Comparison of screening for preeclampsia at 31-34 weeks' gestation by the sFLT to PLGF ratio and a method combining maternal factors with sFLT-1 and PLGF

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Running head: Third trimester screening for preeclampsia

Key words: Placental growth factor, Soluble fms-like tyrosine kinase-1, Preeclampsia, Pyramid of antenatal care

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Acknowledgement: This study was supported by a grant from the Fetal Medicine Foundation (Charity No: 1037116). The reagents and equipment for the measurement of serum placental growth factor and soluble fms-like tyrosine kinase-1 were provided by Roche Diagnostics Limited.

ABSTRACT

Objective: To estimate the patient-specific risk of preeclampsia (PE) at 31-34 weeks' gestation by a combination of maternal characteristics and medical history with multiple of the median (MoM) values of serum placental growth factor (PLGF) and serum soluble fms-like tyrosine kinase-1 (sFLT-1) and compare the performance of screening to that achieved by the sFLT-1 to PLGF ratio.

Methods: This was a prospective observational study in women attending for a third-trimester ultrasound scan at 31-34 weeks as part of routine pregnancy care. We estimated the performance of screening of PE with delivery within four weeks of assessment (PE at <4 weeks) and PE from four weeks after assessment and up to 40 weeks' gestation (PE at 4w-40GW) in screening by the sFLT-1 to PLGF ratio and by a method utilizing Bayes theorem to combine maternal factors and MoM values of sFLT-1 and PLGF. The significance of difference in performance of screening between the method utilising Bayes theorem and that of the sFLT-1 to PLGF ratio was assessed by comparison of the areas under the receiver operating characteristic curves (AUROC).

Results: The study population of 8,063 singleton pregnancies included 231 (2.9%) that subsequently developed PE. In the prediction of delivery with PE at <4 weeks the performance of the method utilising Bayes theorem was similar to that of the sFLT-1 to PLGF (AUROC: 0.987, 95%CI 0.979-0.995 vs. 0.988, 95%CI: 0.981-0.994; p=0.961). and at fixed screen positive rate (SPR) of 3.9% the detection rate (DR) was 87.1% for both methods. In contrast, the performance of screening for delivery with PE at 4w-40GW was better with the method utilising Bayes theorem than with the sFLT-1 to PLGF ratio (AUROC: 0.884, 95%CI 0.854-0.914 vs. 0.818, 95%CI: 0.775-0.860 ; p<0.0001) and at total fixed SPR of 25.7% the DRs were 84.4% vs. 73.0%.

Conclusion: At 31-34 weeks' gestation the performance of screening for PE at <4 weeks from assessment by the method utilising Bayes theorem is similar to that of the sFLT-1 to PLGF ratio, but the former is superior to the latter in prediction of PE at ≥4 weeks.

Introduction

In women with preeclampsia (PE) the maternal serum concentration of the angiogenic placental growth factor (PLGF) is decreased and the level of the anti-angiogenic soluble fms-like tyrosine kinase 1 (sFLT-1) is increased.[1,2] There is also evidence that the altered levels of PLGF and sFLT-1 precede the clinical onset of the disease and measurement of these biomarkers can be used for the prediction of PE.[2-9] Our approach to screening for PE is to use Bayes theorem to derive the *posterior* risk by combining the *prior* risk from maternal characteristics and medical history with multiple of the median (MoM) values of biomarkers.[8,10-15] Others, advocate the use of the simpler sFLT-1 to PLGF ratio.[4,9]

We have recently proposed an approach for stratification of pregnancies into high-, intermediate- and low-risk management groups based on the results of assessment of risk for PE at 32 weeks' gestation (Figure 1).[16] The high-risk group would require intensive monitoring from the time of the initial assessment and up to 40 weeks' gestation, the intermediate-risk group would require reassessment four weeks after the initial assessment or intensive monitoring starting from four weeks and up to 40 weeks' gestation and the low-risk group would be reassessed only at 40 weeks' gestation. The performance of screening at 32 weeks is poor for prediction of PE at >40 weeks' gestation [8] and it would therefore be necessary to reassess all remaining pregnancies at 40 weeks to decide the best time and method of delivery.

The objective of this study is to compare the performance of screening by the method utilising Bayes theorem to that of the sFLT-1 to PLGF ratio in the prediction of delivery with PE at <4 weeks from assessment (PE at <4 weeks) and delivery with PE at four weeks from assessment and up to 40 weeks' gestation (4w-40GW).

