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Dysregulation of circulating autoantibodies against VEGF-A, VEGFR-1 and PlGF in preeclampsia - a role in placental and vascular health?

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Abstract:

Objectives:

Preeclampsia is a state of antiangiogenesis, with high levels of maternal circulating sVEGFR-1 (soluble vascular endothelial growth factor receptor 1, also named sFlt1) and low levels of PlGF (placenta growth factor). Various autoantibodies have been detected in preeclamptic patients. We hypothesize that circulating autoantibodies against VEGF-A (AA-VEGF-A), VEGFR-1 (AA-VEGFR-1) and PlGF (AA-PlGF) are present in preeclamptic women, with different levels from pregnant women with normotensive pregnancies. Secondly, we wanted to analyze if autoantibody levels are associated to sFlt1 or PLGF levels.

Study design:

Retrospective cross sectional study of 88 women with singleton pregnancies who delivered at Oslo University Hospital of whom 46 had preeclampsia and 42 had uncomplicated

normotensive pregnancies. Novel immunoassays for IgG-autoantibodies against VEGFA, VEGFR-1 and PlGF were developed and serum samples were assayed.

Main outcome measures and results:

AA-VEGF-A, AA-VEGF-R1 and AA-PlGF were significantly lower in preeclamptic pregnancies (n=42) compared to normotensive pregnancies (n=46) ($p<0.05$). On unadjusted analysis, only AA-VEGFA and AA-VEGFR-1 were predictors of PE, but none were independent predictors after adjusting for BMI (body mass index) and parity. In the subgroup of normotensive and PE women with overlapping sVEGFR-1/PlGF-ratios, AA-VEGF was a significant predictor of PE with AUC: 0.735.

Conclusion:

IgG autoantibodies against VEGF-A VEGFR-1 and PlGF can be found in pregnant women. They are dysregulated in preeclampsia. The roles of these autoantibodies are unknown, but this study suggests they play a protective role in pregnancy. The levels of AA against VEGF-A, VEGFR-1and PlGF might be important factors contributing to anti-angiogenesis regulation.

Keywords: Pregnancy, preeclampsia, VEGF, sFlt, autoantibody

Abbreviations

AA: autoantibody

ACOG: American College of Obstetricians and Gynecologists

AT1: angiotensin II type-1

CVD: Cardiovascular Disease

E. Coli: Escherichia Coli

GW: Gestational Week

PE: Preeclampsia

SGA: Small for Gestational Age

sVEGFR-1: Soluble Vascular Endothelial Growth Factor Receptor 1

PlGF: Placental Growth Factor

VEGF: Vascular Endothelial Growth Factor

VEGF-A: Vascular Endothelial Growth Factor A

VEGF-B: Vascular Endothelial Growth factor B

VEGFR-1: Vascular Endothelial Growth Factor Receptor 1

VEGFR-2: Vascular Endothelial Growth Factor Receptor 2

1. Introduction

Preeclampsia (PE) is a pregnancy-specific disease, traditionally defined as de novo hypertension (>140/90) after 20th gestational week accompanied by proteinuria, although other PE associated findings like new onset thrombocytopenia and renal impairment may substitute for new onset proteinuria in the latest definitions (1). It affects about 1-7% of all pregnant women (2), causing considerable fetal and maternal morbidity.

Dysregulation with high levels of maternal circulating sVEGFR-1 (soluble vascular endothelial growth factor receptor 1, also named sFlt1) and low levels of PlGF (placenta growth factor) has been detected in PE (3). The circulating levels of sVEGFR-1 and PlGF are mainly placentally derived during pregnancy (4). A high ratio of sVEGFR-1/PlGF may also predict the development of PE in many (5-11), but not all studies (12). Failure to detect a correlation between an elevated sVEGFR-1/PlGF ratio and PE may reflect the heterogeneous nature of the syndrome, including variations in the placental contribution (4, 13) and potential involvement of other vascular related factors.

