



# Accurate Prediction of Total PIGF (Placental Growth Factor) From Free PIGF and sFlt-1 (Soluble Fms-Like Tyrosine Kinase-1): Evidence for Markedly Elevated PIGF Levels in Women With Acute Fatty Liver of Pregnancy

Rugina I. Neuman, Langeza Saleh, Koen Verdonk, Anton H. van den Meiracker, Henk Russcher, Herold J. Metselaar, Willy Visser, A.H. Jan Danser

**ABSTRACT:** Acute fatty liver of pregnancy (AFLP) is characterized by elevated circulating sFlt-1 (soluble Fms-like tyrosine kinase-1), although the free circulating levels of its ligand, PIGF (placental growth factor) are not decreased. Here, we hypothesized that women with AFLP exhibit elevated PIGF production in comparison to women with preeclampsia or hemolysis elevated liver enzymes and low platelet count syndrome. Making use of the well-known mathematical formulas describing drug-receptor interactions, we established that serum total PIGF could be accurately predicted from sFlt-1 and free PIGF levels ( $n=42$ ; mean calculated  $K_D$  of 50 pmol/L), yielding similar values as the previously published method of thermal dissociation of the sFlt-1-PIGF complexes ( $r=0.94$ ,  $P<0.0001$ ). We found that median levels of free PIGF were significantly lower in women with preeclampsia ( $n=13$ ; 117 pg/mL) or hemolysis elevated liver enzymes and low platelet count syndrome ( $n=12$ ; 59 pg/mL) compared with women without preeclampsia ( $n=11$ ; 349 pg/mL,  $P<0.0001$ ). In contrast, median total PIGF did not differ between women with no preeclampsia, preeclampsia, and hemolysis elevated liver enzymes and low platelet count syndrome (354 versus 435 versus 344 pg/mL), whereas it was markedly elevated in AFLP compared with all groups (2054 pg/mL,  $P<0.0001$ ). Furthermore, in AFLP, both sFlt-1 and total PIGF declined rapidly postdelivery, with significantly higher predelivery total PIGF ( $n=12$ ; median, 2054 pg/mL) than postpartum levels ( $n=14$ ; median, 163 pg/mL,  $P<0.0001$ ), suggesting that in AFLP, PIGF is largely placenta-derived. Collectively, our findings indicate that like sFlt-1, PIGF production is significantly upregulated in AFLP, mainly originating from the placenta. Importantly, total PIGF can now be easily calculated from already available free PIGF and sFlt-1 levels, allowing subsequent evaluation of other groups in whom PIGF is altered. (**Hypertension. 2021;78:489–498. DOI: 10.1161/HYPERTENSIONAHA.121.17258.**)

**Key Words:** biomarkers ■ fatty liver ■ HELLP syndrome ■ placental growth factor ■ pregnancy

**A**cute fatty liver of pregnancy (AFLP) is a severe liver disorder unique to pregnancy, typically occurring after 30 weeks' gestation.<sup>1</sup> Although uncommon, with an estimated global incidence of 1 in 7000 to 15 000 pregnancies, AFLP is a life-threatening disease for both mother and child.<sup>1</sup> The exact pathogenesis of AFLP remains unclear, but it is generally believed that mitochondrial oxidation defects of the fetus or placenta

lead to a buildup of free fatty acids in the maternal blood and hepatocytes, subsequently causing the detrimental manifestations of the disorder.<sup>1</sup> The most prominent characteristic of AFLP is the presence of hepatic dysfunction, reflected by complications such as hypoglycemia, coagulopathy, and renal failure.<sup>1,2</sup> Despite these distinctive features, differentiating AFLP from other liver diseases of pregnancy, particularly the hemolysis elevated

Correspondence to: A.H. Jan Danser, Department of Internal Medicine, Room Ee-1418B Erasmus Medical Centre, P.O. Box 2040, 3000 CA, Rotterdam, the Netherlands. Email a.danser@erasmusmc.nl

For Sources of Funding and Disclosures, see page 497.

© 2021 The Authors. *Hypertension* is published on behalf of the American Heart Association, Inc., by Wolters Kluwer Health, Inc. This is an open access article under the terms of the [Creative Commons Attribution](https://creativecommons.org/licenses/by/4.0/) License, which permits use, distribution, and reproduction in any medium, provided that the original work is properly cited.

*Hypertension* is available at [www.ahajournals.org/journal/hyp](http://www.ahajournals.org/journal/hyp)

## Novelty and Significance

### What Is New?

- The levels of total PIGF (placental growth factor) can be accurately predicted from free PIGF and sFlt-1 (soluble Fms-like tyrosine kinase-1) levels using the well-established mathematical formulas of drug-receptor interactions.
- Placental synthesis of PIGF is significantly increased in acute fatty liver of pregnancy (AFLP) compared with preeclampsia or hemolysis elevated liver enzymes and low platelet count syndrome.

### What Is Relevant?

- Total PIGF can now be easily calculated from already available free PIGF and sFlt-1 levels, allowing the evaluation of other groups in whom PIGF might be up- or downregulated.

### Summary

Total PIGF levels were determined in women with AFLP compared with women without preeclampsia, preeclampsia, and hemolysis elevated liver enzymes and low platelet count syndrome. Calculation of total PIGF using mathematical formulas of drug-receptor interactions was shown to be accurate and yielded similar levels to the recently described method of thermal dissociation. Although total PIGF in preeclampsia and hemolysis elevated liver enzymes and low platelet count syndrome were similar to that in uneventful pregnancies, maternal total PIGF levels in AFLP were greatly elevated compared with all groups. In AFLP, like sFlt-1, total PIGF declined massively after delivery, suggesting that in AFLP, the increased levels of total PIGF are likely placenta-derived.

## Nonstandard Abbreviations and Acronyms

<b>AFLP</b>	acute fatty liver of pregnancy
<b>ALT</b>	alanine aminotransferase
<b>AST</b>	aspartate aminotransferase
<b>D</b>	nonreceptor-bound drug
<b>DR</b>	drug-occupied receptors
<b>GA</b>	gestational age
<b>HELLP</b>	hemolysis elevated liver enzymes and low platelet count
<b>LDH</b>	lactate dehydrogenase
<b>PIGF</b>	placental growth factor
<b>rhPIGF</b>	recombinant human PIGF
<b>sFlt-1</b>	soluble Fms-like tyrosine kinase-1
<b>sVEGFR</b>	soluble vascular endothelial growth factor receptor

liver enzymes and low platelet count (HELLP) syndrome, remains a challenge in clinical practice.<sup>2,3</sup> An important factor underlying this difficulty might be that up to 20% of women with AFLP are also diagnosed with preeclampsia, a condition characterized by hypertension in the second half of pregnancy along with proteinuria or signs of maternal organ damage, including HELLP syndrome.<sup>1,4,5</sup> Whether preeclampsia and AFLP are merely associated with one another or belong to a spectrum of the same disorder, as some have suggested,<sup>6,7</sup> is uncertain.

