org/cgi/content/full/CIRCULATIONAHA.111.034793/DC1>.

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Circulation is available at <http://circ.ahajournals.org>

DOI: 10.1161/CIRCULATIONAHA.111.034793

acts by inhibiting local VEGF signaling in target organs including the kidney, brain, and liver where VEGF is constitutively expressed.¹⁴ A preeclampsia-like phenotype after anti-VEGF chemotherapies supports this hypothesis.¹⁵ Although other mediators likely exist,¹⁶ an intervention targeting circulating sFlt-1 may benefit women with very preterm preeclampsia. Because circulating sFlt-1 levels are in excess in preeclampsia, rather than add an agent to target sFlt-1, a potentially attractive approach would be to subtract sFlt-1 from maternal circulation.⁴

Removal of toxic circulating factors in preeclampsia has met with limited success. Women undergoing hemodialysis to remove small molecules through convection and diffusion have elevated rates of preeclampsia.¹⁷ Similarly, plasmapheresis to remove circulating antibodies and immune complexes has not prolonged pregnancy in preterm preeclampsia.¹⁸ Because circulating sFlt-1 represents <20% of the total body sFlt-1 burden,¹⁹ we hypothesized that a selective adsorption column would create a concentration gradient and augment its removal. Herein we characterize the charge of sFlt-1, perform *in vitro* experiments to test commercially available devices that could adsorb circulating sFlt-1, and then describe our early experience in treating women with very preterm preeclampsia with the goal of prolonging pregnancy.

Methods

Biochemical Properties of sFlt-1

To characterize the charge of sFlt-1, the isoelectric point (pI) for sFlt-1 protein (gene bank ID AAC50060.1) was calculated using ExPasy software (http://expasy.org/tools/pi_tool.html). As a comparison, we also calculated the pI for 2 abundant plasma proteins, α -fibrinogen and albumin. Although previous mutagenesis studies suggested that the fourth immunoglobulin-like domain of sFlt-1 contributes to its heparin-binding properties,²⁰ more recent studies have revealed that the third immunoglobulin-like domain may play a more important role.²¹ To examine the spatial structure of the heparin-binding epitopes of sFlt-1, we modeled the third immunoglobulin-like domain of the sFlt-1 protein (amino acids 230 to 327) and calculated the electrostatic surface potential using protein structure prediction software (Prime, www.schrodinger.com).

In Vitro Experiments With Negatively Charged Apheresis Columns

Apheresis columns adsorb circulating proteins on the basis of electrostatic interactions. To determine whether commercially available dextran sulfate cellulose (DSC) columns bind sFlt-1 *in vitro*, we spiked 25 μ g of recombinant sFlt-1 protein (R&D Systems, Minneapolis, MN) into 2 U of discarded human whole blood. In another experiment we used 50 mL of human amniotic fluid, which is known to carry endogenous sFlt-1 isoforms.²² Blood was perfused through a DSC column (DL-75, Kaneka, Osaka, Japan); pre- and postcolumn levels were measured using an automated sFlt-1 assay (inter- and intra-assay coefficients of variation <3%; optimal range 10 to 85 000 pg/mL; Elecsys sFlt-1 Assay, Roche Diagnostics, Germany)¹³; and sFlt-1 reduction ratios ((pre-sFlt-1 - post-sFlt-1)/pre-sFlt-1) were expressed as a percentage. Similar studies also were performed with commercially available heparin-based low-density lipoprotein (LDL) columns (B. Braun, Germany).

Development of Apheresis Protocols for Preeclampsia

We modified existing extracorporeal apheresis protocols to address specific features of preeclampsia.²³⁻²⁶ Preeclampsia is

Table 1. Isoelectric Point of sFlt-1 Protein

Name of Human Protein	Protein Size in Amino Acids	Calculated pI
sFlt-1	687	9.51
Alpha fibrinogen	644	8.09
Albumin	609	5.92

pI indicates isoelectric point.

characterized by intravascular volume depletion.²⁷ Therefore, standard flow rates (80 to 150 mL/min) were lowered to 40 to 60 mL/min to avoid hypotension. Heparin anticoagulation is standard to prevent the columns from clotting. However, heparin has been known to release sFlt-1 into the systemic circulation,²⁸ and heparin-sFlt-1 complexes may interfere with binding to DSC columns. Furthermore, DSC columns transiently lower coagulation factors such as plasma fibrinogen.^{23,25} Preeclampsia can progress to a life-threatening condition within hours, necessitating emergent Caesarean section. To maximize sFlt-1 clearance and minimize the risk for excess bleeding should emergent surgery be necessary, we adapted regional citrate anticoagulation protocols from hemodialysis and LDL apheresis.^{29,30}

We then examined maternal and fetal safety and potential efficacy (lowering circulating sFlt-1 levels) of 1 treatment of extracorporeal DSC apheresis in 5 women meeting the definition of very preterm preeclampsia: presenting at <32 weeks of gestation; systolic or diastolic blood pressure ≥ 140 mm Hg or ≥ 90 mm Hg, respectively, on 2 occasions at least 4 hours apart; proteinuria defined as 24-hour total protein excretion of ≥ 300 mg or $\geq 2+$ on dipstick testing, or a protein-to-creatinine ratio ≥ 0.35 g protein/gram creatinine. All demonstrated circulating sFlt-1 levels at least 2 SDs above the upper limit of normal on the basis of published gestational age references.¹³ Other requirements included the absence of intrauterine growth restriction (defined as <5th centile for gestational age), chronic hypertension or preexisting renal disease, and HELLP (hemolysis, elevated liver enzymes, low platelets) syndrome. We finally refined our protocol (online-only Data Supplement Table I) to standardize the intervention for 3 additional women with very preterm preeclampsia and elevated circulating sFlt-1 levels with the goal to prolong pregnancy for at least 14 days.

The potential risks and benefits of the procedure were explained in detail to each patient, and each provided written informed consent. The study was approved by the Cologne Regional Board (Bezirksregierung) in March of 2010, and 2 Institutional Ethics Committees where the study would take place, Cologne University, Cologne, Germany, in January of 2010 (Project Code 09-258) and University Hospital Leipzig, Leipzig, Germany, in April of 2010 (Project Code 108-10-08032010). The study was also approved by the Partners Human Research Committee for the secondary use of research samples and data analysis (Protocol 2010-P-000487).

