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RESEARCH

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Discussion on the evaluation of the therapeutic efficacy of uterine artery blood flow parameters and serum PLGF and sFlt-1 in patients with recurrent spontaneous abortion

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Abstract

Objective To investigate the effects of different drug treatments on uterine artery blood flow parameters, serum placental growth factor (PLGF), soluble fms-like tyrosine kinase-1 (sFlt-1), and sFlt-1/PLGF in patients with recurrent spontaneous abortion and to explore the predictive value of uterine artery blood flow parameters, serum PLGF, sFlt-1, and sFlt-1/PLGF for pregnancy outcomes.

Methods This retrospective cohort study included 173 patients who experienced recurrent spontaneous abortion and 100 control patients. Patients with recurrent spontaneous abortion were divided into an aspirin group (75 patients), aspirin combined with low molecular weight heparin (LMWH) group (68 patients), and non-drug group (30 patients) based on different drug treatments. Uterine artery blood flow parameters at gestational weeks 30-31^{+6} were monitored for the four groups, and serum samples were collected at gestational weeks 30-31^{+6} to measure the levels of serum PLGF and sFlt-1 and calculate the sFlt-1/PLGF ratio.

Results 1. Uterine artery blood flow parameters at gestational weeks 30-31^{+6} were significantly greater in the non-drug group than in the aspirin group, combined drug group, and control group (p<0.05). 2. Serum PLGF levels and the sFlt-1/PLGF ratio at gestational weeks 30-31^{+6} were significantly lower in the non-drug group than in the aspirin group, combined drug group, and control group, while serum sFlt-1 levels were significantly greater in the non-drug group than in the aspirin group, combined drug group, and control group (p<0.05). 3. Serum PLGF, sFlt-1, and sFlt-1/PLGF had lower diagnostic efficiency for predicting hypertensive disorders during pregnancy than the combined diagnostic efficiency of serum PLGF, sFlt-1, and sFlt-1/PLGF with uterine artery blood flow parameters at gestational weeks 30-31^{+6}.

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Conclusion Aspirin and aspirin combined with LMWH can upregulate serum PLGF and decrease serum sFlt-1 levels in patients with recurrent spontaneous abortion, reduce the miscarriage rate, and significantly improve pregnancy outcomes. The combination of serum PLGF, sFlt-1, sFlt-1/PLGF, and uterine artery blood flow parameters can effectively predict hypertensive disorders during pregnancy.

Keywords Recurrent spontaneous abortion, Uterine artery blood flow parameters, PLGF, sFlt-1, Pregnancy outcomes

Introduction

According to the expert consensus on recurrent spontaneous abortion (RSA) in China, recurrent spontaneous abortion is defined as the loss of pregnancy, including biochemical pregnancy, occurring two or more times with the same spouse, all before the 28th week of gestation [1]. The etiology and mechanism of recurrent spontaneous abortion are complex, involving multiple factors, and have been a focus of research. For example, genetic factors, chromosomal abnormalities in embryos, and chromosomal or genetic abnormalities in either spouse; endocrine factors, such as polycystic ovary syndrome in women, insulin resistance, diabetes, thyroid hormone secretion disorders caused by thyroid disease, hyperprolactinemia and so on; anatomical factors; various factors leading to uterine malformations, such as uterine septum, unicornuate uterus, bicornuate uterus, or pathological changes in uterine morphology or function, such as endometrial polyps, uterine fibroids, adenomyosis, cervical insufficiency, etc.; and infection factors, such as bacterial vaginosis, are important causes of early miscarriage or late miscarriage, while other infections, such as fungi, chlamydia, mycoplasma, TORCH, etc., causing vaginitis or cervicitis can also lead to miscarriage. Severe systemic infections caused by viruses or bacteria can also lead to miscarriage in pregnant women; autoimmune diseases, such as antiphospholipid syndrome, systemic lupus erythematosus, etc.; prethrombotic states (PTS), such as hyperhomocysteinemia, antiphospholipid syndrome, etc.; abnormalities in male sperm and seminal vesicles; or infections of the genitourinary system, varicocele, etc. In clinical practice, 40%-50% of patients with recurrent miscarriage in whom the known causes mentioned above are screened out, and the cause of miscarriage is still unclear, referred to as unexplained recurrent spontaneous abortion (URSA), also known as homologous immune-type RSA [2, 3]. Studies have shown that unexplained recurrent spontaneous abortion may be related to the microenvironment at the maternal–fetal interface [4, 5], the pathogenesis of which is complex, and the cause is not yet clear. The incidence of recurrent miscarriage is approximately 1% to 5%, which places great physiological and psychological burdens on couples preparing for pregnancy. If effective monitoring and treatment methods can be provided in early pregnancy, they will be highly important for clinical research on recurrent miscarriage.

Recent studies have shown that the main causes of miscarriage in pregnant women are endothelial dysfunction and placental ischemia-hypoxia [6]. In early pregnancy, trophoblast cells invade the spiral arteries of the uterus, and trophoblastic cells secrete corresponding vascular active factors to promote placental vascular formation. However, due to various stressors, such as inflammatory factor stimulation, oxidative stress, and even mechanical shear stress, the balance of vascular active factors is disrupted, leading to a decrease in vascular growth factors such as placental growth factor (PLGF) and an increase in antiangiogenic factors such as soluble fms-like tyrosine kinase-1 (sFlt-1), which hinder the normal function of the vascular endothelium and the formation of the placental vascular network, thereby affecting uterine artery blood flow perfusion and ultimately leading to placental ischemia-hypoxia.

Uterine artery blood flow perfusion can be indirectly observed clinically through uterine artery blood flow parameters, including the pulsatility index (PI), resistive index (RI), and peak systolic velocity/end diastolic velocity (S/D ratio).

Currently, there is no unified standard for the clinical treatment of recurrent spontaneous abortion. Studies have shown that aspirin can inhibit platelet aggregation, prevent the formation of microthrombi, and have a good antiplatelet effect. LMWH inhibits the activity of coagulation factors, reduces blood viscosity, and improves blood circulation. Both can also reduce trophoblast cell apoptosis. A large number of studies have reported that aspirin and LMWH can improve pregnancy outcomes in patients with recurrent miscarriage. This study aimed to monitor changes in uterine artery blood flow parameters and serum PLGF and sFlt-1 concentrations in patients with recurrent spontaneous abortion receiving different drug treatments, evaluate pregnancy outcomes, and explore the effects of drug treatments on patients with recurrent spontaneous abortion, providing a good clinical approach for the diagnosis and treatment of recurrent spontaneous abortion.

Methods

Study subjects

Data sources

This retrospective cohort study analyzed clinical data from 273 patients who were treated at the First Affiliated Hospital of Soochow University from December 2020 to June 2023. Patients with a history of two or more unexplained pregnancy losses with the same spouse were included in the recurrent spontaneous abortion group, totaling 173 patients. Based on the different clinical drug treatments, the patients were divided into three groups: non-drug group (30 patients), aspirin group (75 patients), and aspirin combined with low molecular weight heparin (LMWH) group (68 patients). A total of 100 patients with no history of pregnancy loss were included in the control group. This study was approved by the Ethics Committee of the First Affiliated Hospital of Soochow University (Approval No. 121 (2022)).

Inclusion and exclusion criteria

To reduce the impact of confounding variables, the following inclusion and exclusion criteria were used:

Inclusion criteria The patients in the RSA group were ① aged 20-39 years; ② had normal ovarian reserve function; ③ had singleton pregnancies; and ④ had a history of two or more pregnancy losses, including biochemical pregnancies, with the same spouse.

The control group included ① patients aged 20-39 years, ② patients with normal ovarian reserve function, ③ patients with singleton pregnancy, and ④ patients with no history of pregnancy loss.

