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Original Article

Predictive value of placental growth factor level for adverse pregnancy outcome in twin pregnancies at advanced maternal age

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Abstract: Objective: To investigate the predictive value of placental growth factor (PlGF) for adverse pregnancy outcome in twin pregnancies at advanced maternal age. Methods: A retrospective analysis was conducted on 387 women with twin pregnancies who delivered at Northwest Women's and Children's Hospital between March 2020 and March 2024. The women were divided into a favorable outcome group (n = 249) and an adverse outcome group (n = 138) based on their pregnancy outcome. Clinical data and laboratory indicators were compared between the two groups. Logistic regression analysis was used to identify independent risk factors for adverse pregnancy outcome. Receiver Operating Characteristic (ROC) curve analysis was performed to evaluate the predictive value of these independent risk factors. Additionally, the interaction between PlGF and other independent risk factors was analyzed. Results: Significant differences were observed between the favorable and adverse outcome groups in terms of age, pre-pregnancy Body Mass Index (BMI), mode of conception, gestational hypertension, and gestational diabetes (all $P < 0.05$). Laboratory indicators revealed that the levels of White Blood Cells (WBC), Neutrophils (Neut), Alpha-Fetoprotein (AFP), Beta Human Chorionic Gonadotropin (β-HCG), and 24-hour urine protein quantification were lower in the favorable outcome group, while the levels of Lymphocytes (Lym) and PlGF were higher (all $P < 0.05$). Multivariate logistic regression analysis identified mode of conception, Neut, Lym, AFP, β-HCG, 24-hour urine protein quantification, and PlGF as independent risk factors for adverse pregnancy outcome (all $P < 0.05$). The Area Under the Curve (AUC) for PlGF in predicting adverse pregnancy outcomes was 0.874. Furthermore, an interaction was found between PlGF and adverse pregnancy outcome ($P < 0.001$) as well as between PlGF and 24-hour urine protein level ($P = 0.035$). Conclusion: PlGF has significant clinical value for predicting adverse pregnancy outcome in twin pregnancies among women of advanced maternal age. Its levels are strongly correlated with pregnancy outcome and may serve as an effective tool for early screening and intervention in high-risk pregnancies.

Keywords: Advanced maternal age, twin pregnancies, placental growth factor, pregnancy outcomes, predictive value

Introduction

Twin pregnancy is a complex condition characterized by simultaneous gestation of two fetuses, which poses heightened risks and challenges for both the mother and the fetuses throughout the pregnancy [1]. Complications associated with twin pregnancies, such as gestational hypertension, gestational diabetes, preterm birth, and placental complications, can significantly affect maternal and neonatal health [2]. The placenta plays a particularly crucial role in twin pregnancies, as its morphologic and functional abnormalities can directly influence fetal growth and pregnancy outcomes [3]. Advanced maternal age further elevates the risk of adverse outcome in twin pregnancies due to the decline in physiological functions [4]. Studies have shown that advanced maternal age is strongly associated with the development of gestational hypertension and preeclampsia, both of which can severely affect

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maternal and neonatal prognosis [4]. Additionally, older mothers are more likely to develop gestational diabetes, complicating the pregnancy and increasing the risk of adverse outcomes, such as fetal growth restriction and preterm labor. Placental abnormalities, including insufficiency and dysfunction, are more prevalent in twin pregnancies among older women, leading to an increased risk of intrauterine distress, preterm labor, and low birth weight infants. These factors collectively contribute to the higher incidence of adverse pregnancy outcome in twin pregnancies among women of advanced maternal age [5]. Therefore, effective management and monitoring of twin pregnancies are particularly important in older women.

Placental Growth Factor (PlGF) is a protein secreted by the placenta that belongs to the vascular endothelial growth factor (VEGF) family [6]. Its primary role during pregnancy is to promote placental angiogenesis and maintain normal placental function, thereby ensuring proper fetal development [7]. PlGF regulates placental blood supply, ensuring that the fetus receives adequate oxygen and nutrients to support healthy growth and development [8]. Research has shown that abnormal PlGF levels are often associated with various pregnancy complications, such as preeclampsia [9] and fetal growth restriction [10]. Low levels of PlGF are closely linked to the development of preeclampsia, a phenomenon extensively studied and validated in singleton pregnancies [11]. Moreover, PlGF levels can serve as an important biomarker for predicting pregnancy outcome, particularly in the context of gestational hypertensive diseases and fetal growth restriction [12]. However, research on PlGF in twin pregnancies is relatively limited, with most studies focusing on PlGF levels in singleton pregnancies and their predictive value for adverse outcomes [13]. The levels of PlGF in twin pregnancies and their predictive significance, particularly in women of advanced maternal age, warrant further investigation.

Therefore, this study aims to explore the predictive value of maternal plasma PlGF levels for adverse pregnancy outcomes in twin pregnancies at advanced maternal age and to analyze their correlation. By monitoring and analyzing PlGF levels, we seek to evaluate its effectiveness in predicting adverse pregnancy outcome, thereby providing a basis for early intervention and management.