Results

Biochemical Properties and Ex Vivo Removal of sFlt-1

Soluble fms-like tyrosine kinase 1 consists of 6 immunoglobulin-like domains, and the second immunoglobulin-like domain makes up its ligand (VEGF and placental growth factor)-binding site.^{31,32} The calculated pI for sFlt-1 protein is 9.51 (Table 1). At physiological pH, therefore, sFlt-1 is positively charged whereas other serum proteins are less positively charged (α -fibrinogen) or negatively charged (albumin). The heparin- and matrix-binding domain of sFlt-1 is largely formed by a stretch of 10 basic amino acids located in the third immunoglobulin-like domain that is distinct from its ligand-binding domain.²¹ The 3-dimensional model of the

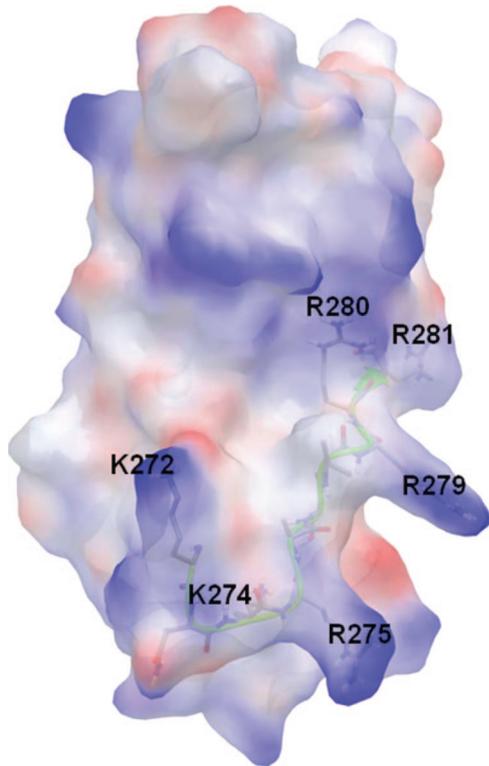


Figure 1. Model of the third immunoglobulin domain of sFlt-1 protein (AAC50060.1). Spatial structure of the heparin-binding epitopes of sFlt-1 is shown using a computer-generated model as described in Methods. A stretch of 10 amino acids from 272 to 281 that constitutes the major heparin-binding domain is highlighted. The positive amino acids (lysine, K, and arginine, R) are labeled. Blue and red region indicate regions of positive and negative charges, respectively.

third immunoglobulin-like loop reveals a patch of basic amino acids around R275 and R279 (Figure 1).

Dextran sulfate, a polyanionic derivative of dextran linking 2 sulfate groups to a single unit of glucose, binds positively charged particles in circulation such as the apolipoprotein B-containing lipoprotein LDL. Low-density lipoprotein apheresis with DSC has been used safely in children and adults with homozygous familial hypercholesterolemia (FH).^{24,33} In pregnant women with FH, treatments have continued without major complications.^{26,34,35} To determine whether negatively charged DSC columns adsorb circulating sFlt-1, we performed ex vivo studies with recombinant sFlt-1 protein spiked into human whole blood. As shown in Table 2, ~80% of the circulating sFlt-1 protein was removed after 2 runs through a DSC column. Because several naturally occurring sFlt-1 isoforms circulate in humans,³⁶ we also used

Table 2. Ex Vivo Removal of Recombinant sFlt-1 Protein by DSC Column

Experiment 1	sFlt-1 in pg/mL	Reduction Ratio, %
Baseline (pre)	4558	
Run 1 (post)*	805.5	82.3
Run 2 (post)*	699.7	84.6

*Each run represents passing of 1 entire blood volume (2 units of discarded human whole blood) through the column.

Table 3. Ex Vivo Removal of Amniotic Fluid sFlt-1 Protein by DSC Column

Experiment With DL75 (Dextran Sulfate)*	sFlt-1 in pg/ml	Reduction Ratio, %
Baseline (pre)	4576	
Run 1 (post)†	2273	50.3
Run 2 (post)†	1611	64.8
Run 3 (post)†	1185	74.1

*Experiment performed as described in Methods with 50 mL of human amniotic fluid spiked into 2 units of discarded human blood.

†Each run represents passing of 1 entire blood volume (2 units of discarded human whole blood) through the column.

amniotic fluid (a rich source of endogenous sFlt-1) in the same experiments and found similar results (Table 3), suggesting that DSC columns would be suitable for sFlt-1 adsorption in vivo.

Single Apheresis Treatment Lowers Circulating sFlt-1

To test whether DSC columns adsorb sFlt-1 in humans, we first performed a single treatment in 5 women and thereafter treated 3 additional women multiple times. Five women with very preterm preeclampsia and elevated circulating sFlt-1 levels underwent a single apheresis treatment (Table 4). During apheresis, the only apparent side effect noted in 3 women was a transient reduction in systolic blood pressure (maximum 20 to 30 mm Hg) within 30 minutes of initiating the procedure. This immediately responded to normal saline infusion, with no woman requiring >0.5 to 1.0 L after this initial episode. No subject experienced bleeding complications, and oxygen saturations remained above 98% throughout. Fetal monitoring was notable for transient (3 to 5 minutes) reduction in fetal heart beats (average 150 to 130/min) concomitant with maternal blood pressure reduction; this also immediately recovered after normal saline infusion. Uterine contractions were not observed, and clinical indications for emergent Caesarean section were absent. After apheresis, plasma fibrinogen levels were reduced by ~20% and returned to baseline levels within 24 hours in all women. Circulating sFlt-1 levels appeared to be lowered in a dose-dependent fashion as the correlation (Spearman) between percentage sFlt-1 reduction and apheresis duration in minutes was 0.80, $P=0.10$. We were unable to use placental growth factor measurements to follow the effects of sFlt-1 reduction during DSC apheresis because certain heparin-binding isoforms of placental growth factor would bind DSC columns, rendering these levels uninterpretable.

