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ORIGINAL RESEARCH

Circulating Growth Differentiation Factor 15 Is Increased Preceding Preeclampsia Diagnosis: Implications as a Disease Biomarker

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BACKGROUND: We investigated the biomarker potential of growth differentiation factor 15 (GDF-15), a stress response protein highly expressed in placenta, to predict preeclampsia.

METHODS AND RESULTS: In 2 prospective cohorts (cohort 1: 960 controls, 39 women who developed preeclampsia; cohort 2: 950 controls, 41 developed preeclampsia), plasma concentrations of GDF-15 at 36 weeks' gestation were significantly increased among those who developed preeclampsia ($P<0.001$), area under the receiver operating characteristic curves (AUC) of 0.66 and 0.71, respectively. In cohort 2 a ratio of sFlt-1/PlGF (a clinical biomarker for preeclampsia) had a sensitivity of 61.0% at 83.2% specificity to predict those who will develop preeclampsia (AUC of 0.79). A ratio of GDF-15×sFlt-1/PlGF yielded a sensitivity of 68.3% at 83.2% specificity (AUC of 0.82). GDF-15 was consistently elevated across a number of international cohorts: levels were higher in placenta and blood from women delivering <34 weeks' gestation due to preterm preeclampsia in Melbourne, Australia; and in the blood at 26 to 32 weeks' gestation among 57 women attending the Manchester Antenatal Vascular Service (MAViS, UK) who developed preeclampsia ($P=0.0002$), compared with 176 controls. In the Preeclampsia Obstetric adVerse Events biobank (PROVE, South Africa), plasma GDF-15 was significantly increased in women with preeclampsia with severe features ($P=0.02$; n=14) compared to controls (n=14).

CONCLUSIONS: We conclude circulating GDF-15 is elevated among women more likely to develop preeclampsia or diagnosed with the condition. It may have value as a clinical biomarker, including the potential to improve the sensitivity of sFlt-1/PlGF ratio.

Key Words: biomarker ■ placental growth factor ■ preeclampsia ■ pregnancy

Preeclampsia is one of the most severe pregnancy complications, affecting 3% to 8% of pregnancies and claiming the lives of many mothers and babies.[1] It is characterised by maternal hypertension and multi-organ involvement resulting from systemic endothelial dysfunction.[2,3] In severe cases, it can

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CLINICAL PERSPECTIVE

What Is New?

- Plasma GDF-15 (growth differentiation factor 15) is consistently elevated among women who later develop preeclampsia and those diagnosed with the condition.
- A ratio of GDF-15×sFlt1/placental growth factor (PlGF) may improve prediction of those at high risk of developing preeclampsia relative to sFlt1/PlGF alone.

What Are the Clinical Implications?

- A ratio of sFlt1/PlGF is being used clinically with excellent negative predictive value, but modest sensitivity.
- Our data at 36 weeks' gestation identify GDF-15 as a promising predictive biomarker that might be combined with sFlt-1 and PlGF to improve the sensitivity of this clinical test, flagging women at increased risk of developing preeclampsia.

Nonstandard Abbreviations and Acronyms

FLAG	fetal longitudinal assessment of growth study
GDF-15	growth differentiation factor 15
MAViS	Manchester antenatal vascular service
PlGF	placental growth factor
PROVE	Preeclampsia Obstetric Adverse Events Biobank

progress to neurological compromise and eclamptic seizures. Among survivors, its legacy is significant maternal and perinatal morbidity. For the mother this can include cardiovascular disease, stroke, and kidney damage, while the baby is more likely to be born preterm, sick, and small.

Measuring maternal blood pressure at every visit to screen for preeclampsia remains a cornerstone of antenatal care. Those suspected of having preeclampsia will undergo resource intensive testing—which often includes hospitalization. Many will never develop the disease, while others at risk can be missed because it might develop between antenatal appointments. As such, a test that can accurately identify at-risk women is desperately needed.

Maternal blood biomarkers may provide an opportunity for improved surveillance. Examples of blood biomarkers that are currently used clinically are soluble fms-like tyrosine kinase-1 (sFlt-1)[4] and placental growth factor (PlGF).[5] It has now been demonstrated that a ratio of sFlt-1/PlGF performs reliably to rule out women at risk of preeclampsia within 4 weeks of the blood test, however its rule in sensitivity performs more modestly.[6] The utility of PlGF alone as a universal screening test for all asymptomatic pregnant women remains to be established in regard to optimum gestational age and threshold for prediction.[7]

Our team has an interest in placental-enriched proteins[8]; molecules very highly expressed in the placenta relative to other tissues, that are released into the maternal circulation. We hypothesise that such proteins may be deranged in diseases of placental dysfunction, such as preeclampsia. One such placental protein is growth differentiation factor 15 (GDF-15), also known as macrophage inhibiting cytokine-1 (MIC-1). GDF-15 is a member of the transforming growth factor β superfamily. Under physiological conditions, its highest expression is in the placenta.[9,10] However, it is also widely reported as a stress-induced molecule, upregulated in response to cellular injury and inflammation.[9,11] GDF-15 also has recently been reported as a potential biomarker for cardiovascular diseases including coronary artery disease, heart failure, and pulmonary hypertension.[12] Indeed, high circulating levels of GDF-15 are associated with an increased risk of developing cardiovascular disease and are believed to result from chronic disease burden. Interestingly, previous groups have measured GDF-15 in the serum of preeclamptic patients, but have found conflicting results, reporting no change,[13] reduced,[14] and significantly increased levels.[15–17]

Given this uncertainty in the current literature, and its high placental expression and links with cardiovascular disease, the purpose of this study was to assess whether GDF-15 is increased in the maternal circulation of women more likely to develop preeclampsia. Here, we report the association between circulating GDF-15 and preeclampsia in multiple international cohorts. This includes 3 cohorts from Australia, a large cohort of high risk pregnant women with vascular conditions (such as chronic hypertension and preexisting diabetes mellitus) attending a clinic in Manchester (UK), and a biobank from women with severe disease collected in Cape Town, South Africa (where there is a high incidence of preeclampsia and eclampsia[18]).

METHODS

Data, Materials, and Code Disclosure

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

The Fetal Longitudinal Assessment of Growth Study

The FLAG (Fetal Longitudinal Assessment of Growth) study was undertaken at the Mercy Hospital for Women, in Melbourne, Australia. It involved the prospective recruitment of pregnant women from whom we obtained 2000 blood samples at 28 (27^{+0}–29^{+0} days) and 36 (35^{+0}–37^{+0}) weeks' gestation. It was designed to identify biomarkers for pregnancy complications such as preeclampsia and fetal growth restriction. Women were screened for eligibility and invited to participate at their oral glucose tolerance test, universally offered to test for gestational diabetes mellitus around 28 weeks' gestation. English-speaking women aged over 18 years, with a singleton pregnancy and normal mid-trimester fetal morphology examination were eligible to participate. Whole blood was collected in 9 mL ethylenediaminetetraacetic acid (EDTA) tubes. Plasma was stored at −80°C until the time of sample analysis. Of the women who provided 36-week blood samples, 4.2% later developed preeclampsia.

The FLAG study samples were divided into 2 consecutively collected cohorts—Cohorts 1 (Table S1, n=960 control, n=39 preeclampsia [PE]) and 2 (Table S2, n=950 control, n=41 PE). GDF-15 was measured in each cohort, while sFlt-1 and PlGF were only measured in Cohort 2 where the median time to delivery after blood sampling was 2.9 weeks (IQR 2.4–3.6).

The FLAG study was approved by the Mercy Health Research Ethics Committee (Ethics Approval Number R14/12) and written informed consent was obtained from all participants.

Preterm Preeclampsia

We also measured GDF-15 in plasma samples collected from a separate cohort of women who had established preterm preeclampsia and delivered <34 weeks' gestation in Melbourne, Australia (Table S3, cohort 3, n=26 control, n=42 PE). Controls for this cohort were pregnant women from whom plasma samples were collected at the same gestation as preeclampsia cases, but who progressed to an uncomplicated delivery of a healthy neonate of normal birthweight at term.

Outcomes and Definitions of Cases

Maternal characteristics and pregnancy outcomes were obtained from review of each participant's medical record, investigation results and hospital database entry. Preeclampsia was defined according to the guidelines published by the American College of Obstetricians and Gynecologists.[19] This included hypertension, defined as systolic blood pressure ^{3}140 mm Hg or diastolic blood pressure ^{3}90 mm Hg on 2 occasions at least 4 hours apart after 20 weeks' gestation with previously normal blood pressure, and proteinuria; or in the absence of proteinuria, new-onset hypertension plus new thrombocytopenia, renal insufficiency, impaired liver function, pulmonary edema, or neurological compromise. Superimposed preeclampsia defined women with preexisting hypertension who developed new onset proteinuria, thrombocytopenia, renal insufficiency, impaired liver function, pulmonary edema, or neurological compromise after 20 weeks.

Manchester Antenatal Vascular Service Cohort

Circulating GDF-15 was also measured in plasma samples obtained from a high-risk cohort in the United Kingdom, the Manchester Antenatal Vascular Service (The MAViS clinic). Women gave written informed consent to donate samples for future research studies. The study was approved by the NRES Committee North West 11/NW/0426.