Immunological dysregulation is believed to be essential in the pathophysiology of PE (14). While the T-cell contributions in PE and pregnancy has been studied extensively , the role of the B-cell population is less explored (15). Some notable exceptions are antiphospholipid antibodies, as reviewed in (16), and antibodies against the angiotensin II type-1(AT1)-receptor, which have been shown to be increased in preeclampsia (17, 18). The latter has been shown to activate the AT1-receptor, and passive transfer of AT1-autoantibodies induces a preeclamptic phenotype in rodents (19, 20). Thus, B-cell mediated immunity through autoantibody activity likely plays a role in the pathophysiology of PE. It is therefore of

interest to examine if levels of other autoantibodies to protein targets involved in the pathophysiology of PE are altered in PE.

VEGF-A is expressed in most tissues and promotes angiogenesis through stimulation of VEGFR-1 and VEGFR-2 on endothelial cells (21, 22). The affinity of VEGF-A to VEGFR-1 is 10-fold higher than to VEGFR-2 although the most potent angiogenic effect is believed to result from the binding of VEGF-A to VEGFR-2(21).

PlGF is expressed mainly in placenta in pregnant woman, but very low levels are also produced in non-pregnant subjects (23). PlGF is suggested to potentiate angiogenesis, partly in concert with other members of the VEGF family, by activating VEGFR-1 and by preventing VEGF-A binding to VEGFR-1 and thereby redistributing VEGF-A to activate VEGFR-2 (24). The interplay between several of the molecules in the VEGF-family is thought to be important for placentation and for the development of excessive maternal vascular inflammation secondary to placental dysfunction (4, 13), and are suggested as important for development of the clinical signs of both early- and late-onset types of preeclampsia.(25)

The aim of this study was to explore if IgG autoantibodies against three important proteins involved in angiogenesis, VEGF-A, VEGFR-1 and PlGF, are present and dysregulated in PE by using an ELISA approach developed by one of the authors (HH) to measure maternal levels of these autoantibodies. Furthermore, we wanted to explore associations between these autoantibodies to circulating levels of sVEFGR-1 and PlGF and to explore if the levels of autoantibodies could be useful biomarkers in the prediction or diagnosis for preeclampsia.

2. Materials and methods

This study included 88 women with singleton pregnancy recruited prior to delivery to the Oslo Pregnancy Biobank, at Oslo University Hospital between 2001 and 2009. The pregnancies were either uncomplicated (named controls, n= 46, all normotensive pregnancies) or complicated by PE (n= 42). All women were fasting, except for three (one control and two women with preeclampsia). None had preexisting hypertension, chronic disease, rupture of membranes, diabetes (gestational or pregestational), clinical signs of infection or were in labor at blood sampling. PE was defined (as well as severity of PE) according to the 2002 criteria of the American College of Obstetricians and Gynecologists (ACOG) including women with new onset hypertension and proteinuria after gestational week 20.

Gestational week (GW) at delivery was defined by routine ultrasound screening at GW 17-20. Newborn gender specific baby weight percentiles were calculated according to Norwegian fetal growth curves (26). Small for gestational age (SGA) was defined as birth weight below the 10th percentile. Obesity was defined as a body mass index (BMI) $\geq$ 30kg/m^2 at the time of delivery. All participating women provided written informed consent. The study was approved by the Regional Committee of Medical Research Ethics in South-Eastern Norway.

Maternal serum blood was obtained prior to delivery from an antecubital vein without intravenous infusion. None of the women were in active labour or had rupture of fetal membranes or signs of infection. After 30-60 minutes at room temperature, serum was obtained by centrifugation at room temperature (10min, 2000x*g*) and stored at -80°C until assay. ~~and stored as previously described .~~ All autoantibodies were measured in serum

samples using a sandwich ELISA kit (CellTrend GmbH Luckenwalde, Germany). The results are presented as Units/mL. The laboratory was blinded to clinical diagnosis.

The microtiter 96-well polystyrene plates ~~were~~ used for the different autoantibodies were coated with a sequence of its protein target which was dissolved in phosphate buffer together with a blocking and ELISA solution buffer according to the manufacturer. For AA-VEGF-A a polypeptide consisting of amino acids 27 to 191 of human VEGF-A derived from cell line SF21, for AA-VEGFR-1 human sVEGFR-1 for AA-PlGF the human PlGF of the sequence of Ala21-Arg149 derived from Escherichia Coli (E. Coli) were used. Supplemental Table 1 shows the amino acid sequences of target proteins.

