Associations Between Soluble fms-Like Tyrosine Kinase-1 and Placental Growth Factor and Disease Severity Among Women With Preterm Eclampsia and Preeclampsia

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BACKGROUND: The angiogenic factors soluble fms-like tyrosine kinase-1 (sFlt-1) and placental growth factor (PlGF) are postulated to be pathogenic disease drivers of preeclampsia. If true, then circulating levels should become more deranged with increasing disease severity.

METHODS AND RESULTS: We investigated the association between circulating sFlt-1 and PlGF levels and severe adverse maternal outcomes among 348 women with preeclampsia. Compared with 125 women with preeclampsia without severe features, 25 women with preeclampsia and any of hemolysis, elevated liver enzymes, low platelet count syndrome, disseminated intravascular coagulation, or severe renal involvement had sFlt-1 levels that were 2.63-fold higher (95% CI, 1.81–3.82), sFlt-1/PlGF levels that were 10.07-fold higher (95% CI, 5.36–18.91) and PlGF levels that were 74% lower (adjusted fold change, 0.26 [95% CI, 0.18–0.39]). Compared with 125 women with preeclampsia without severe features, 37 with eclampsia had sFlt-1 levels that were 2-fold higher (2.02 [95% CI, 1.32–3.09]), sFlt-1/PlGF levels that were 4.71-fold higher (95% CI, 2.30–9.66) and PlGF levels that were 63% lower (0.43-fold change [95% CI, 0.27–0.68]). Compared with those without severe features, preeclampsia with severe hypertension (n=146) was also associated with altered angiogenic levels (sFlt-1, 1.71-fold change [95% CI, 1.39–2.11]; sFlt-1/PlGF, 2.91 [95% CI, 2.04–4.15]; PlGF, 0.59 [95% CI 0.47–0.74]). We also found that sFlt-1 and PlGF levels were altered by the number of maternal complications experienced.

CONCLUSIONS: Further angiogenic imbalance among women with preeclampsia is likely a pathogenic disease driver responsible for the life-threatening maternal complications.

Key Words: antiangiogenic factors ■ eclampsia ■ PlGF ■ preeclampsia ■ severe features ■ sFlt-1
**CLINICAL PERSPECTIVE**

**What Is New?**
- The angiogenic factors, soluble fms-like tyrosine kinase-1 (sFlt-1) and placental growth factor (PIGF), are significantly altered among women who experience severe complications of preeclampsia, including those who experience eclampsia.
- Compared with those who had preeclampsia without severe features, women who experienced hemolysis, elevated liver enzymes, and low platelets syndrome syndrome; disseminated intravascular coagulation; or severe renal involvement had the greatest change in sFlt-1 and PIGF levels, followed by those who experienced eclampsia.

**What Are the Clinical Implications?**
- Our findings implicate sFlt-1 and PIGF in the pathogenesis of adverse outcomes of preeclampsia and provide further evidence to support sFlt-1 as a central driver of disease pathogenesis.
- Our findings support the premise that targeting sFlt-1 is a promising therapeutic strategy to treat the condition.

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If the angiogenic imbalance seen in preeclampsia correlates with these severe outcomes, this would add to the evidence supporting sFlt-1 as a central driver of preeclampsia. However, many of these severe maternal complications are only very rarely seen in high-income settings, where most of the research investigating angiogenic levels has occurred. Thus, the associations between sFlt-1 and PIGF and severe, life-threatening maternal complications of preeclampsia have not been fully explored.

Tygerberg Hospital is a referral center servicing a population of >2 million people in Cape Town, South Africa, and has a high prevalence of preeclampsia and its complications. Thus, it represents a unique opportunity to amass a large cohort of women to investigate severe adverse outcomes of preeclampsia. Using samples collected from women with preeclampsia presenting to Tygerberg Hospital, we investigated whether altered sFlt-1 and PIGF levels were associated with severe maternal complications of preeclampsia.