Exclusion criteria The exclusion criteria were as follows: ① abnormal uterine anatomy, including uterine malformations, uterine fibroids, endometrial polyps, adenomyosis, intrauterine adhesions, etc.; ② chromosomal abnormalities in either spouse; ③ cervical insufficiency; ④ endocrine abnormalities such as hyperthyroidism, hypothyroidism, insulin resistance, polycystic ovary syndrome, diabetes, etc.; ⑤ uterine cervicitis or endometritis caused by chlamydia, mycoplasma, gonorrhea, fungi, etc.; ⑥ use of vasoactive drugs affecting coagulation function in the past six months; ⑦ internal or surgical complications.

Data collection Patients' menstrual history, marital history, reproductive history, history of adverse pregnancy outcomes, past medical history, surgical history, family genetic history, personal history (history of smoking, alcohol abuse, etc.), and history of exposure to harmful environments (chemicals, radiation, etc.) were collected. If the patient has a history of adverse pregnancy outcomes, inquire about the number and timing of adverse pregnancy outcomes, the gestational weeks at termination of pregnancy, the method of termination of pregnancy, and whether the products of conception were sent for genetic testing or chromosomal karyotype analysis.

Treatment methods

Aspirin group for RSA Starting from the month of planned pregnancy, use aspirin (enteric-coated aspirin tablets, Chenxin Pharmaceutical Co., Ltd., 50-75 mg orally per day) was used until one week before termination of the pregnancy.

Combined drug group for RSA Starting from the month of planned pregnancy, aspirin (enteric-coated aspirin tablets, Chenxin Pharmaceutical Co., Ltd., 50-75 mg orally per day) was used until one week before termination of pregnancy, and LMWH (enoxaparin, SANOFI WINTHROP INDUSTRIE, subcutaneous injection 0.4 ml (4000 AXaIU) per day) was used until 24 hours before termination of pregnancy.

Non-drug Group for RSA Due to the lack of standardized treatment for this group, all patients were monitored and treated at our reproductive center. These patients did not receive aspirin or LMWH treatment. Therefore, a small number of such patients were included in this study for comparative analysis.

Detection methods

Uterine artery blood flow parameter detection method

The color Doppler ultrasound instrument GE Voluson E8 was used to measure the bilateral uterine artery hemodynamic parameters of the four groups of patients at gestational weeks 30-31^{+6}, including the RI (resistive index), PI (pulsatility index), and S/D ratio (peak systolic velocity/end diastolic velocity of the uterine artery). During the measurements, the uterine arteries were sampled and transabdominally measured. First, the blood flow of the right uterine artery was measured. The blood flow image was optimized to obtain the best longitudinal scan displaying the curved beam. A blood flow sampling angle θ <30° along the long axis of the vessel corresponds to a stable blood flow spectrum at the neck level. After 3-5 consecutive stable cardiac cycles, the RI, PI, and S/D of the right uterine artery were recorded. This procedure was repeated to measure the RI, PI, and S/D of the left uterine artery. All parameters were measured three times, and the average values were taken and recorded as mRI, mPI,

and mS/D. All procedures were performed by the same senior ultrasound physician.

Serum PLGF and sFlt-1 detection methods

Three to five milliliters of nonanticoagulated venous blood was collected from the elbow vein of pregnant women in the morning on an empty stomach at gestational weeks 30-31^{+6}. After standing at room temperature for 30 minutes, the samples were centrifuged at 1000×g for 15 minutes. Immediately use the supernatant or store it at -80°C to avoid repeated freezing and thawing.

The instructions of the PLGF and sFlt-1 assay kits (purchased from R&D Systems, USA) were followed for operation, with all samples tested in triplicate by the same professional.

Experimental procedure

2.2.1 Before the experiment, the reagents were allowed to equilibrate at room temperature for 20-30 minutes. The required microplate strips were removed according to the experimental volume. The remaining strips were sealed with a desiccant in an aluminum foil bag and stored at 4°C. Use them within one week.

2.2.2 Add samples: Set up zero holes, standard holes, and test sample holes. Then, 100 μL of sample dilution buffer was added to the zero wells, and 100 μL of gradient-diluted standard or test sample was added to the remaining wells. The generation of bubbles was avoided (both the standard and sample should be tested in duplicate to minimize experimental errors, ensuring uninterrupted addition of samples, and complete the addition in 5-10 minutes).

2.2.3 Cover the microplate with a cover film and incubate at 37°C for 2 hours.

2.2.4 Wash:

The plate film was gently removed (to avoid excessive movement that may cause liquid to spill over into adjacent wells), the liquid was discarded, and the plate was patted dry.

The microplate was washed with 350-400 μL of washing solution (1×) per well. After washing, the liquid was shaken off, and the plate was pat-dried. This step was repeated 4 times to prevent foreign matter from entering the wells and the plate from drying out.

2.2.5 Add 100 μL of detection antibody (1×) to each well (refer to the reagent preparation section 2.5.2), cover the plate with a film, and incubate at 37°C for 1 hour.

2.2.6 Repeat step 2.2.4.

2.2.7 Add 100 μL of HRP-labeled streptavidin (1×) to each well, cover the plate with a film, and incubate at 37°C for 40 minutes.

2.2.8 Repeat step 2.2.4.

2.2.9 Color development: 100 μL of TMB chromogenic solution was added to each well, and the plates were incubated at 37°C in the dark for 15-20 minutes (if the color is too light, the incubation time can be extended appropriately, but not more than 30 minutes; if the color changes, the chromogenic substrate is colorless and transparent before the samples are added).

2.2.10 Termination: 100 μL of stop solution was added to each well, and the blue color was changed to yellow. The order of addition of the termination solution and the TMB chromogenic solution should be consistent (note: avoid contact of the eyes and skin with the termination solution).

2.2.11 Reading: A microplate reader was used to measure the optical density (OD) of each well at 450 nm, with 630 nm as the reference wavelength. The samples were read within 5 minutes after the addition of the stop solution. At no 630 nm wavelength, the 450 nm wavelength can also be used.

2.2.12 Data analysis: The OD value of the zero well was subtracted from each standard and sample well, and the average of duplicate samples was taken. The standard curve was used to calculate the concentration of the samples based on the OD values, which were multiplied by the dilution factor to obtain the actual concentration of the samples.

Statistical methods

All the data obtained in this study were analyzed using GraphPad Prism 9 and SPSS 22.0 software. Categorical data are presented as "patients (%)" and were compared using the chi-square test. Normally distributed continuous data are presented as the mean ± standard deviation (x±s) and were analyzed using one-way analysis of variance (ANOVA). When the variance was homogeneous, the Bonferroni (B) test was used; when the variance was heterogeneous, Tamhane's T2 (M) test was applied. Nonnormally distributed continuous data are presented as medians (interquartile ranges) and were analyzed using nonparametric tests. The trend of changes in uterine artery blood flow parameters was fitted using a function, and the fitting process was performed using the curve fitting toolbox in MATLAB R2020b. Missing data were supplemented using the K-nearest neighbor (KNN) method in MATLAB R2020b. The diagnostic

efficacy of uterine artery blood flow parameters and serum PLGF and sFlt-1 for pregnancy outcomes was evaluated using receiver operating characteristic (ROC) curves.

Results

Comparison of general characteristics

Comparisons of age and BMI among the four groups of patients and comparisons of the number of previous miscarriages among the RSA groups:

As shown in Table 1, one-way analysis of variance (ANOVA) revealed no significant differences in age or BMI among the aspirin group, combined drug group, non-drug group, or control group ($p > 0.05$). One-way ANOVA was also used to evaluate the number of previous pregnancy losses among the three groups of patients, and no significant differences were found in the number of previous pregnancy losses among the three groups ($p > 0.05$).

Comparison of uterine artery blood flow parameters

At gestational weeks 30-31^{+6}, the uterine artery blood flow parameters (including the pulsatility index, resistive index, and peak systolic velocity/end diastolic velocity) in the three RSA groups decreased in the following order: non-drug group > aspirin group, combined drug group, and control group. However, there was no significant difference in uterine artery blood flow parameters between the aspirin group, combined medication group, and control group (p>0.05) (Table 2).