Changes in sFlt-1 Levels in Untreated Women

We next examined admission sFlt-1 levels and subsequent prolongation of pregnancy in 7 contemporaneous women with very preterm preeclampsia from the same clinical sites but who did not undergo apheresis treatments (online-only Data Supplement Table II). Admission sFlt-1 values ranged from 8 028 to 18 738 pg/mL, with a mean of 14 123 pg/mL. Prolongation of pregnancy with expectant manage-

Table 4. Characteristics of 5 Women With Very Preterm Preeclampsia Who Underwent a Single Extracorporeal Apheresis Treatment*

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Characteristics on day of apheresis					
Maternal age, y	22	26	37	34	20
Body mass index, kg/m ²	25.9	24.3	29.7	21.8	31.8
Gestational age, weeks+days	29+0	31+3	29+0	24+6	28+3
Systolic blood pressure, mm Hg	186	150	223	211	163
Diastolic blood pressure, mm Hg	96	104	116	104	103
Laboratory tests on day of apheresis					
Hemoglobin, g/L	13.2	12.3	12.0	11.1	10.7
Platelets, ×10 ⁹ /L	276	199	172	203	237
Creatinine, μmol/L	61	53	57	52	37
SGOT, U/L	87	31	20	—	26
SGPT, U/L	64	24	18	39	25
LDH, U/L	265	185	232	186	189
Pre- and post-pheresis measurements					
sFlt-1 before, pg/ml†	14232	13628	12490	18168	8818
sFlt-1 after, pg/ml	12138	11249	10126	14251	6360
% sFlt-1 reduction	14.7	17.5	18.9	21.6	27.9
Urine PC ratio before	446	2983	2568	1168	6583
Urine PC ratio after (nadir)	—	1250	1026	618	2500
Fibrinogen before, g/L	3.5	4.0	3.6	2.0	3.6
Fibrinogen after, g/L	—	3.7	—	1.4	3.0
Volume treated, mL	1916	2500	5900	5459	4300
Apheresis duration, min	60	108	105	134	120
Duration of pregnancy after pheresis, d	2	4	3	0	7
Reason for delivery‡	Worsening preeclampsia	Worsening preeclampsia	HELLP syndrome	HELLP syndrome	Worsening of preeclampsia and evidence of slowing fetal heart rate

*Missing values (—).

†Normal reference values of sFlt-1: median 1449 pg/ml (quartile 1 to quartile 3, 1028–1968 pg/mL) between 24 and 28 weeks of gestation; median 1934 pg/mL (quartile 1 to quartile 3, 1222–2818 pg/mL) between 29 and 33 weeks of gestation. Ninety-fifth centile values at these 2 time periods are 3890 pg/mL and 6688 pg/mL, respectively.¹⁶

‡Worsening preeclampsia included new onset of headache, visual changes, requirement of additional medications to control blood pressure, worsening proteinuria, and worsening edema; HELLP (hemolysis, elevated liver enzymes, low platelets) syndrome included new evidence of hemolysis, rising liver function tests, reduction of platelets, and epigastric pain.

SGOT indicates serum glutamic oxalacetic transaminase; SGPT, serum glutamic pyruvic transaminase; LDH, lactate dehydrogenase; sFlt-1, soluble fms-like tyrosine kinase 1; and PC, protein-to-creatinine ratio.

ment and changes in sFlt-1 levels are shown in Figure 2. Average pregnancy prolongation was 3.6 days (range 1 to 6 days).

Apheresis Treatments to Prolong Pregnancy

To prolong pregnancy for at least 14 days after admission, we then offered >1 treatment to 3 additional women with similar characteristics, namely very preterm preeclampsia. We targeted 5 to 6 L whole blood (≈1.0 to 1.5 times plasma volume)³⁷ exchanges and a 25% to 35% reduction of circulating sFlt-1 levels with each treatment. We used circulating sFlt-1 levels to guide the timing of each treatment. Obstetricians could decide to deliver at any time on the basis of standard indications. The 3 patients are described below.

Patient 1

A 32-year-old gravida 1 para 0 woman (Figure 3A through 3C) presented at 28+0 weeks of gestation with a single fetus, hypertension, and proteinuria. Blood pressure was 170/106 and 147/101 mm Hg (4 hours apart), and urine dipstick demonstrated 3+ proteinuria. She had no history of chronic hypertension or diabetes mellitus, she was a nonsmoker, and prior prenatal visits were unremarkable. Physical examination was notable for pedal edema. Estimated fetal weight was 976 g, which was at the 9th centile for gestational age. Umbilical artery Doppler and amniotic fluid volume measures were normal. Uterine Doppler demonstrated a positive notching sign with an umbilico-placental resistance above the 95th percentile. Fetal heart rate was 140 to 150 bpm. Laboratory findings were normal

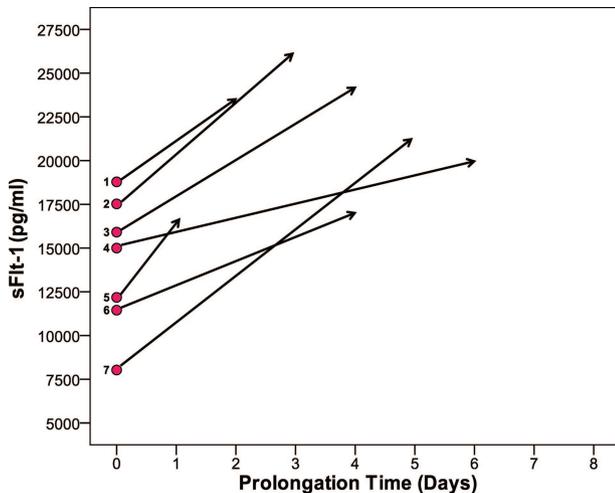


Figure 2. Plot of baseline and predelivery sFlt-1 (pg/mL) levels and prolongation time (days) in 7 untreated patients with very preterm preeclampsia. The red circles represent the baseline levels of sFlt-1 for each patient whereas the black arrowheads represent predelivery levels (y axis) and the days of prolongation (x axis) after admission. sFlt-1 indicates soluble fms-like tyrosine kinase 1.

(Table 5) except for elevated sFlt-1 levels at 12 865 pg/mL. She received 2 doses of betamethasone. Two days later 24-hour urine revealed 6.4 g of protein, and circulating sFlt-1 levels rose to 16 236 pg/mL. She was receiving methyldopa 250 mg 4 times daily.

On hospital day 4, she agreed to undergo 2 extracorporeal apheresis sessions. On average, blood pressure was 186/110 mm Hg, urine protein-to-creatinine ratio was 4.5, and sFlt-1 levels were 18 755 pg/mL. During the apheresis treatment, blood pressure remained stable (range 140/107 to 182/120 mm Hg), and fetal heart rate ranged from 130 to 160 bpm. Over 110 minutes, 6.0 L of whole blood was exchanged. After apheresis, circulating sFlt-1 was lowered by $\approx 30\%$ (to 13 167 pg/mL), and urine protein-to-creatinine ratio fell to 3.4 within 12 hours. Preapheresis plasma fibrinogen was 1.9 g/L. It dropped to 1.6 g/L after apheresis, then returned to 2.0 g/L within 24 hours. The entire procedure was well tolerated.

