INHIBIN-A AUGMENTS PIGF AND THE sFlt-1 / PIGF RATIO IN THE PREDICTION OF PREECLAMPSIA AND / OR FGR NEAR DELIVERY

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**Head:** Inhibin-A augments prediction accuracy of preeclampsia near delivery
ABSTRACT:

Objective: We previously provided evidence to confirm that soluble Fms-like tyrosine kinase-1 (sFlt-1), placental growth factor (PlGF), and their ratio, are useful tools to direct the management of preeclampsia (PE), fetal growth restriction (FGR), and PE+FGR near delivery. In this study we examine the potential additive value of Inhibin-A, a hormone marker of the transforming growth factor family.

Methods: We used a cohort of 125 pregnant women enrolled near delivery at clinics of the University Medical Center of Ljubljana, Slovenia. There were 31 cases of PE, 16 of FGR, 42 of PE+FGR, 15 iatrogenic preterm delivery (PTD), and 21 unaffected controls with delivery of a healthy baby at term. Cases delivered before 34 weeks’ gestation included 13 of PE, 12 of FGR, 22 of PE+FGR, and 6 of PTD. We recorded demographic characteristics and medical history and the levels of PlGF, sFlt-1 and Inhibin-A. The predictive accuracy of each biomarker, their ratios, and combinations was estimated from areas under the curve (AUC) of Receiver Operating Characteristics (ROC) curves. We estimated accuracy by the continuous marker model and a cut-off model.

Results: Combining Inhibin-A with PlGF or with the sFlt-1 / PlGF ratio showed a 10-20% increase in AUCs and 5-15% increase in the detection rate, at 10% false positive rate, of PE, and a lower, but significant, increase for PE+FGR but not for FGR alone. The use of a cut-off model was adequate, although a bit higher accuracy was obtained from the continuous model. Highest correlation was found for PlGF with all three complications.

Conclusion: Inhibin-A improves the accuracy of predicting PE and PE+FGR provided by the angiogenic markers alone, bringing the results to a diagnostic level, thus assisting in directing clinical management. Inhibin-A had no added value for the accuracy of predicting FGR alone.

Keywords: Preeclampsia; Fetal growth restriction; sFlt-1; Inhibin-A; Placental growth factor.
INTRODUCTION

Preeclampsia (PE) is a multisystem disorder unique to pregnancy, characterized by the new onset of hypertension and proteinuria [1-5]. The condition affects 2-7% of pregnancies and worldwide it is accompanied by one maternal death every 8 minutes and a yearly loss of 500,000 fetuses [6-10]. Preeclampsia presents either alone or in combination with fetal growth restriction (PE+FGR) [1]. FGR is another major obstetric complication that develops either independently or together with PE; FGR could be the consequence of impaired blood supply to the placenta and/or due to fetal abnormalities [11-13]. Successful management of PE and/or FGR improves pregnancy outcome and reduces life-long complications [1,2,9-13]. Both PE and FGR can result in preterm delivery (PTD); there are many similarities between early onset PE and/or FGR and PTD itself because all three often require emergency delivery by cesarean section, and they are associated with low birth weight and neonatal complications due to prematurity [14-15].

Several biochemical markers emerged as being useful in the clinical management of women admitted to hospital with suspected PE and/or FGR, including reduced placental growth factor (PlGF) and increased soluble FMF-like tyrosine kinase -1 (sFlt-1) or increased sFlt-1/PlGF ratio [16-22]. Similar results were also found in our dataset [23-25].

In this study we evaluated whether adding Inhibin-A, a glycoprotein hormone belonging to the transforming growth factor family [26-27], could elevate the prediction accuracy. Inhibin-A is abundantly expressed in the placenta, and as we [23] and others [26-27] have previously reported, in cases of PE and/or FGR the level of Inhibin-A is significantly elevated in the placenta, in the uterine vein collecting biomolecules released from the placenta, and in the maternal circulation. We used our Slovenian cohort dataset, and extracted the level of PlGF, sFlt-1, and Inhibin-A from patients’ medical records to explore if there is a potential added value of combining Inhibin-A with PlGF and/or with sFlt-1/PlGF ratio for the accurate prediction of suspected PE and/or FGR.

