



Expression and significance of serum soluble fms-like tyrosine kinase 1 (sFlt-1), CXC chemokine ligand 16 (CXCL16), and lipocalin 2 (LCN-2) in pregnant women with preeclampsia

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Background: To explore the value of serum soluble fms-like tyrosine kinase 1 (sFlt-1), CXC chemokine ligand 16 (CXCL16), and lipocalin 2 (LCN-2) in the diagnosis and grading of preeclampsia (PE).

Methods: A total of 186 patients with PE diagnosed and treated in our hospital were included. According to the disease severity, the patients were divided into the mild PE group (99 cases) and the severe PE group (87 cases). A total of 72 healthy pregnant women who underwent antenatal care were selected as the healthy control group. The levels of serum sFlt-1, CXCL16, and LCN-2 before medication were compared among the patients, and the diagnosis and grading value of the above 3 indicators were analyzed.

Results: For PE patients *vs.* healthy controls, the levels of sFlt-1 (132.71 ± 14.49 *vs.* 68.43 ± 9.28 $\mu\text{g/L}$), CXCL16 (2.15 ± 0.35 *vs.* 0.61 ± 0.12 $\mu\text{g/L}$), and LCN-2 (70.81 ± 8.25 *vs.* 19.22 ± 3.14 $\mu\text{g/L}$) were all significantly higher in PE patients than in the healthy controls ($P < 0.05$). For severe PE *vs.* mild PE, the levels of sFlt-1 (142.16 ± 20.23 *vs.* 124.41 ± 10.36 $\mu\text{g/L}$), CXCL16 (2.87 ± 0.59 *vs.* 1.51 ± 0.28 $\mu\text{g/L}$), and LCN-2 (90.76 ± 10.16 *vs.* 53.27 ± 6.19 $\mu\text{g/L}$) in the severe PE group were higher than those in the mild PE group ($P < 0.05$). Receiver operating characteristic curve (ROC) analysis showed that when the cut-off values of sFlt-1, CXCL16, and LCN-2 were 99.65, 1.36, and 0.84 $\mu\text{g/L}$, respectively, the diagnostic efficacy of PE was the highest. With these cut-off values, the diagnostic sensitivities of sFlt-1, CXCL16, and LCN-2 were 86.67%, 73.33%, and 93.33%, respectively. The specificities of sFlt-1, CXCL16, and LCN-2 were 80.00%, 86.67%, and 60.00%, respectively. The areas under the curves (AUC) of sFlt-1, CXCL16, and LCN-2 were 0.764, 0.769, and 0.831, respectively. When the cut-off values for sFlt-1, CXCL16, and LCN-2 were 135.16, 2.24, and 70.38 $\mu\text{g/L}$, respectively, the efficacy was the highest in distinguishing mild and severe PE. With these cut-off values, the AUC values of sFlt-1, CXCL16, and LCN-2 were 0.837, 0.808, and 0.869, respectively.

Conclusions: sFlt-1, CXCL16, and LCN-2 have certain significance in the diagnosis and grading of PE. Among them, LCN-2 has the highest correlation with the diagnosis and grading of PE.

Keywords: Soluble fms-like tyrosine kinase 1 (sFlt-1); CXC chemokine ligand 16 (CXCL16); lipocalin 2 (LCN-2); preeclampsia (PE)

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Introduction

Preeclampsia (PE) is a multisystem disease that occurs during pregnancy, and often manifests as transient hypertension, systemic endothelial dysfunction, and proteinuria after 20 weeks of pregnancy (1). The incidence of the disease is between 5% and 8% (2). PE patients are usually severely ill and progress rapidly. Therefore, PE has become one of the main causes of death among pregnant women worldwide (3). Currently, it is believed that PE is caused by insufficient placental perfusion-related ischemia and hypoxia, and subsequent secretion of toxic factors into blood vessels (4). Clinically, 24-hour proteinuria and clinical symptoms are used to diagnose and grade the severity of PE. However, the collection of 24-hour proteinuria specimens makes it difficult to diagnose and grade the disease in time. Achieving a fast diagnosis of PE has become a clinical research hotspot (5). In this study, serum soluble fms-like tyrosine kinase 1 (sFlt-1), CXC chemokine ligand 16 (CXCL16), and lipocalin 2 (LCN-2) levels were assessed with enzyme-linked immunosorbent assay (ELISA). The accuracy and efficiency of the 3 indicators in diagnosing and grading PE were discussed. We present the following article in accordance with the STARD reporting checklist (available at <https://dx.doi.org/10.21037/apm-21-1553>).