The inclusion criteria for women in the MAViS study were: (1) chronic hypertension BP ≥140/90 at ≤20 weeks; (2) chronic hypertension requiring antihypertensive treatment ≤20 weeks; (3) pre-gestational diabetes mellitus with evidence of vascular complications (hypertension, nephropathy); (4) history of ischaemic heart disease; and (5) previous early onset preeclampsia.

Women had blood samples taken at ≈4 week intervals. Women recruited to the MAViS cohort are known to have an increased risk of preeclampsia, small for gestational age or fetal growth restriction. A case-cohort of 233 participants recruited between October 2011 and December 2016 with a plasma sample obtained between 24 and 34 weeks and complete outcome data were included in the current study. These 233 participants were selected from an overall cohort of 518 participants and included 176 control women and 57 women who developed preeclampsia. The clinical characteristics are shown in Table S4.

The Preeclampsia Obstetric Adverse Events Cohort

The Preeclampsia Obstetric Adverse Events (PROVE) biobank recruits women with proteinuric preeclampsia, preeclampsia with severe features (pulmonary oedema, intracerebral hemorrhage, cerebral oedema, heart failure or disseminated intravascular coagulation), eclampsia as well as uncomplicated pregnancies at Tygerberg Hospital in Cape Town, South Africa. The biobank was designed to facilitate preeclampsia research (https://doi.org/10.1186/ISRCTN10623443) and has ethical approval from Stellenbosch University Health Research Committee (N17/05/048). All women between 20 to 42 weeks pregnant and present at Tygerberg hospital for delivery were eligible for inclusion. After informed consent was given, clinical information and biological samples were collected. Whole blood was collected in ethylenediaminetetraacetic acid (EDTA) tubes and centrifuged within 1 hour of collection. Plasma was then stored at −80°C until analysis. From April 2018 to March 2020, the PROVE biobank included 230 women and 72 participants were selected from this cohort who had provided blood samples at the time of delivery including 14 control women, 14 proteinuric preeclamptic women, 13 women with preeclampsia with severe features, and 31 with eclampsia. The clinical characteristics are shown in Table S5.

ELISA Measurement Of GDF-15, sFlt-1, and PlGF in Plasma Samples

Plasma GDF-15 was measured in sample cohorts using the GDF-15 Human ELISA kit (Thermo Fisher Scientific, MA) according to the manufacturer's instructions. Maternal plasma levels of sFlt-1 and PlGF were measured with a commercial electrochemiluminescence immunoassay platform (Roche Diagnostics, North Ryde, Australia).

Improving Diagnostic Performance

To determine whether GDF-15 adds to the performance of sFlt-1 and PlGF as a clinical biomarker we performed a classification study using samples from cohort 2 of the FLAG study. We calculated the sensitivity and specificity of several ratiometric biomarker combinations that included GDF-15, sFlt-1, and PlGF. The PROGNOSIS study[6] found that a sFlt-1/PlGF ratio of >38 had a sensitivity of 66.2% and specificity of 83.1% for a rule-in diagnosis of pre-eclampsia within 4 weeks. Therefore, we evaluated the potential of GDF-15 to improve the detection rate of sFlt-1/PlGF at a fixed specificity equal to or >83.1%.

To determine the performance of each ratiometric biomarker combination, the cut-off point for classification was modified in increments of 0.01 units until a specificity of 83.1% or greater was achieved. For PlGF alone, values below the cut-off point were considered as screen positive, whereas for all other biomarker combinations, values above the cut-off point were considered as screen positive.

Placental Tissue Collection

Women presenting to the Mercy Hospital for Women gave informed written consent for placental tissue collection. Human Ethics approval was obtained for this study from the Mercy Health Human Research Ethics Committee (R11/34). We measured GDF-15 expression in placentas from pregnancies complicated by preterm preeclampsia delivered at <34 weeks' gestation and in gestation-matched control placentas from pregnancies not affected by preeclampsia. Indications for preterm birth in the preterm control cohort were preterm labor, vasa praevia, or antepartum haemorrhage. Controls did not have any evidence of infection on histopathological examination of the placentas or of hypertensive disease. All participants where placental specimens were obtained were delivered by caesarean section. Patient characteristics are outlined in Tables S6 and S7.

Placental tissue was obtained immediately following delivery. Maternal and fetal surfaces were removed and the samples were washed in ice-cold sterile phosphate-buffered saline (PBS). Samples for protein extraction were frozen within 15 minutes of delivery and stored at −80 °C, and samples for RNA or protein collected in RNA Later™ stabilization solution. Placenta was also fixed in 10% buffered formalin for histology.

qRT-PCR to Measure Human GDF-15 mRNA Expression

RNA was extracted from 20 to 30 mg of RNAlater preserved frozen human placental samples by homogenization using a RNeasy mini-kit (Qiagen, Hilden, Germany). One µg of RNA was converted to cDNA using Applied Biosystems high capacity cDNA Reverse Transcriptase Kit (Life Technologies, Carlsbad, CA). Taqman gene expression assays (Life Technologies) for human GDF-15 (Assays ID: Hs00171132_m1), TOP1 (Assay ID: Hs00243257_m1), and CYC1 (Assay ID: Hs00357717_m1) were used. For comparisons between human placental samples, data were normalized to the geometric mean of 2 housekeepers; TOP1 and CYC1. qRT-PCR was performed on the CFX 384 (Biorad, Hercules, CA) using FAM-labeled Taqman universal PCR master mix (Life Technologies) with the following run conditions: 50 °C for 2 minutes; 95 °C for 10 minutes, 95 °C for 15 seconds, 60 °C for 1 minute (40 cycles).

Immunohistochemical Staining for GDF-15

GDF-15 was localized by immunohistochemistry in placental tissue collected from paraformaldehyde fixed preeclamptic or preterm control pregnancies. In brief, paraffin sections (5 µm) were dewaxed in Xylene and rehydrated through descending grades of ethanol. Sections underwent antigen retrieval via microwaving using 0.01 mol/L sodium citrate buffer (pH 6.0) for 20 minutes and then incubated in the hot buffer for a further 20 minutes. Sections were washed for 10 minutes in Phosphate-buffered saline pH 7.6 (PBS). Following endogenous peroxidase quenching and blocking of non-specific binding, sections were incubated at 37 °C for 1 hour with 1:500 GDF15 Monoclonal Antibody (Sapphire Biosciencei, NSW, Australia) in blocking buffer (DAKO). For isotype controls, primary antibody was substituted with mouse IgG. Staining was visualized using the HRP/DAB Detection IHC Kit (ABCAM, Cambridge, UK), and lightly counterstained with Harris hematoxylin (Sigma Aldrich, MO, USA). Sections were dehydrated and mounted. Staining was visualized and captured using a Leica microscope and camera.

Statistical Analysis

Maternal characteristics and birth outcome data were compared for all women with preeclampsia against controls using a Mann-Whitney *U* test for continuous data and Fisher's exact test for categorical data. Placental and circulating analyte levels were compared using a Mann-Whitney *U* test. Overall discrimination of GDF-15 was assessed with area under the receiver operating characteristic (ROC) curve analysis. Statistical analyses were performed using GraphPad Prism version 9 (GraphPad Software Inc., San Diego, CA) or R version 4.0.5 (R Core Team, 2021).[20] For performing logistic regression, the R packages Hmisc[21] and rms[22] were utilized.

Participants in the MAViS cohort have underlying vascular disease, and both the MAViS and PROVE cohorts were sampled across a range of gestations. We fitted a logistic regression model of preeclampsia status against the natural logarithm of the GDF-15 values in pg/mL (logGDF15), chronic hypertension (MAViS only), renal hypertension (MAViS only), and gestational age at sampling in days as the independent variables. The model incorporated restricted cubic splines for gestation at sampling with 3 knots used for models with <30 samples, 4 knots for <100 samples, and 5 knots for ≥100 samples. No interaction terms were included. Modelling results were presented as odds ratios (95% CI) of preeclampsia, where the odds ratio for the gestational age at sampling is presented for the interquartile range.

RESULTS

Circulating GDF-15 Is Increased at 36 Weeks' Gestation Before a Diagnosis of Preeclampsia

GDF-15 protein was first measured in plasma collected from pregnant women at 36 weeks' gestation where 39 participants developed preeclampsia and 960 did not (Cohort 1). Circulating GDF-15 was significantly increased among women who later developed preeclampsia (median GDF-15 of 377.7 ng/mL; interquartile range [IQR] 260.5–465) compared to controls (median GDF-15 of 276.5 ng/mL [IQR 187.6–395.3]; P=0.007; see Figure 1A). The area under the receiver operating characteristic curve (AUC) was 0.66 (Figure 1A).

We next sought to validate this finding in cohort 2, a second cohort of samples collected at 36 weeks' gestation where 41 who developed preeclampsia and 950 did not (cohort 2 was collected consecutively after cohort 1 as part of the FLAG study; cohorts 1 and 2 represent 2 independent groups to discover, then validate biomarkers). In cohort 2 GDF-15 was significantly increased in the women who developed preeclampsia, with a median GDF-15 of 174.7 ng/mL (IQR 127.8–211.9) compared with controls (median GDF-15 126.1 ng/mL [IQR 98–159.8]; P<0.0001; Figure 1B), and an AUC of 0.71. Absolute GDF-15 levels are likely to have varied between cohorts due to batch variations associated with research grade ELISAs.

