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Prospective Evaluation of Maternal Serum sFlt-1, PlGF, and the sFlt-1/PlGF Ratio in Placenta Accreta Spectrum and Their Correlation With Placental Immunohistochemical Expression

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Abstract

Background

Placenta accreta spectrum (PAS) is associated with substantial maternal morbidity and mortality, particularly because of severe obstetric haemorrhage. Although antenatal imaging remains the cornerstone of diagnosis, additional biomarkers may have potential value, particularly where access to advanced imaging is limited. This study evaluated maternal serum soluble fms-like tyrosine kinase-1 (sFlt-1), placental growth factor (PlGF), and the sFlt-1/PlGF ratio in women with PAS and examined their relationship with placental immunohistochemical expression.

Methods

This prospective case–control study was conducted at Vardhman Mahavir Medical College and Safdarjung Hospital, New Delhi, in collaboration with the ICMR–National Institute of

Pathology. Women with singleton pregnancies beyond 28 weeks with confirmed or clinically diagnosed PAS were enrolled as cases, while age- and gravidity-matched women undergoing caesarean delivery without PAS served as controls. Maternal plasma sFlt-1 and PlGF concentrations were measured using enzyme-linked immunosorbent assays. Placental tissue was evaluated by histopathology and immunohistochemistry for sFlt-1 and PlGF. Diagnostic performance was assessed using receiver operating characteristic (ROC) analysis, and multivariable logistic regression was performed to account for baseline clinical differences.

Results

Sixty women were included in the final analysis, comprising 40 PAS cases and 20 controls. Median plasma sFlt-1 concentrations were lower in PAS cases than controls (23.5 vs. 29.8 ng/mL; $p = 0.034$), whereas PlGF concentrations were higher (270.5 vs. 48.2 pg/mL; $p = 0.006$). The median sFlt-1/PlGF ratio was significantly lower in PAS cases (88.1 vs. 561.1; $p = 0.001$). ROC analysis demonstrated an area under the curve (AUC) of 0.670 for sFlt-1, 0.716 for PlGF, and 0.753 for the sFlt-1/PlGF ratio. At the selected cut-offs, sFlt-1 showed 100% sensitivity and 65% specificity, PlGF showed 62.5% sensitivity and 100% specificity, and the sFlt-1/PlGF ratio showed 62.5% sensitivity and 95% specificity. None of the three biomarkers differed significantly according to histopathological depth of invasion. In multivariable logistic regression, the sFlt-1/PlGF ratio was not independently associated with PAS after adjustment for maternal age, parity, previous caesarean delivery, and previous placenta previa ($p = 0.110$). Placental PlGF immunohistochemical expression showed a significant positive association with plasma PlGF concentration ($p < 0.001$), whereas sFlt-1 tissue expression was uniformly weak.

Conclusions

Maternal plasma sFlt-1, PlGF, and the sFlt-1/PlGF ratio were significantly different in women with PAS compared with controls and demonstrated moderate discriminatory performance. The sFlt-1/PlGF ratio showed the highest AUC among the biomarkers evaluated; however, it was not independently associated with PAS after adjustment for clinical factors. Plasma PlGF correlated significantly with placental PlGF immunohistochemical expression, supporting a biological relationship between circulating and placental PlGF. Larger prospective studies are required to determine whether these biomarkers can provide clinically useful adjunctive information for PAS screening and diagnosis.

Keywords:placenta accreta spectrum; sFlt-1; PlGF; sFlt-1/PlGF ratio; immunohistochemistry; biomarkers; placenta accreta; PAS

INTRODUCTION

Placenta accreta spectrum (PAS), formerly referred to as morbidly adherent placenta, comprises a spectrum of abnormal placental adherence and invasion, including placenta accreta, increta, and percreta. In placenta accreta, chorionic villi attach abnormally close to or directly onto the myometrium because of deficient or absent decidualization; in placenta increta, villi invade the myometrium; and in placenta percreta, invasion extends through the uterine serosa and may involve adjacent pelvic organs [1].

The incidence of PAS has increased substantially over recent decades, paralleling the rising rates of cesarean delivery [2]. Major risk factors include placenta previa and previous cesarean delivery, with the risk of PAS increasing progressively with the number of prior cesarean deliveries in women with placenta previa [3,4]. Other reported risk factors include previous uterine surgery, advanced maternal age, multiparity, prior uterine artery embolization, manual removal of the placenta, and previous puerperal infection [1,5]. PAS is associated with considerable maternal morbidity, primarily because of severe obstetric hemorrhage. Complications include massive blood loss, blood transfusion, intensive care unit admission, infection, prolonged hospitalization, urinary tract and other pelvic organ injury, and maternal mortality [4,6]. Peripartum hysterectomy is frequently required in cases of extensive placental invasion.

Antenatal diagnosis of PAS facilitates planned delivery at appropriately equipped tertiary care centres with multidisciplinary expertise, advanced imaging facilities, and adequate blood-bank support. Ultrasonography remains the principal imaging modality for antenatal assessment, although diagnostic performance may vary according to operator expertise, placental location, and the extent of disease. Magnetic resonance imaging (MRI) can provide additional anatomical information in selected cases but does not consistently resolve diagnostic uncertainty [7]. Consequently, there remains a need for complementary biomarkers that may provide additional information in women at increased risk of PAS, particularly when imaging findings are equivocal.

Several circulating biomarkers associated with inflammation, angiogenesis, and trophoblastic function have been investigated in PAS, including vascular endothelial growth factor (VEGF), interleukins, and tumour necrosis factor-alpha [8]. Among angiogenic factors, placental growth factor (PlGF) is a pro-angiogenic member of the VEGF family that contributes to placental vascular development, whereas soluble fms-like tyrosine kinase-1 (sFlt-1), a soluble anti-angiogenic receptor derived predominantly from placental trophoblasts, binds and antagonizes PlGF and other pro-angiogenic ligands. Alterations in the balance between pro- and anti-angiogenic factors may therefore reflect changes in placental vascular development and trophoblastic invasion.

Previous studies have reported altered maternal circulating concentrations of sFlt-1 and PlGF in women with PAS, including lower sFlt-1, higher PlGF, and a reduced sFlt-1/PlGF ratio compared with women without PAS [8,9]. These observations suggest that angiogenic imbalance may be associated with abnormal placentation. However, the extent to which circulating biomarkers can discriminate PAS from non-PAS placentation, and whether their concentrations correlate with the local placental expression of these factors, remains

incompletely established. Furthermore, available evidence regarding their ability to differentiate different depths of placental invasion is limited and inconsistent.

Data evaluating the relationship between circulating angiogenic biomarkers and placental tissue expression in women with PAS are particularly limited in the Indian population. Establishing such a relationship may provide biological support for the use of circulating biomarkers as adjunctive indicators of abnormal placentation.

