Hypertension Research (2017) 40, 5–14

www.nature.com/hr

REVIEW SERIES

Prediction and prevention of hypertensive disorders of pregnancy

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The most common classifications of hypertensive disorders of pregnancy consist of chronic hypertension, gestational hypertension, preeclampsia (PE) and superimposed PE. A common final pathophysiology of PE is endothelial dysfunction. The most successful translational research model for explaining the cause–effect relationship in the genesis of PE is the angiogenic/angiostatic balance theory, involving soluble fms-like tyrosine kinase 1 (sFlt-1), placental growth factor (PlGF) and soluble endoglin (sEng). In a systematic review of articles on the prediction of early-onset PE using angiogenesis-related factors, we revealed that the prediction of early-onset PE in the first trimester is clinically possible, but the prediction of early-onset PE in the early third trimester might be ideal. In addition, an onset threshold or a serial approach appeared to be clinically useful for predicting the imminent onset of PE, with onset at <4 weeks after blood sampling in the second and early third trimesters, because the positive likelihood ratio was >10 and the positive predictive value was $>20\%$. The National Institute for Health and Care Excellence guidelines state that the Triage PlGF testing and Elecsys immunoassay for the sFlt-1/PlGF ratio could help to exclude PE in women with suspected PE at 20–34 weeks of gestation. Until now, we have not found any effective therapies to prevent PE. However, low-dose aspirin treatment starting at $\leqslant 16$ weeks of gestation might be associated with a marked reduction in PE. In addition, early statin treatment might prevent the occurrence of PE. Currently, a clinical trial using pravastatin for the prevention of PE is ongoing.
Hypertension Research (2017) **40,** 5–14; doi:10.1038/hr.2016.107; published online 18 August 2016

Keywords: aspirin; hypertensive disorders of pregnancy; prediction; preeclampsia; prevention

INTRODUCTION

Preeclampsia (PE) is a major complication of pregnancy associated with significant morbidity and mortality for both the fetus and mother.[1,2] Hypertensive disorders of pregnancy (HDP) consist of the following four diseases: chronic hypertension, gestational hypertension (GH), PE and superimposed PE; however, PE has been mostly studied for its prediction and prevention. In this review, we focused on the risk factors for PE in antepartum booking, prediction of PE using uterine artery flow velocity waveforms or circulating levels of angiogenesis-related factors and prevention of PE.

CLASSIFICATION OF HYPERTENSIVE DISORDERS OF PREGNANCY

There are several guidelines for the management of hypertensive disorders in pregnancy. Between 2013 and 2014, four major societies, including the American College of Obstetricians and Gynecologists, the Society of Obstetricians and Gynaecologists of Canada, the Society of Obstetric Medicine of Australia and New Zealand, and the International Society for the Study of Hypertension in Pregnancy (ISSHP), revised their guidelines.[3–6] The common points were as follows: (1) the use of the generic term 'hypertensive disorders of pregnancy'; (2) the inclusion of chronic hypertension in the classification of HDPs; (3) the inclusion of a clinical definition of PE, such as GH and impaired liver function but not new proteinuria; and (4) the inclusion of a clinical definition of superimposed PE, such as chronic hypertension and impaired liver function but not new proteinuria. Although the Japan Society for the Study of Hypertension in Pregnancy (JSSHP) classified pregnancy-induced hypertension in 2004, the four common aforementioned points in the recent guidelines were not considered.[7,8] The JSSHP has established a committee for the revision of the guidelines for the management of pregnancy-induced hypertension; in May 2016, the committee decided to change the name of pregnancy-induced hypertension to HDPs and include chronic hypertension in the classification of HDPs. The details of the guidelines for the management of HDPs are provided chronologically in Supplementary Table S1.

The most common classifications of HDPs consist of the following four diseases: chronic hypertension, GH, PE and superimposed PE;[3–6,9–12] however, the Japanese classification of HDP does not include chronic hypertension,[7,8] and the National Institute for Health and Care Excellence (NICE) classification of HDP does not include

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Received 8 June 2016; revised 6 July 2016; accepted 9 July 2016; published online 18 August 2016

superimposed PE.[13] The definitions of edema, proteinuria and hypertensive disorders in pregnancy, childbirth and the puerperium by the International Statistical Classification of Diseases and Related Health Problems (ICD)-10 have also been used in several clinical research studies,[14] and these definitions also include the four common classifications.[15] Therefore, in this review, we defined HDPs as chronic hypertension, GH, PE and superimposed PE. Recent classifications of HDPs have commonly defined PE as hypertension with a new onset of at least one of the following: proteinuria, renal insufficiency, impaired liver function, neurological complications or hematological complications (clinical definition of PE),[3–6,9,12] although the old ISSHP guidelines, JSSHP classification, NICE guidelines and ICD-10 classification used narrow definitions of PE, *de novo* hypertension, and *de novo* proteinuria.[7,8,11,13,15] However, in the current review, we did not distinguish between the clinical definition of PE and the narrower definitions of PE.

