

# Maternal Serum EG-VEGF as A First Trimester Predictor of Hypertensive Disorders of Pregnancy: A Prospective Cohort Study

**Shiyu Zeng**

Second Xiangya Hospital of Central South University

**Ling Yu**

Second Xiangya Hospital of Central South University

**Yiling Ding**

Second Xiangya Hospital of Central South University

**Mengyuan Yang** (✉ [zengshiyu1997@csu.edu.cn](mailto:zengshiyu1997@csu.edu.cn))

Second Xiangya Hospital of Central South University

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## Research Article

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# Abstract

## Background

This study aims to explore whether plasma endocrine gland-derived vascular endothelial growth factor (EG-VEGF) in the first trimester can be used as a predictor of hypertensive disorders of pregnancy (HDP), and compare it with placental growth factor (PIGF) and soluble fms-like tyrosine kinase-1 (sFlt-1) to evaluate its prediction of HDP value.

## Methods

This is a prospective cohort study that records the medical history of the pregnant women included in the study at 11–13 weeks' gestation, and analyzes serum biochemical markers including EG-VEGF, PIGF, sFlt-1 and sFlt-1/PIGF. The predictive values of these tests were determined. We used the receiver operating characteristic (ROC) curve to find the optimal cut-off value for each biomarker and compare the operating characteristics (sensitivity, specificity). Logistic regression analysis was used to create a prediction model for HDP based on maternal characteristics and maternal biochemistry.

## Results

Data were obtained from 205 pregnant women. 17 cases were diagnosed with HDP, the incidence rate was 8.2% (17/205). Women who developed HDP had a significantly higher body mass index (BMI) and mean arterial pressure (MAP). Serum EG-VEGF levels in the first trimester are significantly higher in pregnant women with HDP. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of serum EG-VEGF levels more than 227.83 pg/ml for predicting HDP were 43%, 93%, 86% and 62%, respectively. We established a prediction model in the first trimester include maternal BMI, MAP, and EG-VEGF, with an AUC of 0.8861 (95%CI: 0.7905–0.9818), which is better than using EG-VEGF alone (AUC: 0.66).

## Conclusion

This study demonstrated that serum EG-VEGF is a promising biomarker for predicting HDP in the first trimester. It has better predictive performance compared with the currently used biomarkers like PIGF and sFlt-1. Combining maternal clinical characteristics and biochemical tests at 11–13 weeks can effectively identify women at high risk of HDP.

## Introduction

Hypertensive disorders of pregnancy (HDP) are pregnancy-related diseases including gestational hypertension (GH) and preeclampsia (PE) that constitute one of the leading causes of maternal and

perinatal mortality worldwide [1]. In Latin America and the Caribbean, hypertensive disorders account for nearly 26% of maternal deaths, whereas in Africa and Asia they are responsible for 9% of deaths [2]. Currently, the only treatment for HDP is delivery. However, this approach usually increases the incidence of preterm birth and the risk of neonatal morbidity.

Previous studies have shown that if clinicians can predict the risk of HDP in advance and take corresponding preventive measures, the resulting mortality can be greatly reduced [3, 4]. Therefore, in recent years, considering its epidemiological and clinical importance, people have tried to find specific and practical biomarkers to predict and evaluate HDP. Recent studies have shown that a large number of serum circulating factors including soluble endoglin, placental growth factor (PlGF), soluble fms-like tyrosine kinase 1 (sFlt-1) are related to the occurrence of HDP [5]. However, there is still no indicator that can effectively predict HDP.

Endocrine gland derived vascular endothelial growth factor (EG-VEGF), also known as Prokineticin 1 (PROK1), belongs to a novel family of angiogenic mitogens [6]. It is mainly expressed in the endocrine organs, including the placenta, and the main target is endothelial cells (EC) derived from these endocrine tissues [6]. The biological activity of EG-VEGF is mediated by two G protein-coupled prokineticin receptors (PROKR), termed PROKR1 and PROKR2. Its receptors, PROKR1 and PROKR2 are highly expressed in trophoblasts and placental endothelial cells [7]. EG-VEGF is not only a uterine receptor marker and key protein during embryo implantation, but also a key endocrine factor that controls placental development after pregnancy [8]. The development of the placenta in normal early pregnancy is mainly controlled by two key steps: first, angiogenesis period (8–10 weeks): trophoblast proliferation and differentiation, and angiogenesis; second, vascular recasting period (11–20 weeks): The cytotrophoblast differentiates into extravillous trophoblast cells (EVTs) with high invasion ability. The moderate infiltration of EVT makes the maternal spiral arteries change from high-resistance, low-discharge to low-resistance, high-discharge vessels, and the uterine spiral arteries are recast to establish the placental vascular bed. The expression of EG-VEGF can be upregulated by hypoxia and  $\beta$ HCG, both of which are factors that are highly related to the development of HDP [9]. Although higher levels of EG-VEGF has been demonstrated during preeclampsia [10], there is still no investigation of this cytokine before the disease onset and its predictive potential for this disorder.