SAMPLE AND METHODS

Sample

Patients were enrolled between 2012 and 2015 after obtaining approval of the National Medical Ethics Committee of the Republic of Slovenia (Approval No. 104/04/12). Recruitment after signing on the informed consent was made at the outpatient clinics of high-risk pregnancies at the Department of Perinatology of the University Medical Centre of Ljubljana, Slovenia. All patients were not in labor when included in the study, and their gestational age was 24 weeks or more. The cohort included patients 18 years old and above with singleton viable pregnancy without major fetal anomalies, or pre-existing renal, hematological, or autoimmune conditions. Gestational age was determined from ultrasound measurements of the fetal crown-rump length in the first trimester of pregnancy [28].

The study population included 31 cases of PE, 16 of FGR, 42 PE+FGR cases, 15 of iatrogenic PTD, and 21 unaffected cases who delivered a healthy baby at term. The cases that delivered at <34 weeks included 13 of PE, 12 of FGR, 22 of PE+FGR (22) and 6 of PTD in the absence of PE and/or FGR or placental abruption as was previously described [23, 25].

Outcome measures

Preeclampsia was defined as hypertension > 140/90 systolic/diastolic mmHg blood pressure developed after 20 weeks’ gestation in a previously normotensive woman, accompanied by elevated urine protein (300 mg/dL and above or > 1+ on dipstick [1,29]). Fetal growth restriction was defined as sonographic estimated fetal weight below the 10th percentile and abnormal blood flow patterns demonstrated by Doppler ultrasound in the uterine, umbilical or middle cerebral arteries [13].
Biochemical and biophysical markers

Serum PLGF and sFlt-1 were measured by the Elecsys analyzer (Cobas e411 system, Roche Diagnostics, Germany) according to the manufacturer instructions [17-19]. Inhibin-A was measured by the Access 2 immunoassay analyzer (Beckman Coulter, Pasadena, California) according to the manufacturer's instructions [23]. Blood Pressure was measured according to the guidelines of the Fetal Medicine Foundation using a calibrated electronic device and the mean arterial blood pressure (MAP) was calculated as (systolic + diastolic *2) / 3 [33].

Statistical analyses

The median with 95% Confidence Interval [95% CI] were calculated for each marker and each adverse pregnancy outcome group was compared to results from the normal term delivery group using Mann-Whitney test. Kruskal-Wallis analysis was performed with the SPSS package version 24 (SPSS Inc., Chicago, IL, USA) to calculate the difference among all study groups. Box-Plot graphs provided the graphic description of medians and quartile distribution. Receiver operating characteristic (ROC) curves were used to calculate the area under the curve (AUC) from marker values or from their ratios with 95% CI and to calculate the detection rate at 10% fixed false positive rate (FPR). Cut-offs were marked as X on the ROC curves. The positive predictive value (PPV) was calculated as true cases at the cut-off divided by all cases at the cut-off, and the negative predictive value (NPV) was calculated as all true negative cases at the cut-off divided by all negative cases at the cut-off. In the continuous model the AUCs were extracted from the ROC curves. In the cut-off model AUC, and detection rate were extracted from cut-offs. Combined analysis was performed by combining percentiles of individual marker values for each FPR. Where possible, we used curve fitting by polynomial calculation to smooth ROC curves.

Cohort characteristics:

Cohort features were previously described [23]. Groups had similar maternal age and parity and they were all of Caucasian origin. The groups of PE and PE+FGR had higher body mass index, in the PE and FGR groups there was a higher incidence of conception by in-vitro fertilization and in the PE group there was a higher incidence of patients with history of previous PE, diabetes mellitus, or polycystic ovary syndrome. The blood pressure at presentation was 150/94 in the PE group, 151/94 in the PE+FGR group, 131/80 in the FGR group, 119/76 in the PTD group, and 112/71 in the unaffected controls. Gestational age at delivery was 34.2, 31.7, 32.0, and 33.8 weeks in the PE, FGR, PE+FGR and PTD groups, respectively, compared to 39.1 for control group. Birthweights were 2,306, 1,306, 1,449 and 2,207 grams in the PE, FGR, PE+FGR and PTD groups, respectively, compared to 3,300 grams for the control group.