Methods

Participants

A total of 186 PE patients who received treatment in our hospital from January 2019 to December 2020 and 72 healthy parturients who underwent antenatal care during the same period were enrolled. The inclusion criteria for PE patients were as follows: diagnosed with PE (6); singleton pregnancy; natural conception. The exclusion criteria were as follows: patients with coagulation dysfunction; had a history of hypertension or heart disease; under blood pressure control-related treatments; incomplete clinical data.

All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the ethics committee of Sichuan Provincial People's Hospital. Written informed consent was signed by all participants.

PE degree classification standard

The 186 cases of PE patients were all in the PE group,

and the PE patients were divided into the mild and severe PE group according to the disease severity (7). Mild PE was defined as: blood pressure $\geq 140/90$ mmHg measured 4 hours after 20 weeks of pregnancy; random proteinuria (+) or urine protein ≥ 300 mg/24 h. Other potential symptoms in mild PE patients included headache and abdominal discomfort. Severe PE was defined as: blood pressure measurement $\geq 160/110$ mmHg; random proteinuria \geq (++) or urine protein ≥ 2.0 g/24 h. Other symptoms of severe PE patients included persistent upper abdominal discomfort or persistent headache and visual disturbance.

Sample collection and tests

Five mL fasting blood from the anterior elbow vein of PE patients before treatment was collected, and 5 mL fasting blood from the anterior elbow vein of healthy pregnant women on the day of physical examination was collected. After centrifugation at 3,200 r/min, the serum was separated and placed in a freezer at -80 °C until use. The ELISA method was used to determine sFlt-1, CXCL16, and LCN-2 levels. The kit was taken out from the refrigerator 15 minutes before the test and placed at room temperature. Sample diluent (50 μ L) was added to each 10 μ L serum sample. After mixing well, the samples were incubated at 37 °C for 15 min. After incubation, the liquid was discarded, and the wells were dried with absorbent papers. The wells were then washed 3 times by adding the washing solution for 30 s, pouring off the liquid, then drying with absorbent papers. Then, 50 μ L of enzyme-labeled working solution was added to each well and incubated at 37 °C for 15 minutes. After the incubation, the plate washing was repeated 3 times as mentioned. After drying, 50 μ L of chromogenic agent A and B were added sequentially. After mixing, the wells were incubated in a dark environment at 37 °C for 20 min. Finally, 50 μ L of stop solution was added, and the absorbance value (OD value) of each well was measured within 10 minutes after termination. The OD values of the blank control and standards were tested to generate a standard curve. The final sample concentrations were calculated as tested values \times the dilution ratio.

Statistical analysis

The data obtained were analyzed by SPSS 22.0 software. Measurement data were expressed as $\bar{x} \pm s$, the comparison between groups was performed by the *t* test, and the general

Table 1 General data of patients

Group	n	Age (year)	BMI (kg/m ²)	Gestation time (week)	Systolic pressure (mmHg)	Diastolic pressure (mmHg)
Healthy	72	30.11±2.16	24.89±2.54 [#]	32.41±2.06	110.19±8.12 [#]	75.13±5.91 [#]
Mild PE	99	29.98±2.55	27.79±3.05*	33.15±2.58	141.61±10.39*	95.12±3.64*
Severe PE	87	30.47±2.07	27.88±2.94*	32.68±2.64	157.46±15.14* [#]	103.15±2.16* [#]
<i>F</i>		1.109	27.133	1.993	327.784	988.984
<i>P</i>		0.331	<0.01	0.138	<0.01	<0.01

Compared to healthy controls, **P*<0.05; compared to the mild PE group, [#]*P*<0.05. BMI, body mass index; PE, preeclampsia.

