Diagnostic accuracy of the soluble Fms-like tyrosine kinase-1/placental growth factor ratio for preeclampsia with the use of a common cut-off value

Vasilios Pergialiotis[1,2], Anastasia Prodromidou[1], Alexandros Sotiriadis[3], Nikolaos Papantoniou[1,2]

[1] Laboratory of Experimental Surgery and Surgical Research N.S. Christeas, National and Kapodistrian University of Athens, Greece
[2] Third dpt of Obstetrics and Gynecology, Attikon University Hospital, National and Kapodistrian University of Athens, Greece
[3] Second Department of Obstetrics and Gynecology, "Hippokrateion" General Hospital, Aristotle University of Thessaloniki, Greece

Correspondence
Vasilios Pergialiotis, MD, M.Sc., Ph.D, 6, Danaidon str., Chalandri 152 32 - Greece
E - mail: pergialiotis@yahoo.com

Abstract

Background: To date, predictive markers of preeclampsia have not been established in the field of prenatal care. The Soluble fms-like tyrosine kinase-1/Platelet Growth Factor (sFlt-1/PlGF) ratio has been extensively studied in the literature and seems to be promising. However, consensus regarding its efficacy is lacking.
Objective: The purpose of the present meta-analysis is to evaluate the sensitivity and specificity of sFlt-1/PlGF ratio by including studies that applied the most commonly studied cut-off (85) for the diagnosis of preeclampsia.
Methods: We conducted a systematic review searching the Medline (1966-2016), Scopus (2004-2016), Clinical Trials.gov (2008-2016), and Cochrane Central Register of Controlled Trials CENTRAL (1999-2016) databases together.
Results: Five studies were included that provided data concerning the sFlt-1/PlGF ratio 85 for 850 control pregnant women and 461 patients with preeclampsia. The sensitivity of sFlt-1/PlGF in detecting preeclampsia was 75.1% (95% confidence interval (CI), 70.9-78.9%) and the specificity 90.1% (95% CI, 87.8-92.0%). Additionally, the average positive likelihood ratio was 5.889 (95% CI, 2.473-14.022) and the negative likelihood ratio was 0.281 (95% CI, 0.167-0.473). The diagnostic odds ratio was 25.702 (95% CI, 7.567-87.305) and the area under the SROC curve 0.9173.
Conclusions: The diagnostic cut-off 85 of sFlt-1/PlGF ratio is of mild clinical importance, mainly due to the fact that studies in the field did not limit its application on the first trimester of pregnancy. However, given its high sensitivity and specificity, at least in the case of early onset preeclampsia, it seems to be promising as a tool.

Key words: sFlt-1; PlGF; preeclampsia; Kryptor; Elecsys

Introduction

Preeclampsia complicates 3-5% of pregnancies worldwide and 0.4-2.8% of pregnancies in Europe and is related with significant maternal and fetal morbidity and mortality[1-4]. Risk factors include primiparity, advanced maternal age (>35 years), previous history of PE or gestational hypertension (GH), family history of hypertension, high pre-pregnancy body mass index (BMI) and bilateral notching (BN) in the uterine artery[5, 6]. The exact pathophysiological processes, however, still remain unclear.

Delivery still remains the gold standard in the treatment of the disease, as there is no accurate test which could potentially screen for the disease; thus permitting early therapeutic intervention. Several factors have been investigated in the field of preeclampsia, including mediators of inflammation, angiogenic and anti-angiogenic factors[7-10]. The most commonly tested factors are the soluble fms-like tyrosine kinase 1 (sFlt-1) and the placental growth factor (PlGF) which are both released from the placenta and the maternal endothelium, the soluble endoglin (sEng) and (17-b), dehydrogenase 1(HSD17B1), metalloprotease 12 (ADAM-12), inhibin-A and pregnancy associated plasma protein A (PAPP-A)[11-13]. The imbalance between those angiogenic and antiangiogenic factors seem to play a crucial role in the development of preeclampsia[14, 15].