RESULTS

Median marker levels in the outcome groups

In all the cases in the PE group, FGR group, and PE+FGR group, compared to the unaffected controls and PTD <37 weeks, the median Inhibin A, sFlt-1/PIGF ratio, and Inhibin A / PIGF ratio were significantly higher, and PIGF was significantly lower (Figure 1 and Table 1). There was good separation between affected and unaffected pregnancies at a cut-off of 1,000 pg/ml for Inhibin A, 200 pg/ml for PIGF, 38 for the sFlt-1/PIGF ratio, and 7 for the Inhibin A / PIGF ratio. Similarly, in the cases of PE, FGR, and PE+FGR delivered <34 weeks, compared to the group with PTD <34 weeks, the median Inhibin A, sFlt-1/PIGF ratio, and Inhibin A / PIGF ratio were significantly higher and PIGF was lower.

Performance of screening
The AUCs and detection rates at 10% FPR for PE, FGR and PE+FGR are shown in Table 2. Table 3 and Figures 2 and 3 demonstrate that a combination of PIGF and Inhibin A was superior to PIGF alone in the prediction of all PE, all FGR, all PE+FGR and PE <34 weeks, but not FGR or PE+FGR <34 weeks. Similarly, a combination of sFlt-1/PIGF ratio plus Inhibin A was superior to sFlt-1/PIGF ratio alone in the prediction of all PE, all FGR, all PE+FGR, PE <34 weeks and PE+FGR <34 weeks, but not FGR <34 weeks.

Multiple regression

Multiple regression analysis was used to assess whether gestational age (GA), birthweight (BW), mean arterial blood pressure (MAP), and PE, FGR and PE+FGR can predict the marker level. The equations used were:

\[
\text{Inhibin-A} = -46.66 - 0.61\times\text{BW} - 16.04\times\text{MAP} + 104.06\times\text{GA} + 1692\times\text{PE} + 661\times\text{FGR} + 1165.94\times(\text{FGR}+\text{PE})
\]

\[
\text{PIGF} = 1101 + 0.17\times\text{BW} + 0.79\times\text{MAP} - 27.88\times\text{GA} - 424\times\text{FGR} - 381\times(\text{FGR}+\text{PE})
\]

\[
\text{sFlt-1/PIGF} = 1297 - 0.05\times\text{BW} - 6.08\times\text{MAP} - 16.05\times\text{GA} + 240\times\text{PE} + 118\times\text{FGR} + 210\times(\text{FGR}+\text{PE})
\]

The regression yielded statistical significance ($R^2 = 0.28$, $F(6,63) = 3.63$, $P<0.01$; $R^2 = 0.40$, $F(6,63) = 6.40$, $P<0.001$; $R^2 = 0.54$, $F(6,63) = 11.22$, $P<0.001$, for PE, FGR and PE+FGR, respectively). At all three markers, the parameters of GA and BW were not significant predictors ($p>0.05$). MAP was negatively and significantly associated only with sFlt-1/PIGF ($\beta = -0.33$, $P<0.01$) (Table 4).

For Inhibin-A, there were positive significant correlations with PE and FGR+PE ($\beta = 0.49$, $P = 0.001$ and $\beta = 0.39$, $P<0.05$, respectively). There were also positive correlations between sFlt-1/PIGF ratio and PE and FGR+PE ($\beta = 0.39$, $P < 0.01$ for both). For PIGF there was a negative significant correlation with each of the three complications of PE, FGR and PE+FGR ($P < 0.01$).