Therefore, this exploratory study was undertaken to evaluate maternal plasma concentrations of sFlt-1, PlGF, and the sFlt-1/PlGF ratio in women with PAS compared with non-PAS controls, to assess their diagnostic performance using receiver operating characteristic analysis, and to examine the relationship between circulating biomarker concentrations and placental immunohistochemical expression. A secondary objective was to determine whether these biomarkers differed according to the histopathological depth of placental invasion.

Materials and Methods

Study Design and Setting

This prospective case-control study was conducted over a period of 18 months in the Department of Obstetrics and Gynaecology, Vardhman Mahavir Medical College and Safdarjung Hospital, New Delhi, in collaboration with the Department of Pathology, ICMR–National Institute of Pathology. The study included women with singleton pregnancies beyond 28 weeks of gestation who were either suspected antenatally to have placenta accreta spectrum (PAS) on ultrasound or magnetic resonance imaging (MRI), or were diagnosed with PAS intraoperatively.

Women fulfilling the eligibility criteria were recruited prospectively. Cases comprised women with clinically suspected or intraoperatively confirmed PAS. Controls comprised age- and gravidity-matched women without clinical evidence of adherent placentation who underwent cesarean delivery during the same study period. Controls were restricted to women undergoing cesarean delivery because surgical assessment provided an opportunity for clinical assessment of placental separation and, where clinically indicated, placental bed tissue evaluation, thereby reducing the possibility of differential verification associated with postpartum assessment after vaginal delivery.

Women with pre-eclampsia, gestational diabetes mellitus, fetal growth restriction, known infectious diseases, or multiple pregnancies were excluded.

Sample Size

The initial sample size was calculated using data from Lumbanraja et al. (2022), who reported a sensitivity of 67% for the sFlt-1/PlGF ratio in identifying PAS. The sample size for diagnostic accuracy was estimated using the standard formula based on an anticipated sensitivity of 0.67, a proportion of subjects with the target condition of 0.50, a desired confidence-interval width of 0.15, and a two-sided type I error of 5% (Z = 1.96).

Using these parameters, the estimated sample size was 75.5 participants. This was rounded to 76 participants, corresponding to approximately 38 participants per group. To allow for potential losses and to provide a balanced study population, the planned recruitment target was subsequently increased to 80 participants, comprising 40 PAS cases and 40 controls.

However, the final analytical cohort consisted of 60 participants (40 PAS cases and 20 controls). Although ELISA kits were initially available for 80 samples, repeat testing performed in duplicate or triplicate resulted in insufficient reagents for the remaining 20 control samples. Therefore, these 20 controls could not undergo biomarker analysis and were not included in the final analytical cohort. The 20 controls included in the analysis were the first 20 consecutive eligible controls recruited chronologically. No participants were selectively excluded on the basis of clinical characteristics or biomarker results. The reduction in the control sample size was considered in the interpretation of the findings and is acknowledged as a limitation because it reduced statistical precision and power.

Clinical Data and Sample Collection

Maternal demographic and clinical characteristics, including maternal age, gravidity, parity, gestational age at delivery, previous cesarean delivery, previous uterine procedures, placenta previa, antepartum bleeding, and hemoglobin concentration, were recorded using a standardized data collection proforma.

Maternal venous blood (3 mL) was collected in ethylenediaminetetraacetic acid (EDTA) tubes immediately before cesarean delivery. Samples were centrifuged for plasma separation and stored at −20 °C until biochemical analysis.

Plasma concentrations of sFlt-1 and PlGF were measured using enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions. The Human PlGF Quantikine ELISA Kit (Catalog No. DPG00; R&D Systems) was used for PlGF estimation, and the Human VEGFR1/Flt-1 Quantikine ELISA Kit (Catalog No. DVR100C; R&D Systems) was used for sFlt-1 estimation. The sFlt-1/PlGF ratio was subsequently calculated from the measured plasma concentrations.

Laboratory personnel performing the ELISA assays were blinded to the clinical diagnosis and reference-standard classification of the participants.

Placental Histopathology and Immunohistochemistry

Placental tissue samples were obtained from hysterectomy specimens in women undergoing cesarean hysterectomy for PAS and from representative placental sections in controls. Tissue samples were submitted for histopathological examination and immunohistochemical (IHC) analysis.

Paraffin-embedded tissue sections were examined using hematoxylin and eosin (H&E) staining. Histopathological examination was performed to assess placental adherence and invasion and to classify PAS according to the depth of placental invasion.

Immunohistochemical staining for PlGF and sFlt-1 was performed using the Human PlGF Antibody (Catalog No. MAB2642; R&D Systems) and Human VEGFR1/Flt-1 Antibody (Catalog No. AF321; R&D Systems), respectively. Tissue sections underwent deparaffinization, rehydration, antigen retrieval using citrate buffer, and blocking of endogenous peroxidase activity, followed by incubation with the primary antibodies. Immunoreactivity was visualized using diaminobenzidine (DAB) chromogen, followed by hematoxylin counterstaining.

IHC expression was assessed semi-quantitatively according to the proportion of positively stained cells and categorized as negative (0), weak (<10% of cells), moderate (10–50% of cells), or high (>50% of cells).

Two pathologists independently reviewed the histopathological and IHC findings while being blinded to the clinical diagnosis and plasma biomarker results. In cases of disagreement, the slides were jointly reviewed and a consensus score was assigned.

Classification of PAS

The final clinical classification of PAS was based on the antenatal and intraoperative findings, supported by histopathological examination where tissue was available. PAS cases were categorized according to the depth of invasion into placenta accreta, increta, and percreta. FIGO intraoperative grading was also recorded where applicable.

Two cases that had been clinically and intraoperatively diagnosed as PAS showed no histopathological evidence of PAS in the available tissue specimens. These cases were retained in the primary clinical PAS cohort but were excluded from analyses specifically examining histopathological PAS subtype and depth of invasion.

Correlation of Plasma and Tissue Biomarkers

Plasma concentrations of sFlt-1 and PlGF were compared with their corresponding placental IHC expression scores. Associations between biomarker concentrations and histopathological depth of placental invasion were also assessed.

Statistical Analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS), version 21 (IBM Corporation, Armonk, NY, USA). Data were initially coded and entered into Microsoft Excel and subsequently transferred to SPSS for analysis.

The distribution of continuous variables was assessed using the Shapiro–Wilk test. Normally distributed continuous variables were summarized as mean ± standard deviation (SD), whereas non-normally distributed variables were summarized as median with interquartile range (IQR). Categorical variables were expressed as frequencies and percentages.

For comparison between the PAS and control groups, the independent-samples t-test was used for normally distributed continuous variables and the Mann–Whitney U test for non-normally

distributed variables. For comparisons involving more than two groups, one-way analysis of variance (ANOVA) with Tukey's honestly significant difference (HSD) post-hoc test was used for normally distributed variables, while the Kruskal–Wallis test was used for non-normally distributed variables.

Associations between categorical variables were assessed using the chi-square test or Fisher's exact test, as appropriate. Pearson or Spearman correlation coefficients were used to assess associations between continuous variables according to the underlying data distribution.

