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Treatment of very preterm preeclampsia via heparin-mediated extracorporeal LDL-precipitation (H.E.L.P.) apheresis: The Freiburg preeclampsia H.E.L.P.-Apheresis study

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ABSTRACT

Objective: Soluble Fms-like tyrosine kinase-1 (sFlt-1) is thought to be causative in the pathogenesis of preeclampsia (PE) and specific removal of sFlt-1 via dextran sulfate cellulose (DSC)-apheresis was suggested as cure to allow prolongation of pregnancy in preterm PE. However, in addition a deranged lipoprotein metabolism may impact endothelial and placental function in PE. Lipoprotein-apheresis by heparin-mediated extracorporeal LDL-precipitation (H.E.L.P.) was previously applied and has been shown to alleviate symptoms in PE. This clinical trial reevaluates the clinical efficacy of H.E.L.P.-apheresis in PE considering sFlt-1. *Study design:* Open pilot study assessing the prolongation by H.E.L.P.-apheresis in 6 women (30–41 years) with very preterm PE (24 + 4 to 27 + 0 gestational weeks (GW)) (NCT01967355) compared to a historic control-group matched for GW at admission (< 28 GW; n = 6). Clinical outcome of mothers and babies, and pre- and post H.E.L.P.-apheresis levels of sFlt-1 and PlGF were monitored. *Main outcome measures:* In apheresis patients (2–6 treatments), average time from admission to birth was 15.0 days (6.3 days in controls; p = 0.027). Lung maturation was induced in all treated cases, and all children were released in healthy condition. Apheresis reduced triglycerides and LDL-cholesterol by more than 40%. Although H.E.L.P.-apheresis induced a transient peak baseline levels did not change and rather stabilized sFlt-1 levels at pre-apheresis levels throughout treatments, with sFlt-1/PLGF ratio remaining unaffected. *Conclusions:* H.E.L.P.-apheresis proved again to be safe and prolongs pregnancies in PE. However, without changing sFlt-1 levels below baseline lowering lipids or other yet undefined factors appear to be of more relevance than reducing sFlt-1.

1. Introduction

Preeclampsia (PE) and other hypertensive disorders of pregnancy affect around 2–8% of pregnancies. They are major causes of maternal and fetal morbidity and mortality [1]. Mothers are at risk of vascular endothelial damage and disorders in multiple organ systems [2]. Control of clinical symptoms is attempted by administering antihypertensive drugs and magnesium sulphate to prevent seizures [3], yet with limited success. To date, the only causative therapy consists in preterm delivery by Caesarean section (CS). Especially before 32 weeks of gestation, the risk of fetal immaturity is often inevitable. Therefore, any safe and effective pregnancy-prolonging treatment would be appreciated.

The pathophysiology of PE remains unclear [4] probably because it is a multifactorial disease: inflammatory markers [5], antiangiogenic factors [6], as well as changes in lipid metabolism [7], all have been observed and are intensely discussed.

An imbalance of pro- and anti-angiogenic proteins like placenta growth factor (PlGF) and soluble FMS-like tyrosine kinase-1 (sFlt-1) have been proposed as key factors in the pathogenesis. While sFlt-1 itself has poor predictability, the ratio of sFlt-1/PlGF appears to yield a reliable risk score at least in the second trimester [8]. This implies that a metabolic response to an imbalance of antiangiogenic and proangiogenic factors rather than a toxic effect by either sFlt-1 or PlGF alone

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appears to be associated with PE [9].

During normal pregnancy, the lipid profile changes into an atherogenic phenotype with even more dramatic changes in PE [10–12]. A recent meta-analysis of 24 case-control studies (2720 women) revealed that high triglyceride levels are associated with a 4-fold increased prevalence for PE [7]. This finding was expanded upon and confirmed in five cohort studies that recruited 3147 women in the second trimester before onset of PE proving that hypertriglyceridemia precedes the onset of PE [13].

In hypertriglyceridemia, apolipoprotein (Apo) C-II is involved in the remodeling of triglyceride (TG)-rich remnant particles associated with increased risk of premature cardiovascular disease [14]. Furthermore, apo C-II was very recently described as a relevant component identifying those pregnancies complicated by intrauterine growth restriction [15].

Earlier work of our group has demonstrated that triglyceride-metabolism, namely the triglyceride-content of remnant-like intermediate-dense-lipoproteins correlates well with diastolic blood pressure, proteinuria, and infant birthweight throughout normal pregnancies and those complicated by PE. This previous study also revealed that Apo C-II was highly discriminating between PE and healthy pregnancies. Consequently, back then we already suggested lipid-modifying interventions like lipid-apheresis as a potential therapeutic approach [12].

