Development of preeclampsia within four weeks of sFLT to PLGF ratio >38: Comparison of performance at 31-34 versus 35-37 weeks' gestation

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ABSTRACT

Objective: To compare the performance of screening of the soluble fms-like tyrosine kinase 1 (sFLT-1) to placental growth factor (PLGF) ratio >38 in the prediction of delivery with preeclampsia (PE) at <1 and at <4 weeks from assessment when the test is carried out at 31-34 versus 35-37 weeks' gestation.

Methods: This was a prospective observational study in women attending for a third-trimester ultrasound scan as part of routine pregnancy care; in the first phase of the study the visit was at 30-34 weeks' gestation and in the second phase the visit was at 35-37 weeks. Serum sFlt-1 and PlGF were measured and their ratio calculated. We estimated the detection rate (DR) and false positive rate (FPR) of sFLT-1/PLGF >38 for prediction of delivery with PE at <1 and at <4 weeks after assessment and compared the performance of screening when the test is carried out at 31^{+0}-33^{+6} versus 35^{+0}-36^{+6} weeks' gestation.

Results: The study population included 8,063 singleton pregnancies that were examined at 31-34 weeks' gestation and 3703 examined at 35-37 weeks; PE at <1, <4 and ≥4 weeks occurred in 5 (0.1%), 29 (0.4%) and 202 (2.5%) of the former and 7 (0.2%), 39 (1.1%) and 21 (0.6%) of the latter. In the non-PE group, the median sFLT-1/PLGF increased with gestational age at screening and the ratio of 38 was just below the 99th percentile at 32 weeks' gestation and just below the 90th percentile at 36 weeks. In the two gestational windows the DR of PE at <4 weeks was similar (75.9%, 95% CI 56.5, 89.7 vs. 79.5%, 95% CI 63.5, 90.7), but the FPR was substantially lower at 31-34 than at 35-37 weeks (1.7%, 95% CI 1.4, 2.0 vs. 9.6%, 95% CI 8.7, 10.6). The number of cases with PE at <1 week was small, but as above, in the two gestational windows the DR was similar (80.0%, 95% CI 28.4, 99.5 vs. 85.7%, 42.1, 99.6), but the FPR was substantially lower at 31-34 than at 35-37 weeks (1.9%, 95% CI 1.6, 2.2 vs. 10.2%, 95% CI 9.3, 11.3).

Conclusion: The performance of sFLT-1/PLGF >38 in prediction of delivery with PE at <1 and <4 weeks is substantially different when assessment is carried out at 31-34 weeks compared to assessment at 35-37 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 a ratio of sFLT-1 to PLGF can be used in the assessment of women presenting to specialist clinics with signs or symptoms of hypertensive disorders to help distinguish between those that will develop PE in the subsequent 1-4 weeks from those that will not.[3-8]

A prospective study in 1050 women with singleton pregnancies presenting with signs or symptoms of hypertensive disorders at 24-37 (median 32) weeks' gestation, reported that sFLT-1/PLGF $\leq$38 was the best ratio to predict absence of PE at <1 week from assessment and a value >38 was the best ratio to predict development of PE at <4 weeks from assessment.[8] However, in normal pregnancy at 30-40 weeks serum sFLT-1 increases and PLGF decreases with gestational age;[2,9,10] consequently, the sFLT-1 to PLGF ratio would normally increase with gestational age and in screening with the single cut-off point of 38 there would be an inevitable increase in false positive rate (FPR) with gestational age.

The objective of this screening study in women undergoing routine clinical care at 31-37 weeks' gestation is to compare the performance of screening of sFLT-1/PLGF >38 in the prediction of delivery with PE at <1, <4 and $\geq$4 weeks from assessment when the test is carried out at 31-34 versus 35-37 weeks' gestation.