Over the ensuing days, blood pressure remained stable as did her blood pressure medication requirements. She reported improved pedal edema. By hospital day 8, circulating sFlt-1 and protein-to-creatinine ratio climbed to 18 934 pg/mL and 6.2, respectively. Given the rise in sFlt-1 to pretreatment levels, she underwent a second apheresis treatment. Starting blood pressure was 160/118 mm Hg. At ≈ 30 minutes into the treatment, blood pressure reached a nadir of 125/74 mm Hg but recovered with 500 mL of normal saline to 134/75 mm Hg (within 5 minutes) and 142/103 (within 15 minutes). Fetal heart rate went from 160 to 130 bpm, but this too immediately recovered with normal saline. No further reductions in blood pressure or fetal heart rate were noted, and apheresis continued uninterrupted, exchanging 6.0 L of whole blood. After apheresis, circulating sFlt-1 levels were lowered by 34% (to 12 475 pg/mL), and within 12 hours protein-to-creatinine ratio decreased to 3.2. Plasma fibrinogen levels

went from 2.3 g/L to 2.0 g/L after apheresis and returned to 2.2 g/L within 12 hours.

On hospital day 11, estimated fetal weight was 1097 g. By hospital day 15, at 30+0 weeks of gestation, circulating sFlt-1 levels and protein-to-creatinine ratio climbed to 25 415 pg/mL and 6.1, respectively. In the face of an acute 4-kg weight gain, headaches, and rising diastolic blood pressures (120 mm Hg) despite increasing medications, she was delivered by Caesarean section. At delivery, Apgar scores were 7/9/9, and the newborn girl weighed 995 g. Twelve hours after delivery, maternal blood pressure remained elevated at 177/115 mm Hg, and at 48 hours, when circulating sFlt-1 levels were 2118 pg/mL, blood pressure was 158/123 mm Hg. Within 24 hours, however, urine protein-to-creatinine ratio dropped to 2.6. She was discharged on day 25. The newborn required minimal monitoring and remained in the neonatal intensive care unit for 55 days, after which she was discharged in excellent condition without any notable abnormalities. At her 4-week follow-up, her blood pressures were normal and her newborn weighed 1465 g and had achieved expected developmental milestones.

Patient 2

A 40-year-old gravida 2 para 0 woman (Figure 4A through 4C) presented at 30+0 weeks with significant hypertension, proteinuria, and pedal edema. She was carrying dichorionic-diamniotic twins after receiving in vitro fertilization therapy. Blood pressure was 140/90 mm Hg and 161/92 mm Hg (4 hours apart), and urine dipstick revealed 2+ protein. She was admitted for very preterm preeclampsia. Her past medical history was unremarkable, and family history was only notable for paternal diabetes mellitus. She was a nonsmoker, and prenatal visits routinely demonstrated normal blood pressure and urine dipstick results. She was taking prenatal vitamins. Physical examination was notable for hypertension and pedal edema. Ultrasound showed 2 fetuses, estimated weights 1329 and 1327 g, with normal growth, amniotic fluid volume, and umbilical perfusion. Fetal heart rates were 130 to 150 bpm in each with normal oscillation and sporadic accelerations, uterine contractions were absent, and there was no evidence of decelerations. Laboratory findings revealed no evidence of HELLP syndrome (Table 5). Two doses of betamethasone were administered. Blood sFlt-1 levels were 29 068 pg/mL. A 24-hour urine collection demonstrated 786 mg of protein, and a urine protein-to-creatinine ratio of 1.0. Blood pressures were stable.

On hospital day 6, she agreed to undergo 2 extracorporeal apheresis treatments. Blood pressure was 187/81 mm Hg. Fetal ultrasound revealed normal arterial perfusion in both umbilical cords, and cardiotocography was otherwise normal. By 30 minutes into apheresis, her blood pressure dropped to 90/50 mm Hg, which recovered to 144/60 mm Hg within 5 minutes after 1.0 L of normal saline. Fetal ultrasound and cardiotocography did not reveal alterations. She continued with uninterrupted apheresis for 120 minutes, exchanging 5.1 L of whole blood. For the remainder of the treatment, blood pressure

