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TITLE

sFlt-1/PlGF ratio at 24 weeks gestation in twin pregnancies as a predictor of preeclampsia or fetal growth restriction

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RUNNING TITLE

sFlt-1/PlGF ratio at 24 weeks in twin pregnancies

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MINI-SUMMARY

- **What does this study add to current knowledge?** The present study reveals that an sFlt-1/PlGF ratio ≥17 at 24 weeks in twin pregnancies is associated with a significant increase in the frequency of preeclampsia (odds ratio, 37.13 [95% confidence interval, 4.78 – 288.25]; p=0.002), and FGR (odds ratio, 39.58 [95% confidence interval, 6.31 – 248.17]; p<0.001). Moreover, the addition of maternal characteristics (age, nulliparity, and obesity), and mean pulsatility index of the uterine arteries to the sFlt-1/PlGF ratio at 24 weeks enhance the identification of patients with a twin pregnancy who develop preeclampsia or FGR.
- **What are the main clinical implications?** The sFlt-1/PlGF ratio at 24 weeks in twin pregnancies could select patients who develop preeclampsia or FGR. These patients could benefit from a close follow-up in order to avoid maternal-fetal adverse outcomes.

ABSTRACT

Introduction: the objective was to elucidate if sFlt-1/PlGF ratio at 24 weeks in twin pregnancies could be useful to select patients who subsequently develop diseases related to placental dysfunction, such as preeclampsia or fetal growth restriction (FGR).

Methods: prospective study among all twin pregnancies followed up at a tertiary Hospital. The sFlt-1/PlGF ratio was determined at 24 weeks.

Results: a total of 108 patients with a twin gestation were included. Pregnant women who developed preeclampsia and/or FGR displayed a significantly higher sFlt-1/PlGF ratio at 24 weeks, compared to those who did not develop these diseases (20.3 *vs* 4.3, p=0.002). Mean sFlt-1/PlGF ratio was not significantly different between patients who subsequently developed preeclampsia compared with those that developed FGR (29.8 *vs* 18.45, p=0.42).

An sFlt-1/PlGF ratio ≥17 at 24 weeks is associated with a significant increase in the frequency of preeclampsia (odds ratio, 37.13 [95% confidence interval, 4.78–288.25]; p=0.002), and FGR (odds ratio, 39.58 [95% confidence interval, 6.31–248.17]; p<0.001).

The addition of maternal characteristics, and mean pulsatility index of the uterine arteries to the sFlt-1/PlGF ratio at 24 weeks enhance the identification of patients who develop preeclampsia or FGR.

Conclusion: sFlt-1/PlGF ratio at 24 weeks in twin pregnancies, combined with mean pulsatility index of the uterine arteries and maternal characteristics, could select patients who develop preeclampsia or FGR. These patients might benefit from a close follow-up in order to avoid maternal-fetal adverse outcomes.

INTRODUCTION

Preeclampsia has been classically defined by the new development of hypertension associated with proteinuria from the 20th week of pregnancy [1-7]. It has been recently described that some patients may develop hypertension and other signs or symptoms of preeclampsia in the absence of proteinuria [7-9]. This pregnancy-specific disease affects 2-8% of all gestations and constitutes a foremost cause of maternal and perinatal morbidity and mortality [1-7, 10, 11].

Preeclampsia has been associated with fetal growth restriction (FGR) and placental abruption due to the presence of an underlying placental dysfunction [12-14]. This condition is characterized by a failure of physiological transformation of the myometrial segment of the spiral arteries. Additionally, nontransformed spiral arteries are prone to atherosis, which may further prevent an adequate blood flow to the placenta [6, 14]. Placental dysfunction has been associated with elevated concentrations in the maternal circulation of anti-angiogenic factors released by the dysfunctional hypoxic placenta, such as soluble fms-like tyrosine kinase-1 (sFlt-1), and with reduced bioavailability of proangiogenic factors, such as placental growth factor (PlGF), since it is captured and inhibited by sFlt-1 [13, 14].

The sFlt-1/PlGF ratio has been described as useful to establish the risk of developing preeclampsia among singleton gestations with clinical suspicion of this pregnancy-related disease [15, 16]. Actually, among patients with suspected preeclampsia between 24^{+0}-36^{+6} weeks gestation, sFlt-1/PlGF ratio ≤38 has a negative predictive value within 1 week of 99.3% (95% confidence interval [CI] 97.9-99.9), and a positive predictive value in the subsequent 4 weeks of 36.7% (95%CI, 28.4-45.7) [16]. Moreover, the usefulness of the sFlt-1/PlGF ratio to diagnose preeclampsia has also been shown [17]. A sFlt-1/PlGF ratio >85 detects early-onset

preeclampsia and >110 diagnoses late-onset preeclampsia, with a specificity of 99.5% (95%CI, 97.7-100) and 95.5% (95%CI, 92.9-100), respectively [17]. The cutoff points of ≤38 and >85 of the sFlt-1/PlGf ratio have also been used to assess the risk of FGR and to diagnose FGR, respectively [18].

In uneventful twin pregnancies, the reference ranges for the sFlt-1/PlGF ratio below 29 weeks gestation are similar to those of singleton pregnancies, being higher in twin pregnancies thereafter [19]. The sFlt-1/PGF ratio ≤38 to predict the absence of preeclampsia is not applicable to twin pregnancies [20]. Furthermore, in twin pregnancies with preeclampsia, the cutoff value of sFlt-1/PlGF ratio 85 established for the detection of this disease in singleton pregnancies associates a sensitivity of 83.3% and a specificity of 80.6% [21]. However, a cutoff 53 has a sensitivity of 94.4% and a specificity of 74.2% for the diagnosis of preeclampsia in twin pregnancies [21].

