

ASSOCIATION OF MATERNAL ANGIOGENIC MARKERS WITH ADVERSE NEONATAL OUTCOMES IN PREGNANCIES WITH ESTIMATED FETAL WEIGHT BELOW THE 10TH PERCENTILE

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Abstract

Objective: To evaluate the association between maternal angiogenic markers and neonatal outcomes in pregnancies with an estimated fetal weight (EFW) below the 10th percentile.

Methods: This prospective study included 95 singleton pregnancies with EFW below the 10th percentile. Maternal serum placental growth factor (PlGF) and soluble fms-like tyrosine kinase-1 (sFlt-1) were measured, and the sFlt-1/PlGF ratio was calculated. Neonatal outcomes included birth weight, respiratory distress syndrome (RDS), neonatal sepsis, intraventricular hemorrhage (IVH), neonatal death, and duration of neonatal intensive care unit (NICU) hospitalization. Associations were assessed using Spearman's rank correlation.

Results: PlGF was positively correlated with birth weight ($\rho = 0.5654$, $p < 0.001$), whereas sFlt-1 ($\rho = -0.4644$, $p < 0.001$) and the sFlt-1/PlGF ratio ($\rho = -0.5693$, $p < 0.001$) were negatively correlated with birth weight. RDS, neonatal sepsis, and IVH were significantly associated with lower PlGF and higher sFlt-1 concentrations and sFlt-1/PlGF ratios. Lower PlGF levels were weakly associated with neonatal mortality ($\rho = -0.2159$, $p = 0.036$), while sFlt-1 and the sFlt-1/PlGF ratio were not. No significant correlations were observed with NICU hospitalization duration.

Conclusion: Maternal angiogenic imbalance was associated with lower birth weight and increased neonatal morbidity in pregnancies with fetal growth below the 10th percentile. Angiogenic biomarkers may complement conventional fetal assessment in identifying pregnancies at increased risk of adverse neonatal outcomes.

Keywords: fetal growth restriction; small for gestational age; PlGF; sFlt-1; sFlt-1/PlGF ratio; neonatal outcomes

Introduction

Fetal growth restriction (FGR) is characterized by impaired fetal growth resulting from the fetus's inability to achieve its genetically determined growth potential during intrauterine life. It is one of the leading causes of perinatal morbidity and mortality and remains a major challenge in contemporary obstetric practice due to its association with stillbirth, neonatal complications, and adverse long-term health outcomes (1,2,3).

The etiology of FGR is multifactorial. The condition most commonly results from placental insufficiency but may also be associated with fetal chromosomal or structural abnormalities, congenital infections, and maternal exposure to harmful substances or underlying maternal diseases (4). Regardless of the underlying cause, impaired placental function is the predominant mechanism responsible for the majority of FGR cases (5).

A fetus is considered small for gestational age (SGA) when its estimated fetal weight (EFW) is below the 10th percentile for gestational age. However, not all small fetuses are growth-restricted. SGA fetuses have an EFW below the 10th percentile with normal fetal and placental Doppler findings and are generally considered constitutionally small. In contrast, fetal growth restriction describes a pathological condition in which fetal growth is impaired and is accompanied by Doppler evidence of placental or fetal circulatory compromise, reflecting placental dysfunction.

Traditionally, SGA fetuses were regarded as constitutionally small and at relatively low risk for adverse outcomes. Increasing evidence, however, indicates that even fetuses with an SGA below the 10th percentile are at a significantly higher risk of perinatal morbidity and mortality than appropriately grown fetuses (6,7). Population-based studies have demonstrated that the lowest risk of perinatal death occurs among fetuses with birth weights between the 70th and 90th percentiles, whereas the risk increases progressively below the 60th percentile (6). Furthermore, large cohort studies have shown a gradual rise in stillbirth rates among fetuses with estimated fetal weight below the 25th percentile, with fetuses below the 10th percentile being at an approximately five-fold higher risk of intrauterine fetal death compared with those with appropriate growth (8,9).

Accurate identification of fetuses at increased risk of adverse perinatal outcomes is essential for optimal pregnancy management. Current assessment strategies combine ultrasound estimation of fetal size, Doppler evaluation of the uteroplacental and fetoplacental circulation, and, increasingly, maternal serum biomarkers that reflect placental function (10,11). These methods aim to distinguish constitutionally small fetuses from those with pathological growth restriction who require closer surveillance and timely delivery.

Currently, there is no effective treatment that can reverse fetal growth restriction once placental insufficiency has developed (10,11). Therefore, the cornerstone of management is careful fetal surveillance and determining the optimal timing of delivery (12). This decision requires balancing the risks of prematurity due to early delivery against the increasing risk of fetal hypoxia, acidosis, and intrauterine fetal death with prolonged pregnancy.

Among the emerging biomarkers of placental dysfunction, placental growth factor (PlGF) and soluble fms-like tyrosine kinase-1 (sFlt-1) have received considerable attention. Maternal serum concentrations of these angiogenic markers, particularly the sFlt-1/PlGF ratio, correlate with the severity of placental insufficiency and the clinical manifestations of placental disease, including preeclampsia and fetal growth restriction (13). Consequently, these biomarkers may provide valuable information for identifying pregnancies at increased risk of adverse neonatal outcomes and for improving risk stratification in fetuses with estimated fetal weight below the 10th percentile.

Materials and Methods

A prospective observational cohort study was conducted at the University Clinic for Gynecology and

Obstetrics, Skopje. The study population consisted of 95 pregnant women carrying a singleton fetus with an ultrasound-estimated fetal weight (EFW) below the 10th percentile for gestational age.