Methods

The data for this study were derived from prospective screening for adverse obstetric outcomes in women attending for a 32 week routine hospital visit at King's College Hospital, London or Medway Maritime Hospital, Gillingham, UK between March 2012 and January 2014. This visit included recording of maternal demographic characteristics and medical history, ultrasound examination for fetal anatomy and growth, and measurement of serum concentration of PLGF and sFLT-1 in pg/mL by an automated biochemical analyzer within 10 minutes of blood sampling and results being available 30 minutes later (Cobas e411 system, Roche Diagnostics, Penzberg, Germany). Gestational age was determined by the measurement of fetal crown-rump length at 11-13 weeks or the fetal head circumference at 19-24 weeks.[17,18]

The women gave written informed consent to participate in the study, which was approved by the NHS Research Ethics Committee. The inclusion criteria for this study were singleton pregnancies examined at 31^{+0}-33^{+6} weeks' gestation and delivering a non-malformed live birth or stillbirth at $\geq$31 weeks' gestation. We excluded pregnancies with aneuploidies and major fetal abnormalities. The study population was included in our previous report.[8]

Data on pregnancy outcome were collected from the hospital maternity records or the general medical practitioners of the women. The obstetric records of all women with pre-existing or pregnancy associated hypertension were examined to determine if the

condition was PE, as defined by the International Society for the Study of Hypertension in Pregnancy.[19]

Statistical analysis

Patient-specific risks of delivery with PE at <4 weeks from assessment and at <40 weeks' gestation were calculated using the competing risks model to combine the *prior* risk for PE from maternal characteristics and medical history with MoM values of PLGF and sFLT-1.[8,10-15] Pregnancies were allocated to the high-risk group if their risk for PE at <4 weeks was above a specific high-risk threshold and they were allocated to the low-risk group if their risk for PE at <40 weeks' gestation was below a specified low-risk threshold. Otherwise, they were allocated to the intermediate risk group.[16] Different risk cut-offs were used to vary the proportion of the population stratified into each risk category and performance was assessed in terms of the distribution of pregnancy outcomes by risk group. In order to compare stratification based on risks utilising Bayes theorem with that based on sFLT-1 to PLGF ratios we computed the cut-offs for stratification on the basis of ratios that would give the same proportions in the high-, intermediate- and low-risk groups as those obtained by the risks. We also examined the performance of sFLT-1/PLGF >38, because this ratio was previously reported as being useful in the prediction of PE at <4 weeks in high-risk pregnancies.[9]

The significance of difference in performance between the method utilising Bayes theorem and that of the sFLT-1 to PLGF ratio was assessed by comparison of the areas under the receiver operating characteristic curves (AUROC).[20]

The statistical software package R was used for data analyses.[21]

Results

The study population of 8,063 singleton pregnancies included 231 (2.9%) that subsequently developed PE. Maternal and pregnancy characteristics of the study population are summarized in Table 1.

Comparison of screening performance

The ROC curves for performance of screening for PE at <4 weeks and PE at 4w-40GW by the method utilising Bayes theorem and that of the sFLT-1 to PLGF ratio are shown in Figure 2. There was no significant difference in performance of screening for PE at <4 weeks between the method utilising Bayes theorem and that of the sFLT-1 to PLGF ratio of (AUROC: 0.987, 95%CI 0.979-0.995 vs. 0.988, 95%CI: 0.981-0.994; p=0.961). In contrast, the performance of screening for PE at 4w-40GW by utilising Bayes theorem was significantly better than that of the sFLT-1 to PLGF ratio (AUROC: 0.884, 95%CI 0.854-0.914 vs. 0.818, 95%CI: 0.775-0.860 ; p<0.0001).

Performance of SFLT-1 to PLGF ratio >38

The sFLT-1 to PLGF ratio was >38 in 1.9% of the population and this group contained 75.9% of pregnancies with PE at <4 weeks and 24.1% of those with PE at 4w-40GW (Table 2). In the method utilising Bayes theorem, the risk cut-off for PE at <4 weeks allocating 2.0% of pregnancies to the high-risk group was 1 in 8.5 and the 2.0% of

pregnancies selected by this method contained 80.6% of pregnancies with PE at <4 weeks and 29.1% of those with PE at 4w-40GW.

Stratification of the population into high-, intermediate- and low-risk groups

The allocation of pregnancies to risk group by pregnancy outcome is given in Table 2.

In the study population there were 29 pregnancies that were delivered with PE at <4 weeks. At risk cut-off of 1 in 3 for PE at <4 weeks, 74.2% of pregnancies with PE at <4 weeks were allocated to the high-risk group which comprised of 1.2% of all pregnancies. The proportion of all pregnancies and those with PE at <4 weeks allocated to the high-risk group increased from 1.2 and 74.2%, respectively, if the risk cut-off was 1 in 3 to 5.9 and 93.5% if the risk cut-off was 1 in 150.

The cut-off in sFLT-1 to PLGF ratio that would be equivalent to a risk cut-off of 1 in 3 in allocating 1.2% of pregnancies to the high-risk group was 56.88; at this cut-off, 67.7% of pregnancies with PE at <4 weeks were allocated to the high-risk group. The cut-off in sFLT-1 to PLGF ratio that would be equivalent to a risk cut-off of 1 in 150 in allocating 5.9% of pregnancies to the high-risk group was 16.67; at this cut-off 100% of pregnancies with PE at <4 weeks were allocated to the high-risk group. For the same proportion of all pregnancies allocated to the high-risk group by the method utilizing Bayes theorem and the one by the sFLT-1 to PLGF ratio, the proportion of pregnancies with PE at <4 weeks contained within this group was similar (proportion of the population 1.2%: proportion of PE at <4 weeks 74.2% vs 67.7%; population 2.1%: PE 80.6% vs 77.4%; population 3.9%: PE 87.1% for both; population 5.0%: PE 87.1% vs 90.3%; population 5.9%: PE 93.5% vs 100%).

