Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 December 2020 doi:10.20944/preprints202012.0819.v1

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

[1]Sharabi-Nov Adi*, [2,3]Premru Sršen Tanja*, [4,7]Kumer Kristina*, [2]Fabjan Vodušek Vesna, [4,7]Fabjan Teja,· [3,8] Tul Nataša, [5]Meiri Hamutal, [6]Nicolaides Kypros H*, [4,7]Osredkar Joško*.

*Equal Contribution

1 Tel Hai College and Ziv Medical Center, Safed, Israel
2. Department of Perinatology, Division of Obstetrics and Gynecology, University Medical Center Ljubljana, Slovenia
3. University of Ljubljana, Faculty of Medicine, Slovenia
4 Institute of Clinical Chemistry and Biochemistry, University Medical Centre Ljubljana, Slovenia
5 TeleMarpe Ltd., Tel Aviv, Israel
6 The Fetal Medicine Research Institute, King's College Hospital, London, UK.
7 University of Ljubljana, Faculty of Pharmacy, Slovenia
8 Women's hospital Postojna, Slovenia

Corresponding author:
Dr. Hamutal Meiri,
TeleMarpe Ltd. 41 Beit El St.,
Tel Aviv, Israel
hamutal62@hotmail.com;
+972-54-7774762

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

© 2020 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 December 2020 doi:10.20944/preprints202012.0819.v1

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.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 December 2020 doi:10.20944/preprints202012.0819.v1

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].

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 December 2020 doi:10.20944/preprints202012.0819.v1

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/PlGF ratio, and Inhibin A / PlGF ratio were significantly higher, and PlGF 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 PlGF, 38 for the sFlt-1/PlGF ratio, and 7 for the Inhibin A / PlGF 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/PlGF ratio, and Inhibin A / PlGF ratio were significantly higher and PlGF was lower.

Performance of screening

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 December 2020 doi:10.20944/preprints202012.0819.v1

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 PlGF and Inhibin A was superior to PlGF 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/PlGF ratio plus Inhibin A was superior to sFlt-1/PlGF 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:

Inhibin-A = -46.66 - 0.61*BW - 16.04*MAP + 104.06*GA + 1692*PE + 661*FGR + 1165.94*(FGR+PE)

PlGF = 1101 + 0.17*BW + 0.79*MAP - 27.88*GA - 401*PE - 424*FGR - 381*(FGR+PE)

sFlt-1/PlGF = 1297 - 0.05*BW - 6.08*MAP - 16.05*GA + 240*PE + 118*FGR + 210*(FGR+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/PlGF (β = -0.33, P<0.01) (Table 4).

For Inhibin-A, there were positive significant correlations with PE and FGR+PE (β = 0.49, P = 0.001 and β = 0.39, P<0.05, respectively). There were also positive correlations between sFlt-1/PlGF ratio and PE and FGR+PE (β = 0.39, P < 0.01 for both). For PlGF 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 PlGF and the sFlt-1 / PlGF 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 PlGF, compared to PlGF 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 PlGF, compared to PlGF 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 PlGF in the prediction of FGR alone and this is consistent with the finding of a high correlation between PlGF 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 / PlGF ratio, compared to the sFlt-1 / PlGF 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 / PlGF ratio, compared to the sFlt-1 / PlGF 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

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 December 2020 doi:10.20944/preprints202012.0819.v1

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 PlGF and an increase in the level of sFlt-1 and of the sFlt-1 / PlGF ratio [23-25]. In this study we also examined the potential value of Inhibin-A / PlGF 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 PlGF and the sFlt-1 / PlGF 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/PlGF ratio. The high correlation was found for increased Inhibin-A and sflt-1 / PlGF ratio for PE and PE+FGR, but not for FGR alone, whereas the decrease of PlGF 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 PlGF and of sFlt-1 / PlGF 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 PlGF.