Therefore, This study aims to explore the concentration of EG-VEGF in the plasma of pregnant women before HDP onset, and use the maternal characteristics of early pregnancy, MAP and EG-VEGF and other biomarkers alone or in combination for effective prediction of HDP. In order to verify if EG-VEGF is a potential biomarker for HDP prediction.

## Materials And Methods

This prospective observational study was conducted in the Department of Obstetrics and Gynecology, The Second Xiangya Hospital of Central South University between June 2019 to June 2020. The study protocol was approved by the ethical board of The Second Xiangya Hospital of Central South University.

All participants gave written informed consent. Women with singleton pregnancy at 11–13 + 6 weeks' gestational were invited into the study. Gestational age(GA) was calculated from the first day of their last menstrual period and confirmed by ultrasound scanning of the crown-lump length (CRL) in the first trimester. The exclusion criteria were chromosomal aneuploidy, fetal malformation, miscarriage before 24 weeks of pregnancy, termination of pregnancy, and fetal death. All operations (measurement, data recording) are performed according to routine clinical practice. Regular follow-up of all eligible women, record their demographic characteristics, delivery methods, and perinatal outcomes were recorded.

**Serum biomarkers:** Collect blood samples for biochemical marker measurements at 11 – 13 + 6 weeks. Separated serum was stored frozen (-80°C) until required for assay. Serum EG-VEGF was measured by an enzyme-linked immunosorbent assay (ELISA) kit (Thermo Fisher,EHPROK1,USA) with standard range between 13.72-10,000 ng/mL. Placental growth factor (PIGF) and soluble fms-like tyrosine kinase-1 (sFlt-1) were measured by immunofluorescence method (KeyGEN BioTECH, K02010102, CHINA) according to the company instruction.

**Definition of pregnancy outcome:** Gestational hypertension is defined as a systolic blood pressure of 140 mm Hg or more or a diastolic blood pressure of 90 mm Hg or more, or both, in previously normotensive women on two occasions at least 4 hours apart after 20 weeks of gestation. Preeclampsia was diagnosed according to the guidelines of the American College of Obstetricians and Gynecologists, based on systolic blood pressure  $\geq$  140 mmHg and/or diastolic blood pressure  $\geq$  90 mmHg on two recordings at least 4 hours apart, with the presence of proteinuria of  $\geq$  0.3g/24h or two readings of at least ++ on dipstick analysis or other maternal end-organ damage[2].

## Statistical Analysis

SPSS 26.0 version(IBM, Armonk, NY, USA)was used for statistical analysis.The suitability of the quantitative data for normal distribution was tested by the Kolmogorov–Smirnov test and the

Shapiro–Wilk test, The two-sample t-test was used for the comparison of two groups of quantitative data showing normal distribution, and the Mann–Whitney U test was used for the comparison of two groups of data with abnormal distribution. The Pearson chi-square test or Fisher test was used to compare qualitative data. We tried to use the receiver operating characteristic (ROC) curve to find the optimal cut-off value for each biomarker and compare the operating characteristics (sensitivity, specificity). Logistic regression analysis was used to create a prediction model for HDP based on maternal characteristics and maternal biochemistry. ROC curve was obtained to assess the performance of this model.

## Result

This study recruited 226 singleton pregnant women during the first trimester from June 2019 to June 2020. We excluded a total of 21 cases (during the follow-up period, four participant withdrew consent for the study, nine miscarried after twelve weeks' gestation, three chose termination of pregnancy, three fetuses that later demonstrated morphological anomalies, two with proven aneuploidy). Of the remaining

205 pregnancies, 188 women were normotensive at delivery (91.7%) and 17 developed HDP (8.3%). Of the 17 pregnancies affected by hypertension, 13 developed GH (without PE) and four developed PE; 7.6% and 2.3% of total cohort respectively. Due to the small number of PE-affected pregnancies, GH and PE-affected pregnancies were combined for analysis.