The JSSHP defined GH or PE occurring at <32 weeks of gestation as the early-onset type,[7,8] because the distributions of severe early-onset pregnancy-induced hypertension and severe late-onset pregnancy-induced hypertension showed bimodal distributions with peaks at 30–31 and 34–35 weeks of gestation, respectively.[16] In a recent statement about the definition of severe and early-onset PE, most investigators considered early-onset PE to occur before 34 weeks of gestation.[17] Early-onset PE differs from late-onset PE in terms of maternal mortality, pathophysiology, recurrence in later pregnancies, risk of later cardiovascular disease and death, and the occurrence of fetal growth restrictions.[18] In addition, it was disclosed that an abnormal increase in soluble fms-like tyrosine kinase 1 (sFlt-1) and an abnormal decrease in placental growth factor (PlGF) frequently occurred in preeclamptic women with an onset at <34 weeks of gestation.[19,20]

RISK FACTORS ON ANTENATAL BOOKING

There are many risk factors for the occurrence of PE. The early detection of high-risk factors for PE could allow for improvement of the outcome by increasing patient surveillance or by prescribing drugs to prevent the occurrence of PE. Therefore, it is very important to know whether a pregnant woman has some risk factors for PE at the first visit for an antenatal maternal checkup. In a systematic review of the risk factors for PE on antenatal booking, using 52 cohort and case–control studies from 1966 to 2002, the risk factors (unadjusted relative risk (RR)) were as follows: antiphospholipid antibodies (9.7), preexisting diabetes (3.6), previous PE (7.2), family history of PE (2.9), nulliparity (2.9), twins (2.9), obesity (2.5), high systolic blood pressure $\geqslant 130$ mm Hg (*vs.* <130 mm Hg); (2.4), age $\geqslant 40$ (multipara); (2.0) and age $\geqslant 40$ (primipara); (1.7).[21] Recently, 14 clinical risk factors at $\leqslant 16$ weeks of gestation for the development of PE were evaluated involving almost 25 million pregnancies in 92 cohort studies from 2000 to June 2015.[14] The risk factors (unadjusted RR) were as follows: prior intrauterine growth restriction (1.4, but not significant), systemic lupus erythematosus (2.5), nulliparity (2.1), age $\geqslant 35$ (1.2), age $\geqslant 40$ (1.5), prior stillbirth (2.4), chronic kidney disease (1.8), assisted reproductive technology (1.8), prepregnancy body mass index $\geqslant 25$ (2.1), prepregnancy body mass index $\geqslant 30$ (2.8), multifetal pregnancy (2.9), prior placental abruption (2.0), pregestational diabetes (3.7), prior PE (8.4), chronic hypertension (5.1) and antiphospholipid antibodies (2.8). The results of the recent systematic review mostly confirmed the previous results for nulliparity, age $\geqslant 40$, obesity, diabetes, prior PE and antiphospholipid antibodies, and it additionally revealed that systematic lupus erythematosus, prior stillbirth, chronic kidney disease, assisted reproductive technology, prior placental abruption and chronic hypertension were high-risk factors for PE.

Although nulliparity is a well-known risk factor for PE, the multiparous effect disappeared if the time between pregnancies was 10 years or more.[22] In addition, several studies have reported that the incidence of PE in a second pregnancy with a new partner is almost the same as that in the first pregnancy with the previous partner.[23] However, the incidence rate of PE in the second pregnancy is very different in women with/without PE in the first pregnancy. Then, we systemically reviewed whether pregnant women with a new partner in the second pregnancy developed PE more frequently than those with the same partner in the second pregnancy who did not develop PE in the first pregnancy.[24] In the four cohorts with 601 365 pregnant women who did not have PE in the first pregnancy, women with a new partner developed PE at a rate of 2.0%, whereas women with the same partner developed PE at a rate of 1.5% ($P<0.001$),[25–28] indicating that a change of paternity could increase the occurrence of PE.

PATHOPHYSIOLOGY OF PE

Endothelial dysfunction

A common final pathophysiology of PE is endothelial dysfunction. Many observations have confirmed the presence of endothelial dysfunction in PE.[29] Increased sensitivity to angiotensin II precedes the clinical manifestation of PE by weeks to months.[30] Glomerular endotheliosis is morphological evidence of endothelial injury.[31] The levels of von Willebrand factor and fibronectin were increased in women with later occurrence of PE than in normal pregnant women in the second and third trimesters.[32] Actually, serum samples obtained before delivery in women with PE affected the endothelial cell function in human umbilical vein endothelial cells in culture.[29] Tsukimori *et al.*[33] investigated endothelial cell injury by the release of radiolabeled chromium from human umbilical vein endothelial cells into culture medium; although the release of chromium 51 in PE was almost twice that in normal pregnancy, the levels in GH and chronic hypertension were not significantly different from those in normal pregnancy. Tsukimori *et al.*[34] also demonstrated that neutrophils from women with PE adhered more markedly to human umbilical vein endothelial cells than those from nonpregnant or normal pregnant women; the increased neutrophil-endothelial adhesion in PE was inhibited by pretreatment with anti-CD11b, the expression of which in neutrophils in PE was significantly increased compared with that in normal pregnancy, indicating an important role of CD11b in the adhesion of neutrophils to endothelial cells. It was demonstrated that neutrophils actually infiltrate resistance-sized vessels in women with PE.[35] Vessel reactivity to angiotensin II in endothelium-intact omental arteries obtained from preeclamptic women was significantly increased, compared with that in normal pregnant women; however, the enhanced vessel reactivity to angiotensin II in PE was blocked by pretreatment with superoxide dismutase/catalase or RhoA kinase inhibitor.[36] These results suggested a role of activated neutrophils in the genesis of endothelial dysfunction and enhanced vasoconstriction in PE. However, it is not known whether increased sFlt-1 levels in PE are related to the activation of neutrophils.

