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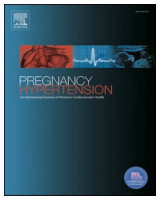
Pregnancy Hypertension: An International Journal of Women's Cardiovascular Health 36 (2024) 101111

Contents lists available at ScienceDirect

Pregnancy Hypertension: An International Journal of Women's Cardiovascular Health

journal homepage: www.elsevier.com/locate/preghy



Full Length Article

The addition of the sFlt-1/PlGF ratio to the protein/creatinine ratio in multiple pregnancy: Post-hoc analysis of the PREPARE cohort study

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ARTICLE INFO

Keywords:
Pre-eclampsia
Multiple pregnancy
Biomarkers
sFlt-1/PlGF ratio

ABSTRACT

Objective: To assess the predictive accuracy of the sFlt-1/PlGF ratio cut-off 38 in addition to the standard-of-care spot urine protein/creatinine ratio (PCr) for multiple pregnancies in women with suspected pre-eclampsia.
Study design: Post-hoc analysis of a prospective cohort study.
Main outcome measures: Primary outcome was the occurrence of pre-eclampsia in one and four weeks after presentation with suspected pre-eclampsia. Test characteristics with 95% confidence intervals (CI) were calculated on pre-eclampsia development in one and four weeks.
Results: Twenty-three multiple pregnancies with suspected pre-eclampsia between 20 and 37 weeks gestation were included for analysis. Women who eventually developed pre-eclampsia had a significantly higher PCr (34.0 vs. 16.5, $p = 0.015$), sFlt-1 (17033 vs. 5270 pg/ml, $p = 0.047$) and sFlt-1/PlGF ratio (99 vs. 25, $p = 0.033$) at baseline. Furthermore, PCr $\geq$ 30 and sFlt-1/PlGF ratio $>$ 38 was respectively seen in 1/16 (6.3 %) and 3/16 (18.8 %) of the women who did not develop pre-eclampsia. For predicting pre-eclampsia within one week the sFlt-1/PlGF ratio sensitivity was 75.0 % [95 % CI 19.4–99.4] and the negative predictive value 93.8 % [73.0–98.8], while no pre-eclampsia developed when PCr was $<$ 30. Consequently, the combination of these tests did not lead to an improvement in test characteristics, with non-significant differences in positive predictive value (50.0 % [29.5–70.5] versus 80.0 % [37.3–96.4]) compared to PCr alone for pre-eclampsia development in one week.
Conclusions: In addition to standard-of-care spot urine PCr measurements, this study has not been able to demonstrate that the sFlt-1/PlGF ratio cut-off 38 is of added value in the prediction of pre-eclampsia in multiple pregnancy.
Trial registration: Netherlands Trial Register (NL8308).

1. Introduction

Diagnostics assessments, such as blood pressure measurements and the spot urine protein/creatinine ratio (PCr), perform poorly in the prediction of pre-eclampsia and related complications [1]. Pre-eclampsia remains a leading cause of maternal and perinatal mortality and morbidity worldwide. Therefore, timely accurate prediction and diagnosis are essential to limit adverse outcomes [1–3]. Since multiple pregnancy is associated with a three-to-four fold higher chance for development of pre-eclampsia compared to singletons, evidence on prediction of pre-eclampsia and related complications is even more essential [4].

Abbreviations: PREPARE, PREdiction of Pre-eclampsia and AdveRse Events; LUMC, Leiden University Medical Centre; sFlt-1, Soluble FMS-like tyrosine kinase-1; PlGF, Placental growth factor; PCr, spot urine protein/creatinine ratio; ALT, alanine aminotransferase; AST, aspartate aminotransferase; FGR, fetal growth restriction; NICU, neonatal intensive care-unit; ISSHP, International Society for the Study of Hypertension in Pregnancy; IQR, interquartile range; CI, confidence interval; SPSS, Statistical Package for the Social Sciences.