The maternal and pregnancy characteristics of both groups are summarized in Table 1. Women who developed HDP had a significantly higher body mass index (BMI) and mean arterial pressure (MAP) in comparison to normotensive pregnancies ( $P = 0.000$ ). With the exception of preterm birth rate there was no difference in pregnancy outcome between normotensive and hypertensive pregnancies ( $P < 0.05$ ). There was no significant difference in clinical information such as the age of pregnant women, gestational week of delivery, mode of delivery, and newborn weight. Because of the small number of pregnant women with chronic hypertension, antiphospholipid antibody syndrome, type 1 or type 2 diabetes, there was no statistical difference between the two groups.

**Table 1** Maternal characteristics, medical history and current pregnancy characteristics in the two groups of pregnancy outcome (n=205)

Characteristic	Normotensive (n=188)	Hypertensive (n=17)	P
Age(years)	30.12(29.49-30.76)	31.65(30.09-33.20)	0.104
BMI(kg/m <sup>2</sup> )	21.39(20.78-22.00)	23.94(22.20-25.68)	0.001
MAP(mmHg)	87.40(85.65-89.15)	96.26(92.14-100.37)	0.000
Parity			
Nulliparous	96(51.1%)	11(64.7%)	0.281
Parous-no previous PE	1(0.5%)	1(5.9%)	0.159
Parous-previous PE	91(48.4%)	5(29.4%)	0.105
Conception			
Spontaneous	179(95.2%)	13(76.5%)	0.015
Ovulation induction	2(1.1%)	0	0.841
In-vitro fertilation	7(3.7%)	4(23.5%)	0.008
Medical history			
Chronic hypertension	1(0.5%)	1(5.9%)	0.159
Diabetes mellitus	1(0.5%)	0	0.917
Systemic lupus erythematosus	1(0.5%)	2(11.8%)	0.019
Antiphospholipid syndrome	1(0.5%)	0	0.917
Renal disease	3	0	0.770
Delivery outcome			
Gestational age at delivery(wk)	38.51(38.25-38.77)	37.57±1.727	0.305
Cesarean section	86(45.8%)	9(52.9%)	0.569
Birth weight(g)	3220.09(3130.01-3310.16)	3029.34±523.49	0.211
Preterm < 34 week	2(1.1%)	2(11.8%)	0.035
IUGR	1(0.5%)	1(5.9%)	0.159

Data are given as n (%) or mean (95% confidence interval). Comparison between each hypertensive disorder group and controls was by chi square or Fisher's exact test for categorical variables and Mann-Whitney-U test for continuous variables.

*BMI* body mass index, *kg* kilogram, *MAP* mean arterial pressure, *wk* week, *IUGR* Intrauterine growth restriction.

All analytes exhibited concentration differences between groups as shown in Table 2. The performance characteristics for prediction of HDP for all biomarkers are shown in Table 3. EG-VEGF value of hypertensive group was significantly higher than that of normotensive group (270.47 (95% CI185.02-355.91) vs. 86.54 (95%CI: 72.13-100.95),  $p = 0.000$ ;  $P < 0.05$ ). Based on this significance, it was found that the presence of HDP was significantly correlated with the serum cut-off value of 227.83 pg/mL ( $p < 0.05$ ). The sensitivity and specificity of EG-VEGF in predicting HDP at the 227.83 pg/mL cut-off point were 93% and 43%, respectively; the positive predictive value (PPV) was 86%, and the negative predictive value (NPV) was 62% (Table 3). Although in hypertensive group, PIGF was lower than normotensive group (83.50 (95%CI: 62.00-105.00) vs. 97.55 (95%CI: 85.28-109.82)), sFlt-1 and sFlt-1/PIGF were higher than normotensive group (1651.09 (95 %CI: 1488.10-1814.08) vs. 1503.38 (95%CI: 1435.799-1570.97; 25.66 (95%CI: 19.46–31.86) vs. 20.93 (95%CI: 19.22–22.64)), but there is no significant difference between two groups ( $P > 0.05$ ). The sensitivity, specificity, PPV and NPV of serum PIGF levels less than 104.31 pg/ml for predicting HDP were 33%, 70%, 52% and 51%, respectively. The sensitivity, specificity, PPV and NPV of serum sFlt-1 levels more than 1111.21 pg/ml for predicting HDP were 95%, 35%, 59% and 87%, respectively.