**METHODS**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

**Study Population**

Maternal plasma samples were obtained antenatally from women with preeclampsia who were prospectively recruited to three large studies at Tygerberg Hospital, Cape Town, South Africa, from 2016 to 2019. For each study, patients provided informed consent before recruitment. The first study was the PIE (Preeclampsia Intervention With Esomeprazole) trial that randomized 120 women diagnosed with preterm preeclampsia (26+0–31+6 weeks’ gestation) to 40 mg esomeprazole or placebo daily (trial registration number: PACTRPACTR201504000771349). The second study was the PI 2 (Preeclampsia Intervention 2) trial that recruited 180 women with preterm preeclampsia to 3 grams of metformin extended release or placebo daily in divided doses (trial registration number: PACTR201608001752102). Both trials assessed prolongation of pregnancy following randomization and collected maternal plasma samples at randomization and twice weekly until delivery. The third study was the PROVE (Preeclampsia Obstetric Adverse Effects) study, a biobank collecting biological samples from women with preeclampsia or normotensive pregnancies. Women recruited to the PROVE study were sampled at inclusion, during hospital stay, and at delivery. Women across all 3 studies with a confirmed diagnosis of preeclampsia (defined per the International Society for the Study of Hypertension in Pregnancy guidelines

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Nonstandard Abbreviations and Acronyms

| Acronym | Description |
|---------|-------------|
| HELLP syndrome | hemolysis, elevated liver enzymes, and low platelets syndrome |
| PI 2 | Preeclampsia Intervention 2 |
| PIE | Preeclampsia Intervention With Esomeprazole |
| PIGF | placental growth factor |
| PROVE | Preeclampsia Obstetric Adverse Effects |
| sFlt-1 | soluble fms-like tyrosine kinase-1 |
with significant proteinuria defined as ≥0.3 g/24 h on a 24-hour urine collection, a urine protein creatinine ratio ≥30 mg/mmol (0.3 mg/mg) or urine dipstick >1+ if a polymerase chain reaction was not done) who provided a blood sample during pregnancy were included in the present study, with the sample closest to delivery and after the onset of severe features assayed. Importantly, all 3 studies were undertaken in recent years at the one center and led by the same chief investigator.

Whole blood samples were collected in 9-mL EDTA tubes and centrifuged, and the resulting plasma collected and stored at −80 °C until assayed. Samples were shipped to the Department of Obstetrics and Gynecology, University of Melbourne, where they were assayed by research staff blinded to group allocations. All women had completed their pregnancies before biomarker assessment. Maternal plasma levels of sFlt-1 and PIGF were measured with a commercial electrochemiluminescence immunoassay platform (Roche Diagnostics).

**Exposures**

We divided our cohort into (1) women with preeclampsia without severe features and (2) those with preeclampsia with severe features. Women with severe features were further categorized, using adapted American College of Obstetrics & Gynecology guidelines for defining severe features10 as (1) eclampsia; (2) pulmonary edema; (3) any of hemolysis, elevated liver enzymes, or low platelet count syndrome (defined as platelet count <100×10^9/L, aspartate aminotransferase >70 μL/L, and hemolysis as demonstrated by lactate dehydrogenase >600 μL/L or hemolysis on a peripheral blood smear), DIC (defined as international normalized ratio >2) or severe renal involvement (creatinine ≥120 μL/M/L); and (4) severe hypertension (systolic blood pressure ≥160 or diastolic blood pressure ≥110 mmHg). Where >1 adverse outcome occurred, the hierarchy of the listed outcomes above was followed. All maternal adverse outcomes occurred antenatally.

Women were also categorized by the number of adverse outcomes experienced; 0, 1, or ≥2. In addition to the outcomes above, several women within our cohort also experienced left ventricular failure, stroke, and coma. These additional complications were also considered within analyses by number of adverse outcomes.

Finally, we assessed angiogenic levels by birth weight percentile, calculated using World Health Organization growth charts. Infants deemed <10th and <3rd birthweight percentile were compared with those born at ≥10th and ≥3rd percentiles, respectively.