DISCUSSION

Main findings

The study has investigated the potential value of Inhibin A, both alone and in addition to PIGF and the sFlt-1 / PIGF ratio, in the prediction of PE and / or FGR near the time of delivery. We found that first, Inhibin A is a moderately good biomarker of PE and PE+FGR, but not of FGR alone; second, combining Inhibin-A with PIGF, compared to PIGF alone, was associated with a 13% and 41% improvement in the AUC and detection rate at 10% FPR of all PE with respective values 10% and 37% for early PE; third, combining Inhibin-A with PIGF, compared to PIGF alone, was associated with a 6% and 29% improvement in the AUC and detection rate at 10% FPR of all PE+FGR, respectively, but there was no benefit in the prediction of early PE+FGR; fourth, the addition of Inhibin-A had low or no added value to PIGF in the prediction of FGR alone and this is consistent with the finding of a high correlation between PIGF and all three complications, whereas Inhibin-A was correlated with PE and PE+FGR, but not FGR alone; fifth, combining Inhibin-A with the sFlt-1 / PIGF ratio, compared to the sFlt-1 / PIGF ratio alone, was associated with a 6% and 8% improvement in the AUC and detection rate at 10% FPR of all PE with respective 6% and 16% increase for early PE; seventh, combining Inhibin-A with the sFlt-1 / PIGF ratio, compared to the sFlt-1 / PIGF ratio alone, there was a minimal impact on the prediction of all or early FGR or PE+FGR and this is consistent with the results of multiple regression analysis where both
markers showed a high correlation with PE, but a small or no correlation with FGR or PE+FGR.

**Interpretation of results and comparison with findings of previous studies**

Inhibin-A is a glycoprotein hormone that is abundantly expressed in the placenta and its levels in both the placental and circulating maternal levels are increased in cases of PE, and the increase is apparent from the second trimester of pregnancy [23,26,27]. Inhibin-A was initially identified as a second trimester marker of chromosomal abnormalities [37] and was subsequently reported as a second and third trimester marker of PE [37-42]. We found that Inhibin-A level is considerably higher in early than late PE the magnitude of increase was greater in PE alone rather than PE+FGR or FGR alone. Yet, our regression analysis showed no correlation of Inhibin-A level with gestational age or birth weight, which are classical parameters to define PE severity. These findings are consistent with previously reported results [40,41].

We have previously reported that in PE and / or FGR there is a reduction in the level of PIGF and an increase in the level of sFlt-1 and of the sFlt-1 / PIGF ratio [23-25]. In this study we also examined the potential value of Inhibin-A / PIGF ratio, but this appeared to be less powerful than any of the other measures. Our results are consistent with large-scale, high-quality studies by others [43-51]. We added to the above the finding and quantification of the added value of Inhibin-A on top of the angiogenic markers and showed its value mainly in augmenting the accuracy of predicting PE. Neuman et al [42] were the first to examine the added value of Inhibin-A to that of angiogenic markers and reported that this was beneficial mainly for early rather than late PE. We found that Inhibin-A had an additive value to both PIGF and the sFlt-1 / PIGF ratio in the prediction of both early and late PE and to a lesser extent of PE+FGR. We also checked the markers by multiple regressions and the results indicated no correlations with gestational age or birth weight and marginal correlation with the MAP for sFlt-1 / PIGF ratio. The high correlation was found for increased Inhibin-A and sflt-1 / PIGF ratio for PE and PE+FGR, but not for FGR alone, whereas the decrease of PIGF yielded high correlations with each of the three complications. Hence, we are expanding the conclusions of Neuman et al [42].

In the past, cut-off values of PIGF and of sFlt-1 / PIGF ratio were used to predict the short-term absence or presence of PE for clinical management of pregnancy-related complications [17-23]. In our study Inhibin-A brough the accuracy to diagnostic level in the complications of pure PE and for PE+FGR for both the continuous and the cut-off models, bringing the NPVs and the PPV to above 93% in the case of pure PE. Thus, although the literature argues for the superiority of the continuous model [18, 49], we concluded that although the continuous model might be a little more accurate, acting by cut-offs was very adequate especially for combining Inhibin-A with PIGF.

