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Prediction of superimposed pre-eclampsia by serum glycosylated fibronectin and angiogenic factors in women with chronic hypertension

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CONTRIBUTION

What are the novel findings of this work?

In women with chronic hypertension, the predictive performance for delivery with preeclampsia (PE) within the subsequent 2 weeks of maternal serum glycosylated fibronectin (GlyFn) is similar to that of serum placental growth factor (PlGF) and the soluble fms-like tyrosine kinase-1 (sFLT-1) / PlGF ratio, with detection rate of about 25%, at false positive rate of 5%.

What are the clinical implications of this work?

GlyFn is a simple point-of-care test without the need of a laboratory and can provide results within 10 minutes of testing. In this respect it may potentially replace the other tests that are currently used in the prediction of imminent PE in high-risk women. However, in the case of chronic hypertension the predictive performance for superimposed PE is poor for all tests and neither GlyFn nor angiogenic factors are likely to improve the management of such women.

ABSTRACT

Objective: To compare the predictive performance for delivery with preeclampsia (PE) within 2 weeks of assessment in women with chronic hypertension at 24-41 weeks' gestation between serum glycosylated fibronectin (GlyFn) concentration, serum placental growth factor (PlGF) concentration and soluble fms-like tyrosine kinase-1 (sFLT-1) / PlGF concentration ratio.

Methods: This was a prospective study in 104 women with singleton pregnancies and chronic hypertension presenting at 24-41 weeks' gestation. In 26 (25.0%) cases there was superimposed PE within 2 weeks from sampling. We compared the predictive performance for superimposed PE between GlyFn, PlGF and sFLT-1 / PlGF at fixed screen positive rates of approximately 10%.

Results: The median gestational age at sampling was 34.1 (31.5, 35.6) weeks and in 84.6% (88/104) of cases it was <36 weeks. The predictive performance for superimposed PE of the three methods of screening was similar with detection rates of about 23-27%, at screen positive rate of 11% and false positive rate of about 5%.

Conclusion: GlyFn is a simple point-of-care test without the need of a laboratory and can provide results within 10 minutes of testing. In this respect it may potentially replace the angiogenic markers that are currently used in the prediction of imminent PE in high-risk women. However, neither GlyFn nor angiogenic factors are likely to improve the management of women with chronic hypertension, because their predictive performance for superimposed PE is poor.

INTRODUCTION

Chronic hypertension affects about 1% of pregnancies and 20-30% of such cases develop superimposed preeclampsia (PE)[1,2]. In the last decade two strategies have evolved for prediction and prevention of PE. The first involves assessment of risk by the Fetal Medicine Foundation (FMF) competing risks model, which combines maternal demographic characteristics and elements from the medical history with the biomarkers mean arterial pressure (MAP), uterine artery pulsatility index, and placental growth factor (PlGF) at 11-13 weeks' gestation (first-trimester triple test)[3,4]. This approach identifies about 90% of early-PE with delivery at <32 weeks' gestation and 75% of preterm-PE with delivery at <37 weeks, at screen positive rate of 10%[3,4]. Treatment of the high-risk group with aspirin (150 mg/day from 12 to 36 weeks) reduces the rates of early- and preterm-PE by about 90% and 60%, respectively[5]. The second strategy, involves assessment of risk at 36 weeks' gestation by a combination of maternal risk factors with MAP, PlGF and soluble fms-like tyrosine kinase-1 (sFLT-1), with detection rate of term-PE with delivery at ≥37 weeks, of about 70% at screen positive rate of 10% (third-trimester triple test)[6-8]. A randomized trial is currently evaluating timed birth based on personalized risk of PE, with the potential of this strategy to decrease the rate of term-PE by about 60%[9]. In two recent studies we have reported that glycosylated fibronectin (GlyFn) is a new biomarker that can potentially replace angiogenic markers in first- and third-trimester screening for preterm- and term-PE, respectively[10,11].

In addition to first- and third-trimester routine screening for subsequent development of PE, assessment of risk for PE has also been proposed for women presenting to specialist clinics with signs or symptoms of hypertensive disorders. In such cases cut-offs in the concentration of PlGF or the ratio of the concentrations of sFLT-1 and PlGF have been used to predict the development of PE within the subsequent 1-4 weeks[12-14]. More recently, the use of GlyFn has been proposed

as a useful biomarker in the prediction of imminent PE in women presenting with signs and symptoms of the disease[15,16]. However, in a study of 409 women with singleton pregnancies presenting with new onset hypertension at 24-41 weeks' gestation we found that the predictive performance for delivery with PE within 2 weeks of presentation, which occurred in 23% of cases, was poor; the performance of GlyFn was similar to that of PlGF and the sFLT-1 / PlGF ratio, with detection rate was about 60%, at screen positive rate of 46% and false positive rate of 42%[17].