Materials and methods

Ethical statement

This study was conducted in accordance with the Declaration of Helsinki and was approved by the Medical Ethics Committee of Northwest Women's and Children's Hospital.

Case source and grouping

A retrospective cohort study was performed on women with twin pregnancies who delivered at Northwest Women's and Children's Hospital between March 2020 and March 2024. A total of 387 eligible cases were identified based on predefined inclusion and exclusion criteria. The participants were categorized into two groups based on their pregnancy outcomes: a favorable outcome group (n = 249) and an adverse outcome group (n = 138). Grouping was determined by reviewing medical records for specific maternal and neonatal outcomes, ensuring consistent and accurate classification.

Inclusion and exclusion criteria

Inclusion criteria: (1) Confirmed twin pregnancy [14]; (2) Age ≥ 35 years; (3) Gestational age of 14-28 weeks; (4) All prenatal examinations conducted at Northwest Women's and Children's Hospital; (5) Complete clinical data.

Exclusion criteria: (1) Presence of congenital diseases; (2) Concurrent malignant tumors; (3) Congenital cardiovascular diseases; (4) Presence of underlying diseases or reproductive system diseases; (5) Use of corticosteroids or other medications during pregnancy that may affect serum marker levels.

Definition of adverse pregnancy outcome

Adverse pregnancy outcomes were meticulously defined to include both maternal and neonatal complications. For maternal outcomes, adverse events included preterm birth (delivery before 37 weeks of gestation), premature rupture of membranes (PROM), and postpartum hemorrhage (blood loss exceeding 500 mL within 24 hours after delivery). For neonatal outcomes, adverse events included intrauterine distress (non-reassuring fetal status), neo-

natal asphyxia (Apgar score of 3 or less at 1 minute or 5 or less at 5 minutes), and neonatal death (death within the first 28 days of life) [15].

Collection of clinical data

Clinical data and laboratory indicators were collected from the hospital's electronic medical record system. The collected clinical data encompassed a wide range of variables, including maternal age, parity (number of deliveries), gravidity (number of pregnancies), history of miscarriage, pre-pregnancy Body Mass Index (BMI), gestational weight gain, education level, mode of conception (assisted reproduction/ natural conception), family history of diabetes, gestational hypertension, gestational diabetes, and chorionicity (monochorionic or dichorionic twins). Laboratory indicators included complete blood counts (White Blood Cells [WBC], Platelets [PLT], Neutrophils [Neut], Lymphocytes [Lym]), serum levels of Alpha-Fetoprotein (AFP), Beta Human Chorionic Gonadotropin (β-HCG), Hemoglobin (Hb), and Placental Growth Factor (PlGF). Blood routine indicators, AFP, and PlGF were measured between 16-20 weeks of gestation, while 24-hour urine protein quantification was performed between 24-28 weeks, and β-HCG was measured between 20-28 weeks if an abnormal pregnancy was suspected.

Detection methods

Venous blood samples (5 mL) were collected from each participant before delivery and processed immediately to ensure the accuracy of laboratory results. Blood routine indicators were analyzed using a Sysmex XN-3000 automatic hematology analyzer, following standard operating procedures. Serum AFP levels were determined through chemiluminescence immunoassay using a Roche Cobas80 analyzer, which is known for its high sensitivity and specificity. β-HCG levels were measured using a double antibody sandwich method, with reagents provided by Shanghai Initial Biotechnology Co., Ltd. Serum PlGF levels were quantified using an enzyme-linked immunosorbent assay (ELISA), with kits supplied by Shanghai Enzyme-linked Biotechnology Co., Ltd. All measurements were carried out in strict accordance with the manufacturers' protocols, and quality control was consistently maintained to ensure reliable data.

Outcome measures

Primary outcome: The independent risk factors for adverse pregnancy outcome were identified through logistic regression analysis. Receiver Operating Characteristic (ROC) curve analysis was utilized to evaluate the predictive value of these risk factors, with a particular focus on PlGF. Additionally, potential interactions between PlGF and other independent risk factors were assessed to explore their combined effect on pregnancy outcome.

Secondary outcomes: The clinical data and laboratory indicators were compared between women with favorable and adverse pregnancy outcome groups. The study flow chart is presented in **Figure 1**.

Statistical analysis

Statistical analyses were performed using SPSS version 26.0 (IBM Corp, Armonk, NY). Categorical data were compared using the chi-square test. The distribution of continuous data was evaluated using the Kolmogorov-Smirnov test. For normally distributed data, independent sample t-tests were employed, and results were reported as mean ± standard deviation (SD). Non-normally distributed data were analyzed using the rank-sum test, with results expressed as median [interquartile range]. Multivariate logistic regression analysis was conducted using the stats package in R (version 4.3.2) to identify independent risk factors for adverse pregnancy outcomes. The interaction between PlGF and other independent risk factors was analyzed using the rms package in R software. ROC curve analysis was performed with the pROC package to determine the cut-off values for predicting adverse outcome. Data visualization was carried out using the ggplot2 package. Statistical significance was set at $P < 0.05$ for all analyses.