A first pilot study proved this concept by means of heparin-mediated extracorporeal LDL-precipitation (H.E.L.P.)-apheresis in severe PE more than a decade ago [16]. This study with 9 patients (24–32 gestational week (GW)) showed a reasonable clinical benefit by prolonging pregnancies on average for 17.7 (3–49) days after admission [16]. Meanwhile other apheresis techniques were reported to prolong complicated pregnancies: immunoapheresis was successfully applied in women with antiphospholipid syndrome [17], and in a recent pilot study in 3 patients with very preterm PE (27–30 GW) with another lipid-lowering apheresis technique using a dextran sulfate cellulose (DSC) it was claimed that lowering circulating sFLT-1 by -17% to -34% prolonged pregnancies by 19 (15–23) days [18]. The prolongation of pregnancy in PE by DSC-apheresis was confirmed by comparing 11 women (23–32 GW) undergoing apheresis (prolongation 11 (2–21) days) to a contemporaneous control group (n = 22) of women with PE (prolongation 3 (0–14) days) [19].

Although similar clinically effective the working hypotheses of DSC-apheresis and H.E.L.P.-apheresis are quite different: while Thadhani et al. assigned the positive effects of DSC apheresis exclusively to the removal of sFlt-1 [18], H.E.L.P.-apheresis lowers sFlt-1 concentrations only marginally [18]. We therefore questioned the hypothesis that the clinical benefit of DSC-apheresis may be solely attributed to the removal of sFlt-1 [20].

To substantiate the clinical value of H.E.L.P.-apheresis regarding prolongation of severe PE we compared the clinical course of women with PE treated with H.E.L.P.-apheresis with a similar historic control group of preeclamptic women not undergoing apheresis with focus on sFlt-1.

2. Materials and methods

2.1. Patients and study protocol

This monocentric open pilot study was initiated in line with the study of Wang et al. [16] to reassess the clinical benefit of H.E.L.P.-apheresis in early onset PE focusing on the angiogenetic and anti-angiogenetic factors PlGF and sFlt-1. Primary outcome measure was the prolongation of pregnancy (time from admission to delivery/CS) via H.E.L.P.-apheresis (ClinicalTrials.gov NCT01967355). The prolongation of pregnancy was compared to a historic control group of similar patients with PE not undergoing apheresis. This study was approved by the Ethics Review Committee of the University of Freiburg and informed consent was obtained from each subject; all procedures were compliant with the Helsinki Declaration of 1975, revised 2008.

Inclusion Criteria: mothers aged > 18 years with early-onset PE (< 32 week of pregnancy). PE was defined according to the criteria of the German Society of Obstetrics and Gynecology [21] with arterial hypertension during pregnancy (BP > 140/90 mmHg), proteinuria (≥1 + dipstick or > 300 mg/24 h), and/or intrauterine growth restriction (IUGR). Between 5/2013 and 05/2014, six patients with preterm PE (individual A1 through A6; Table 1) underwent apheresis during their pregnancy.

For comparison we refrained from enrolling contemporaneous patients for logistic and ethical reasons. Further, theoretically eligible patients who are in poor clinical condition may not be scheduled for apheresis but rather sent to deliver immediately. This may result in a selection bias with shortened time to delivery, an effect already described by Wang et al. [16].

Instead, we decided to identify control subjects in the time period immediately before enrolling the first patient (5/2013) from the records between 4/2009 and 4/2013, when apheresis was not considered a therapeutic option.

In the birth registry, 5993 women presented for delivery in the Department of Obstetrics at Freiburg Medical School. One-hundred and fifteen patients were pre-term (≤34th GW) and admitted with PE or possible PE. Sixty patients were admitted to the hospital before the 32nd GW (≤31 + 6), and 41 patients were confirmed as having definitively been suffering from PE. Of those, 4 were gemini, 6 suffered from diabetes mellitus, and one patient was younger than 18 years. Furthermore, 9 patients were excluded a) because of requiring immediate delivery (eclampsia, epilepsia because of drug abuse, intrauterine fetal death, and retina dissection because of hypertensive crisis); b) fetal malformation; c) hemostaseologic problems (status after lupus erythematodes and thrombosis), and d) multiple morbidity.

Thus, after excluding another 20 patients because of the aforementioned medical reasons, 21 otherwise healthy patients diagnosed with pre-term PE (≤31 + 6) were eligible. However, by selection bias all 6 apheresis-treated patients were admitted to the hospital before the 28th GW. To account for this, we finally specified a subgroup of 6 control patients also presenting before the 28th GW.