In the study population there were 141 pregnancies that delivered with PE at 4w-40GW. The allocation of these cases into the high-, intermediate- and low-risk groups is shown in Table 2. For example, the high-risk group defined by a risk cut-off of 1 in 50 for PE at <4 weeks constituted 3.9% of the population and contained 44.0% (62/141) of pregnancies with PE at 4w-40GW. Using this risk cut-off of 1 in 50 for PE at <4 weeks and a risk cut-off of 1 in 150 for PE at <40 weeks' gestation, 29.1% of pregnancies were allocated to the intermediate-risk group which contained 43.3% (61/141) of pregnancies with PE at 4w-40GW. Consequently, for these particular risk cut-offs, 33.0% of pregnancies were allocated to the high- or intermediate-risk group and the combination of these groups contained a total of 83.3% (123/141) of pregnancies with PE at 4w-40GW.

The cut-off in sFLT-1 to PLGF ratio that would be equivalent to a risk cut-off of 1 in 50 for PE at <4 weeks allocating 3.9% of pregnancies to the high-risk group was 22.38; at this cut-off, 40.4% of pregnancies with PE at 4w-40GW were allocated to the high-risk group, compared to 44.0% when the group of 3.9% of pregnancies was selected by the method utilizing Bayes theorem. The combination of risk cut-off of 1 in 50 for PE at <4 weeks (sFLT-1 to PLGF ratio 22.38) and risk cut-off of 1 in 150 for PE at <40 weeks' gestation (sFLT-1 to PLGF ratio 4.35) allocated 29.1% of pregnancies to the intermediate-risk group; this group contained 41.1% of pregnancies with PE at 4w-40GW when selection of the group was by sFLT-1 to PLGF ratio, compared to 43.3% with selection by the method utilizing Bayes theorem. Consequently, for these particular risk or ratio cut-offs, 33.0% of pregnancies were allocated to the high- or intermediate-risk group and the combination of these groups contained a total of 81.5% of pregnancies with PE at 4w-40GW when the groups were selected by sFLT-1 to PLGF ratio, compared to 83.3% with selection by the

method utilizing Bayes theorem.

Discussion

Main findings

The study has demonstrated how assessment of risk for PE at 31-34 weeks' gestation can be used to stratify the population into high- intermediate- and low-risk groups. Two approaches were applied to achieve such stratification and their performance was compared. The first approach utilised Bayes theorem to combine maternal factors with MoM values of sFLT-1 and PLGF and derive patient-specific risks and the second approach used a simple division of the measured concentration of sFLT-1 by that of PLGF. The advantage of the ratio is simplicity in clinical practice. However, such approach does not take into account the *prior* risk of the individual patient in the study population and ignores the effects of maternal characteristics on the measured serum concentrations and their interrelations in both normal and pathological pregnancies.[10,14,15]

We found that the performance of screening for PE at <4 weeks of assessment was similar by the two methods, but the method utilising Bayes theorem was superior to that of the sFLT-1 to PLGF ratio in prediction of PE at ≥4 weeks. These findings confirm that the sFLT-1 to PLGF ratio is a very strong predictor of imminent PE and the contribution of the *prior* risk from maternal factors in identifying the high-risk group is relatively small. With increasing intervals between sampling and development of PE the contribution of maternal factors in prediction of PE becomes more apparent as the contribution of the sFLT-1 to PLGF ratio becomes weaker.

The proportion of the population stratified into high- intermediate- and low-risk groups and the proportion of each strata developing PE with delivery at <4 weeks, at 4w-40GW and at >40GW would inevitably depend on the risk cut-offs used for defining the groups. In order to compare stratification based on risks utilising Bayes theorem with that based on sFLT-1 to PLGF ratios we computed the cut-offs for stratification on the basis of ratios that would give the same proportions in the high-, intermediate- and low-risk groups as those obtained by the risks. The cut-offs in the sFLT-1 to PLGF ratio for identifying the high-risk group for delivery with PE at <4 weeks ranged from 56.88 to 16.67 with respective proportions of the population allocated to the high-risk group ranging from 1.2 to 5.9% and proportion of cases with PE at <4 weeks contained in this group varying from 67.7-100%. A previous study advocated the use of the specific ratio of >38 to identify a group at high-risk of developing PE within the subsequent four weeks;[9] in this study, 2.0% of the population fulfilled this criterion and this group contained 77.4% of pregnancies with PE at <4 weeks and 24.1% of those with PE at 4w-40GW

Strengths and limitations

The strengths of this study are first, examination of a large population of pregnant women attending for routine care in a gestational age range which is widely used for assessment of fetal growth and wellbeing, second, recording of data on maternal characteristics and medical history to define the *prior* risk, third, use of automated machines to provide accurate measurement within 40 minutes of sampling of maternal serum concentration of PLGF and sFLT-1, fourth, expression of the values of the biomarkers as MoMs after adjustment for factors that affect the measurements and use of Bayes theorem to

combine the *prior* risk from maternal factors with biomarkers to estimate patient-specific risks and the performance of screening for PE delivering at different stages of pregnancy and fifth, direct comparison of the performance of screening for PE by a method utilizing Bayes theorem to that of the sFLT-1 to PLGF ratio.