Results

Comparison of clinical data between different outcome groups

Patients were grouped into a favorable outcome group and an adverse outcome group based on their pregnancy outcome. The comparison of clinical data between these two groups revealed no significant differences in

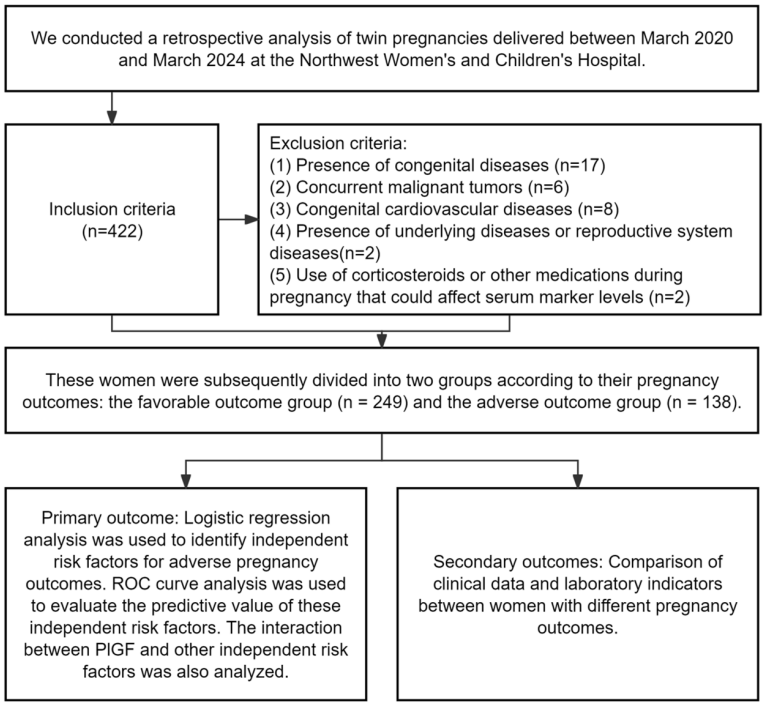

Figure 1. Study flow chart.

parity (P = 0.206), gravidity (P = 0.883), history of miscarriage (P = 0.485), gestational weight gain (P = 0.181), education level (P = 0.201), family history of diabetes (P = 0.482), or chorionicity (P = 0.295) (**Table 1**). However, significant differences were observed in age (P = 0.003), pre-pregnancy BMI (P = 0.002), mode of conception (P = 0.011), gestational hypertension (P = 0.025), and gestational diabetes (P = 0.018) between the two groups, suggesting that these factors may influence pregnancy outcome (**Table 1**).

Comparison of laboratory indicators between different outcome groups

Laboratory indicators were also compared between the favorable and adverse outcome groups. The analysis showed that levels of WBC (P = 0.047), Neut (P < 0.001), AFP (P < 0.001), β-HCG (P < 0.001), and 24-hour urine protein quantification (P < 0.001) were significantly lower in the favorable outcome group. In contrast, higher levels of Lym (P < 0.001) and PlGF (P < 0.001) were observed in the favorable outcome group compared to the adverse outcome group (**Table 2**). These findings suggest that these laboratory indicators may be associated with pregnancy outcome.

Independent risk factors for adverse pregnancy outcome

Multivariate logistic regression analysis was conducted on the indicators that showed significant differences in the univariate analysis, including mode of conception, Neut, Lym, AFP, β-HCG, 24-hour urine protein quantification, and PlGF. Continuous variables were dichotomized using the cut-off values obtained from the ROC curve (**Table 3**). The results of the multivariate analysis identified mode of conception (P = 0.007, OR = 2.606), Neut (P < 0.001, OR = 5.595), Lym (P = 0.002, OR = 0.261), AFP (P < 0.001, OR = 4.261), β-HCG (P = 0.001, OR = 3.268), 24-hour urine protein quantification (P < 0.001, OR = 8.859), and PlGF (P < 0.001, OR = 0.044) as independent risk factors for adverse pregnancy outcome (**Table 4**).

Predictive value of independent risk factors for adverse pregnancy outcome

ROC curve analysis was performed to assess the predictive value of independent risk factors for adverse pregnancy outcome. The results revealed that the Area Under the Curve (AUC) for PlGF in predicting adverse pregnancy outcome was 0.874, which was significantly higher than that of other indicators (all P < 0.001, **Figure 2**; **Tables 5** and **6**). The optimal cut-off value for PlGF at 208.595 pg/ml provided a sensitivity of 80.43% and a specificity of 80.72%, indicating that PlGF has a strong predictive ability for adverse outcome in this patient population.