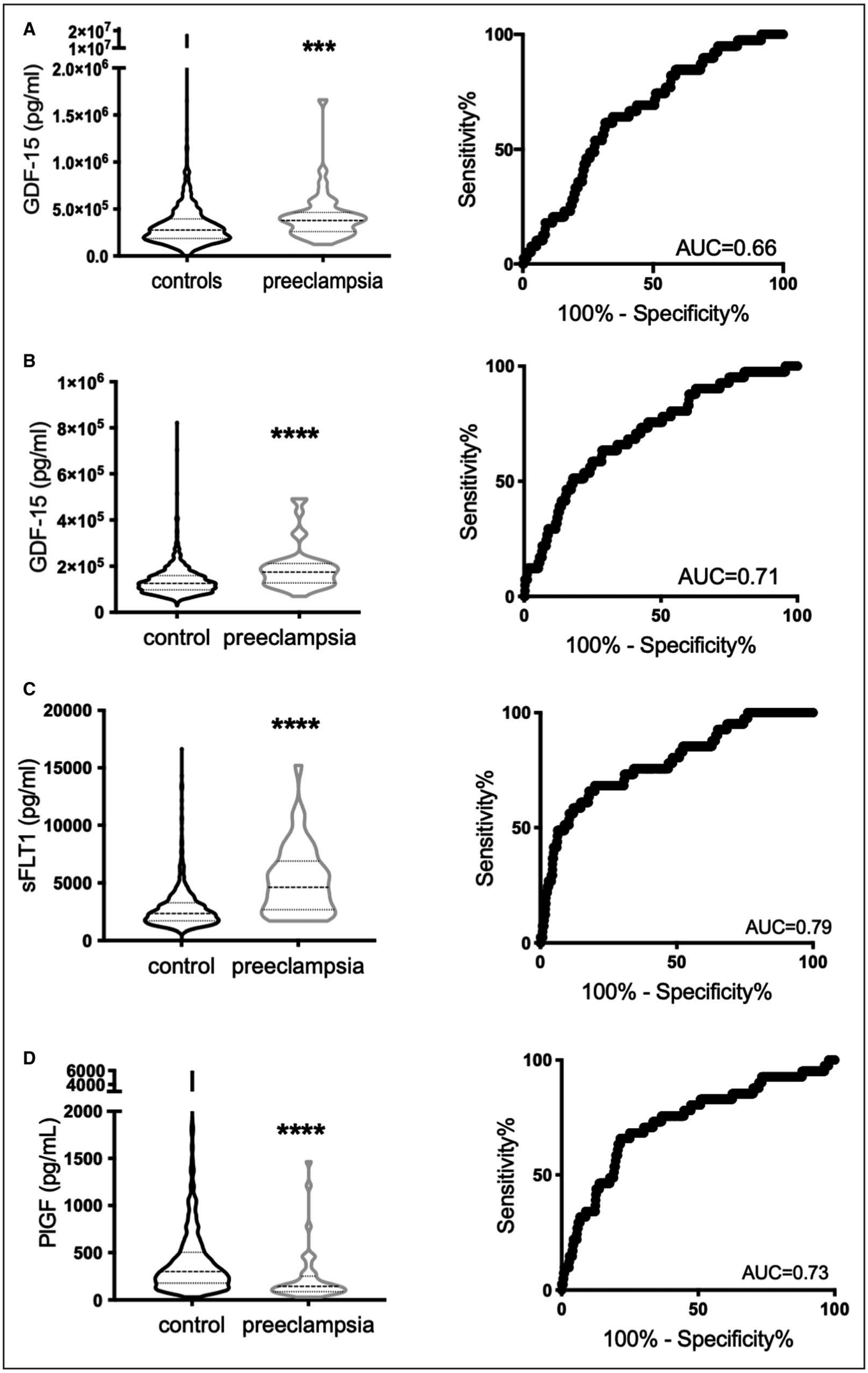
Combining GDF-15 With sFlt-1 and PlGF to Generate Prospective Diagnostic Tests

The landmark PROGNOSIS study showed the sFlt-1/PlGF ratio has excellent performance in identifying women who are unlikely to develop preeclampsia in 1 or 4 weeks (excellent negative predictive value). sFlt/PlGF ratio is now offered as part of clinical care to rule out the likelihood of preeclampsia for women who present where there is clinical uncertainty.[6,23] Conversely, the PROGNOSIS study showed the ratio is more modest in identifying women more likely to develop preeclampsia (sensitivity). Given this, we examined whether adding plasma GDF-15 concentrations may improve the sensitivity of the sFlt-1/PlGF ratio measured at 36 weeks' gestation in identifying who are more likely to develop preeclampsia. We did this using the data we generated in cohort 2 and combined GDF-15 in several ratio combinations with sFlt-1 and PlGF, before calculating diagnostic performances. Given a specificity of 83.1% was used for the PROGNOSIS[6] study, we selected a minimum specificity of 83.1% for our analyses so we could compare different ratio combinations.

We first measured sFlt-1 and PlGF in cohort 2. As expected, at 36 weeks' gestation sFlt-1 was significantly increased in women who developed preeclampsia with a median of 4623 pg/mL (IQR 2668–6884) compared to controls (median 2349 pg/mL [IQR 1715–3273]; P<0.0001), with an AUC of 0.79 (Figure 1C). PlGF at 36 weeks' gestation was significantly reduced in women who developed preeclampsia, with a median of 144.3 pg/mL (IQR 88.1–250.6) compared to 298.9 pg/mL (IQR 117.4–504.6) in controls (P<0.0001). The AUC for PlGF was 0.73 (Figure 1D).

GDF-15 or PlGF alone performed modestly, both with sensitivities of 46.3% (Table 1). sFlt-1 alone, or as a ratio to PlGF yielded a sensitivity of 61% for predicting preeclampsia after 36 weeks. A ratio of GDF-15/PlGF resulted in a sensitivity of 65.9%, while GDF-15×sFlt-1/PlGF resulted in the highest sensitivity of 68.3%.

To assess the rule-in and rule-out performance of the combination of GDF-15×sFlt-1/PlGF for detecting preeclampsia at varying specificity, we performed a supplementary analysis at a fixed 80% and 90% specificity for all



ratiometric biomarker combinations (Tables S8 and S9). This supplementary analysis revealed that the combination of GDF-15×sFlt-1/PlGF achieved the highest rule-in and rule-out performance for all assessed ratiometric combinations with a PPV of 13.6% at 90% specificity and a NPV of 97.8% at 80% specificity.

Figure 2 provides graphical representation of the ratiometric data. The sFlt-1/PlGF ratio was significantly

Figure 1. Circulating GDF-15 and sFlt-1 are increased at 36 weeks' gestation, while circulating PlGF is reduced.
Circulating plasma growth differentiation factor 15 (GDF-15) was measured in 2 cohorts of samples and assessed according to whether women developed preeclampsia at term. In cohort 1 circulating GDF-15 levels were significantly increased in women who later developed preeclampsia relative to controls (**A**; n=960 controls, n=39 preeclampsia) with an area under the receiver operating characteristic curve (AUC) of 0.66. We subsequently measured the levels of GDF-15 in cohort 2, a parallel cohort collected at 36 weeks' gestation (**B**; n=950 controls, n=41 preeclampsia). Similar to cohort 1, GDF-15 was significantly increased in the women who later developed preeclampsia, with an AUC of 0.72. We next assessed soluble fms-like tyrosine kinase 1 (sFlt-1) (**C**) and placental growth factor (PlGF) (**D**) in cohort 2. As expected, sFlt-1 was significantly increased in women who later developed preeclampsia with an AUC of 0.79, while PlGF was significantly reduced, with an AUC of 0.74. The difference in medians between the 2 cohorts likely reflect our use of research grade ELISA that vary in absolute concentrations from batch to batch, and thus the importance of analysis within each cohort, rather than between. Data expressed as median±interquartile range, with each symbol representing a single patient. ****P*<0.011, *****P*<0.0001.

increased (*P*<0.0001) in the women who later developed preeclampsia with an AUC of 0.79 (Figure 2A). The GDF-15/PlGF ratio (Figure 2B) and the combination of GDF-15×sFlt-1/PlGF (Figure 2C) trended toward an increase in identifying those who will develop preeclampsia, with AUCs of 0.78 and 0.82, respectively. Thus, we conclude that adding GDF-15 to the sFlt/PlGF ratio may enhance its performance as a clinical biomarker, although large validation studies with adequate power would be required to confirm this observation.

GDF-15 Is Increased in the Plasma and Placentas of Women Delivering at <34 Weeks' Gestation

We next sought to determine whether there was an association between plasma GDF-15 and preterm preeclampsia. Plasma GDF-15 was significantly increased (*P*<0.01) among 42 women who delivered <34 weeks' gestation with preterm preeclampsia, compared to 26 controls (where bloods where taken around similar gestation as cases, but they progressed to term gestation and did not develop pregnancy complications; Figure 3A).