Participants were enrolled according to predefined eligibility criteria. Women were eligible if they were aged ≥ 18 years, had an EFW below the 10th percentile, were at $\geq 24+0$ weeks of gestation at the time of assessment, were available for follow-up through delivery and neonatal outcome assessment, and provided written informed consent for participation. Women were excluded if they had preeclampsia diagnosed before enrollment, a structural fetal anomaly diagnosed either antenatally or after birth, a multiple pregnancy, a confirmed chromosomal or genetic abnormality, or were in the active phase of labor at enrollment. Pregnancies in which the newborn had a birth weight above the 10th percentile were also excluded from the final analysis.

For each participant, clinical information was recorded using a standardized data collection form. Maternal demographic characteristics included age, weight, and height. Obstetric history included the number and the outcomes of previous pregnancies and a history of preeclampsia, eclampsia, HELLP syndrome, or fetal growth restriction in previous pregnancies. Current pregnancy data included the last menstrual period, gestational age, and number of fetuses.

Gestational age was determined by first-trimester ultrasound dating using crown–rump length (CRL) measurements obtained between 8+0 and 13+6 weeks of gestation (14). For women without available first-trimester ultrasound measurements, gestational age was determined using head circumference (HC) measurements obtained between 14+0 and 21+6 weeks of gestation when the discrepancy between menstrual dating and fetal biometric assessment exceeded 10 days (15).

All participants underwent a standardized ultrasound examination for fetal biometric assessment and evaluation of maternal and fetoplacental Doppler blood flow. Examinations were performed transabdominally using a 4–6 MHz probe on Voluson E8, Voluson E10, or Voluson E6 ultrasound systems (GE HealthCare), with compatible software platforms. Fetal biometry comprised measurements of the biparietal diameter (BPD), head circumference (HC), abdominal circumference (AC), and femur length (FL). All biometric measurements were obtained in accordance with standardized ISUOG recommendations (15). EFW was calculated using the Hadlock formula (16) and expressed as a percentile according to the Hadlock fetal growth reference (17).

Doppler assessment included the pulsatility index (PI) of the umbilical artery (UA), the pulsatility index of the middle cerebral artery (MCA), and the cerebroplacental ratio (CPR), calculated as the MCA PI divided by the UA PI. Doppler measurements were obtained in accordance with the ISUOG recommendations for Doppler assessment in obstetrics (18).

Maternal venous blood samples were collected and processed in the Biochemical Laboratory of the University Clinic for Gynecology and Obstetrics. Maternal serum concentrations of soluble fms-like tyrosine kinase-1 (sFlt-1) and placental growth factor (PlGF), together with the sFlt-1/PlGF ratio, were determined using a fully automated electrochemiluminescence immunoassay (ECLIA) platform (Cobas e 411).

The timing and mode of delivery were determined based on the clinical condition of the mother and fetus and in accordance with established recommendations for the surveillance and management of

pregnancies complicated by fetal growth restriction. Indications for delivery included significant abnormalities in fetal or placental Doppler parameters, cardiotocographic evidence suggestive of fetal compromise, a positive fetal stress test, a biophysical profile score <4 , and oligohydramnios.

The neonatal outcomes assessed included birth weight, duration of neonatal intensive care unit (NICU) stay, respiratory distress syndrome (RDS), neonatal sepsis, intraventricular hemorrhage (IVH), and neonatal death. RDS was defined as respiratory morbidity requiring respiratory support with supplemental oxygen for at least 4 hours, including cases diagnosed as transient tachypnea of the newborn.

Statistical Analysis

Statistical analysis of the data obtained from the study was performed using the Statistical Package for the Social Sciences (SPSS Inc., Chicago, Illinois), version 25.0. The Shapiro–Wilk W test was used to assess data distribution. Qualitative variables are presented as absolute and relative frequencies. Quantitative variables are presented as mean, minimum and maximum values, standard deviation, median, and interquartile range. Spearman's rank correlation coefficient was used to analyze the correlation between placental angiogenic markers and selected variables. Statistical significance was defined as $p < 0.05$.

Results

A total of 95 pregnant women from the University Clinic for Gynecology and Obstetrics in Skopje were included in the study. All participants were carrying a fetus with an ultrasound-estimated fetal weight below the 10th percentile. Maternal age ranged from 18 to 56 years, with a mean age of 28.9 ± 6.1 years and a median of 28 years (IQR = 7). Body mass index (BMI) ranged from 17.6 to 41 kg/m², with a mean of 28.2 ± 5.4 kg/m² and a median of 27 kg/m² (IQR = 6.6). The cohort was predominantly composed of pregnancies complicated by late-onset fetal growth restriction, accounting for 71 cases (74.74%). More than half of the participants were nulliparous, with 54 (56.84%) being in their first pregnancy. Regarding parity, the most common category was a history of one previous delivery, reported in 32 women (33.68%). A total of 24 participants (25.26%) were smokers. Thromboprophylaxis was administered to 27 women (28.42%), while 9 (9.47%) received aspirin therapy. Regarding comorbidities, 22 participants (23.16%) had a history of hypertension, while diabetes mellitus was reported in 6 (6.32%) participants. A previous pregnancy complicated by preeclampsia was reported in 5 women (5.26%), while 13 (13.68%) had a history of a pregnancy complicated by fetal growth restriction. The median gestational age at the time of assessment was 35.4 weeks (IQR = 5).

Maternal demographic and clinical characteristics of the study population are presented in Table 1.