Animal models of PE

The most successful model of translational research from bench to bed for explaining the cause–effect relationship in the genesis of PE is the angiogenic/angiostatic balance theory involving sFlt-1, PlGF and sEng. In 2003, the role of sFlt-1 in the genesis of PE was discovered in a rat

model,[37] followed by the discovery of sEng having an additive effect on the genesis of PE in a rat model.[38] Soon after these two basic research studies, it was discovered that sFlt-1, PlGF and sEng showed changes 3–4 months before the clinical onset of PE. The circulating levels of sFlt-1 were increased 9–11 weeks before the onset of PE.[19] The circulating levels of PlGF were decreased 9–11 weeks before the onset of PE and showed a marked decrease during the 5 weeks before the onset of PE.[19] In addition, the circulating levels of sEng were increased 9–11 weeks before the onset of preterm PE and 12–14 weeks before the onset of term PE, although the magnitude of the increase was less marked in term than in preterm PE.[39] However, the balance theory has a serious limitation because these markers do not have high positive likelihood ratios (LR+) for predicting late-onset PE, especially at $\geqslant$ 36 weeks of gestation,[40] and because the increased levels of sFlt-1 and sEng were not observed in some cases of PE.[20,41] Therefore, we searched for other animal models of PE and investigated whether there were some new candidates for biomarkers that are possible causes of the animal models of PE.[42] We collected 64 articles on animal models of PE, consisting of 25 different methods. Angiotensinogen in the first trimester, prorenin at 8 weeks of gestation in women with Type 1 diabetes and plasma prorenin receptor in early pregnancy might predict the occurrence of PE. Low galectin-1 levels during the second trimester might predict the later occurrence of PE. Interleukin-10 levels did not change before the onset of PE. Maternal plasma asymmetric dimethylarginine in the second and third trimesters was higher in women with PE. However, we could not find any cohort or nested case–control studies of complement component C1q, 2-methoxyoestradiol/catechol-O-methyltransferase or heme oxygenase-1.

Two-stage model

PE occurs mainly during pregnancy and rarely immediately after delivery, and it disappears soon after the cessation of pregnancy, indicating that the placenta is the main cause of the development of PE. In the current understanding of the genesis of PE, there is the two-stage model proposed by Roberts and Hubel[43] as follows: the first stage is reduced placental perfusion owing to abnormal implantation or other pathological disorders, and the second stage is the production of endothelial dysfunction, and the appearance of maternal hypertension and proteinuria caused by a maternal response to the reduced placental perfusion. This model almost completely applies to the development of early-onset PE, which was associated with abnormal uterine artery Doppler (UAD) findings in the first and second trimesters,[44] and was also associated with an abnormal decrease in PlGF or an abnormal increase in the sFlt-1/PlGF ratio in the second and third trimesters.[40] In contrast, late-onset PE often lacks abnormal uterine Doppler findings in the first and second trimesters,[44] or sometimes shows normal PlGF or a normal sFlt-1/PlGF ratio at the onset of PE.[20] Therefore, regarding late-onset PE, the novel concept of 'maternal PE' was proposed by Redman and Sargent.[45] Arterial diseases owing to autoimmune disorders, chronic hypertension, obesity or preexisting diabetes involve microvascular lesions accompanied by low-grade systemic inflammation, which could contribute to the development of endothelial dysfunction during pregnancy. However, in most cases of PE, maternal and placental factors are mixed.

PREDICTION

Prediction of PE in the first, second and early third trimesters

In this review of risk factors in the first, second and early third trimesters, we considered an LR+ of $\geqslant$ 10 and positive predictive value (PPV) of $\geqslant$ 20% to be clinically useful predictive markers.

Failure of extra-trophoblast invasion of the uterine spiral arteries has often been observed in PE.[46–49] Shallow invasion of the extra trophoblasts into the spiral arteries has actually been associated with increased resistance by UAD velocity.[50] The detection rates of the abnormal uterine artery pulsatility index at 23 weeks of gestation were increased when the weeks at delivery were earlier in women with PE, indicating that screening for early-onset PE using UAD might be more accurate than screening for late-onset PE.[51] Thereafter, many studies for predicting early-onset PE using the various findings of UAD in the first and second trimesters have been reported.

When early-onset PE was not distinguished from other types of PE, it is disappointing that the screening properties for various Doppler indices in the first and second trimesters showed LR+ of $<$10 as

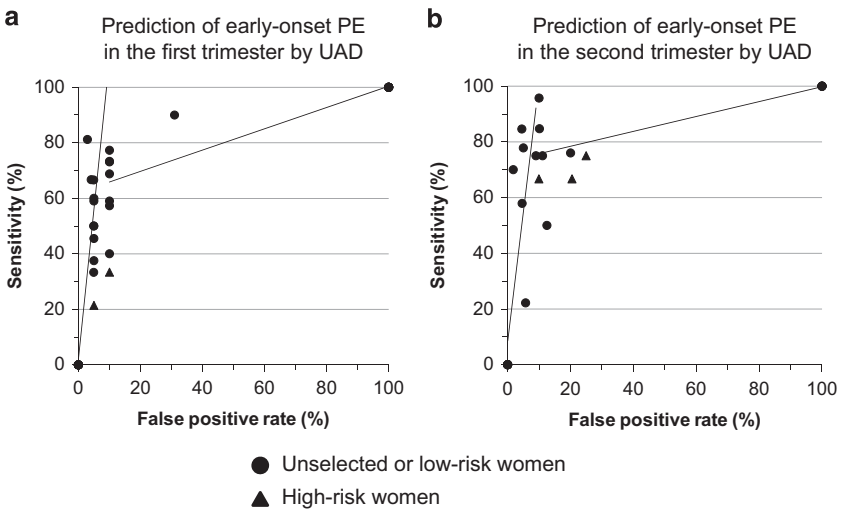

Figure 1 Scatter plot showing the relationship between the false-positive rate (FPR) and the sensitivity to predict early-onset preeclampsia (PE) by uterine artery Doppler (UAD) alone in the first trimester (**a**) and UAD alone in the second trimester (**b**). Closed circles indicate unselected or low-risk women, and closed triangles indicate high-risk women. When the FPR was $<$10%, the regression line of the sensitivity of FPR, including 0% FPR and 0% sensitivity, was plotted in unselected or low-risk women, and, when the FPR was $\geqslant$10%, the regression line of the sensitivity on FPR, including 100% FPR and 100% sensitivity, was plotted in unselected or low-risk women.