2.2. Heparin-induced extracorporeal LDL precipitation (H.E.L.P.)

H.E.L.P.-apheresis is based on complex-building and precipitation of the apoB containing lipoproteins VLDL, IDL and LDL, as well as Lp(a) with heparin at acidic pH [22]. H.E.L.P. apheresis was done by the Plasmat Futura® system according to the manufacturer's instructions (B. Braun, Melsungen; Germany). Apheresis time and separated plasma volume averaged 106 min and 2874 ml, respectively.

2.3. Clinical biochemistry

Laboratory measurements were done by standard laboratory procedures at the Institute of Clinical Chemistry and Laboratory Medicine at the University of Freiburg. sFlt-1 and PLGF were determined by electro chemi-luminescence immune assay (ECLIA). Cholesterol (CH) and triglycerides (TG) were determined enzymatically with the CHOD-PAP and the GPO-PAP method. LDL- and HDL-cholesterol were determined by homogenous assays. All tests were done on the cobas 8000 analyzer platform (Roche Diagnostics, Mannheim, Germany).

2.4. Statistical analysis

The patients' clinical and biochemical characteristics were expressed as mean ± standard deviation or as median and minimum/maximum, as indicated. Differences were tested for significance by the parametric paired and unpaired *t*-test, or the non-parametric paired Wilcoxon signed ranks test, as appropriate. Changes were considered statistically significant if the p-value was < 0.05. The SPSS 22.0.0.0

Table 1
Clinical characteristics at admission of apheresis-treated and control pregnant women < 28th gestational week.

Patient	Age [years]	Gestational Age Admission [weeks]	Estimated Fetal Weight at Admission [percentile]	Syst. RR at Admission [mm Hg]	Diast. RR at Admission [mm Hg]	Protein Excretion [mg/24 h]	BMI Admission [kg/m^2]
A1	41	24.6	73.6	170	100	573.5	28.4
A2	35	24.6	5.9	157	89*	387.5**	29.8
A3	30	25.3	0.2	152	97	462	26.7
A4	31	25.4	–	148	97	312	24.1
A5	30	26.6	2.1	142	92	360	29.7
A6	32	27.0	3.8	147	88*	252**	20.4
(A1–A6) Mean ± SD or median (min/max)	33.2 ± 4.3	25.6 ± 1.0	17.1 ± 31.6	153 ± 10	94 ± 5	391.2 ± 113.9	26.5 ± 3.7
C1	21	24.4	13.9	160	106	4032	21.8
C2	28	26.4	19.5	150	85*	2436	48.1
C3	27	26.7	42.4	164	93	6744	34.9
C4	30	27.1	11.5	150	80*	770	40.6
C5	42	27.3	–	130*	80*	721.5	22.2
C6	23	27.9	39.3	145	105	300***	25.9
Mean (C1–C6) ± SD	28.5 ± 7.4	26.6 ± 1.2	25.3 ± 14.5	150 ± 12	92 ± 12	2500.6 ± 2504.3	32.3 ± 10.8
p-value Apheresis (A1–A6) vs Contr. < 28 GW (C1–C6)	0.210	0.124	0.613	0.665	0.665	0.066	0.245

Patient	Total-CH Admission [mmol/L]	TG Admission [mmol/L]	LDL-CH Admission [mmol/L]	HDL-CH Admission [mmol/L]	sFlt-1 Admission [pg/mL]	PLGF Admission [pg/mL]	sFlt-1/PlGF Admission
A1	7.0	2.6	3.9	2.4	10,432	63	166
A2	5.7	2.6	2.8	1.7	18,794	27	696
A3	6.9	2.7	3.9	2.1	5889	13	453
A4	8.4	2.3	4.4	3.5	10,485	35	300
A5	6.8	1.9	3.7	2.6	14,965	11	1360
A6	4.7	0.9	1.8	2.7	28,086	14	2006
(A1–A6) Mean ± SD or median (min/max)	6.6 ± 1.2	2.4 (0.9/2.7)	3.4 ± 1.0	2.5 ± 0.6	14775 ± 7869	27 ± 20	830 ± 713
C1	–	–	–	–	–	–	–
C2	–	–	–	–	–	–	–
C3	–	–	–	–	–	–	–
C4	–	–	–	–	–	–	–
C5	–	–	–	–	–	–	–
C6	–	–	–	–	–	–	–
Mean (C1–C6) ± SD	–	–	–	–	–	–	–
p-value Apheresis (A1–A6) vs Contr. < 28 GW (C1–C6)	–	–	–	–	–	–	–

Fetal weight at admission was estimated by routine ultrasound examinations using the Hadlock-formula 2 [23].