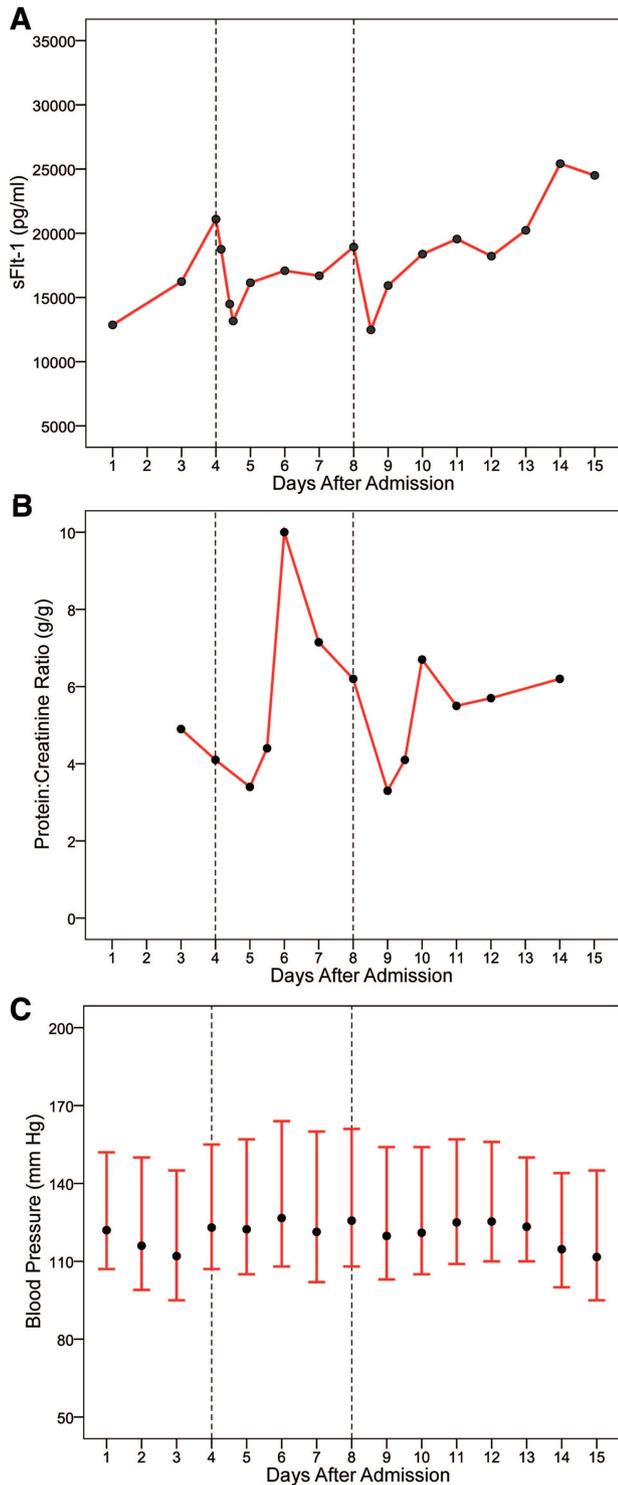


Figure 3. Changes in maternal circulating sFlt-1 levels (pg/mL) (A), protein-creatinine ratio (g/g) (B), and blood pressure (mm Hg) (C) in patient 1 over the course of 15 days of inpatient hospitalization starting at gestational age 28 weeks. Dotted reference lines indicate days on which apheresis was performed. Bars on panel C show average daily systolic blood pressure (top whisker), average daily diastolic blood pressure (bottom whisker), and average daily mean arterial pressure [(2×diastolic blood pressure+systolic blood pressure)/3] (black circle). Normal reference values for sFlt-1: median 1449 pg/mL (quartile 1 to quartile 3, 1028 to 1968 pg/mL) between 24 and 28 weeks of gestation; median 1934 pg/mL (quartile 1 to quartile 3, 1222 to

Table 5. Characteristics of 3 Patients With Very Preterm Preeclampsia Who Underwent ≥ 2 Extracorporeal Apheresis Treatments

	Patient 1	Patient 2	Patient 3
Characteristics prior to pheresis			
Maternal age, y	32	40	35
Body mass index, kg/m ²	26.5	27.4	28.4
Gestational age, weeks+days	28+3	30+0	27+5
Systolic blood pressure, mm Hg	186	187	175
Diastolic blood pressure, mm Hg	110	81	100
Laboratory tests prior to pheresis			
Hemoglobin, g/L	12.9	11.2	11.1
Platelets, $\times 10^9$ /L	234	222	244
Creatinine, μ mol/L	67	41	57
SGOT, U/L	36	53	64
SGPT, U/L	27	43	62
LDH, U/L	247	195	263

SGOT indicates serum glutamic oxalacetic transaminase; SGPT, serum glutamic pyruvic transaminase; and LDH, lactate dehydrogenase.

averaged 150/65 mm Hg and fetal heart rates averaged between 130 and 150 bpm for both fetuses. Circulating sFlt-1 levels were lowered by 26% (to 17 933 pg/mL) after apheresis, and protein-to-creatinine ratio dropped to 0.55 within 24 hours. Plasma fibrinogen levels went from 3.2 g/L to 2.7 g/L after apheresis, then rose to 3.0 g/L within 12 hours.

She received no blood pressure medications, and she had no complaints. By hospital day 11, circulating sFlt-1 levels and urine protein-to-creatinine ratio climbed to 23 075 pg/mL and 1.9, respectively. Given the rise in sFlt-1 levels, she underwent a second treatment. She experienced no episodes of hypotension (nadir 166/72 mm Hg at 30 minutes) or changes in fetal heart rates. Estimated fetal weights were 1674 and 1626 g. Apheresis continued for 120 minutes, exchanging 6.0 L of whole blood. After the second treatment, circulating sFlt-1 levels were lowered by 34% (to 15 197 pg/mL), and within 12 hours urine protein-to-creatinine ratio decreased to 0.79. Plasma fibrinogen levels went from 3.4 g/L to 2.8 g/L after apheresis, then returned to 3.3 g/L within 12 hours.

Over the next 7 days, she remained stable, requiring no blood pressure medications. However, circulating sFlt-1 levels climbed in conjunction with proteinuria. At 32+5 weeks of gestation, 19 days after hospital admission, her placental membranes spontaneously ruptured and she underwent uncomplicated Caesarean section. Five-minute Apgar scores were 9 for fetus 1 and 7 for fetus 2, and fetal weights were 1498 and 1485 g, respectively. At 12 hours after delivery, circulating sFlt-1 levels dropped to 226 pg/mL, yet blood pressure remained elevated: 155/85 mm Hg at 12 and 160/80 mm Hg at 24 hours. By day 7,

2818 pg/mL) between 29 and 33 weeks of gestation. Ninety-fifth centile values at these 2 time periods are 3890 pg/mL and 6688 pg/mL, respectively.¹³ sFlt-1 indicates soluble fms-like tyrosine kinase 1.