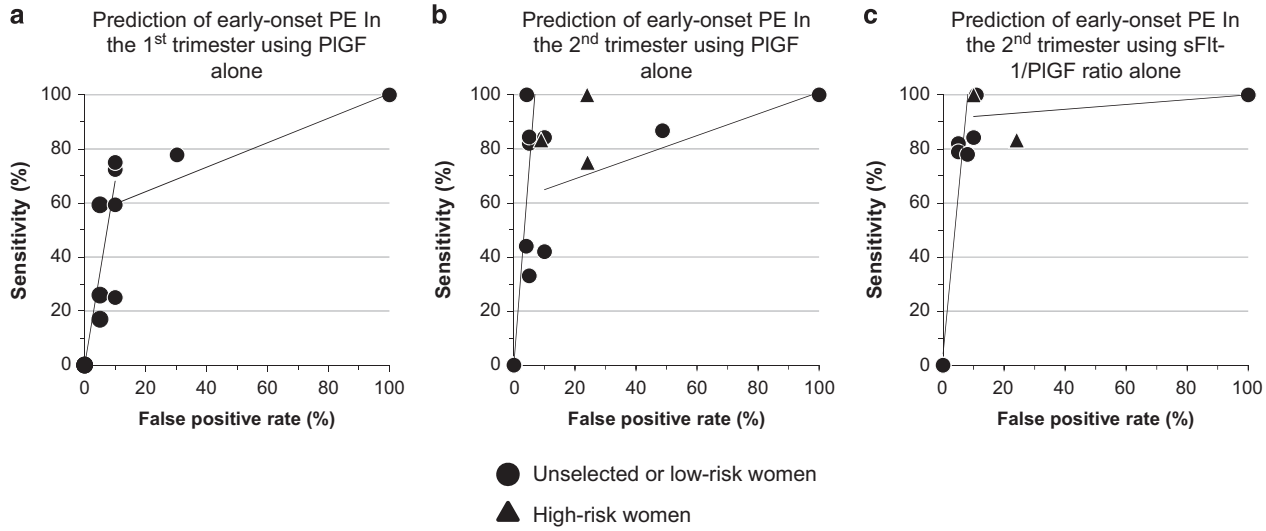

Figure 2 Scatter plot showing the relationship between FPR and sensitivity to predict early-onset PE by placental growth factor (PlGF) alone in the first trimester (**a**), PlGF alone in the second trimester (**b**) and the soluble fms-like tyrosine kinase 1 (sFlt-1)/PlGF ratio alone in the second trimester (**c**). Closed circles indicate unselected or low-risk women, and closed triangles indicate high-risk women. When the FPR was $<10\%$, the regression line of the sensitivity of FPR, including 0% FPR and 0% sensitivity, was plotted in unselected or low-risk women, and, when the FPR was $\geqslant 10\%$, the regression line of the sensitivity on FPR, including 100% FPR and 100% sensitivity, was plotted in unselected or low-risk women. FPR, false-positive rate; PE, preeclampsia.

predictive all-PE in the meta-analysis.[52] Because abnormal UAD findings are frequently observed in women with a later occurrence of early-onset PE, it is more logical to apply the UAD screening to predict only early-onset PE. We summarized the prediction of early-onset PE using UAD alone in a systematic review.[44] In this systematic review, 40 articles with sensitivity and false-positive rate (FPR) data were finally used to calculate LR+, and post-test probability for predicting early-onset PE. In unselected low-risk women, UAD alone could predict early-onset PE in the first and second trimesters with an LR+ of $\geqslant 10$ (Supplementary Table S2).[53–62] In particular, UAD alone in the second trimester in high-risk women yielded a post-test probability of >0.20 in one study, and in another study, the post-test probability was close to 0.20 (0.194); (Supplementary Table S2).[63,64] We constructed a regression line between FPR and sensitivity in the first and second trimesters; at a cutoff level of 5% FPR in unselected or low-risk women, the average LR+ in the first and second trimesters was 11.2 and 11.0, respectively (Figure 1).

Until now, many molecules have been investigated as candidate biomarkers for predicting the onset of PE.[65–67] The conclusions of a recent systematic review were all pessimistic because no single biomarkers could predict the occurrence of HDP or PE with a high LR+.[65,66] However, Forest *et al.*[67] stated that combinations of antenatal risk factors, clinical parameters, and biophysical and biochemical markers were promising. For example, Akolekar *et al.*[68] reported that the sensitivity to predict early, intermediate and late PE under conditions of an FPR of 5% was 91.0%, 79.5% and 60.9%, respectively, when maternal factors, two biophysical markers (uterine artery pulsatility index and mean arterial pressure) and eight biomarkers (pregnancy-associated plasma protein-A, PlGF, placental protein-13, sEng, inhibin-A, activin-A, pentraxin-3 and p-selectin) at 11–13 weeks of gestation were used in a multivariate model. In this model, the LR+ was always >10 for predicting early, intermediate and late PE, indicating that all cases of PE could be detected if we used multiple risk factors simultaneously. However, in light of clinical practice, the fewer the combinations of risk factors that there are, the less expense the cost of screening will be.