Interaction analysis between PlGF and other independent risk factors

Finally, an interaction analysis was performed to explore the relationship between PlGF and other independent risk factors. The results showed significant interactions between PlGF and adverse pregnancy outcome (P < 0.001, **Figure 3A**, **3G**), as well as between PlGF and

Table 1. Comparison of clinical data between the favorable and adverse outcome groups

Factor	Favorable outcome group (n = 249)	Adverse outcome group (n = 138)	χ^2 Value	*P* Value
Age				
≥ 40 years	105 (42.17%)	80 (57.97%)	8.886	0.003
< 40 years	144 (57.83%)	58 (42.03%)		
Parity				
Primiparous	115 (46.18%)	73 (52.9%)	1.602	0.206
Multiparous	134 (53.82%)	65 (47.1%)		
Gravidity				
≥ 2 times	137 (55.02%)	77 (55.8%)	0.022	0.883
< 2 times	112 (44.98%)	61 (44.2%)		
History of miscarriage				
Yes	85 (34.14%)	52 (37.68%)	0.488	0.485
No	164 (65.86%)	86 (62.32%)		
Pre-pregnancy BMI				
< 18 kg/m^2	47 (18.88%)	19 (13.77%)	12.232	0.002
18-24.9 kg/m^2	162 (65.06%)	76 (55.07%)		
≥ 25 kg/m^2	40 (16.06%)	43 (31.16%)		
Gestational weight gain				
< 15 kg	70 (28.11%)	50 (36.23%)	3.42	0.181
15-20 kg	119 (47.79%)	63 (45.65%)		
> 20 kg	60 (24.1%)	25 (18.12%)		
Education level				
High school or below	63 (25.3%)	39 (28.26%)	3.212	0.201
College/Undergraduate	164 (65.86%)	80 (57.97%)		
Graduate or above	22 (8.84%)	19 (13.77%)		
Mode of conception				
Assisted reproduction	129 (51.81%)	90 (65.22%)	6.5	0.011
Natural conception	120 (48.19%)	48 (34.78%)		
Family history of diabetes				
Yes	20 (8.03%)	14 (10.14%)	0.495	0.482
No	229 (91.97%)	124 (89.86%)		
Gestational hypertension				
Yes	12 (4.82%)	15 (10.87%)	5.008	0.025
No	237 (95.18%)	123 (89.13%)		
Gestational diabetes				
Yes	15 (6.02%)	18 (13.04%)	5.609	0.018
No	234 (93.98%)	120 (86.96%)		
Chorionicity				
Dichorionic twins	209 (83.94%)	110 (79.71%)	1.095	0.295
Monochorionic twins	40 (16.06%)	28 (20.29%)		

Note: BMI, Body Mass Index.

24-hour urine protein level (P = 0.035) as well. However, no significant interactions were observed between PlGF and other factors such as mode of conception, Neut, Lym, AFP, or β-HCG (all P > 0.05, **Figure 3B-F**; **Table 7**). These findings suggest that PlGF, in conjunction with certain other factors, may play a critical role in predicting adverse pregnancy outcome.

Discussion

The combination of advanced maternal age and twin pregnancy significantly increases the

Table 2. Comparison of laboratory indicators between the favorable and adverse outcome groups

Indicator	Favorable outcome group (n = 249)	Adverse outcome group (n = 138)	Statistical Value	*P* Value
WBC (×10^9/L)	9.80 ± 1.96	10.20 ± 1.89	1.993	0.047
PLT (×10^9/L)	224.13 ± 55.15	231.85 ± 43.01	1.525	0.128
Neut (×10^9/L)	7.07 ± 0.64	7.56 ± 0.96	5.416	< 0.001
Lym (×10^9/L)	1.90 ± 0.54	1.62 ± 0.51	-5.068	< 0.001
AFP (ng/mL)	93.82 ± 7.92	98.87 ± 6.62	6.687	< 0.001
β-HCG (mU/L)	3.34 ± 0.51	3.70 ± 0.50	6.671	< 0.001
24 h urine protein quantification (g/L)	2.05 [1.56, 2.66]	2.62 [2.13, 3.10]	5.242	< 0.001
Hb (g/L)	115.13 ± 12.04	115.73 ± 15.59	0.393	0.695
PlGF (pg/ml)	225.49 ± 19.65	195.74 ± 17.72	-15.211	< 0.001

Note: WBC, White Blood Cells; PLT, Platelets; Neut, Neutrophils; Lym, Lymphocytes; AFP, Alpha-Fetoprotein; β-HCG, Beta Human Chorionic Gonadotropin; Hb, Hemoglobin; PlGF, Placental Growth Factor.