Table 2 Comparison of the levels of sFlt-1, CXCL16, and LCN-2 between the PE group and the healthy group ($\bar{x}\pm s$)

Group	n	sFlt-1 (μg/L)	CXCL16 (μg/L)	LCN-2 (μg/L)
Healthy	72	68.43±9.28	0.61±0.12	19.22±3.14
PE	186	132.71±14.49	2.15±0.35	70.81±8.25
<i>t</i>		34.947	36.477	51.583
<i>P</i>		<0.01	<0.01	<0.01

sFlt-1, soluble fms-like tyrosine kinase 1; CXCL16, CXCL chemokine ligand 16; LCN-2, lipocalin 2; PE, preeclampsia.

Table 3 Comparison of the levels of sFlt-1, CXCL16, and LCN-2 between the mild PE group and the severe PE group ($\bar{x}\pm s$)

Group	n	sFlt-1 (μg/L)	CXCL16 (μg/L)	LCN-2 (μg/L)
Mild PE	99	124.41±10.36	1.51±0.28	53.27±6.19
Severe PE	87	142.16±20.23	2.87±0.59	90.76±10.16
<i>t</i>		7.663	20.467	30.789
<i>P</i>		<0.01	<0.01	<0.01

sFlt-1, soluble fms-like tyrosine kinase 1; CXCL16, CXCL chemokine ligand 16; LCN-2, lipocalin 2; PE, preeclampsia.

data of the 3 groups of patients were analyzed by one-way analysis of variance. The sensitivity and specificity of sFlt-1, CXCL16, and LCN-2 in the diagnosis and grading of PE were analyzed with the receiver operating characteristic (ROC) curve. *P*<0.05 indicated that the difference was statistically significant.

Results

General information of participants

There were 99 cases in the mild PE group and 87 cases in the severe PE group. The body mass index (BMI), systolic blood pressure, and diastolic blood pressure of the PE patients were higher than those of the healthy group (*P*<0.05), and the systolic and diastolic blood pressures of the severe PE group were higher than those of the mild group (*P*<0.05). No significant difference was observed in the age and gestational age of the 3 groups of participants (*P*>0.05; *Table 1*).

Comparison of sFlt-1, CXCL16, and LCN-2 levels between the PE group and the healthy group

The levels of sFlt-1, CXCL16, and LCN-2 in the PE

group were higher than those in the healthy group, and the difference was statistically significant (*P*<0.05; *Table 2*).

Comparison of sFlt-1, CXCL16, and LCN-2 levels between the mild PE group and the severe PE group

The levels of sFlt-1, CXCL16, and LCN-2 in the severe PE group were all higher than those in the mild PE group, and the difference was statistically significant (*P*<0.05; *Table 3*).

The value of sFlt-1, CXCL16, and LCN-2 in the diagnosis of PE

The ROC curve showed that when the sFlt-1 cut-off value was 99.65 μg/L, the area under the curve (AUC), sensitivity, and specificity in diagnosing PE were 0.764, 86.67%, and 80.00%, respectively. When the CXCL16 cut-off value was 1.36 μg/L, the AUC, sensitivity, and specificity in diagnosing PE were 0.769, 73.33%, and 86.67% respectively. When the LCN-2 cut-off value was 0.84 μg/L, the AUC, sensitivity, and specificity in diagnosing PE were 0.831, 93.33%, and 60.00%, respectively, as shown in *Figure 1* and *Table 4*.