During preeclampsia, poor placentation triggers the excessive release of the sVEGFR (soluble vascular endothelial growth factor receptor; also known as sFlt-1 [soluble Fms-like tyrosine kinase-1]), which binds its free circulating ligands VEGF and PIGF (placental growth factor).<sup>8,9</sup> The

ensuing angiogenic imbalance is thought to contribute significantly to the clinical manifestations of this disorder.<sup>10</sup> Interestingly, our group has recently discovered that women with AFLP also display increased levels of sFlt-1 in their maternal circulation.<sup>11</sup> Yet, despite the markedly elevated sFlt-1 levels, women with AFLP exhibited higher free levels of PIGF, in contrast to what is observed in HELLP syndrome.<sup>11</sup>

Based on our previous observations, we hypothesized that women with AFLP display elevated PIGF production in comparison to preeclampsia/HELLP syndrome. Recently, Lecarpentier et al<sup>12</sup> established a novel method to measure total PIGF in maternal blood because commercial immunoassays only detect the unbound (free) form of PIGF.<sup>12</sup> Hence, in the present study, we intended to (1) validate this method in a small population of women with a low and high sFlt-1/free PIGF ratio; (2) determine whether a more simple approach, by calculating total PIGF mathematically from sFlt-1 and free PIGF, would prove equally reliable as thermal dissociation; and (3) compare serum total PIGF levels in women with AFLP to women with no preeclampsia, preeclampsia or HELLP syndrome. In addition, we explored the origin of the angiogenic markers in AFLP and whether they could derive from the placenta.

## METHODS

All data and supporting materials have been provided within the published article.

### Human Participants

#### Participants With AFLP

Human serum samples from a database of women with singleton pregnancies who had a clinical diagnosis of AFLP at the

Erasmus Medical Center, Rotterdam, the Netherlands between 2005 and 2020 were utilized. Serum samples were collected at the time of AFLP diagnosis (both during and after pregnancy) and later archived at  $-80^{\circ}\text{C}$  as part of routine care. Residual material with enough volume for the analysis of sFlt-1, free and total PIGF was collected for this study if the patients did not object against use of this material. The use of these samples for the purposes of this study was exempted from approval by the local institutional Medical Ethics Committee according to the Dutch Medical Research with Human Participants Law (MEC-2020-0668). All laboratory assays were undertaken masked to the clinical diagnosis. Clinicians had no knowledge of the angiogenic measurements at time of AFLP diagnosis. The diagnosis of AFLP was suspected when a pregnant woman had symptoms of nausea, vomiting, fatigue, and anorexia at the end of the second or third trimester in combination with jaundice and elevated liver enzymes. The diagnosis of AFLP was confirmed when a woman fulfilled  $\geq 6$  out of 15 Swansea criteria,<sup>13</sup> and the treating physician found the clinical diagnosis of AFLP as most likely in comparison to other disorders, such as HELLP syndrome. Pregnancy characteristics and outcome were obtained from the digital medical files.

### Participants With No Preeclampsia, Preeclampsia, or HELLP Syndrome

We aimed to compare all women with AFLP to 12 gestational age (GA) matched women with either no preeclampsia, confirmed preeclampsia, or HELLP syndrome, given that the values of sFlt-1, free, and total PIGF alter with advancing gestation.<sup>14</sup> Available residual serum samples from these three groups were collected from a previously conducted prospective cohort study in which the sFlt-1/free PIGF ratio was measured in singleton pregnancies with suspected or confirmed preeclampsia, between 2013 and 2016 at 3 hospitals in the Netherlands. This study was approved by the research ethics committee (MEC-2013-202), and written informed consent was obtained from all participants. Venous blood was taken at study entry only and was stored at  $-80^{\circ}\text{C}$  until analysis, which was conducted at the end of the study, to avoid influence on decision making of the obstetricians.<sup>9</sup> Preeclampsia was defined as the presence of new-onset hypertension (systolic blood pressure of  $\geq 140$  mm Hg or diastolic blood pressure of  $\geq 90$  mm Hg) and proteinuria (protein-to-creatinine ratio  $\geq 30$  mg/mmol or  $\geq 300$  mg/24 hours or 2+ dipstick) at or after 20 weeks' gestation, according to the 2001 International Society for the Study of Hypertension in Pregnancy definition, which was in effect at the time of study initiation.<sup>15</sup> HELLP syndrome was defined as a reduction of platelet count  $< 100 \times 10^9/\text{L}$ , an elevation of ALT (alanine aminotransferase) or AST (aspartate aminotransferase) 2-fold the upper limit of normal, and an elevated LDH (lactate dehydrogenase; 2-fold the upper reference limit or  $> 650$  IU/L) according to the International Society for the Study of Hypertension in Pregnancy 2013 definition.<sup>16</sup> Women who had a partial HELLP syndrome ( $\geq 2$  of the HELLP criteria) at time of blood sampling but later developed HELLP syndrome were also considered as HELLP syndrome. Women who were initially suspected of preeclampsia but did not fulfill the diagnosis of gestational hypertension, preeclampsia, or HELLP syndrome throughout their pregnancy were defined as no preeclampsia.

For the validation studies, we selected serum samples from patients with a low angiogenic imbalance (sFlt-1/free PIGF

ratio  $\leq 38$ ) versus a high angiogenic imbalance (sFlt-1/free PIGF ratio  $\geq 85$ ) from the abovementioned cohort study. These cutoff values were previously reported to predict the short-term absence of preeclampsia (sFlt-1/free PIGF ratio  $\leq 38$ )<sup>17</sup> or a high risk of preeclampsia-related adverse outcomes (sFlt-1/free PIGF ratio  $\geq 85$ ).<sup>18</sup> All pregnancy characteristics and outcome were obtained from digital medical files.

### Participants With Acute Liver Failure

Available serum samples from nonpregnant women with acute liver failure requiring immediate liver transplantation at the Erasmus Medical Center, Rotterdam, the Netherlands, were selected at random, for the measurement of sFlt-1 and total PIGF. The use of these samples for this study was exempted from approval by the local institutional Medical Ethics Committee according to the Dutch Medical Research with Human Participants Law (MEC-2014-060).

## Measurement of sFlt-1, Free PIGF, and Total PIGF

### Validation Studies

rhPIGF (human recombinant PIGF; 264-PGB-010/CF, R&D Systems, Minneapolis, MN) was dissolved in PBS containing 0.1% BSA (PBS). This solution was mixed 1:3 with either PBS or serum to reach a final rhPIGF concentration of 1693 pg/mL. For comparison, serum samples were also mixed 1:3 with PBS not containing rhPIGF. Serum was obtained from pregnant patients with either a low ( $\leq 38$ ) or a high ( $\geq 85$ ) sFlt-1/free PIGF ratio. All samples (rhPIGF-containing PBS, serum with PBS, and serum with rhPIGF-containing PBS) were incubated for 30 minutes at room temperature to allow rhPIGF to bind to sFlt-1. Next, sFlt-1 and PIGF were measured in all samples before and after heating.