Methods

The data for this study were derived from prospective screening for adverse obstetric outcomes in women attending for a third-trimester routine hospital visit at King's College Hospital, London or Medway Maritime Hospital, Gillingham, UK. In the first phase of the service the visit was at 30-34 weeks' gestation and in the second phase the visit was at 35-37 weeks. The visits included recording of maternal demographic characteristics and medical history, ultrasound examination for fetal anatomy and growth, and measurement of serum PLGF and sFLT-1 in pg/mL by an automated biochemical analyzer within 10 minutes of blood sampling with 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.[11,12]

Written informed consent was obtained from the women agreeing to participate in the study, which was approved by the NHS Research Ethics Committee. The inclusion criteria for this study were singleton pregnancies delivering a non-malformed live birth or stillbirth at $\geq$30 weeks' gestation in which sFLT-1 and PLGF were measured either at 31^{+0}-33^{+6} or at 35^{+0}-36^{+6} weeks' gestation. We excluded pregnancies with aneuploidies and major fetal abnormalities. The study population was included in two previous reports.[13,14]

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.[15]

Percentiles were estimated using quantile regression[16]. We estimated and compared the detection rate (DR) and FPR, with their 95% confidence intervals, of sFLT-1 to PLGF >38 in the prediction of PE leading to delivery at <1, <4 and ≥4 weeks after assessment at 31^{+0}-33^{+6} versus 35^{+0}-36^{+6} weeks' gestation.

The statistical software package R was used for data analyses[17] with the quanteg package[18] for quantile regression.

Results

The study population included 8,063 singleton pregnancies that were examined at 31-34 weeks' gestation and 3,703 examined at 35-37 weeks; PE at <1, <4 and ≥4 weeks occurred in 5 (0.1%), 29 (0.4%) and 202 (2.5%) of the former and 7 (0.2%), 39 (1.1%) and 21 (0.6%) of the latter. Maternal and pregnancy characteristics of the study population are summarized in Table 1.

The relationship between gestational age at assessment and the sFLT-1 to PLGF ratio is shown in Figure 1. In the non-PE group, the median sFLT-1 to PLGF ratio increased significantly with gestational age at screening ($p<0.0001$). The sFLT-1 to PLGF ratio of 38 was just below the 99th percentile at 32 weeks' gestation and just below the 90th percentile at 36 weeks.

The DR and FPR of sFLT-1 to PLGF >38 in the prediction of PE at <1, <4 and ≥4 weeks after assessment at 31-34 weeks' gestation are compared to those after assessment at 35-37 weeks in Table 2. In the two gestational windows the DR of PE at <4 weeks was similar (75.9% vs. 79.5%), but the FPR was substantially lower at 31-34 than at 35-37 weeks (1.7% vs. 9.6%). The number of cases with PE at <1 week was small, but as above, in the two gestational windows the DR was similar (80.0% vs. 85.7%), but the FPR was substantially lower at 31-34 than at 35-37 weeks (1.89% vs. 10.23%). In the prediction of PE at ≥4 weeks the DR and FPR at 31-34 weeks (17.8 and 1.3%) were lower than at 35-37 weeks (38.1 and 9.5%).

Discussion

Principal findings

The findings of this study demonstrate that in normal pregnancy there is a gestational age related increase in the sFLT-1 to PLGF ratio between 31 and 37 weeks' gestation. Consequently, in screening for PE at a fixed cut-off point in sFLT-1/PLGF there was an inevitable increase in FPR with gestational age; there was a 5-fold increase in FPR with screening at 35-37 weeks compared to screening at 31-34 weeks. In contrast, in pregnancies delivering with PE at <1 and <4 weeks from assessment, there was a similar increase in sFLT-1/PLGF from a fixed cut-off and therefore the DR with screening at 31-34 weeks was similar to that with screening at 35-37 weeks. The performance of screening for PE at ≥4 weeks was poor both at 31-34 and at 35-37 weeks.