Patients with twin pregnancies are twice prone to develop preeclampsia compared to those with singleton pregnancies [19, 22, 23]. It has been reported that sFlt-1/PlGF ratio may be helpful to predict selective FGR (sFGR) in twin pregnancies with preeclampsia and/or HELLP [24]. Nevertheless, there is a lack of data regarding sFlt-1/PlGF ratio in uneventful twin pregnancies to predict the development of diseases related to placental dysfunction. Thus, we ought to elucidate if the determination of sFlt-1/PlGF ratio at 24 weeks gestation in twin pregnancies could be useful to select those patients at risk of subsequent development of diseases related to placental dysfunction, such as preeclampsia or fetal growth restriction.

MATERIALS AND METHODS

This was a prospective study among all twin pregnancies at 24 weeks gestation that were followed up at the University and Polytechnic Hospital La Fe (Valencia, Spain), from

January 2018 to August 2021. Given that the threshold of periviable birth has been classically considered 24 weeks [25], this gestational age was selected to determine the sFlt-1/PlGF ratio. The study was approved by the Ethics Committee of the Health Research Institute Hospital La Fe (IIS La Fe). All pregnant women signed the informed consent before participation. The sFlt-1/PlGF ratio was determined at 24 weeks gestation in an outpatient setting through Elecsys® immunoassay sFlt-1/PlGF ratio (Roche Diagnostics, Basel, Switzerland). Serum samples were immediately analyzed (<6 hours) after the collection. Data of patients during pregnancy and delivery were collected from the digital clinical history of the Hospital.

All patients included in the study were 18 years of age or older. Women who already had a diagnosis of preeclampsia, HELLP syndrome, or FGR prior to 24 weeks gestation were not included. Preeclampsia was defined by new-onset hypertension (repeated measurement of systolic blood pressure ≥140 mmHg and diastolic blood pressure ≥90 mmHg) after 20 weeks of pregnancy and the coexistence of one or both of the following new-onset conditions: proteinuria (urine protein:creatinine ratio ≥30 mg/mmol, or albumin:creatinine ratio ≥8 mg/mmol, or ≥1 g/L [2+] on dipstick testing, or 300 mg protein in a 24-hour urine collection), or other maternal organ dysfunction, including renal, liver, neurological or haematological complications, or uteroplacental dysfunction (such as fetal growth restriction, abnormal umbilical artery Doppler waveform analysis, or stillbirth) [6, 9, 16, 21]. HELLP syndrome was characterized by hemolysis (increased lactate dehydrogenase concentrations >2 upper reference threshold), elevated liver-enzyme levels (alanine aminotransferase, aspirate aminotransferase concentrations >2 upper reference threshold), and low platelet counts (<100.000/µl) [16, 21]. FGR was determined by the combination of an estimated fetal weight (EFW) <10th centile with either an abnormal Doppler of the umbilical artery, cerebroplacental

ratio, uterine arteries, or an EFW <3rd centile [26]. The EFW from the measurements of head circumference, abdominal circumference as well as femoral length was derived from the formula reported by Hadlock *et al.* [27]. FGR was classified as either of both twins or selective if it was present in only one twin. sFGR was defined when a fetus had an EFW <10th centile and the intertwin EFW discordance was >25% [28]. The 95th centile of the mean pulsatility index of uterine arteries at 24 weeks was defined as ≥ 1.35, as previously described [29].

Statistics

R version 4.0.3 (The R Foundation for Statistical Computing) has been used for the statistical analysis. Quantitative data were shown as means and standard deviations, while categorical data were presented as absolute and relative frequencies. Comparisons between the characteristics of the groups were performed using student's t-test or Kruskal-Wallis test for continuous variables, and Fisher's exact testing for categorical variables. The sensitivity and specificity for different thresholds of marker levels were calculated and used to estimate the receiver-operating characteristics (ROC) curves and assess the performance of the predictive variables. Logistic regression was used to include two or more variables in the models, and the predicted probabilities of each outcome were used to assess the area under the ROC curve.

RESULTS

A total of 108 patients with a twin gestation were included in the study. All patients signed the informed consent and underwent a subsequent follow-up of the pregnancy as well as a delivery at the tertiary University and Polytechnic Hospital La Fe (Valencia, Spain). In these 108 twin pregnancies, sFlt-1/PlGF ratio was determined at 24 weeks gestation. Data of the baseline characteristics of the study population, comparing pregnant women that did not

develop preeclampsia and/or FGR with those who developed these diseases, are displayed in Table 1. No significant differences were found between groups.

Data regarding maternal characteristics during pregnancy and perinatal outcomes, of twin gestations that developed preeclampsia and/or FGR compared to those who did not develop these diseases, are presented in Table 2. Pregnant women who developed preeclampsia and/or FGR displayed a significantly higher sFlt-1/PlGF ratio at 24 weeks gestation, compared to those who did not develop these diseases related to placental dysfunction (shown in Fig. 1). The mean pulsatility index of uterine arteries at 24 weeks was not statistically different between groups. sFlt-1/PlGF displayed a weak correlation with the mean pulsatility index of uterine arteries (Pearson's correlation coefficient r=0.1355). Additionally, the gestational age at delivery as well as the newborns' weight were significantly lower in patients with preeclampsia and/or FGR compared with pregnant women who did not develop these diseases.