The objective of this study is to compare the predictive performance for superimposed PE between serum GlyFn concentration, serum PlGF concentration and sFLT-1 / PlGF concentration ratio in women with chronic hypertension.

METHODS

Study design and participants

This was a prospective study in women with singleton pregnancies and diagnosis of chronic hypertension, defined as systolic blood pressure ≥140 mmHg and / or diastolic blood pressure ≥90 mmHg on at least two occasions four hours apart, and documented before pregnancy or before 20 weeks' gestation.[18] The women attended Kings' College Hospital, London, UK (between February 2019 and January 2023) for antenatal care and they were managed in a specialist hypertension clinic. The management, which was consistent with that recommended by the National Institute for Health and Care Excellence[19], included: regular visits at 20, 24, 28 and 32 weeks' gestation and every two weeks thereafter until delivery. In these visits the blood pressure was measured using validated automated devices[20], serum creatinine concentration, serum aspartate transaminase, platelet count and 24-hour urine protein concentration or protein to creatinine ratio were measured and serum was stored at -80ºC for subsequent research studies. Ultrasound scans were carried out at 28, 32 and 36 weeks' gestation to monitor fetal growth, by measurement of estimated fetal weight, and assessment of fetal oxygenation by Doppler to examine flow velocity waveforms in the umbilical arteries, ductus venosus and middle cerebral arteries. Antihypertensive medication was given as necessary to maintain the blood pressure at <140 / 90 mmHg. Timing and method of delivery were based on the maternal and fetal condition; if the hypertension was well controlled and the fetal growth and wellbeing were normal, delivery was carried out at 40 weeks' gestation.

The database of the hypertension clinic was searched to identify women with chronic hypertension who had stored serum samples. In those with superimposed PE a sample was selected that was taken within 2 weeks of the diagnosis of superimposed PE. For each case of superimposed PE we then selected three cases of chronic hypertension without PE that were

sampled at the same gestational age as the case of PE.

Gestational age was determined by the measurement of fetal crown-rump length at 11-13 weeks or the fetal head circumference at 19-24 weeks[21,22]. Included in the study were singleton pregnancies delivering a non-malformed liveborn or stillborn at ≥24 weeks' gestation. Excluded were pregnancies with aneuploidies and major fetal abnormalities.

Measurement of angiogenic factors and glycosylated fibronectin

The frozen serum samples were thawed and then analyzed for PlGF and sFLT-1 in pg/mL by an automated biochemical analyzer (BRAHMS KRYPTOR compact PLUS, Thermo Fisher Scientific, Hennigsdorf, Germany). Serum GlyFn concentration was measured using a point-of-care test (LumellaTM PE test, DiabetOmics, Inc., Hillsboro, OR, USA). Briefly, 5 µl of serum was diluted 1:350 in running buffer and 120 µl of diluted serum was added to a test strip and inserted into a hand-held LumellaTM reader system. Test strips were configured with monoclonal antibodies against GlyFn labelled with gold particles. The GlyFn concentration is displayed on the reader at the end of 10 minutes.

The measurements of PlGF, sFLT-1 and GlyFn were not made available to the obstetricians managing the pregnancies, but they were part of a research project which was approved by the NHS Research Ethics Committee (REC reference: 02-03-033).

Outcome measure

Data on pregnancy outcome were collected from the hospital maternity records of the women. The outcome measure was superimposed PE, which was diagnosed as defined by the 2019 American College of Obstetricians and Gynecologists criteria: hypertension, with development of one or more of the following: new-onset proteinuria (≥300 mg/24h or protein to creatinine

ratio ≥30 mg/mmoL), serum creatinine >97 μmol/L in the absence of underlying renal disease, serum aspartate transaminase more than twice normal (≥65 IU/L for our laboratory), platelet count <100,000/μL, headache or visual symptoms, or pulmonary edema.[23]

Statistical analysis

Data were summarized by median and interquartile range (IQR) for continuous variables, and n and percentage for categorical variables. Students t-test, and chi-squared or Fisher's exact tests, were used for comparing outcome groups for continuous and categorical data, respectively.

The following three steps were used to compare the predictive performance for delivery with PE within 2 weeks from presentation of FlyFn, PlGF and the sFLT-1 / PlGF ratio. First, we examined the distribution of GlyFn concentration, PlGF concentration and sFLT-1 / PlGF concentration ratio in the group with superimposed PE and those with no PE. Second, we defined screen positive groups using the recommended cut-off of 85 for the sFLT-1 / PlGF ratio[24] and, to allow for comparison, 75 pg/mL for PlGF and 510 μg/mL for GlyFen were used as previously described.[17] We found great disparity between the three tests in screen positive rates and to allow for a fair comparison between the tests we found the cut-off for each test corresponding to a screen positive rate of approximately 10%. Third, we compared the areas under the receiver operator characteristic curves of the three tests.

