Predictive Performance of sFlt-1, PlGF and the sFlt-1/PlGF Ratio for Preeclampsia: A Systematic Review and Meta-Analysis

Guifeng Ding¹

¹The Xinjiang Uygur Autonomous Region Maternal and Child Health Care Hospital

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Abstract

Background: It is difficult to evaluate whether monitoring serum sFlt-1, PlGF, or sFlt-1/PlGF in pregnant women who are suspected of having PE can significantly shorten the PE diagnosis time. Objectives: To estimate the accuracy of sFlt-1, PlGF and sFlt-1/PlGF in preeclampsia prediction. Search Strategy: Databases including PubMed, Web of Science, Medline, CNKI, SinoMed, VIP Journal, and Wanfang Data were searched for eligible studies published until October 7, 2020. Selection Criteria: The research subjects were pregnant women with or without PE. The research types were case-control studies and cohort studies. This was an original study involving the detection of at least one of the following in the blood, serum or plasma: sFlt-1, PlGF, and sFlt-1/PlGF. Data Collection and Analysis: Meta-Disc 1.4 was employed, using the Sen, Spe, PLR, NLR, and DOR to plot SROC, and subgroup analysis and meta-regression were conducted. Main Results: Meta-analysis showed that for sFlt, PlGF and sFlt-1/PlGF, the Sen was 0.811 (95% CI: 0.783-0.837), 0.735 (95% CI: 0.713-0.757), and 0.779 (95% CI: 0.763-0.795), respectively; the Spe was 0.786 (95% CI: 0.769-0.802), 0.731 (95% CI: 0.721-0.741), and 0.885 (95% CI: 0.881-0.889), respectively. Conclusions: The sFlt-1/PlGF ratio showed better predictive performance for preeclampsia than sFlt-1 or PlGF alone. However, the predictive value of the latter two cannot be ignored. Funding: Xinjiang Uygur Autonomous Region "Tianshan Innovation Team Plan"(2020D14010) Keywords: gestational hypertension; hypertension; meta-analysis; preeclampsia; predictive; PlGF; sFlt-1, PlGF, systematic review

Introduction

Preeclampsia (PE) is a multiorgan disease that is common in pregnancy after 20 weeks of gestation and mainly presents as signs and symptoms of newly developed hypertension with proteinuria or other end-organ dysfunction¹. Worldwide, approximately $3\%^{5}\%$ of pregnancies are complicated with PE²; PE is associated with high morbidity and mortality, accounting for $5\%^{7}\%$ of all pregnant women's deaths³, and it mainly occurs in low- and middle-income countries ⁴. The fetuses and newborns of pregnant women with PE may experience growth restriction ⁵, respiratory distress, eclampsia, HELLP syndrome, renal failure, death, or other adverse outcomes ⁶. PE is often complicated with other conditions, such as renal insufficiency ⁷, impaired liver function ⁸, and neurological disorders⁹, and patients with PE are also at risk of postpartum recurrence and developing cardiovascular and cerebrovascular diseases¹⁰, diabetes ¹¹, end-stage renal disease ¹², dementia ¹³, and others.

At present, the biggest problem in clinical practice is failure to identify patients with preeclampsia early. Patients are already in the middle or late stages of the disease when treated, often have multiple concurrent organ complications, and need referral to a tertiary care center or multidisciplinary treatment¹⁴. Fortunately, if we can detect and identify high-risk PE patients early and instruct them to take a small dose of aspirin in the first trimester to extend the gestational age, we may be able to reduce the incidence of PE and prevent the occurrence of maternal and infant complications¹⁵. Due to the complex pathophysiological characteristics and clinical unpredictability of PE and the limited evidence for the detective performance of

different diagnostic methods, there has been no accurate and reliable diagnostic method for predicting PE to date ^{16, 17}.

The etiology of PE has not been fully elucidated. There is evidence that maternal endothelial dysfunction due to placental factors plays a significant role in the pathogenesis of PE 18,19 . Soluble fms-like tyrosine kinase-1 (sFlt-1), an anti-angiogenic factor secreted by the placenta, binds to vascular endothelial growth factor (VEGF) and placental growth factor (PIGF) in the maternal circulation. In the bound form, sFlt-1 interacts with membranous tyrosine kinase, which is critical to the biological activity of sFlt-1. High concentrations of antiangiogenic factors (e.g., sFlt-1) and low concentrations of proangiogenic factors (e.g., VEGF and PIGF) can produce an antiangiogenic state, leading to general maternal vascular dysfunction $^{20, 21}$ and eventually to hypertension, proteinuria, and other clinical manifestations of PE²².

The study population, gestational age, cut-off value, detection and analysis platform, and disease subtypes such as early-onset preeclampsia(EO-PE) and late-onset preeclampsia(LO-PE) were included in studies from different countries ^{23, 24} are diverse, and the observed diagnostic sensitivity and specificity are still highly variable and controversial. This lack of consistency makes it difficult to evaluate whether monitoring serum sFlt-1, PlGF, or sFlt-1/PlGF in pregnant women who are suspected of having PE can significantly shorten the PE diagnosis time. An increasing number of large-scale, multicenter studies about the detection of sFlt-1, PlGF, and sFlt-1/PlGF concentrations in predicting PE have been published, which has prompted us to conduct this systematic review and meta-analysis to determine whether sFlt-1, PlGF or sFlt-1/PlGF can better predict PE than existing clinical indicators, enabling early screening and timely intervention, avoiding further disease progression, and improving maternal and infant morbidity and mortality.

Methods

A systematic review and meta-analysis were performed according to the PRISMA-DTA statement ²⁵. The meta-analysis was registered in Prospero (CRD42021218579).