Data of patients who developed preeclampsia (5 out of 108, 4.6%) and/or FGR (11 out of 108, 10.2%) are shown in Supplementary Table 1. Two patients developed early preeclampsia (≤34 weeks), one of them with associated sFGR. Three patients developed late preeclampsia, one of them with a prior diagnosis of early sFGR. The mean sFlt-1/PlGF ratio at 24 weeks was not significantly different between patients with twin pregnancies who subsequently developed early preeclampsia, compared with those who developed late preeclampsia (61 *vs* 9, p=0.11). Regarding FGR, 8 pregnant women developed early sFGR (≤34 weeks), 2 patients displayed early FGR of both fetuses, and only 1 patient developed late sFGR. The mean sFlt-1/PlGF ratio at 24 weeks in patients with twin pregnancies who subsequently developed early FGR was 20.1, compared with the sFlt-1/PlGF ratio of 2 of the patient who developed late sFGR. In addition, the mean sFlt-1/PlGF ratio at 24 weeks was not

significantly different between patients with twin pregnancies who subsequently developed preeclampsia compared with those that developed FGR (29.8 *vs* 18.45, p=0.42).

A cutoff sFlt-1/PlGF ratio ≥17 at 24 weeks is associated with a sensitivity of 60% & 45%, a specificity of 96% & 98%, a positive predictive value (PPV) of 42.9% & 71.4%, and a negative predictive value (NPV) of 98.0% & 94.1%, for the identification of patients with a twin pregnancy who develop preeclampsia or FGR, respectively. Accordingly, the sFlt-1/PlGF ratio ≥17 at 24 weeks is associated with a significant increase in the frequency of preeclampsia (odds ratio, 37.13 [95% confidence interval, 4.78 – 288.25]; p=0.002), and FGR (odds ratio, 39.58 [95% confidence interval, 6.31 – 248.17]; p<0.001). The mean pulsatility index of the uterine arteries ≥1.35 at 24 weeks is associated with an increase in the frequency of preeclampsia (odds ratio, 2.69 [95% confidence interval, 0.27 – 27.02]; p=0.263) and FGR (odds ratio, 5.13 [95% confidence interval, 1.07 – 24.50]; p=0.019).

Neither sFlt-1 or PlGF values separately, nor mean pulsatility index of the uterine arteries at 24 weeks gestation in twin pregnancies, associate a higher predictive power for the subsequent development of preeclampsia and/or FGR, compared with the sFlt-1/PlGF ratio (shown in Fig. 2-3). Although the addition of the mean pulsatility index of the uterine arteries to the sFlt-1/PlGF ratio at 24 weeks gestation does not improve the identification of patients that develop preeclampsia, it slightly improves the selection of patients who develop FGR. Ultimately, the addition of maternal characteristics (age, nulliparity, and obesity), and mean pulsatility index of the uterine arteries to the sFlt-1/PlGF ratio at 24 weeks clearly enhance the identification of patients with twin pregnancies who develop preeclampsia or FGR (shown in Fig. 4).

DISCUSSION

The present study reveals that an sFlt-1/PlGF ratio ≥17 at 24 weeks in twin pregnancies is associated with a significant increase in the frequency of preeclampsia (odds ratio, 37.13 [95% confidence interval, 4.78 – 288.25]; p=0.002), and FGR (odds ratio, 39.58 [95% confidence interval, 6.31 – 248.17]; p<0.001). The cutoff point of 17 of the sFlt-1/PlGF ratio in twin pregnancies at 24 weeks is in line with prior findings of De La Calle *et al.*, who have described the 5th percentile, median, and 95th percentile of the sFlt-1/PlGF ratio in uneventful twin pregnancies at 24^{+0}-28^{+6} weeks gestation of 1.33, 3.88 and 19.0 respectively [19]. Furthermore, in twin pregnancies between 28^{+0} and 30^{+6} weeks, an sFlt-1/PlGF ratio ≤22.2 has been associated with the absence of preeclampsia within four weeks [30].

In singleton pregnancies with high-risk of preeclampsia or FGR, defined by maternal history and second-trimester uterine artery Doppler, the sFlt-1/PlGF ratio at 24-28 weeks provides an accurate prediction of preeclampsia or FGR [31]. Actually, the sFlt-1/PlGF ratio associates an AUC with a 95% CI of 0.98 (0.97-1.00) for the detection of early preeclampsia or FGR, and the ratio >95th centile shows a sensitivity of 100% (95%CI, 78.5-100) and a specificity of 80.6% (95%CI, 75.0-85.2) [31]. Patients with twin pregnancies are twice as likely to develop preeclampsia, compared with women with singleton pregnancies [19, 22, 23]. Thus, the determination of sFlt-1/PlGF ratio at 24 weeks gestation in twin pregnancies could enhance the prediction of preeclampsia or FGR, diseases associated with placental dysfunction. The present study reveals an AUC of the sFlt-1/PlGF ratio ≥17 at 24 weeks in twin gestations for the detection of preeclampsia or FGR of 0.814 and 0.759, respectively. Due to the specificity of 96% and 98% of the sFlt-1/PlGF ratio ≥17 at 24 weeks in twin pregnancies to detect preeclampsia or FGR, respectively, patients at risk of developing these diseases could benefit from a close follow-up in order to avoid an adverse pregnancy outcome. As a matter of fact, it has been described a positive correlation between sFlt-1/PlGF ratio and the likelihood of

pregnancy complications [32, 33]. Nonetheless, the sensitivity of 60% and 55% for detecting preeclampsia or FGR, respectively, could lead to an elevated requirement of health resources.