In the case of FGR, the accuracy level of PIGF alone and to a lower extent of the sFlt-1 / PIGF ratio was exceptionally high to begin with, and hence added value by Inhibin-A was negligible, or none. This is likely to be the consequence of our diagnostic criteria of FGR which included the presence of small for gestational age fetuses with abnormal arterial and venous Doppler indices.

**Implications for clinical practice**

Inhibin A has additive value to PIGF in the prediction of PE and PE+FGR and the performance of screening is similar or better than that achieved by the sFlt-1 / PIGF ratio. If these findings are confirmed in larger studies, then measurement of Inhibin A may be an alternative to that of sFlt-1. The described immunodiagnostic methods can be completed within 60-90 minutes and the assays are suitable for points of care both in maternity hospitals and community clinics.

**Limitations of the study**
The main limitations of the study are: first, the biomarkers were not measured at fixed time points, but when the patients were admitted to the hospital or were seen in outpatient clinics, but this reflects clinical reality; and second, the design of the study was such that we did not perform repeated measurements during pregnancy, which were shown to improve the prediction accuracy.

Conclusion

Inhibin-A augments the accuracy of pro-and-anti-angiogenic markers in the prediction of suspected PE and PE+FGR around delivery. Further studies are warranted with larger cohorts of pregnant women to define the exact role of Inhibin A in the prediction of these pregnancy complications.

Declaration of Interest: The authors report no conflict of interest. The authors alone are responsible for the content and writing of the paper.

Acknowledgments: This study was supported in part by the ASPRE project (EU, FP7 # 601852, HM, KHN), and by the Graduate school of the University Medical Center, Ljubljana, Slovenia (KK, OJ).

Author Contributions: All authors were involved in writing and editing the manuscript. The study was conceived by Josko Osredkar, Kristina Kumer, Tanja Premru Srsen and Natasha Tul, who wrote the study protocol and obtained ethics committee approval for the study. Tanja Premru Srsen, Vesna Fabjan Vodusek and Natasha Tul enrolled the patients to the study, recorded their demographic characteristics and medical history, and performed the obstetric and sonographic evaluations. Analysis of samples was carried out by Kristina Kumer, Teja Fabjan and Josko Osredkar. The database was built and completed by Kristina Kumer, Adi Sharabi-Nov, and Hamutal Meiri, who developed the mathematical models and performed the statistical analyses together with Kypros H. Nicolaides, and Josko Osredkar.
ABBREVIATIONS:

ALT - Alanine transaminase
AST - Aspartate transaminase
BMI - Body mass Index
BP - Blood pressure
dBP - diastolic blood pressure
DR- detection rate (sensitivity)
FMF – Fetal Medicine Foundation
FPR- False Positive rate (1-specificity)
ISUOG – International Society of ultrasound in Obstetrics and Gynecology
FGR - Intrauterine growth restriction
IVF - In-vitro fertilization
LDH- Lactate dehydrogenase
MAP - Mean arterial blood pressure
NPV- Negative predictive value
PE - Preeclampsia
PPV- positive predictive value
PSF- Peak systolic flow
PIGF- Placenta growth factor
PSV - Peak systolic velocity index
PTD - Preterm delivery
RHI - Reactive hyperemia index
RI - Uterine artery resistance index
UTPI - Uterine artery pulsatility index
sBP- systolic blood pressure
sFlt-1- soluble Fms-like tyrosine kinase 1
VEGF- Vascular endothelial growth factor
95% CI - 95% Confidence Interval
FIGURE LEGENDS

**Figure 1.** Box and whisker plots of Inhibin A, PIGF and sFlt-1/PIGF ratio in all cases of PE, FGR and PE+FGR. The horizontal lines indicate the cut-offs that separate the affected cases (grey histograms) from unaffected controls (white histograms).

**Figure 2.** Receiver operating characteristic curves of sFlt-1 / PIGF ratio (blue lines) and sFlt-1 / PIGF combined with Inhibin-A (orange lines) in the prediction of PE, FGR and PE+FGR.