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https://doi.org/10.1016/j.preghy.2024.101111
Received 31 October 2023; Received in revised form 14 January 2024; Accepted 11 February 2024
Available online 21 February 2024

In singleton pregnancy, the value of (anti-)angiogenic factors soluble FMS-like tyrosine kinase-1 (sFlt-1)/placental growth factor (PlGF) ratio with cut-off ≤38 has been firmly established to predict the absence of pre-eclampsia in the upcoming week [5–7]. However, in multiple pregnancies, the reference range of (anti-)angiogenic factors seems to differ from singletons [8–12]. This difference could be contributed to the increased placental mass or to the relative placental ischemia compared to singleton pregnancy [11,12]. Unfortunately, caused by its relative rarity, studies on angiogenic factors in multiple pregnancy are sparce with inevitable small sample size. Furthermore, available evidence is conflicting: several studies demonstrated that the sFlt-1/PlGF ratio cut-off of ≤38 in women suspected for pre-eclampsia may not be applicable in multiple pregnancies for ruling out the disease [13–15]. On the other hand, the study with the largest cohort of non-singleton pregnancies till date showed promising results in ruling out delivery because of pre-eclampsia with the sFlt-1/PlGF ratio cut-off of ≤38 in twin pregnancies [16]. In order to compile an overview of reported cut-offs of the sFlt-1/PlGF ratio in multiple pregnancies we summarized the published evidence on test characteristics for clinical outcomes in Additional file 1 [9,13,14,16–21]. Importantly, none of these studies described the sFlt-1/PlGF ratio on top of standard-of-care spot urine PCr in relation to pre-eclampsia diagnosis or clinical outcomes. Before implementing the sFlt-1/PlGF ratio as a routine assessment in daily clinical practice, there is a need to better understand whether the sFlt-1/PlGF ratio test is of added value to standard-of-care analyses performed in women with multiple pregnancies.

In the PREPARE cohort study, we recently demonstrated that the sFlt-1/PlGF ratio in addition to the spot urine PCr in women with a singleton pregnancy and suspected pre-eclampsia, may lead to improved selection of women at risk and a reduction in redundant health care usage [22]. In this study, we performed a post-hoc analysis of the PREPARE cohort study to assess the predictive accuracy of the sFlt-1/PlGF ratio in addition to standard-of-care spot urine PCr on pre-eclampsia in multiple pregnancy.

2. Materials and methods

2.1. Study design and population

This was a post-hoc analysis of the PREPARE cohort (PREdiction of Pre-eclampsia and AdveRse Events), a prospective cohort study in a Dutch tertiary referral center (Leiden University Medical Center (LUMC)) [22]. In this study, spot urine PCr, sFlt-1 and PlGF sampling was performed in women with suspected pre-eclampsia between December 2017 and February 2020. Women with multiple pregnancy, age ≥16 years and presenting with suspected pre-eclampsia between a gestational age ≥20 or <37 weeks at first visit were included for this analysis. Exclusion criterium was pre-eclampsia diagnosis before baseline day.

After the clinician specified the reason for inclusion, 20 mL blood was sampled by venipuncture which was stored at an independent laboratory at −80 °C. Analysis of the biomarkers was performed on the fully automated Elecsys® system (Cobas® analyzers, Roche Diagnostics International Ltd.) after study completion. As clinicians were unaware of the angiogenic factor levels, all women received follow-up and treatment according to local protocol following usual care. The PREPARE study was registered in the Netherlands Trial Register (NL8308) and approval for the study was obtained by the Medical Ethical Committee of the LUMC. Written informed consent was obtained from all patients.

2.2. Outcomes

For this analysis, the primary outcome was the occurrence of pre-eclampsia in the first week after presenting with suspected pre-eclampsia. Based on PCr result cut-off < 30 (mg/mmol) combined with the sFlt-1/PlGF ratio cut-off ≤38, four groups were described: a double negative result, group A−/−; a negative PCr and positive sFlt-1/PlGF ratio, group B−/+; a positive PCr and negative sFlt-1/PlGF ratio, group C+/−; and a double positive result, group D+/+. Furthermore, separate and combined test characteristics of the PCr and sFlt-1/PlGF ratio on pre-eclampsia development in one and four weeks after first presentation at baseline were calculated. For the calculations of the combined characteristics we considered group A−/− as negative and the other groups (B−/+, C+/− and D+/+) as positive results.