The diagnostic performance of plasma sFlt-1, PlGF, and the sFlt-1/PlGF ratio for discriminating PAS from non-PAS controls was evaluated using receiver operating characteristic (ROC) curve analysis. The area under the ROC curve (AUC), sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), together with corresponding 95% confidence intervals where applicable, were calculated. Optimal cut-off values were determined from the ROC analysis.

A multivariable logistic regression model was additionally explored to assess whether the sFlt-1/PlGF ratio remained associated with PAS after accounting for selected baseline clinical variables. Maternal age, parity, previous cesarean delivery, previous placenta previa, and the sFlt-1/PlGF ratio were included in the model. Because of the small sample size and the strong association between several clinical variables and PAS status, the model demonstrated quasi-complete separation for some categorical predictors. Consequently, the corresponding odds-ratio estimates were considered statistically unstable and were interpreted cautiously rather than as definitive independent effects.

A two-sided p-value <0.05 was considered statistically significant.

Ethical Approval and Consent

Ethical approval for this study was obtained from the Institutional Ethics Committee of Vardhman Mahavir Medical College and Safdarjung Hospital, New Delhi(IEC approval no. IEC/VMMC/SJH/Thesis/2023/CC-178; approved on 27 March 2023). The study was conducted in accordance with the principles of the Declaration of Helsinki (2013 revision). Written informed consent was obtained from all participants before enrolment, and confidentiality of participant information was maintained throughout the study.

Funding and Assay Procurement

The study was supported by institutional funding from the medical store allocation of Vardhman Mahavir Medical College and Safdarjung Hospital (Sanction Order No. O-7-MS/2023-1509). No external commercial funding was received. The ELISA kits and antibody reagents were procured through approved institutional administrative channels.

Results

A total of 60 women were included in the final analysis, comprising 40 women with PAS and 20 controls. Plasma biomarker analysis was completed for all participants, while

immunohistochemical analysis was performed on all available placental tissue specimens. The final case-to-control ratio was 2:1 because of the limited availability of ELISA reagents after completion of the initial case and control samples.

Baseline characteristics

The mean maternal age was significantly higher among women with PAS than among controls (28.6 ± 4.3 vs. 25.8 ± 4.4 years; p= 0.024). However, the distribution across predefined age groups did not differ significantly (p= 0.158). Gravidity distribution was also not significantly different between the groups (p= 0.144) **(Table 1).**

Women with PAS delivered at a significantly earlier gestational age than controls (33.6 ± 3.2 vs. 37.2 ± 2.2 weeks; p< 0.001). Previous caesarean delivery was significantly more frequent among PAS cases, with a higher median number of previous LSCS (p< 0.001). Previous dilatation and curettage was also more frequent among women with PAS (p= 0.029). Antenatal haemoglobin concentration did not differ significantly between groups (p= 0.300).

A history of antepartum bleeding was significantly more common among PAS cases (p= 0.001). Postoperative hospital stay was also longer among PAS cases, with a median duration of 7 days compared with 4 days among controls (p< 0.001).

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

Parameter	PAS cases (n=40)	Controls (n=20)	*p*value
Maternal age, years, mean ± SD	28.60 ± 4.34	25.80 ± 4.36	0.024
Age group, n (%)			0.158
18–25 years	9 (22.5)	10 (50.0)	
26–30 years	17 (42.5)	7 (35.0)	
31–35 years	11 (27.5)	3 (15.0)	
>35 years	3 (7.5)	0 (0)	
Gestational age at delivery, weeks, mean ± SD	33.59 ± 3.22	37.19 ± 2.22	<0.001
Gravidity, median (IQR)	3 (2–4)	2.5 (2–3)	0.144
Previous LSCS, median (IQR)	1 (1–2)	0 (0–1)	<0.001
Haemoglobin, g/dL, mean ± SD	10.10 ± 1.56	10.54 ± 1.49	0.300
Postoperative stay, days, median (IQR)	7 (6–8)	4 (3–4)	<0.001

Abbreviations:IQR, interquartile range; LSCS, lower-segment caesarean section; PAS, placenta accreta spectrum; SD, standard deviation.

Ultrasound, operative findings and maternal outcomes

Placenta previa was identified in 85% of women with PAS and in none of the controls ($p<$ 0.001). Among PAS cases, 57.5% were classified as FIGO grade 3 and 27.5% as grade 4.

Estimated blood loss was substantially higher among PAS cases than controls (2835 ± 1360 vs. 528 ± 130 mL; $p<$ 0.001). Higher blood loss was associated with postoperative complications ($p=$ 0.039) and longer ICU/HDU stay ($p=$ 0.009). Median postoperative hospital stay was significantly longer in the PAS group ($p<$ 0.001).

Half of the PAS cases underwent emergency caesarean hysterectomy. Emergency procedures were more frequent among unbooked women ($p=$ 0.045) and those delivering at earlier gestational ages ($p=$ 0.020). The mean number of blood products transfused was significantly higher among PAS cases than controls (9.75 ± 5.30 vs. 1.60 ± 1.34 units; $p=$ 0.002). Additional haemostatic procedures were required in 57.5% of PAS cases, most commonly bilateral internal iliac artery ligation.

One maternal death occurred in the PAS group because of massive haemorrhage and multiorgan dysfunction. No maternal deaths occurred among controls.

Fetal outcomes

Live birth rates did not differ significantly between groups ($p=$ 1.000). Mean birth weight was significantly lower among infants born to women with PAS than among controls (2.00 ± 0.64 vs. 2.52 ± 0.57 kg; $p=$ 0.002). Birth weight did not differ significantly according to accreta, increta, or percreta subtype ($p=$ 0.566).

Plasma biomarker findings

Median plasma sFlt-1 concentrations were significantly lower in PAS cases than controls (23.5 [IQR 23.3–23.6] vs. 29.8 [18.0–43.5] ng/mL; $p=$ 0.034). In contrast, median PlGF concentrations were significantly higher among PAS cases (270.5 [32.4–555.4] vs. 48.2 [30.0–87.9] pg/mL; $p=$ 0.006).

The median sFlt-1/PlGF ratio was significantly lower among PAS cases than controls (88.1 [42.4–691.6] vs. 561.1 [241.6–1013.0]; $p=$ 0.001) **(Table 2).**

Table 2. Plasma concentrations of sFlt-1, PlGF and the sFlt-1/PlGF ratio

Biomarker	PAS cases (n=40)	Controls (n=20)	*p*value
sFlt-1, ng/mL, median (IQR)	23.5 (23.3–23.6)	29.8 (18.0–43.5)	0.034
PlGF, pg/mL, median (IQR)	270.5 (32.4–555.4)	48.2 (30.0–87.9)	0.006
sFlt-1/PlGF ratio, median (IQR)	88.1 (42.4–691.6)	561.1 (241.6–1013.0)	0.001

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

Association with depth of placental invasion

Among the 40 PAS cases, histopathological examination confirmed accreta in 20 (50.0%), increta in 15 (37.5%), and percreta in 3 (7.5%); two specimens showed no histopathological evidence of PAS.