Comparison of serum PLGF, sFlt-1, and pregnancy outcomes

Comparison of serum PLGF, sFlt-1, and sFlt-1/PLGF

The serum levels of PLGF, sFlt-1, and sFlt-1/PLGF in the three groups of RSA patients and the control group were statistically analyzed using one-way ANOVA. The results showed heteroscedasticity among the groups. Further comparisons between the groups were conducted using Tamhane's T2 (M) test. The results showed that the serum PLGF level in the non-drug group was significantly lower than that in the aspirin group, combined drug group, and control group. The serum sFlt-1 and sFlt-1/PLGF levels in the non-drug group were significantly greater than those in the aspirin group, combined drug group, and control group ($p < 0.05$). However, there were no significant differences in the PLGF, sFlt-1, or sFlt-1/PLGF ratio among the aspirin group, combined drug group, and control group ($p > 0.05$) (Table 3 and Fig. 1).

Table 1 Comparison of general characteristics

	Aspirin group(n=75)	Combined drug group(n=68)	Non-drug group(n=30)	Control group(n=100)	p
Age (years)	29.71±3.14	30.71±4.01	29.33±3.36	29.79±3.39	0.079
BMI (kg/m^2)	22.22(21.18, 23.26)	22.44(21.28, 23.28)	22.88(20.86, 23.83)	22.29(20.52, 23.68)	0.648
Previous Miscarriages	2(2,2)	2(2,2)	2(2,2)	-	0.384

* Values are presented as mean ± standard deviation for Age, and as median (interquartile range) for BMI and Previous Miscarriages

* p>0.05 indicates no significant difference between the groups

Table 2 Comparison of uterine artery blood flow parameters

	Aspirin group(n=75)	Combined drug group(n=68)	Non-drug group(n=30)	Control group(n=100)
mRI	0.43 ± 0.03◊	0.43 ± 0.04◊	0.52 ± 0.04▲□*	0.44 ± 0.08◊
mPI	0.62 ± 0.07◊	0.61 ± 0.08◊	0.75 ± 0.09▲□*	0.62 ± 0.08◊
mS/D	1.75 ± 0.14◊	1.73 ± 0.20◊	2.06 ± 0.14▲□*	1.78 ± 0.24◊

▲ indicates $p < 0.05$ compared to the control group; □ indicates $p < 0.05$ compared to the aspirin group; * indicates $p < 0.05$ compared to the combined drug group; ◊indicates $p < 0.05$ compared to the non-drug group

Table 3 Serum PLGF, sFlt-1 and sFlt-1/PLGF levels at 30-31^{+6} weeks of pregnancy

Group	Numbers	sFlt-1(ng/ml)	PLGF(ng/ml)	sFlt-1/PLGF
aspirin group	57	2234.71±888.80▲	371.86±175.01▲	7.59±4.75▲
combined drug group	63	2278.39±879.62▲	422.93±194.87▲	6.96±5.53▲
non-drug group	20	3262.38±941.48	175.57±50.47	20.24±8.09
control group	95	2215.95±737.58▲	366.43±162.48▲	7.91±5.44▲

▲ indicates $p < 0.05$ compared to the non-drug group

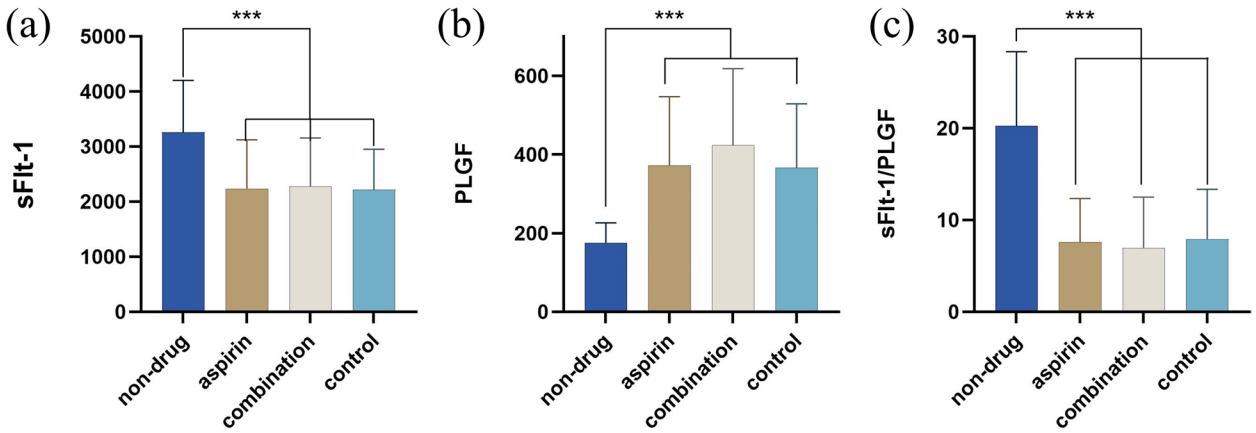

Fig. 1 **a** shows the serum sFlt-1 levels at gestational weeks 30-31^{+6}. The sFlt-1 level in the non-drug group was significantly greater than that in the aspirin group, combined drug group, and control group ($p < 0.05$). **b** Serum PLGF levels at gestational weeks 30-31^{+6}. The PLGF level in the non-drug group was significantly lower than that in the aspirin group, combined drug group, and control group ($p < 0.05$). **c** Serum sFlt-1/PLGF levels at gestational weeks 30-31^{+6}. The sFlt-1/PLGF ratio in the non-drug group was significantly greater than that in the aspirin group, combined drug group, and control group ($^{p} < 0.05$)

Comparison of pregnancy complications

Hypertensive disorders of pregnancy SPSS 25.0 was used to perform a statistical analysis of the occurrence of hypertensive disorders during pregnancy (including gestational hypertension and preeclampsia) among the three groups of RSA patients and the control group. The results showed a significant difference in the incidence of hypertensive disorders during pregnancy ($p < 0.05$). Further subgroup analysis using the chi-square test revealed that the incidence of gestational hypertension and preeclampsia in the non-drug group was greater than that in the aspirin group, combined medication group, and control group, with significant differences ($p < 0.0083$) (Table 4).

Other pregnancy complications Using SPSS 25.0, chi-square tests were performed to compare other pregnancy complications among the three RSA groups and

Table 4 Comparison of pregnancy outcomes among the four groups

	Aspirin group (*n*=75) □	Combined drug group (*n*=68) ⋆	Non-drug group (*n*=30) ◊	Control group (*n*=100) ▲	P
Live birth	57(76) ⋆▲	63(92.6) □◊	20(66.7) ▲⋆	95(95) □◊	<0.001
Miscarriage	18(24) ⋆▲	5(7.4) □◊	10(33.3) ▲⋆	5(5) □◊	
Gestational hypertension	2(3.5)	1(1.6)	6(30) □⋆▲	4(4.2)	0.003
Preeclampsia	1(1.8)	0	4(20) □⋆▲	2(2.1)	0.002
Liver function damage	3(5.3)	5(7.9)	2(10)	3(3.2)	0.136
Gestational diabetes	7(12.3)	5(7.9)	3(15)	10(10.5)	0.731
Premature birth	3(5.3)	4(6.3)	1(5.0)	4(4.2)	0.974
Fetal growth retardation	2(3.5)	1(1.6)	1(5.0)	3(3.2)	0.720
Placenta previa	1(1.8)	0	0	2(2.1)	0.730
Hypothyroidism	9(15.8)	7(11.1)	4(20)	12(12.6)	0.680
Placental abruption	1(1.8)	0	1(5.0)	2(2.1)	0.460
Neonatal asphyxia	2(3.5)	3(4.8)	3(15.0)	5(5.3)	0.298
Postpartum hemorrhage (ml)	283±157	279±133	244±112	216±98	0.568
Gestational age at delivery(week)	39.1 ± 2.5	39.0 ± 1.4	39.5± 1.7	40.2 ± 1.4	0.437
Newborn length (cm)	49.7 ± 2.3	49.6 ± 1.5	49.1 ± 2.6	49.9 ± 1.3	0.881
Newborn weight (g)	3220.0±464.5	3310.5±410.8	3209.3±479.2	3316.3±493.2	0.152
One-minute Apgar score	9.84± 0.7	9.92 ± 0.5	9.90 ± 0.61	9.97 ± 0.5	0.112
Ten-minute Apgar score	10	10	10	10	-

▲ indicates $p < 0.008$ compared to the control group; □ indicates $p < 0.008$ compared to the aspirin group; ⋆ indicates $p < 0.008$ compared to the combined drug group; ◊ indicates $p < 0.008$ compared to the non-drug group

the control group. There were no significant differences in the incidence rates of liver function damage, gestational diabetes, premature birth, fetal growth retardation, placenta previa, hypothyroidism, placental abruption, or neonatal asphyxia between the RSA group and the control group ($p > 0.05$) (Table 4).