To maintain the conformational epitopes of the target proteins~~receptor~~, 1 mM calcium chloride was added to every buffer. Duplicate patient samples of a 1:100 serum dilution were incubated at 4°C for 2 hours. After washing steps, plates were incubated for 60 minutes with a 1:20.000 dilution of horseradish-peroxidase–labeled goat anti-human IgG (Company, Jackson, USA) used for detection. The ELISAs were validated according to the FDA's "Guidance for industry: Bioanalytical method validation" (27).

Standard curves were obtained by incubating plates with test sera from a patient positive for the autoantibody in question. To set a standard for the concentrations of the autoimmune antibodies, standard curves were generated for all autoantibodies by diluting serum samples of a systemic sclerosis patient, the concentrations corresponding to different standard points for each aAb measured (Supplemental Table 2). Each standard point was performed in duplicates and the optical densities were determined.

Serum levels of sVEGFR-1 and PlGF were determined using Elecsys immunoassays (Roche Diagnostics, Rotkreuz, Switzerland) utilizing a fully automated electrochemoiluminiscence immunoassay platform (Cobas E analyzer, Roche Diagnostics).

Statistical analyses were performed with the Statistical Package for the Social Sciences (PASW Statistics 18). Differences in continuous variables between groups were tested by non-parametric Mann-Whitney tests. Fischer exact test was used to calculate correlation coefficients. Logistic regression was used for the adjusted analysis. In the first step, each baseline variable was analyzed. Variables which differed significantly were included in the second step where these variables were analyzed simultaneously in multivariate logistic regression analysis. A probability level of <0.05 was considered statistically significant.

3. Results

Clinical characteristics are presented in Table 1.

Table 1

There were no significant differences between the PE and control group regarding median age at delivery, smoking, ethnicity, gravidity or fetal sex. The PE group had in median value a significantly higher BMI, shorter pregnancy duration at blood sampling and delivery, lower fetal and placental weight and were more often primiparous compared to controls. Twenty of the PE patients delivered prior to GW 37 as compared to 2 of the controls. One of the controls, and 13 of the PE patients delivered prior to GW 34. Following clinical standards, 19 women received antenatal steroids for fetal lung maturation due to prematurity. Seventeen of

the PE patients had severe disease. Seventeen women delivered a SGA baby, 16 in the PE group and 1 in the control group.

All three autoantibodies could be detected in the serum of pregnant women. As shown in Figure 1, 2 and 3 the PE group had statistically significant lower levels of all the three studied AAs compared to controls.

Figure 1

Figure 2

Figure 3

Association between AA and a PE diagnosis according to parity and obesity: two major traditional risk factors for preeclampsia

In nulliparous women with PE, levels of AA-VEGFR-1 and AA-PlGF were significantly lower than in controls. In parous women AA-VEGFR-1 was significantly lower in women with PE compared to controls. In non-obese women levels of AA-VEGFR-1 and AA-PlGF were significantly lower in women with PE compared to controls. In obese women with PE levels of AA-VEGFA and AA-VEGFR-1 were significantly lower than in controls. (See Table 2)

Table 2

Adjusted analyses of the association between AA and a preeclampsia diagnosis

AA-VEGFA (OR: 0.992, 95%CI: 0.985-1.000, p=0.040) and AA-VEGR-1 (OR: 0.985, 95%CI: 0.974-0.997, p=0.012) were statistically associated with PE on unadjusted analysis, while AA-PLGF was not (OR: 0.964, 95%CI: 0.906-1.026, p=0.254. When adjusted for BMI at delivery and parity, none of the AAs were statistically significantly associated with PE (data not shown).

In nulliparous women, levels of AA-VEGFR-1 (OR: 0.980, 95%CI: 0.962-0.997, p=0.025) and AA-PlGF (OR: 0.807, 95%CI: 0.658-0.990, p=0.040) were associated with PE. This was also the case after adjusting for BMI. When adjusted for sVEGFR-1 and PlGF, AA-VEGFR-1 and AA-PlGF were not independently associated with PE (data not shown). In parous women, none of the AAs were associated with PE (data not shown).