In the case of FGR, the accuracy level of PlGF alone and to a lower extent of the sFlt-1 / PlGF 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 PlGF in the prediction of PE and PE+FGR and the performance of screening is similar or better than that achieved by the sFlt-1 / PlGF 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

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 December 2020 doi:10.20944/preprints202012.0819.v1

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.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 December 2020 doi:10.20944/preprints202012.0819.v1

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

PlGF- 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

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 December 2020 doi:10.20944/preprints202012.0819.v1

FIGURE LEGENDS

Figure 1. Box and whisker plots of Inhibin A, PlGF and sFlt-1/PlGF 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 / PlGF ratio (blue lines) and sFlt-1 / PlGF combined with Inhibin-A (orange lines) in the prediction of PE, FGR and PE+FGR.

Figure 3. Receiver operating characteristic curves of PlGF ratio (blue lines) and PlGF combined with Inhibin-A (orange lines) in the prediction of PE, FGR and PE+FGR.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 December 2020 doi:10.20944/preprints202012.0819.v1

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
All Participants						
Inhibin A	724 [491-904]	330 [261-928]	2,097 [1,546-2,660] *	1,269 [760-2,348] *	1,876 [1,239-2,295] *	<0.001
sFlt-1 / PlGF	5 [3-31]	6 [2-9]	177 [106-301] *	195 [55-310] *	265 [168-382] *	<0.001
PlGF	524 [223-681]	693[a] [308-980]	101[b] [69-153] *	76 [43-117] *	62 [48-87] *	<0.001
Inhibin A / PlGF	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		(n=6)	(n=10)	(n=12)	(n=28)	
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 / PlGF		0.090 [0-0.182]	0.009 [0-0.025] **	0.002 [0-0.004] **	0.003 [0-0.005] **	<0.001
PlGF		762 [182-1,343]	215 [0-479] *	70 [27-113] **	103 [39-167] **	0.009
Inhibin A / PlGF		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, PlGF- placental growth factor.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 December 2020 doi:10.20944/preprints202012.0819.v1

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	300 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

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 December 2020 doi:10.20944/preprints202012.0819.v1

Table 3. Comparison of performance of screening by PlGF plus Inhibin A versus PlGF alone and between sFlt-1/PlGF ratio plus Inhibin A versus sFlt-1/PlGF ratio alone.

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

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 December 2020 doi:10.20944/preprints202012.0819.v1

Table 4. Multiple regression model to predict Inhibin-A, PlGF, and sFlt-1/PlGF ratio.

	Inhibin A			**PlGF**			**sFlt-1/PlGF**		
Variables	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	.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****
$F_{(6,63)}$		**3.63****			**6.40*****			**11.22*****	
R^2		**0.28**			**0.40**			**0.54**	

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.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 December 2020 doi:10.20944/preprints202012.0819.v1