PlGF belongs to the VEGF family. It is released from the placenta and the maternal endothelium and is claimed to exert a proangiogenic function in the maternal circulation[16]. Its levels are normally increased during the first and second trimester and decrease during the third trimester. On the contrary, decreased levels of PIGF have been detected early in pregnant women about to develop preeclampsia[17].On the other hand, sFIt-1 is an antagonist of vascular endothelial growth factor alpha (VEGF-A). When its serum concentration rises it reduces the effect of VEGF and PIGF in the maternal circulation and it promotes an antiangiogenic function which is triggered by lowering the systemic nitric oxide and increasing the levels of endothelin[8]. Increased placenta expression and secretion of sFIt-1 have been implicated in the pathogenesis of preeclampsia[18]. In early onset preeclampsia a profound dysregulation of placental proteins, including sFIt-1 PIGFs has been observed by previous investigators[19, 20].

During the last years two commercially available kits which measure the sFlt-1/PlGF ratio have been produced (Elecsys® (Cobas, Hoffman-La Roche, Basel, Switzerland) and KRYPTOR (BRAHMS, GmbH, Hennigsdorf, Germany)). In their previous systematic review Liu et al suggested that the pooled diagnostic sensitivity and specificity of the sFlt-1/PlGF ratio were 0.78 and 0.84 with an area under the curve (AUC) of 0.88[21]. However, the methodological heterogeneity of studies included precludes firm results, mainly because they did not use a specific cut-off value for the sFlt-1/PlGF ratio. The aim of the present systematic review is to evaluate the sensitivity and specificity of the sFlt-1/PlGF ratio by including studies that applied the most commonly studied cut-off (85) for the diagnosis of early and late preeclampsia.

Methods

Study design

The present study was designed according to the PRISMA guidelines[22]. Eligibility criteria were predetermined by the authors. No language or date restrictions were applied during the literature search. Studies that investigated the efficacy of sFlt-1/PlGF ratio with a specific cut-off value (85) in detecting preeclampsia were held eligible for inclusion. The studies were selected in three consecutive stages. The titles and/or abstracts of all electronic articles were screened to assess their eligibility. All the articles that met or that were presumed to meet the criteria were retrieved in full text. In the final stage, after carefully reading the full text articles the authors selected for tabulation all clinical observational studies (both prospective and retrospective) which evaluated the efficacy of an sFlt-1/PlGF ratio of 85 to detect preeclampsia. Case reports and review articles were excluded from tabulation and analysis of results. Animal studies were also ex-

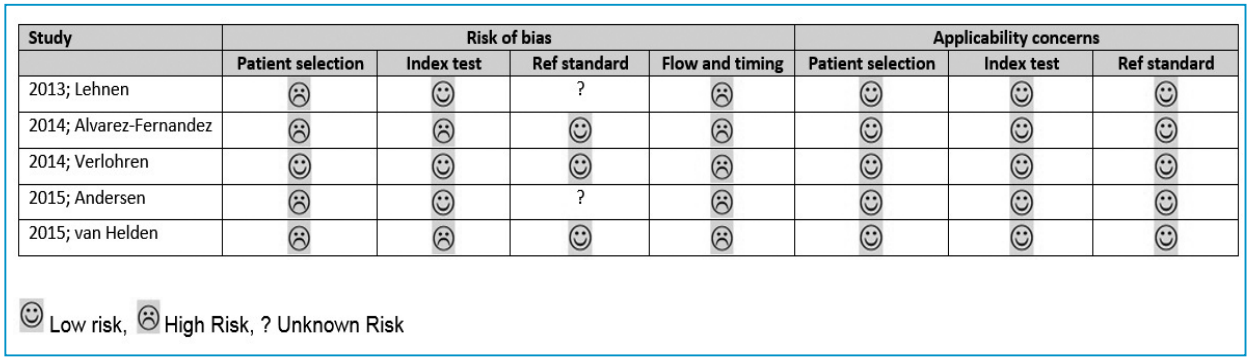

Figure 1: QUADAS-2 assessment tool for studies of diagnostic accuracy

cluded from tables. Vasilios Pergialiotis and Anastasia Prodromidou tabulated the selected indices in structured forms. Any discrepancies in the methodology, retrieval of articles and statistical analysis were resolved by the consensus of all authors.

Literature search and data collection

We used the Medline (1966-2016), Scopus (2004-2016), ClinicalTrials.gov (2008-2016) and Cochrane Central Register of Controlled Trials *CENTRAL* (1999-2016) databases in our primary search, along with the reference lists of electronically retrieved full text papers.