**Statistical Analysis**

Maternal characteristics and birth outcome data were compared between women with adverse outcomes and those with preeclampsia without severe features using Fisher’s exact test or chi-square test for categorical data and Kruskal-Wallis test for continuous data. sFlt-1 and PIGF concentrations and the ratio of sFlt-1/PIGF were assessed for normality and subsequently logarithmically transformed. Angiogenic factors were analyzed using a mixed-effect linear model to account for unequal variance. Comparisons between groups were performed with a Bonferroni correction and the difference between groups presented as fold-change with 95% CIs. All analyses were performed both unadjusted and adjusted for gestational age at sampling, birth of a small for gestational age infant and original study. Statistical analysis was performed via Stata/MP V6.

Ethical approval was obtained from the Health Research Ethics Committee of Stellenbosch University (Federal Wide Assurance Number 00001372; IRB0005239; PIE M14/09/038; PI 2 M16/09/037; PROVE N17/05/048).

**RESULTS**

**Study Population**

A total of 348 women with preeclampsia were included in this study. Among these, 125 did not experience adverse maternal outcomes, while 37 had eclampsia; 15 had pulmonary edema; and 25 had any of HELLP, DIC, or severe renal involvement. The remaining 146 women experienced severe hypertension (systolic blood pressure ≥160 or diastolic blood pressure ≥110 mmHg). Compared with women with preeclampsia without severe features, those with adverse outcomes were younger, had a lower body mass index, gave birth at earlier gestations, and had blood samples taken at a slightly earlier gestational age. Mode of birth and birth weight were not significantly different among the groups (Table 1).

**Circulating Angiogenic Levels and Adverse Outcomes of Preeclampsia**

Maternal plasma samples obtained closest to birth were assayed for sFlt-1 and PIGF levels. Across the total population, mean sFlt-1 and PIGF levels were 10673.8 pg/mL (95% CI, 9618.1–11845.4) and 41.7 pg/mL (95% CI, 36.7–47.4), respectively (Figure S1, Figure S2), which resulted in a ratio of sFlt-1 to PIGF of 255.9 (95% CI, 208.8–313.6).

Given samples were obtained across 3 studies, including 2 treatment trials, we assessed the circulating angiogenic levels in the plasma samples relative to respective treatment (PIE: esomeprazole versus placebo and PI 2: metformin extended release versus placebo). Compared with women randomly assigned to the
placebo groups, the circulating levels of angiogenic factors were not significantly altered among women randomly assigned to esomeprazole or metformin extended release (Table S1). Assessing angiogenic factor levels by gestational age at sampling, we found sFlt-1 levels increased and PlGF levels decreased with gestational age at sampling.

Investigating severe maternal complications, we found that compared with women with preeclampsia without severe features, eclampsia was associated with a significantly altered angiogenic profile, with an adjusted 2.02-fold change in sFlt-1 (95% CI, 1.32–3.09), 57% lower (0.43 [95% CI, 0.27–0.68]) PlGF and 4.71-fold (95% CI, 2.30–9.66) change in sFlt/PlGF. Conversely, compared with women with preeclampsia without severe features, those with pulmonary edema did not have a significantly different angiogenic profile (sFlt-1 1.33-fold [95% CI, 0.81–2.19]; PlGF, 0.66-fold [95% CI, 0.38–1.12]; sFlt-1/PlGF, 2.03-fold change [95% CI, 0.87–4.72]). While women experiencing any of the potentially life-threatening complications of HELLP, DIC, or severe renal involvement had significantly altered angiogenic levels, with an adjusted fold change in circulating sFlt-1 of 2.63 (95% CI, 1.81–3.82), 74% lower PlGF (0.26 [95% CI, 0.18–0.39]), and a 10.07-fold (95% CI, 5.36–18.91) higher sFlt-1/PlGF (Table 2). Compared with women with eclampsia, those women experiencing any of HELLP, DIC, or severe renal involvement had a greater change sFlt-1 levels and the ratio of sFlt-1/PlGF (P values=0.001 and <0.001, respectively). Severe hypertension was also associated with higher sFlt-1 (1.71-fold [95% CI, 1.39–2.11]) and sFlt-1/PlGF (2.91-fold [95% CI, 2.04–4.15]) and lower PlGF (0.59-fold [95% CI, 0.47–0.74]).