Interestingly, the addition of mean pulsatility index of the uterine arteries to sFlt-1/PlGF ratio at 24 weeks gestation at the present study does not improve the sensitivity or specificity to identify patients with a twin pregnancy who subsequently develop preeclampsia. Nevertheless, the identification of patients that develop FGR is slightly improved with the addition of the mean pulsatility index of the uterine arteries to sFlt-1/PlGF ratio at 24 weeks. It has been previously reported an improvement of the detection of early preeclampsia and FGR with the addition of the mean uterine artery pulsatility index and the sFlt-1/PlGF ratio [31, 34]. Actually, the mean uterine artery pulsatility index at 20 weeks associates a 60-80% sensitivity and 90-95% specificity for the detection of early preeclampsia and FGR, but with PPV of 10-20% [31, 35, 36]. The sFlt-1/PlGF ratio provides optimal PPV in selected populations [16, 31, 37]. As previously reported, the addition of maternal risk factors to develop diseases related to placental dysfunction (age, nulliparity, and obesity) [6, 7, 38-40], and mean pulsatility index of the uterine arteries to sFlt-1/PlGF ratio at 24 weeks enhance the identification of patients with twin pregnancies who develop preeclampsia or FGR.

Our study has also shown that the mean sFlt-1/PlGF ratio is not significantly different at 24 weeks gestation between patients with twin pregnancies who develop preeclampsia compared with those that develop FGR (29.8 *vs* 18.45, p=0.42). This is concordant with the findings of Herraiz *et al.*, who elegantly showed that singleton pregnancies with preeclampsia or FGR displayed a no significantly different elevated maternal sFlt-1/PlGF ratio [41]. The underlying placental dysfunction of both preeclampsia and FGR is responsible for the elevated concentrations in the maternal circulation of the anti-angiogenic sFlt-1, and with reduced bioavailability of the proangiogenic PlGF [41]. sFlt-1/PlGF ratio is particularly higher in

pregnancies with early-onset preeclampsia or FGR, compared with those with a later onset. In the present study, the mean sFlt-1/PlGF ratio at 24 weeks gestation was higher in patients with twin pregnancies who subsequently developed early preeclampsia or FGR, compared with those who developed late preeclampsia or FGR, although the difference was not statistically significant (61 *vs* 9, p=0.11).

Regarding mode of conception, it has been previously described that singleton pregnancies conceived through *in vitro* fertilization display an increased antiangiogenic profile (elevated sFlt-1 and reduced PlGF) throughout gestation compared with spontaneously conceived pregnancies, even when controlled for preeclampsia [42]. Notwithstanding approximately half of the twin pregnancies included in the present study were conceived by assisted reproductive techniques, these gestations did not display an elevated sFlt-1/PlGF ratio at 24 weeks compared with spontaneously conceived twin pregnancies (5.73 *vs* 6.90, p=0.30). Similarly, twin pregnancies conceived specifically by *in vitro* fertilization did not show a higher sFlt-1/PlGF ratio compared with twin pregnancies conceived spontaneously or through artificial insemination (5.39 *vs* 6.98, p=0.32). Thus, further studies are required to shed light on the influence of the mode of conception of twin pregnancies on their angiogenic markers.

The main strength of the study is the addition of information to the scarce data of the sFlt-1/PlGF ratio in uneventful twin pregnancies at 24 weeks to identify patients at risk to develop preeclampsia and/or FGR. Limitations of the study include the restricted sample size of patients with a twin gestation, the reduced number of patients with preeclampsia or FGR, as well as the scant incidence of preeclampsia in our area. At the study Hospital, the reported incidence of preeclampsia is below 2% in singleton gestations, and the present study has revealed an incidence of 4.6% in twin pregnancies. These limitations may be responsible for

the lack of improvement of the sensitivity or specificity to identify patients who subsequently develop preeclampsia with the addition of the mean pulsatility index of the uterine arteries to the sFlt-1/PlGF ratio at 24 weeks gestation. The abovementioned limitations may be also responsible for the no significantly different mean sFlt-1/PlGF ratio at 24 weeks in patients with twin pregnancies who develop early *versus* late preeclampsia or FGR. Another constraint of the study due to the restricted sample size is that twin pregnancies that develop sFGR do not show a different sFlt-1/PlGF ratio at 24 weeks compared with gestations that develop FGR of both fetuses (20.7 *vs* 8.5, p=0.48). An additional limitation is that patients included in the study did not undergo a first trimester combined screening test to detect the risk of early-onset preeclampsia, and those patients with a potential high risk did not receive acetylsalicylic acid (150 mg daily, initiated before 16 weeks, given at night) to prevent the disease [43].

Future studies are needed to elucidate whether the combined first trimester screening test for early-onset preeclampsia in twin pregnancies, followed by an assessment at 24 weeks with the sFlt-1/PlGF ratio, the mean pulsatility index of the uterine arteries and maternal characteristics, further improves the selection of patients at risk of developing the disease. Additionally, supplementary studies are required to assess if monochorionic and dichorionic twin pregnancies display a similar sFlt-1/PlGF ratio throughout both uneventful gestations and those with preeclampsia or FGR. Further studies could also clarify whether twin pregnancies that develop sFGR display a dissimilar sFlt-1/PlGF ratio compared to those that develop FGR in both fetuses.