2.3. Definitions

Suspected pre-eclampsia was defined as one or more of following symptoms identified by the clinician: new onset of elevated blood pressure (systolic blood pressure (SBP) ≥ 140 mmHg and/or a diastolic blood pressure (DBP) ≥ 90 mmHg) or proteinuria (positive dipstick or PCr ≥ 30 performed earlier), aggravation of pre-existing hypertension or proteinuria, epigastric pain, excessive edema, headache, visual disturbances, sudden weight gain, low platelets ($<150 \times 10^9$/L), elevated liver transaminases (alanine aminotransferase (ALT) or aspartate aminotransferase (AST) > 40 IU/L (laboratory reference range 0–34 U/L)) or suspicion of fetal growth restriction (FGR, estimated fetal weight < 10th centile).

Pre-eclampsia was defined according to 2018 guidelines of the International Society for the Study of Hypertension in Pregnancy (ISSHP): gestational hypertension, plus one or more of the following conditions after 20 weeks of gestation: proteinuria, maternal organ dysfunction or uteroplacental dysfunction [23]. Superimposed pre-eclampsia was defined as chronic hypertension with a new onset proteinuria or development of any other maternal organ failure linked to pre-eclampsia. HELLP syndrome was defined by lactate dehydrogenase (LDH) > 250 U/L (laboratory reference range 0–247 U/L), AST/ALT > 40 U/L, and thrombocyte count $< 150 \times 10^9$/L, respectively.

Maternal adverse outcomes were adapted from full PIERS including death, stroke, eclampsia, blindness, uncontrolled hypertension (requiring administration of three or more different parenteral antihypertensive agents within a 12 h period), the use of inotropic agents, pulmonary edema (diagnosed clinically with one/more of oxygen saturation <95 %, diuretic treatment or x-ray confirmation), respiratory failure (needing intubation), myocardial ischemia or infarction, hepatic dysfunction (leading to disseminated intravascular coagulation), hepatic hematoma or rupture (confirmed by imaging or at laparotomy), renal failure (serum creatinine > 200 μmol/L), transfusion of any blood products, severe hypertension (SBP > 160 and/or DBP > 110 mmHg), and thromboembolic events (arterial, venous or small vessel thrombosis, other than superficial venous thrombosis, in any tissue or organ) [24]. Perinatal adverse outcomes were defined as fetal growth restriction (FGR, birthweight < 10th centile) [25], selective fetal growth restriction, (sFGR, at out institution defined as estimated fetal weight < 10th centile and intertwin discordance of 20 %), admission to the neonatal intensive care-unit (NICU) or perinatal death.

2.4. Statistical analysis

Baseline characteristics and outcomes were summarized using descriptive statistics, frequency (%) or median ± interquartile range (IQR) depending on data type. Comparisons between groups were analysed with Mann-Whitney *U* test for numerical and $\chi 2$ test for categorical variables. For the assessment of the primary outcome sensitivity, specificity, negative predictive values (NPV), positive predictive value (PPV) and likelihood ratios (LR) with 95 % confidence intervals (CI) were calculated. For the analysis of data Statistical Package for the Social Sciences (SPSS) version 25.0 was used.

3. Results

3.1. Baseline characteristics

From the 365 pregnant women in the source population with suspected pre-eclampsia that were sampled between December 2017 and February 2020, 23 women had a multiple pregnancy between 20 and 37 weeks of gestation (Fig. 1). No multiple pregnancies were excluded or were lost to follow-up. Baseline characteristics are described in Table 1. The most common reasons for the clinical suspicion of pre-eclampsia were new onset of hypertension (60.9 %), excessive oedema (34.8 %), fetal growth restriction (30.4 %) and headache (26.1 %). Furthermore, as can be seen in Table 2, the women who eventually developed pre-eclampsia had a significantly higher median PCr (34.0 vs. 16.5, p = 0.015), sFlt-1 (17033 vs. 5270 pg/ml, p = 0.047) and sFlt-1/PlGF ratio (99 vs. 25, p = 0.033) at baseline. Furthermore, PCr ≥ 30 and sFlt-1/PlGF ratio > 38 was seen in 4/7 (57.1 %) of the women who developed pre-eclampsia at any time and respectively in 1/16 (6.3 %) and 3/16 (18.8 %) of the women who did not develop pre-eclampsia.