Using SPSS 25.0 Using SPSS 25.0, one-way ANOVA was conducted to compare postpartum hemorrhage, gestational age at delivery, newborn length, newborn weight, one-minute Apgar score, and ten-minute Apgar score among the three RSA groups and the control group. There were no significant differences in postpartum hemorrhage, gestational age at delivery, newborn length, newborn weight, one-minute Apgar score, or ten-minute Apgar score among the four groups ($p > 0.05$) (Table 4).

Predictive value of uterine artery blood flow parameters and PLGF, sFlt-1, and sFlt-1/PLGF ratio for hypertensive disorders of pregnancy

Predictive value of PLGF, sFlt-1, and sFlt-1/PLGF for hypertensive disorders of pregnancy

(1) In the aspirin group, the predictive value of PLGF, sFlt-1, and the sFlt-1/PLGF ratio for hypertensive disorders of pregnancy (HDP) was evaluated.

The area under the curve (AUC) for diagnosing HDP was 0.182 for PLGF alone, indicating poor diagnostic performance. For sFlt-1 alone, the AUC was 0.870, indicating good diagnostic performance. When using sFlt-1/PLGF, the AUC was 0.926, indicating superior diagnostic performance compared to that of sFlt-1 alone (Table 5 and Fig. 2).

Table 5 Predictive value of PLGF, sFlt-1, and sFlt-1/PLGF in the aspirin group

Parameter	Region	Standard error[a]	Asymptotic sig[b]	95% confidence interval	
				(Lower)	(Upper)
PLGF	0.182	0.054	0.066	0.077	0.287
sFlt-1	0.870	0.052	0.032	0.768	0.972
sFlt-1/PLGF	0.926	0.043	0.014	0.841	1.000

[a] indicates that this standard error is calculated under the assumption that the null hypothesis is true, specifically under the assumption that the area under the curve (AUC) equals 0.5 (i.e., the performance of a random classifier)

[b] denotes that the *p*-value is calculated under the assumption that the AUC equals 0.5

(2) In the combined drug group, the predictive value of PLGF, sFlt-1, and sFlt-1/PLGF for hypertensive disorders of pregnancy (HDP) was evaluated.

In the combined drug group, the area under the curve (AUC) for diagnosing HDP was 0.097 when using PLGF alone, indicating poor diagnostic performance. When using sFlt-1 alone, the AUC was 0.517, indicating a relatively good diagnostic performance. When using sFlt-1/PLGF alone, the AUC was 0.890, indicating that the diagnostic performance of sFlt-1 was superior to that of sFlt-1 alone (Table 6 and Fig. 3).

(3) In the non-drug group, the predictive value of PLGF, sFlt-1, and sFlt-1/PLGF for hypertensive disorders of pregnancy (HDP) was evaluated.

In the non-drug group, the area under the curve (AUC) for diagnosing HDP was 0.265 when using PLGF alone, indicating poor diagnostic performance. When using sFlt-1 alone, the AUC was 0.850, indicating a relatively good diagnostic performance. When using sFlt-1/PLGF alone, the AUC was 0.810, indicating a diagnostic performance similar to that of sFlt-1 alone (Table 7 and Fig. 4).

(4) In the control group, the predictive value of PLGF, sFlt-1, and sFlt-1/PLGF for hypertensive disorders of pregnancy (HDP) was evaluated.

In the control group, the area under the curve (AUC) for diagnosing HDP was 0.198 when using PLGF alone, indicating poor diagnostic performance. When using sFlt-1 alone, the AUC was 0.664, indicating relatively poor diagnostic performance. When using sFlt-1/PLGF alone, the AUC was 0.801, indicating relatively good diagnostic performance (Table 8 and Fig. 5).

The combination of sFlt-1/PLGF and uterine artery blood flow parameters at 30-31^{+6} weeks has a predictive value for hypertensive disorders of pregnancy (HDP)

(1) The predictive value of sFlt-1/PLGF and uterine artery blood flow parameters at 30-31^{+6} weeks for HDP in the aspirin group.

In the aspirin group, the AUC for using sFlt-1/PLGF alone to diagnose HDP was 0.926, indicating good diagnostic performance. When using uterine artery blood flow parameters (UA-BFPs) at 30-31^{+6} weeks alone, the AUC was 0.833, indicating acceptable diagnostic performance. However, when sFlt-1/PLGF was combined with UA-BFP, the AUC was 0.944, indicating superior

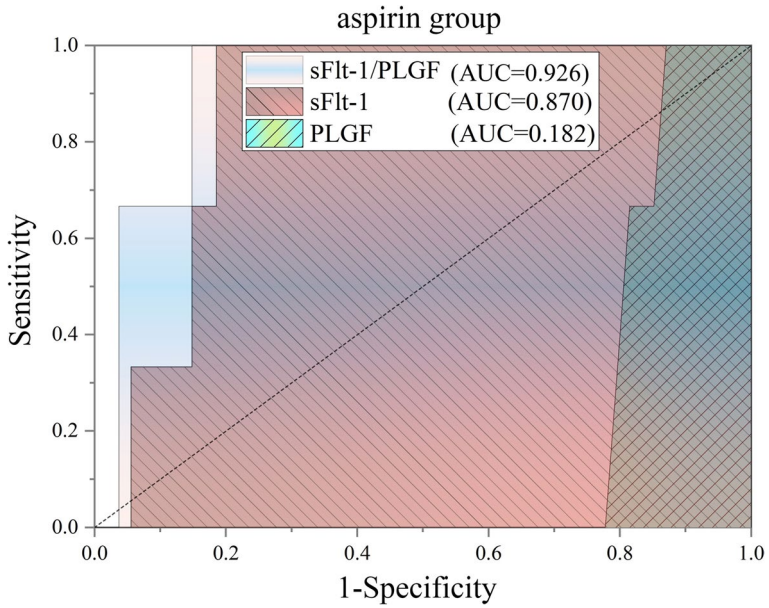

Fig. 2 Predictive value of PLGF, sFlt-1, and the sFlt-1/PLGF ratio for hypertensive disorders of pregnancy in the aspirin group

Table 6 Predictive value of the PLGF, sFlt-1, and sFlt-1/PLGF ratio in the combined drug group

Parameter	Region	Standard error[a]	Asymptotic sig[b]	95% confidence interval	
				(Lower)	(Upper)
PLGF	0.097	0.042	0.007	0.016	0.179
sFlt-1	0.517	0.099	0.910	0.322	0.712
sFlt-1/PLGF	0.890	0.042	0.010	0.808	0.972

[a] indicates that this standard error is calculated under the assumption that the null hypothesis is true, specifically under the assumption that the area under the curve (AUC) equals 0.5 (i.e., the performance of a random classifier)

[b] denotes that the *p*-value is calculated under the assumption that the AUC equals 0.5

diagnostic performance compared to the use of either parameter alone (Table 9 and Fig. 6).

(2) The predictive value of sFlt-1/PLGF and uterine artery blood flow parameters at 30-31^{+6} weeks for predicting HDP in the combined drug group.