### Heating Procedure and Biochemical Measurements

For the thermal dissociation of all sFlt-1-PIGF complexes, serum samples were placed in a heating block at  $70^{\circ}\text{C}$  for 10 minutes, as described by Lecarpentier et al.<sup>12</sup> Measurements of sFlt-1 and PIGF before and after heating were performed using the automated Elecsys immunoassay from Roche Diagnostics (Cobas 6000, e-module; Rotterdam, the Netherlands).

## Calculation of Total PIGF from Free PIGF and sFlt-1

In blood plasma, an equilibrium exists between free sFlt-1, PIGF, and their complex, described by the following equation:  $[\text{sFlt-1}] + [\text{PIGF}] \rightleftharpoons [\text{sFlt-1-PIGF}]$ . This equals classic drug-receptor interaction, with [sFlt-1] resembling the total number of receptors  $[\text{R}]_{\text{total}}$ , free PIGF ( $[\text{PIGF}]_{\text{free}}$ , ie, the PIGF level measured without heating) resembling the amount of nonreceptor-bound drug [D], and [sFlt-1-PIGF] resembling the number of drug-occupied receptors [DR]. The dissociation constant  $K_D = [\text{D}] \times [\text{R}] / [\text{DR}]$ . Given that  $[\text{R}]_{\text{total}} = [\text{R}] + [\text{DR}]$ , this formula can be rewritten as  $K_D = -[\text{D}] + [\text{D}] \times [\text{R}]_{\text{total}} / [\text{DR}]$ . Since [DR] can be calculated by subtracting  $[\text{PIGF}]_{\text{total}}$  (ie, the PIGF level obtained after heating) from  $[\text{PIGF}]_{\text{free}}$  and considering that the molecular weights of PIGF and sFlt-1 are 34 and 100 kD, respectively, it is now possible to calculate  $K_D$ . This approach was followed in all samples where free and total PIGF levels were available,

with total PIGF being higher than free PIGF, allowing us to calculate a mean  $K_D$  on the basis of 42 samples. With this  $K_D$  we were able to predict  $[PIGF]_{total}$  by first calculating  $[DR]$ , that is, the amount of PIGF bound to sFlt-1, as follows:

$$[DR] = \frac{[D] \times [R]_{total}}{[D] + K_D} \text{ and by then adding up } [D] \text{ and } [DR].$$

Since  $[D]=[PIGF]_{free}$  and  $[R]_{total}=[sFlt-1]$ , this translates to

$$[PIGF]_{total} = [PIGF]_{free} + \frac{[PIGF]_{free} \times [sFlt-1]}{[PIGF]_{free} + K_D}$$

## Statistical Analysis

Data are presented as median (interquartile range) or number (percentage). To evaluate whether continuous variables had a normal distribution, the Shapiro-Wilk normality test was used. To compare groups, the Student *t* test or Mann-Whitney *U* test in case of non-normally distributed data were applied. For the comparison of continuous variables between >2 groups, 1-way ANOVA, or Kruskal-Wallis test, in the case of nonparametric distributions was applied, with a Dunnett or Bonferroni correction for multiple testing. Spearman Rho was applied to calculate correlation coefficients. A *P* value of <0.05 was considered to be statistically significant. Statistical analysis was performed with GraphPad Prism (version 8.0, La Jolla, CA) and SPSS (version 25.0, SPSS Chicago, IL) on Windows.

## RESULTS

### Thermal Dissociation of sFlt-1-PIGF Complex Using Recombinant Human PIGF

To confirm the method of thermal dissociation, we added rhPIGF (recombinant human PIGF) to serum samples from pregnancies with a low ( $\leq 38$ ) or high sFlt-1/free PIGF ratio ( $\geq 85$ ), and measured sFlt-1 and PIGF before and after heating, with and without rhPIGF. Five participants with a ratio  $\leq 38$  and 6 participants with a ratio  $\geq 85$  were evaluated, whose clinical characteristics and pregnancy outcomes are shown in Table 1. In participants with a ratio  $\leq 38$ , serum PIGF levels marginally (*P*=nonsignificant) increased after thermal dissociation. Heating did not affect the detection of rhPIGF, and also after adding rhPIGF to serum we did not detect higher levels after heating than before (Figure 1A).

In contrast, in participants with a ratio  $\geq 85$ , serum PIGF levels were significantly higher after thermal dissociation than before (270 versus 36 pg/mL). Furthermore, when adding a fixed amount of rhPIGF to serum in this group (ratio  $\geq 85$ ), the levels detected in the absence of heating were significantly lower than the amount that was added (1634 versus 2221 pg/mL). This confirms binding by sFlt-1. Subsequent heating allowed the detection of a PIGF amount that equaled the sum of the added level and the endogenous level (2372 pg/mL; Figure 1A). Finally, as expected, the sFlt-1 levels were much higher in the ratio  $\geq 85$  group, whereas heating greatly diminished the amount of sFlt-1 that could be detected. These data

**Table 1. Participant Characteristics at Time of Blood Sampling of the Validation Cohort Based on sFlt-1/Free PIGF Ratio**

Parameter	Ratio $\leq 38$ (n=5)	Ratio $\geq 85$ (n=6)
Maternal age, y	32 (27–33)	30 (27–33)
GA at blood sampling, wk <sup>±d</sup>	32 <sup>±4</sup> (31 <sup>+0</sup> –33 <sup>+4</sup> )	32 <sup>+0</sup> (31 <sup>+3</sup> –38 <sup>+1</sup> )
Nulliparity, n	2	5
Ethnic background, n		
White	1	4
African/Afro-Caribbean/Black	2	1
Other	2	1
Clinical parameters		
SBP, mmHg	120 (115–145)	140 (129–149)
DBP, mmHg	80 (73–95)	93 (89–100)
uPCR, mg/mmol	61 (23–174)	45 (18–309)
Pregnancy outcome		
GA at delivery, wk <sup>±d</sup>	37 <sup>±4</sup> (33 <sup>+3</sup> –38 <sup>+3</sup> )	32 <sup>+5</sup> (32 <sup>+0</sup> –38 <sup>+4</sup> )
Male, n	3	4
Birth weight, g	2684 (2120–3538)	1605 (1453–2880)
Birth weight percentile <10, n	1	1

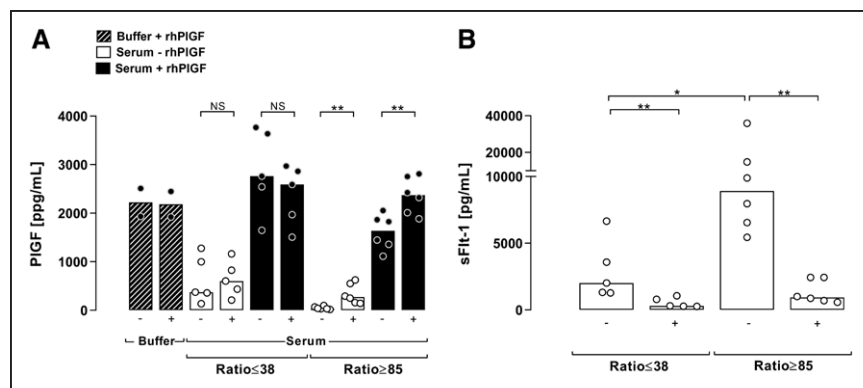
Values are median (interquartile range) or n. DBP indicates diastolic blood pressure; GA, gestational age; PIGF, placental growth factor; SBP, systolic blood pressure; sFlt-1 indicates soluble Fms-like tyrosine kinase-1; and uPCR, urinary protein-to-creatinine ratio.

confirm that heating selectively destroys sFlt-1, without affecting PIGF (Figure 1B), thus allowing the quantification of total PIGF.