Information sources and search strategy

Two researchers, Luhan Zhang and Yuanyuan Li, independently searched English (PubMed, Web of Science, and Medline) and Chinese (China National Knowledge Infrastructure (CNKI), Wanfang Data, Weipu Journal Resources, and Chinese Biomedical Literature) databases. The databases were searched from inception to October 7, 2020, for journal articles that were publicly published. At the same time, the references listed in the obtained documents were manually searched to ensure that no documents had been omitted in the electronic search. The English keywords used for retrieval were preeclampsia and soluble fms-like tyrosine kinase-1, preeclampsia and sFlt-1, preeclampsia and placental growth factor, and preeclampsia and PlGF. The Chinese keywords used for retrieval were 'zixianqianqi' and sFlt-1, 'zixianqianqi' and soluble fms-like tyrosine kinase 1, 'zixianqianqi' and PlGF, 'zixianqianqi' and placental growth factor.

Eligibility criteria

The inclusion criteria were as follows: 1) The research subjects were pregnant women with or without PE; 2) The research types were case-control studies and cohort studies; 3) The studies showed results for the diagnostic standard for PE; 4) The research was an original study involving the detection of at least one of the following in the blood, serum or plasma: sFlt-1, PlGF, and sFlt-1/PlGF; 5) The research data were valid and reliable, and the 2×2 table (true positive number, false positive number, false negative number, and true negative number) could be extracted completely or could be calculated from existing data; and 6) There were exact cut-off values for sFlt-1, PlGF, and sFlt-1/PlGF in blood, serum, or plasma for PE prediction.

The exclusion criteria included the following: 1) abstracts, reviews, duplicate publications, and repeated literature studies were excluded; and 2) studies on nonsingleton pregnancies, pregnancies resulting in death, or other complications were excluded.

Study selection

All the documents were manually and independently screened by two researchers, Luhan Zhang and Yuanyuan Li, and then reviewed according to the preset literature inclusion and exclusion criteria. The full texts of the obtained studies were reviewed in depth to determine whether the studies should be included or excluded. Disagreement was resolved by discussion and negotiation or a definitive opinion given by a third authoritative expert, Qi Sun, or the author was contacted to obtain original information.

Data extraction

According to a preset extraction table, the data from documents that met the inclusion criteria, including the first author, publication year, study design type, study population characteristics, number of cases and controls, gestational age at the time of sampling, cut-off value, disease subtypes (early-onset and late-onset disease), assay used, number of true positives, number of false positives, number of false negatives, number of true negatives, sensitivity, and specificity, were extracted independently by Luhan Zhang. Controversial data encountered during the data extraction process were addressed in conjunction with a second researcher, Wenjing Li, through discussion and negotiation. If no consensus was reached, the problem was left to a third authoritative expert, Ying Feng, for resolution.

Assessment of risk of bias

The methodological quality of the included studies was examined by two independent reviewers, Luhan Zhang and Weiwei Xing, by using the Cochrane Collaboration Diagnostic Test Methodology Quality Assessment Guide and Quality Review of Diagnostic Accuracy Studies-2 $(QUADAS-2)^{26}$ to assess the deviation risk. A third researcher, Yuanyuan Li, gave the final opinion on disagreements during the screening process. The risk of deviation was mainly applied in the following five aspects: case selection, trials to be evaluated, diagnostic standard, case flow and progression. The first three aspects also judged the clinical applicability of the method to be tested.

Diagnostic accuracy evaluation

The number of true positives, true negatives, false positives, and false negatives from all studies were extracted, and the combined sensitivity (Sen), specificity (Spe), positive likelihood ratio (PLR), negative likelihood ratio (NLR), and diagnostic odds ratio (DOR) and their 95% confidence intervals (CIs) were calculated. The Sen and Spe of the included studies were used to construct symmetric receiver operating characteristic (SROC) curves and to calculate the area under the curve (AUC) for each variable. The combined effect was determined based on each study.

Data synthesis

A meta-analysis was performed on all data using Review Manager 5 (Version 5.0 Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2008) and Meta-Disc 1.4 (XI Cochrane um, Barcelona, Spain). First, the receiver operating characteristic (ROC) curve was drawn, and whether the figure was "shoulder-arm-shaped" was observed. Then, the Spearman correlation coefficient of the sensitivity logarithm and (1-specificity) logarithm was calculated to determine whether there was a threshold effect. The Cochran Q test and I^2 statistic were used to assess heterogeneity. When there was significant heterogeneity among studies, a random effects model (P < 0.05 or $I^2 > 50\%$) was used. Otherwise, the fixed-effects model for data pooling was applied. Subgroup analysis was conducted based on presets, and meta-regression was also used to explore the source of statistical heterogeneity. All statistical tests were two-sided, and P < 0.05 was considered statistically significant.

Results

Study selection

Figure S1 summarizes the literature search and selection process. A total of 1,716 articles were identified; among them, 563 duplicate articles were excluded, leaving 1,153 articles that potentially met the criteria. After reviewing the titles and abstracts of the articles, 1,024 articles were excluded; the full texts of the remaining 129 articles were read, 71 did not meet the inclusion criteria (for reasons including poor outcome

prediction, insufficient information in the 2×2 table, lack of a control group, and poor quality of literature), and 58 studies were finally included in the study.

Study characteristics

All 58 studies, 39 in English and 19 in Chinese, were published between 2001 and 2020 and came from different parts of the world. A total of 33,558 patients were enrolled, including 3,661 cases in the case group (all were PE patients according to the diagnostic standard) and 29,897 in the control group (all were non-PE patients). It was known from the included literature that the case group included all singleton PE patients without other hypertensive disorder complications; the control group included pregnant women with singleton pregnancies and normal prenatal examinations during the same period. Among them, 19 studies were prospective studies, and the other 39 studies were retrospective studies. The included studies were nostly case-control studies and a few cohort studies. The gestational age measured by each test index were not the same, so the cut-off values used were different. There were 16 studies that determined the concentration of sFlt-1 to predict PE, 28 that determined the PIGF concentration to predict PE, and 41 that determined the sFlt-1/PIGF ratio to predict PE. There were 3 reports of sFlt-1 alone predicting EO-PE, 5 of PIGF that alone predicting EO-PE, 2 of sFlt-1 alone predicting LO-PE, 12 of sFlt-1/PIGF alone predicting EO-PE, and 7 of PIGF alone predicting LO-PE. When the same article had different cut-off values, the concentration of each index was measured and included in the subsequent meta-analysis (Table S1).