The statistical software package R, with packages PropCIs and pROC were used for data analyses.[25]

RESULTS

Study participants

The study population of 104 singleton pregnancies with chronic hypertension, included 26 (25.0%) that developed superimposed PE. The diagnosis of superimposed PE was based on the development of new-onset proteinuria in 21 cases, proteinuria plus elevated liver enzymes in two, elevated liver enzymes alone in two and increased creatinine in one.

Maternal and pregnancy characteristics of the study population are summarized in Table 1. There were no significant differences between those with superimposed PE, compared to the unaffected pregnancies, in maternal and pregnancy characteristics, except for black ethnicity, which was significantly more common in the superimposed PE group. In the superimposed PE group there was a significantly higher median systolic and diastolic blood pressure, GlyFn and sFLT-1 / PlGF ratio and lower PlGF.

Association between biomarkers

The association between the biomarkers in those who developed PE and those who did not is shown in Table 2. There were significant associations between GlyFn and PlGF, GlyFn and sFLT-1, sFLT-1 and PlGF and systolic and diastolic blood pressure in both the PE and non-PE groups. There were also significant associations between PlGF and systolic and diastolic blood pressure in the non-PE but not in the PE group and between sFLT-1 and diastolic blood pressure in the PE group, but not in the non-PE group. The highest associations were between GlyFn and sFLT-1 in both groups.

Prediction of superimposed PE

The distribution of GlyFn, PlGF and sFlt-1 / PlGF ratio in the group with superimposed PE and those without PE is shown in Figure 1, the predictive performance of the three methods of screening is shown in Table 3 and the receiver operating characteristics curves are shown in Figure 2.

As shown in Figure 1 there is considerable overlap between the cases of superimposed PE and unaffected pregnancies in all three methods of screening. The predictive performance of the three methods at risk cut-offs of 85 for the sFLT-1 / PlGF ratio, 75 pg/mL for PlGF and 510 µg/mL for GlyFn is shown on the top panel of Table 3. The screen positive rate was 5.8% for sFLT-1 / PlGF, 8.7% for PlGF and 24.0% for GlyFn and the respective detection rates for superimposed PE were 19.2%, 23.1% and 46.2%. Consequently, new cut-offs were selected to fix the screen positive rate of the three methods at approximately 10%. The predictive performance of the three methods, as shown in the bottom panel of Table 3, was similar and the detection rate varied between 23% and 27%, at screen positive rate of 10.6% and the false positive rates varied between 5.1% and 6.4%. The overall results for all 26 cases of superimposed PE were similar to those in the 22/26 cases where blood sampling was carried out at <36 weeks' gestation.

DISCUSSION

Main findings

This study in women with chronic hypertension has demonstrated that the predictive performance for superimposed PE within 2 weeks of examination by GlyFn is similar to that of PlGF and the sFLT-1 / PlGF ratio. At cut-offs corresponding to a screen positive rate of approximately 10%, the detection rate of superimposed PE was about 25% and the false positive rate was 5%. There were significant associations between GlyFn and PlGF and GlyFn and sFLT-1 in both the PE and non-PE groups.

Interpretation of results and comparison to previous studies

In a previous study of women presenting with new onset hypertension at 24-41 weeks' gestation we found that the predictive performance for delivery with PE within 2 weeks of presentation by GlyFn was similar to that of PlGF and the sFLT-1 / PlGF ratio, with a detection rate of about 60%, at screen positive rate of 46% and false positive rate of 42%[17]. Although, the screen positive and false positive rates in the women with new onset hypertension are much higher than in those with chronic hypertension, the predictive performance is similar, because as shown in the receiver operating characteristics curves in Figure 2, the detection rate of superimposed PE by GlyFn in women with chronic hypertension is about 75%, at false positive rate of 42%.

Very few studies have reported on the use of angiogenic factors for prediction of imminent PE in women with chronic hypertension. A study of 42 women with chronic hypertension, including 30 with superimposed PE and 12 with uncontrolled hypertension at 21-37 weeks' gestation, reported that the sFLT-1 / PlGF ratio was significantly higher and PlGF was lower in the superimposed PE group[26]. The authors did not report the predictive accuracy of the test but

claimed that the sFLT-1 / PlGF ratio is an excellent tool for emergency rooms for differentiating between superimposed PE and uncontrolled hypertension[26].

