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Soluble fms-like tyrosine kinase-1, placental growth factor and their ratio as a predictor for pre-eclampsia in East Asians

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ABSTRACT

Objective: To assess the clinical utility of the sFlt-1:PlGF ratio rule-in/rule-out pre-eclampsia either directly or after correcting each marker for gestation and maternal weight.

Methods: This was a prospective cohort study. sFlt-1, PlGF were measured in 965 women randomized to undergo a single blood withdraw between 20 and 39 weeks of gestation. sFlt-1, PlGF and the sFlt-1:PlGF ratio temporal relationship was determined. sFlt-1 and PlGF were converted to multiples of the expected gestational median (MoM) and adjusted for maternal weight. The 90th centile of the adjusted sFlt-1MoM:PlGFMoM ratio was determined. Clinical utility of the sFlt-1:PlGF ratio (≥ 38) to rule in/rule–out pre-eclampsia (PE) after 20 weeks of gestation versus that of the sFlt-1MoM:PlGFMoM 90th percentile was assessed in 81 women admitted for management of antenatal hypertension.

Results: The sFlt-1:PlGF ratio had quadratic relationship with gestation whereas the sFlt-1MoM:PlGFMoM ratio log distribution that was Gaussian with a mean of zero and a standard deviation of 0.85 with a 90th percentile equal to 1.08. Thirty-four (42%) of the 81 women admitted for management of their antenatal hypertension had PE, 26 (76.4%) had a sFlt-1:PlGF ratio ≥ 38. Four of the remaining 8 PE affected pregnancies with sFlt-1:PlGF ratio < 38 delivered within 7 days, 3 were preterm. Two of the 3 preterm PE pregnancies had sFlt-1MoM:PlGFMoM exceeding 90th percentile.

Conclusion: The relative level of the sFlt-1 to PlGF carries prognostic value. A sFlt-1MoM:PlGFMoM ratio exceeding the 90th centile resulted in additional detection of pregnancies which developed PE compared to the conventional sFlt-1:PlGF ratio.

1. Introduction

Levels of angiogenic and anti-angiogenic factors produced by placental trophoblasts have been shown to be reduced or increased in women who have or are developing pre-eclampsia (PE) [1–7] The ratio of soluble fms-like tyrosine kinase-1 (sFlt-1) and placental growth factor (PlGF), the sFlt-1:PlGF ratio is actively being proposed and assessed as a clinical management aide to both rule in and out PE and as a potential means to monitor for a deteriorating maternal condition [6–10] The Prediction of Short-Term Outcome in Pregnant Women with Suspected Preeclampsia Study (PROGNOSIS) study indicated that the sFlt-1:PlGF ratio had both high positive and negative predictive values [10]. Participants in the PROGNOSIS study were predominantly 95% Caucasian or Afro-Caribbean reflecting the geographic regions of the world in which the study was conducted [10].

The sFlt-1:PlGF ratio is currently not adjusted for gestation at testing or maternal weight. It has recently been advocated that gestational adjustment should be performed ('MoMing') [11,12]. One potential reason for not 'MoMing' would be that the sFlt-1:PlGF is presumably stable and independent of gestation and that the impact of inter- and intra-population differences in maternal height and weight on the measured levels of sFlt-1 and PlGF is identical and thus not directly impacting on the ratio as they cancel each other out. Blood volume and hence degree of haemodilution and haemoconcentration has been shown to be dependent on total blood volume which in turn is a function of both height and weight [13]. The average adult in Asia has been reported as 13-15Kg and 23Kg lighter than their European/Latin American and North American counterparts respectively whilst Central/Northern Europe and North America are generally taller than their Asian counterparts [14,15]. The variation in underlying populations may explain why previous studies have reported that the performance of the sFlt-1:PlGF ratio was lower in multiparas and women with high body mass index and in early versus later gestations as well as being different between different analytical platforms [16–19]. One way of

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standardizing would be to adopt MoMing.

Whether the sFlt-1:PlGF ratio, and specifically the rule-in/rule-out level, has similar characteristics and diagnostic performance in Asian populations remains to be demonstrated. The objective of the current study was to assess the potential clinical utility of the sFlt-1:PlGF ratio rule-in or rule-out pre-eclampsia either directly or after correcting each marker for gestation and maternal weight by MoMing.

2. Methods

2.1. sFlt-1, PlgF and sFlt-1:PlGF gestational levels in non-pre-eclampsia pregnancies

A 10 ml maternal blood sample was collected by venipuncture and left to clot at room temperature for approximately 30 min before undergoing centrifugation at 2000*g* from Chinese pregnant women having a singleton spontaneously conceived pregnancy. The gestational age at blood taking was determined using the crown rump length (CRL) measured at 12 weeks of gestation using a Chinese specific crown rump length dating formula [20,21]. The method of recruitment, inclusion and exclusion criteria's, number of study participants returning for their study visit are described in detail in Cheng et al. [22] The separated serum was stored at −80 °C until all the samples had been collected. The PlGF and sFlt-1 concentrations were measured in parallel in all stored samples and reported in picograms per milliliter (pg/ml) using a commercially available fully automated Elecsys assays on an electrochemiluminescence immunoassay platform (Cobas e411, Roche Diagnostics, Switzerland). The analyzer measurement range for PlGF and sFlt-1 concentrations determination is 3–10,000 pg/ml and 10–85,000 pg/ml respectively. Fresh quality-control samples were used to determine assay coefficients of variation. The between run coefficients of variation for Elecsys assays lower and upper quality control samples were respectively 4.76% and 1.95% for sFlt-1 and 3.65% and 4.45% for PlGF. Concentrations were reported 953 of the 965 serum samples.