The date of last search was 20 July 2016. Our search was restricted to a minimum number of keywords in order to assess an eligible number that could be hand searched, minimizing the potential loss of articles. All the articles that met or were presumed to meet the inclusion criteria were retrieved in full text. Search strategies and results are shown in Figure 1.

Our search strategy included the words "sFlt-1, PlGF, preeclampsia, Elecsys, KRYPTOR".

Quality assessment

The diagnostic accuracy of the test was assessed with QUADAS-2 which is a tool for the quality assessment of studies of diagnostic accuracy included in systematic reviews (Figure 1)[23]. The test consists of four domains including evaluation of patient selection, index test, reference standards and flow and timing.

Statistical analysis

Statistical meta-analysis was performed using the MetaDisc 1.4 software[24]. Confidence intervals were set at 95%. Pooled odds ratios (OR) and 95% confidence interval (CI) for all primary and secondary outcomes were calculated, using the DerSimonian-Laird random effect model due to the significant heterogeneity in the methodological characteristics of included studies[25]. Publication bias was not tested due to the relatively small number of studies included in the present meta-analysis[26].

Results

Excluded studies

The overall search retrieved 672 articles. After removing duplicates 295 articles were assessed for their eligibility. Thirty-one studies met the potential criteria for inclusion in the present meta-analysis. Twenty-seven studies were excluded from the present meta-analysis after reading the full text[27-53]. One of them investigated the effectiveness of sFlt-1/PlGF in predicting adverse outcomes in preeclampsia; however, the study did not include a control group[36]. Another study compared the ratio among twin and singleton pregnancies and thus did not investigate the outcomes assessed in the present meta-analysis[37]. In another study sFlt-1/PIGF ratio was only mentioned in the full text, rather than investigated[38]. In the remaining twenty four studies the cut-off value was not set at 85 which was the predefined cut-off point for the present analysis[27-35, 39-53].

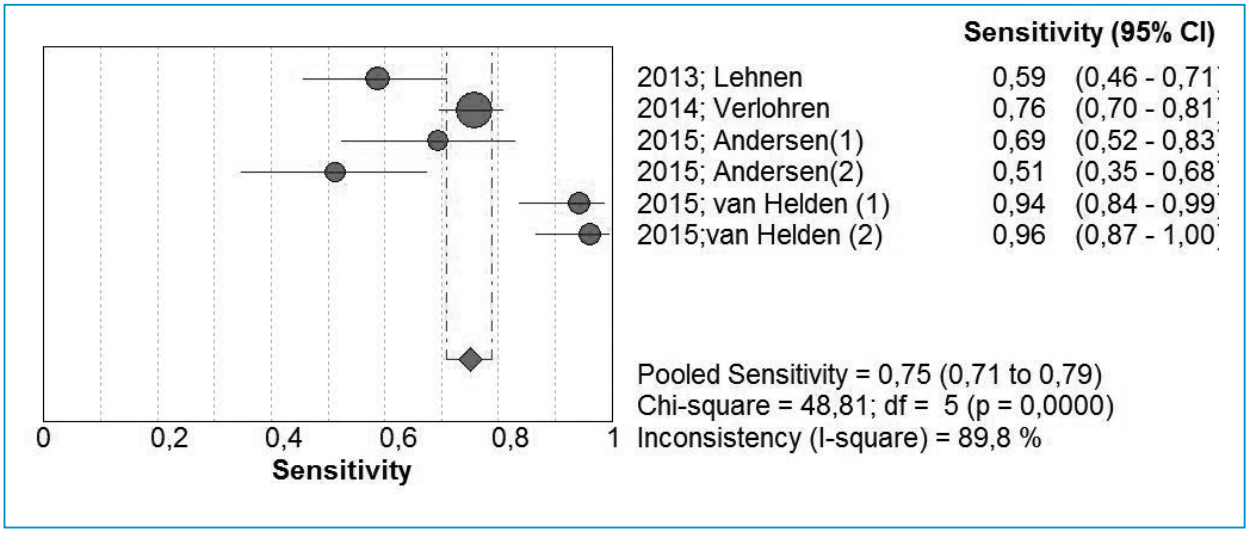

Figure 2

Included studies

Five studies were identified which provided data concerning the sFlt-1/PIGF ratio 85 for 850 control pregnant women and 461 preeclamptic patients. In the latter group 184 women were diagnosed with early onset preeclampsia while 277 had late onset preeclampsia.

