Screening for preeclampsia by the sFLT to PLGF ratio cut-off of 38 at 30-37 weeks' gestation

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Running head: sFLT to PLGF ratio in screening for preeclampsia

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ABSTRACT

Objective: To evaluate the soluble fms-like tyrosine kinase 1 (sFLT-1) to placental growth factor (PLGF) ratio cut-off of 38 for prediction of preeclampsia (PE) in routine assessment in singleton pregnancies at 30-37 weeks' gestation.

Methods: This was a prospective observational study in women attending for a third-trimester ultrasound scan at 30-37 weeks as part of routine pregnancy care. Serum sFlt-1 and PlGF were measured and their ratio calculated. We estimated the detection rate (DR), false positive rate (FPR), positive predictive value (PPV) and negative predictive value (NPV) of sFLT-1/PLGF >38 for prediction of delivery with PE at <1, <4 and ≥4 weeks after assessment.

Results: The study population of 12,305 singleton pregnancies was examined at a median of 32.4 (range 30.0-36.9) weeks and included 14 (0.11%), 77 (0.63%) and 227 (1.84%) that subsequently delivered with PE at < 1, < 4 or ≥ 4 weeks' after assessment, respectively. The DR, FPR, PPV and NPV of sFLT-1/PLGF >38 in the prediction of delivery with PE at <1 week were 78.6%, 4.5%, 1.9% and 99.97%, respectively. The respective values for delivery with PE at <4 weeks were 76.6%, 4.1%, 10.4% and 99.85% and for delivery with PE at ≥4 weeks were 20.7%, 4.3%, 8.3% and 98.47%.

Conclusion: In routine screening of singleton pregnancies, the performance of sFLT-1/PLGF >38 is modest for prediction of delivery with PE at <1 and <4 weeks and poor for prediction of PE at ≥4 weeks. The sFLT-1/PLGF >38 predicted 79% of cases with PE at <1 week at FPR of 4.5%; consequently, a policy of hospitalizing patients with this ratio would potentially lead to unnecessary hospitalization in 4.5% of pregnancies and a ratio of ≤38 would falsely reassure one fifth of the women that will deliver with PE at <1 week from assessment.

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 the 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.[3-9]

A prospective study in 500 women with singleton pregnancies in whom PE was suspected 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.[9] In a subsequent validation study among an additional 550 women, including 98 (17.8%) that developed PE, sFLT-1/PLGF $\leq$38 had a reassuring negative predictive value (NPV) suggesting that 99.3% of these women will not develop PE at <1 week; the detection rate (DR) of sFLT-1/PLGF >38 was 80.0% and false positive rate (FPR) was 21.7%. The positive predictive value (PPV) of sFLT-1/PLGF >38 for diagnosis of PE at <4 weeks was 36.7%, with DR of 66.2% and FPR of 16.9%.[9] However, the performance of sFLT-1/PLGF would inevitably depend on the prevalence of PE among the heterogeneous group of women presenting with a suspicion of PE; inclusion in the study was the presence of any one of the following: new onset hypertension or aggravated pre-existing hypertension, new onset proteinuria or aggravated pre-existing proteinuria, epigastric pain, headache, visual disturbance, severe swelling of the face, hands or feet, weight gain of >1 kg/week in the third trimester, low platelets, elevated liver transaminases, suspected intrauterine growth restriction, or second trimester uterine artery Doppler demonstrating mean pulsatility index (UTPI) >95th percentile or presence of bilateral notching.[9]

The objective of this screening study is to investigate the potential value of sFLT-1/PLGF >38 as part of routine clinical care at 30-37 weeks' gestation in the prediction of subsequent development of PE.

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 between March 2012 and December 2014. In the first phase of the study the visit was at 30^{+0}-34^{+6} weeks' gestation and subsequently at 35^{+0}-36^{+6} 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.[10,11] The population for this study was included in two previous reports.[12,13]

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 ≥30 weeks' gestation. We excluded pregnancies with aneuploidies and major fetal abnormalities.

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.[14]

We estimated the DR, FPR, PPV, NPV, 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.

Results

The study population of 12,305 singleton pregnancies was examined at a median of 32.4 (range 30.0-36.9) weeks and included 14 (0.11%), 77 (0.63%) and 227 (1.84%) that subsequently developed PE and were delivered at <1, <4 and ≥4 weeks after assessment, respectively. Maternal and pregnancy characteristics of the study population are summarized in Table 1.

The median sFLT-1/PLGF was significantly higher in the pregnancies developing PE and delivering at <1, <4 and ≥4 weeks compared to those not developing PE within these intervals (Figure 1). In the PE group there was a significant inverse association between sFLT-1/PLGF and interval between assessment and delivery (Figure 2); sFLT-1/PLGF >38 predicted 78.6% (11/14) cases of delivery with PE at <1 week after assessment, 76.6% (59/77) of delivery with PE at <4 weeks and 20.7% (47/227) of PE at ≥4 weeks.