Risk of bias of included studies

The 58 included studies were evaluated and scored according to the following three criteria: "Yes", "No" and "Unclear". Among the quality items, there were 3 studies at high risk of deviation in the 1st item, which addressed the disease spectrum; in the 3rd item, on the acceptableness of the test interval, there was 1 study at high risk of deviation; in the 8th item, the standard diagnostic blinding method, there was 1 study at high risk of deviation; in the 9th item, addressing relevant clinical information, there were 13 studies at high risk of deviation; and in the 10th item, whether to explain the unexplainable/intermediate result report, there were 14 studies at high risk of deviation. Figure 2 summarizes the quality assessment of these studies. In most studies, there were great reports, including a full description of the selection criteria, patient profiles, tests, and use of appropriate reference standards (Figure S2).

Synthesis of results

Diagnostic accuracy evaluation

The results from the Meta-Disc 1.4 software showed that in the 16^{27-42} , $28^{-27-32, 34-41, 43-54}$, and $41^{27, 30-33, 35-37, 39-42, 50, 56-64, 66-8081-83}$ studies using sFlt-1, PlGF, and the sFlt-1/PlGF ratio, respectively, to predict PE, the Spearman correlation coefficients were -0.222, -0.171, and -0.118, respectively, and the *P* values were 0.408, 0.384, and 0.464, respectively, indicating that there was no threshold effect. The results for the heterogeneity tests showed P < 0.001 and $I^2 > 75\%$, indicating that the heterogeneity among different studies was great. A random effects model was used for the meta-analysis, and the results showed that the overall combined Sen was 0.811 (95% CI: 0.783-0.837), 0.735 (95% CI: 0.713-0.757), and 0.779 (95% CI: 0.763-0.795), respectively; the PLR was 5.097 (95% CI: 3.498-7.426), 4.053 (95% CI: 3.150-5.214), and 6.385 (95% CI: 4.847-8.410), respectively; the NLR was 0.265 (95% CI: 0.164-0.430), 0.341 (95% CI: 0.275-0.423), and 0.241 (95% CI: 0.192-0.303), respectively; the DOR was 21.092 (95% CI: 10.857-40.976), 14.150 (95% CI: 8.972-22.315), and 31.431 (95% CI: 19.681-50.197), respectively; and the AUC was 0.9005, 0.8582, and 0.9065, respectively (Figure S3, S5-S7).

Subgroup analysis and metaregression

The forest plot of the combined DOR for sFlt-1 was obtained by Meta-Disc 1.4 software. The DORs of each study and the combined DOR were not distributed along the same straight line. Meanwhile, Cochran's Q=99.16, P < 0.001, indicating that there was heterogeneity caused by non-threshold effects. A subgroup analysis of 16 articles was conducted according to study design (prospective or retrospective), sample size

([?]50 or <50), and literature quality ("Unclear"[?]4 or "Unclear">4), and 3 articles ^{31, 32, 40} were left. The overall combined Sen was 0.826 (95% CI: 0.780-0.866), $I^2 = 51.3\%$; the combined Spe was 0.691 (95% CI: 0.649-0.730), $I^2 = 52.9\%$; the combined PLR was 2.661 (95% CI: 2.316_3.056), $I^2 = 0.0\%$; the combined NLR was 0.256 (95% CI: 0.199-0.329), $I^2 = 3.10\%$; and the combined DOR was 11.251 (95% CI: 7.872-16.081). The chi-squared for heterogeneity was 0.11, P = 0.947, $I^2 = 0.0\%$, indicating that there was no heterogeneity among the studies, and thus, a fixed-effects model was used to combine the study results. The AUC was 0.8366. The combined DOR, the results, and the forest plot are shown in Figure 4 and Table S2.

In the forest plot of the combined DOR for PlGF and sFlt-1/PlGF, the DORs of each study and the combined DOR were not distributed along the same straight line. At the same time, Cochran's Q=247.74, P < 0.001, and Cochran-Q=632.89, P < 0.001, respectively, demonstrating that there was heterogeneity elicited by non-threshold effects. Meta-regressions of 29 and 43 data points were conducted separately according to study design (prospective or retrospective), sample size ([?]30 or <30), literature quality ("Unclear")?]4 or "Unclear">>4), PlGF cut-off value ([?]100 or <100), detected gestational week ([?]14 w or >14 w to parturition), sFlt-1/PlGF cut-off value ([?]30 or <30), and detected gestational week ([?]20 w or >20 w to parturition). The heterogeneity might be related to the sample size and cut-off values (Table S2).

According to the data for the EO-PE and LO-PE classification in the literature, the two entities were divided into subgroups. There were 3 reports of EO-PE detected by sFlt-1 alone $^{33, 37, 39}$, the combined DOR was 13.160 (95% CI: 1.952-88.713), and the AUC was 0.9217. There were 5 reports of PlGF alone predicting EO-PE^{33, 37, 39, 50, 55}, the combined DOR was 13.108 (95% CI: 1.865-92.146), and the AUC was 0.8754. There were two reports of PlGF alone predicting LO-PE $^{50, 55}$, the combined DOR was 8.572 (95% CI: 2.254-32.603). sFlt-1/PlGF alone predicted EO-PE in 12 cases $^{33, 37, 50, 55, 65, 79, 81, 83}$, the combined DOR was 230.24 (95% CI: 63.956-828.82), and the AUC was 0.9806. There were seven data points that predicted LO-PE $^{50, 55, 65, 79, 81}$, the combined DOR was 20.997 (95% CI: 5.947-74.132), and the AUC was 0.8877. The results are shown in Figures S8-S13 and Table S3.