* RR values > 140/90 mmHg were recorded in the patients' documentation.

** IUGR, platelet count 93,000/μl; hemoglobin 117 g/L; ASAT 65 U/L.

*** Dipstick 1 + at admission. Comparison between treated and control patients were performed by the parametric unpaired *t*-test. P-values < 0.05 were considered significant.

Table 2
Clinical outcome of the children of apheresis-treated and control pregnant women < 28th gestational week.

Infant	No. of Apheresis	Gender of Child	Gestational Age at Birth [weeks]	APGAR-5′	APGAR-10′	pH (umb. cord)	BE	Birth Weight [g]	Time Admission-Birth [days
A1	3	m	27.7	7	7	7.28	−6.6	860	22
A2	5	m	26.9	7	7	7.31	−5.0	660	16
A3	4	f	27.6	7	8	7.31	−3.9	430	16
A4	6	f	28.0	6	8	7.22	−6.6	835	18
A5	3	f	28.0	6	7	7.35	−2.1	530	10
A6	2	f	28.1	7	8	7.26	−5.3	520	8
A1–A6 Mean ± SD	3.8 ± 1.5		27.7 ± 0.5	6.7 ± 0.5	7.5 ± 0.5	7,29 ± 0.05	−4.9 ± 1.7	639 ± 177	15.0 ± 5.2
p-value Fetal Weight Gain									
C1	–	f	24.7	5	8	7.32	−5.4	510	2
C2	–	m	29.1	8	9	7.26	−4.6	935	19
C3	–	f	27.1	7	7	7.31	−3.2	870	3
C4	–	m	28.0	6	9	7.20	−3.4	790	6
C5	–	m	28.0	7	8	7.31	−4.5	880	5
C6	–	m	28.3	5	7	7.33	−1.8	1125	3
C1–C6 Mean ± SD			27.5 ± 1.5	6.3 ± 1.2	8.0 ± 0.9	7.29 ± 0.05	−3.8 ± 1.3	852 ± 202	6.3 ± 6.4
p-value Fetal Weight Gain									
p-value Apheresis (A1–A6) vs Contr. < 28 GW (C1–C6)			0.804	0.549	0.270	1.000	0.235	0.081	0.027

Differences between groups were tested by the parametric unpaired *t*-test. P-values < 0.05 were considered significant.

statistical package (IBM Corp. Amrok, NY, USA) was used for all analyses.

3. Results

Clinical characteristics of patients receiving H.E.L.P.-apheresis (A1 to A6) and the non-apheresis group (C1 to C6) are presented in Table 1. The treated patients' average age was 33.2 years, the controls' 28.5 years (p = 0.210). The gestational age at admission did not differ between treated (25.6 weeks) and control subjects (26.6 weeks; p = 0.124); this also applied to blood pressure, body mass index (BMI) and 24 h-protein-excretion, although proteinuria was more severe in the control group, but this was not significant. Proteinuria was low in individual A6, however, this patient was included because of thrombocytopenia, elevated liver enzymes, and IUGR superimposed to chronic hypertension. Fetal weight percentiles at admission by routine ultrasound using the Hadlock-formula 2 [23] were in average 17.1% in apheresis and 25.3% in control subjects (p = 0.613). Four apheresis-patients were below the 10th percentile. Total cholesterol, TG, LDL-and HDL-cholesterol as well as sFLt-1, PLGF and the ratio of s-Flt-1to PLGF were only recorded in the treated preeclamptic patients but not available for controls. However, in the treated patients values were in the expected elevated range [8].

A total of 23 H.E.L.P.-apheresis treatments, between 2 and 6 times per individual, were performed prior to delivery. Reasons for preterm delivery were pathologic cardio-tokogramm (CTG) (A1, A3); centralized Doppler (A1, A3, A4, A5, A6); fetal distress (A1, A6); and maternal aszites (A2). The time-schedule for apheresis treatments was not predefined but tried to separate subsequent treatments by at least 2 days. The clinician in charge orientated on the individual clinical condition and laboratory tests. Apheresis treatments were well tolerated and only minor complications were observed: one had to be terminated due to an extravasat; during two, patients suffered temporarily from a drop in blood pressure; and at the end of one apheresis we noted that a clot had formed in the tubing.

Pregnancy time from admission to birth averaged 15.0 days in patients who underwent apheresis (A1–A6). In contrast, the 6 control subjects' pregnancies (C1–C6) lasted an average of 6.3 days (p = 0.027). However, unexpectedly one pregnancy in the control group (C2) lasted over 19 days when CTG-worsening lead to delivery by CS (Table 2 and Fig. 1).