There were no statistically significant differences in plasma sFlt-1, PlGF, or the sFlt-1/PlGF ratio according to histopathological subtype.

Table 3. Plasma biomarker concentrations according to histopathological PAS subtype

Biomarker	Accreta (n=20)	Increta (n=15)	Percreta (n=3)	χ^2	*p*value
sFlt-1, ng/mL, median (IQR)	23.49 (23.11–23.60)	23.61 (23.29–23.73)	23.61 (23.57–23.62)	1.511	0.680
PlGF, pg/mL, median (IQR)	236.10 (29.88–507.25)	350.23 (99.08–502.63)	555.04 (293.88–566.58)	2.118	0.548
sFlt-1/PlGF ratio, median (IQR)	105.59 (47.18–750.16)	67.72 (48.42–381.62)	42.38 (41.61–382.02)	2.118	0.548

This analysis included the 38 histopathologically confirmed PAS cases and excluded the two specimens without histopathological evidence of PAS.

Table 3 presents the plasma concentrations of sFlt-1, PlGF, and the sFlt-1/PlGF ratio according to the histopathological subtype of placenta accreta spectrum (PAS) among the 38 histopathologically confirmed cases. The analysis included 20 cases of accreta, 15 cases of increta, and 3 cases of percreta, while the two specimens without histopathological evidence of PAS were excluded.

The median sFlt-1 concentration was comparable across the three PAS subtypes, ranging from 23.49 ng/mL in accreta to 23.61 ng/mL in percreta, with no statistically significant difference (χ^2=1.511, p=0.680). Median PlGF concentrations showed an increasing trend from 236.10 pg/mL in accreta to 350.23 pg/mL in increta and 555.04 pg/mL in percreta; however, this difference was not statistically significant (χ^2=2.118, p=0.548). Similarly, the median sFlt-1/PlGF ratio decreased from 105.59 in accreta to 67.72 in increta and 42.38 in percreta, but the difference did not reach statistical significance (χ^2=2.118, p=0.548). Overall, none of the evaluated plasma biomarkers demonstrated a statistically significant difference across the histopathological PAS subtypes.

Diagnostic performance

ROC analysis demonstrated an AUC of 0.670 for sFlt-1, 0.716 for PlGF, and 0.753 for the sFlt-1/PlGF ratio. At the prespecified optimal cut-offs, sFlt-1 demonstrated the highest sensitivity, whereas PlGF demonstrated the highest specificity**. (Table 4 & Fig 1-3)**

Table 4. Diagnostic performance of maternal plasma biomarkers for PAS

Parameter	AUC (95% CI)	Cut-off	*p*value	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
sFlt-1, ng/mL	0.670 (0.47–0.87)	≤23.9	0.034	100% (91–100)	65% (41–85)	85.1% (72–94)	100% (75–100)
PlGF, pg/mL	0.716 (0.584–0.848)	≥144.5	0.007	62.5% (46–77)	100% (83–100)	100% (86–100)	57.1% (39–74)
sFlt-1/PlGF ratio	0.753 (0.631–0.874)	≤163.0	0.002	62.5% (46–77)	95% (75–100)	96.2% (80–100)	55.9% (38–73)

Abbreviations:AUC, area under the curve; CI, confidence interval; NPV, negative predictive value; PAS, placenta accreta spectrum; PlGF, placental growth factor; PPV, positive predictive value; sFlt-1, soluble fms-like tyrosine kinase-1.

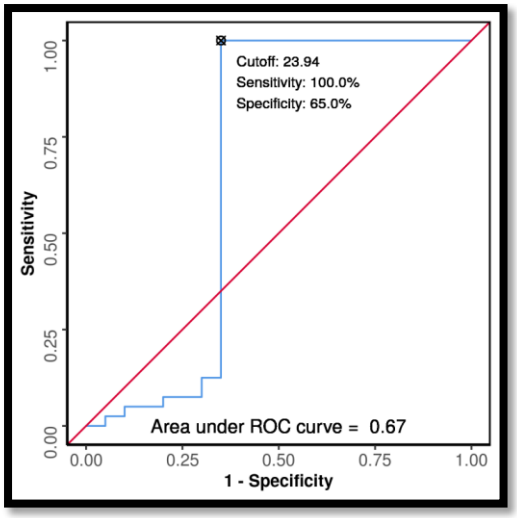


Fig 1

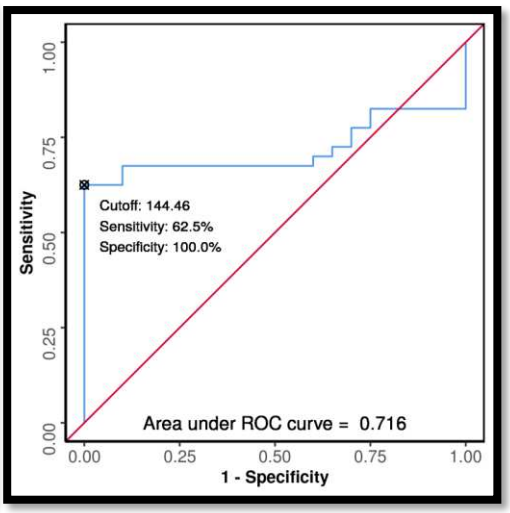


Fig 2

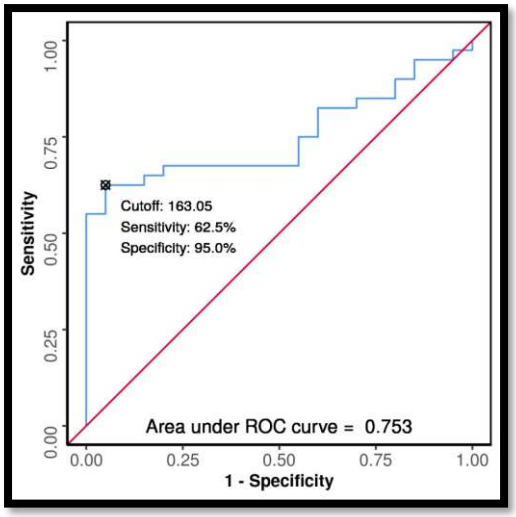


Fig 3

Multivariable logistic regression

Multivariable logistic regression was performed to examine whether the association between the sFlt-1/PlGF ratio and PAS remained after adjustment for maternal age, parity, previous LSCS, and previous placenta previa.

The overall model was statistically significant ($\chi^2(7) = 20.06$, $p = 0.005$), with a C-statistic of 0.824 and acceptable calibration according to the Hosmer–Lemeshow test ($p = 0.197$). However, quasi-complete separation was observed for several clinical variables because some categories occurred exclusively among PAS cases or controls. Consequently, the corresponding odds-ratio estimates were extremely large and associated with very wide or non-estimable confidence intervals and should not be interpreted as stable effect estimates.