This study shows that the sFlt-1/PlGF ratio ≥17 at 24 weeks in twin pregnancies is associated with a significant increase in the frequency of preeclampsia and FGR. The sFlt-1/PlGF ratio at 24 weeks in twin pregnancies, combined with the mean pulsatility index of the uterine arteries and maternal characteristics, could select patients at risk to develop diseases

related to placental dysfunction, such as preeclampsia or FGR. These patients might benefit from a close follow-up in order to avoid maternal-fetal severe adverse outcomes.

STATEMENT OF ETHICS

This study protocol was reviewed and approved by the Ethics Committee of the Health Research Institute Hospital La Fe (IIS La Fe), approval *Ref: 2018/0164, P.I. Exp. 2018_0164_PP_DE_DIAGO*. Written informed consent was obtained from each patient before her inclusion in the study.

CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to declare.

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No funding has been received to carry out this study.

AUTHOR CONTRIBUTIONS

Alicia Martínez-Varea and Vicente Diago-Almela have contributed to the conception and design of the work. Alicia Martínez-Varea, Sagrario Monfort-Pitarch, Julia Desco-Blay, María Hueso, and Vicente Diago-Almela have contributed to the inclusion of patients in the study and the clinical management of the patients. Alicia Martínez-Varea and Clara Martínez-Sáez have contributed to the acquisition of data for the study. Josep Domenech, Clara Martínez-Sáez, Vicente Diago-Almela, and Alicia Martínez-Varea have analyzed and interpreted data for the work. Alicia Martínez-Varea has drafted the work. Alicia Martínez-Varea, Clara Martínez-Sáez, Josep Domenech, Sagrario Monfort-Pitarch, Julia Desco-Blay,

María Hueso, and Vicente Diago-Almela have critically revised the manuscript and have given the final approval of the version to be published.

DATA AVAILABILITY STATEMENT

The data in this study was obtained from the clinical program of La Fe University and Polytechnic Hospital. Further enquiries can be directed to the corresponding author.

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Fig. 1. sFlt-1/PlGF ratio at 24 weeks gestation between patients who do not develop preeclampsia and/or fetal growth restriction, compared to those who develop these pregnancy-specific diseases related to placental dysfunction. Fig. 1a) All twin pregnancies (n=108).

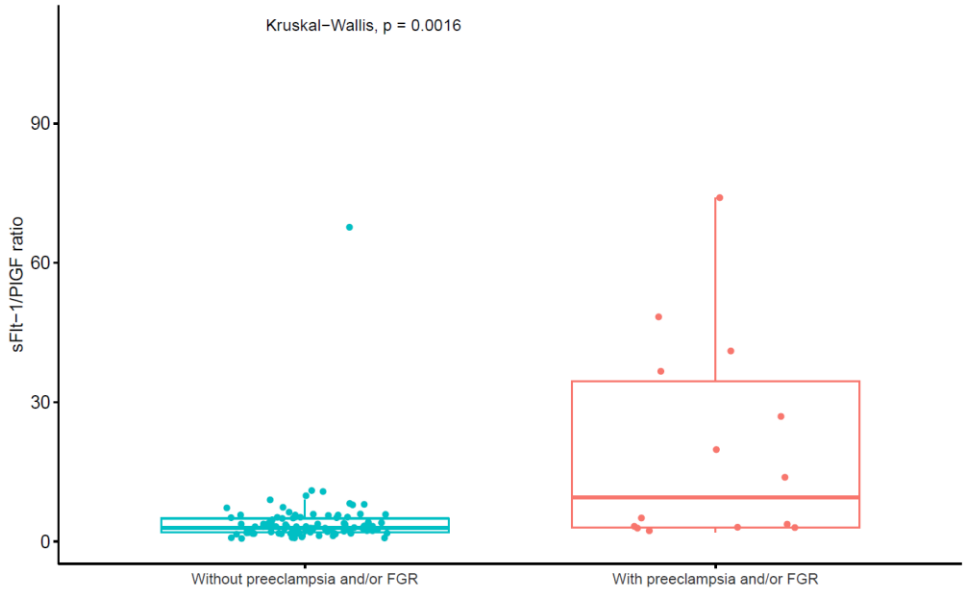


Fig. 1b) Comparison between monochorionic (n=21) and dichorionic (n=87) twin pregnancies.