When we compared patients diagnosed with preeclampsia, both early and late onset, the sensitivity of sFlt-1/PlGF was 75.1% (95% confidence interval (CI), 70.9-78.9%) (Figure 2) and the specificity 90.1% (95% CI, 87.8-92.0%) (Figure 3). Additionally, the average positive likelihood ratio was 5.889 (95% CI, 2.473-14.022) and the negative likelihood ratio was 0.281 (95% CI, 0.167-0.473). The diagnostic odds ratio was 25.702 (95% CI, 7.567-87.305) and the area under the SROC curve 0.9173 (Figure 4).

In the case of early onset preeclampsia (<34 weeks of gestation) the sensitivity of the test was 85.8% (95% confidence interval (CI), 80.7-90.0%) and the specificity 97.0% (95% CI, 95.2-98.2%). The positive likelihood ratio for early onset preeclampsia was 27.666 (95% CI, 8.692-88.063) and the negative likelihood ratio was 0.182 (95% CI, 0.084-0.397). The diagnostic odds ratio was 273.38 (95% CI, 110.10-678.77) and the area under the SROC curve 0.9818.

In the case of late onset preeclampsia (>34 weeks of gestation) the test was not as accurate. Specifically, its sensitivity was 54.9% (95% confidence interval (CI), 47.4-62.2%) with a specificity of 76.7% (95% CI, 72.6-80.4%). Additionally, the average positive likelihood ratio was 2.690 (95% CI, 1.469-4,927) and the negative likelihood ratio 0.637 (95% CI, 0.457-0.889). The diagnostic odds ratio was 4.885 (95% CI, 1.874 - 12.730) and the area under the SROC curve 0.79.

Discussion

Preeclampsia results in severe maternal and perinatal morbidity and mortality. Its early form (<34 weeks of gestation) is the most severe. Various models have been proposed for the detection of the disease. One of the best is the one proposed by Akolekar et al which suggests that the evaluation of uterine artery PI, MAP, PAPP-A and PlGF has a detection rate for early preeclampsia (<34 weeks of gestation) of 93.4 when implementing 5% false positive rates respectively[54].

However, its detection rates drops to 61% for pregnancies delivered <37 weeks. According to the findings of our study the diagnostic cut-off value of 85 of the serum sFlt/PlGF ratio reaches a 97% specificity and 85.8% sensitivity for cases with early preeclampsia. However, it also fails to provide an