Maternal age was significantly associated with PAS in univariable analysis (OR 1.17, 95% CI 1.02–1.35; $p = 0.028$), but this association was attenuated after adjustment (adjusted OR 1.11, 95% CI 0.96–1.31; $p = 0.187$). The sFlt-1/PlGF ratio was not independently associated with PAS in the multivariable model ($p = 0.110$).

Table 5. Univariable and multivariable logistic regression analysis of factors associated with PAS

Variable	Univariable OR (95% CI), *p*value	Multivariable OR (95% CI), *p*value
Maternal age, per year	1.17 (1.02–1.35), $p = 0.028$	1.11 (0.96–1.31), $p = 0.187$
Parity	Not reliably estimable due to quasi-complete separation	Not reliably estimable due to quasi-complete separation
Previous LSCS	Not reliably estimable due to quasi-complete separation	Not reliably estimable due to quasi-complete separation
Previous placenta previa	Not reliably estimable due to quasi-complete separation	Not reliably estimable due to quasi-complete separation
sFlt-1/PlGF ratio	1.00 (1.00–1.00), $p = 0.115$	1.00 (1.00–1.00), $p = 0.110$

Model fit:$\chi^2(7) = 20.06$, $p = 0.005$; pseudo-$R^2 = 0.26$; AIC = 72.3; C-statistic = 0.824; Hosmer–Lemeshow $\chi^2(8) = 11.08$, $p = 0.197$.

Note: Quasi-complete separation occurred for several categorical clinical variables because some categories were represented exclusively or predominantly in one outcome group. Therefore, reliable odds-ratio estimates and confidence intervals could not be obtained for these variables. The sFlt-1/PlGF ratio was entered as a continuous variable in its original measurement scale.

Multivariable logistic regression demonstrated quasi-complete separation for several categorical clinical variables, including parity, previous LSCS, and previous placenta previa, resulting in unstable or non-estimable odds-ratio estimates. Maternal age was significantly associated with PAS in the univariable analysis (OR 1.17, 95% CI 1.02–1.35; $p = 0.028$), but this association was no longer statistically significant after adjustment (adjusted OR 1.11, 95% CI 0.96–1.31; $p = 0.187$). The sFlt-1/PlGF ratio was not independently associated with PAS in the multivariable model (adjusted $p = 0.110$). Given the small sample size and quasi-complete separation, these adjusted estimates should be interpreted cautiously **(Table 5).**

Histopathology and immunohistochemistry

Among the 40 PAS cases, 20 were classified as accreta, 15 as increta, 3 as percreta, and 2 showed no histopathological evidence of PAS.

sFlt-1 immunohistochemical expression was weak in all placental specimens. PlGF expression varied from weak to high across the PAS subgroups, but its distribution did not differ significantly according to histopathological subtype ($p = 0.978$).

Table 6. Immunohistochemical expression of PlGF and sFlt-1 according to histopathological findings

IHC expression	Accreta (n=20)	Increta (n=15)	Percreta (n=3)	No evidence (n=2)	*p*value
PlGF					0.978
Weak	7 (35.0%)	4 (26.7%)	1 (33.3%)	1 (50.0%)	
Moderate	8 (40.0%)	8 (53.3%)	1 (33.3%)	1 (50.0%)	
High	5 (25.0%)	3 (20.0%)	1 (33.3%)	0 (0%)	
sFlt-1					1.000
Weak	20 (100%)	15 (100%)	3 (100%)	2 (100%)	

Abbreviations: IHC, immunohistochemistry; PlGF, placental growth factor; sFlt-1, soluble fms-like tyrosine kinase-1.

Table 6 summarizes the immunohistochemical (IHC) expression of PlGF and sFlt-1 according to histopathological findings. The analysis included 40 specimens: 20 accreta, 15 increta, 3 percreta, and 2 specimens without histopathological evidence of PAS.

For PlGF, weak, moderate, and high expression was observed across all PAS subtypes as well as in the two specimens without PAS. Moderate expression was the most frequent pattern in both accreta (40.0%) and increta (53.3%), whereas the three percreta cases showed an equal distribution of weak, moderate, and high expression (33.3% each). Among specimens without evidence of PAS, PlGF expression was either weak or moderate (50.0% each), with no high expression. However, there was no statistically significant difference in PlGF expression across the histopathological groups (p=0.978).

For sFlt-1, all specimens demonstrated weak IHC expression, including 100% of accreta, increta, percreta, and non-PAS specimens. Accordingly, there was no statistically significant variation in sFlt-1 expression between the groups (p=1.000).

Correlation of plasma PlGF with placental IHC expression

Plasma PlGF concentration differed significantly according to the intensity of placental PlGF immunohistochemical expression. Mean plasma PlGF concentrations increased progressively from weak to moderate and high tissue expression (21.59 ± 11.67, 319.39 ± 148.68, and 763.05 ± 202.26 pg/mL, respectively; p< 0.001) **(Table 7)**

Table 7. Plasma PlGF concentration according to placental PlGF immunohistochemical expression

PlGF IHC score	N	Mean plasma PlGF ± SD (pg/mL)
Weak (+1)	12	21.59 ± 11.67
Moderate (+2)	17	319.39 ± 148.68
High (+3)	9	763.05 ± 202.26

Kruskal–Wallis χ^2 = 33.102; p< 0.001.

This finding demonstrates a significant association between placental PlGF immunohistochemical expression and circulating plasma PlGF concentration. In contrast, no significant association was observed between placental sFlt-1 immunohistochemical expression and circulating sFlt-1 concentration.

Fig 4,5 and **6** below show representative H&E images and IHC images of placenta accreta, increta and percreta respectively, as found in this study.

Fig 4 a)Haematoxylin and eosin stain of placenta accreta histopathology(4x magnification). b)IHC stain showing moderate expression for PLGF in the same placenta accreta section (10x magnification). c)IHC stain showing weak expression for sFlt-1 in placenta accreta (4x magnification)