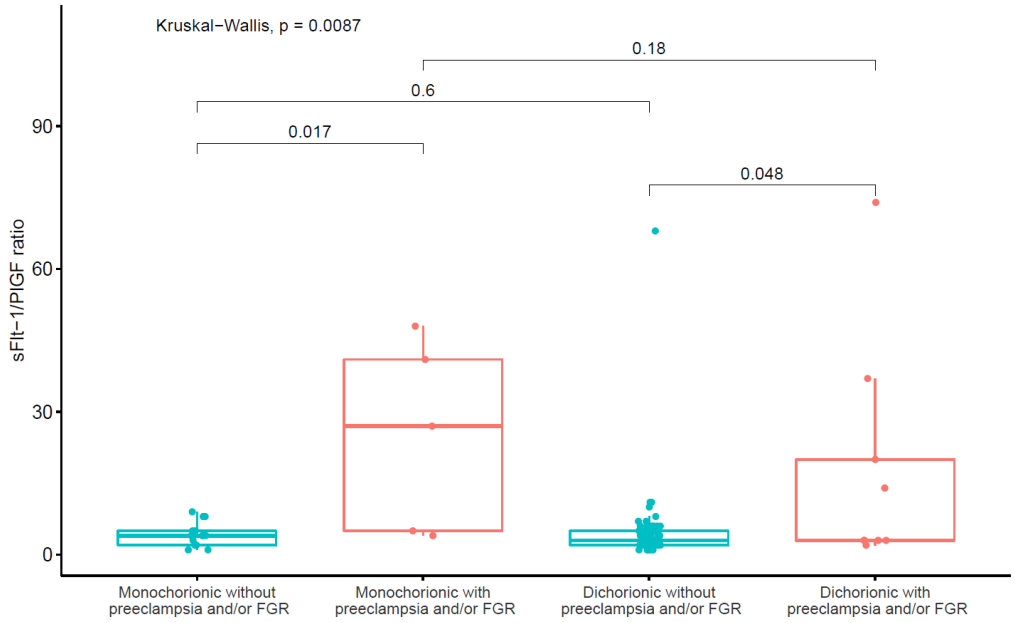


Fig. 2. Area under the curve of the sFlt-1/PlGF ratio at 24 weeks gestation for the detection of patients with twin pregnancies who subsequently develop preeclampsia.

Mean pulsatility index of the uterine arteries (Mean PI UtA).

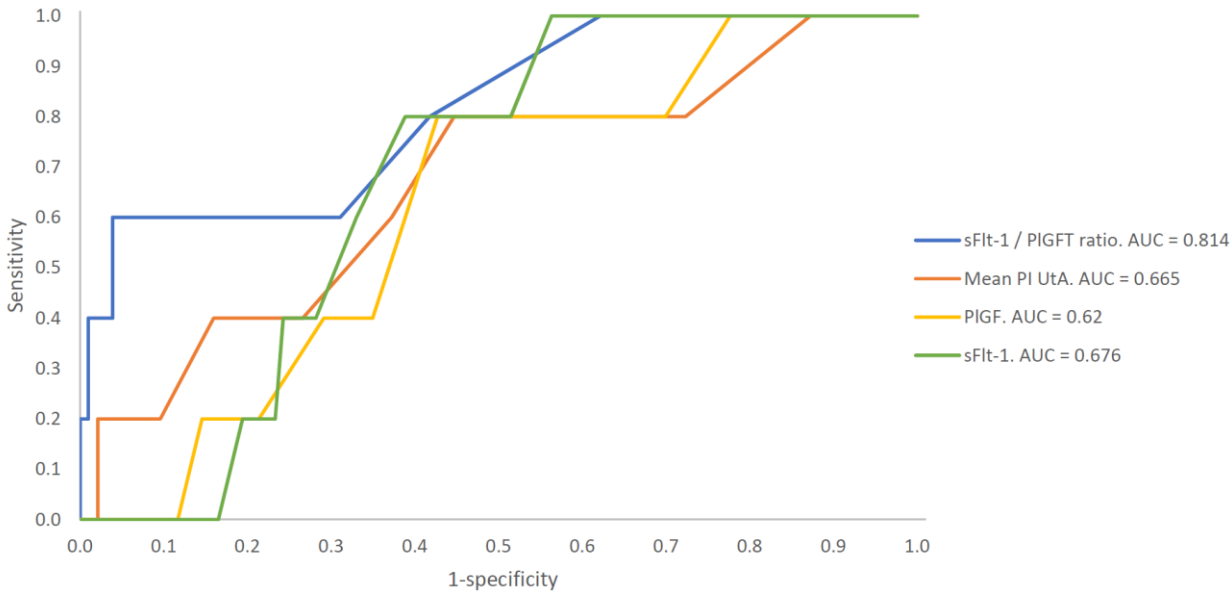


Fig. 3. Area under the curve of the sFlt-1/PlGF ratio at 24 weeks gestation for the detection of patients with twin pregnancies who subsequently develop fetal growth restriction (FGR). Mean pulsatility index of the uterine arteries (Mean PI UtA).