Table 3. Value assignment table

Factor	Assignment Contents
Age	≥ 40 years = 1, < 40 years = 0
Pre-pregnancy BMI	< 18 kg/m^2 = 0, 18-24.9 kg/m^2 = 1, ≥ 25 kg/m^2 = 2
Mode of conception	Assisted Reproduction = 1, Natural Conception = 0
Gestational hypertension	Yes = 1, No = 0
Gestational diabetes	Yes = 1, No = 0
WBC (×10^9/L)	≤ 9.145 = 0, > 9.145 = 1
Neut (×10^9/L)	≤ 7.725 = 0, > 7.725 = 1
Lym (×10^9/L)	≤ 2.055 = 0, > 2.055 = 1
AFP (ng/mL)	≤ 96.04 = 0, > 96.04 = 1
β-HCG (mU/L)	≤ 3.405 = 0, > 3.405 = 1
24 h urine protein quantification (g/L)	≤ 2.095 = 0, > 2.095 = 1
PlGF (pg/ml)	≤ 208.595 = 0, > 208.595 = 1
Pregnancy Outcome	Favorable Outcome = 0, Adverse Outcome = 1

Note: BMI, Body Mass Index; WBC, White Blood Cells; Neut, Neutrophils; Lym, Lymphocytes; AFP, Alpha-Fetoprotein; β-HCG, Beta Human Chorionic Gonadotropin; PlGF, Placental Growth Factor.

risks for both the mother and the fetuses [16]. The decline in physiological function of the uterus and placenta in older pregnant women further elevate the risk of adverse outcomes in twin pregnancies [17]. Therefore, predicting adverse pregnancy outcome in twin pregnancies at an advanced maternal age is crucial for improving maternal and neonatal health, formulating personalized interventions, and reducing the incidence of pregnancy complications. Effective predictive tools can help clinicians identify high-risk pregnancies early and implement timely interventions.

The results of this study indicate that PlGF levels play a significant role in predicting adverse outcome in twin pregnancies at advanced maternal age. Specifically, there were significant differences in PlGF levels between the favorable outcome group and the adverse outcome group. The study also found that the AUC for PlGF in predicting adverse pregnancy outcome was 0.874, significantly higher than other indicators such as Neut, Lym, AFP, β-HCG, and 24-hour urine protein quantification. This suggests that PlGF has higher sensitivity and specificity in predicting adverse outcome. PlGF is an important angiogenic factor that plays a critical role in placental angiogenesis and maintaining placental function [18]. Elevated levels of PlGF can promote placental vascular formation, ensuring that the fetus receives adequate oxygen and nutrients, thereby supporting healthy fetal development [19]. In twin pregnancies,

Table 4. Multivariate logistics regression analysis

Factor	Estimate	Std. Error	*P* Value	OR	Lower 95 CI	Upper 95 CI
Age	0.428	0.345	0.214	1.535	0.781	3.036
Pre pregnancy BMI	0.499	0.273	0.068	1.647	0.970	2.844
Mode of conception	0.958	0.353	0.007	2.606	1.321	5.307
Gestational hypertension	0.421	0.663	0.526	1.523	0.424	5.711
WBC	0.254	0.373	0.495	1.290	0.622	2.700
Neut	1.722	0.386	< 0.001	5.595	2.677	12.224
Lym	-1.342	0.423	0.002	0.261	0.110	0.585
AFP	1.449	0.350	< 0.001	4.261	2.180	8.662
β-HCG	1.184	0.359	0.001	3.268	1.639	6.731
24 h urine protein quantification	2.181	0.394	< 0.001	8.859	4.229	19.946
PlGF	-3.120	0.372	< 0.001	0.044	0.020	0.089

Note: BMI, Body Mass Index; WBC, White Blood Cells; Neut, Neutrophils; Lym, Lymphocytes; AFP, Alpha-Fetoprotein; β-HCG, Beta Human Chorionic Gonadotropin; PlGF, Placental Growth Factor.

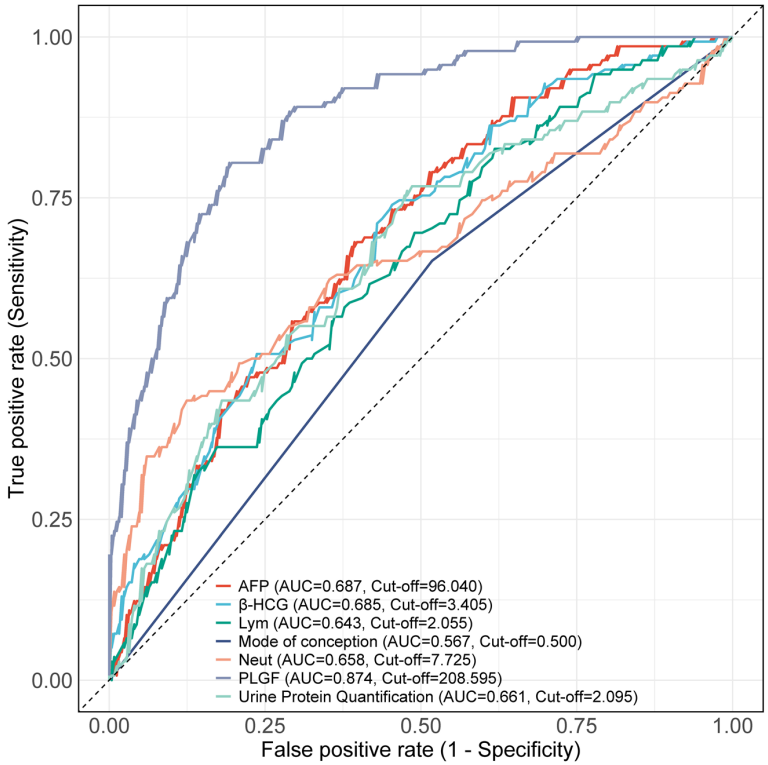

Figure 2. ROC curves of independent risk factors for predicting adverse pregnancy outcome Note: Neut, Neutrophils; Lym, Lymphocytes; AFP, Alpha-Fetoprotein; β-HCG, Beta Human Chorionic Gonadotropin; PlGF, Placental Growth Factor.