### Circulating Angiogenic Levels and Number of Maternal Complications

Next, we investigated angiogenic levels by the number of severe maternal complications experienced (0, 1, ≥2). Of 348 women, 223 (64%) experienced ≥1 adverse outcome, with 67 experiencing ≥2. Altered angiogenic levels were associated with the number of complications experienced (Table 3). Compared with women without severe features, those with preeclampsia and ≥2 adverse outcomes had significantly altered levels of sFlt-1 (2.26 [95% CI, 1.67–3.07]), PlGF (0.39 [95% CI, 0.29–0.55]), and sFlt-1/PlGF (6.12 [95% CI, 3.53–10.61]). PlGF and the ratio of sFlt-1/PlGF were significantly higher among women with ≥2 adverse outcomes when compared with those with only 1 adverse outcome (P=0.002 and 0.028, respectively).

### Antiangiogenic Levels and Birth Weight

We next investigated the association between angiogenic levels and birthing a small-for-gestational-age infant. Within our cohort, 67.8% (236/348) of women gave birth to a small-for-gestational-age infant (birth weight less than the 10th percentile according to

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**Table 1. Maternal Characteristics**

|                      | Preeclampsia without severe features n=125 | Eclampsia n=37 | Pulmonary edema n=15 | Other (HELLP, DIC, renal) n=25 | Severe hypertension n=146 | P value |
|----------------------|-------------------------------------------|----------------|----------------------|-------------------------------|---------------------------|---------|
| Age, y               | 28 (23–34)                                | 21 (17–24)     | 28 (21–34)           | 25 (24–27)                    | 30 (24–33)                | <0.001  |
| Body mass index, kg/m² | 31.1 (25.8–35.6)                           | 24.2 (22.7–26.8) | 27.0 (22.6–31.0)     | 29.9 (28–33.9)                | 28.9 (24.0–35.2)         | <0.001  |
| Race, n (%)          |                                           |                |                      |                               |                           |         |
| Black                | 83 (66.4)                                 | 23 (62.2)      | 9 (60.0)             | 10 (40.0)                     | 93 (63.7)                 | 0.223   |
| Other*               | 41 (31.8)                                 | 14 (37.8)      | 6 (40.0)             | 15 (60.0)                     | 53 (36.3)                 |         |
| White                | 1 (32.8)                                  | 0              | 0                    | 0                             | 0                         |         |
| Nulliparous, n (%)   | 46 (36.8)                                 | 29 (78.4)      | 5 (33.3)             | 9 (36.0)                      | 47 (32.2)                 | <0.001  |
| Mode of birth, n (%) |                                           |                |                      |                               |                           |         |
| Vaginal birth        | 31 (24.8)                                 | 13 (35.1)      | 2 (13.3)             | 3 (12.0)                      | 18 (12.4)                 | 0.002   |
| Elective CS          | 26 (20.8)                                 | 2 (5.4)        | 1 (6.7)              | 5 (20.0)                      | 17 (11.7)                 |         |
| Emergency CS         | 88 (54.4)                                 | 22 (59.4)      | 12 (80)              | 17 (68)                       | 110 (75.9)                |         |
| Gestation at delivery, wks | 33.1 (30.1–34.1)                      | 32.4 (30.4–36.1) | 30.9 (28.9–33.7)     | 31.3 (29.9–31.9)              | 31.9 (30.1–34.0)         | <0.001  |
| Gestation at sampling, wks+d 31.8 (29.1–33.6) | 32.4 (30.1–36.1) | 30.9 (28.9–33.7) | 30.5 (29.4–31.7) | 31.2 (28.9–33.4) | 0.001 |
| Days from sampling to birth 3 (1–5) | 0 (0–0) | 1 (0–2) | 2 (1–3.5) | 2 (6–1) | 0.009 |
| Birthweight, g        | 1590 (1090–2100)                          | 1750 (1200–2635) | 1240 (1130–1715)     | 1270 (1060–1376)              | 1347.5 (1110–1720)       | 0.108   |
| Highest systolic BP, mm Hg | 150 (145–156)                       | 167 (152–181)  | 167 (160–185)        | 168 (160–174)                 | 166 (160–171)            | <0.001  |
| Highest diastolic BP, mm Hg | 92 (85–96)                          | 107 (99–119)   | 103 (99–116)         | 104 (98–110)                  | 102 (100–107)            | <0.001  |