REFERENCES

1. Brown MA, Magee LA, Kenny LC, Karumanchi SA, McCarthy FP, Saito S, Hall DR, Warren CE, Adoyi G, Ishaku S. International Society for the Study of Hypertension in Pregnancy. The hypertensive disorders of pregnancy: ISSHP classification, diagnosis & management recommendations for international practice. Pregnancy Hypertension 2018; 13: 291–310.
2. Rana S, Lemoine E, Granger JP, Karumanchi SA. Preeclampsia: Pathophysiology, challenges, and perspectives. Circ Res 2019; 124: 1094–1112.
3. Sibai B, Dekker G, Kupferminc M. Pre-eclampsia. Lancet 2005; 365: 785–799.
4. Zhang J, Klebanoff MA, Roberts JM. Prediction of adverse outcomes by common definitions of hypertension in pregnancy. Obstet Gynecol 2001; 97: 261–267.
5. Huppertz B. "Molecular and Cellular Mechanisms of Preeclampsia"-Editorial. Huppertz B. Int J Mol Sci. 2020; 21: 4801.
6. https://www.preeclampsia.org/what-is-preeclampsia
7. Awuah SP, Okai I, Ntim EA, Bedu-Addo K. Prevalence, placenta development, and perinatal outcomes of women with hypertensive disorders of pregnancy at Komfo Anokye Teaching Hospital. PLoS One 2020; 15: e0233817.
8. WHO Managing Complications in Pregnancy and Childbirth: A guide for midwives and doctors.2017. http://apps.who.int/iris/bitstream/handle/10665/255760/9789241565493-eng.pdf;jsessionid=2BF1F3D8022566FA91FE021D76D50256?sequence=1
9. ACOG guidelines. Hypertension disorders in pregnancy. 2013. https://www.acog.org/~/media/Task%20Force%20and%20Work%20Group%20Reports/public/HypertensioninPregnancy.pdf. 100 pages. 2013
10. NICE guidelines, Hypertension in pregnancy. 2010. https://www.nice.org.uk/guidance/cg107/resources/hypertension-in-pregnancy-diagnosis-and-management-pdf-35109334011877
11. Salomon LJ, Alfirevic Z, Da Silva Costa F, Deter RL, Figueras F, Ghi T, Glanc P, Khalil A, Lee W, Napolitano R, Papageorghiou A, Sotiriadis A, Stirnemann J, Toi A, Yeo G. ISUOG Practice Guidelines: ultrasound assessment of fetal biometry and growth. Ultrasound Obstet Gynecol 2019; 53: 715-723.
12. Khalil A, Gordijn SJ, Beune IM, Wynia K, Ganzevoort W, Figueras F, Kingdom J, Marlow N, Papageorghiou AT, Sebire N, Zeitlin J, Baschat AA. Essential variables for reporting research studies on fetal growth restriction: a Delphi consensus. Ultrasound Obstet Gynecol 2019; 53: 609-614.
13. Figueras F, Gratacos E. An integrated approach to fetal growth restriction. Best Pract Res Clin Obstet Gynaeco 2017; 38: 48-58.
14. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. 2008. Lancet 2008; 5; 371: 75-84
15. Romero R, Dey SK, Fisher SJ. Preterm labor: one syndrome, many causes. Science 2014; 345: 760-765.
16. Levine RJ, Maynard SE, Qian C, Lim KH, England LJ, Yu KF, Schisterman EF, Thadhani R, Sachs BP, Epstein FH, Sibai BM, Sukhatme VP, Karumanchi SA.

Circulating angiogenic factors and the risk of preeclampsia. N Engl J Med 2004; 12; 350: 672-683