Binder *et al*.[27], examined 142 women with chronic hypertension who had suspected superimposed PE (worsening hypertension, epigastric pain, new-onset edema, dyspnea or neurological symptoms). The best prediction of superimposed PE (significant proteinuria, creatinine ≥1mg/dL, transaminase levels >40 IU/L, platelet count <150 000/μL or neurological symptoms)[28], diagnosed within 1 week after assessment was provided by maternal serum sFLT-1 / PlGF, compared to serum PlGF alone. The authors provided receiver operating characteristic curves and for the sFLT-1 / PlGF ratio the detection rate was 85% at false positive rate of 30%. In our study, the detection rate of sFLT-1 / PlGF ratio, at false positive rate of 30%, was about 70%. Possible explanations for the apparent superior predictive performance for superimposed PE in the Binder *et al*.[27] study, compared to our results, include first, they examined patients with suspected superimposed PE rather than all patients with chronic hypertension; second, their prediction was for PE within 1 week vs 2 weeks in our study; third, 58% of their patients were sampled at <32 weeks' gestation vs 27% in our study; fourth, Binder *et al.*, who recruited patients in Austria did not report the ethnicity of their patients and presumably they were totally or mostly white, whereas 77% of our patients with superimposed PE and 44% of those without PE were black and such women have higher mean serum PlGF concentrations and lower sFLT-1/PlGF concentration ratio values, regardless of whether they go on to develop PE[29]; and fifth, in both studies the number of patients was small and there is considerable uncertainty concerning the accuracy of the tests.

Implications for clinical practice

Chronic hypertension is associated with increased risk of stillbirth, PE, gestational diabetes, and birth of small for gestational age neonates[1]. Consequently, pregnancies with chronic

hypertension merit routine intensive antenatal care[19] and the potential value of biomarkers, such as PlGF, sFLT-1 and GlyFn, in the management of such pregnancies needs to be assessed against such background of intensive care. The use of cut-offs in measured concentration of biomarkers or their ratio to define clinical management has the advantage of simplicity. However, such simplicity would be truly advantageous only if there was no overlap in the distributions of biomarkers between women that would from those that would not develop imminent PE; in such case the test would be diagnostic. However, in reality, as confirmed by the findings of this study, this is not so for the proposed biomarkers.

A high proportion of women with chronic hypertension that would develop superimposed PE within the subsequent 1-2 weeks would have a false negative result, even when they present with suspected superimposed PE[27], and many of those that would not develop this complication would have a false positive result. It would therefore be inappropriate to rely on the results of angiogenic markers and GlyFn for clinical decisions on hospitalization and intensity of monitoring rather than continue with routine regular measurement of blood pressure and simple assessment for development of proteinuria or measurement of serum creatinine, liver enzymes and platelet count in those with increasing hypertension and certain symptoms suggestive of superimposed PE.

Strengths and limitations

The main strength of this study is examination of a population of pregnant women with chronic hypertension, rather than a heterogeneous group with signs and / or symptoms of hypertensive disorders, as in previous studies advocating the use of angiogenic markers, where measurement of blood pressure and demonstration of hypertension was not an inclusion criterion[12-14]. All our women had chronic hypertension and we wanted to examine the predictive performance of GlyFn, by comparison with angiogenic markers, in the prediction of subsequent development of

PE.

We found no substantive differences between GlyFn and the other screening methods in their predictive performance for superimposed PE within the subsequent 2 weeks, but the number of cases is small and inevitably the confidence intervals around the predicted detection rates are wide. However, the data demonstrate that none of the tests are diagnostic and detection rates are low for relatively high false positive rates.

Conclusions

GlyFn is a simple point-of-care test which can be carried out without the need of a laboratory and can provide results within 10 minutes of testing. In this respect it may potentially replace the angiogenic markers that are currently used in the prediction of imminent PE in high-risk women. However, neither GlyFn nor angiogenic factors are likely to improve the management of women with chronic hypertension, because their predictive performance for superimposed PE is poor.

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REFERENCES

1. Panaitescu AM, Syngelaki A, Prodan N, Akolekar R, Nicolaides KH. Chronic hypertension and adverse pregnancy outcome: a cohort study. *Ultrasound Obstet Gynecol* 2017; **50**: 228–35.

2. Bramham K, Parnell B, Nelson-Piercy C, Seed PT, Poston L, Chappell LC. Chronic hypertension and pregnancy outcomes: systematic review and meta-analysis. *BMJ* 2014; **348**: g2301.

3. O'Gorman N, Wright D, Syngelaki A, Akolekar R, Wright A, Poon LC, Nicolaides KH. Competing risks model in screening for preeclampsia by maternal factors and biomarkers at 11-13 weeks' gestation. *Am J Obstet Gynecol* 2016; **214**: 103.e1-103.e12.