3.2. Main results

In Table 3, a case overview is presented of pregnancy outcomes with the baseline PCr and sFlt-1/PlGF ratio values. The overall prevalence of pre-eclampsia in this cohort was 30.4 %, of which 4/23 (17.4 %) developed in one week and 6/23 (26.1 %) in four weeks after baseline. Two out of 15 (13 %) in group A−/− (negative PCr and negative sFlt-1/PlGF ratio), developed pre-eclampsia more than a week after baseline. Eleven out of 15 (73 %) had a kind of maternal or perinatal adverse outcome later in pregnancy, not necessarily related to pre-eclampsia. None of the 18 women with suspected pre-eclampsia and a negative PCr at baseline, group A−/− and B−/+, developed pre-eclampsia or adverse outcomes within a week. Interestingly, of the three women in group B−/+, one woman with a sFlt-1/PlGF ratio of 43.1 had a spontaneous preterm birth at 32 + 3 weeks, one day after baseline. The only woman in group C+/− developed pre-eclampsia on baseline day with severe features such as low platelets and hemolysis while the sFlt-1/PlGF ratio was 33.0. She delivered three days later at 32 + 5 weeks. In the four women in group D+/+, three were diagnosed with pre-eclampsia shortly after presentation and all had adverse pregnancy outcomes. The one woman in this group that did not develop pre-eclampsia, despite a sFlt-1/PlGF ratio of 143.2, eventually delivered two growth restricted neonates, of which one was diagnosed with Tetralogy of Fallot.

Group A−/− = PCr < 30 and sFlt-1/PlGF ≤ 38. Group B−/+ = PCr < 30 and sFlt-1/PlGF > 38. Group C+/− = PCr ≥ 30 and sFlt-1/PlGF ≤ 38. Group D+/+ = PCr ≥ 30 and sFlt-1/PlGF > 38. PCr, protein creatinine ratio; MCDA, monochorionic diamniotic twin; DCDA, dichorionic diamniotic twin; DCTA, dichorionic triamniotic triplet; TCTA trichorionic triamniotic triplet; GA, gestational age; HT, hypertension; LDH, lactate dehydrogenase; FGR, fetal growth restriction < 10th centile; sFGR, selective fetal growth restriction (estimated fetal weight < 10th centile and intertwin discordance of 20 %); ToF, Tetralogy of Fallot.

Table 1
Baseline characteristics of the women with suspected pre-eclampsia.

Patients (N)	Multiple pregnancies *N = 23*
General characteristics	
Maternal age*	31 (28–34)
Gestational age*	32.6 (29.3–34.4)
White	22 (95.7)
Smoking during pregnancy	1 (4.3)
Pre-pregnancy BMI (kg/m^2)*	24.7 (21.5–27.3)
Reason of inclusion ‡	
New onset of hypertension	14 (60.9)
Aggravation of pre-existing hypertension	1 (4.3)
Epigastric pain	4 (17.4)
Headache	6 (26.1)
Excessive oedema	8 (34.8)
Visual disturbances	3 (13.0)
Haemolysis	2 (8.7)
Elevated liver transaminases	2 (8.7)
Low platelets	2 (8.7)
Estimated Fetal Weight < 10th centile	7 (30.4)
Gestational characteristics	
Nulliparous	14 (60.9)
History of pre-eclampsia	1 (4.3)
Chronic hypertension	1 (4.3)
Twin pregnancy	21 (91.3)
Monochorionic	9 (39.1)
Triplet pregnancy	2 (8.7)
Clinical characteristics	
Systolic blood pressure (mmHg)*	138 (120–150)
Diastolic blood pressure (mmHg)*	85 (80–92)

Data depicted as numbers (%) unless otherwise specified. * Median (IQR). † Shows a significant difference with two-sided α < 0.05. ‡ Combination of reasons possible.