As shown in the 'Animal models of PE' section, the angiogenic/angiostatic balance theory involving sFlt-1, PlGF and sEng has been the most successful model explaining the genesis of PE. Therefore, many clinical studies to predict PE using sFlt-1, PlGF and sEng have been reported. In a recent review of angiogenesis-related factors for predicting PE, it was concluded that the test accuracy of these biomarkers was too low for the accurate prediction of PE in clinical practice.[69] However, the increase in sFlt-1 and sEng, and decrease in PlGF are phenomena mainly related to early-onset PE.[20] Therefore, we summarized the predictions of early-onset PE using angiogenesis-related factors alone, or the combined use of angiogenesis-related and other risk factors.[40] Twenty-one articles with sensitivity and FPR data were used. For the prediction of early-onset PE with angiogenesis-related factors alone, in unselected or low-risk women, PlGF alone in the first and second trimesters could predict early-onset PE with an LR+ of $\geqslant 10$;[70–74] the sFlt-1/PlGF ratio at 19–25 weeks of gestation[71] and at 24 week of gestation[73] could predict early-onset PE with an LR+ of $\geqslant 10$ (Supplementary Table S3). In high-risk women, the sFlt-1/PlGF ratio at 23 weeks of gestation,[63] sEng at 19–24 weeks of gestation[64] and sFlt-1 at 28–31 weeks of gestation[75] could predict early-onset PE with an LR+ of $\geqslant 10$ (Supplementary Table S3). We constructed a regression line between FPR and sensitivity in various settings; with a cutoff level of 5% FPR in unselected or low-risk women, PlGF in the first trimester, PlGF in the second trimester, and the sFlt-1/PlGF ratio in the second trimester yielded an average LR+ of 6.8, 14.6 and 12.7, respectively (Figure 2). Regarding the prediction of early-onset PE with the combination of angiogenesis-related and other risk factors, in unselected or low-risk women, PlGF with other risk factors in the first trimester could predict early-onset PE with an LR+ of $\geqslant 10$;[54,68,70,76–80] and the sFlt-1/PlGF ratio with other risk factors in the second or early third trimester could predict early-onset PE with an LR+ of $\geqslant 10$ (Supplementary Table S4).[72,73] We constructed a regression line between FPR and sensitivity for the combined methods in the first, second and early third trimesters; at the cutoff level of 5% FPR in unselected or low-risk women, the average LR+ in the first, second and early third trimesters was 13.2, 14.6 and 25.4, respectively

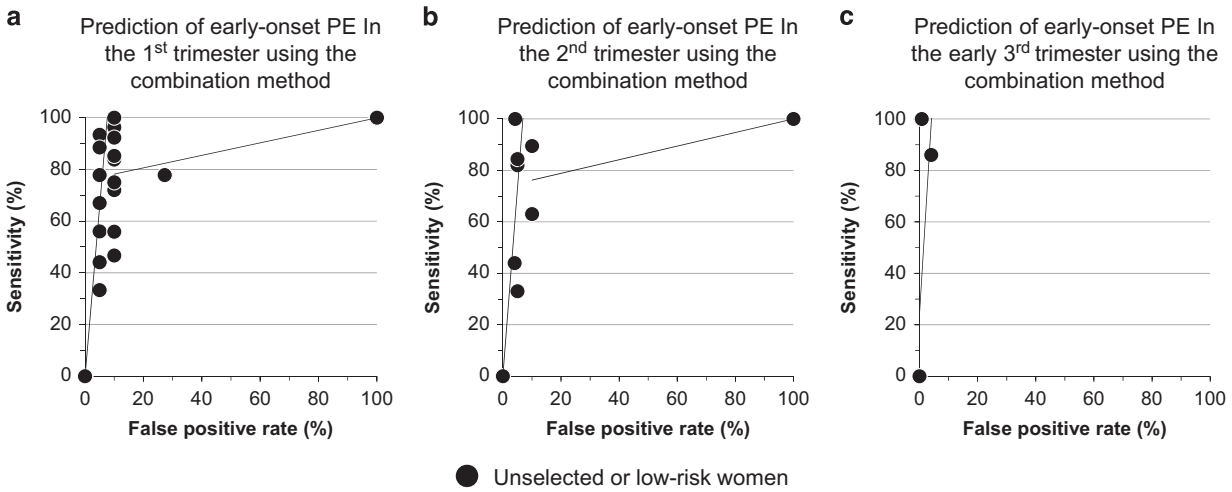

Figure 3 Scatter plot showing the relationship between FPR and the sensitivity to predict early-onset PE by the combined use of angiogenesis-related factors and other risk factors in the first trimester (**a**), the second trimester (**b**) and the early third trimester (**c**). Closed circles indicate unselected or low-risk women. When the FPR was $<10\%$, the regression line of the sensitivity of FPR, including 0% FPR and 0% sensitivity, was plotted in unselected or low-risk women, and, when the FPR was $\geqslant 10\%$, the regression line of the sensitivity on FPR, including 100% FPR and 100% sensitivity, was plotted in unselected or low-risk women. FPR, false-positive rate; PE, preeclampsia.