Gestation specific references for sFlt-1, PlGF and their ratio were constructed using the 'R' statistical software package and the Generalized Additive Models for Location, Scale and Shape (GAMLSS) [23]. Prior to modelling the measurements were transformed to their natural log equivalents. The final selected model was chosen on basis of simplicity and goodness of fit and used to determine percentile values for the 50th, 10th and 90th percentiles using the expression $\mu \times (1 + z_p \cdot \upsilon\sigma)^{1/\upsilon}$ where z_p is the centile of interest and μ, υ and σ are respectively the median, skewness and coefficient of variation time covariates dependent on gestation [23].

The derived median models for sFlt-1 and PlGF were used to transform measured sFlt-1 and PlGF into Multiples of their expected Median (MoM). Multiple regression analysis was then performed to assess the effect of maternal weight on the natural log transformed MoM levels of each marker. The ratio of the sFlt-1 MoM: PlGF MoM was determined for comparative assessment to that of the standard sFlt-1:PlGF ratio.

2.2. Assessment of sFlt-1 to PlGF ratio clinical utility

Women had systolic blood pressure (SBP) ≥140 mmHg and/or diastolic blood pressure (DBP) ≥90 mmHg on at least two or more episodes four hours admitted to the antenatal ward after 20 weeks of gestation were invited to participate. sFlt-1 and PlGF were measured in blood samples taken from women who agreed to participate in the study. All women were managed by the attending obstetricians in accordance with internally published Departmental protocols and remained blinded to the level of the sFlt-1:PlGF ratio. PE was diagnosed if de novo hypertension occurred after 20 weeks of gestation with a systolic blood pressure (SBP) ≥140 mmHg and/or diastolic blood pressure (DBP) ≥90 mmHg on at least two separate occasions four hours apart together with significant proteinuria (≥300 mg in 24 h or urine dipstick of ≥2+) in accordance with the International Society for the Study of Hypertension in Pregnancy (ISSHP) criteria [24].

Admitted pregnancies were stratified as having either a sFlt-1:PlGF ratio ≥38 using the PROGNOSIS study criteria [10] Women with a sFlt-1 MoM:PlGF MoM ratio above the lower 95% CI of the ≥90th percentile in non-PE pregnancies were stratified as having PE for analysis purposes. The 90th percentile was chosen as this would be expected to have a false positive rate of 10% in non-PE pregnancies similar to that expected when screening for PE in the 1st trimester [25]. ROC curves were calculated and the difference between the areas under the curve (AUC) was assessed using Delong et al. to determine if the difference in rule-in/rule-out overall test performance was significant [26]. The ratio approach was used to allow direct comparison with existing published studies.

The study was approved by the Joint Chinese University of Hong Kong – New Territories East Cluster Clinical Research Ethics Committee (CREC – 2014.507).

3. Results

965 of the 1154 women recruited at $11\text{-}13^{+6}$ weeks attended for their blood withdraw procedure. Their mean (Standard Deviation (SD)) maternal age, height and weight at recruitment were 32 years (SD 4.1), 157 cm (SD 9.1) and 54.4 kg (SD 8.1) respectively and 526 (54.23%) were nulliparous [22]. Twenty participants (2.1%) developed gestational hypertension of which 4 had pre-eclampsia. All 4 women who developed pre-eclampsia were asymptomatic at the time of their study visit and were included in the derivation of the gestational reference. All fetuses were liveborn with a mean birthweight and gestational age at delivery of 3118 g (SD: 418 g) and 274 days (SD: 9.9 days) respectively [22].

Table 1 reports the best fit models for median, coefficient of variation and skewness for sFlt-1 and PlGF and their ratio in the non-PE participants. All three measurements exhibited a quadratic relationship with gestational age. There was a significant linear relationship between sFlt-1 and PlGF and maternal weight after expressing measured sFlt-1 and PlGF in MoMs and transforming them to their natural log equivalent values (Table 2). After correcting for maternal weight there was a negative correlation between log sFlt-1 and log PlGF MoMs which was statistically significant (r = −0.13; p = .004). Fig. 1 (a) reports the distribution of the log of the sFlt-1 MoM:PlGF MoM ratio. The overall log distribution was Gaussian with a mean of zero and a standard deviation of 0.85. The log (sFlt-1 MoM:PlGF MoM) ratio had both a mean and median of zero except for all gestations except for at 39 weeks as shown in Fig. 1(b). The smoothed 90th percentile of the log (sFlt-1

Table 1
Gestation specific estimated median level of soluble fms-like tyrosine kinase-1 and placental growth factor in non-pre-eclamptic Chinese pregnant women. GA is for gestational age, in exact weeks and the log function denotes the natural logarithm.

Log Estimated soluble fms-like tyrosine kinase-1 (sFlt-1)	
Median	$\mu(GA) = 10.890402 - 0.273709 \times GA + 0.005324 \times GA^2$
Coefficient Variation	$\sigma\ (GA) = exp^{-2.383858 - 0.015231 \times GA}$
Skewness	$\upsilon = -0.178482$
Log Estimated placental growth factor (PlGF) concentrations in	
Median	$\mu(GA) = -1.541919 + 0.559749 \times GA - 0.009884 \times GA^2$
Coefficient Variation	$\sigma\ (GA) = exp^{-3.364230 + 0.038246 \times GA}$
Skewness	$\upsilon = 1.753628$
Log estimated sFLT-1/PlGF ratio	
Median	$\mu(GA) = 12.323282 - 0.823470 \times GA + 0.015004 \times GA^2$
Coefficient Variation	$\sigma\ (GA) = exp^{-2.800136 + 0.030796 \times GA}$
Skewness	$\upsilon = 0.384198$