We also measured GDF-15 expression in placental samples from women who delivered with preterm preeclampsia, compared to gestationally matched pregnancies (without a diagnosis of hypertension during pregnancy). Both placental GDF-15 mRNA (Figure 3B) and protein (Figure 3C) expression were significantly (*P*<0.0001) increased in preeclamptic placentas, relative to gestation matched control placentas. Immunohistochemistry showed GDF-15 was localized to the syncytiotrophoblast layer in both preterm control and preeclamptic placentas (Figure 3D).

Thus, GDF-15 is increased in the circulation and the placenta of women with a diagnosis of preterm preeclampsia.

Plasma GDF-15 Is Increased at 24 to 34 Weeks' Gestation Before a Clinical Diagnosis of Preeclampsia Among Women Attending a High-Risk Pregnancy Clinic

We next measured GDF-15 in a nested case-cohort collection of plasma from women at 24 to 34 weeks' gestation presenting to the Manchester Antenatal Vascular Service (MAViS clinic, Manchester, United Kingdom). Women referred to the MAViS clinic are at high risk of preeclampsia, having previously had preeclampsia in a previous pregnancy, have chronic hypertension or preexisting diabetes mellitus.

After adjusting for gestation at sampling and the presence of underlying hypertensive disease, logGDF15 values in preeclampsia cases were associated with a 2.70 odds ratio (*P*=0.0002, 95% CI, 1.60–4.56) relative to controls (Table 2).

Plasma GDF-15 Is Increased Among Women With Preeclampsia With Severe Features

We also examined circulating GDF-15 levels in a biobank of plasma collected in South Africa, the PROVE cohort. Samples were collected between 21 and 41 weeks' gestation from women with the following subtypes of preeclampsia: proteinuric preeclampsia (no severe features), preeclampsia with severe features or with eclampsia. We also collected controls which were pregnancies without a diagnosis of hypertensive disorders. For all comparisons we adjusted for gestation at sampling.

For cases of proteinuric preeclampsia, logGDF15 values produced an odds ratio of 1.68 (95% CI 0.96–2.96) compared to controls, however the difference was not significant (*P*=0.07; Table 2). For preeclampsia with severe features, logGDF15 values were associated with an odds ratio of 3.28 (95% CI 1.19–9.06) relative to controls (*P*=0.02; Table 2). In eclampsia, logGDF15 values produced an odds ratio of 2.40 (95% CI 0.87–6.57) compared to controls, however the difference was not significant (*P*=0.09; Table 2). Thus, we have confirmed in a cohort from South Africa that GDF15 is significantly elevated in women who develop preeclampsia with severe features, with further study required to confirm the effect in cases of proteinuric preeclampsia or eclampsia.

DISCUSSION

Preeclampsia is a severe disease of the maternal vascular system characterized by placental insufficiency, maternal hypertension, and multi-organ dysfunction. It

Table 1. Predictive Performance for Each Biomarker Combination in Cohort 2 at the Indicated Cut-Off Point, Chosen to Provide a Specificity of 83.1% or Greater

	Cut-off point (pg/mL)	Sensitivity % (95% CI)	Specificity % (95% CI)	Positive predictive value % (95% CI)	Negative predictive value % (95% CI)
GDF-15 alone	176 586.63	46.3 (30.7–62.6)	83.2 (80.6–85.5)	10.6 (7.7–14.5)	97.3 (96.4–98.0)
PlGF alone	135.00	46.3 (30.7–62.6)	83.2 (80.6–85.5)	10.6 (7.7–14.5)	97.3 (96.4–98.0)
sFlt-1 alone	3806.00	61.0 (44.5–75.8)	83.2 (80.6–85.5)	13.5 (10.5–17.2)	98.0 (97.1–98.6)
sFlt-1/PlGF	23.91	61.0 (44.5–75.8)	83.2 (80.6–85.5)	13.5 (10.5–17.2)	98.0 (97.1–98.6)
GDF-15/PlGF	1009.82	65.9 (49.4–79.9)	83.2 (80.6–85.5)	14.4 (11.5–18.0)	98.3 (97.4–98.9)
GDF-15×sFlt-1/PlGF	3 424 354.50	68.3 (51.9–81.9)	83.2 (80.6–85.5)	14.9 (12.0–18.4)	98.4 (97.5–99.0)

While cut-offs are presented, note the GDF-15 is a research grade ELISA so absolute numbers may vary between batches. Cohort 2 contained 41 preeclampsia cases and 950 controls, resulting in a prevalence of ≈4.1%. All cut-off points calculated with biomarker measurements in pg/mL. GDF-15 indicates growth differentiation factor 15; PlGF, placental growth factor; and sFlt-1, soluble FMS-like tyrosine kinase-1.

can also develop to neurological compromise and eclampsia. In this study we report consistent increases in circulating GDF-15 both preceding term diagnosis and in preterm disease. Finally, we have undertaken analyses in women with underlying vascular disease and in a biobank of increasing disease severity, including eclampsia. Our novel data suggest that GDF-15 should be considered in combination with well-known placental biomarkers, sFlt-1 and PlGF, to predict preeclampsia in future validation studies.

GDF-15 is a circulating protein that has recently gained much interest as a suppressor of food intake in mice, that binds to the GDNF family receptor-α-like (GFRAL), a receptor only found in the hindbrain.[24,25] Interestingly, GDF-15 is also highly expressed in placenta, and thus it has previously been measured in preeclampsia. However, the current consensus within the literature is conflicted. Chen and others[14] suggest that serum GDF-15 is reduced in the circulation of women with preeclampsia, Marjorno and others[13] report no difference, and Sugulle et al[15] and Temel Yuksel et al[16] report increased GDF-15 in patients with preeclampsia. A fifth recent report[17] examined GDF-15 in case-cohorts across the second and third trimesters and only reported significant increases in the preeclamptic cohort between 30 and 34 weeks' gestation for women who developed preterm disease. An important difference between these prior studies and our studies is that they all utilized serum, compared to our measurement of plasma GDF-15. In addition to this difference in starting sample, there were also differences in the ELISA platforms used to measure the serum GDF-15, with none of the prior studies utilizing the Thermo-Fisher Scientific Human GDF-15 kit employed in this study. We measured GDF-15 levels in a large prospective collection at 36 weeks' gestation preceding term preeclampsia diagnosis, a cohort in which we also characterised sFlt-1 and PlGF levels. A significant strength of our study is the large cohorts in which we measured sFlt-1, PlGF and GDF-15, numbering just under 1000 women, with preeclampsia patients considered at population rates, rather than as a case-cohort, which may enrich findings. Although no significant differences were detected between groups in regard to sensitivity, our data provide strong trends to suggest GDF-15 may indeed be additive to sFlt-1 and PlGF for predicting term preeclampsia, and thus warrants future validation.

Circulating GDF-15 was also increased in women with preterm preeclampsia who delivered <34 weeks' gestation, and we also identified significantly increased circulating GDF-15 levels in women with underlying cardiovascular disease who later developed preeclampsia (MAViS clinic patients). This indicates that those more likely to develop preeclampsia have further elevations in this stress-related biomarker beyond the levels already apparent in women with hypertension and underlying vascular disease.[12] Moreover, our analyses in the smaller PROVE biobank, suggest that GDF-15 levels might increase with disease severity in preeclamptic women, with more significant alterations observed in women with preeclampsia and severe features relative to controls, compared to the alterations observed in the women with proteinuric preeclampsia.

While GDF-15 is measurable in many conditions outside pregnancy, our placental data from women with preterm preeclampsia suggest that the increase in circulating concentrations is likely placental in origin. Indeed, being localized to the syncytiotrophoblast layer that separates the maternal and fetal circulation provides a site of release directly into the maternal circulation. Whether high GDF-15 plays an important role in the pathogenesis of preeclampsia or is a bystander, requires further investigation. While epidemiological