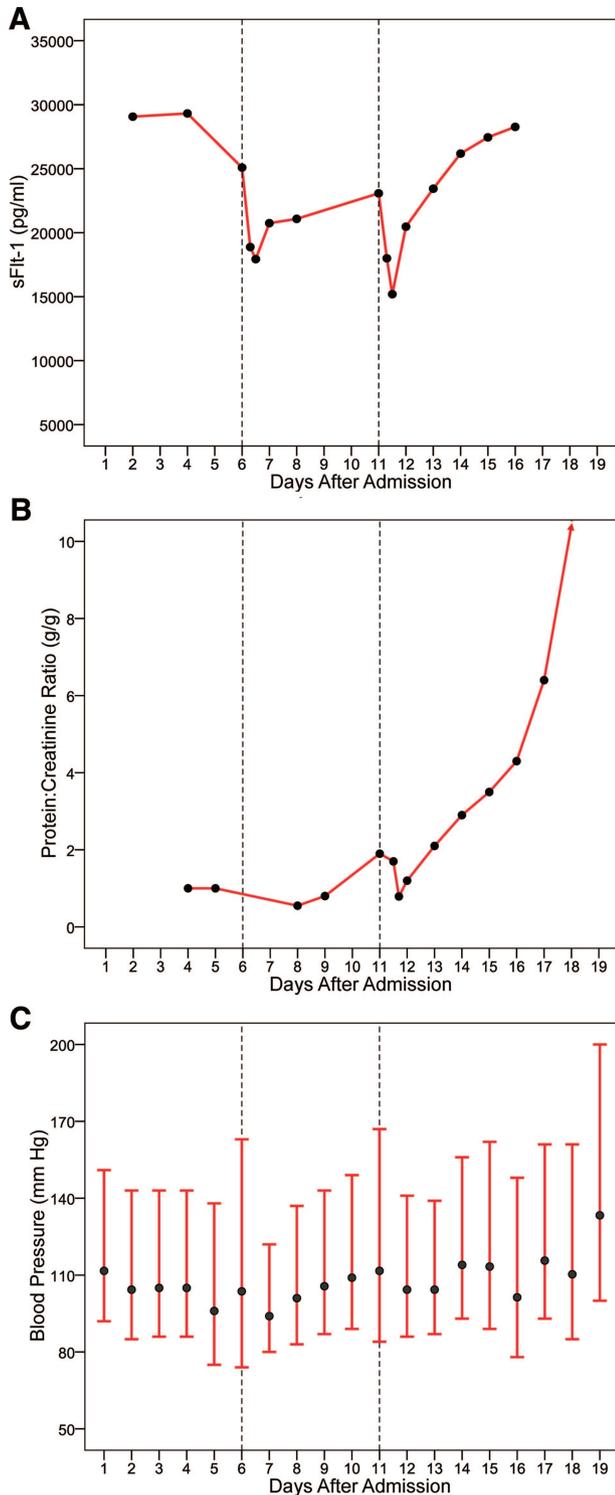


Figure 4. Changes in maternal circulating sFlt-1 levels (pg/mL) (A), protein-creatinine ratio (g/g) (B), and blood pressure (mm Hg) (C) in patient 2 over the course of 19 days of inpatient hospitalization starting at gestational age 30 weeks. Dotted reference lines indicate days on which apheresis was performed. Bars on panel C show average daily systolic blood pressure (top whisker), average daily diastolic blood pressure (bottom whisker), and average daily mean arterial pressure [$(2 \times \text{diastolic blood pressure} + \text{systolic blood pressure})/3$] (black circle). see Figure 3 legend for sFlt-1 reference ranges. sFlt-1 indicates soluble fms-like tyrosine kinase 1.

blood pressure was 135/80 mm Hg. In contrast, urine protein-to-creatinine ratio just before delivery was 18.1, and within 24 hours after delivery it was 1.4. Neither newborn required ventilator support other than continuous positive airway pressure. On follow-up at 5 weeks, their weights were 2330 and 2300 g, and their expected developmental milestones were achieved.

Patient 3

A 35-year-old gravida1 para 0 woman (Figure 5A through 5C) presented at 27+4 weeks with hypertension, proteinuria, and pedal edema. Initial blood pressure was 177/95 mm Hg, which was confirmed several hours later, and urine protein-to-creatinine ratio was 2.3. She was admitted for very preterm preeclampsia. Her medical history was notable for Hashimoto's thyroiditis, and her family history was notable for paternal and maternal hypertension. She was a nonsmoker, and previous prenatal visits demonstrated normal blood pressure and urine dipstick results. She had no history of chronic hypertension. Physical examination was notable for hypertension and pedal edema. Ultrasound showed a singleton fetus in breech presentation with an estimated weight of 811 g. Amniotic fluid volume and placenta appeared normal. Doppler ultrasound revealed an abnormal uterine Doppler with increased resistance but no notching. Fetal perfusion in the umbilical artery, middle cerebral artery, and ductus venosus was normal. Fetal heart rate was 140 to 160 bpm, and there was no evidence of uterine contractions or decelerations. Laboratory findings revealed no evidence of HELLP syndrome (Table 5). Two doses of betamethasone were administered.

On hospital day 3, she agreed to undergo serial extracorporeal apheresis treatments. Blood pressure was 175/105 mm Hg without antihypertensive medications. Fetal ultrasound revealed normal umbilical perfusion, and cardiotocography was otherwise normal. By 23 minutes into treatment, her blood pressure dropped to 93/50 mm Hg, which recovered to 126/51 mm Hg (within 5 minutes) after 1.0 L of normal saline. She continued with uninterrupted apheresis, but after 90 minutes of treatment her blood pressure dropped again. Apheresis was then stopped after exchanging a total 3.2 L of whole blood. Ultrasound examination showed unchanged umbilical artery perfusion values and normal fetal movements. After apheresis, sFlt-1 levels dropped together with her urine protein-to-creatinine ratio. Her blood pressure remained stable with values of $\approx 175/100$ mm Hg without medications. By hospital day 7 (28+3 weeks), sFlt-1 levels and urine protein-to-creatinine ratio climbed.

A second treatment was performed on day 7. This time, 1 to 2 L of saline were administered slowly at the start of the treatment. The procedure was better tolerated with no pronounced hypotensive episodes. Apheresis continued for 110 minutes, exchanging a total of 4.0 L of whole blood. After the second treatment, circulating sFlt-1 levels were lowered to 10 345 pg/mL, and within 12 hours, urine protein-to-creatinine ratio also decreased. Plasma fibrinogen levels went from 4.4 g/L to 3.1 g/L after apheresis,