The DR, FPR, PPV and NPV of sFLT-1/PLGF >38 in the prediction of delivery with PE at <1 week were 78.6%, 4.5%, 1.9% and 99.97%, respectively (Table 1). The values for delivery with PE at <4 weeks were 76.6%, 4.1%, 10.4% and 99.85% and for PE at ≥4 weeks were 20.7%, 4.3%, 8.3% and 98.47%.

Discussion

Principal findings

The findings of this study demonstrate that in singleton pregnancies that develop PE the serum sFLT-1 to PLGF ratio is higher than in non-PE pregnancies and the increase is inversely related to the interval between assessment and delivery. The sFLT-1 to PLGF ratio was >38 in 79, 77 and 21% of pregnancies that delivered with PE at <1, <4 and ≥4 weeks after assessment at FPR of about 4.5%. Consequently, the performance of sFLT-1/PLGF >38 is modest for prediction of delivery with PE at <1 and <4 weeks and poor for prediction of PE at ≥4 weeks.

A low sFLT-1/PLGF of ≤38 was potentially reassuring because 99.97% of women with this value did not deliver with PE at <1 week from assessment. However, in our

population of 12,305 pregnancies only 14 developed PE at <1 week and even if in all such cases the sFLT-1/PLGF was ≤38, the NPV of sFLT-1/PLGF >38 would still be very high at 99.91%.

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 [9].

A potential limitation of the study relates to the objective of comparing the performance of sFLT-1/PLGF >38 in an unselected population to that in women presenting to specialist clinics with signs or symptoms of hypertensive disorders. The two types of studies would inevitably differ in the prevalence of PE and consequently PPV and NPV, but not in DR. The FPR could also be affected by the prevalence of the disease because of the way the outcome measures were defined. For example, a woman with sFLT-1/PLGF >38 that develops PE at 2 or 5 weeks will be classified as false positive for PE at <1 and at <4 weeks, respectively; consequently, the FPR would be higher in a population with high- than low-prevalence of PE. The effect of increasing prevalence of a disease on increasing FPR has also been observed in data from a study of 23 meta-analyses.[15]

Comparison with previous studies

In this screening study, where the prevalence of PE was 2.6%, the DR of sFLT-1/PLGF >38 of PE with delivery at <1 week after testing was similar to that of a previous study in high-risk pregnancies in which the prevalence of PE was 17.8% (79% vs. 80%), but the DR of PE at <4 weeks in our study was higher (77% vs. 66%); however, the biggest difference was in FPR which was substantially lower in our study (FPR at <1 week 4.5% vs 21.7% and FPR at <4 weeks 4.1% vs. 16.9%).[9]

Implications for clinical practice

The sFLT to PLGF ratio has been proposed as a useful test in women presenting to specialist clinics with signs or symptoms of hypertensive disorders to help distinguish between those that will develop PE in the subsequent few weeks from those that will not.[9] The authors proposed that a ratio of ≤38 rules out the development of PE during the subsequent one week thereby avoiding hospitalization, whereas a ratio of >38 identifies a group at high-risk of developing PE at <4 weeks; it is implied that certainly for those at high-risk of developing PE at <1 week the appropriate management is hospitalization, but no management plan was proposed for those at high-risk of developing PE at 1-4 weeks.[9]

However, such arguments are flawed. First, in the heterogeneous groups of patients who apparently presented with signs suggestive of PE only 18% actually developed PE and it is unlikely that many obstetricians would consider hospitalizing a patient with a diagnosis of impending PE in the absence of hypertension and significant proteinuria purely because she had epigastric pain or headache or visual disturbance or swelling of the feet or suspected intrauterine growth restriction or high uterine artery PI in the second

trimester. Indeed, widespread acceptability of the test is likely to further dilute the prevalence of PE in the populations considered to be at 'high-risk' of developing PE towards the rate of 2.6% observed in our general population. Second, the DR of sFLT-1/PLGF >38 for PE at <1 week was only 80% [9] and therefore 20% of patients that will develop PE within this time interval will have sFLT-1/PLGF ≤38 and they will be falsely reassured that development of PE at <1 week is unlikely. This problem is even greater when considering the prediction of PE at <4 weeks where the DR of sFLT-1/PLGF >38 was only about 70%. Third, the FPR of sFLT-1/PLGF >38 for PE at <1 week was 22% [9] and therefore many patients will be hospitalized unnecessarily.

The appropriate management of patients presenting with one or more symptoms or signs that are also observed in PE is not hospitalization, but to obtain a medical history, measure blood pressure and examine for proteinuria for the diagnosis of PE and if this is absent to review in a few days or weeks as necessary. The management of patients with suspected intrauterine growth restriction in the absence of PE is to perform an ultrasound examination for fetal anatomy and size and if the fetus is small to determine whether this is constitutional or due to a fetal abnormality or uteroplacental insufficiency; in the latter case the decision on timing of delivery would be based on fetal heart rate patterns and / or Doppler findings in the umbilical artery, middle cerebral artery and ductus venosus, rather than the sFLT-1 to PLGF ratio. The management of patients with high UTPI in the second trimester should be based on the estimated risk for PE derived from a combination of maternal factors, UTPI, MAP and PLGF that would define the timing and content of subsequent visits in the second and third trimesters.[13,16]

In third-trimester screening for PE, serum sFLT-1 and PLGF are powerful biomarkers and their individual performance of screening is superior to that of UTPI and MAP 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.[12,17,18] 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. However, a ratio of ≤38 does not rule out the development of PE during the subsequent one week and a ratio of >38 has only a modest performance in identifying women that will develop PE within the subsequent four weeks.