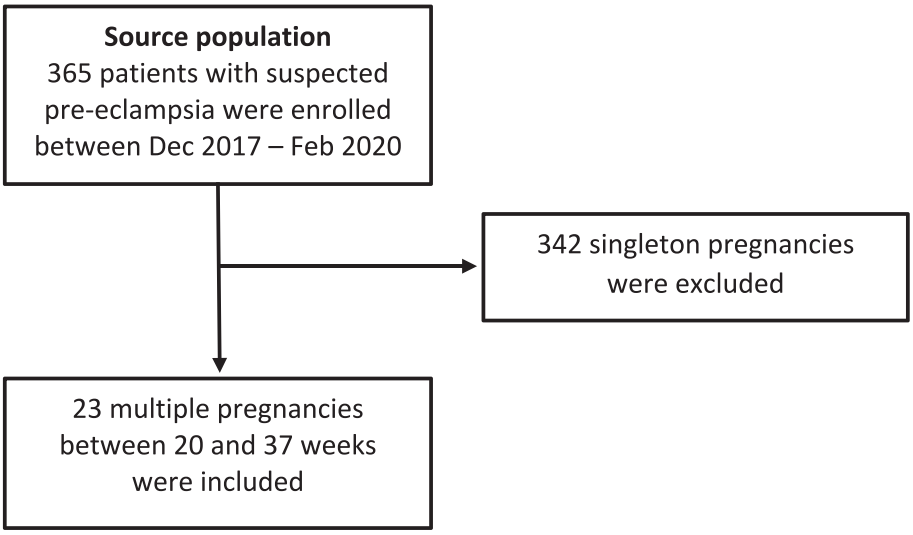

Fig. 1. Flowchart inclusions.

Table 2
Laboratory characteristics of the women with suspected pre-eclampsia at baseline.

Patients (N)	No pre-eclampsia *N = 16*	Pre-eclampsia at any time *N = 7*
Protein/creatinine ratio (mg/mmol)*	16.5 (13.2–19.3)	34 (20.4–143.5) †
Protein/creatinine ratio ≥ 30	1 (6.3)	4 (57.1) †
sFlt-1 (pg/ml) *	5270 (3640–9095)	17,033 (5850–25118) †
PlGF (pg/ml) *	296 (192–504)	154 (129–254)
sFlt-1/PlGF ratio *	25 (8–36)	99 (25–180) †
sFlt-1/PlGF ratio > 38	3 (18.8)	4 (57.1)

Data depicted as numbers (%) unless otherwise specified. * Median (IQR). † Shows a significant difference with two-sided $\alpha < 0.05$.

In Table 4, test characteristics of the spot urine PCr and sFlt-1/PlGF ratio are summarized separately. The spot urine PCr had overall better test characteristics compared to the sFlt-1/PlGF ratio, notably with respect to test sensitivity of 100.0 % [95 % CI 39.8–100.0] versus 75.0 % [19.4–99.4] and positive predictive value of 80.0 % [37.3–96.4] versus 42.9 % [21.0–67.9]. For the prediction of pre-eclampsia in four weeks sensitivity was 66.7 % [22.3–95.7] of both PCr and sFlt-1/PlGF ratio, and the negative predictive value was 88.9 % [71.9–96.2] and 87.5 % [68.9–95.7] respectively. Consequently, the combination of these tests did not lead to an improvement in test characteristics, showing a non-significant difference in specificity (79.0 % [54.4–94.0] versus 94.7 % [74.0–99.9]; 82.4 % [56.6–96.2] versus 94.1 % [71.3–99.9]) and positive predictive value (50.0 % [29.5–70.5] versus 80.0 % [37.3–96.4]; 62.5 % [36.0–83.2] versus 80.0 % [35.5–96.7]) comparing to PCr alone in the prediction of pre-eclampsia in respectively one and four weeks after baseline.