Strengths and limitations

The strengths of this screening study for PE in the third-trimester of pregnancy are first, examination of a large population of women attending for routine care, second, measurement of serum sFLT-1 and PLGF by automated machines that provide reproducible results within 40 minutes of sampling so that complete assessment and counseling can potentially be undertaken in the same hospital visit, and third use of sFLT-1 to PLGF cut-off that was previously proposed and validated for prediction of PE at <1 and <4 weeks after assessment at 24-37 weeks' gestation.[8]

A potential limitation of the study relates to the objective of comparing the performance of sFLT-1/PLGF >38 in an unselected population, where the prevalence of PE was about 3%, to that in women presenting to specialist clinics with signs or symptoms of hypertensive disorders in which the prevalence of PE was 18%[8]. However, the DR of the test for PE at <1 and <4 weeks was similar because the DR is not affected by the prevalence of PE.

Comparison with previous studies

The finding that in normal pregnancy during the third-trimester the sFLT-1 to PLGF ratio increases with gestational age is compatible with previous reports.[2,9,10]

A previous study in 1050 singleton pregnancies presenting with signs or symptoms of hypertensive disorders at 24-37 weeks' gestation, selected the sFLT-1 to PLGF ratio with the single cut-off point of 38 to predict PE at <1 and <4 weeks because of its simplicity and because its performance was apparently similar to that of first, a model with two cut-off points, one for the earlier gestational phase of 24 to <34 weeks and one for the later gestational phase of ≥34 weeks, and second, a model with a different cut-off point for each gestational week.[8] However, the study did not provide details on how the performance of the three models was evaluated and found to be similar. Our study showed that although the DR of PE at <1 and <4 weeks in screening by sFLT-1 to PLGF of >38 was similar with screening at 31-34 and at 35-37 weeks, the FPR at 35-37 weeks was five times higher than at 31-34 weeks.

Implications for clinical practice

In third-trimester screening for PE, serum sFLT-1 and PLGF are powerful biomarkers for PE at <4 weeks and their individual performance of screening is superior to that of uterine artery pulsatility index and mean arterial pressure, which are the other two useful biomarkers; however, the performance of a model that combines maternal characteristics and medical history with all four biomarkers is superior to that of a combination of only sFLT-1 and PLGF.[13,14,19]

The sFLT-1 to PLGF ratio as a method of screening for PE both in the general population and in high-risk pregnancies is attractive because of its simplicity and the performance of screening for PE at <1 and <4 weeks is similar to that of our preferred method of utilizing Bayes theorem to combine the *prior* risk from maternal characteristics and medical history with multiple of the median (MoM) values of sFLT-1 and PLGF to derive the patient-specific *posterior* risks. However, if sFLT-1/PLGF, rather than the method utilizing Bayes theorem, was to be applied a high performance in screening necessitates the use of a variable rather than a fixed ratio. A precondition for effective use of a fixed cut-off is

that the distribution of values in both the PE and unaffected cases does not change with gestation; as demonstrated in this study this is not the case for unaffected pregnancies. Consequently, use of sFLT to PLGF ratio of >38 in identifying a high-risk group in need for intensive monitoring in the subsequent four weeks would lead to five times more pregnancies being falsely classified as high-risk when screening is carried out at 35-37 weeks than at 31-34 weeks.