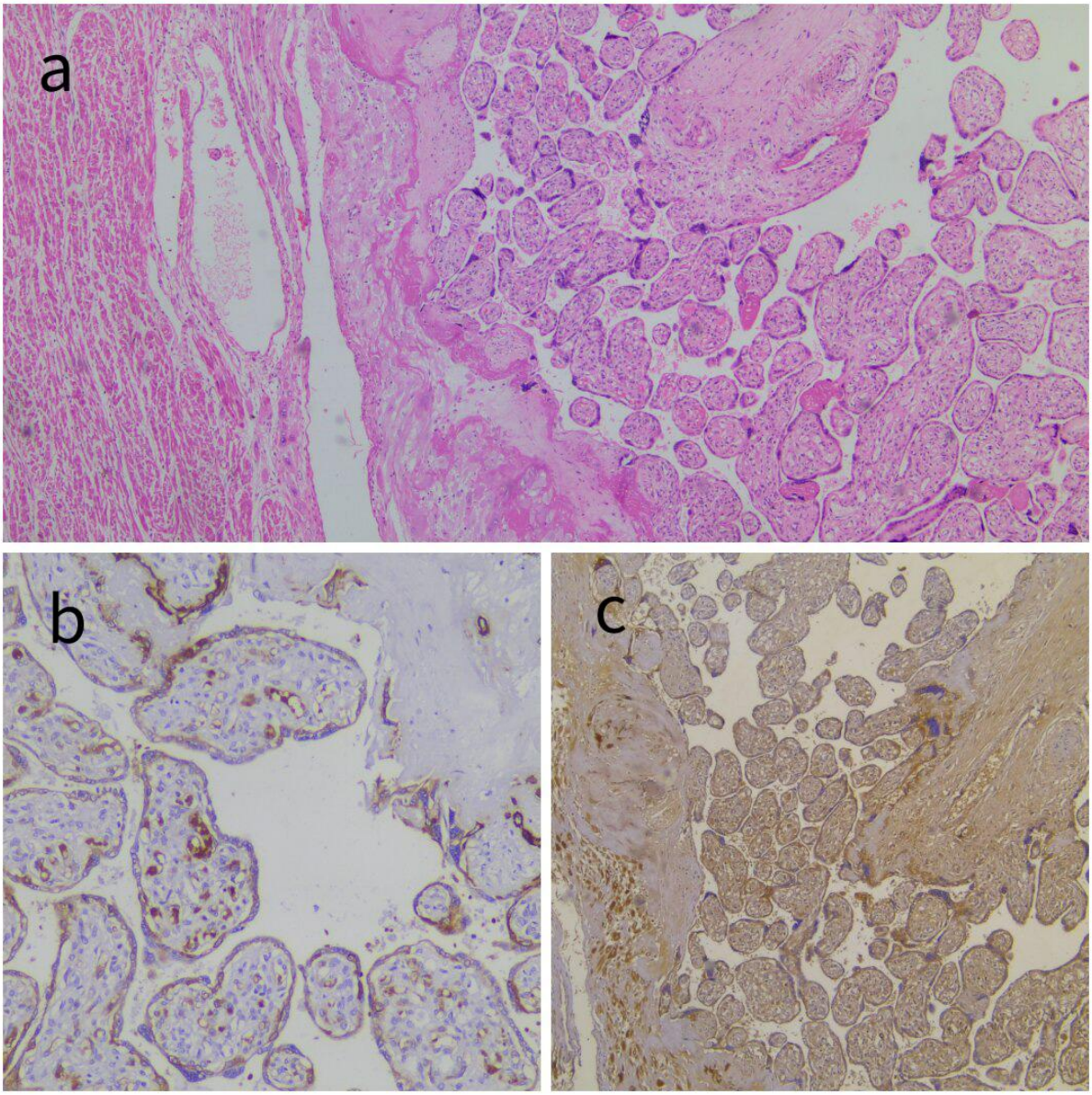


Fig 4

Fig 5 a)Haematoxylin and eosin stain of placenta increta histopathology(4x magnification). b)IHC stain showing strong expression for PLGF in the placenta increta (10x magnification). c) IHC stain showing weak expression for sFlt-1 in placenta increta (4x magnification)

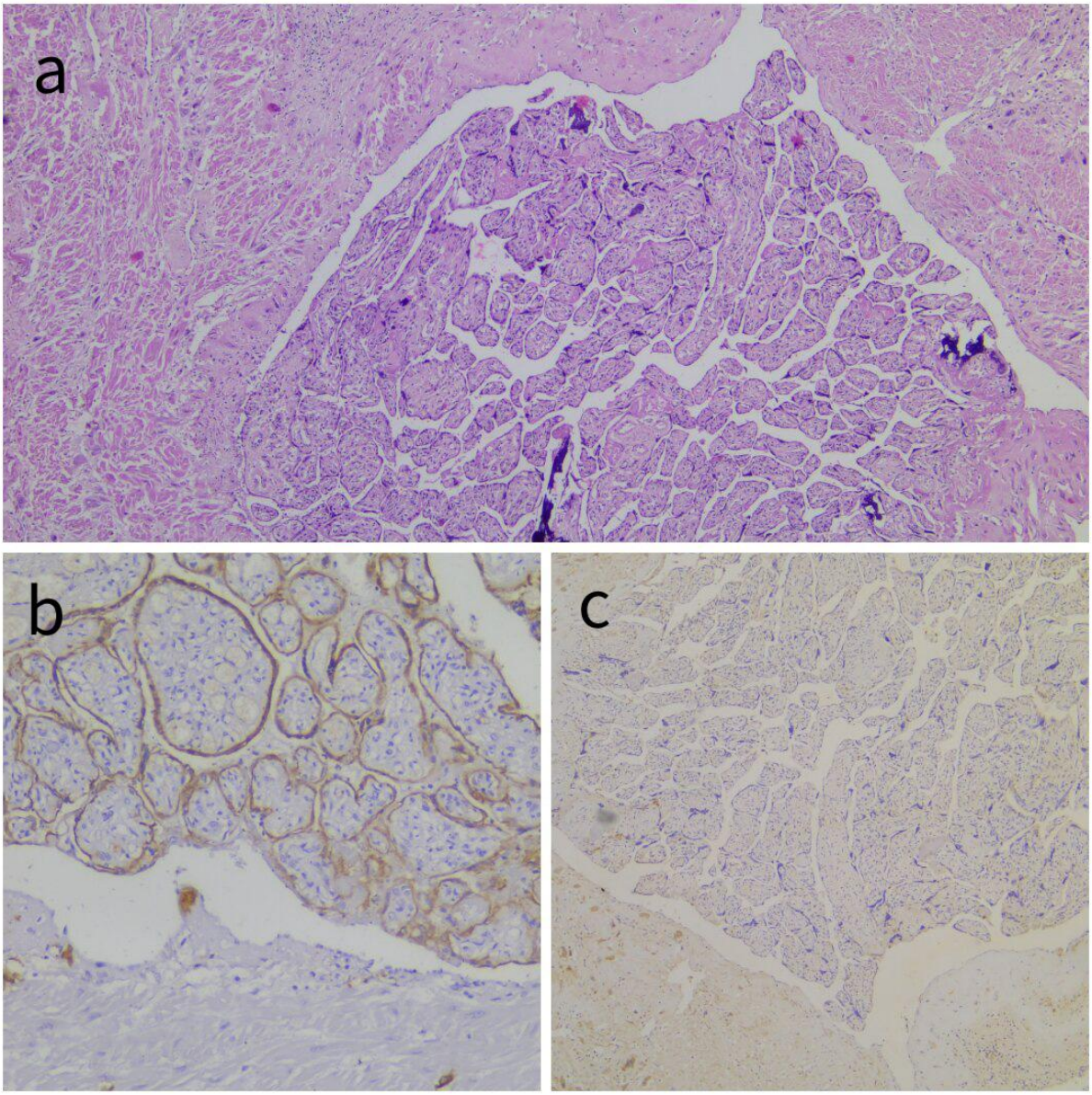


Fig 5

Fig 6 a)Haematoxylin and eosin stain of placenta percreta histopathology(4x magnification). b)IHC stain showing strong expression for PLGF in the same placenta accreta section (4x magnification). c)IHC stain showing weak expression for sFlt-1 in placenta percreta (4x magnification)