17. Levine RJ, Lam C, Qian C, Yu KF, Maynard SE, Sachs BP, Sibai BM, Epstein FH, Romero R, Thadhani R, Karumanchi SA; CPEP Study Group. Soluble endoglin and other circulating antiangiogenic factors in preeclampsia. N Engl J Med 2006; 355: 992-1005
18. Saleh L, Vergouwe Y, van den Meiracker AH, Verdonk K, Russcher H, Bremer A, Versendaal HJ, Steegers EAP, Danser AHJ, Visser W. Angiogenic Markers Predict Pregnancy Complications and Prolongation in Preeclampsia: Continuous Versus Cutoff Values. Hypertension 2017; 70: 1025-1033.
19. Zeisler H, Llurba E, Chantraine F, Vatish M, Staff AC, Sennstrom 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.
20. Panaitescu A, Ciobanu A, Syngelaki A, Wright A, Wright D, Nicolaides KH. Screening for pre-eclampsia at 35-37 weeks' gestation. Ultrasound Obstet Gynecol 2018; 52: 501-506.
21. Dragan I, Wright D, Fiolna M, Leipold G, Nicolaides KH. Development of pre-eclampsia within 4 weeks of sFlt-1 / PlGF ratio > 38: comparison of performance at 31-34 vs 35-37 weeks' gestation. Ultrasound Obstet Gynecol 2017; 49: 209-212.
22. Frampton GK, Jones J, Rose M, Payne L. Placental growth factor (alone or in combination with soluble fms-like tyrosine kinase 1) as an aid to the assessment of women with suspected pre-eclampsia: systematic review and economic analysis. Health Technol Assess 2016; 20: 1-160.
23. Sharabi-Nov A, Kumar K, Fabjan Vodušek V, Premru Sršen T, Tul N, Fabjan T, Meiri H, Nicolaides KH, Osredkar J. Establishing a differential marker profile for pregnancy complications near delivery. Fetal Diagn Ther 2020; 47: 471-484.
24. Kumer K, Premru-Sršen T, Fabjan-Vodušek V, Tul N, Fabjan T, Osredkar J. Peripheral arterial tonometry and angiogenic biomarkers in preeclampsia. Hypertens Pregnancy 2018; 4: 1-17
25. Kumer K, Sharabi-Nov A, Fabjan Vodušek V, Premru Sršen T, Tul N, Fabjan T, Meiri, Nicolaides KH Osredkar J. Pro-and-anti-angiogenic markers as clinical tool for suspected preeclampsia with and without FGR near delivery. Submitted to Reproductive Medicine.
26. Phupong V, Paiwattananupant K, Honsawek S. Inhibin A levels and severity of preeclampsia. Arch Gynecol Obstet 2009; 280:183-6.
27. Paiwattananupant K, Phupong V. Serum inhibin A level in preeclampsia and normotensive pregnancy. Hypertens Pregnancy 2008; 27: 337-343.
28. Hadlock FP, Shah YP, Kanon DJ, Lindsey JV. Fetal crown-rump length: reevaluation of relation to menstrual age (5–18 weeks) with high-resolution real-time US. Radiology 1992; 182: 501-505.
29. Arnaldo Cassia M, Daminelli G, Zambon M, Cardellicchio M, Cetin I, and Gallieni M. Proteinuria in pregnancy: Clinically driven considerations. Nephrology @ Point of Care 2008; 4: 1–5.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 December 2020 doi:10.20944/preprints202012.0819.v1

30. Jodkowska A, Martynowicz H, Kaczmarek-Wdowiak B, Mazur G. Trombocytopenia in pregnancy - pathogenesis and diagnostic approach. Postepy Hig Med Dosw (online), 2015; 69: 1215-1221
31. Burwick RM, Rincon M, Beeraka SS, Gupta M, Feinberg BB. Evaluation of hemolysis as a severe feature of preeclampsia. Hypertension 2018; 72: 460-465.
32. Ekun OA, Olawumi OM, Makwe CC, Ogidi NO. Biochemical assessment of renal and liver function among preeclamptics in Lagos Metropolis. Int J Reprod Med 2018; 1594182.
33. Poon LC, Kametas NA, Valencia C, Chelemen T, Nicolaides KH. Hypertensive disorders in pregnancy: screening by systolic diastolic and mean arterial pressure at 11-13 weeks. Hypertens Pregnancy 2011; 30: 93-107.
34. Hadlock F, Harrist R, Martinez-Poyer J. In utero analysis of fetal growth: a sonographic weight standard. Radiology 1991; 181: 129-133.
35. Hadlock F, Harrist R, Sharman R, Deter R, Park S. Estimation of fetal weight with the use of head, body, and femur measurements—a prospective study. Am J Obstet Gynecol 1985; 151: 333–337.
36. Salomon LJ, Alfirevic Z, Berghella V, Bilardo C, Hernandez-Andrade E, Johnsen SL, Kalache K, Leung KY, Malinger G, Munoz H, Prefumo F, Toi A, Lee W; ISUOG Clinical Standards Committee. Practice guidelines for performance of the routine mid-trimester fetal ultrasound scan. Ultrasound Obstet Gynecol 2011; 37: 116-126.
37. Kang JH, Farina A, Park JH, Kim SH, Kim JY, Rizzo N, Elmakky A, Jun HS, Hahn WB, Cha DH. Down syndrome biochemical markers and screening for preeclampsia at first and second trimester: Correlation with the week of onset and the severity. Prenat Diagn 2008; 28:704–709.
38. Ree PH, Hahn WB, Chang SW, Jung SH, Kang JH, Cha DH, Kang MS, Huh JY. Early detection of preeclampsia using inhibin A and other second-trimester serum markers. Fetal Diagn Ther 2011; 29(4):280-6.
39. Davidson EJ, Riley SC, Roberts SA, Shearing CH, Groome NP, Martin CW. Maternal serum activin, inhibin, human chorionic gonadotrophin, and alpha-fetoprotein as second trimester predictors of pre-eclampsia. BJOG 2003; 110: 46-52.
40. Muttukrishna S, North RA, Morris J, Schellenberg JC, Taylor RS, Asselin J, Ledger W, Groome N, Redman CW. Serum inhibin A and activin A are elevated prior to the onset of pre-eclampsia. Hum Reprod 2000; 15: 1640–1645.
41. Muttukrishna S, Hyett J, Paine M, Moodley J, Groome N, Rodeck C. Uterine vein and maternal urinary levels of activin A and inhibin A in pre-eclampsia patients. Clin Endocrinol (Oxf) 2006; 64: 469-473.
42. Neuman RI, Alblas van der Meer MM, Nieboer D, Saleh L, Verdonk K, Kalra B, Kumar A, Alpadi K, van den Meiracker AH, Visser W, Danser AHJ. PAPP-A2 and Inhibin A as Novel Predictors for Pregnancy Complications in Women with suspected or confirmed Preeclampsia. J Am Heart Assoc 2020; 9: e018 219.
43. Rana S, Burke SD, Karumanchi SA. Imbalances in circulating angiogenic factors in the pathophysiology of preeclampsia and related disorders. Am J Obstet Gynecol 2020; 20: S0002-9378(20)31196-0.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 December 2020 doi:10.20944/preprints202012.0819.v1