Continuous data presented as median (interquartile range). BP indicates blood pressure; CS, cesarean section; DIC, disseminated intravascular coagulation; and HELLP, hemolysis, elevated liver enzymes, low platelet syndrome.

*Other includes those who identified as mixed race.
World Health Organization reference charts) and 51% (178/348) birthed an infant less than the 3rd percentile. Compared with pregnancies with a birth weight greater than the 10th percentile, birthing an infant less than the 10th percentile was associated with an adjusted fold change in sFlt-1 levels that were 2.52-fold higher (95% CI, 2.06–3.09), PIGF levels that were 68% less [95% CI, 0.25–0.41] and sFlt/PIGF ratios that were 7.94-fold higher. Similarly, angiogenic levels were significantly altered among women who birthed an infant with a birth weight less than the 3rd percentile (sFlt-1, 2.03 [95% CI, 1.67–2.45]; PIGF, 0.41 [95% CI, 0.33–0.45]; sFlt/PIGF, 4.89 [95% CI, 3.44–6.96]; Table 4).

**DISCUSSION**

This study demonstrates that the angiogenic factors, sFlt-1 and PIGF, are significantly altered among women who experience severe complications of preeclampsia, including severe complications that are life-threatening. Compared with those who had preeclampsia without severe features, women who experienced HELLP syndrome, DIC, or severe renal involvement had the greatest change in sFlt-1 and PIGF levels, followed by those who experienced eclampsia. Angiogenic levels were not significantly altered among women who experienced pulmonary edema but were increasingly altered by the number of maternal complications experienced.

Within our study, 64% (n=223) of women with preeclampsia experienced at least 1 adverse outcome and, of these, 10% had eclampsia. This high incidence of maternal complications allowed us to investigate disease severity by the presence and type of maternal complication. Although there have been large studies investigating the utility of angiogenic markers for predicting severe complications, there has been little investigation among women with established severe complications. A previous study, undertaken in Haiti, examined angiogenic levels among women with adverse outcomes, including eclampsia, and found angiogenic levels to be correlated with adverse outcomes. However, this study was small, with only 35 women with preeclampsia, and the exact number of women who experienced each adverse outcome is not clear. Additionally, that study compared women with adverse outcomes to normotensive women, not those with preeclampsia without severe feature. Furthermore, a study by Leaños-Miranda et al examined serum angiogenic levels among 689 women with hypertensive disorder, which also found the greatest change among women with HELLP and eclampsia. However, within this study only 17 women developed eclampsia, and they were analyzed with those who developed HELLP syndrome.

Given that sFlt-1 is hypothesized to have a casual role in the pathogenesis of preeclampsia, our
findings are biologically plausible. Further placental secretion of sFlt-1 and subsequent angiogenic imbalance, may result in increased damage to the endothelium (beyond that of preeclampsia without severe features) and the onset of severe maternal complications, including HELLP syndrome, eclampsia, and severe hypertension. This hypothesis may also explain why angiogenic levels were not further perturbed among women who birthed an infant with a birth weight less than the 3rd percentile compared with less than the 10th percentile, whereby birth weight may reflect overall placental function rather than angiogenic imbalance and resulting endothelial dysfunction and the onset of severe maternal complications.

The significant implications of our findings are that our observations support quenching sFlt-1 as a therapeutic strategy to treat preeclampsia. In fact, our work suggests that quenching its release may be able to counter the development of the most life-threatening maternal complications, such as eclampsia, HELLP syndrome, DIC, and others. It suggests that novel approaches to remove sFlt-1 from the circulation, including via apheresis or medications that reduce secretion, are plausible therapeutic strategies. However, further studies investigating these strategies are needed.