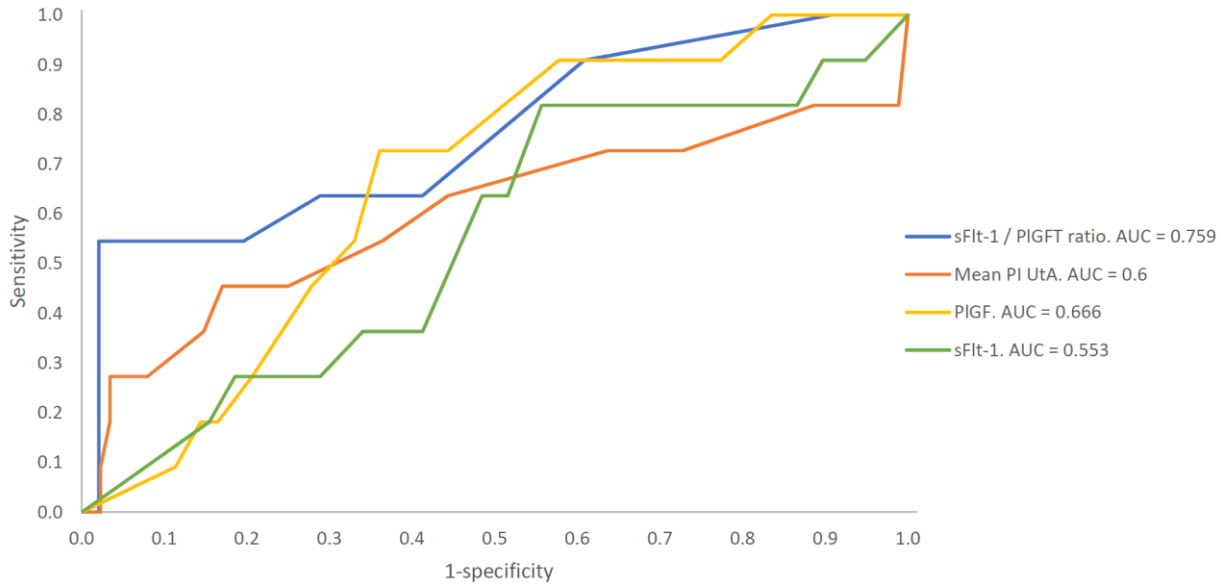
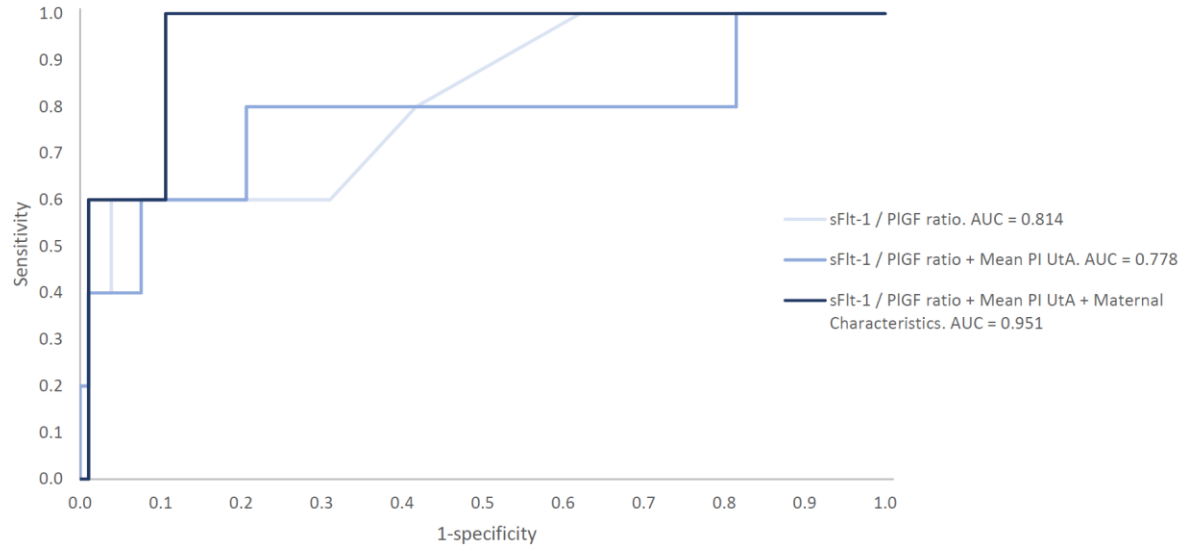
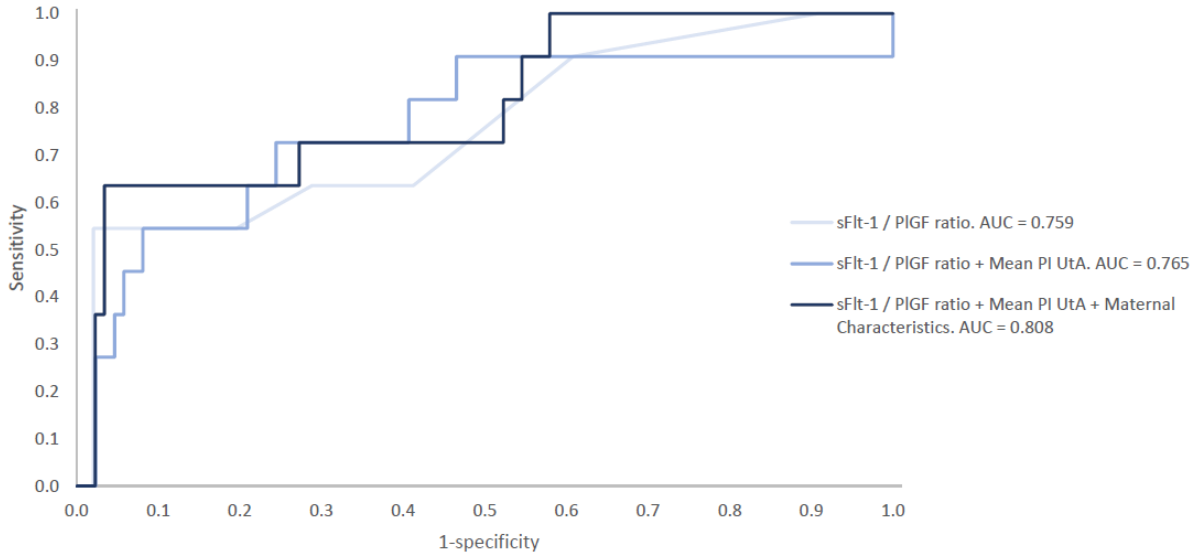


Fig. 4. Area under the curve of the sFlt-1/PlGF ratio at 24 weeks in twin pregnancies with the addition of the mean pulsatility index of the uterine arteries, as well as maternal age, nulliparity, and obesity (body max index ≥30), for the detection of patients who subsequently develop (Fig. 4a) preeclampsia or (Fig. 4b) fetal growth restriction (FGR).