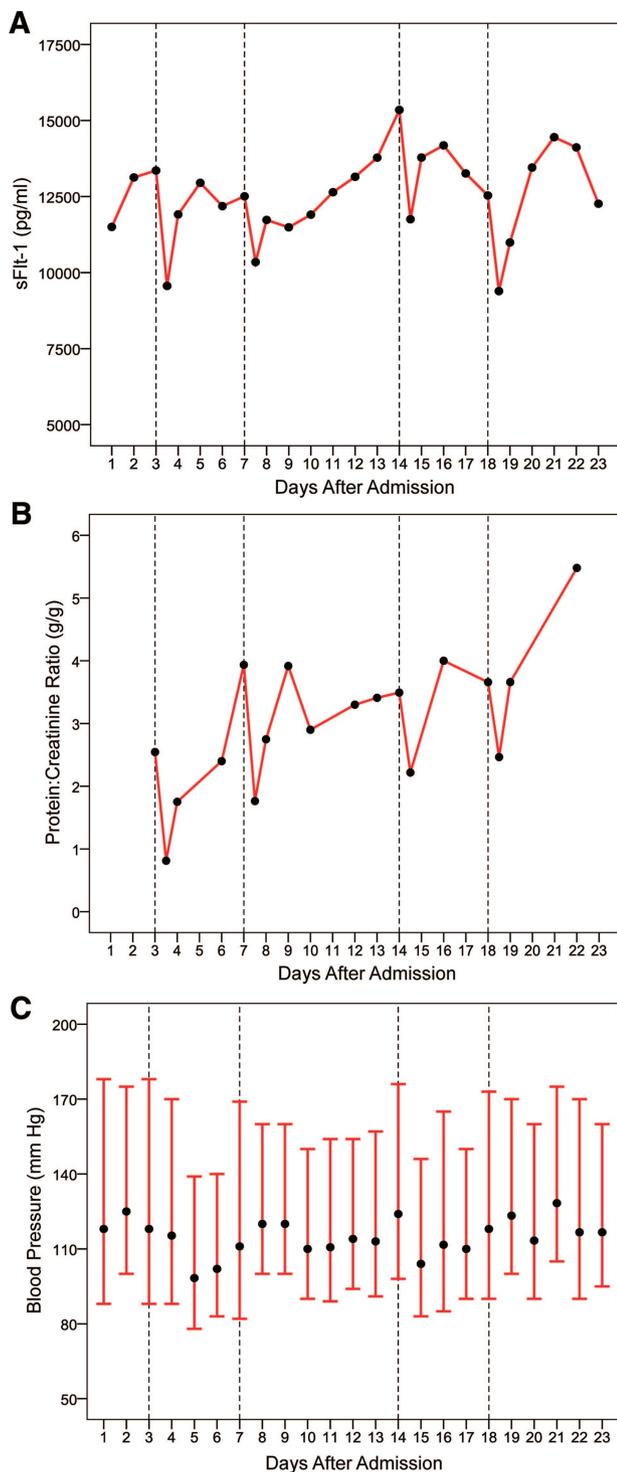


Figure 5. Changes in maternal circulating sFlt-1 levels (pg/mL) (A), protein-creatinine ratio (g/g) (B), and blood pressure (mm Hg) (C) in patient 3 over the course of 23 days of inpatient hospitalization starting at gestational age 27 weeks+4 days. Dotted reference lines indicate days on which apheresis was performed. Bars on panel C show average daily systolic blood pressure (top whisker), average daily diastolic blood pressure (bottom whisker), and average daily mean arterial pressure $[(2 \times \text{diastolic blood pressure} + \text{systolic blood pressure})/3]$ (black circle). see Figure 3 legend for sFlt-1 reference ranges. sFlt-1 indicates soluble fms-like tyrosine kinase 1.

then increased to 3.4 g/L within 12 hours. Ultrasound demonstrated fetal weight gain (estimated at 1013 g). Amniotic fluid amount and fetal perfusion indices were within the normal range. Over the next 7 days, she was without complaints. Beginning at week 29+1, she received a daily dose of 32 mg methylprednisolone intravenously because of slightly increased liver enzymes. Pedal edema was present but stable.

A third treatment was performed on day 14. She experienced no drops in blood pressure, and apheresis continued for 115 minutes, exchanging 4.0 L of whole blood. After treatment 3, circulating sFlt-1 levels were lowered by 23% (to 11 755 pg/mL), and within 12 hours, urine protein-to-creatinine ratio also decreased from 3.5 to 2.2. Plasma fibrinogen levels went from 5.2 to 3.7 g/L after apheresis, then climbed to 3.9 g/L within 12 hours. A fourth uncomplicated treatment was performed on day 18. Apheresis continued for 126 minutes, exchanging 4.5 L of whole blood. Circulating sFlt-1 levels were lowered by 25%, and within 12 hours, urine protein-to-creatinine ratio was lowered from 3.7 to 2.5.

Twenty-three days after hospital admission, at 30+6 weeks of gestation, she developed regular contractions with cervical effacement. She underwent uncomplicated Caesarean section. Five-minute Apgar score was 7/9/9, and the fetal weight was 1140 g. At 12 hours after delivery, circulating sFlt-1 levels dropped to 1464 pg/mL. However, her blood pressure remained elevated at 144/98 mm Hg. Blood pressure returned to normal values (110/75 mm Hg) by 24 to 48 hours after delivery. Her newborn received positive airway pressure support in the neonatal intensive care unit for 5 days, after which he was sent to a regular neonatal care bed. The patient was discharged 4 days after delivery. The newborn was discharged approximately 9 weeks later, weighing 2710 g, and with normal hearing, respiratory, cardiac, and neurological development.

Discussion

The antiangiogenic factor sFlt-1 is one possible candidate involved in mediating the clinical manifestations of preeclampsia. By understanding the charged nature of sFlt-1 and modifying existing extracorporeal apheresis methods to optimize its removal, we demonstrated that apheresis with DSC columns reduces circulating sFlt-1 in a dose-dependent fashion in women with very preterm preeclampsia. In 3 additional patients with very preterm preeclampsia, repeated apheresis treatments reduced circulating levels of sFlt-1 and proteinuria, stabilized blood pressure, and potentially prolonged pregnancy without apparent adverse events occurring to either mother or fetus.

We chose extracorporeal methods to remove sFlt-1. However, other strategies to target circulating sFlt-1 include administration of VEGF (its natural ligand) or a neutralizing antibody or inhibition of its translation with small interfering RNA technologies.⁴ Preeclampsia does not occur naturally in animals. Therefore, encouraging results in animals may not immediately translate into successful human therapeutics. Because each extra week in utero markedly lowers fetal morbidity and mortality,³⁸ any short-term intervention for very preterm preeclampsia

should aim to prolong pregnancy without compromising maternal safety. The intervention should stabilize or improve maternal and fetal parameters without leaving residual effects. The intervention should be easily titrated given the potential for acute deterioration of mother, fetus, or both. Extracorporeal adsorption of circulating sFlt-1 fulfills several of these criteria. This strategy also does not deplete VEGF, which does not circulate to any large extent and which is critical in preeclampsia in maintaining local vascular integrity.^{39,40}

Maternal blood pressure appeared to be stabilized but not markedly decreased after apheresis therapy. In addition to apheresis itself, withholding medications before treatments and saline administration during treatments also may have modified blood pressure. In experimental sFlt-1-induced preeclampsia, immediate VEGF-121 administration lowers blood pressure only after several days.³⁹ In humans, delivery of the placenta normalizes sFlt-1 levels almost immediately,¹¹ yet maternal blood pressure returns to normal only after days to weeks. This lag was evident in our 3 patients. Maternal hypertension in preeclampsia may result from renal endothelial cell damage, and the time required to heal this lesion may explain the delayed response. In women treated serially, sFlt-1 levels were lowered by 17% to 34%. Although further sFlt-1 reductions may have lowered maternal blood pressure, we were concerned about fetal growth retardation from aggressive blood pressure reduction.⁴¹ Perceiving the need for more blood flow in preeclampsia, the fetal-placental unit may release excess sFlt-1 into the maternal circulation to sustain blood pressure.⁴² This likely explains the rebound in circulating sFlt-1 levels in the days after each apheresis.

The role of sFlt-1 in normal placentation remains unknown. Genetically engineered mice that lack placental sFlt-1 do not develop placental abnormalities,⁴³ and case reports highlight natural reductions in circulating sFlt-1 with continuation of human pregnancy to term.^{44,45} Thus, lowering sFlt-1 to levels near normal does not appear to be harmful. Regardless, we designed our methods to avoid significant reduction in circulating sFlt-1, maternal blood pressure, and placental blood flow, and in this context, maternal blood pressure stabilized, fetal compromise was clinically absent, and fetal weight gain was evident.