A limitation of the study is that fitting of the risk model [8] and development and assessment of risk stratification were on the same data, which induces a degree of optimistic bias into the results. However, our risk model[4] is a parsimonious one with just two parameters for the mean log MoM value for each of the markers and a pooled estimate of an assumed common covariance matrix and this limits the degree of bias induced. Nevertheless, prospective evaluation using an independent test data set is needed to validate the results.

Comparison with previous studies

Our findings are compatible with those of a previous screening study for PE at 30-34 weeks' gestation which included 118 cases of PE and 3,734 unaffected pregnancies; in the cases of PE the sFLT-1 MoM to PLGF MoM ratio was increased and the deviation from normal was inversely related to the interval between sampling and the gestational age at delivery.[7] Our findings are also compatible with those of previous studies investigating high-risk pregnancies which reported that the sFlt-1 to PLGF ratio is highly accurate in identifying the subgroup that will develop severe PE requiring delivery within the subsequent few weeks.[3-6,9]

A study investigating serum PLGF at 11-13, 19-25, 30-34 and 35-37 weeks' gestation, demonstrated that in pregnancies that develop PE, serum PLGF in all four gestational age groups was decreased, but the separation in MoM values from normal was greater when the interval between sampling the development of PE was closer; the performance of screening for PE at <37 weeks' gestation was superior with screening at 32 than at 22 or 12 weeks and the performance of screening for PE at ≥37 weeks was superior with screening at 36 weeks than at earlier gestations.[22] A similar study demonstrated that in pregnancies that develop PE, serum sFLT-1 is increased and the separation in MoM values from normal was greater when the interval between sampling the development of PE was closer; however, unlike PLGF, sFLT-1 at 11-13 weeks was not a useful marker of PE.[23]

Clinical implications of the study

In the traditional approach to prenatal care, screening and diagnosis of PE is based on the demonstration of elevated blood pressure and proteinuria during a routine clinical visit in the late second- or third-trimester of pregnancy.

In a proposed new pyramid of pregnancy care, the timing and content of clinical visits should be defined by the patient-specific risk of developing PE.[25] This study provides the framework for subsequent management of pregnancies based on the results of screening by maternal factors and the measurements of serum sFLT-1 and PLGF at 30-34 weeks' gestation. A small high-risk group can be monitored by measurement of blood pressure and urinalysis at least on a weekly basis, a bigger intermediate-risk group would either be reassessed in four weeks or will undergo intensive monitoring from four weeks after the initial assessment, whereas patients in the large low-risk group can be reassured that development of PE before 40 weeks is very unlikely.

The best approach for stratification of risks for development of PE is the one that takes into account the *prior* risk of the individual patient based on maternal characteristics and medical history and defines the *posterior* risk by adjusting the measured serum concentrations of sFLT-1 and PLGF for those maternal characteristics that affect these measurements. [10,14,15] This approach also allows incorporation into the risk algorithm of additional potentially useful biomarkers, such as uterine artery pulsatility index and mean arterial pressure.[8] The alternative approach for allocation of patients into management groups is the simple ratio of the measured concentrations of sFL-1 and PLGF; this appears to be equally good to that utilizing Bayes theorem in identifying the group at high-risk of developing PE at <4 weeks from assessment. However, in this respect the best ratio is not >38,[9] but varies according to the desired proportion of the population allocated into the different management groups. The method utilizing Bayes theorem is superior to the ratio in identifying pregnancies at high-risk of developing PE at $\geq$4 weeks from assessment.

The cut-offs in risks or sFLT-1 to PLGF ratios to define the proportion of the population stratified into each of the three management groups and the protocols for such management will inevitably vary according to local preferences and health economic considerations. Future studies will examine whether the implementation of such protocols could improve perinatal outcome.

References

1. Maynard SE, Min JY, Merchan J, Maynard SE, Min JY, Merchan J, Lim KH, Li J, Mondal S, Libermann TA, Morgan JP, Sellke FW, Stillman IE, Epstein FH, Sukhatme VP, Karumanchi SA.. 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.

2. Levine RJ, Maynard SE, Qian C, Lim KH, England LJ, Yu KF, Schisterman EF, Thadhani R, Sachs BP, Epstein FH, Sibai BM, Sukhatme VP, Karumanchi SA. Circulating angiogenic factors and the risk of preeclampsia. *N Engl J Med* 2004; **350**: 672-683.