Supplementary Table 1. Maternal and perinatal outcomes of patients with twin pregnancies who developed preeclampsia and/or FGR, both pregnancy-specific diseases related to placental dysfunction.

Patient	Maternal age (years)	Nulliparity	Chorionicity and amnionicity	Mode of conception	BMI	First trimester bHCG mUI/mL	First trimester PAPPA mUI/mL	sFlt-1/PlGF at 24 weeks gestation	Mean pulsatility index of uterine arteries at 24 weeks gestation	Cervical length at 24 weeks gestation (mm)	Diseases during pregnancy	Gestational age at diagnosis of diseases (weeks^{+days})	Gestational age at delivery (weeks $^{+}$ ^{days})	Mode of delivery	Gender of first newborn	Weight of first newborn (grams)	pH of umbilical artery of first newborn	Gender of second newborn	Weight of second newborn (grams)	pH of umbilical artery of second newborn
1	38	Yes	Dichorionic diamniotic	Spontaneous	27,9	91,6	3,2	3	1,54	44	• sFGR	• 24^{+1}	37^{+0}	Cesarean section	Female	2370	7,28	Female	1700	7,27
2	40	Yes	Dichorionic diamniotic	IVF-ED	na	188	7,54	20	0,89	32,5	• Gestational diabetes • sFGR • Preeclampsia	• 22^{+5} • 27^{+3} • 35^{+0}	35^{+0}	Cesarean section	Female	1580	7,21	Male	2080	7,20
3	30	Yes	Dichorionic diamniotic	Spontaneous	na	81,7	9	74	0,99	44	• Preeclampsia and threatened preterm labor	• 33^{+5}	35^{+1}	Cesarean section	Male	2160	7,35	Male	2095	7,42
4	38	Yes	Dichorionic diamniotic	IVF	27	48,9	1,81	2	0,355	36	• sFGR	• 36^{+0}	36^{+2}	Cesarean section	Male	2490	7,36	Female	2205	7,27
5	34	No	Dichorionic diamniotic	Spontaneous	23	na	na	14	0,325	45	• FGR	• 29^{+1}	37^{+4}	Cesarean section	Male	2080	7,34	Male	1960	7,33
6	41	Yes	Dichorionic diamniotic	IVF-ED	27,5	120	16,9	3	1,22	33	• sFGR	• 34^{+5}	37^{+3}	Vaginal delivery	Male	2000	7,31	Female	2550	7,28
7	32	Yes	Monochorionic diamniotic	Spontaneous	19	24,2	4,43	27	0,57	24	• sFGR • Threatened preterm labor	• 25^{+1} • 30^{+0}	32^{+1}	Cesarean section	Female	1640	7,32	Female	880	7,24
8	34	Yes	Dichorionic	AI	21.5	na	na	37	1,14	41	• sFGR	• 24^{+3}	31^{+2}	Cesarean	Male	1645	7,37	Male	1195	7,36

			diamniotic								• PPROM	• 30^{+4}		section						
9	38	Yes	Dichorionic diamniotic	IVF	27	29,8	6,74	3	1,25	39	• Gestational diabetes • Preeclampsia	• 28^{+0} • 36^{+2}	36^{+3}	Cesarean section	Male	2285	7,38	Male	2200	7,30
10	43	Yes	Monochorionic diamniotic	IVF	26,2	226	5,92	4	0,5	45	• Preeclampsia • Cholestasis	• 35^{+6} • 34^{+6}	35^{+6}	Cesarean section	Male	2340	7,35	Male	2035	7,37
11	24	Yes	Monochorionic diamniotic	Spontaneous	23	na	na	41	1,61	38	• sFGR	• 25^{+3}	31^{+0}	Cesarean section	Female	1375	7,28	Female	950	7,26
12	39	No	Monochorionic diamniotic	Spontaneous	21	88,7	4,49	48	1,79	29	• Preeclampsia and sFGR	• 28^{+5}	29^{+3}	Cesarean section	Male	1290	7,31	Male	910	7,23
13	37	No	Monochorionic diamniotic	Spontaneous	20,2	141	14,3	5	0,74	30	• Covid-19 • sFGR	• 20^{+2} • 28^{+3}	36^{+0}	Cesarean section	Female	1765	7,38	Female	2085	7,37
14	36	Yes	Dichorionic diamniotic	IVF	27	86	3,76	3	0,92	33	• Gestational diabetes • FGR • Threatened preterm labor	• 26^{+3} • 26^{+3} • 26^{+3}	36^{+1}	Cesarean section	Female	2450	7,34	Female	1875	7,29

Data are given as mean (standard deviation) for continuous variables and n (%) for categorical variables. Statistical analysis was made comparing cases and controls.

bHCG: beta-subunit of human chorionic gonadotropin; PAPPA: pregnancy associated plasma protein A; FGR: fetal growth restriction of both twins; sFGR: selective FGR of one twin; IVF: *in vitro* fertilization; IVF-ED: *in vitro* fertilization with egg donation; AI: artificial insemination; na: data no available; PPROM: preterm premature rupture of membranes.