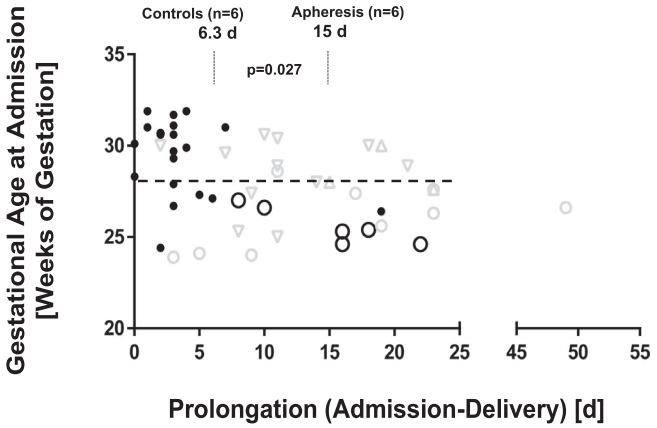

Fig. 1. Time from admission to delivery (prolongation of pregnancy) according to gestational age at admission. Black open circles: apheresis patients A1–A6. Black closed circles: control subjects; below 28th GW control subjects C1–C6. Reference apheresis trials in preeclampsia are shown with gray symbols: Open gray circles represent data by Wang et al. [16]; open upright [18] and downright [19] gray triangles represent prolongation times via DSC-apheresis reported by Thadhani et al. Horizontal dotted line represents the admission threshold at the 28th GW. Only patients reporting before the 28th GW were considered for evaluation.

Fig. 1 shows the prolongation times of apheresis and control patients according to GA at admission. Although low in number, apheresis appears to be more effective in those patients presenting with earlier GA. To enable comparison with other apheresis trials in PE either with H.E.L.P-[16], or DSC-apheresis [18,19], the respective prolongation times are shown. In general, apheresis treatment resulted in similar prolongation times irrespective of the technique applied.

As expected, apheresis significantly reduced levels of total cholesterol (−33%), triglycerides (−43%), and LDL-cholesterol (−44%), with a minor reduction in HDL-cholesterol of 8% (Table 3).

sFlt-1 increased about four-fold initially after H.E.L.P.-apheresis (Table 4) but rapidly dropped to pre-apheresis levels (Fig. 2). However, we estimated the H.E.L.P-mediated change of sFlt-1 independent from the initial heparin-induced peak. From each treatment we considered the last sFlt-1 value before apheresis and took the earliest value after sFlt-1 reached baseline again but considered only values between 10 and 24 h after treatment has started. This appears reasonable because in non-pregnant individuals it was shown that the heparin-induced sFlt-1 rise returns to baseline within 10 h [24] and on the other hand the continuous increase of sFlt-1 immanent to preeclampsia may cause a bias beyond 24 h. Eighteen out of a total of 23 apheresis treatments qualified for evaluation. Heparin-independent changes of sFlt-1 show large heterogeneity from a decrease of −36.6% to an increase of 41.8%. However, the mean change was −0.32 ± 19.99% (median −4.6%, p = 0.292, data not shown). Thus, in this study H.E.L.P.-apheresis had no significant effect on sFlt-concentrations after the heparin-induced peak has passed. Of note sFlt-1 levels appear to stabilize during the course of repetitive H.E.L.P.-treatments (Fig. 2). Similar effects were observed for PLGF and thus the sFlt-1/PLGF ratio remained unaffected (Table 4).

We observed no significant difference in APGAR scores at 5 and 10 min, or in pH or BE of the umbilical cord (Table 2). Prematurity complications such as respiratory distress syndrome were similar in both groups. There were no cases of neonatal mortality and all infants were released in healthy conditions.

4. Discussion

Apheresis has been suggested as therapeutic option for the treatment of PE to prolong pregnancy [12] and hence to reduce prematurity-related morbidity and mortality for the newborn. Especially before the 28th GW prolongation of pregnancy may result in a 2–3% lower mortality risk for the unborn per gained day [25]. In the present study lipid-apheresis via the H.E.L.P.-technique resulted in pregnancies being prolonged in average by 15 days after patients were admitted to hospital, and by 9 days when compared to a control group. This may have translated into a calculated 20% lower fetal mortality risk compared to untreated pregnancies.