4. Tan MY, Syngelaki A, Poon LC, Rolnik DL, O'Gorman N, Delgado JL, Akolekar R, Konstantinidou L, Tsavdaridou M, Galeva S, Ajdacka U, Molina FS, Persico N, Jani JC, Plasencia W, Greco E, Papaioannou G, Wright A, Wright D, Nicolaides KH. Screening for pre-eclampsia by maternal factors and biomarkers at 11-13 weeks' gestation. *Ultrasound Obstet Gynecol* 2018; **52**: 186-195.

5. Rolnik DL, Wright D, Poon LC, O'Gorman N, Syngelaki A, de Paco Matallana C, Akolekar R, Cicero S, Janga D, Singh M, Molina FS, Persico N, Jani JC, Plasencia W, Papaioannou G, Tenenbaum-Gavish K, Meiri H, Gizurarson S, Maclagan K, Nicolaides KH. Aspirin versus placebo in pregnancies at high risk for preterm preeclampsia. *N Engl J Med* 2017; **377**: 613-622.

6. 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.

7. Döbert M, Wright A, Varouxaki AN, Mu AC, Syngelaki A, Rehal A, Delgado JL, Akolekar R, Muscettola G, Janga D, Singh M, Martin-Alonso R, Dütemeyer V, De Alvarado M, Atanasova V, Wright D, Nicolaides KH. STATIN trial: predictive performance of competing-risks model in screening for pre-eclampsia at 35-37 weeks' gestation. *Ultrasound Obstet Gynecol* 2022; **59**: 69-75.

8. Schiattarella A, Magee LA, Wright A, Syngelaki A, Akolekar R, Von Dadelszen P, Nicolaides KH. Prediction of hypertensive disorders after screening at 35-36 weeks' gestation: comparison of angiogenic markers with competing-risks model. *Ultrasound Obstet Gynecol* 2023 Jun 17. doi: 10.1002/uog.26291.

9. Magee LA, Wright D, Syngelaki A, von Dadelszen P, Akolekar R, Wright A, Nicolaides KH. Preeclampsia prevention by timed birth at term. *Hypertension* 2023; **80**: 969-978.

10. Sokratous N, Bednorz M, Sarli P, Morillo Montes OE, Syngelaki A, Wright A, Nicolaides KH. Screening for pre-eclampsia by maternal serum glycosylated fibronectin at 11-13 weeks' gestation. *Ultrasound Obstet Gynecol* 2023 Jul 4. doi: 10.1002/uog.26303.

11. Sokratous N, Wright A, Syngelaki A, Kakouri E, Laich A, Nicolaides KH. Screening for pre-eclampsia by maternal serum glycosylated fibronectin and angiogenic markers at 36 weeks' gestation. *Ultrasound Obstet Gynecol* 2023; in press.

12. Chappell LC, Duckworth S, Seed PT, Griffin M, Myers J, Mackillop L, Simpson N, Waugh J, Anumba D, Kenny LC, Redman CW, Shennan AH. Diagnostic accuracy of placental growth factor in women with suspected preeclampsia: a prospective multicenter study. *Circulation* 2013; **128**: 2121-31.

13. Zeisler H, Llurba E, Chantraine F, Vatish M, Staff AC, Sennström M, Olovsson M, Brennecke SP, Stepan H, Allegranza D, Dilba P, Schoedl M, Hund M, Verlohren S. Predictive value of the sFlt-1:PlGF ratio in women with suspected preeclampsia. *N Engl J Med* 2016; **374**: 13-22.

14. Stepan H, Hund M, Gencay M, Denk B, Dinkel C, Kaminski WE, Wieloch P, Semus B, Meloth T, Dröge LA, Verlohren S. A comparison of the diagnostic utility of the sFlt-1/PlGF ratio versus PlGF alone for the detection of preeclampsia/HELLP syndrome. *Hypertens Pregnancy* 2016; **35**: 295-305.

15. Huhn EA, Hoffmann I, Martinez De Tejada B, Lange S, Sage KM, Roberts CT, Gravett MG, Nagalla SR, Lapaire O. Maternal serum glycosylated fibronectin as a short-term predictor of preeclampsia: a prospective cohort study. *BMC Pregnancy Childbirth* 2020; **20**: 128.

16. Nagalla SR, Janaki V, Vijayalakshmi AR, Chayadevi K, Pratibha D, Rao PV, Sage KM, Nair-Schaef D, Bean E, Roberts CT Jr, Gravett MG. Glycosylated fibronectin point-of-care test for diagnosis of pre-eclampsia in a low-resource setting: a prospective Southeast Asian population study. *BJOG* 2020; **127**: 1687-1694.

17. Sokratous N, Bbednorz M, Wright A, Nicolaides KH, Kametas NA. Prediction of imminent preeclampsia by serum glycosylated fibronectin in women with new onset hypertension. *Ultrasound Obstet Gynecol* 2023; in press.