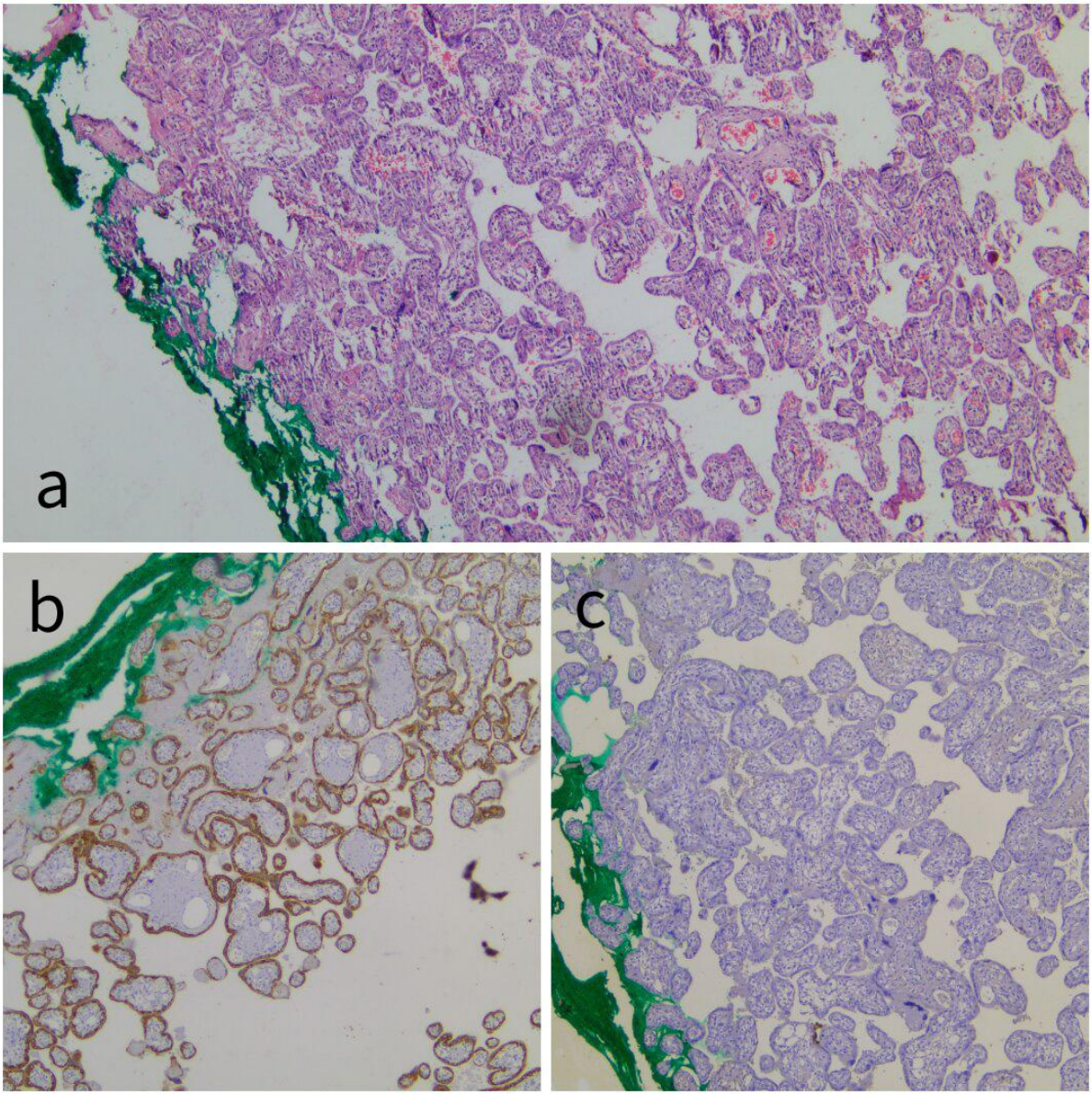


Fig 6

DISCUSSION

In this study, we evaluated maternal plasma sFlt-1, PlGF, and the sFlt-1/PlGF ratio in women with placenta accreta spectrum (PAS) and examined their relationship with placental immunohistochemical expression. Women with PAS had significantly lower sFlt-1, higher PlGF, and a lower sFlt-1/PlGF ratio than controls. These findings are consistent with altered angiogenic signalling in PAS and suggest that these biomarkers may provide additional biological information in women at risk of abnormal placentation **(Fig 4-6).**

Our findings are consistent with those of Zhang et al., who reported lower sFlt-1 and higher PlGF concentrations in women with PAS, resulting in a reduced sFlt-1/PlGF ratio [9]. The authors proposed that increased pro-angiogenic activity and reduced anti-angiogenic signalling may be associated with abnormal trophoblastic invasion and vascular remodelling at the

placental implantation site. The direction of these changes differs from the angiogenic profile typically described in preeclampsia, in which increased sFlt-1 and reduced PlGF are associated with an elevated sFlt-1/PlGF ratio. The concordance between our findings and previous PAS studies supports a possible association between altered angiogenic signalling and invasive placentation, although the present study cannot establish causality [10-13]

The sFlt-1/PlGF ratio demonstrated an area under the ROC curve of 0.753 in our cohort, with a sensitivity of 62.5% and specificity of 95% at a cut-off of ≤163. Lumbanraja et al. reported a sensitivity of approximately 67% and specificity of 97% for the sFlt-1/PlGF ratio in PAS [13]. The broadly comparable diagnostic estimates support the potential value of the ratio as an adjunctive biomarker. However, the confidence intervals in the present study were relatively wide because of the small sample size, and the diagnostic performance should therefore be considered preliminary and requires validation in larger cohorts.

The higher plasma PlGF concentration observed among PAS cases is consistent with the findings of Arakaza et al., who reported increased PlGF expression in invasive placentation [14]. In our study, placental PlGF immunohistochemical expression was significantly associated with circulating plasma PlGF concentration. This tissue–plasma relationship provides evidence of biological concordance between local placental expression and circulating PlGF levels. However, the association does not establish that increased PlGF production originates exclusively from invasive trophoblasts. In contrast, placental sFlt-1 expression was weak across the PAS subgroups, and no significant association was observed between tissue sFlt-1 expression and circulating sFlt-1 concentrations. Collectively, these findings are compatible with a relative shift towards pro-angiogenic signalling in PAS.