Recently suggested and actively debated is whether specific removal of sFlt-1 may be a useful therapeutic target in preeclampsia. It is presumed that the favorable clinical effects of DSC-apheresis – another lipid-apheresis technique – is exclusively attributable to the removal of sFlt-1 [18,19]. Thadhani and colleagues thus proposed that rather than the initial DSC-a more specific antibody-based sFlt-1-apheresis should be applied [19]. In preparation of their attempts to remove sFlt-1 via apheresis the study group also explored *ex vivo* the ability of H.E.L.P.-apheresis to remove sFlt-1, and found that H.E.L.P. was almost 85% less effective to remove sFlt-1 compared to DSC [18].

However, since already in the study of Wang et al. [16] H.E.L.P. apheresis was clinically similar effective the hypothesis that the clinical benefit of DSC-apheresis in PE may be attributed exclusively to the removal of sFlt-1 was doubted [20]. This objection has now been proven in a therapeutic setting: although not reducing circulating sFlt-1 levels H.E.L.P.-apheresis showed again to be clinically effective.

All our patients initially presented with baseline sFlt-1/PlGF ratios > 85, a value predictable for adverse outcome. In the study by

Table 3
Treatment effect of apheresis on lipid parameters.

Patient	number of Apheresis treatments per patient	CH [mmol/L] before	after	%-change	TG [mmol/L] before	after	%-change	LDL-C [mmol/L] before	After	%-change	HDL-C [mmol/L] before	After	%-change
A1	3	6.7	4.3	−36	2.4	1.4	−39	3.6	2.0	−44	2.4	2.2	−11
A2	5	4.0	2.5	−39	1.7	0.9	−46	1.8	0.8	−57	1.5	1.4	−7
A3	4	5.7	3.2	−43	2.5	1.3	−49	3.1	1.5	−51	1.7	1.5	−16
A4	6	5.2	3.7	−30	2.6	1.8	−30	2.1	1.2	−43	2.2	2.0	−10
A5	3	6.8	5.3	−21	5.6	3.4	−39	2.9	2.3	−18	2.1	2.1	0
A6	2	5.0	3.7	−27	1.1	0.6	−49	1.5	0.8	−49	2.8	2.6	−6
Mean ± SD or Median (min/max)		5.6 ± 1.04	3.8 ± 1.0	−33 ± 8	2.4 (1.1/5.6)	1.4 (0.6/3.4)	−43 (−30/−49)	2.5 ± 0.8	1.4 ± 0.7	−44 ± 13	2.1 ± 0.5	1.9 ± 0.5	−8 ± 5
p for change				< 0.001			0.027*			0.002			0.011

Changes were tested for significance by the paired *t*-test or the paired non-parametric Wilcoxon-test as indicated by *. Values represent averaged concentrations before and after apheresis as mean ± SD or median (min/max). P-values < 0.05 were considered significant.

Table 4
Treatment effect of apheresis on sFlt-1 and PLGF.

Patient	number of Apheresis treatments per patient	sFlt-1 [pg/mL] before	after	%-change	PlGF [pg/mL] before	after	%-change	sFlt-1/PlGF ratio before	after	%-change
A1	3	10,639	22,755	114	47	100	113	226	228	1
A2	5	36,148	75,173	108	40	99	148	904	759	-16
A3	4	7218	53,086	635	16	101	531	451	526	17
A4	6	8888	28,208	217	22	61	177	404	462	14
A5	3	13,116	75,824	478	17	56	229	772	1354	75
A6	2	32,004	> 85,000*	> 166*	18	87	383	1778**	–	–
Mean ± SD		18002 ± 12671	51009 ± 25110	311 ± 236	27 ± 13	84 ± 20	264 ± 162	551 ± 279**	666 ± 429	18 ± 35
p for change				0.017*			0.001			0.406

Changes were tested for significance by the paired *t*-test. Values represents averaged concentrations before and after apheresis as mean ± SD.
* Denotes above sFlt-1 detection limit and was not considered for further calculation.
** As consequence the corresponding value before apheresis was not considered to calculate the average sFlt-1/PlGF ratio before apheresis and the % change of sFlt-1/PlGF ratio due to apheresis. P-values < 0.05 were considered significant.

Rana et al. 3 in 4 patients with a sFlt-1/PlGF ratio > 85 needed delivery within 7 days [26]. However, none of our patients were delivered within 7 days even with sFlt-1/PlGF ratios of more than 1000.