18. American College of Obstetricians and Gynecologists (ACOG) Practice Bulletin no. 203: Chronic hypertension. *Obstet Gynecol* 2019; **133**: e26-50.

19. NICE guideline. Hypertension in pregnancy: diagnosis and management. Published: 25 June 2019. Last updated: 17 April 2023 www.nice.org.uk/guidance/ng133

20. Clark K, Snowball O, Nzelu D, Kay P, Kametas NA. Validation of the Microlife WatchBP Home blood pressure device in pregnancy for medium and large arm circumferences. *Blood Press Monit* 2018; **23**: 171-174.

21. Robinson HP, Fleming JE. A critical evaluation of sonar crown rump length measurements. *Br J Obstet Gynaecol* 1975; **82**: 702-710.

22. Snijders RJ, Nicolaides KH. Fetal biometry at 14-40 weeks' gestation. *Ultrasound Obstet Gynecol* 1994; **4**: 34-38.

23. ACOG Practice Bulletin No. 202: Gestational hypertension and preeclampsia. *Obstet Gynecol* 2019; **133**: 1.

24. National Institute for Health and Care Excellence. PLGF-based testing to help diagnose suspected preterm pre-eclampsia. NICE guideline No 49. Published: 27 July 2022. www.nice.org.uk/guidance/dg49

25. R Core Team 2020 R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/. 2020.

26. Hernandez-Pacheco JA, Rosales-Zamudio CI, Borboa-Olivares H, Espejel-Nunez A, Parra-Hernandez S, Estrada-Gutierrez G, Camargo-Marin L, Medina-Bastidas D, Guzman-Huerta M. The sFlt-1/PlGF ratio as a triage tool to identify superimposed preeclampsia in women with chronic hypertension in emergency rooms. *Pregnancy Hypertens* 2020; **21**: 38-42.

27. Binder J, Kalafat E, Palmrich P, Pateisky P, Khalil A. Should angiogenic markers be included in diagnostic criteria of superimposed pre-eclampsia in women with chronic hypertension? *Ultrasound Obstet Gynecol* 2022; **59**: 192-201.

28. 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 P. Hypertensive Disorders of Pregnancy: ISSHP Classification, Diagnosis, and Management Recommendations for International Practice. *Hypertension* 2018; **72**: 24–43.

29. Wright A, von Dadelszen P, Magee LA, Syngelaki A, Akolekar R, Wright D, Nicolaides KH. Effect of race on the measurement of angiogenic factors for prediction and diagnosis of pre-eclampsia. *BJOG* 2023; **130**: 78-87.

FIGURE LEGENDS

Figure 1. Distribution of GlyFn, PlGF and sFLT-1 / PlGF ratio in the group with superimposed PE (red circles) and those without PE (black circles).

Figure 2. Receiver operating characteristic curves for prediction of superimposed PE in women with chronic hypertension by GlyFn (blue), PlGF (red) and sFLT-1 / PlGF (black).

Table 1. Maternal and pregnancy characteristics of the study population.

Characteristic	No PE (n=78)	PE (n=26)	p-value
Maternal age (years)	35.0 (33.0, 37.0)	35.0 (33.0, 38.0)	0.465
Maternal weight (kg)	88.0 (76.0, 99.0)	94.5 (84.5, 104.0)	0.101
Maternal height (cm)	165 (160, 170)	164 (160, 170)	0.811
Body mass index (kg/m^2)	32.1 (28.2, 36.3)	35.4 (31.8, 38.6)	0.068
Gestational age (weeks)	34.1 (31.5, 35.6)	34.0 (31.5, 35.4)	0.878
Antihypertensive medication	59 (75.6)	25 (96.2)	0.044
Ethnicity			0.030
White	33 (42.3)	6 (23.1)	
Black	34 (43.6)	19 (73.1)	
South Asian	11 (14.1)	1 (3.9)	
Diabetes mellitus Type 2	1 (1.3)	0 (0.0)	1
Smoker	1 (1.3)	0 (0.0)	1
Method of conception			
Natural	73 (93.6)	26 (100)	0.427
In vitro fertilization	5 (6.4)	26 (100)	0.427
Parity			0.942
Nulliparous	28 (35.9)	10 (38.5)	
Parous, no previous PE	42 (53.9)	13 (50.0)	
Parous, previous PE	8 (10.3)	3 (11.5)	
Interpregnancy interval (years)	3.3 (1.7, 6.8)	4.0 (3.0, 5.5)	0.509
Systolic blood pressure	126.0 (121.0, 135.0)	138.0 (130.0, 145.0)	0.0001
Diastolic blood pressure	82.0 (76.3, 87.0)	88.5 (81.3, 95.8)	0.002
GlyFen (µg/mL)	319.0 (244.3, 422.3)	478.50 (360.3, 667.3)	<0.0001
PlGF (pg/mL)	374.4 (162.0, 597.9)	202.0 (78.3, 324.8)	0.009
sFLT-1 / PlGF	3.77 (2.27, 13.43)	15.71 (7.66, 43.84)	<0.0001

Values are presented as n (%) and median (interquartile range). PE, preeclampsia; sFLT-1, soluble fms-like tyrosine kinase-1; PlGF, placental growth factor; GlyFn, glycosylated fibronectin. Comparisons between outcome groups were by chi-square or Fisher exact test for categorical variables and Mann Whitney-U test for continuous variables.