Data are given as percentage (with 95 % CI). PPV, positive predictive value; NPV, negative predictive value; AUC, area under the curve; LR+, positive likelihood ratio; LR-, negative likelihood ratio. Group A−/− = PCr < 30 and sFlt-1/PlGF ≤ 38. Group B−/+ = PCr < 30 and sFlt-1/PlGF > 38. Group C+/− = PCr ≥ 30 and sFlt-1/PlGF ≤ 38. Group D+/+ = PCr ≥ 30 and sFlt-1/PlGF > 38.

4. Discussion

This study demonstrated that adding the sFlt-1/PlGF ratio in multiple pregnancy to spot urine PCr did not contribute to the prediction of pre-eclampsia. Therefore, implementation of the sFlt-1/PlGF ratio with cut-off ≤38 for ruling out pre-eclampsia development in multiple pregnancy may not be of added value to the current standard-of-care [22]. The present study adds to the sparce reports on the sFlt-1/PlGF ratio in multiple pregnancy, helping clinicians in daily clinical practice to identify the women at risk for development of pre-eclampsia.

Given the acknowledged limitation of proteinuria as a poor predictor of adverse outcomes, we hypothesized that the incorporation of the sFlt-1/PlGF ratio could enhance the identification of at-risk multiple pregnancies [26]. However, false negative test results allude to the safety of implementing the sFlt-1/PlGF ratio in multiple pregnancies. Despite recent conclusions in a large cohort by Binder et al. [16] where the sFlt-1/PlGF ratio with cut-off 38 was able to rule out delivery related to pre-eclampsia in 2 weeks, several other studies demonstrated the occurrence of pre-eclampsia in up to two-third of twin pregnancies with a sFlt-1/PlGF ratio ≤38 [13,14]. In line with the latter, also in our study, one woman (Table 3, case 1 in group C+/−) developed pre-eclampsia with severe features within 24 h despite a sFlt-1/PlGF ratio ≤38. Conversely, it must be noted that also a woman with negative PCr testing at baseline developed early severe pre-eclampsia together with fetal growth restriction. Furthermore, Binder et al. and Karge et al. also reported disappointing AUC values of the sFlt-1/PlGF ratio of 0.69 [0.60–0.77] and 0.62 [0.39–0.85] for respectively maternal adverse outcomes and neonatal adverse outcomes [16,19]. Although women in groups B−/+,

Table 3
Overview of pre-eclampsia outcomes in multiple pregnancies.

	Case	Chorionicity	PCr (mg/mmol)	sFlt-1/PlGF ratio	GA at baseline	Time to PE	GA at diagnosis	Maternal and perinatal adverse outcomes (number of affected neonates)	GA at delivery
A−/−	1	MCDA	0.1	1.3	22.5	–	–	FGR (2)	35.0
	2	MCMA	14.0	4.7	26.6	–	–	Severe HT	32.6
	3	DCDA	15.8	5.6	25.3	–	–	FGR (2)	37.0
	4	DCDA	26.4	7.0	35.4	–	–	–	36.5
	5	TCTA	16.6	9.2	27.4	–	–	FGR (2), NICU (3)	30.0
	6	DCDA	17.9	10.2	30.2	–	–	–	37.2
	7	DCDA	13.7	17.4	33.6	–	–	FGR (1)	37.0
	8	MCDA	17.3	19.9	33.1	–	–	sFGR	36.1
	9	DCTA	21.4	23.0	29.3	≤4 weeks	31.2	Severe HT + low platelets, NICU (3)	31.6
	10	DCDA	8.6	25.2	27.2	>4 weeks	33.5	HELLP	34.0
	11	DCDA	12.5	30.2	35.1	–	–	FGR (1)	36.4
	12	DCDA	17.0	30.7	35.6	–	–	FGR (1)	38.0
	13	DCDA	21.5	34.7	34.2	–	–	–	37.0
	14	DCDA	13.0	35.8	36.0	–	–	–	37.0
	15	DCDA	19.8	36.5	34.3	–	–	FGR (1)	36.6
B−/+	1	MCDA	8.5	43.1	32.2	–	–	sFGR, NICU (2)	32.3
	2	MCDA	16.4	71.8	30.3	–	–	FGR (1), NICU (1)	33.1
	3	DCDA	20.4	98.6	29.5	≤4 weeks	31.4	Severe HT + low platelets, FGR (1), NICU (1)	36.5
C+/−	1	MCDA	148.0	33.0	32.2	≤1 week	32.2	Low platelets + ↑LDH, sFGR	32.5
D+/+	1	MCDA	34.0	123.3	34.4	≤1 week	34.4	Low platelets	36.1
	2	MCDA	30.3	143.2	32.6	–	–	FGR (2), NICU (2), ToF	35.1
	3	DCDA	143.5	180.3	35.3	≤1 week	35.3	↑LDH, FGR (1), NICU (1)	35.5
	4	MCMA	69.0	393.4	33.0	≤1 week	33.0	Severe HT + ↑ liver enzymes, sFGR, NICU (2)	33.0