Table 1. Maternal and Pregnancy Characteristics of the Study Population (n = 95)

Variable	Value
Maternal age, years	
Mean $\pm$ SD (range)	28.9 ± 6.1 (18–56)
Median (Q1–Q3)	28 (25–32)
Body mass index, kg/m ²	
Mean $\pm$ SD (range)	28.2 ± 5.4 (17.6–41)

Variable	Value
Median (Q1–Q3)	27 (25–31.6)
Fetal growth restriction, n (%)	
Early-onset FGR	24 (25.26)
Late-onset FGR	71 (74.74)
Parity, n (%)	
0	54 (56.84)
1	32 (33.68)
2	4 (4.21)
3	5 (5.26)
Smoking, n (%)	24 (25.26)
Aspirin therapy, n (%)	9 (9.47)
Thromboprophylaxis, n (%)	27 (28.42)
Hypertension, n (%)	22 (23.16)
Diabetes mellitus, n (%)	6 (6.32)
Preeclampsia/eclampsia/HELLP syndrome during current pregnancy, n (%)	3 (3.16)
Previous pregnancy complicated by preeclampsia, n (%)	5 (5.26)
Previous pregnancy complicated by FGR, n (%)	13 (13.68)
Gestational age at assessment, weeks	
Median (Q1–Q3)	35.4 (32–37)

Abbreviations: FGR, fetal growth restriction; HELLP, hemolysis, elevated liver enzymes, and low platelet count; Q1, first quartile; Q3, third quartile; SD, standard deviation.

The median values of the analyzed angiogenic markers were as follows: PlGF, 155 pg/mL (IQR = 322); sFlt-1, 3837 pg/mL (IQR = 5386); and the sFlt-1/PlGF ratio, 25.3 (IQR = 107.8). The distribution of angiogenic marker concentrations is presented in Table 2.

Table 2. Maternal Serum Angiogenic Marker Levels in the Study Population (n = 95)

Variable	Median (Q1–Q3)
PlGF, pg/mL	155 (56–378)
sFlt-1, pg/mL	3837 (1902–7288)
sFlt-1/PlGF ratio	25.3 (5.2–113)

Abbreviations: PlGF, placental growth factor; sFlt-1, soluble fms-like tyrosine kinase-1; Q1, first quartile; Q3, third quartile; IQR, interquartile range.

Neonatal birth weight ranged from 600 to 2950 g, with a mean birth weight of 2059.9 ± 592.9 g. The first birth-weight percentile was the most frequent category, observed in 48 neonates (50.53%). The median Apgar score at 5 minutes was 9 (Q1–Q3: 8–9), with 60 neonates (63.16%) having an Apgar score of 9.

The median duration of NICU stay was 31 days (Q1–Q3: 21.5–48). Respiratory distress syndrome

occurred in 21 neonates (22.11%), neonatal sepsis in 14 (14.74%), and intraventricular hemorrhage in 9 (9.47%). Neonatal death occurred in 4 cases (4.21%). An adverse neonatal outcome was recorded in 50 cases (52.63%).

Table 3. Neonatal Morbidity and Outcomes

Variable	Value
NICU stay, days, median (Q1–Q3)	31 (21.5–48)
Respiratory distress syndrome, n (%)	21 (22.11)
Neonatal sepsis, n (%)	14 (14.74)
Intraventricular hemorrhage, n (%)	9 (9.47)
Neonatal death, n (%)	4 (4.21)
Adverse neonatal outcome, n (%)	50 (52.63)

Abbreviations: NICU, neonatal intensive care unit; Q1, first quartile; Q3, third quartile.

The duration of NICU stay was not significantly correlated with PLGF ($\rho = -0.2339$, $p = 0.23$), sFlt-1 ($\rho = 0.1266$, $p = 0.52$), or the sFlt-1/PLGF ratio ($\rho = 0.1871$, $p = 0.34$).

Table 4. Correlation Between Maternal Angiogenic Markers and Duration of Neonatal Intensive Care Unit Hospitalization

Duration of NICU stay	Spearman ρ	p-value
PLGF (pg/ml)	-0.2339	0.23
sFlt-1 (pg/ml)	0.1266	0.52
sFlt-1/PLGF	0.1871	0.34

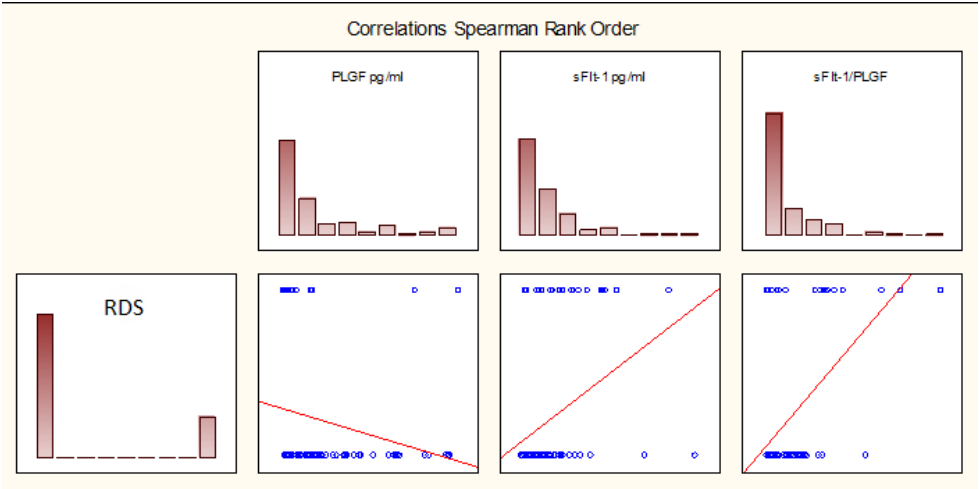
Abbreviations: NICU, neonatal intensive care unit; PLGF, placental growth factor; sFlt-1, soluble fms-like tyrosine kinase-1

Correlation analysis demonstrated a significant moderate negative correlation between respiratory distress syndrome (RDS) and PLGF concentration ($\rho = -0.4007$, $p = 0.0004$). In contrast, RDS was significantly and positively correlated with sFlt-1 concentration ($\rho = 0.3312$, $p = 0.001$) and with the sFlt-1/PLGF ratio ($\rho = 0.4107$, $p < 0.001$).