### sFlt-1, Free, and Total PIGF in Women With AFLP

Twelve women with AFLP were compared with women with no preeclampsia (n=11), confirmed preeclampsia (n=13), or HELLP syndrome (n=12). Because most of the women with HELLP syndrome from the previously conducted cohort<sup>9</sup> had a lower GA at blood sampling, we were unable to adequately match the AFLP group to women with HELLP syndrome at a similar GA. All participant characteristics and pregnancy outcomes according to clinical diagnosis are shown in Table 2. Women with AFLP displayed higher median sFlt-1 levels (77 762 [45 044–116 657] pg/mL) compared with women with no preeclampsia, preeclampsia, and HELLP syndrome (2518 [1744–3903], 8772 [6410–10 736] and 14 572 [5641–20 056] pg/mL, respectively; Figure 2A). When comparing free PIGF levels, women diagnosed with preeclampsia and HELLP syndrome displayed lower PIGF values than women with no preeclampsia, whereas free PIGF levels in women with HELLP syndrome were also significantly decreased in comparison to women with AFLP (59 [39–97] pg/mL versus 208 [106–293] pg/mL; Figure 2B). In contrast, total PIGF levels (measured after thermal dissociation) did not differ between women with no preeclampsia, preeclampsia, and HELLP





**Figure 1. Protein level measurements performed before and after heating.** PIGF (placental growth factor; **A**) and sFlt-1 (soluble Fms-like tyrosine kinase-1; **B**) levels in buffer (n=2) or serum measured before [–] and after [+] heating at 70 °C for 10 min, with or without the addition of rhPIGF (human recombinant PIGF; mixed 1:3 in either PBS or serum to reach a final rhPIGF concentration of 1693 pg/mL). Serum was obtained from pregnant women with an sFlt-1/free PIGF ratio  $\leq 38$  (n=5) or  $\geq 85$  (n=6). Data are presented as individual values and median (bar). \*P<0.05; \*\*P<0.01.

syndrome, whereas they were significantly increased in AFLP in comparison to all groups (Figure 2B). None of the women were on heparin treatment at time of blood sampling.

### Prediction of Total PIGF and Comparison With Its Measured Value

$K_D$  was calculated in all samples in which we determined both free and total PIGF. This resulted in an average  $K_D$  ( $\pm$ SEM) value of  $50 \pm 6.4$  pmol/L. Next, we predicted total PIGF in each sample making use of this  $K_D$  value, and compared it with the actually measured total PIGF value. As can be seen in Figure 2C, this yielded a relationship that was not different from the line of identity ( $r=0.94$ ,  $P<0.0001$ ; Figure 2C).

### Antepartum Versus Postpartum Levels of sFlt-1, Total PIGF, and ALT

In 12 women with AFLP, measurements of sFlt-1 and total PIGF were performed antepartum. In 5 of these 12 women, postpartum levels were additionally measured, and in 3 of these 5, postpartum measurements were performed at 2 separate time-points. Additionally, there were 66 women with AFLP in which the values of sFlt-1 and total PIGF were determined postpartum only. Figure 3A and 3B show that the levels of sFlt-1 decreased by  $>80\%$  within 2 days after delivery, whereas for total PIGF, the drop was even larger, with  $<10\%$  remaining after 2 days. This pattern was fully confirmed when simply comparing the median levels before delivery with those after, irrespective of the sampling moment (Figure 3C). The postpartum course of ALT values in AFLP patients was much more gradual (Figures 3E and 3F).

### sFlt-1 and Total PIGF Levels in Participants With Acute Liver Failure in Need of Liver Transplantation

Median levels for sFlt-1 were available in 7 patients, whereas total PIGF was measured in 8 patients with

liver failure in need of liver transplantation. The clinical characteristics of these patients are shown in Table 3. All patients were female with a median (interquartile range) of 28 (24–31) years. The most common reason for liver transplantation was toxic or drug-induced liver failure (n=4). Median total PIGF levels of these patients were 22 (12–51) pg/mL, which is comparable to the reference values for free PIGF in healthy nonpregnant women (16 [14–18] pg/mL; Figure 3D).<sup>19</sup> In contrast, median sFlt-1 levels (446 [211–1414] pg/mL) were  $\approx 6$ -fold above the normal range in healthy nonpregnant women (76 [67–84] pg/mL).

## DISCUSSION

In the present study, we established that total PIGF levels in serum can be accurately calculated from the sFlt-1 and free PIGF levels, making use of the well-known mathematical formulas describing drug-receptor interaction. This approach yielded the same levels as the previously published method of thermal dissociation. We confirmed that total PIGF in preeclampsia is similar to that in uneventful pregnancy, although this is also true for HELLP. Yet, in AFLP, maternal total PIGF levels were greatly elevated, both versus women with preeclampsia and HELLP syndrome. Furthermore, in AFLP, total PIGF and sFlt-1 declined rapidly after delivery, suggesting the placenta as their most likely source.

A potential role for sFlt-1 and PIGF in the pathophysiology of AFLP had not been suggested until recently, when our group and others<sup>11,20</sup> noticed abundantly high circulating sFlt-1 levels in women with this disorder. sFlt-1 is a well-recognized feature of preeclampsia, in which its dramatic rise causes a significant reduction of free circulating PIGF levels.<sup>10</sup> Although a decrease in placental PIGF production has also been suggested to contribute to the low PIGF levels in preeclamptic disease,<sup>21,22</sup> we observed no differences in total PIGF when comparing women without preeclampsia, preeclampsia, or HELLP syndrome in the current study. This agrees with the view of Lecarpentier et al<sup>12</sup> that decreased free PIGF levels in preeclampsia / HELLP are a consequence of higher