**Figure 3.** Receiver operating characteristic curves of PIGF ratio (blue lines) and PIGF combined with Inhibin-A (orange lines) in the prediction of PE, FGR and PE+FGR.
Table 1. Median (95% CI) of biochemical marker levels in different groups of patients classified according to pregnancy outcome.

| Marker             | Term delivery (n=20) | Birth <37 weeks (n=12) | Preeclampsia (PE, n=29) | Fetal growth restriction (FGR, n=16) | PE+FGR (n=41) | p-value |
|--------------------|----------------------|------------------------|-------------------------|--------------------------------------|---------------|---------|
| Inhibin A          | 724 [491-904]        | 330 [261-928]          | 2,097 [1,546-2,660] *   | 1,269 [760-2,348] *                  | 1,876 [1,239-2,935] * | <0.001  |
| sFlt-1 / PlGF      | 5 [3-31]             | 6 [2-9]                | 177 [106-301] *         | 195 [55-310] *                       | 265 [168-382] *   | <0.001  |
| PIGF               | 524 [223-681]        | 693^b [308-980]        | 101^b [69-153] *        | 76 [43-117] *                        | 62 [48-87] *     | <0.001  |
| Inhibin A / PIGF   | 3.1 [0.7-3.6]        | 1.2 [0.2-1.2]          | 41.0 [10.2-39.4] **     | 36.3 [11.2-50.8] **                  | 45.0 [19.5-44.1] **| <0.001  |
| Birth < 34 weeks   |                      |                        |                         |                                     |               |         |
| Inhibin A          | 457 [0-1,015]        | 3,216 [2,212-4,220] ** | 1,503 [1,019-1,987] *   | 2,384 [1,711-3,057] **               |               | 0.001   |
| sFlt-1 / PIGF      | 0.090 [0-0.182]      | 0.009 [0-0.025] **     | 0.002 [0-0.004] **      | 0.003 [0-0.005] **                   |               | <0.001  |
| PIGF               | 762 [182-1,343]      | 215 [0-479] *          | 70 [27-113] **          | 103 [39-167] **                      |               | 0.009   |
| Inhibin A / PIGF   | 1.1 [0.2-3.5]        | 75.0 [17.1-114.2] **   | 44.8 [11.2-70.3] **     | 57.7 [19.8-66.0] **                  |               | 0.004   |

P values between all groups were calculated with Kruskal Wallis test. In addition, each complication was compared by Mann Whitney test to term delivery in the upper part and to birth <34 weeks in the lower part. *P<0.05, **P<0.01.

sFlt-1: Soluble Fms-like tyrosine kinase 1, PIGF- placental growth factor.
Table 2. Prediction of all PE, all FGR, all PE+FGR and PE, FGR and PE+FGR <34 weeks, by Inhibin A, PlGF, sFlt-1/PlGF ratio and Inhibin A / PlGF ratio.