Table 2  
Biomarker serum levels

Parameter	Normotensive(n = 188)	Hypertensive(n = 17)	P
EG-VEGF(pg/ml)	86.54(72.13-100.95)	270.47(185.02-355.91)	0.000
PIGF(pg/ml)	97.55(85.28-109.82)	83.50(62.00-105.00)	0.426
sFlt-1(pg/ml)	1503.38(1435.799-1570.97)	1651.09(1488.10-1814.08)	0.137
S/P	20.93(19.22–22.64)	25.66(19.46–31.86)	0.074
MeanUAPI	1.64(1.55–1.73)	1.51(1.12–1.90)	0.398
MeanUARI	0.72(0.70–0.74)	0.76(0.61–0.91)	0.314
Data are given as mean (95% confidence interval).Biomarker distributions are compared via Wilcoxon rank sum test.			
<i>EG-VEGF</i> endocrine gland-derived vascular endothelial growth factor, <i>PIGF</i> placental growth factor, <i>sFlt-1</i> soluble fms-like tyrosine kinase-1			

Table 3

Performance characteristics of biomarkers for prediction of Hypertensive disorders of pregnancy

	<b>AUC</b>	<b>P-value</b>	<b>Cutoff</b>	<b>sensitivity</b>	<b>specificity</b>	<b>PPV</b>	<b>NPV</b>
EGVEGF(pg/ml)	0.66	0.001	227.83	43%	93%	86%	62%
PIGF(pg/ml)	0.54	0.549	104.31	33%	70%	52%	51%
sFLT(pg/ml)	0.60	0.108	1111.21	95%	35%	59%	87%
S/P	0.59	0.137	23.81	62%	69%	66%	64%
<i>EG-VEGF</i> endocrine gland-derived vascular endothelial growth factor, <i>PIGF</i> placental growth factor, <i>sFlt-1</i> soluble fms-like tyrosine kinase-1							

We combined use of maternal clinical characteristics and biomarkers to establish prediction models including: BMI, EG-VEGF, MAP(Table 4). The regression analysis results of the area under the ROC curve (AUC) are shown in (Fig. 1). For a fixed false positive rate, the sensitivity, negative value and predictive value of the final model are shown in Table 5.

Table 4

Logistic regression analysis of the parameters used to predict hypertensive disorders of pregnancy

<b>parameter</b>	<b><math>\beta</math></b>	<b>P</b>	<b>OR(CI)</b>
BMI	0.271	0.022	1.311(1.040–1.652)
EGVEGF	0.006	0.005	1.006(1.002–1.010)
MAP	0.080	0.069	1.083(0.9941.181)
Constant	-16.529	0.001	0.000
The data were analysed using binary logistic regression analysis. Model R <sup>2</sup> = 0.048; P < 0.0001			
Outcome variable: normotensive = 0, hypertensive = 1			
Explanatory parameters: all fitted as continuous variables. <i>OR</i> Odds ratio, <i>CI</i> confidence interval,			
<i>BMI</i> body mass index, <i>MAP</i> mean arterial pressure, <i>EG-VEGF</i> endocrine gland-derived vascular endothelial growth factor			

Table 5  
Diagnostic performance of the combined model

FPR	sensitivity	specificity	PPV	NPV	LR+	LR-
5%	0.444	0.95	0.897	0.629	8.8	0.589
10%	0.556	0.90	0.846	0.666	5.5	0.5
20%	0.889	0.80	0.814	0.869	4.4	0.15
Data for sensitivity are given as mean (95% confidence interval).						
<i>FPR</i> , False positive rate; <i>PPV</i> , positive predictive value; <i>NPV</i> , negative predictive value; <i>LR+</i> , positive likelihood ratio; <i>LR-</i> , negative likelihood ratio						

## Discussion

In this prospective cohort study, we found that the BMI and MAP of pregnant women who developed HDP were significantly higher than that of normotensive, which indicates that the risk of HDP is greater in women with high BMI and MAP. The research is consistent with Rahman [11]. In addition, the pregnancy method and previous SLE history of pregnant women also suggested that it would affect the occurrence of HDP. Whether other clinical features have an impact on HDP needs further research.