44. Verlohren S, Dröge LA. The diagnostic value of angiogenic and antiangiogenic factors in differential diagnosis of preeclampsia. Am J Obstet Gynecol 2020; 28: S0002-9378(20)31169-8.
45. Suresh S, Mueller A, Salahuddin S, Naseem H, Dhir R, Rana S. Evaluation of angiogenic factors in the decision to admit women with suspected preeclampsia. Pregnancy Hypertens 2020; 21: 124-131.
46. 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.
47. Barton JR, Woelkers DA, Newman RB, Combs CA, How HY, Boggess KA, Martin JN Jr, Kupfer K, Sibai BM; PETRA (Preeclampsia Triage by Rapid Assay) Trial. Placental growth factor predicts time to delivery in women with signs or symptoms of early preterm preeclampsia: a prospective multicenter study. Am J Obstet Gynecol 2020; 222: 259.e1-259.e11.
48. Yong HE, Murthi P, Borg A, Kalionis B, Moses EK, Brennecke SP, Keogh RJ. Increased decidual mRNA expression levels of candidate maternal pre-eclampsia susceptibility genes are associated with clinical severity. Placenta 2014; 35: 117-124.
49. Saleh L, van den Meiracker AH, Geensen R, Kaya A, Roeters van Lennep JE, Duvekot JJ, Verdonk K, Steegers EAP, Russcher H, Danser AHJ et al. Soluble fms-like tyrosine kinase-1 and placental growth factor kinetics during and after pregnancy in women with suspected or confirmed pre-eclampsia. Ultrasound Obstet Gynecol 2018; 51:751–757.
50. Yu J, Shixia CZ, Wu Y, Duan T. Inhibin A, activin A, placental growth factor and uterine artery doppler pulsatility index in the prediction of pre-eclampsia. Ultrasound Obstet Gynecol 2011; 37:528–533.
51. Boucoiran I, Thissier-Levy S, Wu Y, Wei SQ, Luo ZC, Delvin E, Fraser WD, Audibert F, Group MS. Risks for preeclampsia and small for gestational age: Predictive values of placental growth factor, soluble fms-like tyrosine kinase-1, and inhibin a in singleton and multiple-gestation pregnancies. Am J Perinatol 2013; 30:607–612.