**Table 2. Pregnancy Characteristics of All 48 Participants According to Clinical Diagnosis**

Parameter	No PE	PE	HELLP	AFLP
N	11	13	12	12
Maternal age, y	32 (28–35)	30 (28–33)	29 (26–32)	30 (28–34)
GA at blood sampling, wk <sup>±d</sup>	35 <sup>+4</sup> (34 <sup>+0</sup> –37 <sup>+1</sup> )	35 <sup>+3</sup> (32 <sup>+2</sup> –38 <sup>+1</sup> )	32 <sup>+0</sup> (30 <sup>+4</sup> –33 <sup>+4</sup> )*†	36 <sup>+1</sup> (35 <sup>+1</sup> –38 <sup>+0</sup> )‡
Nulliparity, n (%)	4 (36)	9 (69)	12 (100)*	9 (75)
Ethnic background, n (%)				
White	6 (54)	9 (69)	10 (83)	10 (83)
African/Afro-Caribbean/Black	2 (18)	1 (8)	0 (0)	1 (8)
Other	3 (27)	3 (23)	2 (17)	1 (8)
Clinical findings				
SBP, mmHg	120 (111–125)	140 (140–168)*	140 (128–144)*	127 (115–156)
DBP, mmHg	79 (74–85)	100 (90–100)*	91 (86–96)	85 (73–91)
uPCR, mg/mmol	15 (9–50)	84 (40–178)*	166 (24–662)*	29 (18–54)
Creatinine, μmol/L	56 (54–63)	63 (59–69)	68 (61–83)	158 (128–206)*†‡
ALT, U/L	17 (13–25)	15 (9–25)	170 (107–415)*†	372 (129–944)*†
LD, U/L	178 (160–195)	224 (193–253)	409 (275–870)*†	475 (367–753)*†
Platelet count, 10 <sup>9</sup> /L	230 (182–269)	181 (146–245)	103 (66–156)*	215 (167–258)‡
Pregnancy outcome				
GA at delivery, wk <sup>±d</sup>	39 <sup>+0</sup> (37 <sup>+4</sup> –40 <sup>+2</sup> )	37 <sup>+0</sup> (34 <sup>+4</sup> –38 <sup>+2</sup> )*	32 <sup>+2</sup> (30 <sup>+5</sup> –35 <sup>+2</sup> )*†	36 <sup>+1</sup> (35 <sup>+1</sup> –38 <sup>+0</sup> )‡
Male, n (%)	5 (45)	8 (62)	4 (33)†	6 (50)
Birth weight, grams	3410 (2915–3700)	2950 (2205–3372)	1568 (1163–1968)*†	2660 (2165–3300)‡
Birth weight percentile <10	1 (9)	1 (8)	3 (25)	1 (8)
Angiogenic markers				
sFlt-1, pg/mL	2518 (1744–3903)	8772 (6410–10 736)	14 572 (5641–20 056)*	77 762 (45 044–116 657)*†‡
Free PIGF, pg/mL	349 (174–420)	117 (61–161)*	59 (39–97)*	208 (106–293)‡
Total PIGF, pg/mL	354 (284–406)	435 (302–497)	344 (265–604)	2054 (863–2597)*†‡
sFlt-1/free PIGF ratio	13 (5–19)	110 (43–240)	334 (90–523)*	452 (202–563)*

Values represent median (interquartile range) or n (%). AFLP indicates acute fatty liver of pregnancy; ALT, alanine aminotransferase; DBP, diastolic blood pressure; GA, gestational age; HELLP, hemolysis elevated liver enzymes and low platelet count; LD, lactate dehydrogenase; PE, preeclampsia; PIGF, placental growth factor; SBP, systolic blood pressure; sFlt-1, soluble Fms-like tyrosine kinase-1; and uPCR, urinary protein-to-creatinine ratio.

\* $P < 0.05$  for comparison with no PE.

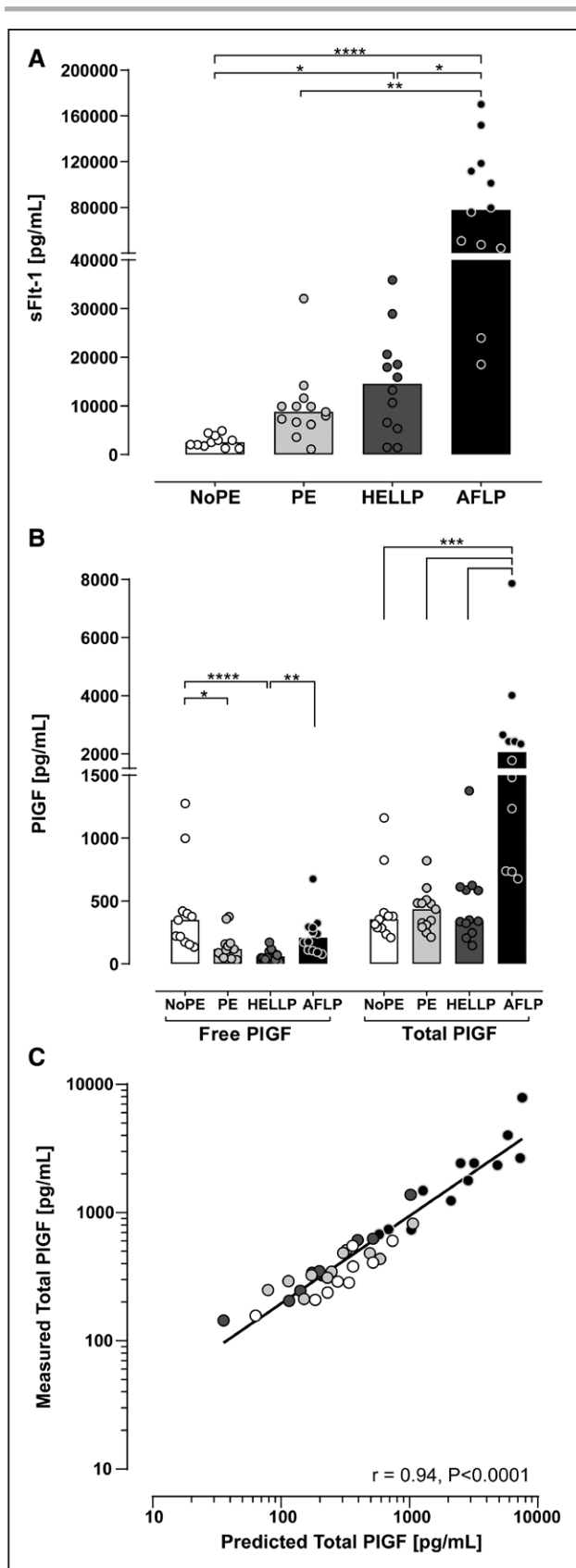
† $P < 0.05$  for comparison with PE.

‡ $P < 0.05$  for comparison with HELLP syndrome.

sFlt-1 rather than decreased PIGF production of the placenta.<sup>12</sup> With this perspective in mind, our previous observation that despite greater sFlt-1 elevation, free PIGF levels were not reduced in women with AFLP in comparison to women with preeclampsia, was quite surprising.<sup>11</sup> Our present finding that total PIGF levels are significantly raised in women with AFLP now explains this observation.