(Figure 3). These results suggested that the prediction of early-onset PE in the first trimester was clinically possible, but the prediction of early-onset PE in the early third trimester might be the best. We recently reported the effect of the onset threshold, on the basis of the distribution of biomarkers in women with PE but not the distribution of biomarkers in normal pregnant women, on the prediction of imminent onset of PE within 4 weeks after blood sampling.[71] The sensitivity, specificity, LR+ (95% confidence interval (CI)), PPV and negative predictive value (NPV) of the sFlt-1/PlGF ratio over the onset threshold for predicting PE with an onset at <4 weeks after blood sampling at 19–25 weeks were 100%, 100%, >1000, 100%, and 100%, respectively; those for predicting PE with an onset at <4 weeks after blood sampling at 26–31 weeks were 83%, 99.4%, 132 (95% CI: 51–339), 50%, and 99.9%, respectively, indicating that the onset threshold, on the basis of distribution of the sFlt-1/PlGF ratio in women with PE, but not the abnormal threshold on the basis of normal reference range of sFlt-1/PlGF ratio in normal pregnant women, was a promising cutoff level for predicting the imminent onset of PE soon after blood sampling in the second and early third trimesters (Figure 4). We also devised a novel prediction model on the basis of a three-step approach by sequential selection using maternal factors, including a past history of PE/GH or blood pressure levels $\geqslant 120/80$ mm Hg at 16–23 weeks (first step), followed by plasma levels of PlGF in the <5th percentile (second step) and plasma levels of sFlt-1 in the $\geqslant 95$th percentile (third step).[81] The sensitivity, specificity, LR+ (95% CI), PPV and NPV of the three-step approach for predicting PE with an onset at <4 weeks after blood sampling at 19–25 weeks were 100%, 99.8%, 599 (150–2390), 50% and 100%, respectively; those at 26–31 weeks were 83%, 99.1%, 94 (42–214), 42% and 99.9%, respectively (Figure 5). These results indicated the following: (1) the three-step approach markedly improved the LR+ from 2.6 (first step) to 94 (third step) and the PPV from 1.9% (first step) to 42% (third step); (2) the three-step approach could exclude almost 98% of women with very high NPV of 99.9–100%; and (3) the three-step approach yielded a cost reduction of 78%, compared with an approach for measuring both sFlt-1 and PlGF in all subjects. Taken together, the onset threshold or three-step approach appeared to be clinically useful for predicting the imminent onset of PE with onset at <4 weeks after blood sampling in the second and early third trimesters because the LR+ was >10, and the PPV was $>20\%$.

Prediction of PE in women with suspected PE

The most important issue for the prediction of PE is whether the method of prediction is clinically useful, in other words, whether there are any effective treatments to prevent the occurrence of PE. However, almost all of the large clinical trials evaluating the prevention of PE with several drugs have failed to identify any clinically significant values.[82–84] In addition, practitioners' interest has shifted to the use of the biomarkers to triage high-risk pregnant women, such as those with gestational proteinuria or GH. Rana *et al.*[85] found that a circulating sFlt-1/PlGF ratio (Elecsys immunoassay, Roche Diagnostics, Penzberg, Germany) of $\geqslant 85$ could predict women who will show adverse outcomes within 2 weeks among women with suspected PE. Interestingly, the ability of the sFlt-1/PlGF ratio to stratify the risk of PE was marked in women with suspected PE at <34 weeks of gestation, whereas it was very weak in those with suspected PE at $\geqslant 34$ weeks of gestation.[85] Álvarez-Fernández *et al.*[86] investigated whether a cutoff point of 85 of the sFlt-1/PlGF ratio was clinically useful to triage women with suspected PE. In women with suspected PE at <34 weeks of gestation, a cutoff point of 23 of the sFlt-1/PlGF ratio could exclude PE within 3 weeks with NPV of 94%, whereas a cutoff point of 85 could predict PE within 3 weeks with PPV of 93%. Recently, the results of the PROGNOSIS study were reported, which is a prospective, multicenter, observational study to derive and validate a serum level of the sFlt-1/PlGF ratio for predicting PE within 1 week and 4 weeks after the onset of suspected PE at 24–36 weeks of gestation.[87] A cutoff value of 38 of the sFlt-1/PlGF ratio could exclude PE within 1 week with NPV of 99% and could predict PE within 4 weeks with PPV of 39%. These results indicated that the sFlt-1/PlGF ratio in women with suspected PE at <37 weeks of gestation might be clinically useful to triage very high-risk women with suspected PE.

Alere Triage PlGF is also promising for predicting the imminent onset of PE in women with suspected PE. Duckworth *et al.*[88] investigated whether Alere Triage PlGF could predict the delivery owing to PE within 2 weeks in women with suspected PE. In women with suspected PE at <34 weeks of gestation, the PlGF level of the