In the combined drug group, the AUC for the ability of sFlt-1/PLGF alone to predict HDP was 0.887, indicating a fair diagnostic performance. When using uterine artery blood flow parameters (UA-BFPs) at 30-31^{+6} weeks gestation alone, the AUC was 0.839, also indicating a fair diagnostic performance. However, when sFlt-1/PLGF was combined with UA-BFP for diagnosing HDP, the AUC was 0.919, indicating that the diagnostic performance of sFlt-1/PLGF was superior to that of either parameter used alone (Table 10 and Fig. 7).

(3) The predictive value of sFlt-1/PLGF and uterine artery blood flow parameters at 30-31^{+6} weeks for predicting HDP in the non-drug group.

In the non-drug group, the AUC for the ability of sFlt-1/PLGF alone to predict HDP was 0.810, indicating a fair diagnostic performance. When using uterine artery blood flow parameters (UA-BFPs) at 30-31^{+6} weeks gestation alone, the AUC was 0.550, indicating poor diagnostic performance. However, when sFlt-1/PLGF was combined with UA-BFP for diagnosing HDP, the AUC was 0.940, indicating that the diagnostic performance of sFlt-1/PLGF was superior to that of either parameter used alone (Table 11 and Fig. 8).

(4) The predictive value of sFlt-1/PLGF and uterine artery blood flow parameters at 30-31^{+6} weeks for HDP in the control group.

In the control group, the AUC for sFlt-1/PLGF alone in diagnosing HDP was 0.801, indicating a fair diagnostic performance. When using uterine artery blood flow parameters (UA-BFPs) at 30-31^{+6} weeks gestation alone, the AUC was 0.681, indicating poor diagnostic performance. However, when sFlt-1/PLGF was combined with UA-BFP for diagnosing HDP, the AUC was 0.969, indicating that the diagnostic performance of sFlt-1/PLGF was superior to that of either parameter used alone (Table 12 and Fig. 9).

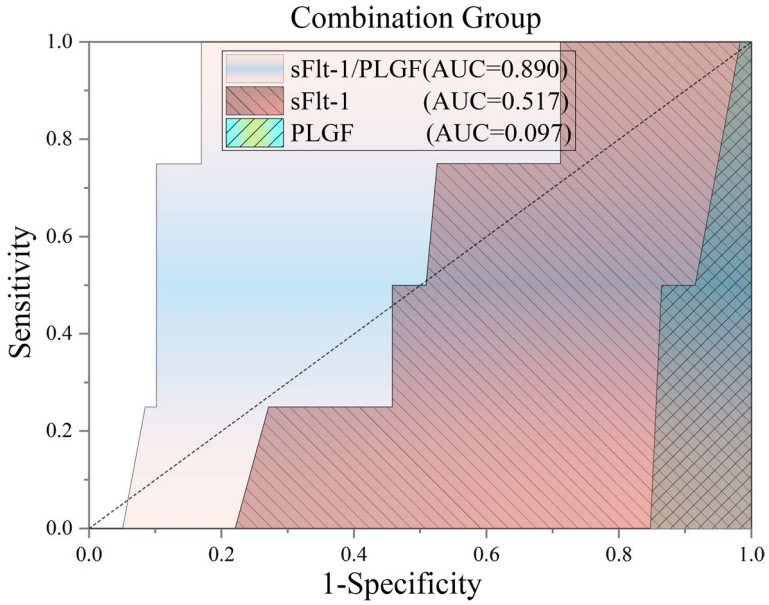

Fig. 3 Predictive value of PLGF, sFlt-1, and sFlt-1/PLGF for hypertensive disorders of pregnancy in the combined drug group

Table 7 Predictive value of the PLGF, sFlt-1, and sFlt-1/PLGF ratio in the non-drug group

Parameter	Region	Standard error[a]	Asymptotic sig[b]	95% confidence interval	
				(Lower)	(Upper)
PLGF	0.265	0.115	0.076	0.039	0.491
sFlt-1	0.850	0.090	0.008	0.674	1.000
sFlt-1/PLGF	0.810	0.103	0.019	0.607	1.000

[a] indicates that this standard error is calculated under the assumption that the null hypothesis is true, specifically under the assumption that the area under the curve (AUC) equals 0.5 (i.e., the performance of a random classifier)

[b] denotes that the *p*-value is calculated under the assumption that the AUC equals 0.5

Discussion

Recurrent spontaneous abortion is a complex condition with multifactorial etiology, and its specific causes and pathogenesis have been a hot topic of research. Currently, many theories suggest that endothelial dysfunction and placental ischemia-hypoxia are important factors leading to spontaneous miscarriage in pregnant women [7, 8]. The function of endothelial cells is regulated mainly by proangiogenic factors and antiangiogenic factors. Numerous studies have shown that placental growth factor (PLGF) and soluble fms-like tyrosine kinase-1 (sFlt-1) play important roles in pregnancy and in adverse pregnancy outcomes. Placental growth factor (PLGF) is a member of the vascular endothelial growth factor (VEGF) family. It is mainly secreted by syncytiotrophoblasts and is also expressed in the villous stroma and villous vessels. It is an important angiogenic factor. Due to its high homology with VEGF, PLGF binds to VEGF receptor-1 (VEGFR-1) and activates signaling pathways through autophosphorylation to exert its biological effects. VEGF receptor-1 (VEGFR-1) is divided into membrane-bound and soluble forms. The soluble form, known as soluble fms-like tyrosine kinase-1 (sFlt-1), is expressed in the placenta. sFlt-1 is an endogenous antagonist of VEGF that blocks receptor tyrosine kinase activation and plays an important role in endothelial dysfunction. Excess sFlt-1 in the circulation binds with high affinity to VEGF and PLGF, neutralizing their effects and reducing their concentrations in the circulation.

In the normal process of pregnancy, syncytiotrophoblasts invade the spiral arteries of the uterus early in pregnancy, and syncytiotrophoblasts secrete proangiogenic factors, thereby promoting placental vascular formation and remodeling and facilitating the normal development of the placenta and embryo. In the 1970s, some scholars believed that the impairment of trophoblast invasion and spiral artery remodeling was the cause of uteroplacental circulation defects and subsequent placental ischemia [9]. In 2009, Burton *et al.* successfully simulated damage to the chorionic villi due to failure of spiral artery remodeling [10], demonstrating that true placental ischemia may only occur late in the disease. Most current theories [11, 12] have suggested that various stressors, such as oxidative stress, inflammation, and possibly mechanical shear stress, contribute to the release of sFlt-1. When excess sFlt-1 enters the maternal circulation, it antagonizes VEGF-A and PlGF, causing endothelial dysfunction.

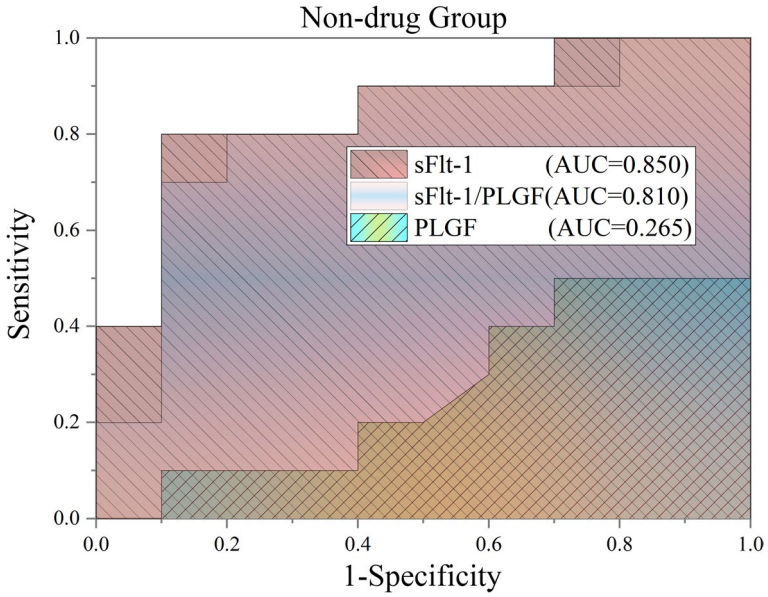

Fig. 4 Predictive value of PLGF, sFlt-1, and sFlt-1/PLGF for hypertensive disorders of pregnancy in the non-drug group