Our study has several strengths. With a large referral area, high prevalence of preeclampsia, and an established biobanking facility, recruitment from Tygerberg Hospital provided a unique opportunity to investigate rare maternal complications that have not been previously well characterized. Notably, our study included one of the largest cohorts of biological samples from women with eclampsia and pulmonary edema. Additionally, angiogenic factor levels were measured in maternal plasma via a commercial platform, which reduced the potential for interassay variability. There are some limitations to our study. First, these angiogenic factors have been previously shown to change with increasing gestation; thus, given that plasma samples were obtained across a range of gestational ages within our study, this may have implications with interpretation of our findings. To overcome this, we included gestational age at sampling as a covariate within our adjusted statistical model. The other potential limitation is that all women recruited to the PIE and PI2 trials were delivered by 34 weeks' gestation (as per trial protocol); thus, it is plausible that these women may have gone on to develop additional complications without the intervention of delivery. To account for this and potential variation across studies, we also included each study as a covariate within our adjusted model. Additionally, given that an adapted version of the American College of Obstetrics & Gynecology criteria was used to categorize adverse outcomes, our findings may not be generalizable to those using standard criteria. Our study was not aimed at predicting

| Number of Severe Complications | sFlt-1 (Fold change (95% CI)) | Crude | Adjusted | P-value | Crude | Adjusted | P-value |
|--------------------------------|-----------------------------|-------|----------|---------|-------|----------|---------|
| 0                              | 1.92 (1.54–2.39)            | 1.69 (1.37–2.08) | <0.001 | 0.27 (0.20–0.38) | 0.19 (0.13–0.28) | <0.001 |
| 1                              | 1.98 (1.49–2.61)            | 2.26 (1.67–3.07) | <0.001 | 0.39 (0.29–0.50) | 0.07 (0.05–0.09) | <0.001 |
| ≥2                             | 1.96 (1.56–2.43)            | 2.26 (1.67–3.07) | <0.001 | 0.39 (0.29–0.50) | 0.07 (0.05–0.09) | <0.001 |

Table 3. Fold Change of Maternal Antiangiogenic Levels by Number of Maternal Complications Experienced

Women with preeclampsia without severe features (reference group) were compared with those with 1 or ≥2 severe complications. Adjusted analyses include gestation at sampling, birth weight less than the third percentile, and study from which samples were obtained from (PIE, PI2, PROVE) as covariates. DIC indicates disseminated intravascular coagulation; HELLP, hemolysis, elevated liver enzymes, low platelet syndrome; PlGF, placental growth factor; sFlt-1, soluble fms-like tyrosine kinase-1.
severe maternal outcomes, and thus, further large prospective studies are needed to determine the utility of angiogenic factors in predicting these rare and severe maternal complications.

CONCLUSIONS

Circulating sFlt-1 and PlGF are significantly altered among women who experience severe complications of preeclampsia. Given their role in the pathophysiology of preeclampsia, the associations between sFlt-1 and PlGF levels and serious adverse maternal outcomes are biologically plausible. Our findings implicate sFlt-1 and PlGF in the pathogenesis of adverse outcomes of preeclampsia and provide further evidence to support sFlt-1 as a central driver of disease pathogenesis. Our findings support the premise that targeting sFlt-1 is a promising therapeutic strategy to treat the condition.

ARTICLE INFORMATION

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Disclosures
None.