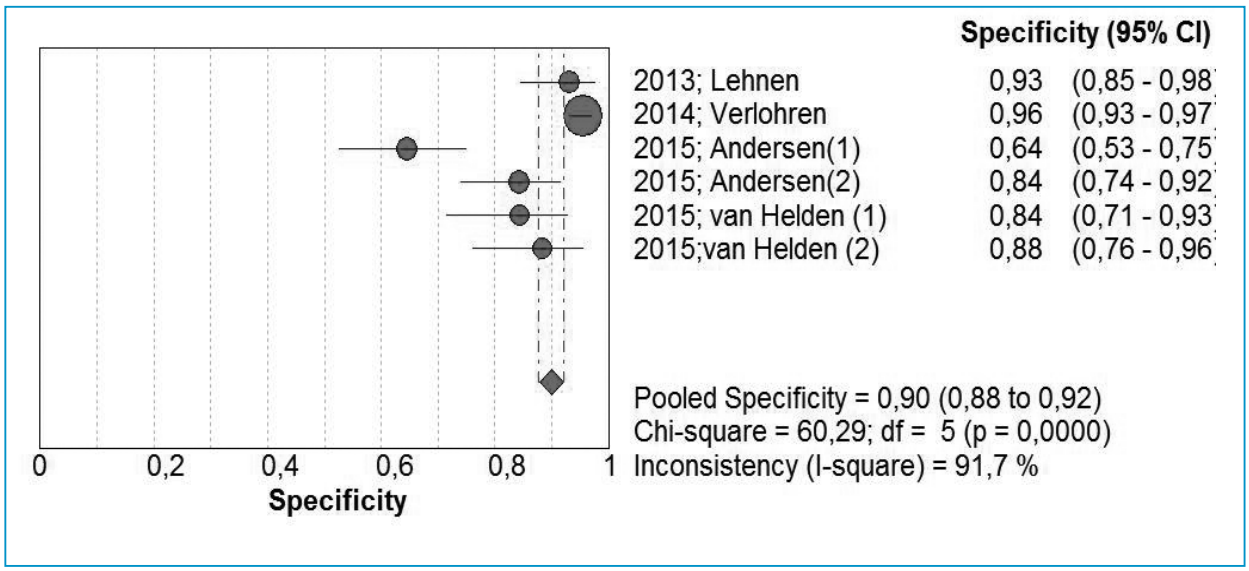

Figure 3

advantage, compared to other screening strategies, for the detection of late preeclampsia.

In their previous meta-analysis Liu et al found comparable results to those of our meta-analysis, despite the fact that they did not opt for a specific cut-off value during the study inclusion phase[21]. Specifically, they observed that the overall diagnostic sensitivity and specificity were 78% and 84%. This was, however, increased, in the case of early onset preeclampsia (94% in both cases).

Strengths and weaknesses of our study

Our study is based on meticulous review of the literature and introduces in the statistical analysis, studies with a predetermined cut-off, therefore, limiting the possibility of bias.

However, the clinical significance of our findings is limited by the fact that the test was performed at different weeks of gestation including the first, second and early third trimester of gestation.

Therefore, its actual value as a screening tool remains debatable, as its accuracy in the first trimester of pregnancy remains unknown. This limits, of course, its significance, because preventive measures for the occurrence of preeclampsia are limited at the time, since impaired placentation has already taken place.

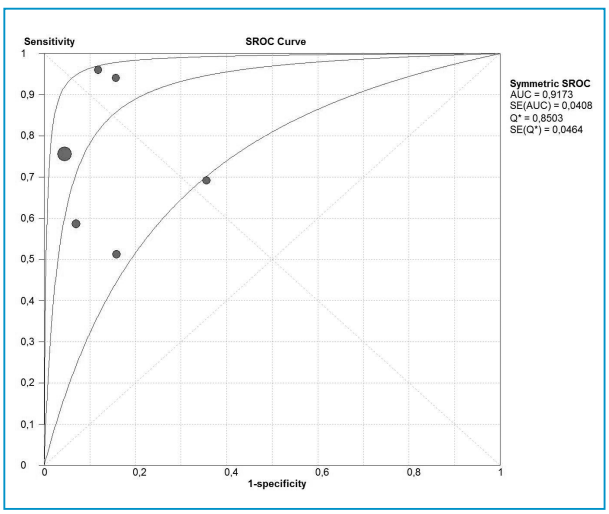

Figure 4

Implications for current clinical practice and future research

To date the use of sFlt-1/PlGF ratio as a screening tool for the detection of preeclampsia does not seem to be justified. Although its sensitivity and specificity for the detection of early onset preeclampsia seem to be high, its clinical importance seems to be significantly influenced by the fact that the specific cut-off has never been tested as a screening marker from the first trimester of pregnancy. In this context, we strongly believe that future studies should

focus on its actual significance early in pregnancy. Should it prove to be adequately efficacious, early intervention with heparin and/or LMWH inside randomized clinical trials should be undertaken to observe their clinical importance among women with increased levels of sFLT-1/PlGF.

Conclusion

According to the findings of our meta-analysis, the diagnostic cut-off 85 of sFlt-1/PlGF ratio is of mild clinical importance, mainly due to the fact that studies in the field did not limit its application on the first trimester of pregnancy. However, given its high sensitivity and specificity, at least in the case of early onset preeclampsia, it seems to be promising as a tool. Future studies during the first weeks of pregnancy are needed to assess its efficacy before impaired placentation takes place.

Conflict of interest

The authors declare no conflict of interest.