Obviously, a key question is whether an elevated production or impaired metabolism accounts for the dramatic increases of PIGF in AFLP. Because the relatively small PIGF (MW 34 kD) can readily cross the glomerular filtration barrier, one could hypothesize that its elimination is compromised as renal function declines in AFLP. The observation that total PIGF in women with this disorder decreased rapidly after delivery, despite the persistence of elevated serum creatinine (data not shown), does not support this argument. Another possibility is

that the ensuing liver injury and inflammation in AFLP drive increased PIGF production either in the liver or elsewhere. However, our observation that total PIGF is diminished by >90% within 2 days postpartum, whereas the liver recovers more gradually after delivery, indicates that a placental origin or placenta-stimulated PIGF synthesis somewhere else is more probable. Consistent with this hypothesis, serum total PIGF levels in nonpregnant patients with acute liver failure were roughly comparable to the free PIGF levels observed in healthy controls (median [interquartile range], 22 [12–51] versus 16 [14–18] pg/mL). Conversely, their sFlt-1 values were 6-fold higher in relation to the reference values of healthy nonpregnant women (76 [67–84] pg/mL). This suggests that liver failure might contribute, at least in part, to the higher sFlt-1 values observed in AFLP, but not to the higher PIGF levels. A partial nonplacental origin of sFlt-1 might



**Figure 2. Serum protein levels according to clinical diagnosis.** Antepartum serum sFlt-1 (soluble Fms-like tyrosine kinase-1; **A**) and PIGF (placental growth factor; free and total; **B**) (Continued)

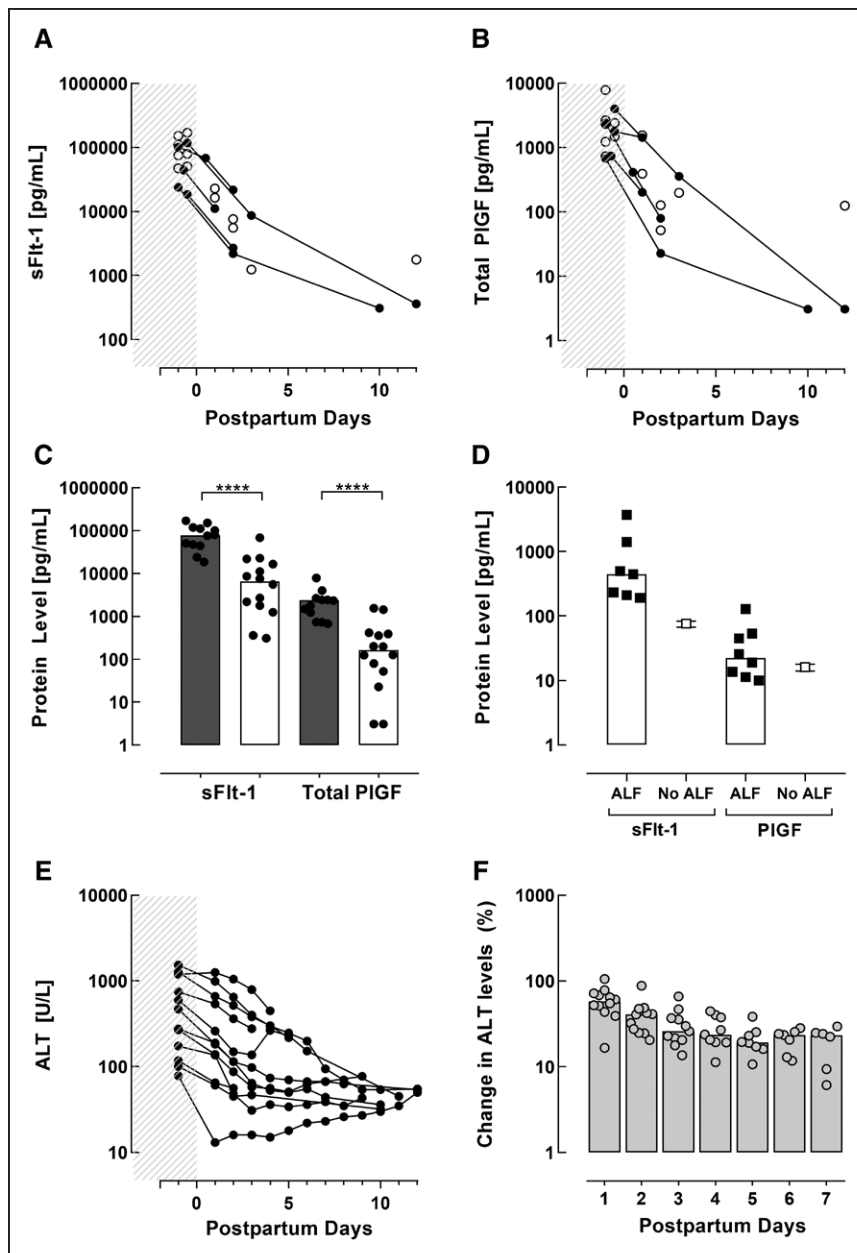
also explain why in AFLP women the drop in sFlt-1 levels after delivery was more modest in comparison to that of total PIGF. In both preeclampsia and HELLP, as we have demonstrated before, the opposite is true: sFlt-1 falls by >90% within 2 days, while free PIGF levels off at around 30% to 40% of its predelivery concentrations. Yet, postpartum, the free PIGF levels reach the same nadir in all groups and resemble the total PIGF levels. The simplest explanation of our findings is therefore that the stronger total PIGF drop in AFLP (exceeding that of sFlt-1) is due to its very high predelivery levels.

The exact contribution of the different PIGF isoforms (1–4) to the levels of total PIGF is unknown. Previous studies have indicated that PIGF-1 and PIGF-2 are the main isoforms found in maternal blood,<sup>23,24</sup> whereas commercial assays mainly measure free PIGF-1. Although differences between assay results may in part be due to different degrees of cross-reactivity, the serum levels of PIGF-1 and PIGF-2 are highly correlated in both normal and pathological pregnancies in all 3 trimesters.<sup>25</sup> This supports their common origin and control mechanisms. It has therefore been suggested that knowledge on the precise contribution of these isoforms may not be of added clinical significance.<sup>25</sup>

Accounting for other sFlt-1 binding ligands, such as VEGF, might further improve the accuracy of the predicted total PIGF levels. Ideally, a competitive binding model is set up that takes into account the interaction of all potential binding partners (in particular the various isoforms of both VEGF and PIGF) with sFlt-1. This would require a wide range of assays to measure all these binding partners, as well as knowledge on their affinities for sFlt-1. In reality, we were able to accurately predict total PIGF from sFlt-1 and free PIGF in pregnant women with a considerable variation in clinical background. This would argue against huge variation in VEGF (and thus its capacity to occupy sFlt-1-binding sites) between these conditions. Moreover, current assays provide the levels of sFlt-1 and PIGF only, and thus a simple model with these 2 readily available parameters remains preferred.

The limitations of our study must be addressed. Although the number of women evaluated with AFLP remains limited, this reflects the rarity of this disorder. In addition, we were unable to find women with HELLP syndrome with a similar GA as the women with AFLP. However, if anything, one would expect free PIGF levels to be further decreased in women with HELLP syndrome

**Figure 2 Continued.** levels in women with no preeclampsia (no PE; n=11), preeclampsia (PE; n=13), hemolysis (elevated liver enzymes and low platelet count (HELLP) syndrome (n=12) and acute fatty liver of pregnancy (AFLP; n=12). Data are presented as individual values and median (bar). \* $P < 0.05$ ; \*\* $P < 0.01$ , \*\*\* $P < 0.001$  and \*\*\*\* $P < 0.0001$ . **C**, Correlations between measured and predicted total PIGF according to no PE (white circles), PE (light gray circles), HELLP (dark gray circles) and AFLP (black circles; n=42,  $r = 0.94$ ,  $P < 0.0001$ ).