We found no statistically significant differences in plasma sFlt-1, PlGF, or the sFlt-1/PlGF ratio according to histopathological depth of invasion. Similar observations have been reported by Lumbanraja et al. and Arakaza et al., suggesting that angiogenic biomarkers may help distinguish PAS from non-PAS placentation but may not reliably discriminate between accreta, increta, and percreta [14,15]. The absence of a significant relationship between biomarker concentration and depth of invasion in our study may indicate that the angiogenic alterations associated with PAS are not directly proportional to the extent of myometrial invasion. Other anatomical, mechanical, vascular, and local microenvironmental factors may contribute to the final depth of placental invasion.

Two clinically diagnosed PAS cases showed no histopathological evidence of PAS in the examined specimens. Histopathological assessment in PAS may occasionally be challenging, particularly when the uterine wall is extensively disrupted or when the relevant implantation site is incompletely represented in the available specimen. Previous reports have described loss or disruption of decidual and myometrial tissue in severe invasive placentation, which may limit pathological confirmation [16]. Therefore, histopathological findings should be interpreted in conjunction with the clinical, imaging, and operative findings when assessing suspected PAS.

The clinical outcomes observed in our PAS cohort were consistent with the substantial maternal morbidity associated with the condition. PAS cases delivered at an earlier gestational age, had

substantially greater blood loss, required more blood products, and more frequently required additional haemostatic procedures than controls [6,17]. Although FIGO grade was not significantly associated with estimated blood loss in our cohort, this finding should be interpreted cautiously given the small number of participants within the individual invasion categories. One maternal death occurred in the PAS group because of massive haemorrhage and multiorgan dysfunction, illustrating the potential severity of maternal complications associated with PAS.

An important strength of this study is the combined assessment of circulating angiogenic biomarkers and placental immunohistochemical expression within the same cohort. This approach enabled assessment of the relationship between systemic biomarker concentrations and local placental expression. Prospective sample collection and standardized ELISA procedures further strengthened the laboratory component of the study. In addition, the study incorporated histopathological assessment of available placental tissue, allowing comparison of biomarker findings with pathological classification.

Several limitations should be considered. First, the final control group consisted of 20 participants compared with 40 PAS cases because the available ELISA reagents were exhausted before analysis of the remaining eligible control samples. This reduction in control numbers may have reduced statistical power and contributed to the relatively wide confidence intervals around the diagnostic estimates. Nevertheless, only samples with valid and reliable assay results were included in the final analysis.

Second, the groups differed in several baseline obstetric characteristics, particularly previous caesarean delivery, placenta previa, and gestational age at delivery. These differences reflect the clinical characteristics associated with PAS but also introduce potential confounding. In particular, the mean gestational age at delivery was substantially lower among PAS cases than controls. Because circulating PlGF and sFlt-1 concentrations vary with gestational age, this difference may have contributed to the observed biomarker differences. Future studies should therefore include gestational-age-matched controls or incorporate appropriate gestational-age adjustment into the study design.

Third, the multivariable logistic regression analysis was affected by quasi-complete separation because several clinical variables, including previous LSCS and placenta previa, were concentrated in one outcome group. This resulted in unstable or non-estimable odds-ratio estimates for some categorical variables. After adjustment, the sFlt-1/PlGF ratio was not independently associated with PAS (p= 0.110). Thus, although the ratio demonstrated discriminatory performance in the ROC analysis, its independent diagnostic contribution beyond established clinical risk factors could not be confirmed in this small cohort.

Fourth, biomarkers were assessed at a single time point close to delivery. Consequently, the present findings do not establish whether sFlt-1, PlGF, or the sFlt-1/PlGF ratio can identify PAS during the first-trimester screening window or predict subsequent development of clinically significant PAS. Serial assessment from early pregnancy through delivery would be necessary to determine the temporal relationship between angiogenic biomarkers and abnormal placentation. Finally, differences between assay platforms, laboratory procedures, and

population characteristics may influence biomarker concentrations and limit direct application of the cut-off values identified in this study to other settings.

The findings nevertheless indicate that angiogenic biomarkers may have a potential role as adjuncts to established clinical and imaging assessment. Ultrasound remains central to antenatal PAS evaluation, while biomarkers could potentially provide complementary information when imaging findings are equivocal or when access to specialist imaging is limited. In the present cohort, PlGF showed high specificity at the selected cut-off, whereas sFlt-1 showed high sensitivity. These estimates should, however, be interpreted in the context of the small sample size and should not be considered sufficient to replace imaging-based diagnosis.

Future studies should include larger, multicentre populations with balanced and appropriately matched controls, standardized assay platforms, and adjustment for gestational age and established PAS risk factors. Prospective longitudinal studies beginning in early pregnancy are particularly important to determine whether angiogenic biomarkers can identify women at increased risk before clinical or sonographic manifestations become evident. Composite models integrating biomarkers with ultrasound findings and established clinical risk factors may provide a more robust approach than individual biomarkers alone. Further mechanistic studies may also help clarify the contribution of angiogenic signalling to the pathogenesis and progression of PAS.

CONCLUSION

This study demonstrated significant alterations in maternal plasma sFlt-1, PlGF, and the sFlt-1/PlGF ratio among women with PAS compared with controls. The significant association between circulating PlGF concentrations and placental PlGF immunohistochemical expression further supports a relationship between systemic and placental angiogenic activity in PAS. The sFlt-1/PlGF ratio showed moderate discriminatory performance (AUROC 0.753); however, it was not independently associated with PAS after adjustment for clinical risk factors in this small cohort.

These findings suggest that angiogenic biomarkers may have potential as adjuncts to established clinical and ultrasound assessment, particularly when imaging findings are equivocal. However, the present results do not support their use as standalone diagnostic tests. Larger, multicentre prospective studies with appropriate gestational-age matching, standardized assays, and adjustment for established obstetric risk factors are required to validate biomarker cut-offs and determine their clinical utility in antenatal PAS risk assessment.

DECLARATIONS

Human Ethics Approval and Consent to Participate

Ethical approval for this study was obtained from the Institutional Ethics Committee of Vardhman Mahavir Medical College and Safdarjung Hospital(IEC approval no.

IEC/VMMC/SJH/Thesis/2023/CC-178; approved on 27 March 2023). The study was conducted in accordance with the principles of the Declaration of Helsinki (2013 revision). Written informed consent was obtained from all participants prior to inclusion in the study.

Consent for Publication

Written informed consent for publication of anonymized clinical and research data was obtained from all participants.

Availability of Data and Materials

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

Competing Interests

The authors declare that they have no competing interests.

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Authors' Contributions

Dr. Rahul Amitabh contributed to conceptualization of the study, patient recruitment, data collection, and manuscript drafting. Dr. Fouzia Siraj contributed to histopathology and immunohistochemistry analysis and manuscript review. Dr. Priyanka Pangtey contributed to clinical data acquisition and analysis. Dr. Sarita Singh contributed to supervision, study design, and critical manuscript revision. Dr. Ritu Singh contributed to pathological assessment and interpretation. Dr. Abhishek Lachyan contributed to literature review, statistical interpretation, manuscript editing, and final approval of the manuscript. All authors read and approved the final manuscript.

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