We noticed a presumably heparin-induced [27] transient 4-fold increase of sFlt-1 with each treatment cycle that rapidly cleared to pre-apheresis levels – obviously without any sign of clinical worsening. However, independent from the transient heparin-induced effect H.E.L.P.-apheresis did not result in a significant immediate change of sFlt-1 levels. Although we are not aware of studies examining the slope of sFlt-1 levels in longitudinal measurements once the cut-off of 85 has been reached, it was implied that sFlt-1 levels increase with ongoing preeclampsia. Of note, repeated H.E.L.P.-apheresis resulted in similar sFlt-1 peak-levels and sFlt-1 levels remained stable with repeated apheresis cycles until termination of pregnancy. Thus, the temporary heparin-induced release of vast amounts of sFlt-1into the circulation appears not to lead to a significant clearance and depletion of sFlt-1. However, even if heparin increases circulating sFlt-1 and may enhance urinary elimination to some extent [27], heparin-treatment in general seems not to result in favorable outcome [28].

The failed expected continuous increase of sFlt-1 during the course of pregnancy needs further consideration. sFlt-1 is a valuable marker for preeclampsia. However, in our study prolongation of preeclamptic pregnancies by H.E.L.P.-apheresis was not associated with a direct treatment-associated change of sFlt-1. Therefore, it seems unlikely that removing sFlt-1 may be the causative factor for the observed prolongation of pregnancy. On the other hand if H.E.L.P.-apheresis induces beneficial effects independent of sFlt-1 this may then in turn be mirrored by sFlt-1. For example elution of toxic factors like lipid peroxides and metabolic active lipids by H.E.L.P. apheresis may ameliorate toxic effects on the placenta and subsequently may reduce sFlt-1 release into the maternal circulation. This would, however, render sFlt-1 from a crucial pathogenic target for apheresis-treatment rather to a marker monitoring the clinical benefit of intervention. This interesting theory is certainly speculative and merits further investigation. However, the question arises as to what factor/s other than sFlt-1 may be relevant for the observed beneficial effects.

Besides lipid-lowering, other pleiotropic effects related to lowering inflammatory markers, and improving rheology are already reported for H.E.L.P.-apheresis in preeclampsia [16]. Improved microcirculation may also be an unspecific but relevant factor. There is evidence that H.E.L.P.-apheresis reduces plasma viscosity by 20% and erythrocyte aggregation by 60%, implying that H.E.L.P.-apheresis could alleviate various microcirculatory disorders like retinal ischemia, critical limb ischemia, and sudden hearing loss [29].

Our group has reported previously on the association between triglyceride-rich remnant lipoproteins and PE [12] and other pathologies in pregnancy like acute fatty liver of pregnancy [30]. Consequently, lipid-modifying therapies like apheresis were suggested as possible therapeutic options to prolong pregnancies complicated by PE [12].

However, other lipid-lowering therapies, namely the use of statins and especially pravastatin have been proposed to treat or prevent such obstetrical complications. Some maintain that the positive effects of pravastatin and other statins may be attributed to some kind of secondary pleiotropic effects, namely the reversal of various pathophysiological pathways associated with preeclampsia, specifically the angiogenetic imbalance of sFlt-1 and PLGF present in preeclampsia [31]. In fact, recently pravastatin was successfully tested as a therapeutic option to prevent complications in antiphospholipid syndrome refractory to anti-thrombotic therapy [32] and in the prevention of preeclampsia in high-risk pregnant women [33].

Like statins, the two lipid-lowering apheresis techniques DSC and H.E.LP. were initially designed to primarily address lipid-metabolism lowering lipid-levels by about 40–70% [20]. However, they obviously differ in their sFlt-1-lowering potential. Consequently, we feel it justified to suggest that rather than specifically reducing sFlt-1 levels, the modulation of other factors like lipids and/or other pro-inflammatory