Table 8 Predictive value of PLGF, sFlt-1, and sFlt-1/PLGF in the control group

Parameter	Region	Standard Error[a]	Asymptotic sig[b]	95% confidence interval	
				(Lower)	(Upper)
PLGF	0.198	0.064	0.014	0.072	0.324
sFlt-1	0.664	0.102	0.180	0.464	0.863
sFlt-1/PLGF	0.801	0.097	0.014	0.610	0.992

[a] indicates that this standard error is calculated under the assumption that the null hypothesis is true, specifically under the assumption that the area under the curve (AUC) equals 0.5 (i.e., the performance of a random classifier)

[b] denotes that the *p*-value is calculated under the assumption that the AUC equals 0.5

The impaired development of vascular endothelial cells leads to impaired formation and branching of placental blood vessels, resulting in poor formation of the placental vascular network and ultimately leading to placental ischemia-hypoxia. This condition makes it difficult to provide a favorable environment for fetal survival, leading to various adverse pregnancy outcomes, including miscarriage, intrauterine fetal demise, preterm birth, pregnancy-induced hypertension, preeclampsia, and intrauterine fetal growth restriction. Similarly, the antiangiogenic state induced by excess production of sFLT-1 from the placenta can be rescued by administration of VEGF-A and PlGF [13].

The uterine artery is the main blood supply to the uterus and placenta, and uterine artery blood flow parameters can directly reflect blood perfusion in the placenta and embryo. Trophoblast invasion and subsequent remodeling of the uterine spiral arteries are important processes in placental formation during pregnancy [14]. The downregulation of PLGF and upregulation of sFlt-1 leading to endothelial damage results in impaired development of the endometrium and the muscle layer near the endometrium. This leads to poor infiltration of trophoblast cells into the muscle layer arteries, causing impaired uterine artery perfusion, ischemia, and subsequent ischemia–reperfusion, leading to a strong oxidative stress response in the embryo and resulting in impaired development, degeneration, stagnation, and, in severe cases, miscarriage. Second, ischemia–reperfusion injury caused by impaired placental blood perfusion affects the generation and release of vascular active factors in the body, and some inflammatory cytokines enter the patient's systemic circulation through the villous gaps, leading to systemic inflammatory reactions in the patient and further damage and destruction of the vascular endothelium, ultimately leading to adverse pregnancy outcomes such as miscarriage, pregnancy-induced hypertension, and preeclampsia.

Clinically, uterine artery blood flow perfusion can be measured by uterine artery blood flow parameters (RI, PI, and S/D). Numerous studies have shown that uterine artery blood flow parameters are significantly greater in patients with recurrent miscarriage than in healthy pregnant women.

Studies have shown that both aspirin and LMWH can reduce trophoblast apoptosis. During pregnancy, these agents can reduce uterine artery resistance parameters, significantly improve pregnancy outcomes, and reduce

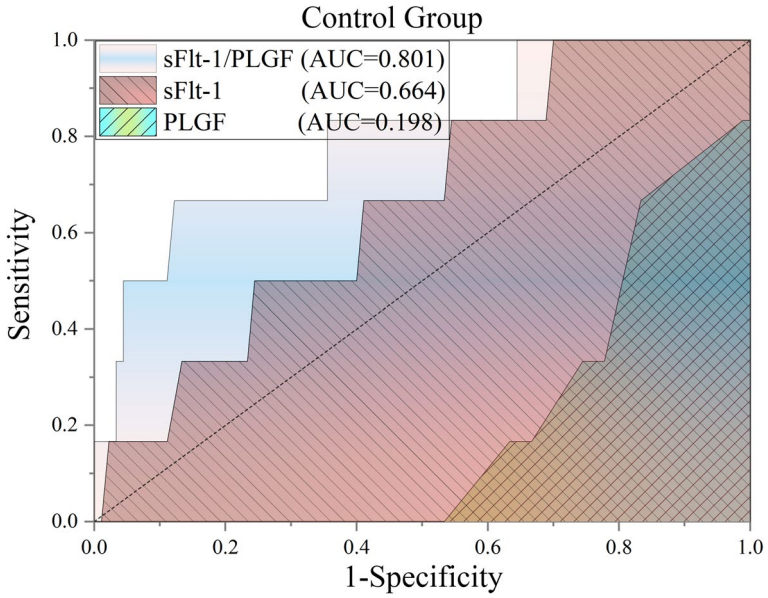

Fig. 5 Predictive value of the PLGF, sFlt-1, and sFlt-1/PLGF ratio for hypertensive disorders of pregnancy in the control group

Table 9 Predictive value of sFlt-1/PLGF and uterine artery blood flow parameters at 30-31^{+6} weeks in the aspirin group

Parameter	Region	Standard Error[a]	Asymptotic Sig[b]	95% Confidence Interval	
				(Lower)	(Upper)
sFlt-1/PLGF	0.926	0.043	0.014	0.841	1.000
UA-BFP at 30-31^{+6} Weeks	0.833	0.072	0.054	0.693	0.974
Combination	0.944	0.031	0.010	0.884	1.000

[a] indicates that this standard error is calculated under the assumption that the null hypothesis is true, specifically under the assumption that the area under the curve (AUC) equals 0.5 (i.e., the performance of a random classifier)

[b] denotes that the *p*-value is calculated under the assumption that the AUC equals 0.5

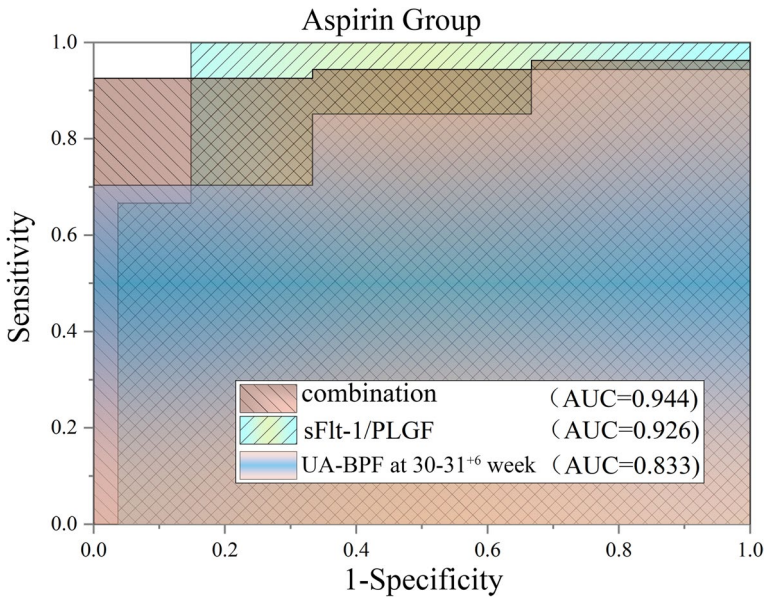

Fig. 6 Predictive value of the sFlt-1/PLGF ratio and uterine artery blood flow parameters at 30-31^{+6} weeks in the aspirin group

Table 10 Predictive value of sFlt-1/PLGF and Uterine artery blood flow parameters at 30-31^{+6} weeks in the combined drug group

Parameter	Region	Standard error[a]	Asymptotic sig[b]	95% confidence interval	
				(Lower)	(Upper)
sFlt-1/PLGF	0.890	0.040	0.187	0.808	0.966
UA-BFP at 30-31^{+6} Weeks	0.909	0.047	0.248	0.747	0.930
Combination	0.941	0.035	0.153	0.852	0.987

[a] indicates that this standard error is calculated under the assumption that the null hypothesis is true, specifically under the assumption that the area under the curve (AUC) equals 0.5 (i.e., the performance of a random classifier)

[b] denotes that the *p*-value is calculated under the assumption that the AUC equals 0.5