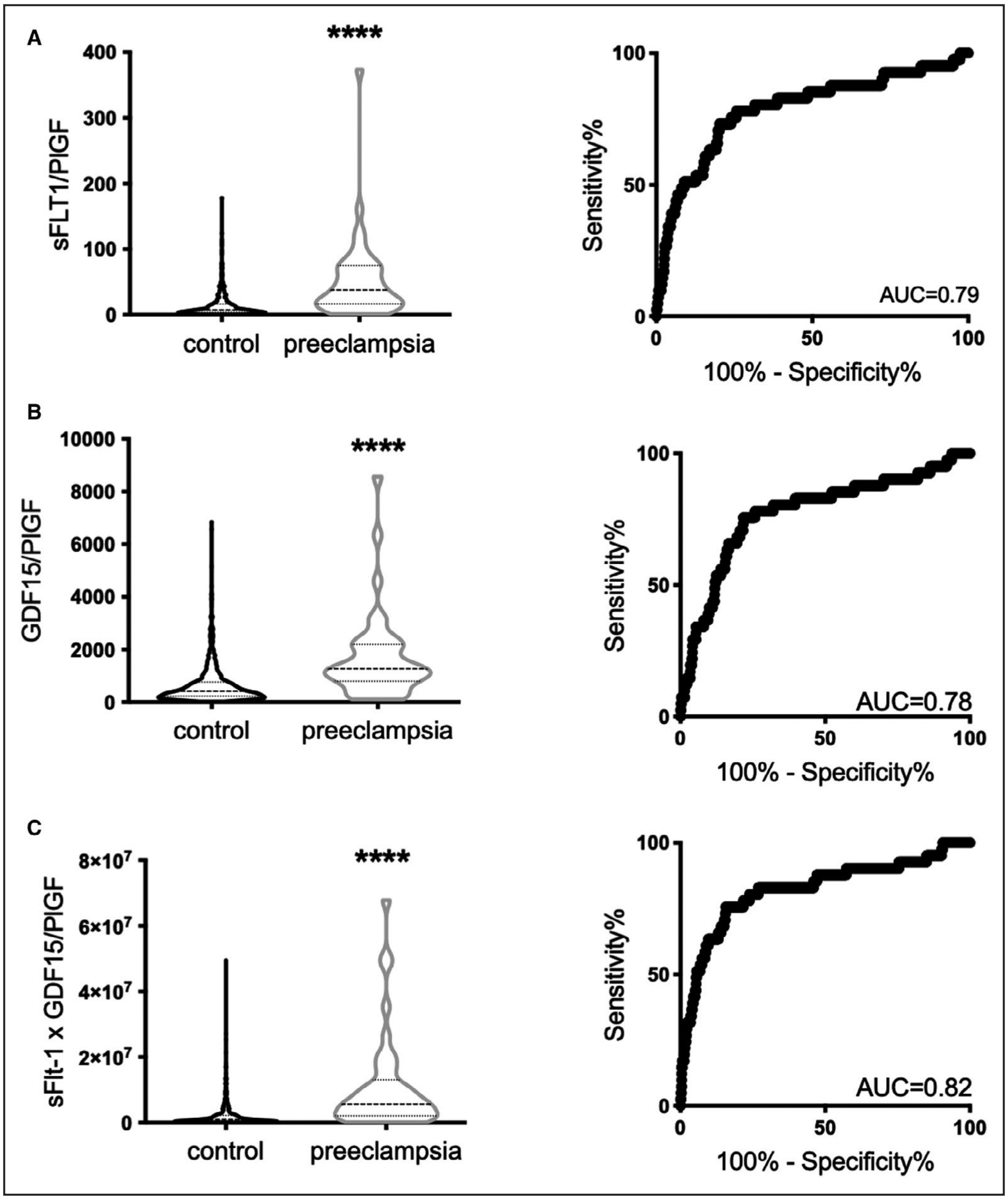


Figure 2. Ratiometric analyses identify a novel combination of sFlt-1, PlGF and GDF-15.
Given a ratio of soluble fms-like tyrosine kinase 1/placental growth factor (sFlt-1/PlGF) is now being used clinically to rule out high-risk women, we assessed the performance of this ratio using data from cohort 2. The sFlt-1/PlGF ratio was significantly increased at 36 weeks' gestation in women who developed preeclampsia at term, with an area under the receiver operator curve (AUC) of 0.79 (**A**). Similarly, a ratio of growth differentiation factor 15 (GDF-15)/PlGF also produced a significant increase in the preeclamptic cohort, with an AUC of 0.78 (**B**). Finally, a novel ratio, where GDF-15 was multiplied by sFlt-1/PlGF also resulted in a significantly increased ratio in the women who later developed preeclampsia, with an AUC of 0.82 (**C**). Data expressed as median±interquartile range, with each symbol representing a single patient. ****P<0.0001.

evidence suggests high circulating GDF-15 levels are strongly associated with worsening cardiovascular prognosis[26–29] outside of pregnancy, other functional and preclinical studies in mice suggest it may be cardioprotective.[30] Similarly, circulating GDF-15 levels also correlate with increased risk of chronic kidney disease progression[31] or worsening albuminuria in patients with type 2 diabetes,[32] while preclinical functional and animal studies suggest that it could be reno-protective.[33–35] These seemingly contradictory findings have led to suggestions that increased GDF-15 might occur as a compensatory response to tissue injury.[30]

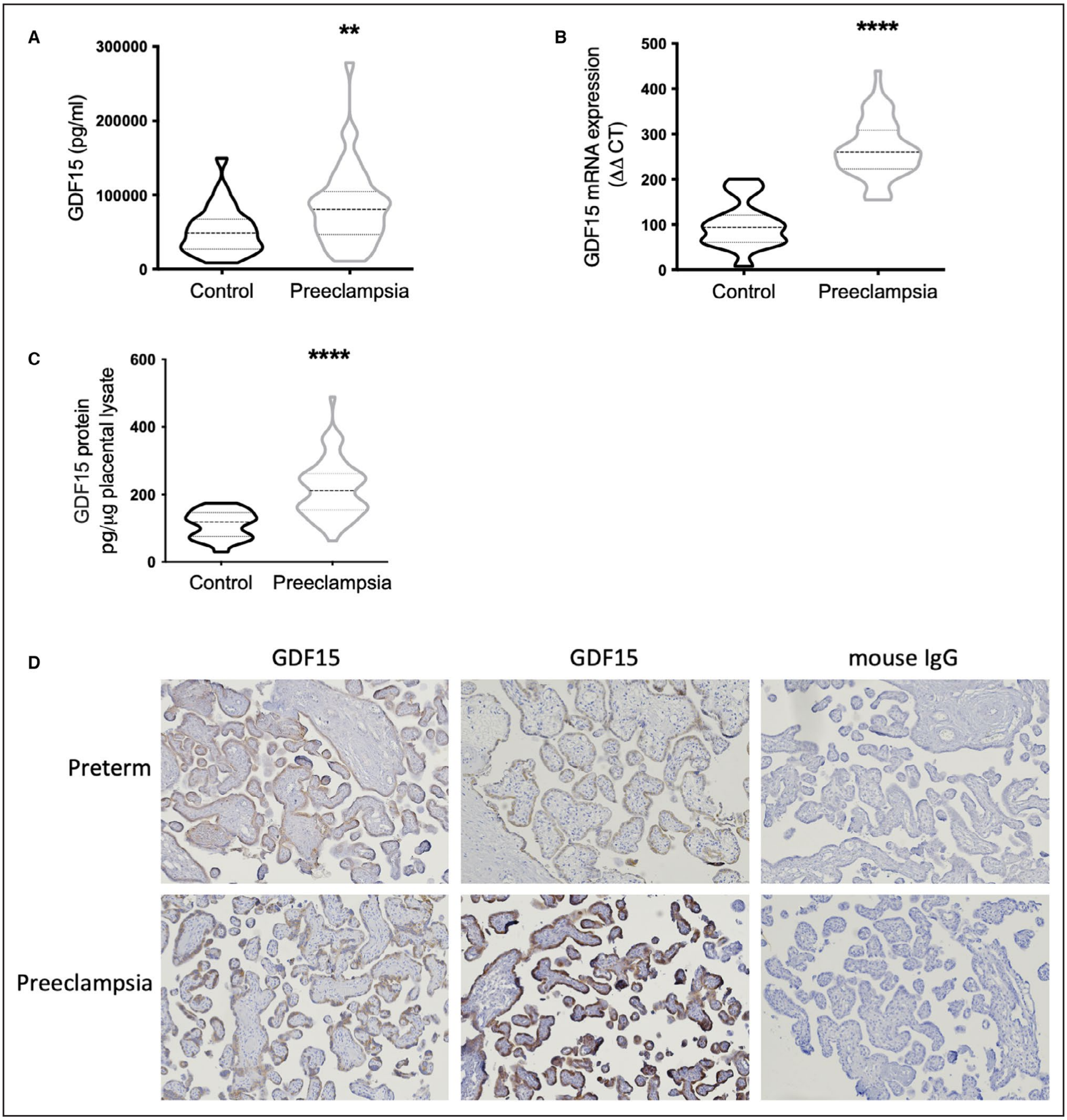


Figure 3. GDF-15 is increased in the placenta and plasma of women who deliver preterm with severe preeclampsia.
Circulating growth differentiation factor 15 (GDF-15) was significantly increased in the plasma of 42 women with preterm preeclampsia relative to 26 gestation matched controls who delivered healthy infants at term (**A**). Similarly, in the placentas of women who had preterm preeclampsia, GDF-15 mRNA expression (n=64 preeclampsia, n=15 controls) (**B**) and GDF-15 protein (n=59 preeclampsia, n=13 controls) (**C**) was significantly increased, relative to women who delivered preterm for other reasons. Immunohistochemistry identified GDF-15 in the syncytiotrophoblast layer of both preterm (PT) and preterm preeclamptic (PE) placentas, with the mouse immunoglobulin G (IgG) control clear of brown staining (**D**). Data expressed as median±interquartile range, with each symbol representing a single patient. $^{**}P<0.01$, $^{****}P<0.0001$.