3. Chaiworapongsa T, Romero R, Savasan ZA, Kusanovic JP, Ogge G, Soto E, Dong Z, Tarca A, Gaurav B, Hassan SS. Maternal plasma concentrations of angiogenic/anti-angiogenic factors are of prognostic value in patients presenting to the obstetrical triage area with the suspicion of preeclampsia. *J Matern Fetal Neonatal Med* 2011; **24**: 1187-1207.

4. Verlohren S, Herraiz I, Lapaire O, Schlembach D, Moertl M, Zeisler H, Calda P, Holzgreve W, Galindo A, Engels T, Denk B, Stepan H. The sFlt-1/PlGF ratio in different types of hypertensive pregnancy disorders and its prognostic potential in preeclamptic patients. *Am J Obstet Gynecol* 2012; **206**: 58.e1-8.

5. Rana S, Powe CE, Salahuddin S, Verlohren S, Perschel FH, Levine RJ, Lim KH, Wenger JB, Thadhani R, Karumanchi SA. Angiogenic factors and the risk of adverse outcomes in women with suspected preeclampsia. *Circulation* 2012; **125**: 911-919.

6. 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.

7. Lai J, Garcia-Tizon Larroca S, Peeva G, Poon LC, Wright D, Nicolaides KH. Competing risks model in screening for preeclampsia by serum placental growth factor and soluble fms-like tyrosine kinase-1 at 30-33 weeks' gestation. *Fetal Diagn Ther* 2014; **35**: 240-248.

8. Tsiakkas A, Saiid Y, Wright A, Wright D, Nicolaides KH. Competing risks model in screening for preeclampsia by maternal factors and biomarkers at 30–34 weeks' gestation. *Am J Obstet Gynecol* 2016; **215**: 87.e1-87.e17.

9. Zeisler H, Llurba E, Chantraine F, Vatish M, Staff AC, Sennström M, Olovsson M, Brennecke SP, Stepan H, Allegranza D, Dilba P, Schoedl M, Hund M, Verlohren S. Predictive Value of the sFlt-1:PlGF Ratio in Women with Suspected Preeclampsia. *N Engl J Med* 2016; **374**: 13-22.

10. Wright D, Syngelaki A, Akolekar R, Poon LC, Nicolaides KH. Competing risks model in screening for preeclampsia by maternal characteristics and medical history. *Am J Obstet Gynecol* 2015; **213**: 62.e1-10.

11. Wright D, Akolekar R, Syngelaki A, Poon LC, Nicolaides KH. A competing risks model in early screening for preeclampsia. *Fetal Diagn Ther* 2012; **32**: 171-178.

14. Tsiakkas A, Duvdevani N, Wright A, Wright D, Nicolaides KH. Serum placental growth factor in the three trimesters of pregnancy: effects of maternal characteristics and medical history. *Ultrasound Obstet Gynecol* 2015; **45**: 591-598.

15. Tsiakkas A, Duvdevani N, Wright A, Wright D, Nicolaides KH. Serum soluble fms-like tyrosine kinase-1 in the three trimesters of pregnancy: effects of maternal characteristics and medical history. *Ultrasound Obstet Gynecol* 2015; **45**: 584-590.

16. Wright D, Dragan I, Syngelaki A, Akolekar R, Nicolaides KH. Proposed clinical management of pregnancies after combined screening for preeclampsia at 30-34 weeks' gestation. *Ultrasound Obstet Gynecol* 2016; in press

17. Robinson HP, Fleming JE. A critical evaluation of sonar crown rump length measurements. *Br J Obstet Gynaecol* 1975; **82**: 702-710.

18. Snijders RJ, Nicolaides KH. Fetal biometry at 14-40 weeks' gestation. *Ultrasound Obstet Gynecol* 1994; **4**: 34-48.

19. Brown MA, Lindheimer MD, de Swiet M, Van Assche A, Moutquin JM. The classification and diagnosis of the hypertensive disorders of pregnancy: Statement from the international society for the study of hypertension in pregnancy (ISSHP). *Hypertens Pregnancy* 2001; **20**: IX-XIV.

20. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. *Biometrics* 1988; **44**: 837-845.

21. R Development Core Team. R. A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2011;ISBN 3-900051-07-0, URL http://www.R-project.org/

22. Tsiakkas A, Cazacu R, Wright A, Wright D, Nicolaides KH. Maternal serum placental growth factor at 12, 22, 32 and 36 weeks' gestation in screening for pre-eclampsia. *Ultrasound Obstet Gynecol* 2016; **47**: 472-477.

23. Tsiakkas A, Mendez O, Wright A, Wright D, Nicolaides KH. Maternal serum soluble fms-like tyrosine kinase-1 at 12, 22, 32 and 36 weeks' gestation in screening for pre-eclampsia. *Ultrasound Obstet Gynecol* 2016; **47**: 478-483.