Table 4
Test characteristics for prediction of pre-eclampsia in one and four weeks.

Test	Pre-eclampsia	Within one week	Within four weeks
Spot urine PCr only	Sensitivity	100.0 (39.8–100.0)	66.7 (22.3–95.7)
	Specificity	94.7 (74.0–99.9)	94.1 (71.3–99.9)
	PPV	80.0 (37.3–96.4)	80.0 (35.5–96.7)
	NPV	100.0	88.9 (71.9–96.2)
	LR+	19.0 (2.8–128.0)	11.3 (1.6–82.4)
	LR−	0	0.4 (0.1–1.1)
sFlt-1/PlGF ratio only	Sensitivity	75.0 (19.4–99.4)	66.7 (22.3–95.7)
	Specificity	79.0 (54.4–94.0)	82.4 (56.6–96.2)
	PPV	42.9 (21.0–67.9)	57.1 (29.2–81.2)
	NPV	93.8 (73.0–98.8)	87.5 (68.9–95.7)
	LR+	3.6 (1.3–10.1)	3.8 (1.2–12.2)
	LR−	0.3 (0.1–1.8)	0.4 (0.1–1.3)
Test scenario *(A−/− vs. B−/+, C+/− and D+/+)*	Sensitivity	100.0 (39.8–100.0)	83.3 (35.9–99.6)
	Specificity	79.0 (54.4–94.0)	82.4 (56.6–96.2)
	PPV	50.0 (29.5–70.5)	62.5 (36.0–83.2)
	NPV	100.0	93.3 (69.8–98.8)
	LR+	4.8 (2.0–11.4)	4.7 (1.6–14.0)
	LR−	0	0.2 (0.0–1.2)

C−/+ and D+/+ in our cohort exhibited an increased risk of adverse outcomes, our study corroborates the current evidence that employing the sFlt-1/PlGF ratio cut-off 38 for multiple pregnancies is highly debatable. In our study, it did not provide added value to current clinical practice, notably PCr testing.

Even though promising overall predictive value of the sFlt-1/PlGF ratio for multiple pregnancies has been reported, differences in sFlt-1 and PlGF reference range values in twins compared to singletons have been described extensively in literature [9–12,14,27,28]. Most of these studies report higher sFlt-1 across all trimesters and similar or higher PlGF concentrations in multiple pregnancies [9–12]. Whilst a higher cut-off of the sFlt-1/PlGF ratio is plausible [14], it remains to be investigated whether this beneficially impacts test characteristics (e.g. positive/negative predictive values an likelihood ratios) in multiple pregnancies. Additionally, from a placental-oriented point of view, comparative evidence on sFlt-1/PlGF ratio values between monochorionic, dichorionic pregnancies and triplets is limited [11,29]. Noteworthy, the two triplets (cases 5 and 9) included in our cohort experienced placenta-related adverse outcomes despite having sFlt-1/PlGF ratio $\leq$38 at baseline. Moreover, applying an adjusted cut-off of the sFlt-1/PlGF ratio as suggested by Dröge et al. in our cohort of multiple pregnancies did not significantly impact the overall results (only one case had a sFlt-1/PlGF ratio between 38 and 53: case 1 in group B−/+) [14]. Taken together, in light of the current evidence, it is not self-evident that the sFlt-1/PlGF ratio will perform as good in multiple pregnancies as in singleton pregnancies. Moreover, given the uncertainty of this new test in multiple pregnancies compared to proteinuria measurement should prompt future studies to focus on identification of the most effective algorithms and group-specific cut-offs [8].