Table 5. Correlation Between Maternal Angiogenic Markers and Neonatal Respiratory Distress Syndrome

Respiratory distress syndrome &	Spearman ρ	p-value
PLGF (pg/ml)	-0.4070	***0.00004
sFlt-1 (pg/ml)	0.3312	**0.001
sFlt-1/PLGF	0.4107	***0.000036

Figure 1. Correlation Between Maternal Angiogenic Markers and Neonatal Respiratory Distress Syndrome

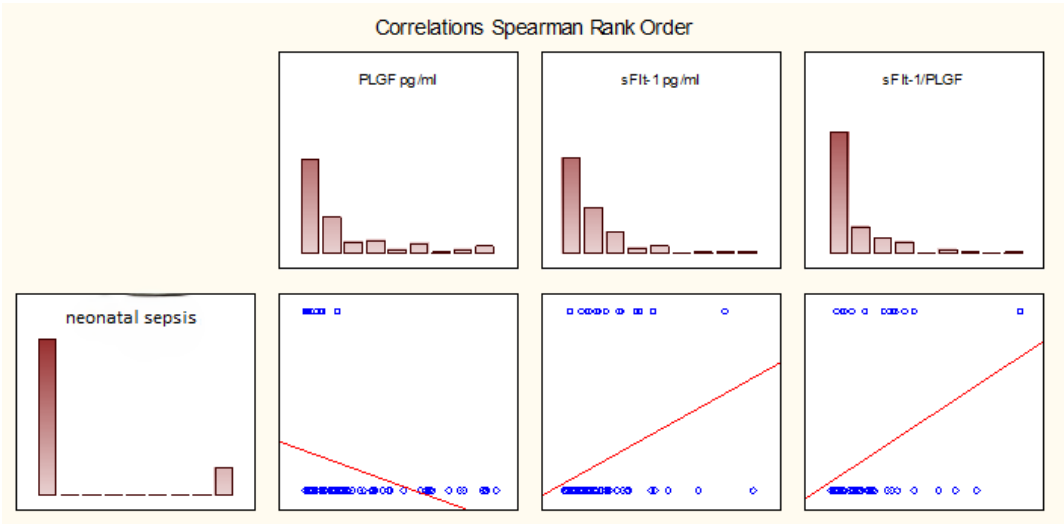


Correlation analysis demonstrated a significant moderate negative correlation between neonatal sepsis and PLGF concentration ($\rho = -0.3677$, $p < 0.001$). In contrast, neonatal sepsis was significantly and positively correlated with sFlt-1 concentration ($\rho = 0.3303$, $p = 0.001$) and with the sFlt-1/PLGF ratio ($\rho = 0.3769$, $p < 0.001$).

Table 6. Correlation Between Maternal Angiogenic Markers and Neonatal Sepsis

Neonatal sepsis &	Spearman ρ	p-value
PLGF (pg/ml)	-0.3677	***0.00025
sFlt-1 (pg/ml)	0.3303	**0.001
sFlt-1/PLGF	0.3769	***0.00017