Table 2. Associations (r values with 95% confidence interval) between the different biomarkers in women who developed preeclampsia (top) and those who did not (bottom).

	log10(GlyFen)	**log10(sFLT-1)**	**log10(PlGF)**	**log10(SBP)**
log10(sFLT-1)	0.636 (0.330, 0.821)			
log10(PlGF)	-0.466 (-0.722, -0.094)	-0.401 (-0.682, -0.016)		
log10(SBP)	0.155 (-0.247, 0.512)	0.303 (-0.096, 0.618)	-0.026 (-0.409, 0.366)	
log10(DBP)	0.047 (-0.347, 0.426)	0.430 (0.051, 0.701)	-0.267 (-0.593, 0.134)	0.510 (0.153, 0.749)

	log10(sFLT-1)	log10(PlGF)	log10(SBP)	log10(DBP)
log10(GlyFen)	0.437 (0.237, 0.601)	-0.377 (-0.553, -0.169)	0.117 (-0.109, 0.331)	0.193 (-0.031, 0.399)
log10(sFLT-1)		-0.339 (-0.522, -0.126)	0.071 (-0.154, 0.289)	0.290 (0.072, 0.481)
log10(PlGF)			-0.335 (-0.519, -0.122)	-0.347 (-0.529, -0.135)
log10(SBP)				0.535 (0.355, 0.677)

GlyFn, glycosylated fibronectin; S, systolic; D, diastolic; BP, blood pressure; sFLT-1, soluble fms-like tyrosine kinase-1; PlGF, placental growth factor.

Table 3. Predictive performance for superimposed preeclampsia in women with chronic hypertension.

	Cut-off	SPR n/N (%)	FPR n/N (%)	DR n/N (%, 95% CI)
sFLT-1/PIGF				
Total	85	6/104 (5.8)	1/78 (1.3)	5/26 (19.2, 6.6-39.4)
Blood sampled < 36 weeks	85	5/88 (5.7)	1/66 (1.5)	4/22 (18.2, 5.2-40.3)
Blood sampled ≥ 36 weeks	85	1/16 (6.2)	0/12 (0.0)	1/4 (25.0, 0.6-80.6)
PIGF				
Total	75 pg/mL	9/104 (8.7)	3/78 (3.8)	6/26 (23.1, 9.0-43.6)
Blood sampled < 36 weeks	75 pg/mL	9/88 (10.2)	3/66 (4.5)	6/22 (27.3, 10.7-50.2)
Blood sampled ≥ 36 weeks	75 pg/mL	0/16 (0.0)	0/12 (0.0)	0/4 (0.0, 0-60.2)
GlyFn				
Total	510 µg/mL	25/104 (24.0)	13/78 (16.7)	12/26 (46.2, 26.6-66.6)
Blood sampled < 36 weeks	510 µg/mL	20/88 (22.7)	10/66 (15.2)	10/22 (45.5, 24.4-67.8)
Blood sampled ≥ 36 weeks	510 µg/mL	5/16 (31.2)	3/12 (25.0)	2/4 (50.0, 6.8-93.2)

	Cut-off	SPR n/N (%)	FPR n/N (%)	DR n/N (%, 95% CI)
sFLT-1/PIGF				
Total	63	11/104 (10.6)	5/78 (6.4)	6/26 (23.1, 9.0-43.6)
Blood sampled < 36 weeks	63	10/88 (11.4)	5/66 (7.6)	5/22 (22.7, 7.8-45.4)
Blood sampled ≥ 36 weeks	63	1/16 (6.2)	0/12 (0.0)	1/4 (25.0, 0.6-80.6)
PIGF				
Total	77 pg/mL	11/104 (10.6)	4/78 (5.1)	7/26 (26.9, 11.6-47.8)
Blood sampled < 36 weeks	77 pg/mL	11/88 (12.5)	4/66 (6.1)	7/22 (31.8, 13.9-54.9)
Blood sampled ≥ 36 weeks	77 pg/mL	0/16 (0.0)	0/12 (0.0)	0/4 (0, 0-60.2)
GlyFn				
Total	669 µg/mL	11/104 (10.6)	4/78 (5.1)	7/26 (26.9, 11.6-47.8)

Blood sampled < 36 weeks	669 μg/mL	10/88 (11.4)	4/66 (6.1)	6/22 (27.3, 10.7-50.2)
Blood sampled ≥ 36 weeks	669 μg/mL	1/16 (6.2)	0/12 (0.0)	1/4 (25.0, 0.6-80.6)

SPR, screen positive rate; FPR, false positive rate; DR, detection rate; sFLT-1, soluble fms-like tyrosine kinase-1; PlGF, placental growth factor; GlyFn, glycosylated fibronectin.