Comment

Main findings

A total of 58 articles were included in this systematic review and meta-analysis. Although the test indicators reported in the articles were different, the standard used was the diagnostic criteria for PE recommended by international guidelines. Meta-analysis showed that the combined Sen of sFlt, PlGF and sFlt-1/PlGF was 0.811 (95% CI: 0.783-0.837), 0.735 (95% CI: 0.713-0.757), and 0.779 (95% CI: 0.763-0.795), respectively; the Spe was 0.786 (95% CI: 0.769-0.802), 0.731 (95% CI: 0.721-0.741), and 0.885 (95% CI: 0.881-0.889), respectively; and the AUC was 0.9005, 0.8582, and 0.9065, respectively. From the individual Sen, Spe, and AUC, we determined that the sFlt-1/PlGF ratio was more effective in PE prediction than sFlt-1 or PlGF alone, which may be due to the sFlt-1/PlGF ratio eliminating the detection error. The efficacy of sFlt-1 alone for predicting PE was similar to that of PlGF alone, and the efficacy of sFlt-1 was slightly better than that of PlGF; however, the heterogeneity of the pooled statistics for each variable was relatively high.

The results from the subgroup analysis showed that the reasons for sFlt-1-related heterogeneity might be the quality of the literature, the size of the sample, or the study design (prospective or retrospective). The high-quality, prospective and large-sample-size references included in the subgroup analysis confirmed that sFlt-1 concentration detection is indeed helpful in predicting PE. Meta-regression analysis including study design, sample size, cut-off value, detected gestational age, and literature quality found that the heterogeneity in PIGF detection might be associated with sample size. We also divided EO-PE and LO-PE into two subgroups. Only EO-PE was detected by sFlt-1, and the combined odds ratio of EO-PE detected by PIGF and sFlt-1/PIGF was higher than the combined odds ratio of LO-PE, indicating that PIGF and sFlt-1/PIGF had better screening performance for EO-PE.

Meta-regression analysis by study design, sample size, cut-off value, detected gestational age, and study quality showed that sFlt-1/ PlGF-related heterogeneity might be associated with the cut-off value.

Strengths and limitations

It is undeniable that our study also has some limitations. First, in terms of study inclusion, although the literature screening was carried out independently by two researchers according to the preset criteria, there was still a certain selection bias. In addition, since the information needed by the 2x2 table could not be directly extracted in most of the studies, there was a slight discrepancy between the results we obtained after calculation and those given in the literature; this might have resulted from the different ways of recording decimal places in the data by the researchers. Second, in terms of the interaction level of the included studies, the heterogeneity of each of the pooled detection indicators was high, the examined range of gestational weeks in some studies was wide, and the distribution of cut-off values was scattered. Some studies have shown that the concentration of each detection indicator changes with gestational age, so many studies use cut-off values related to gestational age, thus resulting in greater heterogeneity. Third, regarding the level of system evaluation, due to language limitations, documents in languages other than Chinese and English were not retrieved, and only foreign articles with English versions were searched.

Comparison with existing literature

The subgroup analysis results from a systematic review and meta-analysis on detecting PlGF alone in PE prediction, published by Swati Agrawal⁸⁴, showed that the accuracy of prediction gradually increased after 19 weeks of pregnancy. This is also in line with the evidence that the concentration of PlGF changes in PE patients during pregnancy ⁸⁵: PlGF increased before 30 weeks of pregnancy and decreased after 34 weeks. When the cut-off value was 80-120 pg/mL, the Sen of PlGF in predicting PE was the highest (0.78); however, the lack of uniform standards for cut-off value division have limited its clinical applicability. We have included original research literature that was published from 2018 to 2020. The difference between our study and the previous meta-analysis lies in what we found through subgroup analysis, i.e., that PlGF concentration detection might be more accurate in PE prediction when considering larger sample sizes. The articles with large sample sizes were mostly multicenter studies with diverse populations, which can better account for differences in genetic, behavioral, and race-related factors, and thus, it can better illustrate that the detection of PlGF can predict PE. This is also a reminder to researchers that high-quality research programs should be designed in the future.

A meta-analysis conducted by Swati Agrawal⁸⁶ divided patients into high-risk and low-risk groups for a subgroup analysis, which showed that the Spe of sFlt-1/PlGF was high (0.80) in high-risk patients. No significant differences were found in other evaluation indicators. One study ⁸⁰ pointed out that it is necessary to consider the effective period the cut-off value in predicting PE, and different cut-off ranges can exclude PE within one week and predict PE within 4 weeks. Another study ⁸⁷ proposed that for early-onset or late-onset PE, sFlt-1/PlGF cut-off values were used to predict the disease. As preeclampsia is a highly heterogeneous disease, it can be seen that PIGF and sFlt-1/PIGF predict a higher DOR for EO-PE than for LO-PE, indicating that PIGF and sFlt-1/PIGF have better performance in screening EO-PE. It may be that preeclampsia (usually in its early stages) has two stages, the first of which is incomplete remodeling of the spiral arteries and the second of which involves uterine placental perfusion disorders and oxidative stress of the placenta. Oxidatively stressed syncytiotrophoblastic cells (STB) secrete proteins that disrupt the balance of angiogenesis in pregnant women and are biomarkers for preeclampsia⁸⁸. Oxidative stress occurs in the late stage, but the gestational ages in most of the included studies are concentrated in the early and middle stages of pregnancy, so the prediction effect for the late stage is not as good as that for the early stage. Since the time interval between the test results and the onset of preeclampsia is not reported in the literature, we cannot analyze this. The research subjects included in our study were not strictly divided according to basic conditions (age, race, pregnancy times, conception method, weight, etc.). In addition, the detection reagents, platforms and methods, and gestational weeks of each study were different. All these factors may have caused differences in the cut-off values. Due to the large differences in the cut-off values among studies. previous meta-analyses have not provided an exact cut-off level. State-of-the-art prediction models for the disease rely on prior risk for disease determined by maternal characteristics and changes in the biomarker data after transformation into the multiple of the median for gestational age and maternal characteristics;

however, classification with the cutoff value obtained from this study was not easy, so we did not include these studies. The specificity in our research was also very high, indicating effective identification of non-PE patients, which relieves the anxiety of low-risk patients and prevents unnecessary expenses incurred by hospitalization monitoring and treatment due to misdiagnosis and other reasons ⁸⁹.