In 2019, the outcomes of the PHOENIX trial[36] provided strong evidence that planned delivery reduces maternal morbidity and severe hypertension compared to expectant management for women with preeclampsia. Thus, a reliable method for detecting at-risk women could result in improved surveillance and personalized care to reduce adverse outcomes for both the mother and baby. We provide consistent data in numerous cohorts to indicate that GDF-15 is increased in preeclampsia and eclampsia. Importantly, our data

Table 2. Logistic Regression Results for the MAViS and PROVE Cohorts

Outcome (cohort)	Variable	Odds ratio (95% CI)	*P* value
Pre-eclampsia (MAViS)	Log transformed GDF15, pg/mL	2.70 (1.60–4.56)	0.0002
	Restricted cubic spline transformed gestation at sampling, d	0.43* (0.17–1.08)	N/A
	Chronic hypertension	0.97 (0.39–2.43)	0.95
	Renal hypertension	0.13 (0.03–0.56)	0.006
Proteinuric pre-eclampsia (PROVE)	Log transformed GDF15, pg/mL	1.68 (0.96–2.96)	0.07
	Restricted cubic spline transformed gestation at sampling, d	0.56* (0.06–5.36)	N/A
Pre-eclampsia with severe features (PROVE)	Log transformed GDF15, pg/mL	3.28 (1.19–9.06)	0.02
	Restricted cubic spline transformed gestation at sampling, d	0.02* (0.001–0.28)	N/A
Eclampsia (PROVE)	Log transformed GDF15, pg/mL	2.40 (0.87–6.57)	0.09
	Restricted cubic spline transformed gestation at sampling, d	0.41* (0.05–3.43)	N/A

Given the wide gestational range at sampling, logistic regression with respect to the log-transformed GDF15 values was performed to account for gestation at sampling in both MAViS and PROVE cohorts. For MAViS, underlying hypertension was also included as part of the regression analysis. GDF-15 indicates growth differentiation factor 15; and N/A, not applicable.

*For restricted cubic spline transformed variables, the odds ratio (95% CI) is reported for the interquartile range.

at 36 weeks' gestation identify GDF-15 as a promising predictive biomarker that might be combined with sFlt-1 and PlGF to improve the sensitivity of this clinical test, flagging women at increased risk of developing preeclampsia.

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Disclosures

None.

Supplementary Material

Tables S1–S9

REFERENCES

1. Young BC, Levine RJ, Karumanchi SA. Pathogenesis of preeclampsia. *Annu Rev Pathol*. 2010;5:173–192. DOI: 10.1146/annurev-pathol-121808-102149.
2. Redman CW, Sargent IL. Latest advances in understanding preeclampsia. *Science*. 2005;308:1592–1594. DOI: 10.1126/science.1111726.
3. Roberts JM, Gammill HS. Preeclampsia: recent insights. *Hypertension*. 2005;46:1243–1249. DOI: 10.1161/01.HYP.0000188408.49896.c5.
4. Maynard SE, Min J-Y, Merchan J, Lim K-H, Li J, Mondal S, Libermann TA, Morgan JP, Sellke 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.
5. Levine RJ, Thadhani R, Qian C, Lam C, Lim KH, Yu KF, Blink AL, Sachs BP, Epstein FH, Sibai BM, et al. Urinary placental growth factor and risk of preeclampsia. *JAMA*. 2005;293:77–85. DOI: 10.1001/jama.293.1.77.
6. Zeisler H, Llurba E, Chantraine F, Vatish M, Staff AC, Sennström M, Olovsson M, Brennecke SP, Stepan H, Allegranza D, et al. Predictive value of the sFlt-1: PlGF ratio in women with suspected preeclampsia. *N Engl J Med*. 2016;374:13–22. DOI: 10.1056/NEJMoa1414838.
7. Agrawal S, Shinar S, Cerdeira AS, Redman C, Vatish M. Predictive performance of PlGF (placental growth factor) for screening preeclampsia in asymptomatic women: a systematic review and meta-analysis. *Hypertension*. 2019;74:1124–1135. DOI: 10.1161/HYPERTENSIONAHA.119.13360.
8. Whigham CA, MacDonald TM, Walker SP, Hannan NJ, Tong S, Kaitu'u-Lino TJ. The untapped potential of placenta-enriched molecules for diagnostic and therapeutic development. *Placenta*. 2019;84:28–31. DOI: 10.1016/j.placenta.2019.02.002.
9. Bootcov MR, Bauskin AR, Valenzuela SM, Moore AG, Bansal M, He XY, Zhang HP, Donnellan M, Mahler S, Pryor K, et al. MIC-1, a novel macrophage inhibitory cytokine, is a divergent member of the TGF-beta superfamily. *Proc Natl Acad Sci USA*. 1997;94:11514–11519.
10. Yokoyama-Kobayashi M, Saeki M, Sekine S, Kato S. Human cDNA encoding a novel TGF-beta superfamily protein highly expressed in placenta. *J Biochem*. 1997;122:622–626.

11. Bauskin AR, Brown DA, Kuffner T, Johnen H, Luo XW, Hunter M, Breit SN. Role of macrophage inhibitory cytokine-1 in tumorigenesis and diagnosis of cancer. *Cancer Res*. 2006;66:4983–4986.
12. Wollert KC, Kempf T, Wallentin L. Growth differentiation factor 15 as a biomarker in cardiovascular disease. *Clin Chem*. 2017;63:140–151. DOI: 10.1373/clinchem.2016.255174.
13. Marjono AB, Brown DA, Horton KE, Wallace EM, Breit SN, Manuelpillai U. Macrophage inhibitory cytokine-1 in gestational tissues and maternal serum in normal and pre-eclamptic pregnancy. *Placenta*. 2003;24:100–106. DOI: 10.1053/plac.2002.0881.
14. Chen Q, Wang Y, Zhao M, Hyett J, da Silva CF, Nie G. Serum levels of GDF15 are reduced in preeclampsia and the reduction is more profound in late-onset than early-onset cases. *Cytokine*. 2016;83:226–230. DOI: 10.1016/j.cyto.2016.05.002.
15. Sugulle M, Dechend R, Herse F, Weedon-Fekjaer MS, Johnsen GM, Brosnihan KB, Anton L, Luft FC, Wollert KC, Kempf T, et al. Circulating and placental growth-differentiation factor 15 in preeclampsia and in pregnancy complicated by diabetes mellitus. *Hypertension*. 2009;54:106–112. DOI: 10.1161/HYPERTENSIONAHA.109.130583.
16. Temel Yuksel I, Mathyk BA, Aslan Cetin B, Turhan U, Okumus ZG, Yetkin Yildirim G, Acar DK. Maternal levels of growth differentiation factor-15 in patients with preeclampsia. *Hypertens Pregnancy*. 2018;37:192–196. DOI: 10.1080/10641955.2018.1524477.
17. Wertaschnigg D, Rolnik DL, Nie G, Teoh SSY, Syngelaki A, da Silva CF, Nicolaides KH. Second and third trimester serum levels of growth-differentiation factor-15 in the prediction of pre-eclampsia. *Ultrasound Obstet Gynecol*. 2020;56:879–884.
18. Moodley J, Soma-Pillay P, Buchmann E, Pattinson RC. Hypertensive disorders in pregnancy: 2019 national guideline. *S Afr Med J*. 2019;109:12723.
19. Apbn S. Gestational hypertension and preeclampsia. *Obstet Gynecol*. 2019;133:211–214.
20. R Core Team. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing; 2021. https://www.R-project.org/.
21. Harrell FE Jr. et al. Hmisc: Harrell Miscellaneous. R package version 4.5-0. 2021. http://CRAN.R-project.org/package=Hmisc.
22. Harrell FE Jr. Rms: regression modeling strategies. R package version 6.2-0. 2021. https://CRAN.R-project.org/package=rms.
23. Zeisler H, Llurba E, Chantraine FJ, Vatish M, Staff AC, Sennstrom M, Olovsson M, Brennecke SP, Stepan H, Allegranza D, et al. The sFlt-1/PlGF ratio: ruling out pre-eclampsia for up to 4 weeks and the value of retesting. *Ultrasound Obstet Gynecol*. 2019.53:367–375. DOI: 10.1002/uog.19178.
24. Mullican SE, Lin-Schmidt X, Chin C-N, Chavez JA, Furman JL, Armstrong AA, Beck SC, South VJ, Dinh TQ, Cash-Mason TD, et al. GFRAL is the receptor for GDF15 and the ligand promotes weight loss in mice and nonhuman primates. *Nat Med*. 2017;23:1150–1157. DOI: 10.1038/nm.4392.
25. Mullican SE, Rangwala SM. Uniting GDF15 and GFRAL: therapeutic opportunities in obesity and beyond. *Trends Endocrinol Metab*. 2018;29:560–570. DOI: 10.1016/j.tem.2018.05.002.
26. Brown DA, Breit SN, Buring J, Fairlie WD, Bauskin AR, Liu T, Ridker PM. Concentration in plasma of macrophage inhibitory cytokine-1 and risk of cardiovascular events in women: a nested case-control study. *Lancet*. 2002;359:2159–2163. DOI: 10.1016/S0140-6736(02)09093-1.
27. Hagström E, Held C, Stewart RAH, Aylward PE, Budaj A, Cannon CP, Koenig W, Krug-Gourley S, Mohler ER III, Steg PG, et al. Growth differentiation factor 15 predicts all-cause morbidity and mortality in stable coronary heart disease. *Clin Chem*. 2017;63:325–333. DOI: 10.1373/clinchem.2016.260570.
28. Hagström E, James SK, Bertilsson M, Becker RC, Himmelmann A, Husted S, Katus HA, Steg PG, Storey RF, Siegbahn A, et al. Growth differentiation factor-15 level predicts major bleeding and cardiovascular events in patients with acute coronary syndromes: results from the PLATO study. *Eur Heart J*. 2016;37:1325–1333. DOI: 10.1093/eurheartj/ehv491.
29. Lindholm D, Hagstrom E, James SK, Becker RC, Cannon CP, Himmelmann A, Katus HA, Maurer G, Lopez-Sendon JL, Steg PG, et al. Growth differentiation factor 15 at 1 month after an acute coronary syndrome is associated with increased risk of major bleeding. *J Am Heart Assoc*. 2017;6:e005580. DOI: 10.1161/JAHA.117.005580.
30. Emmerson PJ, Duffin KL, Chintharlapalli S, Wu X. GDF15 and growth control. *Front Physiol*. 2018;9:1712. DOI: 10.3389/fphys.2018.01712.
31. Nair V, Robinson-Cohen C, Smith MR, Bellovich KA, Bhat ZY, Bobadilla M, Brosius F, de Boer IH, Essioux L, Formentini I, et al. Growth differentiation factor-15 and risk of CKD progression. *J Am Soc Nephrol*. 2017;28:2233–2240. DOI: 10.1681/ASN.2016080919.
32. Hellemons ME, Mazagova M, Gansevoort RT, Henning RH, de Zeeuw D, Bakker SJ, Lambers-Heerspink HJ, Deelman LE. Growth-differentiation factor 15 predicts worsening of albuminuria in patients with type 2 diabetes. *Diabetes Care*. 2012;35:2340–2346. DOI: 10.2337/dc12-0180.
33. Kim YI, Shin HW, Chun YS, Park JW. CST3 and GDF15 ameliorate renal fibrosis by inhibiting fibroblast growth and activation. *Biochem Biophys Res Commun*. 2018;500:288–295. DOI: 10.1016/j.bbrc.2018.04.061.
34. Mazagova M, Buikema H, Landheer SW, Vavrinec P, Buiten A, Henning RH, Deelman LE. Growth differentiation factor 15 impairs aortic contractile and relaxing function through altered caveolar signaling of the endothelium. *Am J Physiol Heart Circ Physiol*. 2013;304:H709–H718. DOI: 10.1152/ajpheart.00543.2012.
35. Mazagova M, Buikema H, van Buiten A, Duin M, Goris M, Sandovici M, Henning RH, Deelman LE. Genetic deletion of growth differentiation factor 15 augments renal damage in both type 1 and type 2 models of diabetes. *Am J Physiol Renal Physiol*. 2013;305:F1249–F1264. DOI: 10.1152/ajprenal.00387.2013.
36. Chappell LC, Brocklehurst P, Green ME, Hunter R, Hardy P, Juszczak E, Linsell L, Chiocchia V, Greenland M, Placzek A, et al. Planned early delivery or expectant management for late preterm pre-eclampsia (PHOENIX): a randomised controlled trial. *Lancet*. 2019;394:1181–1190. DOI: 10.1016/S0140-6736(19)31963-4.