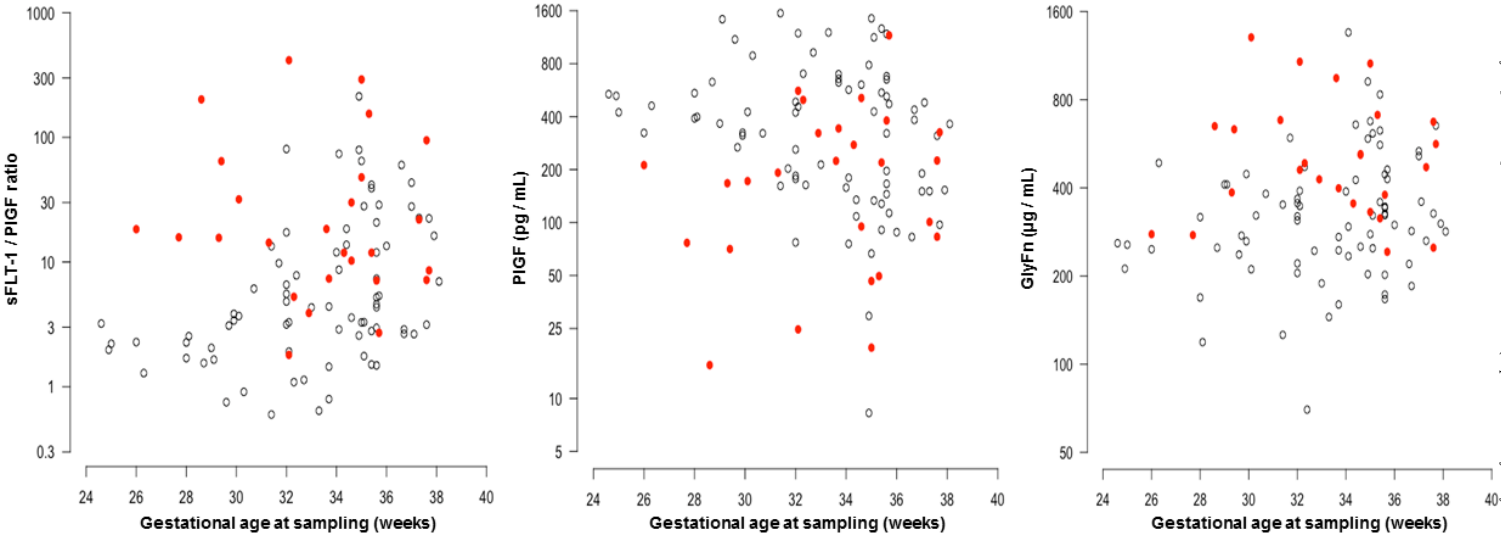

Figure 1

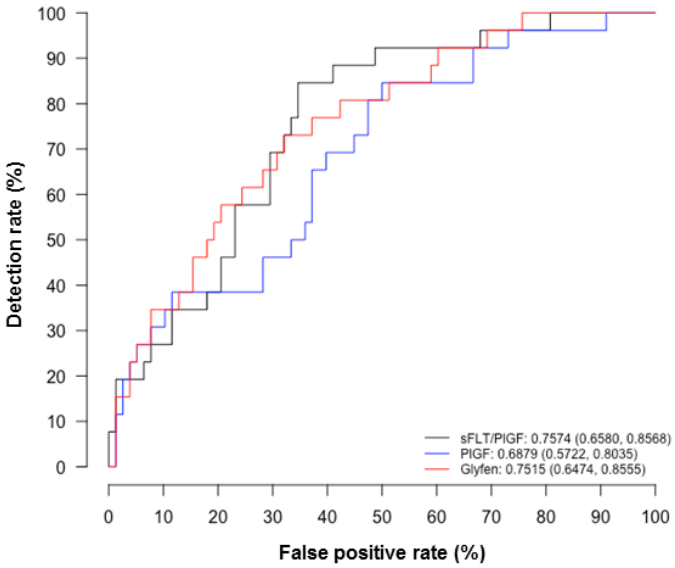

Figure 2