Figure 1. Box and whiskers plot of sFLT-1/PLGF in the pregnancies developing preeclampsia (PE) and delivering at <1, <4 and ≥4 weeks after assessment compared to those not developing PE within these intervals. The bottom and top edges of each box represent the first and third quartiles, respectively, the band within the box represents the median value, the whiskers represent values that are 1.5 times the interquartile range, and the horizontal dotted line represents the cut-off point of 38.

Figure 2. Scatter diagram and regression line for the relationship of sFLT-1/PLGF and interval between assessment and delivery for preeclampsia (PE). The interrupted horizontal line represents the cut-off point of 38 for sFLT-1/PLGF and the horizontal band represents the median and 95th percentile of sFLT-1/PLGF in pregnancies that did not develop PE.

Table 1. Maternal and pregnancy characteristics in pregnancies that delivered with preeclampsia (PE) at <1, <4 and ≥4 weeks after assessment compared with pregnancies that remained normotensive

Maternal characteristics	**No PE (n=12,001)**	**PE < 1 wk (n=14)**	**PE < 4 wks (n=77)**	**PE ≥ 4 wks (n=227)**
Age, median (IQR)	31.2 (26.7-34.9)	34.0 (28.3-37.4)	32.4 (27.7-35.6)	31.6 (27.0-35.2)
Weight, median (IQR)	67.5 (59.5-78.4)	75.1 (66.8-90.7)	70.4 (62.1-85.8)	73.0 (63.6-89.0) *
Height, median (IQR)	1.65 (1.60-1.69)	1.63 (1.60-1.66)	1.63 (1.59-1.69)	1.65 (1.60-1.69)
Racial origin				
Caucasian, n (%)	8,960 (74.7)	8 (57.1)	50 (64.9)	144 (63.4)
Afro-Caribbean, n (%)	2,070 (17.2)	3 (21.4)	19 (24.7)	67 (29.5) *
South Asian, n (%)	442 (3.7)	1 (7.1)	4 (5.2)	10 (4.4)
East Asian, n (%)	230 (1.9)	1 (7.1)	1 (1.3)	4 (1.8)
Mixed, n (%)	299 (2.5)	1 (7.1)	3 (3.9)	2 (0.9)
Method of conception				
Spontaneous, n (%)	11,619 (96.8)	13 (92.9)	72 (93.5)	216 (95.2)
Assisted conception, n (%)	382 (3.2)	1 (7.1)	5 (6.5)	11 (4.8)
Cigarette smoking, n (%)	1,190 (9.9)	0	3 (3.9)	13 (5.7)
Chronic hypertension, n (%)	141 (1.2)	1 (7.1)	9 (11.7)*	29 (12.8)*
SLE / APS, n (%)	25 (0.2)	0	0	0
Diabetes mellitus, n (%)	113 (0.9)	0	0	3 (1.3)
Parity				
Nulliparous, n (%)	5,787 (48.2)	7 (50.0)	48 (62.3)	140 (61.7)
Parous no previous PE, n (%)	5,543 (48.7)	5 (35.7)	18 (23.4) *	56 (24.7) *
Parous previous PE, n (%)	371 (3.1)	2 (14.3)	11 (14.3) *	31 (13.7) *
Family history of PE, n (%)	371 (3.1)	1 (7.1)	7 (9.1) †	10 (4.4)
Inter-pregnancy interval, median (IQR)*	3.1 (2.1-5.1)	7.0 (3.1-9.2) †	4.9 (2.5-8.7)	3.8 (2.4-6.2) †

Post hoc Bonferroni correction for multiple comparisons; † = p< 0.01; * = p< 0.001
* 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.

Prediction	Preeclampsia with delivery after assessment at		
	<1 week	**<4 weeks**	**≥4 weeks**
Detection rate (n/N) (%, 95% CI)	11/14 (78.6%; 57.1-100.0)	59/77 (76.6%; 67.1-86.0)	47/227 (20.7%; 15.4-26.0)
False positive rate (n/N) (%, 95% CI)	554/12,291 (4.5%; 4.1-4.9)	506/12,228 (4.1%; 3.8-4.5)	518/12,078 (4.3%; 3.9-4.7)
Positive predictive value (n/N) (% 95% CI)	11/565 (1.9%; 0.8-3.0)	59/565 (10.4%; 7.9-12.9)	47/565 (8.3%; 6.0-10.6)
Negative predictive value (n/N) (% 95% CI)	11,737/11,740 (99.97%; 99.94-100.0)	11,722/11,740 (99.85%; 99.78-99.92)	11,560/11,740 (98.47%; 98.25-98.69)

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