In non-obese women AA-VEGFR-1 (OR: 0.977 95%CI: 0,959-0,996, p=0.017) and AA-PlGF (OR: 0.759, 95%CI: 0.601-0.958, p=0.020) were significant predictors of PE on unadjusted analysis. When adjusted for BMI, AA-VEGFR-1 (OR: 0.979, 95%CI: 0.961-0.997, p=0.023) and AA-PlGF (OR: 0.776, 95%CI: 0.618-0.975, p=0.029) were still significant predictors of PE. When adjusted for sVEGFR-1 and PlGF, AA-VEGFR-1 and AA-PlGF were not independent predictors of PE (data not shown). In obese women none of the AAs were predictors of PE (data not shown).

Can autoantibodies help identifying preeclampsia pregnancies when circulating PlGF and sFlt1 levels are not dysregulated?

The group of preeclamptic women had lower median level of PlGF (72.4 vs 134.6 pg/mL, p<0.001) and higher median level of sVEGFR-1 (11303 vs 3847 pg/mL, p<0.001) and sVEGFR-1/PlGF-ratios (161.58 vs 25.69, p<0.001) as compared to the control group, but not

all women with PE had dysregulated sFlt of PlGF levels as compared to all controls (Supplemental Figure 1, 2 and 3). This is in accordance with previous studies. (5-11)

Interestingly, as shown in Table 3, controls having similar levels of PlGF as women with PE had significantly higher levels of AA-VEGFR-1, but not AA-VEGFA nor AA-PlGF. Controls having similar levels of sVEGFR-1/PlGF-ratios as women with PE had significantly higher levels of AA-VEGFA and AA-VEGFR-1, but not AA-PlGF. In the group of controls and women with PE having overlapping levels of sVEGFR-1, there were no significant differences in any AA level.

Table 3

In the group of women with PE and controls with overlapping sFlt/PlGF-ratios AA-VEGF-A was significantly associated with PE (OR: 0.987, 95%CI: 0.975-0.998, p=0.023).There were no significant differences in age at delivery, gravidity, parity, BMI, ethnicity, fetal sex and placental weight between controls and women with PE in this group, but fetal weight was significantly different (p=0.007) ROC-analysis for the ability of AA-VEGFA for identifying absence of PE provided AUC=0.735.

4. Discussion

This is the first study showing that circulating autoantibodies against VEGF-A, VEGFR-1 and PlGF can be found in pregnant women. We found that women with PE have lower levels of them as compared to controls using a novel immunoassay. All the three studied autoantibodies are members of the VEGF-family, involved in angiogenesis and vascular health. Dysregulation of sVEGFR-1 and PlGF has previously been shown to be associated

with prediction and diagnosis of especially early-onset PE, and is believed to play a part in the pathogenesis of some, but not all cases of PE.(5-12, 25) Especially at term, the PE group is less often identified with dysregulated maternal circulating PlGF or sVEGFR-1 as compared to controls.(25) Our finding that normotensive women with similar sFlt/PlGF-ratios as the PE group prior to delivery having higher levels of AA-VEGFA than in the PE group, may be of important clinical and pathophysiological importance that needs testing in larger and longitudinal (prospective) pregnancy studies. As a low maternal sFlt/PlGF-ratio has shown in a large prospective study of pregnant women with "suspected PE" to predict the absence of PE,(11) it is interesting to see that higher levels of AA-VEGFA is associated with the absence of PE in the group with overlapping sFlt/PlGF-ratios.

The roles of the studied autoantibodies in PE and vasculatory inflammatory diseases are as yet unknown, precluding any definite conclusions about their effect. We speculate they might to some extent explain why some women with adverse levels of sVEGFR-1 and PlGF do not develop preeclampsia. It has been postulated that increased levels of sVEGFR-1 cause increased binding of circulating free PlGF and free VEGFA to sVEGFR-1, thereby impairing their normal binding to VEGFR-1 and VEGFR-2 and hence reduced vascular relaxation (28) Although other explanations may indeed be possible, we speculate that the autoantibodies against VEGFR-1 and VEGFR-2 may alleviate this process by stimulating the VEGFR-1 and VEGFR-2 in the absence of PlGF and VEGFA and hence stall the development of preeclampsia.