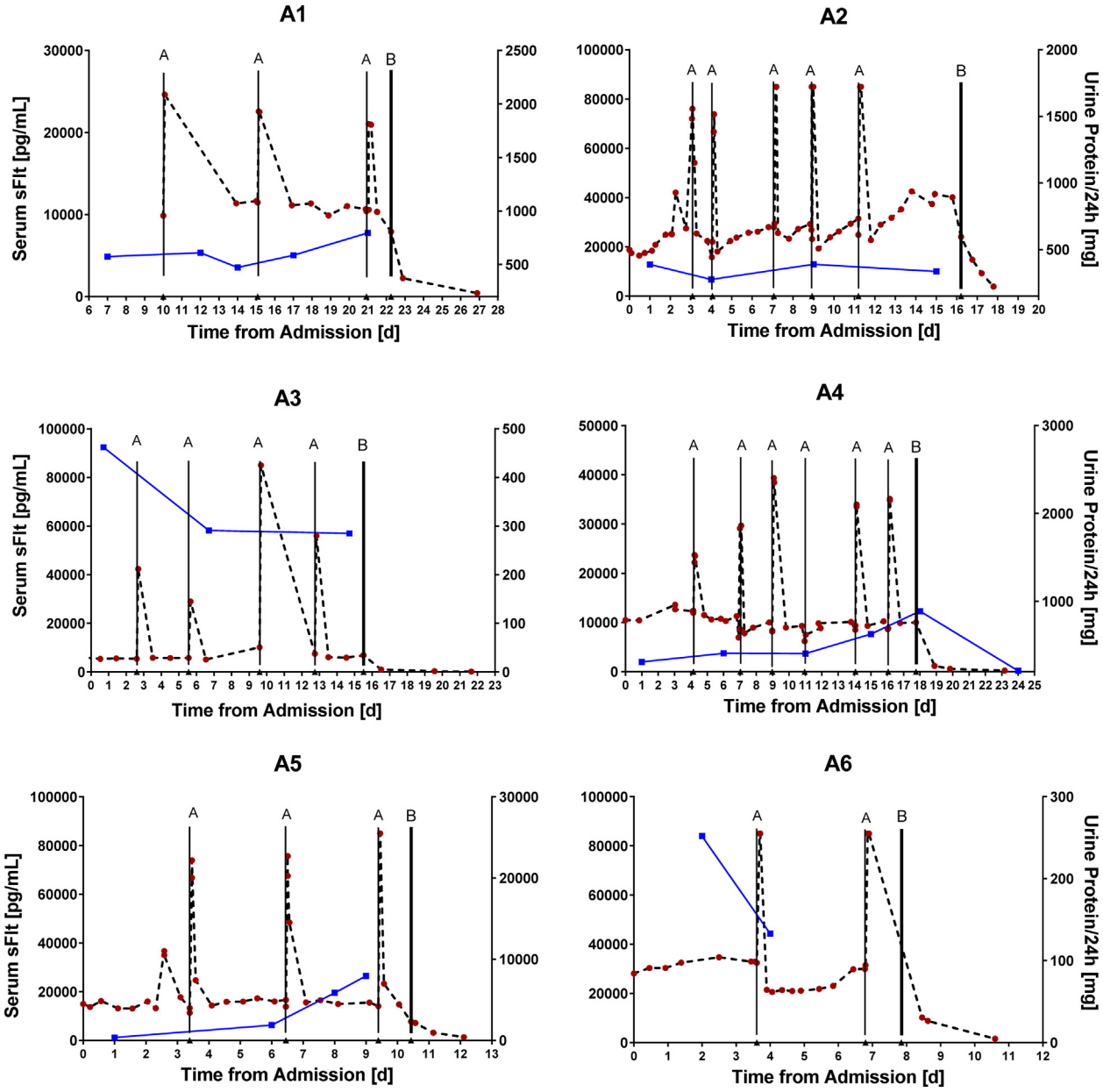

Fig. 2. Individual sFlt-1 levels and urinary 24 h-protein-excretion during the course of multiple apheresis treatments. Panels illustrate individual values of each treated patient A1 through A6 over the observation time. Red circles and black dashed lines represent sFlt-1 measurements, blue squares and solid lines represent urinary 24 h-protein-excretion. Vertical lines marked A represent time of apheresis treatment and vertical lines marked B denote time of delivery. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and rheologic factors may be reasonable candidates that may explain the clinical benefit of apheresis.

However, the interpretation of this study may be limited by the low number of patients included and the historic control group referred to for comparison. It finally may not be ruled out that the clinical benefit of apheresis may rely merely on some kind of a placebo effect. But this may only be addressed by a randomized controlled trial using sham-apheresis for comparison.

5. Author contribution

KW initiated the study and wrote the manuscript. FM-E, MK and HP were in obstetrical charge of preeclamptic women. SZ and BKö organized and supervised the apheresis treatment and BKö was responsible for subsequent amendments and correspondence with the Ethics Review Committee. BKr identified and characterized the control subjects. CC organized the clinical biochemistry of the study and contributed to discussion of the manuscript. GP evaluated the impact of H.E.L.P.-apheresis on sFlt-1 concentrations, contributed to the scientific discussion and edited the manuscript, and DS supplied the data on newborn outcomes. UP contributed to the evaluation strategy, discussion, and reviewed and edited the manuscript. The company B. Braun (Melsungen, Germany) supported this study by providing a Plasmat Futura® system to the maternity room at the Department of Gynecology and Obstetrics, University Medical Center Freiburg. KW was supported by a grant for an independent investigator-initiated trial by B. Braun. The authors have no other conflicts of interest to declare.

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