Figure 2. Correlation Between Maternal Angiogenic Markers and Neonatal Sepsis

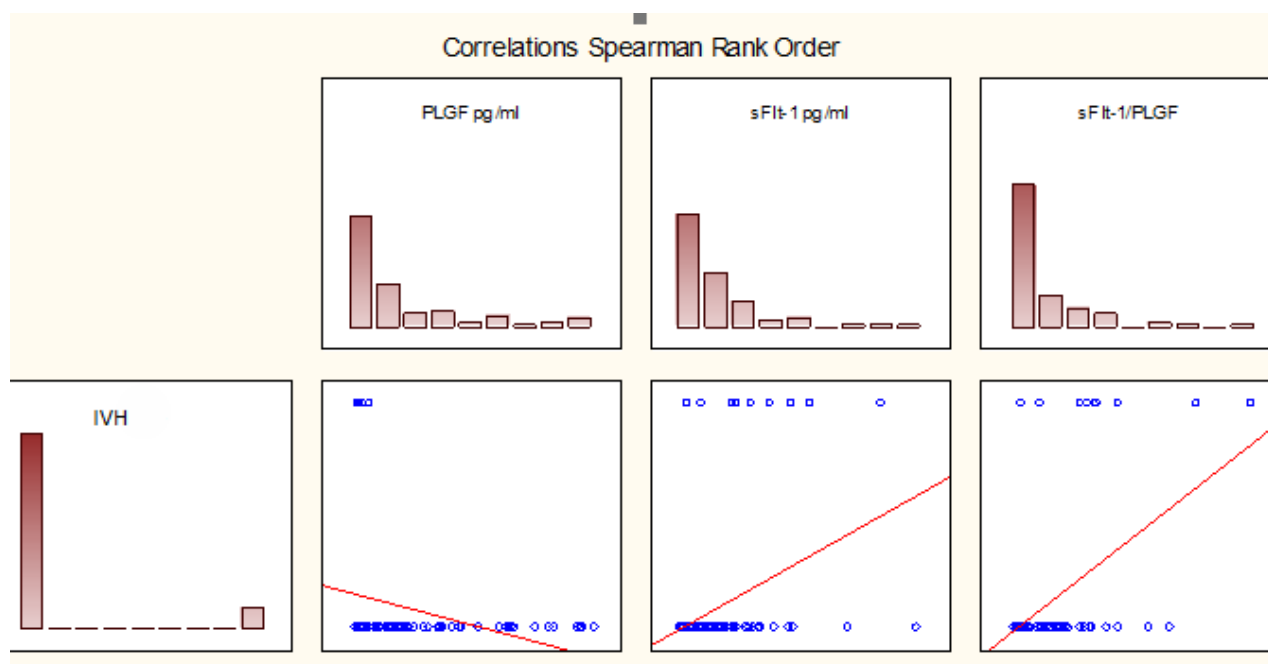


Spearman correlation analysis demonstrated a significant moderate negative correlation between intraventricular hemorrhage (IVH) and PLGF concentration ($\rho = -0.3932$, $p < 0.001$). In contrast, IVH was significantly and positively correlated with sFlt-1 concentration ($\rho = 0.2870$, $p = 0.0048$) and with the sFlt-1/PLGF ratio ($\rho = 0.3723$, $p < 0.001$).

Table 7. Correlation Between Maternal Angiogenic Markers and Neonatal Intraventricular Hemorrhage

Fetal intraventricular hemorrhage &	Spearman ρ	p-value
PLGF (pg/ml)	-0.3932	0.00008
sFlt-1 (pg/ml)	0.2870	0.0048
sFlt-1/PLGF	0.3723	0.0002

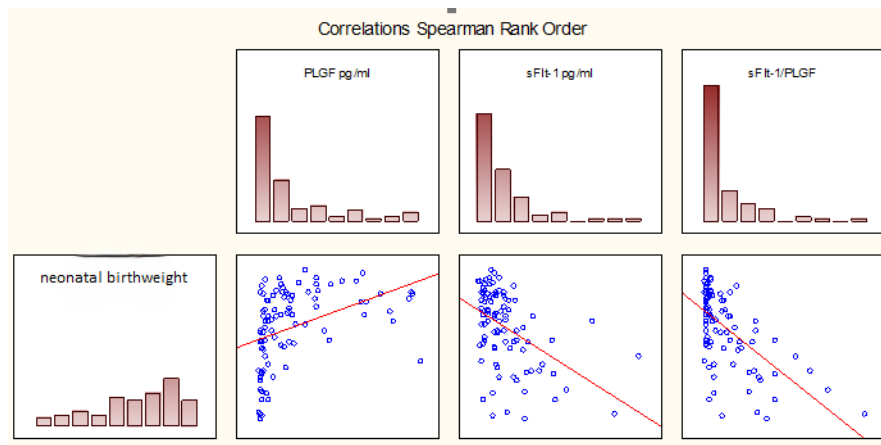
Figure 3. Correlation Between Maternal Angiogenic Markers and Neonatal Intraventricular Hemorrhage



Spearman correlation analysis demonstrated a significant moderate positive correlation between birth weight and PLGF concentration ($\rho = 0.5654$, $p < 0.001$), indicating that higher PLGF concentrations were associated with higher birth weight. In contrast, birth weight was significantly and moderately negatively correlated with sFlt-1 concentration ($\rho = -0.4644$, $p < 0.001$) and with the sFlt-1/PLGF ratio ($\rho = -0.5693$, $p < 0.001$), indicating that higher sFlt-1 concentrations and sFlt-1/PLGF ratios were associated with lower birth weight.

Table 8. Correlation Between Maternal Angiogenic Markers and Neonatal Birth Weight

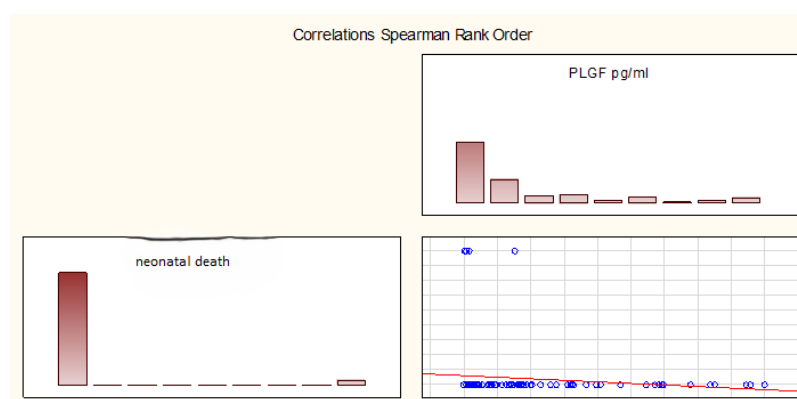
Neonatal birth weight &	Spearman ρ	p-value
PLGF (pg/ml)	0.5654	<0.001
sFlt-1 (pg/ml)	-0.4644	***0.000002
sFlt-1/PLGF	-0.5693	<0.001

Figure 4. Correlation Between Maternal Angiogenic Markers and Neonatal Birth Weight

Spearman correlation analysis demonstrated a significant weak negative correlation between neonatal mortality and PLGF concentration ($\rho = -0.2159$, $p = 0.036$). In contrast, no significant correlations were observed between neonatal mortality and sFlt-1 concentration ($\rho = 0.0937$, $p = 0.37$) or the sFlt-1/PLGF ratio ($\rho = 0.1701$, $p = 0.10$).