The recent ISSHP 2021 definition included angiogenic imbalance (i.e. sFlt-1/PlGF ratio $>$ 38) as an indicative marker for uteroplacental dysfunction and thus the definition of pre-eclampsia [30]. In our cohort of multiple pregnancies, applying this definition would have resulted in 5 (22 %) of 14 (61 %) women, initially presenting with new-onset hypertension, being diagnosed with pre-eclampsia based on the combination of hypertension and angiogenic imbalance. This would have resulted in an additional pre-eclampsia diagnosis in group D+/+ (case 2) and a shorter time to diagnosis in group B−/+ (case 3). It is noteworthy that all five of these women developed adverse outcomes. Implementation of the sFlt-1/PlGF ratio in routine clinical practice may therefore alter management and follow-up of multiple pregnancies. However, integrating the sFlt-1/PlGF ratio in routine clinical practice of monitoring multiple pregnancy, should also demonstrate an improved cost-effectiveness in a setting where women with multiple pregnancies are routinely under more intensive routine antenatal follow-up compared to singleton pregnancies, including a lower threshold for hospital admissions.

It is important to address some limitations of the present study. First, the study was conducted in a third-line referral center for complicated multiple pregnancies and the study size was small. Firm conclusions cannot be drawn based on the current small sample size and the results from this cohort may be less generalizable to the general population. Second and consequentially, only a handful of complications with clinical consequences occurred and rare complications such as pulmonary edema, placental abruption or disseminated intravascular coagulation were not seen. Third, the inclusion of various types of multiple pregnancies may have impacted the sFlt-1/PlGF ratio values. Given the small sample size and consequently low number of events, significant differences between groups regarding occurrence of complications in our cohort might be precluded.

5. Conclusions

The present study has not been able to demonstrate that the addition of sFlt-1/PlGF ratio cut-off 38 to standard-of-care spot urine PCr improves prediction of pre-eclampsia in multiple pregnancy. These results should be considered when implementing the sFlt-1/PlGF ratio in routine clinical care as it remains matter of debate if the sFlt-1/PlGF ratio could contribute to a reduction in maternal/perinatal morbidity and increased cost-effectiveness in the specific sub-group of multiple pregnancies.

6. Ethics approval and consent to participate

The study was registered in the Netherlands Trial Register (NL8308) and approval for the study was obtained by the Medical Ethical Committee of the LUMC. The study was conducted in accordance with the principles of the Declaration of Helsinki, the Medical Research Involving Human Subjects Act (WMO) and other guidelines, regulations and Acts. Written informed consent was obtained from all patients.

7. Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CRediT authorship contribution statement

M. Wind: Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft, Writing – review & editing. **L. Dekker:** Data curation, Conceptualization, Writing – original draft. **M.E. van den Akker-van Marle:** Conceptualization. **B.E.P.B. Ballieux:** Conceptualization. **C.M. Cobbaert:** Conceptualization. **T.J. Rabelink:**

Conceptualization. **J.M.M. van Lith:** Conceptualization. **Y.K.O. Teng:** Conceptualization, Methodology, Writing – review & editing, Supervision. **M. Sueters:** Conceptualization, Methodology, Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Grant support: The work of Y.K. Onno Teng was supported by the Dutch Kidney Foundation (17OKG04). We thank Roche Diagnostics for providing the sFlt-1 and PlGF tests. Furthermore, we would like to thank B.Y. van der Goes (Obstetric Research Coordination LUMC) and J. Plevier (Medical Information Specialist) for their support during the study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.preghy.2024.101111.

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