24. Nicolaides KH. Turning the pyramid of prenatal care. *Fetal Diagn Ther* 2011; **29**: 183-196.

Figure legends

Figure 1. Stratification of pregnancies into high-, intermediate- and low-risk management groups based on screening for preeclampsia at 31-34 weeks' gestation. The high-risk group would require intensive monitoring from the time of the initial assessment and up to 40 weeks' gestation, the intermediate-risk group would require intensive monitoring starting from four weeks after the initial assessment and up to 40 weeks' gestation and the low-risk group would be reassessed only at 40 weeks' gestation.

Figure 2. Receiver operating characteristic curves for prediction of preeclampsia (PE) within four weeks of assessment (left) and PE from four weeks after assessment and up to 40 weeks' gestation (right) by a method utilising Bayes theorem (red line) and by the sFLT-1 to PLGF ratio (blue line).

Table 1. Maternal and pregnancy characteristics in pregnancies that developed preeclampsia (PE) within four weeks from assessment, at four weeks from assessment and up to 40 weeks' gestation and at >40 weeks' gestation, compared with pregnancies that remained normotensive.

Maternal characteristics	Preeclampsia			
	None (n=7,832)	<4 weeks (n=29)	4 w-40GW (n=141)	>40GW (n=61)
Age, median (IQR)	31.0 (26.7-34.7)	31.0 (26.4-34.0)	31.7 (27.5-35.2)	31.0 (24.9-34.8)
Weight, median (IQR)	67.3 (59.5-78.0)	70.4 (60.0-86.0)	76.0 (64.5-89.9)	69.0 (61.0-84.8)
Height, median (IQR)	1.65 (1.60-1.69)	1.60 (1.58-1.65)	1.65 (1.60-1.69)	1.64 (1.60-1.69)
Racial origin				
Caucasian, n (%)	5880 (75.1)	19 (65.5)	80 (56.7)	41 (67.2)
Afro-Caribbean, n (%)	1339 (17.1)	8 (27.6)	50 (35.5) *	17 (27.9)
South Asian, n (%)	295 (3.8)	2 (6.9)	6 (4.3)	2 (3.3)
East Asian, n (%)	145 (1.9)	0	3 (2.1)	1 (1.6)
Mixed, n (%)	173 (2.2)	0	2 (1.4)	0 (0.0)
Method of conception				
Spontaneous, n (%)	7570 (96.7)	27 (93.1)	136 (96.5)	59 (96.7)
Assisted conception, n (%)	262 (3.3)	2 (6.9)	5 (3.5)	2 (3.3)
Cigarette smoking, n (%)	790 (10.1)	1 (3.4)	8 (5.7)	4 (6.6)
Chronic hypertension, n (%)	87 (1.1)	6 (20.7)	23 (16.3)	3 (4.9)
SLE / APS, n (%)	14 (0.2)	0	0	0
Diabetes mellitus, n (%)	76 (1.0)	0	3 (2.1)	0
Parity				
Nulliparous, n (%)	3839 (49.0)	18 (62.1)	68 (48.2)	48 (78.7)
Parous no previous PE, n (%)	3726 (47.6)	8 (27.6)	42 (29.8)	12 (19.7)
Parous previous PE, n (%)	267 (3.4)	3 (10.3)	31 (22.0)	1 (1.6)
Family history of PE, n (%)	234 (3.0)	1 (3.4)	6 (4.3)	2 (3.3)
Inter-pregnancy interval, median (IQR)*	3.1 (2.1-5.1)	7.1 (4.2-7.9)	3.6 (2.4-5.3)	6.5 (2.9-8.2)

* Inter-pregnancy interval reported for parous women

Table 2: Stratification of risk for preeclampsia. The numbers in the grey bands represent the high-risk group and those in the white bands the intermediate-risk group. The cut-offs are given as risks estimated with the use of Bayes theorem to combine maternal characteristics with MoM values of sFLT-1 and PLGF or as sFLT-1 to PLGF ratio. Numbers in parentheses are percentages with 95% confidence intervals.