maintaining effective placental function is particularly important. Low levels of PlGF may lead to placental insufficiency, increasing the risk of adverse outcomes such as gestational hypertension, fetal growth restriction, and preterm birth. Previous research by Ekelund et al., involving 11,758 pregnant women, found that low levels of PlGF were associated with adverse outcome and were an independent risk factor [13]. Additionally, Bligh et al. discovered that lower PlGF levels were related to adverse neonatal outcomes [20]. Binder et al. proposed that the sFlt-1/PlGF ratio is significant in predicting composite adverse perinatal outcome and is superior to some conventional laboratory data [21]. These findings align with our results, further supporting the importance of PlGF in predicting pregnancy outcome. In this study, we demonstrated that PlGF has significant predictive value for pregnancy outcome in twin pregnancies at advanced maternal age. By measuring PlGF levels in these pregnancies, clinicians can identify high-risk pregnancies early and implement timely interventions to improve outcomes, and reduce complications.

This study further identified PlGF, Neut, Lym, AFP, β-HCG, and 24-hour urine protein quantification as important independent risk factors for adverse pregnancy outcome in this population. These risk factors were categorized into routine blood indicators, biomarkers, and urine indicators for discussion. Neut and Lym are

Table 5. ROC curve parameters of independent risk factors in predicting adverse maternal and infant outcomes

Marker	AUC	95% CI	Specificity	Sensitivity	Youden index	Cut off	Accuracy	Precision	F1 Score
Mode of conception	0.567	0.516-0.618	0.4819	0.6522	0.1341	0.5	0.5426	0.6522	0.5042
Neut ($\times10^9$/L)	0.658	0.597-0.720	0.8755	0.4348	0.3103	7.725	0.7183	0.4348	0.524
Lym ($\times10^9$/L)	0.643	0.587-0.699	0.3815	0.8261	0.2076	2.055	0.5401	0.8261	0.5616
AFP (ng/mL)	0.687	0.634-0.741	0.6064	0.6812	0.2876	96.04	0.6331	0.6812	0.5697
β-HCG (mU/L)	0.685	0.631-0.739	0.5462	0.7391	0.2853	3.405	0.615	0.7391	0.5779
24 h urine protein quantification (g/L)	0.661	0.603-0.718	0.5221	0.7609	0.283	2.095	0.6072	0.7609	0.5801
PlGF (pg/ml)	0.874	0.840-0.909	0.8072	0.8043	0.6116	208.595	0.8062	0.8043	0.7475

Note: WBC, White Blood Cells; Neut, Neutrophils; Lym, Lymphocytes; AFP, Alpha-Fetoprotein; β-HCG, Beta Human Chorionic Gonadotropin; PlGF, Placental Growth Factor.

Table 6. Comparison of the AUCs between PlGF and other independent risk factors

Marker1	Marker2	Z value	*P* value	AUC difference	95% CI
Mode of conception	PlGF	-9.423	< 0.001	-0.307	-0.371 - -0.243
Neut	PlGF	-5.8	< 0.001	-0.216	-0.289 - -0.143
Lym	PlGF	-7.077	< 0.001	-0.231	-0.295 - -0.167
AFP	PlGF	-5.685	< 0.001	-0.187	-0.251 - -0.123
B HCG	PlGF	-5.645	< 0.001	-0.189	-0.255 - -0.124
Urine Protein Quantification	PlGF	-6.167	< 0.001	-0.214	-0.281 - -0.146

Note: WBC, White Blood Cells; Neut, Neutrophils; Lym, Lymphocytes; AFP, Alpha-Fetoprotein; β-HCG, Beta Human Chorionic Gonadotropin; PlGF, Placental Growth Factor; AUC, Area under the curve.

important indicators in routine blood tests, reflecting maternal inflammation and immune status [22]. In advanced maternal age twin pregnancies, an increase in neutrophils may indicate chronic inflammation or acute infection in the mother, leading to adverse outcomes such as preterm birth and premature rupture of membranes [23]. Lymphocytes play a vital role immune response, and changes in the immune system during pregnancy significantly affect maternal and fetal health [24]. Low lymphocytes count may indicate impaired maternal immune function, increasing the risk of infection and pregnancy complications, which can result in adverse pregnancy outcomes. Previous studies by Christoforaki et al. found that the ratio of Neut to Lym in early pregnancy can predict adverse pregnancy outcomes [25]. Additionally, Ata et al. found that the neutrophil-to-lymphocyte and platelet-to-lymphocyte ratios can predict threatened miscarriage and early pregnancy loss [26].