In general, a role of IgG autoantibodies is little explored in pregnancy. A 40 year old study by Taylor et al (29) showed that maternal serum from pregnant women prevented trophoblasts lysis by maternal lymphocytes whereas removing IgG from the serum significantly reduced

this *in vitro* effect. Asymmetric IgG antibodies are antibodies that bind to their corresponding antigen without eliciting immunologic responses. Borel et al (30) found that the level of asymmetric IgG antibodies was doubled in sera from pregnant women compared to non-pregnant controls, and that the level was even higher in the placenta. It might therefore be speculated that the circulating autoantibodies we found suppressed in PE are of an inhibitory or asymmetric type, beneficial for the development of an uncomplicated pregnancy. Interestingly, Braicu et al found that autoantibodies against VEGFA and VEGFR-1 were decreased in epithelial ovarian cancer and that lower levels of these autoantibodies were associated with a poorer prognosis, yielding support for a protective effect of these autoantibodies.(31)

We found no significant differences between early- ~~and~~ and late-onset PE (defined as delivery either before or after GW 34/ GW 37) or between clinically severe and non-severe PE. Early- and late-onset PE have been suggested to represent the end-point of different pathways into the PE syndrome, but with significant overlap and with placenta as an important common mediator (13, 25). The studied autoantibodies may be markers of placental dysfunction or syncytiotrophoblast stress in general (25). All except one of the cases with SGA offspring did belong to the PE group, hence the effect of placental dysfunction in more general terms could not be differentiated. Longitudinal studies are needed to establish when in pregnancy the autoantibodies we have studied are altered, as compared to normal pregnancy. Also prepregnancy and postpartum studies are needed to establish whether the dysregulated autoantibody situation in preeclampsia is present prior to pregnancy, and sustained after delivery, without the presence of placenta.

Although fetal growth restriction and placentation problems are more common features of early than late onset PE, all forms of PE are likely to be placentally mediated (13, 25). Interestingly, in relation to the classical two-stage placental model of preeclampsia development, with placental dysfunction as the first important pathophysiological step (25), all proteins targeted by the novel autoantibodies studied in this project are placentally expressed. Their potential pathophysiological role in relation to placental dysfunction may extend beyond preeclampsia, and could be tested in relation to preterm birth, fetal growth restriction and placental abruption.

We found decreased levels of autoantibodies (VEGFR1-AAs and PlGF-AAs) in non-obese women, also after adjusting for BMI, whilst there were no significant findings in obese women in the adjusted analysis. Obesity is associated with lower antibody levels in general and is a risk factor of PE.(32, 33) Hence, we speculate that these autoantibodies might be protective for PE in lean women and that the relative impairment of autoantibody production in obese women may play a part in the PE pathophysiology. Due to lack of power, we could not explore this hypothesis further,

A bulk of studies shows an association between PE and increased risk of cardiovascular disease (CVD). (34, 35) All the protein targets for the autoantibodies we have studied in the present paper have been shown to be implicated in the development of atherosclerotic CVD (36, 37), consistent with common pathways for CVD and PE and also PE as an independent risk factor for CVD. However, the autoantibodies in our study have at present not been evaluated in relation to CVD risk. We therefore suggest exploring a role for these autoantibodies as potential biomarkers for identifying young women at highest risk for long-term CVD after pregnancy complications such as PE.

The strength of this study lies within the uniform procedure of blood sampling, storing, analyzation and a well described clinical cohort in addition to novel assays for of IgG autoantibodies against the VEGF protein families. The AA assays have been validated as documented in the Supplemental Tables and previously tested ~~on~~ on nonpregnant healthy subjects. Further research on circulating autoantibodies against VEGF-A, VEGFR-1 and PlGF is warranted. Isolation of the autoantibodies and further characterization by other techniques, such as surface plasmon resonance to confirm antigen-antibody interaction are important next steps.

This is a first pilot study publishing the presence of these autoantibodies in humans, and therefore also the first report from pregnancy in general and from preeclampsia in particular. Also, our women with PE had developed PE at the time of blood-sampling. Hence, a possible predictive ability of AA-VEGF has to be further evaluated in prospective studies.

The lack of biological understanding regarding the functions of these autoantibodies and a limited study size suggests that further studies are needed to obtain further pathophysiological understanding for their role in pregnancy and placental dysfunction in general and in PE in particular. Although our study found suppressed levels of autoantibodies in preeclamptic pregnancies, their exact action on their target proteins remains unknown. The limited knowledge about the functions of the protein targets of the autoantibodies we have investigated suggests complex interactions.