| Condition      | Marker          | Continuous model            |                      | Cut-off model            |                      |
|----------------|-----------------|-----------------------------|----------------------|--------------------------|----------------------|
|                |                 | AUC (95% CI)                | DR at 10% FPR        | Cut-off                  | AUC (95% CI)          | DR at 10% FPR        | PPV | NPV |
| All PE        | Inhibin A       | 0.91 (0.84-0.98)            | 72                   | 1,000 pg/mL              | 0.80 (0.69-0.92)      | 42                   | 79  | 81  |
|                | PlGF            | 0.85 (0.75-0.95)            | 53                   | 200 pg/mL                | 0.82 (0.71-0.93)      | 43                   | 82  | 83  |
|                | sFlt-1/PlGF     | 0.89 (0.80-0.97)            | 79                   | 38                       | 0.85 (0.74-0.96)      | 68                   | 85  | 83  |
|                | Inhibin A / PlGF| 0.92 (0.85-0.99)           | 79                   | 7                        | 0.83 (0.72-0.94)      | 73                   | 91  | 79  |
| All FGR       | Inhibin A       | 0.82 (0.70-0.95)            | 50                   | 1,000 pg/mL              | 0.75 (0.59-0.91)      | 36                   | 65  | 84  |
|                | PlGF            | 0.95 (0.89-1.00)            | 77                   | 200 pg/mL                | 0.86 (0.75-0.98)      | 68                   | 74  | 94  |
|                | sFlt-1/PlGF     | 0.97 (0.92-1.00)            | 81                   | 38                       | 0.86 (0.73-0.99)      | 69                   | 76  | 91  |
|                | Inhibin A / PlGF| 0.94 (0.88-1.00)           | 75                   | 7                        | 0.84 (0.71-0.98)      | 76                   | 86  | 88  |
| All PE+FGR    | Inhibin A       | 0.87 (0.78-0.95)            | 68                   | 1,000 pg/mL              | 0.80 (0.69-0.90)      | 41                   | 84  | 74  |
|                | PlGF            | 0.92 (0.86-0.98)            | 71                   | 200 pg/mL                | 0.87 (0.78-0.96)      | 71                   | 88  | 85  |
|                | sFlt-1/PlGF     | 0.97 (0.93-1.00)            | 93                   | 38                       | 0.92 (0.84-0.99)      | 80                   | 90  | 91  |
|                | Inhibin A / PlGF| 0.94 (0.88-1.00)           | 85                   | 7                        | 0.90 (0.82-0.98)      | 86                   | 95  | 83  |
| PE <34 w      | Inhibin A       | 0.98 (0.93-1.00)            | 91                   | 400 pg/mL                | 0.90 (0.68-1.00)      | 49                   | 92  | 100 |
|                | PlGF            | 0.89 (0.73-1.00)            | 60                   | 300 pg/mL                | 0.91 (0.76-1.00)      | 53                   | 100 | 71  |
|                | sFlt-1/PlGF     | 0.93 (0.80-1.00)            | 82                   | 120                      | 0.91 (0.76-1.00)      | 82                   | 100 | 71  |
|                | Inhibin A / PlGF| 0.96 (0.88-1.00)           | 91                   | 2                        | 0.86 (0.62-1.00)      | 45                   | 100 | 71  |
| FGR <34 w     | Inhibin A       | 0.90 (0.71-1.00)            | 50                   | 400 pg/mL                | 0.90 (0.68-1.00)      | 50                   | 92  | 100 |
|                | PlGF            | 1.00 (1.00-1.00)            | 100                  | 400 pg/mL                | 1.00 (1.00-1.00)      | 100                  | 100 | 100 |
|                | sFlt-1/PlGF     | 1.00 (1.00-1.00)            | 100                  | 120                      | 0.92 (0.78-1.00)      | 85                   | 100 | 71  |
|                | Inhibin A / PlGF| 0.98 (0.93-1.00)           | 92                   | 2                        | 0.90 (0.68-1.00)      | 50                   | 100 | 71  |
| PE+FGR <34 w  | Inhibin A       | 0.93 (0.80-1.00)            | 67                   | 400 pg/mL                | 0.88 (0.67-1.00)      | 100                  | 100 | 80  |
|                | PlGF            | 0.96 (0.90-1.00)            | 100                  | 300 pg/mL                | 0.96 (0.90-1.00)      | 100                  | 100 | 71  |
|                | sFlt-1/PlGF     | 1.00 (1.00-1.00)            | 100                  | 120                      | 0.91 (0.81-1.00)      | 83                   | 100 | 50  |
|                | Inhibin A / PlGF| 0.99 (0.97-1.00)           | 96                   | 2                        | 0.88 (0.67-1.00)      | 47                   | 100 | 63  |
Table 3. Comparison of performance of screening by PIGF plus Inhibin A versus PIGF alone and between sFlt-1/PIGF ratio plus Inhibin A versus sFlt-1/PIGF ratio alone.