Risk cut off	SFLT-1 / PLGF cut-off	All pregnancies (n=8063)	PE at <4 w (n=29)		PE at 4 w to 40 GW (n=141)		PE at >40 GW (n=61)	
			Combined test	SFLT/PLGF	Combined test	SFLT/PLGF	Combined test	SFLT/PLGF
1 in 3 for PE <4 w	56.88 for PE <4 w	99 (**1.2**; 1.0, 1.5)	23 (**74.2**; 55.4, 88.1)	21 (**67.7**; 48.6, 83.3)	22 (**15.6**; 10, 22.7)	23 (**16.3**; 10.6, 23.5)	1 (**1.6**; 0.0, 8.7)	1 (**1.6**; 0.0, 8.7)
1 in 50 for PE <40 GW	7.41 for PE <40 GW	1268 (**15.6**; 14.8, 16.4)	8 (**25.8**; 11.9, 44.6)	10 (**32.3**; 16.7, 51.4)	89 (**63.1**; 54.6, 71.1)	71 (**50.4**; 41.8, 58.9)	27 (**43.5**; 31.0, 56.7)	22 (**35.5**; 23.7, 48.7)
1 in 100 for PE <40 GW	5.35 for PE <40 GW	1987 (**24.4**; 23.5, 25.4)	8 (**25.8**; 11.9, 44.6)	10 (**32.3**; 16.7, 51.4)	97 (**68.8**; 60.5, 76.3)	80 (**56.7**; 48.1, 65.0)	35 (**56.5**; 43.3, 69.0)	33 (**53.2**; 40.1, 66.0)
1 in 150 for PE <40 GW	4.35 for PE <40 GW	2577 (**31.7**; 30.7, 32.7)	8 (**25.8**; 11.9, 44.6)	10 (**32.3**; 16.7, 51.4)	101 (**71.6**; 63.4, 78.9)	92 (**65.2**; 56.8, 73.1)	42 (**67.7**; 54.7, 79.1)	36 (**58.1**; 44.8, 70.5)
1 in 200 for PE <40 GW	3.73 for PE <40 GW	3085 (**38.0**; 36.9, 39.0)	8 (**25.8**; 11.9, 44.6)	10 (**32.3**; 16.7, 51.4)	106 (**75.2**; 67.2, 82.1)	95 (**67.4**; 59.0, 75.0)	46 (**74.2**; 61.5, 84.5)	39 (**62.9**; 49.7, 74.8)
1 in 10 for PE <4 w	35.63 for PE <4 w	170 (**2.1**; 1.8, 2.4)	25 (**80.6**; 62.5, 92.5)	24 (**77.4**; 58.9, 90.4)	42 (**29.8**; 22.4, 38.1)	36 (**25.5**; 18.6, 33.6)	2 (**3.2**; 0.4, 11.2)	4 (**6.5**; 1.8, 15.7)
1 in 50 for PE <40 GW	7.41 for PE <40 GW	1197 (**14.7**; 14, 15.5)	6 (**19.4**; 7.5, 37.5)	7 (**22.6**; 9.6, 41.1)	69 (**48.9**; 40.4, 57.5)	58 (**41.1**; 32.9, 49.7)	26 (**41.9**; 29.5, 55.2)	19 (**30.6**; 19.6, 43.7)
1 in 100 for PE <40 GW	5.35 for PE <40 GW	1916 (**23.6**; 22.7, 24.5)	6 (**19.4**; 7.5, 37.5)	7 (**22.6**; 9.6, 41.1)	77 (**54.6;** 46.0, 63.0)	67 (**47.5**; 39.1, 56.1)	34 (**54.8**; 41.7, 67.5)	30 (**48.4**; 35.5, 61.4)
1 in 150 for PE <40 GW	4.35 for PE <40 GW	2506 (**30.8**; 29.8, 31.8)	6 (**19.4**; 7.5, 37.5)	7 (**22.6**; 9.6, 41.1)	81 (**57.4**; 48.8, 65.7)	79 (**56.0**; 47.4, 64.4)	41 (**66.1**; 53.0, 77.7)	33 (**53.2**; 40.1, 66.0)
1 in 200 for PE <40 GW	3.73 for PE <40 GW	3014 (**37.1**; 36.0, 38.1)	6 (**19.4**; 7.5, 37.5)	7 (**22.6**; 9.6, 41.1)	86 (**61.0**; 52.4, 69.1)	82 (**58.2**; 49.6, 66.4)	45 (**72.6**; 59.8, 83.1)	36 (**58.1**; 44.8, 70.5)
1 in 50 for PE <4 w	22.38 for PE <4 w	314 (**3.9**; 3.5, 4.3)	27 (**87.1**; 70.2, 96.4)	27 (**87.1**; 70.2, 96.4)	62 (**44.0**; 35.6, 52.6)	57 (**40.4**; 32.3, 49.0)	6 (**9.7**; 3.6, 19.9)	6 (**9.7**; 3.6, 19.9)
1 in 50 for PE <40 GW	7.41 for PE <40 GW	1053 (**13.0**; 12.2, 13.7)	4 (**12.9**; 3.6, 29.8)	4 (**12.9**; 3.6, 29.8)	49 (**34.8**; 26.9, 43.2)	37 (**26.2**; 19.2, 34.3)	22 (**35.5**; 23.7, 48.7)	17 (**27.4**; 16.9, 40.2)