5. Conclusion

To our knowledge, this is the first published study exploring the presence of circulating autoantibodies to the family of VEGF proteins, whether under physiological or pathological conditions. The studied autoantibodies do all bind to members of the VEGF-family which are implicated in angiogenesis and vascular health. Dysregulation of sVEGFR-1 and PlGF has previously been shown to be associated with the development of PE. We found significant lower autoantibody levels in preeclamptic as compared to uncomplicated pregnancies. Furthermore, elevated levels of autoantibodies against VEGFR-1, and VEGFA were found in controls compared to women with preeclampsia, when the levels of sVEGFR-1, PlGF and sVEGFR-1/PlGF-ratios were of similar ranges. This may suggest that autoantibodies against the VEGF family play a protective role against pregnancy hypertensive disorders and placental dysfunction. Our data indicate that other pathways disrupting normal angiogenesis and vascular health in pregnancy may also be involved, including other members of the VEGF family. A potential role for these autoantibodies as biomarkers for PE and as biomarkers for the augmented risk for longterm cardiovascular disease after PE and other placentally related pregnancy complications needs also to be explored.

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FIGURE LEGENDS:

Figure 1: Box plot of levels of autoantibodies (Units/mL) against VEGF-A in normotensive controls and women with preeclampsia.

Figure 2:
Box plot of levels of autoantibodies (Units/mL) against VEGFR-1 in normotensive controls and women with preeclampsia.

Figure 3:
Box plot of levels of autoantibodies (Units/mL) against PlGF in normotensive controls and women with preeclampsia.

Supplemental Figure 1:
Dot blot of levels of PlGF (pg/mL) in normotensive controls and women with preeclampsia. Light grey indicates area of overlapping PlGF-values between normotensive controls and women with preeclampsia.

Supplemental Figure 2:
Dot blot of levels of sFlt (pg/mL) in normotensive controls and women with preeclampsia. Light grey indicates area of overlapping sFlt-values between normotensive controls and women with preeclampsia.

Supplemental Figure 3:
Dot blot of levels of sFlt/PlGF-ratios in normotensive controls and women with preeclampsia. Light grey indicates area of overlapping sFlt/PlGF-ratio-values between normotensive controls and women with preeclampsia.

TABLES:

Table 1: Clinical characteristics of the study population

	Total (n=88)		**Controls (n=46)**		**Preeclampsia (n=42)**		
	Median (Range)	n (%)	Median (Range)	n (%)	Median (Range)	n (%)	p
Age at delivery (years)	33(18-41)		34 (22-41)		33 (18-41)		0.548
BMI prepregnancy/early pregnancy (kg/m^2)	23.8 (18.1-49.0)		23.2 (18.1-39.7)		24.4 (19.7- 49.0)		0.021
Pregnancy duration at delivery (days)	269 (177-293)		270 (177-293)		260 (177-290)		<0.005
Pregnancy duration at blood sampling (days)	267 (170-287)		270 (170-281)		257 (187-277)		<0.005
Fetal weight at delivery (g)	3280 (540-5036)		3581 (856-4548)		2746 (540-5036)		<0.005
Placental weight (g)	595 (130-1200)		645 (420-1200)		545 (130-1100)		<0.005
Smoking at time of blood sampling		2 (2.3)		1 (2.2)		1 (2.4)	0.730
Caucasian ethnicity		81 (91.3)		42 (91.3)		3 (92.9)	0.551
Primiparous		47 (53.4)		18 (39.1)		29 (69.0)	0.006
Primigravida		31 (35.2)		13 (28.3)		18 (42.9)	0.113
Female fetal sex		37 (42.1)		19 (41.3)		18 (42.9)	0.527

p: p-value for difference between women with preeclampsia and pregnant controls on the variable in question. Mann-Whitney test for continuous variables and Fisher's exact for dichotomous variables.