| Condition | Marker               | AUC (95% CI)       | p     | DR at 10% FPR |
|-----------|----------------------|--------------------|-------|--------------|
| All PE    | PIGF                 | 0.85 (0.75-0.95)   | <0.001| 53           |
|           | PIGF + Inhibin A     | 0.98 (0.90-1.00)   | 0.006 | 94           |
|           | sFlt-1/PIGF          | 0.89 (0.80-0.97)   | <0.001| 79           |
|           | sFlt-1/PIGF +Inhibin A | 0.95 (0.91-0.99) | 0.003 | 87           |
| All FGR   | PIGF                 | 0.95 (0.89-1.00)   | <0.001| 77           |
|           | PIGF + Inhibin A     | 0.98 (0.87-1.00)   | 0.002 | 93           |
|           | sFlt-1/PIGF          | 0.97 (0.92-1.00)   | <0.001| 81           |
|           | sFlt-1/PIGF +Inhibin A | 0.99 (0.94-1.00) | <0.001| 90           |
| All PE+FGR| PIGF                 | 0.92 (0.86-0.98)   | <0.001| 71           |
|           | PIGF + Inhibin A     | 0.98 (0.91-0.99)   | <0.001| 90           |
|           | sFlt-1/PIGF          | 0.97 (0.93-1.00)   | <0.001| 93           |
|           | sFlt-1/PIGF +Inhibin A | 0.99 (0.93-1.00) | 0.004 | 95           |
| PE <34 w  | PIGF                 | 0.89 (0.73-1.00)   | 0.015 | 60           |
|           | PIGF + Inhibin A     | 0.99 (0.91-1.00)   | <0.001| 98           |
|           | sFlt-1/PIGF          | 0.93 (0.80-1.00)   | 0.008 | 82           |
|           | sFlt-1/PIGF +Inhibin A | 0.99 (0.89-1.00) | 0.009 | 98           |
| FGR <34 w | PIGF                 | 1.00 (1.00-1.00)   | <0.001| 100          |
|           | PIGF + Inhibin A     | No added value     | No added value |
|           | sFlt-1/PIGF          | 1.00 (1.00-1.00)   | <0.001| 100          |
|           | sFlt-1/PIGF +Inhibin A | No added value   | No added value |
| PE+FGR <34 w | PIGF                 | 0.96 (0.90-1.00)   | <0.001| 100          |
|           | PIGF + Inhibin A     | No added value     | No added value |
|           | sFlt-1/PIGF          | 1.00 (1.00-1.00)   | <0.001| 100          |
|           | sFlt-1/PIGF +Inhibin A | No added value   | No added value |
Table 4. Multiple regression model to predict Inhibin-A, PlGF, and sFlt-1/PlGF ratio.

| Variables              | Inhibin A |          | PIGF   |          | sFlt-1/PlGF |          |
|------------------------|-----------|----------|--------|----------|-------------|----------|
|                        | B        | S.E.     | β      | B        | S.E.        | β        | B        | S.E.     | β          |
| GA (weeks)             | 104.06   | 88.83    | 0.34   | -27.88   | 24.77       | -0.29    | -16.05   | 12.57    | -1.28      |
| BW (g)                 | -0.61    | 0.39     | -0.51  | 0.17     | 0.11        | 0.47     | -0.05    | 0.06     | -0.24      |
| MAP (mm HG)            | -16.04   | 14.16    | -0.16  | 0.79     | 3.86        | 0.26     | -6.08    | 1.96     | -0.33**    |
| PE (vs. unaffected)    | 1692     | 493      | 0.49** | -401     | 135         | -0.37**  | 240      | 69       | 0.39**     |
| FGR (vs. unaffected)   | 661      | 568      | 0.19   | -424     | 157         | -0.40**  | 118      | 80       | 0.19       |
| FGR+PE (vs. unaffected)| 1166     | 506      | 0.39*  | -381     | 139         | -0.41**  | 210      | 70       | 0.39**     |

Multiple regression analysis to assess whether gestational age (GA), birth weight (BW), mean arterial blood pressure (MAP), preeclampsia (PE), fetal growth restriction (FGR) and PE+FGR could predict Inhibin-A, PlGF and sFlt-1/PlGF ratio.
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