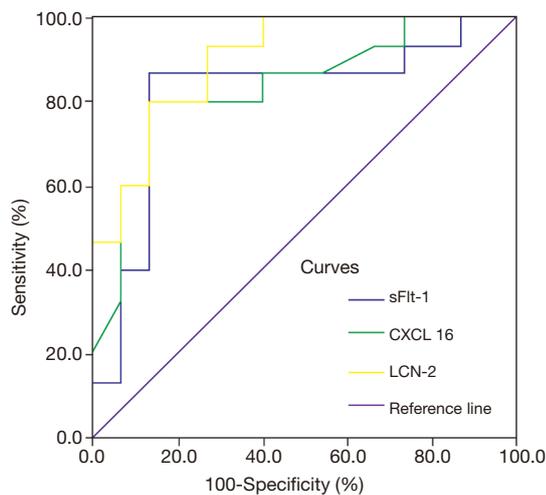


Figure 1 Receiver operating characteristic (ROC) curves of soluble fms-like tyrosine kinase 1 (sFlt-1), CXC chemokine ligand 16 (CXCL16) and lipocalin 2 (LCN-2) in the diagnosis of preeclampsia (PE).

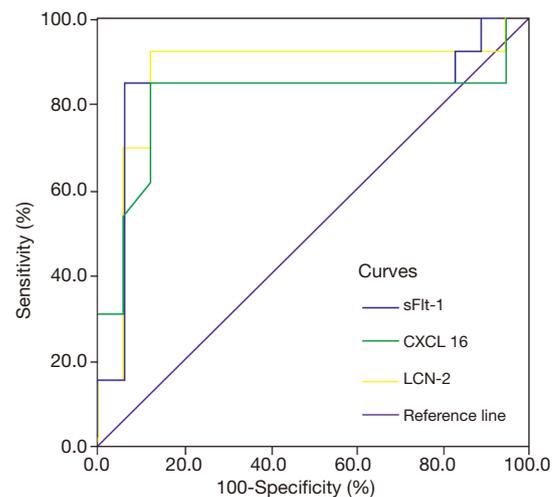


Figure 2 Receiver operating characteristic (ROC) curves of soluble fms-like tyrosine kinase 1 (sFlt-1), CXC chemokine ligand 16 (CXCL16) and lipocalin 2 (LCN-2) in the grading of mild and severe preeclampsia (PE).

Table 4 Value of sFlt-1, CXCL16, and LCN-2 in the diagnosis of PE

Indicator	Cut-off	AUC	Sensitivity (%)	Specificity (%)	Youden index	95% CI
sFlt-1	>99.65	0.764	86.67	80.00	0.667	0.575–0.899
CXCL16	>1.36	0.769	73.33	86.67	0.600	0.580–0.902
LCN-2	>0.84	0.831	93.33	60.00	0.53	0.650–0.942

sFlt-1, soluble fms-like tyrosine kinase 1; CXCL16, CXC chemokine ligand 16; LCN-2, lipocalin 2; PE, preeclampsia; AUC, area under curve; CI, confidence interval.

The diagnostic value of sFlt-1, CXCL16, and LCN-2 in discriminating mild and severe PE

The ROC curve showed that when the cut-off value for sFlt-1 was 135.16 $\mu\text{g/L}$, the AUC, sensitivity, and specificity for distinguishing mild and severe PE were 0.837, 84.62%, and 94.12%, respectively. When the cut-off value for CXCL16 was 2.24 $\mu\text{g/L}$, the AUC, sensitivity, and specificity were 0.808, 84.62%, and 88.24%, respectively. When the cut-off value for LCN-2 was 70.38 $\mu\text{g/L}$, the AUC, sensitivity, and specificity were 0.869, 92.31%, and 88.24%, respectively (Figure 2 and Table 5).

Discussion

PE is a multisystem dysfunction that happens in women after week 20 of gestation (4). Induced by placental hypoperfusion, PE progression leads to changes in placental microenvironment, releasing of cytokines, and

finally resulting in proteinuria and maternal hypertension. There was no targeted treatment for PE, antihypertensive, spasmolysis and sedation are usually used, termination of pregnancy was also a choice to severe conditions.