Table 9. Correlation Between Maternal Angiogenic Markers and Neonatal Mortality

Neonatal mortality &	Spearman ρ	p-value
PLGF (pg/ml)	-0.2159	*0.036
sFlt-1 (pg/ml)	0.0937	0.37
sFlt-1/PLGF	0.1701	0.1

Figure 5. Correlation Between Maternal Angiogenic Markers and Neonatal Mortality

Discussion

The present study evaluated the association between maternal circulating angiogenic markers and neonatal outcomes in pregnancies complicated by fetal smallness, defined by an estimated fetal weight below the 10th percentile. The principal finding was that an altered maternal angiogenic profile, characterized by lower PlGF concentrations and higher sFlt-1 concentrations and sFlt-1/PlGF ratios, was associated with several indicators of adverse neonatal outcome. These associations were particularly evident for birth weight, respiratory distress syndrome, neonatal sepsis, and intraventricular hemorrhage, whereas the duration of NICU stay was not significantly associated with any of the evaluated angiogenic markers.

The relationship between angiogenic imbalance and neonatal morbidity was evident across several neonatal complications. RDS and neonatal sepsis were negatively associated with PlGF and positively associated with both sFlt-1 and the sFlt-1/PlGF ratio. A similar pattern was observed for IVH, with lower PlGF and higher sFlt-1 and sFlt-1/PlGF ratios associated with this complication. Taken together, these findings suggest that greater angiogenic imbalance is associated not only with impaired fetal growth but also with increased neonatal morbidity.

The findings regarding neonatal mortality were less consistent. Lower PlGF was weakly but significantly associated with neonatal mortality ($\rho = -0.2159$, $p = 0.036$), whereas no statistically significant correlations were found for sFlt-1 or the sFlt-1/PlGF ratio. Given that only four neonatal deaths occurred in the study population, this finding should be interpreted with particular caution. The small number of events limits statistical power and results in considerable uncertainty regarding the strength and stability of the observed associations.

In contrast to the associations with specific neonatal morbidities, none of the angiogenic markers was significantly correlated with the duration of NICU stay. NICU length of stay is a multifactorial outcome influenced by gestational age, birth weight, respiratory support, infection, feeding difficulties, and other clinical factors. Therefore, it may be less directly related to the degree of placental angiogenic imbalance than to individual neonatal complications.

A particularly relevant finding was the association between angiogenic markers and neonatal birth weight. Birth weight showed a moderate positive correlation with maternal PlGF concentration ($\rho = 0.5654$, $p < 0.001$), whereas significant moderate negative correlations were observed with sFlt-1 ($\rho = -0.4644$, $p < 0.001$) and the sFlt-1/PlGF ratio ($\rho = -0.5693$, $p < 0.001$). Our finding of a significant negative association between the sFlt-1/PlGF ratio and neonatal birth weight is consistent with previous studies. Kwiatkowski et al. reported a significant negative correlation between the sFlt-1/PlGF ratio and neonatal birth weight across their entire study population ($R = -0.46$, $p < 0.001$). (19) Similarly, Konopka et al. demonstrated a significant negative correlation between the pre-delivery sFlt-1/PlGF ratio and birth-weight centile for gestational age in pregnancies with isolated fetal growth restriction (iFGR; $R = -0.62$, $p = 0.02$) (20). In our cohort, the sFlt-1/PlGF ratio was also significantly and negatively correlated with neonatal birth weight ($\rho = -0.5693$, $p < 0.001$). These findings support an association between increasing angiogenic imbalance and lower neonatal birth weight, suggesting that the sFlt-1/PlGF ratio may reflect the severity of placental dysfunction and impaired fetal growth.

Our findings are consistent with those of Witwicki et al., who reported a higher incidence of respira-

tory disorders, more frequent NICU admission, and longer hospitalization among neonates in the high sFlt-1/PlGF ratio group. Moreover, an sFlt-1/PlGF ratio ≥ 33 predicted a combined adverse neonatal outcome with a sensitivity of 73% and specificity of 82.2%, and remained independently associated with adverse neonatal outcome in multivariable analysis (21). Similarly, in our study, the sFlt-1/PlGF ratio was significantly associated with respiratory distress syndrome ($p = 0.4107$, $p < 0.001$), supporting the association between greater angiogenic imbalance and increased neonatal morbidity. Although Witwicki et al. reported two neonatal deaths in the high-ratio group and none in the low-ratio group, we found no significant correlation between the sFlt-1/PlGF ratio and neonatal mortality ($p = 0.1701$, $p = 0.10$).

The association between angiogenic imbalance and adverse neonatal outcome observed in our study is also supported by Kamphof et al. In a cohort of 440 singleton SGA pregnancies, they found that the sFlt-1/PlGF ratio was significantly associated with composite adverse perinatal outcome (CAPO; $p < 0.001$). Moreover, the sFlt-1/PlGF ratio remained part of the final multivariable prediction model together with estimated fetal weight and the highest umbilicocerebral ratio, achieving an AUC of 0.75 (95% CI, 0.70–0.81) (22). Similarly, our findings demonstrated significant associations between a higher sFlt-1/PlGF ratio and several adverse neonatal outcomes, including respiratory distress syndrome, neonatal sepsis, and intraventricular hemorrhage. These findings further support the potential value of angiogenic markers, particularly the sFlt-1/PlGF ratio, as complementary indicators of neonatal risk in pregnancies complicated by impaired fetal growth.

Hughes et al. investigated the value of angiogenic biomarkers for risk stratification in pregnancies with suspected fetal growth restriction without signs of preeclampsia at recruitment. Positive angiogenic biomarker results, defined as an sFlt-1/PlGF ratio >38 or PlGF <100 pg/mL, were associated with an increased risk of preterm birth and birth weight below the 3rd centile in pregnancies presenting before 32 weeks of gestation, whereas these associations were not observed in pregnancies presenting between 32 and 37 weeks. These findings suggest that angiogenic imbalance may be particularly informative in early-onset FGR (23). This is consistent with our findings showing that lower PlGF concentrations and a higher sFlt-1/PlGF ratio were associated with lower neonatal birth weight and increased neonatal morbidity, further supporting the potential role of angiogenic biomarkers in identifying pregnancies at increased perinatal risk.