Conclusions and Implications

In summary, the sFlt-1/PlGF ratio is more effective than sFlt-1 or PlGF alone in PE prediction. However, the effectiveness of the latter two cannot be ignored, since both tests are beneficial in the early diagnosis of PE, helping to identify high-risk patients and improve adverse outcomes in these patients and their infants. In the future, more prospective multicenter studies will be required to further refine the population, disease subtypes, detected gestational age, testing platforms, and cut-off values. In this way, we can conduct more detailed subgroup analysis to explore the sources of heterogeneity among studies and provide a reference for clinical decision-making.

Author contributions

Guifeng Ding and Qi Sun conceived and designed the analysis. Luhan Zhang and Yuanyuan Li screened studies. Luhan Zhang and Weiwei Xing assessed the studies. Qi Sun and Ying Feng were the third party for the screening and quality assessment of the literature. Luhan Zhang and Wenjing Li extracted and collected data. Luhan Zhang and Yuanyuan Li jointly analyzed the data and reported the results. All authors participated in the manuscript writing and revision.

Figure legend

Figure S1: PRISMA flowchart outlining study selection.

Search strategy and study selection per Preferred Reporting Items for Systematic reviews and Meta-Analyses. Figure S2: Quality of the studies.

Assessment of risk of bias in studies included according to the Cochrane handbook.

Figures S3: Forest plot of sFlt-1 predicting summary sensitivity (A), specificity (B), positive likelihood ratio (C), and negative likelihood ratio (D) of preeclampsia.

Figure S4: sFlt-1 predicting the summary diagnostic odds ratio forest plot (A) and subgroup analysis (B) of preeclampsia.

Figure S5: Forest plot of PlGF predicting summary sensitivity (A), specificity (B), positive likelihood ratio (C), negative likelihood ratio (D), and diagnostic odds ratio (E) of preeclampsia.

Figure S6: Forest plot of sFlt-1/PlGF predicting summary sensitivity (A), specificity (B), positive likelihood ratio (C), negative likelihood ratio (D), and diagnostic odds ratio (E) of preeclampsia.

Figure S7: Symmetric receiver operator characteristic curve of sFlt-1 (A), PlGF (B) and sFlt-1/PlGF (C)

Figure S8: Forest plot of sFlt-1 predicting summary sensitivity (A), specificity (B), positive likelihood ratio (C), negative likelihood ratio (D), and diagnostic odds ratio (E) of EO-PE.

Figure S9: Forest plot of PlGF predicting summary sensitivity (A), specificity (B), positive likelihood ratio (C), negative likelihood ratio (D), and diagnostic odds ratio (E) of EO-PE.

Figure S10: Forest plot of PIGF predicting summary sensitivity (A), specificity (B), positive likelihood ratio (C), negative likelihood ratio (D), and diagnostic odds ratio (E) of LO-PE.

Figure S11: Forest plot of sFlt-1/PlGF predicting summary sensitivity (A), specificity (B), positive likelihood ratio (C), negative likelihood ratio (D), and diagnostic odds ratio (E) of EO-PE.

Figure S12: Forest plot of sFlt-1/PlGF predicting summary sensitivity (A), specificity (B), positive likelihood ratio (C), negative likelihood ratio (D), and diagnostic odds ratio (E) of LO-PE.

Figure S13: Symmetric receiver operator characteristic curve of sFlt-1 predicting EO-PE (A), PlGF predicting EO-PE (B), sFlt-1/PlGF predicting EO-PE (C) and sFlt-1/PlGF predicting LO-PE (D).

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No.	The first author	Published year	Study design	Characteria Study Population	stics: Cases (n)	Characteri Control Population	stics: Control(n)	PE Definition	GA(wk)	measurem
1	Andersen	⁵⁰ 2016	Prospectiv cohort study	ePE	137	Non- PE	1732	ACOG	20-34	PLGF、sl 1/PLGF
2	Andersen	⁵⁰ 2016	Prospectiv cohort study	eEO- PE	18	Non- PE	1732	ACOG	20-34	PLGF 、 sl 1/PLGF

Table S1 Characteristics of the Included Studies

No.	The first author	Published year	Study design	Characteri Study Population	stics: Cases	Characteria Control Population	stics: Control(n)	PE Definition	GA(wk)	measurem
3	Andersen	⁵⁰ 2016	Prospectiv cohort	reLO- PE	119	Non- PE	1732	ACOG	20-34	$PLGF \sim sl$ 1/PLGF
4	Bahlmanı	n ³ 2016	Prospectiv cohort study	vePE	194	Non- PE	390	ISSHP	average 37	sFlt- 1 、 PLGF 1/PLGF
5	Bian ⁶⁴	2019	Case con- trol study	PE	101	Non- PE	599	BJOG2014	420-36	sFlt- 1/PLGF
6	Cai L ⁵⁶	2018	cohort study	PE	34	Non- PE	348	Obstetrics and Gyne- cology (8th edition)	14-18	sFlt- 1/PLGF
7	Chen YM ²⁷	2018	Case con- trol study	PE	35	Non- PE	41	Gestation gesta- tion Guide- lines for the Diag- nosis and Treat- ment of Hy- per- ten- sion (2015)	9-13	sFlt- 1 、 PLGF 1/PLGF
8	$\begin{array}{c} {\rm Chen} \\ {\rm YQ}^{57} \end{array}$	2018	Case con- trol study	high risk PE	11	Non- PE	260	Obstetrics and Gyne- cology (8th edition)	28-34	sFlt- 1/PLGF
9	Chuah ⁶⁵	2018	prospectiv case- control study	eEO- PE	24	Non- PE	18	Have	20- 33+6	sFlt- 1/PLGF
10	Chuah ⁶⁵	2018	prospectiv case- control study	eEO- PE	24	Non- PE	18	Have	20- 33+6	sFlt- 1/PLGF