PlGF, AFP, and β-HCG are key biomarkers identified in this study. PlGF is an important angiogenic factor that promotes placental vascular formation and maintains normal placental function [27]. Low levels of PlGF may lead to placental insufficiency, increasing the risk of gestational hypertension, fetal growth restriction, and preterm birth. Previous study demonstrated that PlGF levels were significantly associated with late preterm birth and adverse perinatal outcome in twin pregnancies, with the PlGF/sFlt-1 ratio showing strong predictive ability at 32 weeks [28]. Another study reported that low levels of PlGF were closely related to composite adverse pregnancy outcome in twin pregnancies and were independent risk factors [29]. AFP is mainly produced by the fetal liver and yolk sac, and its abnormal elevation is often associated with fetal structural abnormalities, such as neural tube defects and abdominal wall defects, thereby increasing the risk of adverse pregnancy outcome [30]. Research indicates that elevated AFP levels may suggest placental dysfunction, increasing the risk of gestational hypertension and fetal development issues [31]. β-HCG is a hormone secreted by the placenta, and fluctuations in its levels can reflect placental function and pregnancy status [32]. Abnormal β-HCG levels may be associated with placental dysfunction or fetal development issues, with both elevated and diminished β-HCG levels indicating adverse pregnancy outcomes, such as placental abrup-