Supplemental Material

Table S1. Maternal characteristics and pregnancy outcomes for FLAG Cohort 1.

	Controls (n=960)	**Preeclampsia (n=39)**	**P value**
Maternal Age (years) Median [IQR]	32.0 [30.0-35.0]	34.0 [30.0-36.0]	0.41
Booking BMI (kg/m^2) Median [IQR]	24.0 [21.7-27.4]	26.8 [23.1-31.3]	0.006
Nulliparous no. (%)	470 (49.0%)	28 (71.8%)	0.005
Current smokers no. (%)	30 (3.1%)	2 (5.1%)	0.36
Gestational diabetes no. (%)	128 (13.3%)	9 (23.1%)	0.09
Onset of labour no. (%)			
Spontaneous	468 (48.8%)	13 (33.3%)	0.12
Induced	327 (34.1%)	19 (48.7%)	
No labour	165 (17.2%)	7 (17.9%)	
Caesarean Section no. (%)	305 (31.8%)	21 (53.8%)	0.005
Gestation at delivery (weeks) Median [IQR]	39.6 [38.7-40.4]	39.3 [37.9-40.4]	0.08
Birthweight (g) Median [IQR]	3460 [3150-3760]	3350 [2867-3650]	0.08

BMI = body mass index. Data presented as median [25th – 75th percentile] and as number (%) if categorical. Mann-Whitney U tests used for comparison of medians. Fisher's exact tests used for categorical variables.

Table S2. Maternal characteristics and pregnancy outcomes for FLAG Cohort 2.

	Controls (n=950)	**Preeclampsia (n=41)**	**P value**
Maternal Age (years) Median [IQR]	32.0 [30.0-35.0]	31.0 [29.5-34.0]	0.25
Booking BMI (kg/m^2) Median [IQR]	24.4 [22.0-27.9]	23.8 [21.5-28]	0.93
Nulliparous no. (%)	407 (42.8%)	29 (70.1%)	0.0006
Current smokers no. (%)	28 (2.9%)	3 (7.3%)	0.13
Gestational diabetes no. (%)	114 (12.0%)	6 (14.6%)	0.62
Onset of labour no. (%) Spontaneous Induced No labour	437 (46.0%) 324 (34.1%) 189 (19.9%)	10 (24.4%) 24 (58.5%) 7 (17.1%)	0.0043
Caesarean Section no. (%)	318 (33.5%)	23 (56.1%)	0.005
Gestation at delivery (weeks) Median [IQR]	39.3 [38.7- 40.3]	39.1 [38.7-39.9]	0.58
Birthweight (g) Median [IQR]	3403 [3110-3710]	3390 [3090-3738]	0.81

BMI = body mass index. Data presented as median [25^{th} – 75^{th} percentile] and as number (%) if categorical. Mann-Whitney U tests used for comparison of medians. Fisher's exact tests used for categorical variables.

Table S3. Severe early onset preeclampsia plasma samples.

	Controls (n=26)	**Preeclampsia (n=42)**	**P value**
Maternal Age (years) Median [IQR]	31.4 [29.6 – 33.8]	32.4 [29.4 – 34.5]	0.47
Gestation at Delivery (weeks) Median [IQR]	36.7 [34.5 – 37.4]	28.6 [27.1 – 30.9]	<0.0001
Gestation at Blood Collection (weeks) Median [IQR]	28.1 [26.7 – 29.8]	29.1 [27.4 – 31.3]	0.08
BMI (kg/m^2) Median [IQR]	24.6 [22 – 28.5]	28 [26 – 33.7]	0.0042
Parity no. (%) 0 1 ≥2	 10 (38.5) 10 (38.5) 6 (23.1)	 28 (66.7) 9 (21.4) 5 (11.9)	0.07
SBP at Delivery (mmHg) Median [IQR]	125 [120 – 130]	171 [166 – 180]	<0.0001
DBP at Delivery (mmHg) Median [IQR]	75 [70 – 80]	100 [100 – 110]	<0.0001
Birth weight (g) Median [IQR]	3583 [3215 – 3783]	1057 [788 – 1455]	<0.0001
Male no. (%)	13 (50)	17 (40.4)	0.46

BMI = body mass index, SBP = systolic blood pressure and DBP = diastolic blood pressure. Data presented as median [25th – 75th percentile] and as number (%) if categorical. Mann-Whitney U tests used for comparison of medians. Fisher's exact tests used for categorical variables. BMI data missing for 3/42 PE samples.

Table S4. Maternal characteristics and pregnancy outcomes for the Manchester Antenatal Vascular Service (MAViS) cohort.

	Controls (n=176)	**Preeclampsia** (n=57)
Ethnicity:		
White	86 (49.71)	21 (36.84)
Asian	26 (15.03)	8 (14.04)
Black	43 (24.86)	23 (40.35)
Other	18 (10.4)	5 (8.77)
Age	34.7 (4.9)	33.7 (5.4)
BMI	29.6 [25.1-34.4]	30.1 [25.7-33.5]
Nullip	45 (25.57)	14 (24.56)
Multip with hx	69 (39.2)	29 (50.88)
Multip no hx	62 (35.23)	14 (24.56)
Smoker	4 (2.55)	1 (1.92)
Chronic hypertension	116 (78.38)	44 (91.67)
Renal hypertension	32 (21.62)	4 (8.33)
No diabetes	105 (76.09)	31 (67.39)
Type 1 diabetes	4 (2.9)	4 (8.7)
Type 2 Diabetes	7 (5.07)	3 (6.52)
GDM	22 (15.94)	8 (17.39)
Labour onset		
spontaneous	30 (17.44)	0 (0)
induced	81 (47.09)	24 (42.86)
no labour	61 (35.47)	31 (55.36)
not known	0 (0)	1 (1.79)
Mode of birth		
Elective Section	61 (34.86)	13 (22.81)
Emergency Section	22 (12.57)	25 (43.86)
Forceps	16 (9.14)	5 (8.77)

Vaginal	76 (43.45)	14 (24.56)
Gestation at birth	270 [266-275]	252 [232-264]
Birthweight	3340 [3040-3560]	2440 [1686-2960]
Customised centile	45.8 [26.0-66.1]	12.505 [3.1-45.5]
SGA		
<5th centile	0 (0)	18 (31.58)
<10th centile	0 (0)	25 (43.86)
Preterm Birth		
Delivered <34weeks	0 (0)	18 (31.58)
Delivered <37weeks	0 (0)	36 (63.16)

Table S5. Maternal characteristics and pregnancy outcomes for the Preeclampsia Obstetric adVerse Events (PROVE) cohort.