	The first	Published	Study	Characteria Study	stics: Cases	Characteria Control	stics:	PE	$C \Lambda (1)$	
No.	author	year	design	Population	. (n)	Population	Control(n)	Definition	GA(wk)	measurem
11	Chuah ⁶⁵	2018	prospective case- control study	eLO- PE	23	Non- PE	12	Have	34- delivery	sFlt- 1/PLGF
12	Chuah ⁶⁵	2018	prospective case- control study	eLO- PE	23	Non- PE	12	Have	34- delivery	sFlt- 1/PLGF
13	De Vivo ³²	2008	prospective case- control study	ePE	52	Non- PE	52	Have	24-28	sFlt- 1 、 PLGF 1/PLGF
14	Diab ³³	2008	Prospectiv cohort study	ePE	33	Non- PE	66	ACOG	23	sFlt- 1 、 PLGF 1/PLGF
15	Diab ³³	2008	Prospectiv cohort study	eEO- PE	8	Non- PE	66	ACOG	23	sFlt- 1、PLGF 1/PLGF
16	Ding ³⁴	2018	Case con- trol study	PE	136	Non- PE	350	ACOG2002	2>20	sFlt- 1 、 PLGF 1/PLGF
17	$Doherty^{66}$	2014	Prospectiv cohort study	eserve PE	6	Non- PE	14	Have	24	m sFlt- $ m 1/PLGF$
18	Dragan ⁶⁷	2017	Prospectiv cohort study	ePE	14	Non- PE	12291	ISSHP	30-37	m sFlt- $1/PLGF$
19	Forest ⁶⁸	2014	Prospectiv nested case- control study	ePE	180	Non- PE	338	Canadian Col- lege of Ob- stetri- cians and Gynecologi	20-32	sFlt- 1/PLGF
20	Gao J ⁶⁸	2014	Case con- trol study	PE	41	Non- PE	88	Obstetrics and Gyne- cology (5th edition)	15- 20,24- 28	sFlt- 1、PLGF
21	Ghosh^{51}	2012	Prospectiv cohort study	ePE	43	Non- PE	467	ISSHP	20-22	PLGF

No.	The first author	Published year	Study design	Characteri Study Population	stics: Cases n (n)	Characteri Control Population	stics: Control(n)	PE Definition	GA(wk)	measureme
22	Hanita ⁶⁹	2014	Prospectiv cohort	ehigh risk PF	12	Non- PE	72	ASSHP	29-36	sFlt- 1/PLGF
23	Hassan ³⁵	2013	Nested case- control studies	PE	83	Non- PE	250	ACOG	16-20	m sFlt- 1 $\ m PLGF$ 1/PLGF
24	Huang R ⁴³	2018	prospectiv case- control study	ePE	60	Non- PE	30	Gestation gesta- tion Guide- lines for the Diag- nosis and Treat- ment of Hy- per- ten- sion (2015)	11-14	PLGF
25	Huang X ⁴⁴	2017	Prospectiv cohort study	теРЕ	12	Non- PE	620	Obstetrics and Gyne- cology (8th edition)	11-14	PLGF
26	Huhn ⁸¹	2018	Case con- trol study	EO- PE	34	Non- PE	64	The "tradi- tional" crite- ria for PE	15-42	sFlt- 1/PLGF
27	Huhn ⁸¹	2018	Case con- trol study	LO- PE	25	Non- PE	45	The "tradi- tional" crite- ria for PE	15-42	sFlt- 1/PLGF
28	Jia D ⁴⁵	2018	Case con- trol study	PE	138	Non- PE	58	Obstetrics and Gyne- cology (8th edition)	average 37	PLGF

No.	The first author	Published year	Study design	Characteri Study Populatior	stics: Cases n (n)	Characteria Control Population	stics: Control(n)	PE Definition	GA(wk)	measurem
29	Jiang F ⁴⁶	2019	Case con- trol study	PE	123	Non- PE	105	Obstetrics and Gyne- cology (8th edition)	Before hospi- tal delivery	PLGF
30	${\rm Ke} \\ {\rm W}^{58}$	2019	Case con- trol study	PE	98	Non- PE	452	Have	20-36	sFlt- 1/PLGF
31	Kim^{70}	2007	Case con- trol study	PE	46	Non- PE	100	Have	14-23	sFlt- 1/PLGF
32	Kusanovic	⁵ 2009	Case con- trol study	PE	62	Non- PE	1560	ACOG	20-25	PLGF
33	Lafuente- Ganuza ⁷¹	2019	Case con- trol study	PE	51	Non- PE	258	ACOG	24-33	sFlt- 1/PLGF
34	Lafuente- Ganuza ⁷¹	2019	Case con- trol study	PE	51	Non- PE	258	ACOG	24-33	m sFlt- $ m 1/PLGF$
35	Lehnen ⁷²	2013	Case con- trol study	PE	63	Non- PE	72	Have	2 to 4 weeks before delivery	m sFlt- $ m 1/PLGF$
36	Madazli ⁵³	2005	Case con- trol study	serve PE	14	Non- PE	108	Have	21-26	PLGF
37	Mayer- Pickel ⁷³	2019	Case con- trol study	PE	38	Non- PE	84	Have	12-40	sFlt- 1/PLGF
38	Nguye ³⁶	2018	Case con- trol study	high risk PE	30	Non- PE	67	Have	24-28	m sFlt- 1 $\sim m PLGF$ 1/PLGF
39	Nikuei ⁸²	2020	Case con- trol study	PE	38	Non- PE	20	Have	-	sFlt- 1/PLGF