PE progresses rapidly. There are currently no specific laboratory indicators to diagnose and grade the severity of PE patients, which may have an impact on the subsequent treatment of patients (8,9). The anti-angiogenic factor sFlt-1 is secreted by the placenta and is involved in vascular regulation. It can cause placental angiogenesis and cause vascular endothelial damage (9,10). CXCL16 is a multifunctional protein which is soluble in the cytoplasm or bound in the membrane. CXCL16 is expressed in T cells, B cells, epithelial cells, macrophages, fibroblasts, endothelial cells, and vascular smooth muscle cells (11). LCN-2 is a widely distributed member of the lipocalin family. LCN-2 can mediate inflammation, inhibit cell apoptosis, and promote tumor cell metastasis. The expression of LCN-2

Table 5 Diagnostic value of sFlt-1, CXCL16, and LCN-2 in grading mild and severe PE

Indicator	Cut-off	AUC	Sensitivity (%)	Specificity (%)	Youden index	95% CI
sFlt-1	>135.16	0.837	84.62	94.12	0.787	0.657–0.946
CXCL16	>2.24	0.808	84.62	88.24	0.729	0.623–0.928
LCN-2	>70.38	0.869	92.31	88.24	0.805	0.695–0.964

sFlt-1, soluble fms-like tyrosine kinase 1; CXCL16, CXC chemokine ligand 16; LCN-2, lipocalin 2; PE, preeclampsia; AUC, area under curve; CI, confidence interval.

is increased in a variety of inflammatory reactions such as enteritis, peritonitis, and atherosclerosis (12,13).

In this study, the levels of sFlt-1, CXCL16, and LCN-2 in the PE group were higher than those in the healthy group. The levels of sFlt-1, CXCL16, and LCN-2 in the severe PE group were higher than those in the mild PE group. The ROC curve results in this study show that sFlt-1, CXCL16, and LCN-2 all have good sensitivity for the diagnosis of PE, among which LCN-2 is the best for the diagnosis of PE, as it reached a diagnostic sensitivity of 93.33% and an AUC of 0.831. Insufficient blood supply to the placenta leads to the increase in sFlt-1 content, and the increase in sFlt-1 leads to angiogenesis dysfunction, damaged endothelial cells, and subsequent inflammation and PE (9). Inflammation and endothelial damage can induce high expression of LCN-2, which can possibly cause vascular sclerosis to aggravate PE (14). LCN-2 can combine with matrix metalloprotein-9 (MMP-9) to form a heterodimer protein (15). The reduced decomposition or activation of MMP-9 during endothelial dysfunction promotes vascular smooth muscle-related vascular remodeling (16), which can aggravate endothelial dysfunction and promote PE. LCN-2 is also increased in insulin-resistant individuals (12), who are often more susceptible to PE (17). Furthermore, LCN-2 has been shown to be an indicator and key player in renal injury (18), and the multiple organ dysfunction syndrome caused by PE may promote renal injury-related LCN-2 expression. All these factors may possibly explain why LCN-2 had a higher AUC and sensitivity for the diagnosis of PE among the 3 indicators. As a chemokine, CXCL16 can participate in inflammatory responses to aggravate vascular endothelial damage and the subsequent inflammatory response (19). CXCL16 has a high affinity with its receptors. The activation of CXCL16 enables recognition of lymphocytes to vascular endothelial cells (20), thus potentially aggravating vascular endothelial damage. We also showed that sFlt-1, CXCL16, and LCN-2

are satisfactory indicators for diagnosing and grading PE, indicating that they are all potential key players in the pathogenesis of PE.

In summary, the levels of sFlt-1, CXCL16, and LCN-2 are significantly higher in PE patients. The 3 indicators can also discriminate between severe PE patients and mild PE patients, among which LCN-2 has the highest correlation with the diagnosis and grade of PE.

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Footnote

Reporting Checklist: The authors have completed the STARD reporting checklist. Available at <https://dx.doi.org/10.21037/apm-21-1553>

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the ethics committee of Sichuan Provincial People's Hospital. Written informed consent was signed by all participants.

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