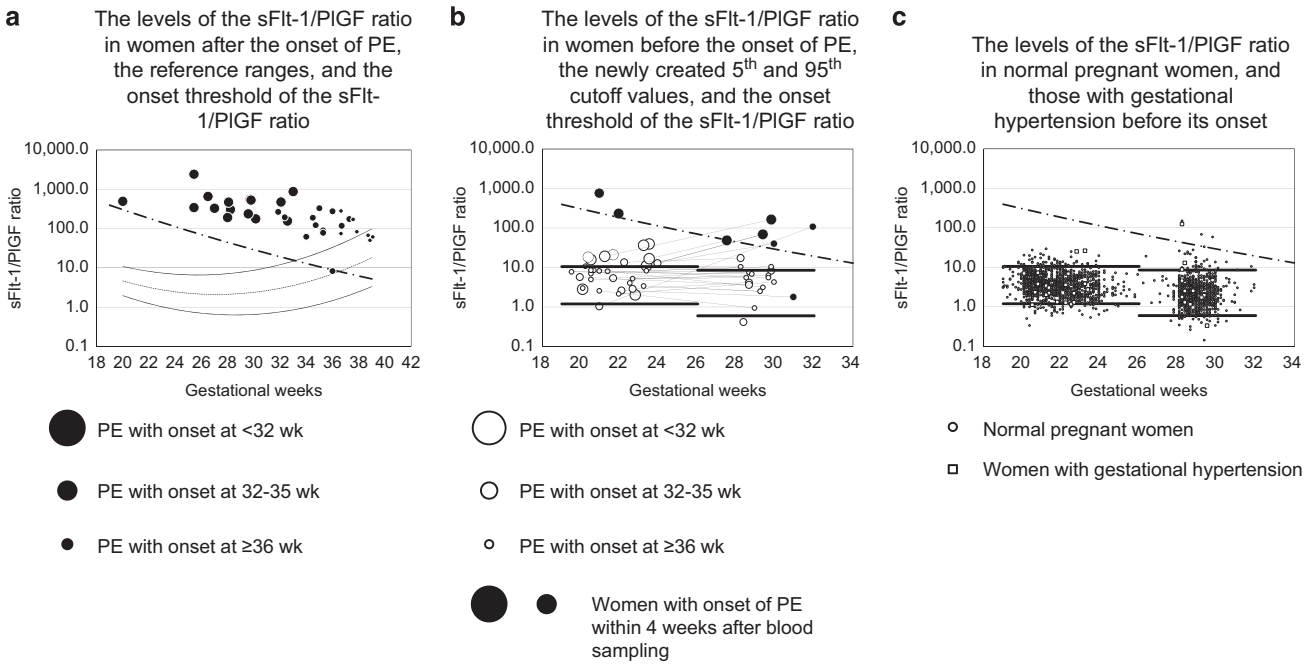

Figure 4 Prediction of the imminent onset of PE using the onset threshold of the sFlt-1/PlGF ratio. The meanings of the symbols are presented in the figure. (**a**) The thin straight curves represent the 5th and 95th percentiles of the reference range of the sFlt-1/PlGF ratio, and the thick curve represents the mean of the normal reference range of the sFlt-1/PlGF ratio. The dashed and dotted curve represents the onset threshold. The raw values of the sFlt-1/PlGF ratio after the onset of PE are shown. (**b**, **c**) Thick straight lines represent the 5th and 95th percentiles of the distribution of $\log_{10}$(sFlt-1/PlGF) in 1155 normal pregnant women at 19–25 weeks of gestation and 769 normal pregnant women at 26–31 weeks of gestation in a prospective cohort study. The dashed and dotted curve represents the onset threshold. In **b**, the raw values of the sFlt-1/PlGF ratio before the onset of PE are shown; cases with the onset of PE within 4 weeks after blood sampling are indicated by closed circles. In **c**, the raw values of the sFlt-1/PlGF ratio in normal pregnant women and those with gestational hypertension are shown. PE, preeclampsia; PlGF, placental growth factor; sFlt-1, soluble fms-like tyrosine kinase 1.

fifth percentile yielded NPV of 98%, whereas the PlGF level of the fifth percentile in women with suspected PE at 34–36 weeks of gestation yielded NPV of only 69%. Thus, Alere Triage PlGF showed favorable ability to exclude PE in women with suspected PE at <34 weeks of gestation.

In 2016, NICE published 'PlGF-based testing to help diagnose suspected preeclampsia (Triage PlGF test, Elecsys immunoassay sFlt-1/PlGF ratio, DELFIA Xpress PlGF 1-2-3 test and BRAHMS sFlt-1 Kryptor/BRAHMS PlGF plus Kryptor PE ratio)'.[89] The committee's recommendations were as follows: the Triage PlGF test and Elecsys immunoassay sFlt-1/PlGF ratio were recommended to help exclude PE in women with suspected PE at 20–34 weeks of gestation; however, there is currently insufficient evidence to recommend their routine use for diagnosing PE, and the DELFIA Xpress PlGF 1-2-3 test and BRAHMS sFlt-1 Kryptor/BRAHMS PlGF plus Kryptor PE ratio are not recommended for routine use.

Prediction of GH

In GH, the pulsatility index of the uterine artery did not differ significantly from that of normal pregnant women, but the mean arterial pressure was higher from 12 weeks onward.[90] Actually, high blood pressure in the second trimester could predict GH.[91] In addition, angiogenesis-related factors in the second or early third trimesters could predict the later occurrence of GH, although all of the LR+ values were <5.[72] The characteristics of GH compared with normal pregnancy in a longitudinal study from the first to third trimesters were as follows: slightly decreased PlGF levels after 26 weeks, slightly increased sFlt-1 levels after 34 weeks and almost identical sEng levels during pregnancy; high blood pressure levels during pregnancy; and enhanced maternal flow-mediated dilatation.[92]

PREVENTION OF PE

No effective measure to prevent PE

Until now, effective therapies to prevent PE have not been found.[82–84] Therefore, it is very important to identify clinically useful biomarkers that could help to triage pregnant women with suspected PE, such as GH or gestational proteinuria. Whenever we diagnose a pregnant woman with PE, we should monitor the mother and her fetus(es) on hospitalization, and we should induce delivery or perform cesarean section when necessary.[93,94] If we could separate low-risk women from those with suspected PE, we could reduce medical costs by reducing bed days.

Early low-dose aspirin treatment for the prevention of PE

After negative reports on the prevention of PE from 1998 to 2010,[82–84] Bujold *et al.*[95,96] offered a ray of hope for solving the problems in the prevention of PE. Several large, multicenter, randomized and controlled trials had failed to demonstrate the clinical efficacy of low-dose aspirin (LDA) for preventing the occurrence of PE.[82,97,98] However, the three large randomized controlled trials (RCTs) included participants at $\geqslant 16$ weeks of gestation. Bujold *et al.*[95,96] found that LDA treatment starting at $\leqslant 16$ weeks of gestation was associated with a marked reduction in PE. In the most recent systematic review, an RR of PE by LDA treatment starting at $\leqslant 16$ weeks of gestation was 0.47 (CI: 0.34–0.65), whereas the same treatment starting after 16 weeks of gestation did not lead to a significant reduction in PE (RR 0.81, 95% CI 0.63–1.03).[96] Recently, an RCT evaluating LDA (100 mg per day)