Table 4. Fold Change of Maternal Antiangiogenic Levels by Birthweight Centile

| sFlt-1 Fold change (95% CI) | PlGF Fold change (95% CI) | sFlt-1/PlGF Fold change (95% CI) |
|---------------------------|------------------------|----------------------------------|
| Crude | Adjusted | Crude | Adjusted | Crude | Adjusted |
| Pre-eclampsia, plus infant with a birth weight <10th percentile, n=236 | 2.75 (2.26–3.35) | 2.52 (2.06–3.09) | 0.27 (0.21–0.34) | 0.32 (0.25–0.41) | 10.39 (7.25–14.88) | 7.94 (5.49–11.48) |
| Pre-eclampsia, plus infant with a birth weight <3rd percentile, n=178 | 2.19 (1.81–2.65) | 2.03 (1.67–2.45) | 0.36 (0.28–0.45) | 0.41 (0.33–0.52) | 6.14 (4.28–8.80) | 4.89 (3.44–6.96) |

Adjusted analyses include gestation at sampling and study from which samples were obtained from (PIE [Preeclampsia Intervention With Esomeprazole], PI 2 [Preeclampsia Intervention 2], PROVE [Preeclampsia Obstetric Adverse Effects]) as covariates. PIGF indicates placental growth factor; and sFlt-1, soluble fms-like tyrosine kinase-1.

CONCLUSIONS

Circulating sFlt-1 and PI GF are significantly altered among women who experience severe complications of preeclampsia. Given their role in the pathophysiology of preeclampsia, the associations between sFlt-1 and PI GF levels and serious adverse maternal outcomes are biologically plausible. Our findings implicate sFlt-1 and PI GF in the pathogenesis of adverse outcomes of preeclampsia and provide further evidence to support sFlt-1 as a central driver of disease pathogenesis. Our findings support the premise that targeting sFlt-1 is a promising therapeutic strategy to treat the condition.

REFERENCES

1. Chappell LC, Cluver CA, Kingdom J, Tong S. Pre-eclampsia. Lancet. 2021;398:341–354. doi: 10.1016/S0140-6736(20)32335-7

2. Maynard S, Min JY, Merchand J, Lim KH, Li J, Mondal S, Libermann TA, Morgan JP, Selke FW, Stillman IE, et al. Excess placental soluble fms-like tyrosine kinase 1 (sflt-1) may contribute to endothelial dysfunction, hypertension, and proteinuria in pre-eclampsia. J Clin Invest. 2003;111:649–658. doi: 10.1172/JCI17189

3. Levine RJ, Maynard SE, Qian C, Lim K-H, Yang L, 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/NEJMoa031984

4. Koga K, Osuga Y, Yoshino O, Hirota Y, Fumio X, Hirata T, Takeda S, Yano T, Tsutsui O, Takeda N, Takeda Y. Elevated serum soluble vascular endothelial growth factor receptor 1 (svegfr-1) levels in women with preeclampsia. J Clin Endocrinol Metab. 2003;88:2348–2351. doi: 10.1210/jc.2002-021942

5. March MI, Geachan C, Weng J, Raghuraman N, Berg A, Haddow H, McKeon BA, Narcisse R, David JL, Scott J, et al. Circulating angiogenic factors and the risk of adverse outcomes among haitian women with preeclampsia. PloS one. 2015;10:e0126815. doi: 10.1371/journal.pone.0126815

6. Cluver CA, Hannan NJ, van Papendorp E, Hiscock R, Beard S, Mol BW, Theron GB, Hall DR, Decloedt EH, Stander M, et al. Esomeprazole to treat women with preterm preeclampsia: a randomized placebo controlled trial. Am J Obstet Gynecol. 2018;219:388.e381–388.e317.

7. Cluver CA, Walker SP, Mol BW, Theron GB, Hooe DR, Hiscock R, Hannan N, Tong S. Double blind, randomised, placebo-controlled trial to evaluate the efficacy of esomeprazole to treat early onset pre-eclampsia (pre-eclamp) (pie trial): a study protocol. BMJ Open. 2015;5:e008211. doi: 10.1136/bmjopen-2015-008211

8. Cluver C, Walker SP, Mol BW, Hall D, Hiscock R, Brownfoot FC, Kaltu'n-Lino TJ, Tong S. A double blind, randomised, placebo-controlled trial to evaluate the efficacy of metformin to treat preterm pre-eclampsia (p2e trial): study protocol. BMJ Open. 2019;9:e025809. doi: 10.1136/bmjopen-2018-025809