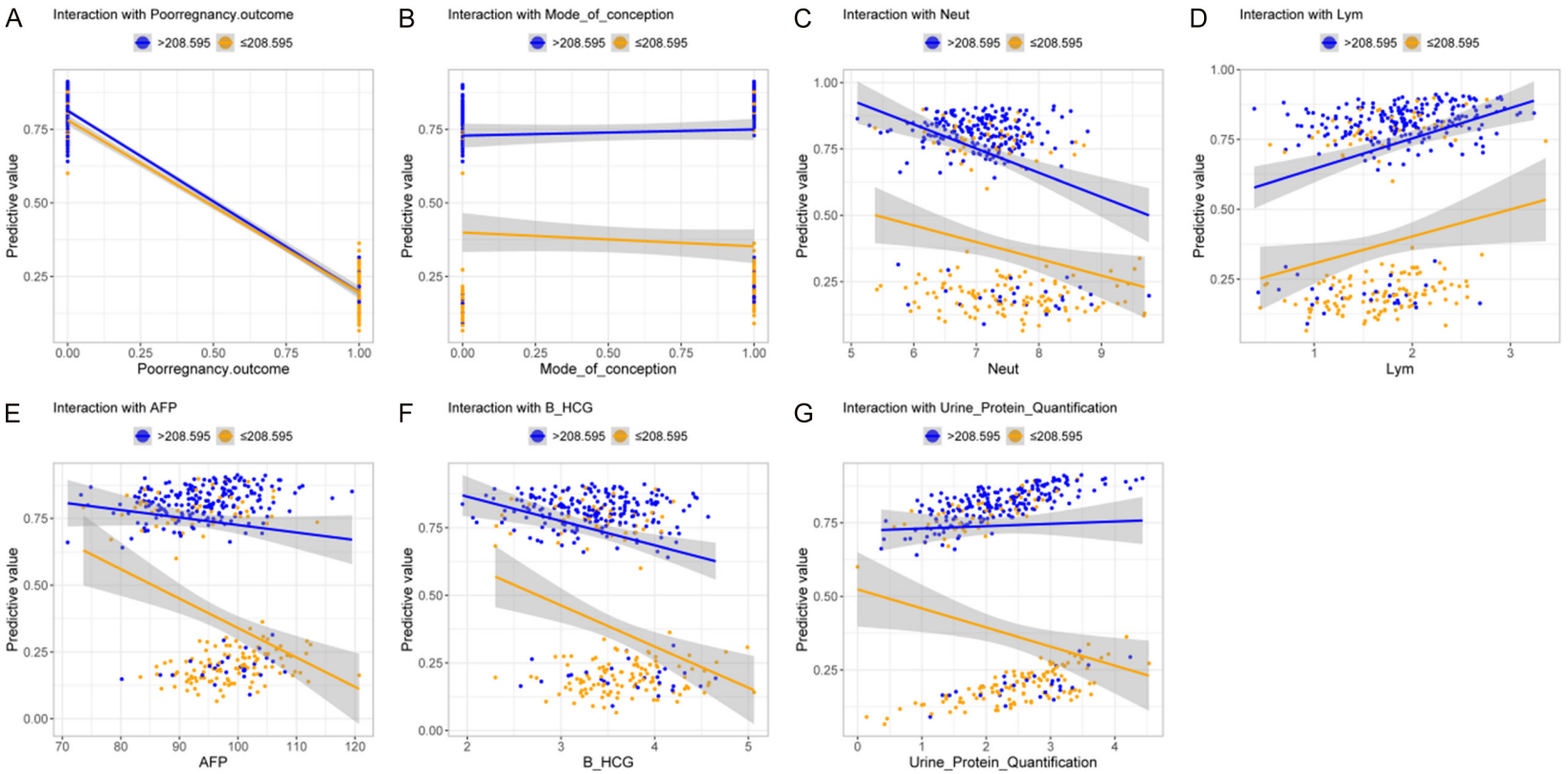

Figure 3. Correlation between PlGF and other independent risk factors. A. Correlation between PlGF and adverse pregnancy outcome. B. Correlation between PlGF and mode of conception. C. Correlation between PlGF and Neut. D. Correlation between PlGF and Lym. E. Correlation between PlGF and AFP. F. Correlation between PlGF and β-HCG. G. Correlation between PlGF and 24 h Urine Protein Quantification. Note: The blue line represents the predictive value when PlGF level is less than or equal to 208.595, while the yellow line represents the predictive value when PlGF level is greater than 208.595. Neut, Neutrophils; Lym, Lymphocytes; AFP, Alpha-Fetoprotein; β-HCG, Beta Human Chorionic Gonadotropin; PlGF, Placental Growth Factor.

Table 7. Correlation between PlGF and other independent risk factors

Factor	Estimate	Std. Error	Z value	*P* value
Pregnancy outcome	-3.186	0.356	-8.962	< 0.001
Mode of conception	0.403	0.270	1.489	0.136
Neut	0.012	0.171	0.069	0.945
Lym	0.168	0.248	0.679	0.497
AFP	0.016	0.018	0.916	0.360
β-HCG	0.016	0.257	0.063	0.949
24 h Urine Protein Quantification	0.369	0.174	2.112	0.035

Note: Neut, Neutrophils; Lym, Lymphocytes; AFP, Alpha-Fetoprotein; β-HCG, Beta Human Chorionic Gonadotropin; PlGF, Placental Growth Factor.

tion and preeclampsia [33]. A systematic review and meta-analysis found that low β-HCG levels were significantly associated with adverse pregnancy outcomes, such as preterm birth, small-for-gestational-age infants, and placental abruption [34]. Another study showed that high levels of β-HCG in the mid-trimester were associated with increased risks of preeclampsia and fetal developmental abnormalities, while changes in β-HCG levels in early pregnancy could help predict early miscarriage and fetal death [35].

The 24-hour urine protein quantification is primarily used to detect kidney function and assess the risk of preeclampsia [36]. Older pregnant women are particularly prone to gestational hypertension and preeclampsia, both of which can lead to elevated urine protein levels [37]. High levels of urine protein indicate kidney damage, which may lead to placental dysfunction, increasing the risk of adverse outcome, such as preterm birth and fetal growth restriction. For example, a study by Lei et al. suggested that in patients with gestational hypertension, a higher 24-hour urine protein level correlated with an increased incidence of adverse outcomes for both the mother and the fetus. This study also pointed out that increased 24-hour urine protein was associated with gestational kidney dysfunction, indicating that kidney damage may compromise placental function and contribute to the risk of adverse pregnancy outcome [38]. Overall, monitoring these indicators can facilitate the early identification of high-risk pregnancies and enable prompt intervention.

Finally, we analyzed the interactions between PlGF and other risk factors. Significant interactions were observed between PlGF and adverse pregnancy outcomes, as well as 24-hour urine protein level. We speculate that decreased PlGF levels and increased urine protein reflect placental and kidney dysfunction, where a low level of PlGF may lead to endothelial dysfunction, increasing the filtration burden on the kidneys and resulting in elevated urine protein [39]. This interaction is significant in the diagnosis, treatment monitoring, and prognosis of of high-risk pregnancies and for early intervention to improve outcome.

Although this study provides valuable insights, it has several limitations. First, the sample size is relatively small, which may affect the representativeness and generalizability of the results. Future studies should include larger sample sizes to enhance the reliability and applicability of the findings. Second, as a retrospective study, there is a possibility of selection bias and information bias, and confounding factors cannot be fully controlled. Future prospective studies can help reduce bias and better control confounding factors. Additionally, this is a single-center study, and the data only come from one medical center, which may limit the external validity of the results and may not fully represent other regions or hospitals. Future multi-center studies are needed to broaden the applicability of the findings. Finally, the study lacks long-term follow-up data, so the impact of PlGF levels on long-term maternal and neonatal health outcomes cannot be assessed. Future studies should conduct longer follow-up to show the impact of PlGF levels on long-term maternal and neonatal health.

Conclusion

PlGF has significant clinical value for predicting adverse pregnancy outcome in twin pregnan-

cies at advanced maternal age. PlGF level is significantly correlated with pregnancy outcome, making it an effective marker for early screening and intervention in high-risk pregnancies.

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Disclosure of conflict of interest

None.

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