Our findings are also consistent with those of Mendoza et al., who demonstrated the value of the sFlt-1/PlGF ratio for risk assessment in early-onset FGR and SGA (24). In their study, the sFlt-1/PlGF ratio was strongly associated with adverse perinatal outcomes, and prediction models incorporating this biomarker generally performed better than models based solely on maternal characteristics and ultrasound findings. Similarly, in our cohort, a higher sFlt-1/PlGF ratio was significantly associated with lower neonatal birth weight and with increased neonatal morbidity, including respiratory distress syndrome, neonatal sepsis, and intraventricular hemorrhage. These findings support the potential value of the sFlt-1/PlGF ratio as an adjunct to conventional ultrasound and Doppler assessment for identifying growth-restricted fetuses at increased risk of adverse perinatal outcome.

Taken together, the present findings suggest that maternal angiogenic markers may provide information about the severity of placental dysfunction and the subsequent risk of adverse neonatal outcomes in pregnancies with fetuses below the 10th percentile. Of the evaluated parameters, the sFlt-1/PlGF ratio showed relatively consistent associations with birth weight, RDS, neonatal sepsis, and IVH.

As the ratio integrates both the antiangiogenic and proangiogenic components of placental dysfunction, it may potentially provide a more comprehensive reflection of angiogenic imbalance than either marker considered separately.

Conclusion

In conclusion, maternal angiogenic imbalance was associated with fetal growth and several important neonatal morbidities in pregnancies with fetuses below the 10th percentile. Lower PlGF concentrations and higher sFlt-1 concentrations and sFlt-1/PlGF ratios were generally associated with less favorable neonatal outcomes, particularly lower birth weight, respiratory distress syndrome, neonatal sepsis, and intraventricular hemorrhage. These findings support further investigation of angiogenic markers as complementary tools for risk stratification in pregnancies complicated by suspected placental dysfunction. Larger prospective studies incorporating multivariable models are needed to determine whether these biomarkers improve prediction of neonatal outcomes beyond established clinical, ultrasound, and Doppler parameters.

Declarations

Ethics approval and consent to participate: The study was approved by the Ethics Committee of the Faculty of Medicine, Ss. Cyril and Methodius University in Skopje, Republic of North Macedonia [No. 03-96/12].

Informed consent: Written informed consent was obtained from all participants prior to inclusion in the study.

Conflict of interest: The authors declare no conflict of interest.

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Availability of data and materials: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors' contributions: All authors reviewed and approved the final version of the manuscript.

Artificial Intelligence (AI) statement: AI was used for language editing and improvement of the clarity and readability of the manuscript.

References:

1. Nohuz E, Riviere O, Coste K, Vendittelli F. Prenatal identification of 'small-for-gestational-age' and risk of neonatal morbidity and stillbirth. *Ultrasound Obstet Gynecol* 2020; 55: 621–628.
2. Lees CC, Marlow N, van Wassenhaer-Leemhuis A, et al. 2 year neurodevelopmental and intermediate perinatal outcomes in infants with very preterm fetal growth restriction (TRUFFLE): a randomised trial. *The Lancet*. 2015;
3. Moraitis AA, Wood AM, Fleming M, Smith GC. Birth weight percentile and the risk of term

- perinatal death. *Obstet Gynecol* 2014; 124: 274–283.
4. Romero R, Kingdom J, Deter R, Lee W, Vintzileos A. Fetal growth: evaluation and management. *Am J Obstet Gynecol*. 2018;218(2 Suppl):S608. doi:10.1016/j.ajog.2018.01.010.
 5. Salafia CM, Minior VK, Pezzullo JC, Popek EJ, Rosenkrantz TS, Vintzileos AM. Intrauterine growth restriction in infants of less than thirty-two weeks' gestation: associated placental pathologic features. *Am J Obstet Gynecol* 1995;173:1049–57.
 6. Moraitis AA, Wood AM, Fleming M, Smith GC. Birth weight percentile and the risk of term perinatal death. *Obstet Gynecol* 2014; 124: 274–283.
 7. Vasak B, Koenen SV, Koster MP, Hukkelhoven CW, Franx A, Hanson MA, Visser GH. Human fetal growth is constrained below optimal for perinatal survival. *Ultrasound Obstet Gynecol* 2015; 45: 162–167.
 8. Iliodromiti S, Mackay DF, Smith GC, Pell JP, Sattar N, Lawlor DA, et al. Customised and noncustomised birth weight centiles and prediction of stillbirth and infant mortality and morbidity: a cohort study of 979,912 term singleton pregnancies in Scotland. *PLoS Med*. 2017;14(1):e1002228.
 9. Pilliod RA, Cheng YW, Snowden JM, Doss AE, Caughey AB. The risk of intrauterine fetal death in the small-for-gestational-age fetus. *Am J Obstet Gynecol* 2012; 207:318.e1–6.
 10. Lees CC, Stampalija T, Baschat A, da Silva Costa F, Ferrazzi E, Figueras F, Hecher K, Kingdom J, Poon LC, Salomon LJ, Unterschedier J. ISUOG Practice Guidelines: diagnosis and management of small-for-gestational-age fetus and fetal growth restriction. *Ultrasound Obstet Gynecol* 2020; 56: 298–312.
 11. Morris RK, Johnstone E, Lees C, Morton V, Smith G; Royal College of Obstetricians and Gynaecologists. Investigation and care of a small-for-gestational-age fetus and a growth-restricted fetus (Green-top Guideline No. 31). *BJOG*. 2024;131(9):e31–e80.
 12. Melamed, N., Baschat, A., Yinon, Y., Athanasiadis, A., Mecacci, F., Figueras, F., Berghella, V., Nazareth, A., Tahlak, M., McIntyre, H.D., Da Silva Costa, F., Kihara, A.B., Hadar, E., McAuliffe, F., Hanson, M., Ma, R.C., Gooden, R., Sheiner, E., Kapur, A., Divakar, H., Ayres-de-Campos, D., Hirsch, L., Poon, L.C., Kingdom, J., Romero, R. and Hod, M. (2021), FIGO (International Federation of Gynecology and Obstetrics) initiative on fetal growth: Best practice advice for screening, diagnosis, and management of fetal growth restriction. *Int J Gynecol Obstet*, 152: 3–57.
 13. Herraiz I, Quezada MS, Rodriguez-Calvo J, Gomez-Montes E, Villala ' 'in C, Galindo A. Longitudinal change of sFlt-1/PlGF ratio in singleton pregnancy with early-onset fetal growth restriction. *Ultrasound Obstet Gynecol* 2018; 52: 631–638.
 14. American College of Obstetricians and Gynecologists. Committee Opinion No. 700: methods for estimating the due date. *Obstet Gynecol*. 2017;129(5):e150–e154. doi:10.1097/AOG.0000000000002046.
 15. Salomon LJ, Alfrevic Z, Da Silva Costa F, Deter RL, Figueras F, Ghi T, Glanc P, Khalil A, Lee W, Napolitano R, Papageorgiou A, Sotiriadis A, Stirnemann J, Toi A, Yeo G. ISUOG Practice Guidelines: ultrasound assessment of fetal biometry and growth. *Ultrasound Obstet Gynecol* 2019; 53: 715–723