**Figure 3. Protein levels pre- and postpartum, and in patients with acute liver failure.**

**A** and **B**, sFit-1 (soluble Fms-like tyrosine kinase-1) and total PIGF (placental growth factor) values in women with acute fatty liver of pregnancy (AFLP) determined before (shaded area;  $n=12$ ) or after delivery ( $n=11$ , 14 measurements total), according to the number of postpartum days. Values are depicted as single ( $\circ$ ) or repeated measurements ( $\bullet$ ). **C**, Median and individual levels of sFit-1 or total PIGF in all women with AFLP before delivery (gray bars;  $n=12$ ) and after delivery (white bars;  $n=14$ ). Antepartum blood was taken  $\leq 2$  d before delivery, whereas postpartum blood was drawn at 0–12 d postpartum.  $***P<0.001$ . **D**, Median and individual levels of sFit-1 ( $n=7$ ) and total PIGF ( $n=8$ ) in nonpregnant women with acute liver failure ( $\blacksquare$ ) in comparison to reference values (median and interquartile range) in healthy nonpregnant women for sFit-1 and free PIGF ( $\square$ ). **E**, Alanine aminotransferase (ALT) values of all 12 women with AFLP determined before (shaded area) and after delivery, according to the number of postpartum days. **F**, Postpartum ALT values of all 12 women with AFLP depicted as percentages of antepartum values in the first week after delivery.

at a later GA because free PIGF levels start declining from 29 to 32 weeks' gestation until the end of pregnancy.<sup>14</sup> Lastly, it should be taken into account that the pregnancies of women defined as no preeclampsia were not entirely healthy, which might have influenced the interpretation of total PIGF in this population.

## PERSPECTIVES

At present, it is not known why PIGF production would be so much increased in AFLP. Oxidative stress in placental mitochondria has already been observed in AFLP,<sup>26</sup> and this is widely accepted to upregulate sFit-1.<sup>10,27</sup> Moreover, the poor uteroplacental perfusion as a consequence of liver failure and the ensuing hypovolemia in AFLP, is likely to have the same consequence.<sup>2</sup>

Yet, in AFLP, unlike preeclampsia,<sup>10</sup> there is no evidence for abnormal placental development. Thus, one plausible theory might be that in AFLP the normal placenta is still able to counteract the increases of sFit-1 by massively upregulating PIGF. As a consequence, the free PIGF levels in this disorder are in the normal pregnancy range. An alternative scenario could be that the inflammatory response and endothelial disruption following liver injury trigger the release of sFit-1 and PIGF. Clearly, potential stimuli of sFit-1 or PIGF, like proinflammatory cytokines and free fatty acids, are worth exploring in AFLP, if possible in liver and placental tissue.

Our study is the first to present a simple mathematical approach to obtain the total PIGF levels. Not surprisingly, our observed  $K_D$  falls within the binding



**Table 3. Characteristics of Nonpregnant Women With Acute Liver Failure**

Parameter	Acute liver failure
N	8
Age, y	28 (24–31)
Female/male	8/0
Parity	
Nulliparous	3
Multiparous	4
Unknown	1
Ethnicity	
White	5
African/Black	1
Other/unknown	2
Reason for liver failure	
Drug-induced or toxic	4
Autoimmune	1
Wilson disease	1
Sepsis	1
Unknown	1
Angiogenic markers	
sFlt-1, pg/mL	446 (211–1414)
Total PIGF, pg/mL	22 (12–51)

Values are median (interquartile range) or n. PIGF indicates placental growth factor; and sFlt-1 indicates soluble Fms-like tyrosine kinase-1.

affinity reported for both PIGF and VEGF in relationship to the Flt-1 receptor ( $\approx 20$ – $200$  pmol/L).<sup>28,29</sup> Consequently, total PIGF can now be easily calculated from already available free PIGF and sFlt-1 levels, allowing the subsequent evaluation of other groups in whom PIGF might be upregulated or downregulated, for instance in preeclamptic women with intrauterine growth restriction. In addition, the increased total PIGF concentrations might aid in distinguishing AFLP from other liver disorders of pregnancy, particularly HELLP syndrome. To evaluate this, future studies should firstly validate our findings in a separate cohort of AFLP pregnancies.

## ARTICLE INFORMATION

Received March 18, 2021; accepted June 1, 2021.

### Affiliations

Division of Pharmacology and Vascular Medicine, Department of Internal Medicine (R.I.N., L.S., K.V., A.H.v.d.M., W.V., A.H.J.D.), Department of Gynecology and Obstetrics (R.I.N., W.V.), Department of Clinical Chemistry (H.R.), and Department of Gastroenterology & Hepatology (H.J.M.), Erasmus MC University Hospital, Rotterdam, the Netherlands.

### Sources of Funding

R.I. Neuman and A.H. van den Meiracker are supported by the Dutch Foundation Lijf en Leven.

### Disclosures

None.