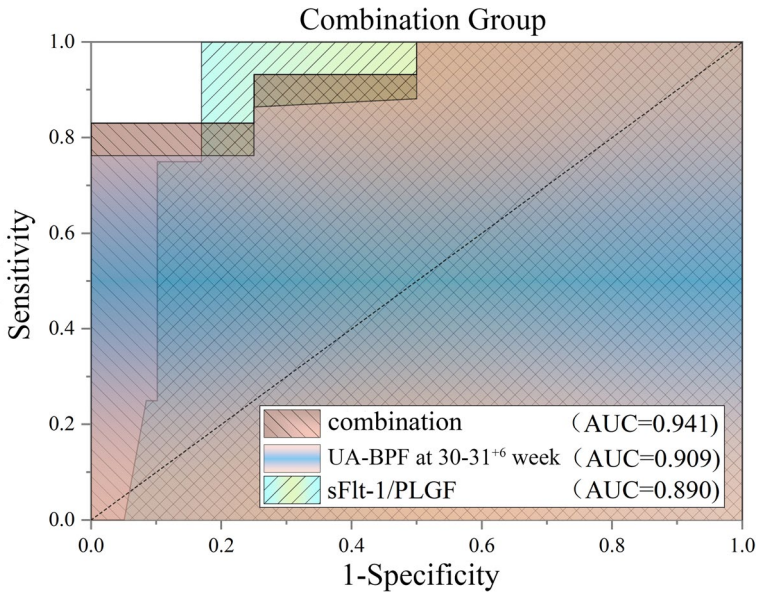

Fig. 7 Prediction value of sFlt-1/PLGF and uterine artery blood flow parameters at 30-31+6 Weeks in the non-drug group

Table 11 Predictive value of sFlt-1/PLGF and uterine artery blood flow parameters at 30-31^{+6} weeks in the non-drug group

Parameter	Region	Standard error[a]	Asymptotic sig[b]	95% confidence interval	
				(Lower)	(Upper)
sFlt-1/PLGF	0.810	0.103	0.019	0.607	1.000
UA-BFP at 30-31^{+6} Weeks	0.550	0.140	0.705	0.276	0.824
Combination	0.940	0.051	0.001	0.840	1.000

[a] indicates that this standard error is calculated under the assumption that the null hypothesis is true, specifically under the assumption that the area under the curve (AUC) equals 0.5 (i.e., the performance of a random classifier)

[b] denotes that the *p*-value is calculated under the assumption that the AUC equals 0.5

miscarriage rates [15]. Aspirin inhibits platelet aggregation and increases prostaglandins, achieving a good anticoagulant effect [16]. LMWH inhibits the activity of coagulation factors [17, 18], reduces blood viscosity, improves blood circulation, prevents microthrombosis, reduces local oxidative stress reactions in the placenta, regulates the intrauterine environment, increases blood supply to the placenta and embryo, reduces miscarriage and fetal death, and improves pregnancy outcomes [19]. Recent studies have also shown that aspirin and LMWH, in addition to their good anticoagulant effects, can promote trophoblast proliferation, invasion, and differentiation; inhibit trophoblast apoptosis; protect vascular endothelium; and promote placental formation [20, 21].

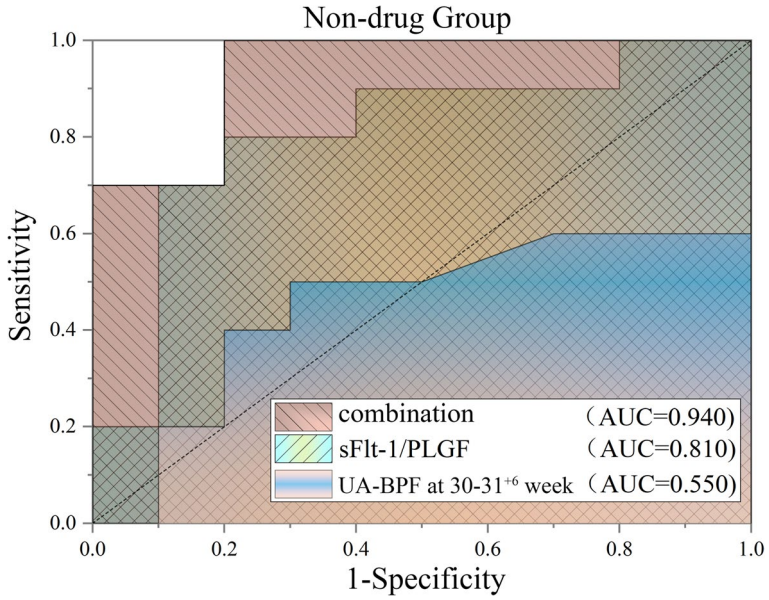

Fig. 8 Prediction value of sFlt-1/PLGF and uterine artery blood flow parameters at 30-31^{+6} weeks in the combined drug group

Table 12 Predictive value of sFlt-1/PLGF and uterine artery blood flow parameters at 30-31^{+6} weeks in the control group

Parameter	Region	Standard error[a]	Asymptotic sig[b]	95% confidence interval	
				(Lower)	(Upper)
sFlt-1/PLGF	0.801	0.097	0.014	0.610	0.992
UA-BFP at 30-31^{+6} Weeks	0.681	0.133	0.140	0.419	0.942
Combination	0.969	0.021	0.000	0.927	1.000

[a] indicates that this standard error is calculated under the assumption that the null hypothesis is true, specifically under the assumption that the area under the curve (AUC) equals 0.5 (i.e., the performance of a random classifier)

[b] denotes that the *p*-value is calculated under the assumption that the AUC equals 0.5

This study analyzed the uterine artery blood flow parameters and serum PLGF and sFlt-1 levels of patients with recurrent spontaneous abortion treated with aspirin alone, aspirin combined with LMWH, or no medication to evaluate their relationship with pregnancy outcomes and explore the impact of medication on patients with recurrent spontaneous abortion.

Uterine artery blood flow parameters

The results of this study revealed changes in uterine artery blood flow parameters between 30 and 32 weeks of pregnancy. There were no significant differences in the data between the aspirin group, combined drug group, and control group, while the non-drug group continued to have higher uterine artery blood flow parameters (including the pulsatility index, resistive index, and peak systolic velocity/end diastolic velocity) than did the other three groups. This may suggest that after 30 weeks of pregnancy, the use of aspirin or aspirin combined with low molecular weight heparin in women with recurrent spontaneous abortion is no different from that in normal pregnant women. However, further research is needed to determine whether discontinuing drug treatment at this time leads to changes in uterine artery blood flow parameters and pregnancy outcomes. On the other hand, in the non-drug group, from before pregnancy to 32 weeks of pregnancy, uterine artery blood flow parameters were all greater than those in normal pregnant women, and there was no change at the 30-week pregnancy mark. This indicates that early and timely treatment is crucial for patients with recurrent miscarriage.