No.	The first author	Published year	Study design	Characteri Study Population	istics: Cases n (n)	Characteri Control Populatior	stics: 1 Control(n)	PE Definition	GA(wk)	measurem
40	Nikuei ⁸²	2020	Case con- trol study	PE	38	Non- PE	20	Have	-	sFlt- 1/PLGF
41	Ohkuchi ⁷⁴	2013	Case con- trol study	$\rm PE$	6	Non- PE	792	Have	26-31	sFlt- 1/PLGF
42	Park ⁷⁵	2014	Case con- trol study	low risk PE	8	Non- PE	254	ACOG	24-27	sFlt- 1/PLGF
43	Phupong ³⁷	2020	Case con- trol study	elderly gravida with PE	14	Non- PE	286	ACOG	16-18	sFlt- 1、PLGF 1/PLGF
44	Phupong ³⁷	2020	Case con- trol	EO- PE	5	Non- PE	286	ACOG	16-18	sFlt- 1、PLGF 1/PLGF
45	Sabria ⁷⁶	2017	Case con- trol	PE	65	Non- PE	130	ISSHP	24-36	m sFlt- $ m 1/PLGF$
46	Saleh ⁷⁷	2016	Case con- trol	PE	62	Non- PE	45	Have	After delivery	sFlt- 1/PLGF
47	$\mathrm{Schmidt}^{54}$	2009	Case con- trol	PE	7	Non- PE	54	ISSHP	15-18	PLGF
48	Shokry^{38}	2010	Nested cohort study	PE	27	Non- PE	213	Have	13-16	sFlt- 1 、 PLGF
49	Sovio ⁷⁸	2017	Prospectiv cohort study	теРЕ	132	Non- PE	3751	ACOG	20-36	m sFlt- $1/ m PLGF$
50	Stepan ⁵⁵	2016	Case con- trol study	EO- PE	83	Non- PE	174	ISSHP	When PE was diagnosed	PLGF、sF 1/PLGF
51	$\rm Stepan^{55}$	2016	Case con- trol study	LO- PE	95	Non- PE	271	ISSHP	When PE was diagnosed	PLGF、sF 1/PLGF

No.	The first author	Published year	Study design	Characteri Study Population	istics: Cases n(n)	Characteri Control Population	stics: Control(n)	PE Definition	GA(wk)	measurem
52	Stubert ³⁹	2014	Case con- trol	PE	12	Non- PE	50	ACOG	19-26	m sFlt- 1 $\sim m PLGF$ 1/PLGF
53	Stubert ³⁹	2014	Case con- trol	EO- PE	9	Non- PE	50	ACOG	19-26	m sFlt- $1 \ PLGF$ 1/PLGF
54	${f Sun} {f W}^{59}$	2020	Case con- trol	PE	33	Non- PE	132	Have	20-26	m sFlt- $ m 1/PLGF$
55	Tarasevici	e 20 46	prospectiv case- control	еРЕ	72	Non- PE	72	ACOG200	225-34	m sFlt- 1 $\sim m PLGF$ 1/PLGF
56	Tardif ⁴¹	2017	Nested case- control	PE	8	Non- PE	59	Have	20-37	sFlt- 1 、 PLGF 1/PLGF
57	Tidwell ⁸¹	2001	case con- trol	PE	14	Non- PE	25	ACOG	16-20	PLGF
58	Verlohren	⁷⁹ 2014	case con- trol	PE	234	Non- PE	468	ISSHP	20-33	m sFlt-1/PLGF
59	Verlohren	⁷⁹ 2014	case con- trol	EO- PE	100	Non- PE	200	ISSHP	20-33	m sFlt- $1/PLGF$
60	Verlohren	⁷⁹ 2014	case con- trol	EO- PE	100	Non- PE	200	ISSHP	20-33	m sFlt-1/PLGF
61	Verlohren	⁷⁹ 2014	case con- trol	LO- PE	134	Non- PE	268	ISSHP	20-33	m sFlt- $ m 1/PLGF$
62	Verlohren	⁷⁹ 2014	study Case con- trol study	LO- PE	134	Non- PE	268	ISSHP	20-33	sFlt- 1/PLGF

No.	The first author	Published vear	Study design	Characteri Study Population	stics: Cases (n)	Characteria Control Population	stics:	PE Definition	GA(wk)	measurem
63	Ye Y ²⁹	2006	Case con- trol study	PE	16	Non- PE	156	Obstetrics and Gyne- cology (6th edition)	26-28	sFlt- 1、PLGF
64	$ You \\ C^{47} $	2018	Case con- trol study	PE	40	Non- PE	40	Obstetrics and Gyne- cology (8th edition)	11-14	PLGF
65	Yu ⁴²	2019	Case con- trol study	$\rm PE$	48	Non- PE	134	Have	12-36	m sFlt- 1 $\sim m sFlt-$ 1/PLGF
66	Yuan X ⁶¹	2010	Case con- trol study	PE	57	Non- PE	200	Obstetrics and Gyne- cology (7th edition)	20-24	sFlt- 1/PLGF
67	Yuan X ⁶⁰	2013	Case con- trol study	PE	122	Non- PE	230	Obstetrics and Gyne- cology (7th edition)	16-20	sFlt- 1/PLGF
68	Zeisler ⁸⁰	2016	Case con- trol study	PE	101	Non- PE	399	ISSHP	24-36	sFlt- 1/PLGF
69	Zhang L ⁴⁸	2018	Case con- trol study	PE	36	Non- PE	58	Obstetrics and Gyne- cology (8th edition)	21-29	PLGF
70	$_{\mathbf{S}^{62}}^{\mathbf{Zhao}}$	2020	cohort	$\rm PE$	39	Non- PE	340	Have	24-36	sFlt- 1/PLCF
71	Zhong Y ³⁰	2019	Prospecti nested case- control study	ivePE	48	Non- PE	134	Obstetrics and Gyne- cology (8th edition)	12-36	sFlt- 1 × PLGF 1/PLGF