References

1. Redman CW and Sargent IL. Latest advances in understanding preeclampsia. *Science 2005*;308:1592-1594.
2. Walker JJ. Pre-eclampsia. *Lancet*2000;356:1260-1265.
3. Murphy DJ and Stirrat GM. Mortality and morbidity associated with early-onset preeclampsia. *Hypertens Pregnancy* 2000; 19: 221-231.
4. MacKay AP, Berg CJ and Atrash HK. Pregnancy-related mortality from preeclampsia and eclampsia. *Obstet Gynecol* 2001; 97: 533-538
5. Pedrosa, AC and Matias A. Screening for pre-eclampsia: A systematic review of tests combining uterine artery Doppler with other markers. *J Perinat Med* 2011; 39: 619-635.
6. Abdelaziz A, Maher MA, Sayyed TM, Bazeed MF, Mohamed NS. Early pregnancy screening for hypertensive disorders in women without a-priori high risk. *Ultrasound Obstet Gynecol* 2012; 40: 398-405.
7. Roberts JM, Pearson G, Cutler J and Lindheimer M. Summary of the NHLBI Working Group on Research on Hypertension During Pregnancy. *Hypertension* 2003; 41: 437-445.
8. Maynard SE, Min JY, Merchan J, et al. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. *J Clin Invest* 2003;111: 649-658.
9. Lau SY, Guild SJ, Barrett CJ, et al. Tumor necrosis factor-alpha, interleukin-6, and interleukin-10 levels are altered in preeclampsia: A systematic review and meta-analysis. *Am J Reprod Immunol* 2013; 70: 412-427.
10. Pergialiotis V, Prodromidou A, Pappa E, Vlachos GD, Perrea DN, Papantoniou N. An evaluation of calprotectin as serum marker of preeclampsia: a systematic review of observational studies. *Inflamm Res* 2016; 65: 95-102.
11. Kar M. Role of biomarkers in early detection of preeclampsia. J *Clin Diagn Res* 2014; 8: BE01-4.
12. Bdolah Y, Karumanchi SA and Sachs BP. Recent advances in understanding of preeclampsia. *Croat Med J* 2005;46:728-736
13. Kleinrouweler CE, Wiegerinck MM, Ris-Stalpers C, et al. Accuracy of circulating placental growth factor, vascular endothelial growth factor, soluble fms-like tyrosine kinase 1 and soluble endoglin in the prediction of pre-eclampsia: A systematic review and meta-analysis. *Bjog* 2012; 119:.778-787.
14. Maynard S, Epstein FH and Karumanchi SA. Preeclampsia and angiogenic imbalance. *Annu Rev Med* 2008; 59: 61-78.
15. Espinoza J. Recent biomarkers for the identification of patients at risk for preeclampsia: the role of uteroplacental ischemia. *Expert Opin Med Diagn* 2012; 6: 121-130.
16. Park JE, Chen HH, Winer J, Houck KA ,Ferrara N. Placenta growth factor. Potentiation of vascular endothelial growth factor bioactivity, *in vitro* and *in vivo*, and high affinity binding to Flt-1 but not to Flk-1/KDR. *J Biol Chem* 1994; 269: 25646-25654.
17. Thadhani R, Mutter WP, Wolf M, et al. First trimester placental growth factor and soluble fms-like tyrosine kinase 1 and risk for preeclampsia. *J Clin Endocrinol Metab* 2004; 89: 770-775.
18. Levine RJ, Maynard SE, Qian C, et al. Circulating angiogenic factors and the risk of preeclampsia. *N Engl J Med* 2004; 350: 672-683.