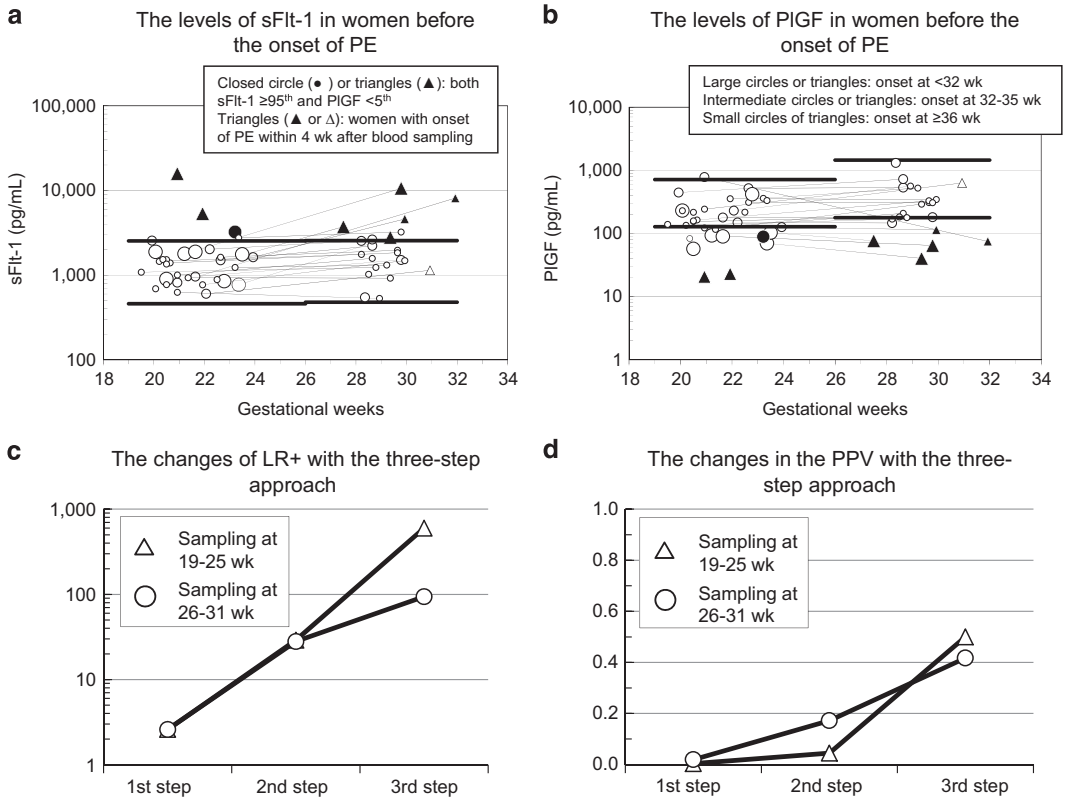

Figure 5 Prediction of the imminent onset of PE using the three-step approach. The meanings of the symbols are presented in the figure. (**a**) True-positive cases for predicting the imminent onset of PE using sFlt-1 levels are shown as closed triangles, whereas false-positive cases are shown as closed circles. Thick straight lines represent the 5th and 95th percentiles of the distribution of $\log_{10}$sFlt-1. (**b**) True-positive cases for predicting the imminent onset of PE using PlGF levels are shown as closed triangles, whereas false-positive cases are shown as closed circles. Thick straight lines represent the 5th and 95th percentiles of the distribution of $\log_{10}$PlGF. (**c**) The changes of LR+ by the three-step approach. The lines show the effects of the three-step approach (maternal factors (first step) → PlGF (second step) → sFlt-1 (third step)) on the marked changes in the LR+ for predicting the imminent onset of PE at 19–25 weeks (open triangles) and 26–31 weeks (open circles). (**d**) The changes in PPV by the three-step approach. The lines show the effects of the three-step approach (first step → second step → third step) on the marked changes in the PPV for predicting the imminent onset of PE at 19–25 weeks (open triangles) and 26–31 weeks (open circles). LR+, positive likelihood ratio; PE, preeclampsia; PlGF, placental growth factor; PPV, positive predictive value; sFlt-1, soluble fms-like tyrosine kinase 1.

starting at 8–10 weeks of gestation for preventing HDP was reported; a total of 164 women participated in this study, and the very early LDA treatment resulted in a marked reduction in HDP (GH and PE) with an RR of 0.07 (CI: 0.01–0.51).[99] Recent animal research using a PE mouse model supported early LDA treatment for preventing PE. Doridot *et al.*[100] created a PE mouse model by crossing transgenic male mice overexpressing human STOX1 with wild-type female mice; the wild-type female mice showed hypertension on days 2.5–18.5 with proteinuria, and hypertension and proteinuria were prevented by adding LDA to drinking water for the entirety of gestation.

Statins for future treatment to prevent PE

In 2007, Cudmore *et al.*[101] discovered that simvastatin treatment of endothelial cells upregulated heme oxygenase-1, which can exert protective effects against oxidative stress, and simvastatin inhibited the release of sFlt-1. Soon after this discovery, two groups demonstrated early statin-based prevention of PE using two different rodent models of PE. Costantine *et al.*[102] used a mouse model created by an adenovirus carrying sFlt-1 on day 8 of gestation, in which the mice developed hypertension and proteinuria on day 18 of gestation. Free drinking of water containing pravastatin reduced the sFlt-1 serum levels, and treatment with pravastatin decreased the contractile responses of the carotid artery to phenylephrine, which is a sympathomimetic amine that acts predominantly on α- adrenergic receptors.[102] Kumasawa *et al.*[103] established a unique experimental model using a lentiviral vector expressing a human sFlt1-mediated placenta-specific expression system; the model mice showed hypertension on day 16.5 of gestation and proteinuria on day 18.5 of gestation, and pravastatin induced PlGF and ameliorated hypertension and proteinuria. However, the protective effect was only observed when started on day 7.5 or day 10.5 of gestation, and it was not observed when the pravastatin was started at day 13.5 or day 16.5 of gestation. Currently, a clinical trial using pravastatin for the prevention of PE is ongoing.[104]

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This work was supported by Grants-in-Aid (18591809, 24390383 and 24592482 to AO) from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

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Supplementary Information accompanies the paper on Hypertension Research website (http://www.nature.com/hr)