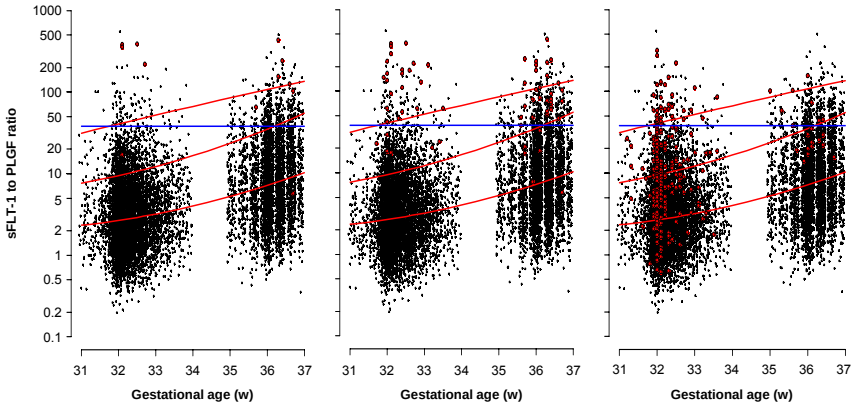

Figure 1. Scatter diagram of sFLT-1 to PLGF ratio in pregnancies that delivered with preeclampsia (red circles) at <1 weeks (left), at <4 week (centre) and at >4 weeks (right) from assessment at 31-34 and 35-37 weeks' gestation. The black circles are from pregnancies that did not develop preeclampsia. The blue horizontal line represents the cut-off point of 38 for sFLT-1/PLGF and the red curved lines represent the 50th, 90th and 99th percentiles of the unaffected pregnancies with gestational age.

Table 1. Maternal and pregnancy characteristics of study population at 31-34 and 35-37 weeks' gestation.

Maternal characteristics	31-34 w (n=8,063)	35-37 w (n=3,703)
Preeclampsia at <1 w, n (%)	5 (0.1)	7 (0.2)
Preeclampsia at <4 w, n (%)	29 (0.4)	39 (1.1)
Preeclampsia at ≥4 w, n (%)	202 (2.5)	21 (0.6)
Age (years), median (IQR)	31.0 (26.7, 34.7)	31.7 (26.9, 35.3)
Weight(kg), median (IQR)	77.0 (68.8, 88.0)	79 (70.8, 89.4)
Height(cm), median (IQR)	165 (160, 169)	164 (160, 169)
Racial origin		
Caucasian, n (%)	6020 (74.7)	2748 (74.2)
Afro-Caribbean, n (%)	1414 (17.5)	629 (17.0)
South Asian, n (%)	305 (3.8)	135 (3.6)
East Asian, n (%)	149 (1.8)	76 (2.1)
Mixed, n (%)	175 (2.2)	115 (3.1)
Method of conception		
Spontaneous, n (%)	7,792 (96.6)	3,593 (97.0)
Assisted conception, n (%)	271 (3.4)	110 (3.0)
Cigarette smoking, n (%)	803 (10.0)	350 (9.5)
Chronic hypertension, n (%)	119 (1.5)	45 (1.2)
SLE / APS, n (%)	14 (0.2)	10 (0.3)
Diabetes mellitus		
Type 1, n (%)	26 (0.3)	14 (0.4)
Type 2, n (%)	53 (0.7)	17 (0.5)
Parity		
Nulliparous, n (%)	3,973 (49.3)	1735 (46.9)
Parous no previous PE, n (%)	3,788 (47.0)	1,877 (50.7)
Parous previous PE, n (%)	302 (3.7)	91 (2.5)
Family history of PE, n (%)	243 (3.0)	133 (3.6)
Inter-pregnancy interval (years), median (IQR)*	3.2 (2.1, 5.2)	3.1 (2.1, 5.1)

* Inter-pregnancy interval reported for parous women

Table 2. Performance of sFLT-1/PLGF >38 in the prediction of delivery with preeclampsia at <1, <4 and ≥4 weeks after assessment at 31-34 or 35-37 weeks' gestation.

Prediction	Gestation at assessment	Preeclampsia with delivery after assessment at:		
		<1 week	<4 weeks	≥4 weeks
Detection rate n/N (%, 95% CI)	31-34 w	4/5 (80.0; 28.4, 99.5)	22/29 (75.9; 56.5, 89.7)	36/202 (17.8; 12.8-23.8)
	35-37 w	6/7 (85.7; 42.1, 99.6)	31/39 (79.5; 63.5, 90.7)	8/21 (38.1; 18.1-61.6)
False positive rate n/N (%, 95% CI)	31-34 w	152/8058 (1.9; 1.6, 2.2)	134/8034 (1.7; 1.40, 2.0)	98/7832 (1.3; 1.0-1.5)
	35-37 w	378/3696 (10.2; 9.3, 11.3)	353/3664 (9.6; 8.7, 10.6)	345/3643 (9.5; 8.5-10.5)

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