19. Egbor M, Ansari T, Morris N, Green CJ, and Sibbons PD. Morphometric placental villous and vascular abnormalities in early- and late-onset pre-eclampsia with and without fetal growth restriction. *BJOG* 2006;113: 580-589.
20. Soto E, Romero R, Kusanovic JP, et al. Late-onset preeclampsia is associated with an imbalance of angiogenic and anti-angiogenic factors in patients with and without placental lesions consistent with maternal underperfusion. *J Matern Fetal Neonatal Med* 2012; 25: 498-507.
21. Liu Y, Zhao Y, Yu A, Zhao B, Gao Y, Niu H. Diagnostic accuracy of the soluble Fms-like tyrosine kinase-1/placental growth factor ratio for preeclampsia: A meta-analysis based on 20 studies. *Arch Gynecol Obstet* 2015; 292: 507-518.
22. Liberati A, Altman DG, Tetzlaff J, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. *J Clin Epidemiol* 2009; 62: e1-34
23. Whiting PF, Rutjes AW, Westwood ME, et al. QUADAS-2: A revised tool for the quality assessment of diagnostic accuracy studies. *Ann Intern Med* 2011;155: 529-536.
24. Zamora J, Abraira V, Muriel A, Khan K, Coomarasamy A. Meta-DiSc: A software for meta-analysis of test accuracy data. *BMC Med Res Methodol* 2006; 6:31.
25. DerSimonian R, R Kacker. Random-effect model for meta-analysis of clinical trials: An update. *Contemp Clin Trials* 2007;28:105-114
26. Souza JP, Pileggi C and Cecatti JG. Assessment of funnel plot asymmetry and publication bias in reproductive health meta-analyses: An analytic survey. *Reprod Health* 2007; 4: 3.
27. Stubert J, Ullmann S, Bolz M, et al. Prediction of preeclampsia and induced delivery at <34 weeks gestation by sFLT-1 and PlGF in patients with abnormal midtrimester uterine Doppler velocimetry: A prospective cohort analysis. *BMC Pregnancy Childbirth* 2014;14: 292.
28. Ohkuchi A, Hirashima C, Takahashi K, et al. A trio of risk factors for the onset of preeclampsia in the second and early third trimesters. *Pregnancy Hypertension: An International Journal of Women's Cardiovascular Health* 2014; 4: 224-230.
29. Ohkuchi A, Hirashima C, Takahashi K, Suzuki H, Matsubara S, Suzuki M. Onset threshold of the plasma levels of soluble fms-like tyrosine kinase 1/placental growth factor ratio for predicting the imminent onset of preeclampsia within 4 weeks after blood sampling at 19-31 weeks of gestation. *Hypertens Res* 2013;36:1073-1080.
30. Ohkuchi A, Ishibashi O, Hirashima C, et al. Plasma level of hydroxysteroid (17-β) dehydrogenase 1 in the second trimester is an independent risk factor for predicting preeclampsia after adjusting for the effects of mean blood pressure, bilateral notching and plasma level of soluble fms-like tyrosine kinase 1/placental growth factor ratio. *Hypertension Research* 2012; 35: 1152-1158.
31. Zeisler H, Llurba E, Chantraine F, et al. Predictive Value of the sFlt-1:PlGF Ratio in Women with Suspected Preeclampsia. *N Engl J Med* 2016; 374:13-22.
32. Forest JC, Theriault S, Masse J, Bujold E and Giguere Y. Soluble Fms-like tyrosine kinase-1 to placental growth factor ratio in mid-pregnancy as a predictor of preterm preeclampsia in asymptomatic pregnant women. *Clin Chem Lab Med* 2014;52: 1169-1178.
33. Hassan MF, Rund NM, Salama AH. An Elevated Maternal Plasma Soluble fms-Like Tyrosine Kinase-1 to Placental Growth Factor Ratio at Midtrimester Is a Useful Predictor for Preeclampsia. *Obstet Gynecol Int* 2013; 2013: 202346.
34. Kienast C, Moya W, Rodriguez O, Jijon A, Geipel A. Predictive value of angiogenic factors, clinical risk factors and uterine artery Doppler for pre-eclampsia and fetal growth restriction in second and third trimester pregnancies in an Ecuadorian population. *J Matern Fetal Neonatal Med* 2016; 29: 537-543.
35. Lim JH, Kim SY, Park SY, Yang JH, Kim MY, Ryu HM. Effective prediction of preeclampsia by a combined ratio of angiogenesis-related factors. *Obstet Gynecol* 2008;111:1403-1409.
36. De Oliveira L, Peracoli JC, Peracoli MT, et al. sFlt-1/PlGF ratio as a prognostic marker of adverse outcomes in women with early-onset preeclampsia. *Pregnancy Hypertens* 2013; 3:191-195.