Table 2: Levels of IgG autoantibodies against VEGFA, VEGFR-1 and PlGF in women with preeclampsia compared to normotensive controls in the total cohort and in groups according to parity and obesity

		Serum VEGFA IgG-autoantibodies (U/mL)			
		Pregnant controls		Preeclampsia	
	n	Median(Range)		Median(Range)	p
Total	88	65.52	(10.60-287.91)	49.71	(12.36-287.91)	0.026
Nulliparous	47	56.23	(10.60-287.91)	55.24	(12.36-194.05)	0.381
Parous	41	67.34	(18.32-287.91)	43.20	(14.64-287.91)	0.051
Non-obese	46	61.68	(10.60-287.91)	48.44	(12.36-194.05)	0.230
Obese	42	67.09	(18.32-255.10)	49.71	(13.56-287.91)	0.049

		Serum VEGFR-1 IgG-autoantibodies (U/mL)			
		Pregnant controls		Preeclampsia	
	n	Median(Range)		Median(Range)	p
Total	88	84.24	(8.29-313.91)	34.82	(8.33-340.93)	<0.001
Nulliparous	47	59.14	(8.29-126.30)	33.69	(8.33-115.38)	0.023
Parous	41	87.17	(26.50-313.91)	49.98	(21.96-340.93)	0.036
Non-obese	46	78.56	(8.29-313.91)	33.40	(8.33-115.38)	0.007
Obese	42	87.17	(26.50-126.30)	43.98	(11.98-340.93)	0.016

		Serum PlGF IgG-autoantibodies (U/mL)			
		Pregnant controls		Preeclampsia	
	n	Median(Range)		Median(Range)	p
Total	88	8.37	(2.71-66.44)	6.08	(2.04-71.09)	0.001
Nulliparous	47	7.80	(2.71-18.24)	5.78	(2.04-12.9)	0.040
Parous	41	8.37	(4.10-66.44)	7.11	(4.91-71.09)	0.080
Non-obese	46	8.31	(2.71-66.44)	5.89	(2.04-12.90)	0.007
Obese	42	8.50	(4.12-18.24)	7.03	(2.94-71.09)	0.070

VEGFA: Vascular endothelial growth factor A. VEGFR-1: Vascular endothelial growth factor receptor 1. PlGF: Placental growth factor. p: p-values when comparing women with preeclampsia to pregnant controls with the Mann-Whitney test in the group (e.g. obese women) in question.

Table 3: Levels of IgG autoantibody in women with PE and controls within similar ranges of sVEGFR-1, PlGF and sVEGFR-1/PlGF-ratio respectively

	sVEGFR-1 (4129-10995pg/mL)		
	Controls (n=19)	Preeclampsia (n=19)	
	Median (Range)	Median(Range)	p
AA-PlGF (U/mL)	7.13 (2.71-30.54)	5.99 (2.89-12.90)	0.258
AA-VEGF-A (U/mL)	82.57 (10.60-287.91)	47.12 (13.56-194.05)	0.091
AA-VEGFR-1 (U/mL)	63.03 (8.29-189.78)	45.96 (11.98-115.38)	0.154
	PlGF (54.7-230.9pg/mL)		
	Controls (n=28)	Preeclampsia (n=28)	
	Median (Range)	Median (Range)	p
AA-PlGF (U/mL)	7.70 (2.71-66.44)	6.08 (2.17-12.90)	0.066
AA-VEGF-A (U/mL)	63.08 (10.60-287.91)	49.71 (12-36-194.05)	0.068
AA-VEGFR-1 (U/mL)	63.27 (8.29-313.91)	34.31 (8.33-115.38)	0.019
	sVEGFR-1/PlGF-ratio (24.1-149.6)		
	Controls (n=21)	Preeclampsia (n=17)	
	Median (Range)	Median (Range)	p
AA-PlGF(U/mL)	7.76 (2.71-66.44)	6.17 (2.17-12.79)	0.060
AA-VEGF-A (U/mL)	89.92 (10.60-287.91)	23.15 (12.36-194.05)	0.012
AA-VEGFR-1 (U/mL)	63.03 (8.29-313.91)	34.00 (8.33-113.27)	0.031

AA-PlGF: Placental Growth Factor IgG autoantibodies. AA-VEGF-A: Vascular Endothelial Growth Factor IgG autoantibodies. AA-VEGFR-1: Vascular Endothelial Growth Factor IgG autoantibodies. p: p-value when comparing the autoantibody in question between controls and women with preeclampsia using the Mann-Whitney test.