Although the etiology of PE is not fully understood, its development is believed to be mainly related to the superficial invasion of the maternal decidua and spiral artery by extravillous trophoblast(EVT). Hoffmann et al. found that EG-VEGF inhibits the migration and invasion of HTR-8 cells (extravillous trophoblast cell line) and the EVT in the early pregnancy villous explant culture system, and proved that EG-VEGF inhibits HTR-8 cell tissue from entering the tubular structure [10]. The data strongly suggests that EG-VEGF can act as an inhibitor of the differentiation of trophoblasts into an aggressive phenotype. EG-VEGF controls the migration and invasion of trophoblast cells, suggesting that these proteins locally control the process of spiral artery remodeling and fetal-maternal circulation establishment. Our previous studies have shown that during normal pregnancy, EGVEGF peaks in the proliferation and differentiation of placental trophoblasts and early angiogenesis (8–10 + 6 weeks), and then drops to a lower level to maintain until the end of placental recasting (11–20 weeks). This clinical finding suggests that the secretion level of EGVEGF is consistent with its physiological function: high levels of EGVEGF in the first trimester mainly exercise its pro-angiogenic function, and then effectively withdraw (low levels) to avoid inhibiting the normal invasion of EVT cells. In the normal placenta model, the down-regulation of EG-VEGF expression at around 11 weeks of gestation promotes EVT differentiation. In our study, we found that compared with the normotensive group, the persistently high levels of EG-VEGF that can be observed in the first trimester of HDP patients. We speculated that the high expression of EG-VEGF at 11–13 weeks' gestation may make EVT invade too shallow and lead to insufficient spiral artery recasting and cause pregnancy-induced hypertension. Sergent et al. have shown that in a mouse model, maintaining EG-VEGF levels more than 11.5 days of pregnancy, which is equivalent to the first trimester of pregnancy, can lead to the development of the pathogenesis of HDP [12]. EG-VEGF has been described as a new actor in human

fertility and plays a major role in the development of the uterus, placenta, and ovaries. During pregnancy, PROK1 can not only promote the development of chorionic villi, but also regulate placental angiogenesis [8, 10, 13–15]. Its deregulation has been reported to be related to various placental pathologies, such as fetal growth restriction and PE [10, 15]. More interestingly, EG-VEGF has recently been confirmed to be involved in the development of tumors in multiple reproductive organs such as testes, prostate [16]. EG-VEGF can promote apoptosis via regulating PI3K/AKT/mTOR pathway [17] and may be involved in the ability of tumor cells to invade other organs [18]. As PROK1 is a well-known actor in cell proliferation and survival [19], one could speculate that PROK1 might then participate with other factors to the development of HDP. However, the exact mechanism by which EG-VEGF causes HDP is unclear, and we still need to make further study.

Since 2012, the focus of biomarker research has been on the prediction accuracy of sFlt-1, PlGF and sFlt-1/PlGF ratio. Previous studies have suggested that in pregnant women who develop HDP the concentration of pro-angiogenic factor PlGF decreases, and the concentration of anti-angiogenic factor sFlt-1 increases [20, 21], which is consistent with our research. Although elevated sFlt-1 and low PlGF are described as predictors of GHD, the predictive validity of these parameters is still worth exploring. The detection rate of PlGF in the first trimester is 41–59% in early-onset PE and 33% in late-onset PE [22]. A recent study of 4,212 singleton pregnancies showed that PlGF cannot improve the screening performance of early-onset preeclampsia [23]. A study by Boucoiran et al. found that sFlt-1 has a specificity of 90% and a sensitivity of 25% [24]. In a multi-center clinical study conducted, it is reported that the DR of the PlGF/sFlt-1 ratio is 82%, and the FPR is 5%, which is especially high for EO-PE (the detection rate is 89%) [22]. Therefore, we hope to compare EG-VEGF with PlGF and sFlt-1 to evaluate its predictive value in this study. Our study found that when EG-VEGF  $\geq 227.83$  pg/ml, the sensitivity, specificity, positive predictive value, and negative predictive value of EG-VEGF for HDP screening were higher than those of PlGF (43% vs. 33%; 93% vs. 70%; 52% vs. 86%; 51% vs. 62%). In addition, the positive predictive value of EG-VEGF is also higher than that of sFlt-1 (86% vs. 59%). In this study, EG-VEGF is more specific than PlGF and sFlt-1. This shows that this indicator has a better ability to correctly diagnose it as a non-patient in people who are not actually sick, reducing the waste of medical resources, and alleviating the patients' unprovoked panic and anxiety. The higher positive predictive value allows more people who screen positive to benefit from preventive interventions, reducing unnecessary exposure of the population.