37. Droge L, Herraiz I, Zeisler H, et al. Maternal serum sFlt-1/PlGF ratio in twin pregnancies with and without pre-eclampsia in comparison with singleton pregnancies. *Ultrasound Obstet Gynecol* 2015; 45: 286-293.
38. Kusanovic JP, Romero R, Chaiworapongsa T, et al. A prospective cohort study of the value of maternal plasma concentrations of angiogenic and anti-angiogenic factors in early pregnancy and midtrimester in the identification of patients destined to develop preeclampsia. *J Matern Fetal Neonatal Med* 2009; 22: 1021-1038.
39. Kim SY, Ryu HM, Yang JH, et al. Increased sFlt-1 to PlGF ratio in women who subsequently develop preeclampsia. *J Korean Med Sci* 2007; 22: 873-877.
40. Stepan H, Unversucht A, Wessel N and Faber R. Predictive value of maternal angiogenic factors in second trimester pregnancies with abnormal uterine perfusion. *Hypertension* 2007; 49: 818-824.
41. De Vivo A, Baviera G, Giordano D, Todarello G, Corrado F and D'Anna R. Endoglin, PlGF and sFlt-1 as markers for predicting pre-eclampsia. *Acta Obstet Gynecol Scand* 2008; 87: 837-842.
42. Diab AE, El-Behery MM, Ebrahiem MA, Shehata AE. Angiogenic factors for the prediction of pre-eclampsia in women with abnormal midtrimester uterine artery Doppler velocimetry. *Int J Gynaecol Obstet* 2008;102: 146-151.
43. Sibai BM, Koch MA, Freire S, et al. Serum inhibin A and angiogenic factor levels in pregnancies with previous preeclampsia and/or chronic hypertension: Are they useful markers for prediction of subsequent preeclampsia? *Am J Obstet Gynecol* 2008;199: 268 e1-9.
44. Molvarec A, Szarka A, Walentin S, Szucs E, Nagy B, Rigo J, Jr. Circulating angiogenic factors determined by electrochemiluminescence immunoassay in relation to the clinical features and laboratory parameters in women with pre-eclampsia. *Hypertens Res* 2010; 33: 892-898.
45. McElrath TF, Lim KH, Pare E, et al. Longitudinal evaluation of predictive value for preeclampsia of circulating angiogenic factors through pregnancy. *Am J Obstet Gynecol* 2012; 207: 407 e1-7.
46. Chen G, Zhang L, Jin X, et al. Effects of angiogenic factors, antagonists, and podocyte injury on development of proteinuria in preeclampsia. *Reproductive Sciences* 2012:1933719112459227.
47. Park HJ, Kim SH, Jung YW, et al. Screening models using multiple markers for early detection of late-onset preeclampsia in low-risk pregnancy. *BMC Pregnancy Childbirth* 2014; 14: 35.
48. Odibo A, Rada C, Cahill A, et al. First-trimester serum soluble fms-like tyrosine kinase-1, free vascular endothelial growth factor, placental growth factor and uterine artery Doppler in preeclampsia. *Journal of Perinatology* 2013; 33: 670-674.
49. Villa PM, Hamalainen E, Maki A, et al. Vasoactive agents for the prediction of early- and late-onset preeclampsia in a high-risk cohort. *BMC Pregnancy Childbirth* 2013;13: 110.
50. Hanita O, Alia NN, Zaleha AM, Nor Azlin MI. Serum soluble FMS-like tyrosine kinase 1 and placental growth factor concentration as predictors of preeclampsia in high risk pregnant women. *Malays J Pathol* 2014; 36: 19-26.
51. Doherty A, Carvalho JC, Drewlo S, et al. Altered hemodynamics and hyperuricemia accompany an elevated sFlt-1/PlGF ratio before the onset of early severe preeclampsia. *J Obstet Gynaecol Can* 2014;36: 692-700.
52. Moore Simas TA, Crawford SL, Bathgate S, et al. Angiogenic biomarkers for prediction of early preeclampsia onset in high-risk women. *J Matern Fetal Neonatal Med* 2014; 27: 1038-1048.
53. Sunderji S, Gaziano E, Wothe D, et al. Automated assays for sVEGF R1 and PlGF as an aid in the diagnosis of preterm preeclampsia: a prospective clinical study. *Am J Obstet Gynecol* 2010; 202: 40 e1-7.
54. Akolekar R, Syngelaki A, Poon L, Wright D, Nicolaides KH. Competing risks model in early screening for preeclampsia by biophysical and biochemical markers. *Fetal Diagn Ther* 2013;33: 8-15.