9. Bergman L, Bergman K, Langenegrer E, Moodley A, Griffith-Richards S, Wikström J, Hall D, Joubert L, Herbst P, Schell S, et al. Pre-eclampsia: obstetric adverse events: establishment of a biobank and database for pre-eclampsia. Cells. 2021;10:958. doi: 10.3390/cells10040959

10. Gestational hypertension and preeclampsia: ACOG practice bulletin, number 222. Obstet Gynecol. 2020;135:1492–1495.

11. von Dadelszen P, Payne B, Li J, Arismerino JM, Broughton Pipkin F, Côté AM, Douglas MJ, Gruulain A, Hutchison JD, Joseph KS, et al. Prediction of adverse maternal outcomes in pre-eclampsia: development and validation of thefulmpliers model. Lancet. 2011;377:219–227. doi: 10.1016/S0140-6736(10)61351-7

Supplemental Material

Table S1

Figures S1–S2

Table S4

Supplemental Material

Figures S1–S2

Table S1

Supplemental Material
12. Leaños-Miranda A, Méndez-Aguilar F, Ramírez-Valenzuela KL, Serrano-Rodríguez M, Berumen-Lechuga G, Molina-Pérez CJ, Isordia-Salas I, Campos-Galicia I. Circulating angiogenic factors are related to the severity of gestational hypertension and preeclampsia, and their adverse outcomes. *Medicine.* 2017;96:e6005. doi: 10.1097/MD.00000000000006005

13. Rana S, Lemoine E, Granger JP, Karumanchi SA. Preeclampsia: pathophysiology, challenges, and perspectives. *Circ Res.* 2019;124:1094–1112. doi: 10.1161/CIRCRESAHA.118.313276

14. Hastie R, Brownfoot FC, Pritchard N, Hannan NJ, Cannon P, Nguyen V, Palmer K, Beard S, Tong S, Kaitu'u-Lino TJ. EGFR (epidermal growth factor receptor) signaling and the mitochondria regulate sFlt-1 (soluble FMS-like tyrosine kinase-1) secretion. *Hypertension.* 2019;73:659–670. doi: 10.1161/HYPERTENSIONAHA.118.12300

15. Thadhani R, Hagmann H, Schaarschmidt W, Roth B, Cingoez T, Karumanchi SA, Wenger J, Lucchesi KJ, Tamez H, Lindner T, et al. Removal of soluble fms-like tyrosine kinase-1 by dextran sulfate apheresis in preeclampsia. *J Am Soc Nephrol.* 2016;27:903–913. doi: 10.1681/ASN.2015020157

16. Tong S, Kaitu'u-Lino TJ, Hastie R, Brownfoot F, Cluver C, Hannan N. Pravastatin, proton-pump inhibitors, metformin, micronutrients, and biologics: new horizons for the prevention or treatment of preeclampsia. *Am J Obstet Gynecol.* 2022;226:S1157–S1170. doi: 10.1016/j.ajog.2020.09.014
SUPPLEMENTAL MATERIAL
Table S1. Fold change in circulating angiogenic levels by treatment received.

|                | sFlt-1 (95% confidence interval) | PI GF (95% confidence interval) | sFlt-1/PI GF (95% confidence interval) |
|----------------|----------------------------------|---------------------------------|----------------------------------------|
| Placebo        | 1 (ref)                          | 1 (ref)                         | 1 (ref)                                |
| Esomeprazole   | 1.03 (0.68, 1.57)                | 0.91 (0.61, 1.35)               | 1.14 (0.57, 2.28)                      |
| Metformin      | 1.10 (0.80, 1.50)                | 1.25 (0.84, 1.86)               | 0.88 (0.45, 1.71)                      |
Figure S1. Maternal sFlt-1 concentration across gestation

A

B

C

D

Gestational age at sampling (weeks)

Log(sFlt-1)

Preeclampsia without severe features
Eclampsia

Preeclampsia without severe features

Pulmonary edema

Severe hypertension

HELLP, DIC, severe renal

Severe hypertension
Figure S2. Maternal PlGF concentration across gestation