## REFERENCES

- Liu J, Ghaziani TT, Wolf JL. Acute fatty liver disease of pregnancy: updates in pathogenesis, diagnosis, and management. *Am J Gastroenterol*. 2017;112:838–846. doi: 10.1038/ajg.2017.54
- Nelson DB, Byrne JJ, Cunningham FG. Acute fatty liver of pregnancy. *Clin Obstet Gynecol*. 2020;63:152–164. doi: 10.1097/GRF.0000000000000494
- Nelson DB, Yost NP, Cunningham FG. Acute fatty liver of pregnancy: clinical outcomes and expected duration of recovery. *Am J Obstet Gynecol*. 2013;209:456.e1–456.e7. doi: 10.1016/j.ajog.2013.07.006
- Vigil-De Gracia P. Acute fatty liver and hellp syndrome: two distinct pregnancy disorders. *Int J Gynaecol Obstet*. 2001;73:215–220
- Brown MA, Magee LA, Kenny LC, Karumanchi SA, McCarthy FP, Saito S, Hall DR, Warren CE, Aoyagi G, Ishaku S; International Society for the Study of Hypertension in Pregnancy (ISSHP). Hypertensive disorders of pregnancy: ISSHP classification, diagnosis, and management recommendations for international practice. *Hypertension*. 2018;72:24–43. doi: 10.1161/HYPERTENSIONAHA.117.10803
- Minakami H, Oka N, Sato T, Tamada T, Yasuda Y, Hirota N. Preeclampsia: a microvesicular fat disease of the liver? *Am J Obstet Gynecol*. 1988;159:1043–1047. doi: 10.1016/0002-9378(88)90407-3
- Riely CA, Latham PS, Romero R, Duffy TP. Acute fatty liver of pregnancy. A reassessment based on observations in nine patients. *Ann Intern Med*. 1987;106:703–706. doi: 10.7326/0003-4819-106-5-703
- Steegers EA, von Dadelszen P, Duvetko JJ, Pijnenborg R. Pre-eclampsia. *Lancet*. 2010;376:631–644. doi: 10.1016/S0140-6736(10)60279-6
- Saleh L, Vergouwe Y, van den Meiracker AH, Verdonk K, Russcher H, Bremer HA, Versendaal HJ, Steegers EAP, Danser AHJ, Visser W. Angiogenic markers predict pregnancy complications and prolongation in preeclampsia: continuous versus cutoff values. *Hypertension*. 2017;70:1025–1033. doi: 10.1161/HYPERTENSIONAHA.117.09913
- Jim B, Karumanchi SA. Preeclampsia: pathogenesis, prevention, and long-term complications. *Semin Nephrol*. 2017;37:386–397. doi: 10.1016/j.semnephrol.2017.05.011
- Neuman RI, Hesselink ERM, Saleh L, van den Meiracker AH, Danser AHJ, Visser W. Angiogenic markers are elevated in women with acute fatty liver of pregnancy. *Ultrasound Obstet Gynecol*. 2020;56:465–466. doi: 10.1002/uog.21912
- Lecarpentier E, Zsengellér ZK, Salahuddin S, Covarrubias AE, Lo A, Haddad B, Thadhani RI, Karumanchi SA. Total versus free placental growth factor levels in the pathogenesis of preeclampsia. *Hypertension*. 2020;76:875–883. doi: 10.1161/HYPERTENSIONAHA.120.15338
- Morton A, Laurie J. Physiological changes of pregnancy and the Swansea criteria in diagnosing acute fatty liver of pregnancy. *Obstet Med*. 2018;11:126–131. doi: 10.1177/1753495X18759353
- Levine RJ, Maynard SE, Qian C, Lim KH, England LJ, Yu KF, Schisterman EF, Thadhani R, Sachs BP, Epstein FH, et al. Circulating angiogenic factors and the risk of preeclampsia. *N Engl J Med*. 2004;350:672–683. doi: 10.1056/NEJMoa031884
- Brown MA, Lindheimer MD, de Swiet M, Van Assche A, Moutquin JM. The classification and diagnosis of the hypertensive disorders of pregnancy: statement from the International Society for the Study of Hypertension in Pregnancy (ISSHP). *Hypertens Pregnancy*. 2001;20:IX–XIV. doi: 10.1081/PRG-100104165
- Tranquilli AL. Introduction to ISSHP new classification of preeclampsia. *Pregnancy Hypertens*. 2013;3:58–59. doi: 10.1016/j.preghy.2013.04.006
- Zeisler H, Llorca E, Chantraine F, Vatis M, Staff AC, Sennström M, Olovsson M, Brennecke SP, Stepan H, Allegranza D, et al. Predictive value of the sFlt-1:PIGF ratio in women with suspected preeclampsia. *N Engl J Med*. 2016;374:13–22. doi: 10.1056/NEJMoa1414838
- Rana S, Powe CE, Salahuddin S, Verloren S, Perschel FH, Levine RJ, Lim KH, Wenger JB, Thadhani R, Karumanchi SA. Angiogenic factors and the risk of adverse outcomes in women with suspected preeclampsia. *Circulation*. 2012;125:911–919. doi: 10.1161/CIRCULATIONAHA.111.054361
- Molvarec A, Szarka A, Walentin S, Szucs E, Nagy B, Rigó J Jr. Circulating angiogenic factors determined by electrochemiluminescence immunoassay in relation to the clinical features and laboratory parameters in women with pre-eclampsia. *Hypertens Res*. 2010;33:892–898. doi: 10.1038/hr.2010.92
- Suzuki H, Nagayama S, Hirashima C, Takahashi K, Takahashi H, Ogoyama M, Nagayama M, Shirasuna K, Matsubara S, Ohkuchi A. Markedly higher sFlt-1/PIGF ratio in a woman with acute fatty liver of pregnancy compared with HELLP syndrome. *J Obstet Gynaecol Res*. 2019;45:96–103. doi: 10.1111/jog.13786

21. Noori M, Donald AE, Angelakopoulou A, Hingorani AD, Williams DJ. Prospective study of placental angiogenic factors and maternal vascular function before and after preeclampsia and gestational hypertension. *Circulation*. 2010;122:478–487. doi: 10.1161/CIRCULATIONAHA.109.895458
22. Romero R, Nien JK, Espinoza J, Todem D, Fu W, Chung H, Kusanovic JP, Gotsch F, Erez O, Mazaki-Tovi S, et al. A longitudinal study of angiogenic (placental growth factor) and anti-angiogenic (soluble endoglin and soluble vascular endothelial growth factor receptor-1) factors in normal pregnancy and patients destined to develop preeclampsia and deliver a small for gestational age neonate. *J Matern Fetal Neonatal Med*. 2008;21:9–23. doi: 10.1080/14767050701830480
23. Frang H, Hurskainen P, Nicolaides K, Sairanen M. PlGF isoform 3 in maternal serum and placental tissue. *Pregnancy Hypertens*. 2019;18:9–13. doi: 10.1016/j.preghy.2019.08.001
24. Yang W, Ahn H, Hinrichs M, Torry RJ, Torry DS. Evidence of a novel isoform of placenta growth factor (PlGF-4) expressed in human trophoblast and endothelial cells. *J Reprod Immunol*. 2003;60:53–60. doi: 10.1016/s0165-0378(03)00082-2
25. Nucci M, Poon LC, Demirdjian G, Darbouret B, Nicolaides KH. Maternal serum placental growth factor isoforms 1 and 2 at 11–13, 20–24 and 30–34 weeks' gestation in late-onset pre-eclampsia and small for gestational age neonates. *Fetal Diagn Ther*. 2014;35:249–257. doi: 10.1159/000358595
26. Natarajan SK, Thangaraj KR, Eapen CE, Ramachandran A, Mukhopadhyaya A, Mathai M, Seshadri L, Peedikayil A, Ramakrishna B, Balasubramanian KA. Liver injury in acute fatty liver of pregnancy: possible link to placental mitochondrial dysfunction and oxidative stress. *Hepatology*. 2010;51:191–200. doi: 10.1002/hep.23245
27. Redman CW, Sargent IL. Placental stress and pre-eclampsia: a revised view. *Placenta*. 2009;30(suppl A):S38–S42. doi: 10.1016/j.placenta.2008.11.021
28. Kendall RL, Wang G, DiSalvo J, Thomas KA. Specificity of vascular endothelial cell growth factor receptor ligand binding domains. *Biochem Biophys Res Commun*. 1994;201:326–330. doi: 10.1006/bbrc.1994.1705
29. Sawano A, Takahashi T, Yamaguchi S, Aonuma M, Shibuya M. Flt-1 but not KDR/Fik-1 tyrosine kinase is a receptor for placenta growth factor, which is related to vascular endothelial growth factor. *Cell Growth Differ*. 1996;7:213–221.