Serum placental growth factor (PLGF) and soluble fms-like tyrosine kinase-1 (sFlt-1)

There are reports in the literature that the serum level of placental growth factor (PLGF) in nonpregnant patients is extremely low. After pregnancy, the PLGF level slowly begins to rise. In normal pregnant women, the serum

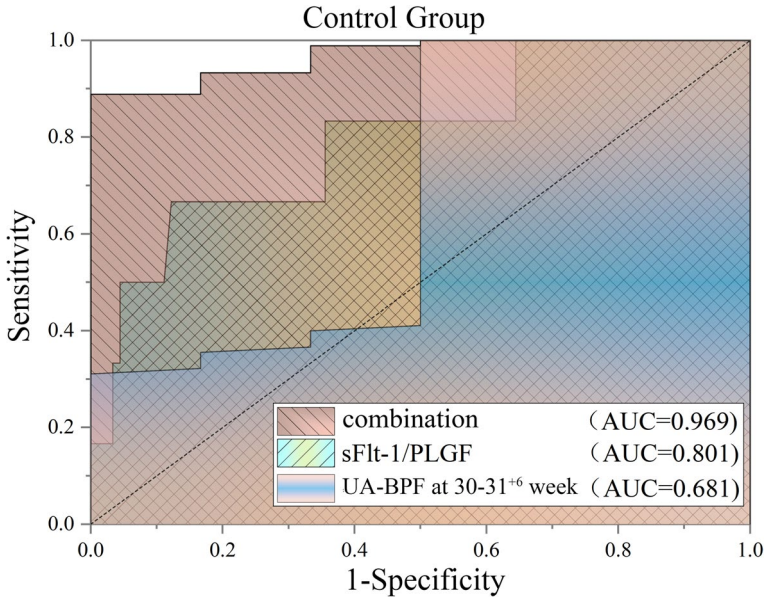

Fig. 9 Predictive value of sFlt-1/PLGF and uterine artery blood flow parameters at 30-31^{+6} weeks in the control group

PLGF concentration gradually increases with gestational age, peaking at approximately 28-30 weeks of gestation, after which it gradually decreases to 55-65 pg/ml in late pregnancy. This is mainly because PLGF is mainly secreted by the syncytiotrophoblast cells of the placenta. In early pregnancy, the placenta is immature, the placental circulation is incomplete, and the placenta is in a hypoxic state, leading to lower PLGF levels. As the placental blood flow circulation improves, the placental vasculature gradually changes from a branching structure to a nonbranching structure, and PLGF regulates the formation of nonbranching blood vessels. When the placenta is most mature, the PLGF also peaks. After 30-32 weeks of gestation, as the placenta matures and begins to age, the PLGF gradually decreases.

On the other hand, soluble fms-like tyrosine kinase-1 (sFlt-1) levels do not change significantly in early to mid-pregnancy but gradually increase in late pregnancy. Therefore, the sFlt-1/PLGF ratio is lowest in mid-pregnancy [22, 23]. This study selected the mid-pregnancy period, specifically at 30-31^{+6} weeks, when the PLGF levels were the highest and the sFlt-1/PLGF ratio was the lowest, to monitor the serum PLGF and sFlt-1 levels and compare the sFlt-1/PLGF ratios. The results showed that the serum PLGF level in the nondrug group was significantly lower than that in the other three groups, while there was no significant difference in the serum PLGF level among the other three groups. The serum sFlt-1 level was significantly greater in the nondrug group than in the other three groups, with no significant difference among the three groups. Studies have shown that monitoring decreased serum PLGF and increased sFlt-1/PLGF in early pregnancy is associated with an increased risk of miscarriage [24]. Similarly, monitoring decreased serum PLGF and increased sFlt-1/PLGF in mid-pregnancy is associated with a significantly increased risk of developing gestational hypertension and preeclampsia. In this study, there was no significant difference in the serum PLGF and sFlt-1/PLGF levels between the aspirin group and the combined therapy group, while the untreated group showed decreased serum PLGF and increased serum sFlt-1 and sFlt-1/PLGF levels, which may indicate that both single-agent aspirin and aspirin combined with low molecular weight heparin can correct these levels to those of normal pregnant women, achieving normal serum factor levels.

Pregnancy outcomes

This study examined the rates of live birth, miscarriage, and pregnancy complications. The miscarriage rates in patients ranked from highest to lowest were as follows: non-drug group > aspirin group > combined drug group > control group. Subgroup comparison analysis revealed a significant difference in the miscarriage rate between the aspirin group and the combined drug group. This demonstrates that aspirin combined with low molecular weight heparin (LMWH) can effectively reduce the miscarriage rate in patients with recurrent spontaneous abortion, and the effect is superior to that of aspirin alone. This result is consistent with the findings of Peter Clark [25] and YU X M [26], who reported that the live birth rate in the combined medication group was greater than that in the aspirin group, suggesting that the

combination of LMWH and aspirin is more effective at improving the miscarriage rate than aspirin alone.

For pregnancy complications, only gestational hypertension and preeclampsia differed among the four groups. The incidence of other pregnancy complications, including liver dysfunction, gestational diabetes, preterm birth, fetal growth restriction, placenta previa, hypothyroidism, placental abruption, and neonatal asphyxia, did not significantly differ among the four groups. The incidence of gestational hypertension and preeclampsia in the nondrug group was greater than that in the other three groups, while there was no significant difference in incidence among the other three groups. This finding is consistent with our findings for mid-pregnancy serum PLGF, sFlt-1, and uterine artery blood flow parameters. Patients with low PLGF, high sFlt-1, and high uterine artery blood flow parameters had a correspondingly increased incidence of gestational hypertension and preeclampsia.

Previous studies by Liu Xiaoning and others have shown that pregnant women with preeclampsia have significantly lower serum PLGF levels than women in the control group in early pregnancy (11-13^{+6} weeks of gestation), and their uterine artery blood flow parameters are significantly greater than those of women in the control group [27–30]. Hasko *et al.* [31] studied serum PLGF, sFlt-1, and sFlt-1/PLGF levels in 164 women with preeclampsia and 36 women with gestational hypertension and reported that the sFlt-1/PLGF ratio in the preeclampsia group was significantly greater than that in the normal control group; thus, the sFlt-1/PLGF ratio could be used as a good diagnostic tool for distinguishing preeclampsia in pregnant women. These results are consistent with the findings of our study.

The predictive value of uterine artery blood flow parameters, serum placental growth factor (PLGF), soluble fms-like tyrosine kinase-1 (sFlt-1), and the sFlt-1/PLGF ratio for pregnancy outcomes

In this study, PLGF showed poor predictive efficacy for predicting hypertensive pregnancy disorders, while sFlt-1 demonstrated good predictive value, with the sFlt-1/PLGF ratio showing even better predictive value than sFlt-1 alone. When uterine artery blood flow parameters (mRI, mPI, mS/D) were combined with serum PLGF, sFlt-1, and sFlt-1/PLGF, the predictive value for pregnancy-induced hypertension and preeclampsia was significantly improved. Reports in the literature indicate that the sFlt-1/PlGF ratio is a better screening tool than serum PLGF or sFlt-1 alone [32–36]. Studies by Liu Tingting and others have shown that combining uterine artery ultrasound with PLGF and other indicators can effectively improve the accuracy of diagnosing preeclampsia in early pregnancy and mid-pregnancy [37, 38]. This is consistent with the results of this study.

One of the primary limitations of our study is its retrospective nature, which inherently carries the risk of selection bias. Patients were not randomly assigned to treatment groups; instead, treatment decisions were made based on clinical indications, physician judgment, and patient preferences. While this reflects real-world practice, it introduces the possibility that unmeasured confounding factors could influence the observed outcomes. We have taken this into account in our interpretation of the results, and we advise caution in generalizing these findings. Future prospective studies are warranted to further explore and confirm the efficacy of these treatment strategies in patients with recurrent spontaneous abortion.

Conclusion

The uterine artery blood flow parameters, serum PLGF and sFlt-1 levels, and incidence of hypertensive disorders during pregnancy in the aspirin group and the combined drug group were similar to those in normal pregnant women. In contrast, the nondrug group had significantly greater uterine artery blood flow parameters, serum sFlt-1 levels, and incidence of hypertension, along with significantly lower serum PLGF levels.

Uterine artery blood flow parameters, serum PLGF, sFlt-1, and the sFlt-1/PLGF ratio can predict the incidence of hypertensive disorders during pregnancy to a certain extent, with a greater predictive value when these indicators are combined.

Clinical trial number

The clinical trial number is: (Approval No. 121 (2022)).

Authors' contributions

Xiaolu Lian and Yanyu Zhong wrote the main manuscript text. Ru Sun, Ying Zhou and Fia Xia prepared figures and tables. All authors reviewed the manuscript.

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Availability of data and materials

The data underlying this article cannot be shared publicly because of the privacy of individuals who participated in the study. The data will be shared upon reasonable request with the corresponding author.

Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

The study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the ethics committee of the first affiliated hospital of Soochow University.(Approval No. 121(2022)). All participants provided written informed consent.

Consent for publication
Not applicable.

Competing interest
The authors declare no competing interests.

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