Protein-to-creatinine ratios rose and fell in conjunction with sFlt-1 levels. Rescue therapy with VEGF-121 in experimental preeclampsia also demonstrates a more immediate improvement in proteinuria, as does discontinuation of anti-VEGF therapies in cancer.^{39,46} Although blood pressure lowering reduces proteinuria, apheresis did not result in sustained blood pressure reduction. Therefore, reduction of circulating sFlt-1 levels with improvement of proteinuria after apheresis suggests a more dynamic effect of circulating sFlt-1 on the integrity of fenestrated glomerular endothelium and proteinuria. However, the specific mechanisms involved remain unknown.⁴⁷ Improvement of proteinuria may become an important early signal for efficacy of this intervention.

Expectant management in preterm preeclampsia increases the risk of maternal and fetal morbidity and

mortality, but in highly selected women this may be appropriate.⁴⁸ Limited data suggest that in very preterm preeclampsia without fetal growth restriction, expectant management extends pregnancy by 7 to 14 days.^{5,48} Among contemporaneous controls that met the criteria for very preterm preeclampsia, the average prolongation of pregnancy was <4 days, similar to our 5 patients treated once. Furthermore, all controls had a singleton pregnancy, but the risk for adverse outcomes is markedly higher in twin pregnancies. A pregnancy with twins is infrequently prolonged beyond a few days with expectant management.⁴⁹ We excluded those with fetal growth restriction (<5th centile for gestational age). With 2 apheresis treatments, the patient with a singleton fetus remained pregnant for 15 days and the patient with twins remained pregnant for 19 days. With 4 treatments, our last patient remained pregnant for 23 days after admission. None showed evidence of fetal compromise. Although it is tempting to speculate that apheresis prolonged pregnancy and that longer or additional treatments would have prolonged pregnancy even further, we treated only a limited number of patients, and only randomized trials with additional patients will allow definitive conclusions on this important end point.

Our work leaves certain questions unanswered. We did not include patients with a severely growth-restricted fetus or with other evidence of fetal compromise, and thus we do not know if this therapy would benefit or harm the fetus in this setting. We cannot exclude the possibility that DSC apheresis removes substances such as LDL cholesterol and C-reactive protein or substances that have a heparin-binding domain and exhibit angiogenic properties (eg, fibroblast growth factors) and that this contributed to our findings. We also do not know if apheresis cleared substances altered in preeclampsia such as auto-antibodies against the angiotensin receptor.⁵⁰ Data from one patient, however, suggested that DSC apheresis does not reduce endothelin-1 but modestly reduces circulating soluble endoglin (online-only Data Supplement Table III), similar to what has been reported in patients with FH.⁵¹ The specificity of sFlt-1 removal may be improved by developing extracorporeal columns with anti-sFlt-1 antibodies covalently bound to beads, similar to methods developed in LDL apheresis.⁵² Such specificity may reduce the frequency of treatments when compared to the 1 to 2 treatments/week we employed to avert further elevations in circulating sFlt-1 levels. Other adsorption therapies may benefit women with preeclampsia. Heparin-mediated LDL apheresis has been attempted in preeclampsia with moderate success.⁵³ Our *ex vivo* studies suggest that heparin-based columns would be inferior to DSC columns in removing circulating sFlt-1 (online-only Data Supplement Table IV).

Novel drugs directed at improving maternal and fetal health are infrequently developed. Risk aversion and limited market size are among the many reasons.⁵⁴ Modifying approved interventions with known safety profiles such as demonstrated here with extracorporeal DSC apheresis is a viable and attractive alternative.⁵⁵ In this hypothesis-generating study we provide 1 potential intervention for very preterm preeclampsia characterized by elevated circulating

sFlt-1. Further studies with additional patients are warranted to fully assess whether the intervention studied herein safely and effectively prolongs pregnancy and improves maternal and fetal outcomes in very preterm preeclampsia.

Acknowledgments

We would like to thank the patients who agreed to participate in this study. We would especially like to thank Drs Samuel O. Thier, Dennis A. Ausiello, and M. Amin Arnaut for their support. Finally, we thank Kathy Lucchesi for her assistance with the protocol, and Rameh Follette with her assistance in early in vitro studies.

Sources of Funding

This work was funded by the Discovery Fund from the Department of Medicine, Massachusetts General Hospital, to Dr Thadhani. Dr Thadhani's salary is supported by several federal and foundation grants. This work also received support from Forschungspool Klinische Studien, Faculty of Medicine, University of Cologne. Dr Cornely is supported by the German Federal Ministry of Research and Education (Bundesministerium für Bildung und Forschung Grant 01KN1106). Dr Benzing received funding from the German Research Foundation (BE2212). Dr Karumanchi is supported by the Howard Hughes Medical Institute.

Disclosures

Dr Thadhani is coinventor of patents related to diagnostics in the prediction of preeclampsia that have been out-licensed to diagnostic companies and has financial interest in Aggamin LLC. Dr Karumanchi is coinventor of multiple patents related to the use of angiogenic proteins for the diagnosis and therapy of preeclampsia. These patents have been licensed to multiple companies. Dr Karumanchi is a consultant to Roche Diagnostics and Beckman Coulter and has financial interest in Aggamin LLC. The remaining authors report no conflicts.

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CLINICAL PERSPECTIVE

Preeclampsia is a devastating medical complication of pregnancy associated with significant maternal and fetal morbidity and mortality. Delivery of the placenta remains the only effective treatment. Although the pathogenesis of preeclampsia remains a mystery, recent developments suggest that elevated levels of circulating soluble fms-like tyrosine kinase 1 (sFlt-1) may contribute to the pathogenesis of preeclampsia. Therapies targeting circulating sFlt-1 may alleviate the signs and symptoms of preeclampsia and potentially prolong pregnancy in women presenting with very preterm (gestational age <32 weeks) preeclampsia. The challenge in pregnancy is to administer an effective therapy that would be safe for both mother and baby. Rather than administering an agent to the mother, we hypothesized that extracorporeal removal of sFlt-1 using a selective adsorption column would create a concentration gradient and augment its removal from maternal circulation and that this form of therapeutic apheresis would be safe. We tested dextran sulfate columns, which exploit the charged nature of circulating proteins and are used clinically for other purposes. We treated women with dextran sulfate cellulose apheresis and found that depletion of circulating sFlt-1 may prolong pregnancy in women with very preterm preeclampsia. The therapies were well tolerated by both mother and baby. However, further clinical studies are warranted to determine if this intervention can safely prolong pregnancy in women with very preterm preeclampsia.