	Controls (n=14)	**Proteinuric preeclampsia (n=13)**	**Preeclampsia with severe features (n=14)**	**Eclampsia (n=31)**	**P value**
Age (years) Median [IQR]	30 [24-33]	24 [21-28]	30.5 [23-34]	20 [17-24]	<0.001
Booking BMI (kg/m^2) Median [IQR]	26.3 [23.7-30.4]	34.6 [26.4-36.5]	26.7 [22.9-29.8]	24.2 [22.5-25.9]	0.004
Nulliparous no. (%)	4 (28.6)	8 (57.1)	3 (23.1)	25 (80.7)	<0.001
Current smokers no. (%)	3 (21.4)	0	1 (7.7)	5 (16.1)	0.36
Gestational diabetes no. (%)	0	0	0	1 (3.2)	1
Mode of delivery, no. (%)					
Spontaneous	3 (21.4)	0	0	1 (3.2)	0.07
Induced	0	4 (28.5)	3 (23.1)	9 (29.1)	
Caesarean Section	11 (78.6)	10 (71.4)	10 (76.9)	21 (67.7)	
Gestation at delivery (weeks) Median [IQR]	37.6 [31-39.7]	34.4 [32.9-37.9]	31.0 [28.9-33.7]	34.1 [30.7-36.6]	0.023
Birthweight (g) Median [IQR]	2720 [2030-3485]	2630 [1820-3150]	1437.5 [1130-1715]	2105 [1210-2975]	0.017
Highest systolic blood pressure (mmHg) Median [IQR]	117.5 [111-123]	168 [155-171]	169 [167-194]	167 [149-187]	<0.001
Highest diastolic blood pressure (mmHg) Median [IQR]	62.5 [56.5-69]	100 [93-110]	103 [97-100]	107 [97-120]	<0.001

BMI = body mass index. Data presented as median [25th – 75th percentile] and as number (%) if categorical. Kruskal-Wallis tests used for comparison of medians. Fisher's exact tests used for categorical variables. BMI and blood pressure data not available for 7 and 2 patients, respectively.

Table S6. Patient characteristics from which placental samples were obtained for mRNA analyses.

	Controls (n=15)	**Preeclampsia (n=64)**	**P value**
Maternal Age (years) Median [IQR]	30.4 [21.8 – 36.4]	31.4 [27.7 – 34.3]	0.42
Gestation at Delivery (weeks) Median [IQR]	30.7 [28.4 – 31.7]	30.2 [28.2-31.9]	0.83
BMI (kg/m^2) Median [IQR]	29.2 [23.7-36.7]	27 [25-35.1]	0.83
Parity no. (%) 0 1 ≥2	 5 (33.3) 7 (46.7) 3 (20)	 47 (73.4) 11 (17.2) 6 (9.4)	0.0081
SBP at Delivery (mmHg) Median [IQR]	125 [112 – 130]	175 [160-180]	<0.0001
DBP at Delivery (mmHg) Median [IQR]	75 [70-80]	102.5 [96.3-110]	<0.0001
Birth weight (g) Median [IQR]	1589 [1318-1886]	1127 [859-1435]	0.0045
Male no. (%)	9 (60)	34 (53)	0.78

BMI = body mass index, SBP = systolic blood pressure and DBP = diastolic blood pressure. Data presented as median [25^{th} – 75^{th} percentile] and as number (%) if categorical. Mann-Whitney U tests used for comparison of medians. Fisher's exact tests used for categorical variables. BMI data missing for 5/15 control samples and 6/64 preeclampsia samples.

Table S7. Patient characteristics from which placental samples were obtained for protein analyses.

	Controls (n=13)	**Preeclampsia (n=59)**	**P value**
Maternal Age (years) Median [IQR]	32 [23 – 37.5]	31 [27 – 35]	0.92
Gestation at Delivery (weeks) Median [IQR]	30 [28 – 31.9]	30.6 [28.9-31.9]	0.56
BMI (kg/m^2) Median [IQR]	30 [24.5-35.2]	30.6 [28.9-31.9]	0.98
Parity no. (%) 0 1 ≥2	 3 (23) 7 (54) 3 (23)	 44 (74.6) 9 (15.3) 6 (10.1)	0.0013
SBP at Delivery (mmHg) Median [IQR]	120 [111 – 132.5]	170 [160-180]	<0.0001
DBP at Delivery (mmHg) Median [IQR]	80 (70-80)	100 [99-110]	<0.0001
Birth weight (g) Median (IQR)	1540 [1145-1861]	1314 [1000-1464]	0.05
Male no. (%)	6 (46)	32 (54)	0.76

BMI = body mass index, SBP = systolic blood pressure and DBP = diastolic blood pressure. Data presented as median [25^{th} – 75^{th} percentile] and as number (%) if categorical. Mann-Whitney U tests used for comparison of medians. Fisher's exact tests used for categorical variables. BMI data missing for 4/13 control samples.

Table S8. Predictive performance for each biomarker combination in Cohort 2 at the indicated cut-off point, chosen to provide a specificity of 80%.

	Cut-off point (pg/ml)	**Sensitivity % (95% CI)**	**Specificity % (95% CI)**	**Positive predictive value % (95% CI)**	**Negative predictive value % (95% CI)**
GDF-15 alone	170925.50	51.2 (35.1 – 67.1)	80.0 (77.3 – 82.5)	10.0 (7.4 – 13.3)	97.4 (96.5 – 98.1)
PlGF alone	148.21	58.5 (42.1 – 73.7)	80.0 (77.3 – 82.5)	11.2 (8.7 – 14.4)	97.8 (96.9 – 98.5)
sFlt-1 alone	3588.99	68.3 (51.9 – 81.9)	80.0 (77.3 – 82.5)	12.8 (10.4 – 15.8)	98.3 (97.4 – 98.9)
sFlt-1/PlGF	20.10	70.7 (54.5 – 83.9)	80.0 (77.3 – 82.5)	13.2 (10.8 – 16.2)	98.4 (97.5 – 99.0)
GDF-15/PlGF	896.09	68.3 (51.9 – 81.9)	80.0 (77.3 – 82.5)	12.8 (10.4 – 15.8)	98.3 (97.4 – 98.9)
GDF-15 x sFlt-1/PlGF	2752313.68	73.2 (57.1 – 85.8)	80.0 (77.3 – 82.5)	13.6 (11.2 – 16.5)	98.6 (97.7 – 99.1)

While cut-offs are presented, note the GDF-15 is a research grade ELISA so absolute numbers may vary between batches. Cohort 2 contained 41 preeclampsia cases and 950 controls, resulting in a prevalence of approximately 4.1%. All cut-off points calculated with biomarker measurements in pg/ml.

GDF-15 = Growth Differentiation Factor 15

sFlt-1 = soluble FMS-like tyrosine kinase-1

PlGF = Placental growth factor

Table S9. Predictive performance for each biomarker combination in Cohort 2 at the indicated cut-off point, chosen to provide a specificity of 90%.

	Cut-off point (pg/ml)	**Sensitivity % (95% CI)**	**Specificity % (95% CI)**	**Positive predictive value % (95% CI)**	**Negative predictive value % (95% CI)**
GDF-15 alone	200806.91	29.3 (16.1 – 45.5)	90.0 (87.9 – 91.8)	11.2 (7.0 – 17.4)	96.7 (96.0 – 97.3)
PlGF alone	106.11	34.1 (20.1 – 50.6)	90.0 (87.9 – 91.8)	12.8 (8.5 – 19.0)	96.9 (96.2 – 97.5)
sFlt-1 alone	4456.99	51.2 (35.1 – 67.1)	90.0 (87.9 – 91.8)	18.1 (13.4 – 24.0)	97.7 (96.9 – 98.3)
sFlt-1/PlGF	34.92	51.2 (35.1 – 67.1)	90.0 (87.9 – 91.8)	18.1 (13.4 – 24.0)	97.7 (96.9 – 98.3)
GDF-15/PlGF	1418.74	41.5 (26.3 – 57.9)	90.0 (87.9 – 91.8)	15.2 (10.6 – 21.3)	97.3 (96.5 – 97.9)
GDF-15 x sFlt-1/PlGF	5569401.64	53.7 (37.4 – 69.3)	90.0 (87.9 – 91.8)	18.8 (14.1 – 24.6)	97.8 (97.0 – 98.4)

While cut-offs are presented, note the GDF-15 is a research grade ELISA so absolute numbers may vary between batches. Cohort 2 contained 41 preeclampsia cases and 950 controls, resulting in a prevalence of approximately 4.1%. All cut-off points calculated with biomarker measurements in pg/ml.

GDF-15 = Growth Differentiation Factor 15

sFlt-1 = soluble FMS-like tyrosine kinase-1

PlGF = Placental growth factor