The current guidelines recommend that preventive use of low-dose aspirin before 16 weeks for pregnant women at high risk of preeclampsia can reduce the risk of early-onset PE by more than 60% [25]. The same aspirin intervention after 20 weeks will not be as effective as the first trimester [26]. Therefore, in order to calculate individualized risks, the current trend in screening involves combining the presence or absence of multiple risk factors at 11–13 + 6 weeks. The prediction model we established in the first trimester include maternal BMI, MAP, and EG-VEGF, with an AUC of 0.8861 (95%CI: 0.7905–0.9818), which is better than using EG-VEGF alone (AUC: 0.66). This suggests that the combination of maternal characteristics of pregnant women and EG-VEGF can improve the effectiveness of the current prediction model. Our research supplements the existing evidence that during pregnancy, the maternal

characteristics routinely measured in clinical practice, combined with the biochemical marker EG-VEGF, can be used to screen the risk of gestational hypertension and preeclampsia in low-risk populations.

This research has the following advantages: In this study, for the first time, EG-VEGF was included in the prediction model of HDP, and the prediction model showed good predictive performance (AUC: 0.886); all measurements were carried out by trained personnel using recognized standardized methods, enabling the comparison of our findings with previous reports; both patients and care providers were blinded to HDP risk calculation, so as not to affect further management.

The potential limitations of this study as follows: first, it is a single-center retrospective study. We hope but have no chance to verify the most suitable model among external populations from more races, different regions, and different geographic regions. There is still a lack of evidence for clinical practice. Therefore, a larger scale multi-center prospective study is still needed to further verify the role of the examined markers in assessing the severity of PE and pregnancy outcome. Second, the combination of the mentioned thresholds of biomarkers as a biomarker test is only a preliminary suggestion. The level of all biomarkers may vary with race, gestational age, and may also depend on parity and smoking status. Therefore, further research is needed to reduce the bias caused by confounding factors.

## **Conclusion**

To our knowledge, this is the first time that EG-VEGF has been included in the HDP prediction model. Serum EG-VEGF levels increase significantly at 11–13 weeks of gestation. We found that EG-VEGF has better screening performance than PIGF and sFlt-1 in the first trimester. In addition, by combining with maternal characteristics, EG-VEGF can effectively predict the occurrence of HDP which provide new insights for HDP prevention strategies.

## **Declarations**

### **Data Availability**

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

### **Funding Information**

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### **Author information**

Affiliations

**Department of Obstetrics and Gynecology, The Second Xiangya Hospital|Central South University|  
Changsha|Hunan 410011|China**

Shiyu Zeng

**Department of Obstetrics and Gynecology, The Second Xiangya Hospital|Central South University|  
Changsha|Hunan 410011|China**

Ling Yu

**Department of Obstetrics and Gynecology, The Second Xiangya Hospital|Central South University|  
Changsha|Hunan 410011|China**

Yiling Ding

**Department of Obstetrics and Gynecology, The Second Xiangya Hospital|Central South University|  
Changsha|Hunan 410011|China**

MengyuanYang

### **Contributions**

Ling Yu(LY), YilingDing(YLD) and MengyuanYang(MYY) conceived the study. Shiyu Zeng(SYZ) and LY recruited the study participants, conducted the observations and participant interviews. YLD and MYY helped with implementation. SYZ conducted the qualitative analysis and drafted the manuscript. MYY reviewed the manuscript. All authors read and approved the submitted manuscript.

### **Corresponding author**

Correspondence to Mengyuan Yang.