1 in 100 for PE <40 GW	5.35 for PE <40 GW	1772 (**21.8**; 20.9, 22.7)	4 (**12.9**; 3.6, 29.8)	4 (**12.9**; 3.6, 29.8)	57 (**40.4**; 32.3, 49.0)	46 (**32.6**; 25.0, 41.0)	30 (**48.4**; 35.5, 61.4)	28 (**45.2**; 32.5, 58.3)
1 in 150 for PE <40 GW	4.35 for PE <40 GW	2362 (**29.1**; 28.1, 30.1)	4 (**12.9**; 3.6, 29.8)	4 (**12.9**; 3.6, 29.8)	61 (**43.3**; 35.0, 51.9)	58 (**41.1**; 32.9, 49.7)	37 (**59.7**; 46.4, 71.9)	31 (**50.0**; 37.0, 63.0)
1 in 200 for PE <40 GW	3.73 for PE <40 GW	2870 (**35.3**; 34.3, 36.4)	4 (**12.9**; 3.6, 29.8)	4 (**12.9**; 3.6, 29.8)	66 (**46.8**; 38.4, 55.4)	61 (43.3; 35.0, 51.9)	41 (**66.1**; 53.0, 77.7)	34 (**54.8**; 41.7, 67.5)
1 in 100 for PE <4 w	18.59 for PE <4 w	406 (**5.0**; 4.5, 5.5)	27 (**87.1**; 70.2, 96.4)	28 (**90.3**; 74.2, 98)	73 (**51.8**; 43.2, 60.3)	63 (**44.7**; 36.3, 53.3)	10 (**16.1**; 8.0, 27.7)	7 (**11.3**; 4.7, 21.9)
1 in 50 for PE <40 GW	7.41 for PE <40 GW	961 (**11.8**; 11.1, 12.5)	4 (**12.9**; 3.6, 29.8)	3 (**9.7**; 2.0, 25.8)	38 (**27.0**; 19.8, 35.1)	31 (**22.0**; 15.5, 29.7)	18 (**29.0**; 18.2, 41.9)	16 (**25.8**; 15.5, 38.5)
1 in 100 for PE <40 GW	5.35 for PE <40 GW	1680 (**20.7**; 19.8, 21.6)	4 (**12.9**; 3.6, 29.8)	3 (**9.7**; 2.0, 25.8)	46 (**32.6**; 25.0, 41.0)	40 (**28.4**; 21.1, 36.6)	27 (**43.5**; 31, 56.7)	26 (**41.9**; 29.5, 55.2)
1 in 150 for PE <40 GW	4.35 for PE <40 GW	2270 (**27.9**; 27.0, 28.9)	4 (**12.9**; 3.6, 29.8)	3 (**9.7**; 2.0, 25.8)	50 (**35.5**; 27.6, 44.0)	52 (**36.9**; 28.9, 45.4)	33 (**53.2**; 40.1, 66.0)	30 (**48.4**; 35.5, 61.4)
1 in 200 for PE <40 GW	3.73 for PE <40 GW	2778 (**34.2**; 33.1, 35.2)	4 (**12.9**; 3.6, 29.8)	3 (**9.7**; 2.0, 25.8)	55 (**39.0**; 30.9, 47.6)	55 (**39.0**; 30.9, 47.6)	37 (**59.7**; 46.4, 71.9)	33 (**53.2**; 40.1, 66.0)
1 in 150 for PE <4 w	16.67 for PE <4 w	476 (**5.9**; 5.4, 6.4)	29 (**93.5**; 78.6, 99.2)	31 (**100**; 88.8, 100)	80 (**56.7**; 48.1, 65.0)	65 (**46.1**; 37.7, 54.7)	12 (**19.4**; 10.4, 31.4)	10 (**16.1**; 8.0, 27.7)
1 in 50 for PE <40 GW	7.41 for PE <40 GW	891 (**11.0**; 10.3, 11.7)	2 (**6.5**; 0.8, 21.4)	0 (**0.0**; 0.0, 11.2)	31 (**22.0**; 15.5, 29.7)	29 (**20.6**; 14.2, 28.2)	16 (**25.8**; 15.5, 38.5)	13 (**21.0**; 11.7, 33.2)
1 in 100 for PE <40 GW	5.35 for PE <40 GW	1610 (**19.8**; 18.9, 20.7)	2 (**6.5**; 0.8, 21.4)	0 (**0.0**; 0.0, 11.2)	39 (**27.7**; 20.5, 35.8)	38 (**27.0;** 19.8, 35.1)	24 (**38.7**; 26.6, 51.9)	24 (**38.7**; 26.6, 51.9)
1 in 150 for PE <40 GW	4.35 for PE <40 GW	2200 (**27.1**; 26.1, 28.0)	2 (**6.5**; 0.8, 21.4)	0 (**0.0**; 0.0, 11.2)	43 (**30.5**; 23.0, 38.8)	50 (**35.5**; 27.6, 44.0)	31 (**50.0**; 37.0, 63.0)	27 (**43.5**; 31, 56.7)
1 in 200 for PE <40 GW	3.73 for PE <40 GW	2708 (**33.3**; 32.3, 34.4)	2 (**6.5**; 0.8, 21.4)	0 (**0.0**; 0.0, 11.2)	48 (**34.0**; 26.3, 42.5)	53 (**37.6**; 29.6, 46.1)	35; (**56.5**; 43.3, 69.0)	30 (**48.4**; 35.5, 61.4)
1 in 8.5 for PE<4	**SFLT-1 / PLGF >38**	159 (**2.0**; 1.7, 2.3)	25 (**80.6**; 62.5, 92.5)	24 (**77.4**; 58.9, 90.4)	41 (29.1; 21.7, 37.3)	34 (**24.1**; 17.0, 31.2)	1 (**1.6**; 0, 8.7)	3 (**4.8**; 0.5, 10.2)