16. Hadlock FP, Harrist RB, Sharman RS, Deter RL, Park SK. Estimation of fetal weight with the use of head, body, and femur measurements--a prospective study. *Am J Obstet Gynecol.* 1985 Feb 1;151(3):333-7. doi:10.1016/0002-9378(85)90298-4 PMID: 3881966.
17. Hadlock FP, Harrist RB, Martinez-Poyer J. In utero analysis of fetal growth: a sonographic weight standard. *Radiology.* 1991;181(1):129-133.
18. Bhide, A., Acharya, G., Baschat, A., Bilardo, C.M., Brezinka, C., Cafci, D., Ebbing, C., Hernandez-Andrade, E., Kalache, K., Kingdom, J., Kiserud, T., Kumar, S., Lee, W., Lees, C., Leung, K.Y., Maling, G., Mari, G., Prefumo, F., Sepulveda, W. and Trudinger, B. (2021), ISUOG Practice Guidelines (updated): use of Doppler velocimetry in obstetrics. *Ultrasound Obstet Gynecol*, 58: 331-339
19. Kwiatkowski S, Bednarek-Jędrzejek M, Ksel J, Tousty P, Kwiatkowska E, Cymbaluk A, Rzepka R, Chudecka-Głaz A, Dołęgowska B, Torbè A. sFlt-1/PIGF and Doppler ultrasound parameters in SGA pregnancies with confirmed neonatal birth weight below 10th percentile. *Pregnancy Hypertens.* 2018 Oct;14:79-85. doi: 10.1016/j.preghy.2018.08.448. Epub 2018 Aug 17. PMID: 30527123.
20. Dymara-Konopka W, Laskowska M, Grywalska E, Hymos A, Leszczyńska-Gorzelak B. Maternal serum Angiogenic profile and its correlations with ultrasound parameters and perinatal results in normotensive and Preeclamptic pregnancies complicated by fetal growth restriction. *J Clin Med.* (2023) 12:4281. doi: 10.3390/jcm12134281
21. Witwicki J, Chaberek K, Szymecka-Samaha N, Krysiak A, Pietruski P, Kosińska-Kaczyńska K. sFlt-1/PIGF ratio in prediction of short-term neonatal outcome of small for gestational age neonates. *Children (Basel).* 2021;8(8):718.
22. Kamphof HD, Marijnen MC, Damhuis SE, Wolf H, Gordijn SJ, Ganzevoort W; DRIGITAT Trial Group. Predictive value of fetal Doppler velocimetry, fetal growth trajectory and maternal serum biomarkers for short-term adverse perinatal outcome: secondary analysis of DRIGITAT study. *Ultrasound Obstet Gynecol.* 2025 Oct;66(4):470-479. doi: 10.1002/uog.29266. Epub 2025 Jul 11. PMID: 40641303; PMCID: PMC12488208.
23. Hughes RCE, Kyle P, Phillips I, Florkowski CM, Gullam J. In suspected fetal growth restriction, sFlt-1/PIGF and PIGF may have value in risk stratification for preterm birth and birthweight <3rd centile: a blinded cohort study. *Aust N Z J Obstet Gynaecol.* 2025 Feb 12. doi:10.1111/ajo.70003. Epub ahead of print.
24. Mendoza M, Hurtado I, Bonacina E, Garcia-Manau P, Serrano B, Tur H, Rodo C, Maiz N, Carreras E. Individual risk assessment for prenatal counseling in early-onset growth-restricted and small-for-gestational-age fetuses. *Acta Obstet Gynecol Scand.* 2021 Mar;100(3):504-512. doi: 10.1111/aogs.14032. Epub 2020 Nov 6. PMID: 33098097