### **Ethics approval and consent to participate**

The Ethics Committee of Second Xiangya Hospital of CSU granted approval to the study (REC 19/181). All participants signed informed consent forms. All experiments protocol for involving humans was in accordance to with the 1964 Helsinki declaration and with the guidelines of the Second Xiangya Hospital of CSU.

### **Consent for publication**

Not applicable.

### **Competing interests**

The authors declare that they have no competing interests.

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## Figures

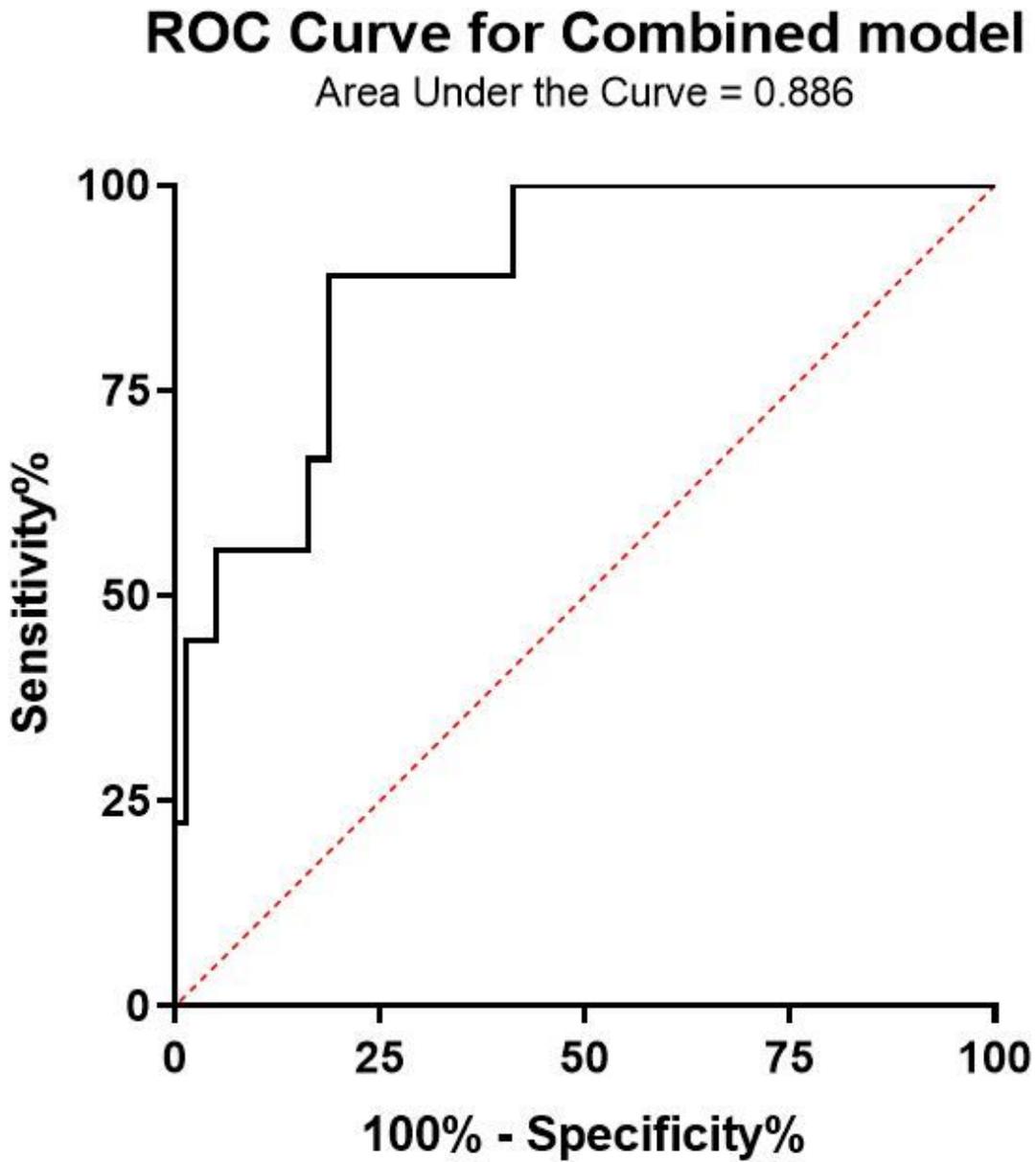


Figure 1

Receiver-operating characteristic curves (AUC) showing the performance for the combined model