	The first	Published	Study	Characteri Study	istics: Cases	Characteris Control	stics:	PE		
No.	author	year	design	Population	n (n)	Population	Control(n)	Definition	GA(wk)	measurem
72	$ m Zhou$ $ m W^{63}$	2017	Case con- trol study	PE	61	Non- PE	115	Obstetrics and Gyne- cology (7th edition)	16-20	sFlt- 1/PLGF
73	$\substack{\text{Zhou}\\\text{X}^{49}}$	2017	Case con- trol study	PE	84	Non- PE	84	Obstetrics and Gyne- cology (8th edition)	11-13	PLGF
74	Zhu X ⁸³	2020	Case con- trol study	EO- PE	30	Non- PE	100	Obstetrics and Gyne- cology (8th edition)	Obstetrics and Gyne- cology (8th edition)	sFlt- 1/PLGF
75	Zhu X ⁸³	2020	Case con- trol study	EO- PE	116	Non- PE	100	Obstetrics and Gyne- cology (8th edition)	Obstetrics and Gyne- cology (8th edition)	sFlt- 1/PLGF

Table S2 Summary of meta analysis results

Indicator	Index	Merger value	95% CI	$I^2(\%)$	Cochran-Q	Р
sFlt-1	Sen	0.811	0.783 - 0.837	86.6	111.71	< 0.001
	Spe	0.786	0.769 - 0.802	96.1	388.12	< 0.001
	PLR	5.097	3.498 - 7.426	92.5	199.92	< 0.001
	NLR	0.265	0.164 - 0.430	92.5	199.81	< 0.001
	DOR	21.092	10.857 - 40.976	84.9	99.16	< 0.001
subgroup	Sen	0.826	0.780 - 0.866	51.3	4.11	0.128
	Spe	0.691	0.649 - 0.730	52.9	4.25	0.119
	PLR	2.661	2.316 - 3.056	0.0	1.64	0.440
	NLR	0.256	0.199 - 0.329	3.1	2.07	0.356
	DOR	11.251	7.872 - 16.081	0.0	0.11	0.947
PLGF	Sen	0.735	0.713 - 0.757	83.1	159.78	< 0.001
	Spe	0.731	0.721 – 0.741	96.1	693.94	< 0.001
	PLR	4.053	3.150 - 5.214	90.6	287.00	< 0.001
	NLR	0.341	0.275 - 0.423	83.1	159.84	$<\!0.001$
	DOR	14.150	8.972 - 22.315	86.2	195.03	$<\!0.001$
sFlt-1/PLGF	Sen	0.779	0.763 – 0.795	86.4	295.18	$<\!0.001$
	Spe	0.885	0.881 - 0.889	98.6	2855.25	$<\!0.001$
	PLR	6.385	4.847 - 8.410	96.5	1136.54	$<\!0.001$
	NLR	0.241	0.192 – 0.303	88.4	345.59	< 0.001

Indicator	Index	Merger value	95% CI	$\mathrm{I}^2(\%)$	Cochran-Q	P
	DOR	31.431	19.681 - 50.197	91.5	470.98	< 0.001

Sen: sensitivity; Spe:specificity; PLR:positive likelihood ratio; NLR:negative likehood ratio; DOR:diagnostic odds ratio; 95% CI:95% confidence interval.

Indicator	Index	Merger value	95% CI	$I^2(\%)$	Cochran-Q	P
sFlt-1	Sen	0.955	0.772-0.999	36.1	3.13	0.209
(EO-PE,n=3)	Spe	0.652	0.603 - 0.698	97.5	78.98	< 0.001
	PLR	2.615	0.735 - 9.304	96.6	59.1	$<\!0.001$
	NLR	0.217	0.059 - 0.798	0	1.23	0.54
	DOR	13.16	1.952 - 88.713	34.8	3.07	0.216
PlGF	Sen	0.862	0.788 - 0.917	87.5	31.91	$<\!0.001$
(EO-PE, n=5)	Spe	0.776	0.758 - 0.793	78.3	18.43	0.001
	PLR	3.401	1.844 - 6.275	85.9	28.28	$<\!0.001$
	NLR	0.259	0.058 - 1.160	92.8	55.58	$<\!0.001$
	DOR	13.108	1.865 - 92.146	88	33.42	$<\!0.001$
PlGF	Sen	0.776	0.714 - 0.830	94.4	17.73	$<\!0.001$
(LO-PE,n=2)	Spe	0.682	0.662 - 0.703	47.9	1.92	0.166
· · · /	PLR	2.331	1.974 - 2.752	54.5	2.2	0.138
	NLR	0.273	0.082 - 0.911	92.1	12.61	$<\!0.001$
	DOR	8.572	2.254 - 32.603	90.4	10.37	0.001
sFlt-1/PlGF	Sen	0.944	0.921 - 0.961	69.1	35.64	$<\!0.001$
(EO-PE, n=12)	Spe	0.805	0.790 - 0.819	96.5	317.71	$<\!0.001$
	PLR	13.751	4.948 - 38.216	96.9	358.78	$<\!0.001$
	NLR	0.084	0.048 - 0.147	55	24.44	0.011
	DOR	230.24	63.956-828.82	79.2	52.93	< 0.001
sFlt-1/PlGF	Sen	0.72	0.680 - 0.757	91.6	71.8	< 0.001
(LO-PE,n=7)	Spe	0.72	0.702 - 0.737	97.9	280.66	$<\!0.001$
. , ,	PLR	6.148	2.717 - 13.912	96.2	156.43	$<\!0.001$
	NLR	0.318	0.194 - 0.522	92.7	81.89	$<\!0.001$
	DOR	20.997	5.947 - 74.132	93.7	95.25	< 0.001

Table S3 Summary of EO-PE and LO-PE meta analysis results

$$\label{eq:constraint} \begin{split} & EO-PE: early onset preeclampsia; LO-PE: late onset preeclampsia; Sen: sensitivity; Spe: specificity; PLR: positive likelihood ratio; NLR: negative likehood ratio; DOR: diagnostic odds ratio; 95\% CI: 95\% confidence interval. \end{split}$$



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