Pregnancy Hypertension 17 (2019) 165–171

Contents lists available at ScienceDirect

Pregnancy Hypertension

journal homepage: www.elsevier.com/locate/preghy

Inter-manufacturer comparison of automated immunoassays for the measurement of soluble FMS-like tyrosine kinase-1 and placental growth factor

Cheng Yvonne Kwun Yue[a], Poon Liona Chiu Yee[a], Shennan Andrew[b], Leung Tak Yeung[a], Sahota Daljit Singh[a,*]

[a] Dept Obstetrics and Gynaecology, The Chinese University of Hong Kong, Hong Kong SAR, China
[b] Dept. of Women and Children's Health, School of Life Course Sciences, FoLSM, Kings College London, United Kingdom

ARTICLE INFO

Keywords:
Immunoassay
Placental growth factor (PlGF)
Soluble FMS-like tyrosine kinase 1 (sFlt-1)
SFlt-1:PlGF ratio
Pre-eclampsia
Hypertensive disorders

ABSTRACT

Objective: To assess inter-manufacturer automated immunoassays for soluble FMS-like tyrosine kinase 1 (sFlt-1) to placental growth factor (PlGF).

Methods: sFlt-1 and PlGF levels were measured using the AutoDelfia PlGF1-2-3 (PerkinElmer Inc. Turku, Finland), BRAHMS Kryptor sFlt-1, PlGF plus and PlGF-2 (BRAHMS ThermoFisher, Germany) and Cobas e411 Elecsys® sFlt-1 and PLGF (Roche Diagnostics GmbH, Mannheim, Germany) in 965 asymptomatic pregnancies between 20 and 39 weeks of gestation and in in-vitro samples with predefined levels of glycosylated PlGF isomers (1, 2 and 3), sFlt-1 in human male serum. Percentage PlGF isoform recovery and cross-reactivities were determined. Paired Bland-Altman and Passing-Bablok analyses were performed to determine bias, precision and accuracy. Inter-manufacturer sFlt-1:PlGF ratio were compared.

Results: PlGF-1 isomer recovery ranged from 36 to 39% for Elecsys® to 52–60% for PlGF plus and PlGF-1-2-3 assays. PlGF-2 and PlGF-3 isoform cross-reactivity was assay dependent, ranging from 10 to 21% and 16–36% respectively. BRAHMS PlGF-2 assay had high cross-reactivity to PlGF-1 (37–41%) and PlGF-3 isomers (48–65%). Elecsys® recovery of sFlt-1 was 13% vs 6% for BRAHMS. Passing-Bablok indicated significant proportional and systematic differences between all paired PlGF assay comparisons. PlGF Bland-Altman percentage biases ranged from 12 to 37% for PlGF and 18% for sFlt-1. A linear relationship existed between log transformed sFlt-1:PlGF ratios. The clinical equivalent of the BRAHMS sFlt-1:PlGF plus to the Elecsys® sFlt-1:PlGF ratios of 38 and 110 are 55 and 188 respectively.

Conclusion: Inter-manufacture immunoassay differences are significantly different. sFlt-1:PlGF rule in/rule out criteria are manufacturer specific, not interchangeable and require separate clinical validation.

1. Introduction

The Prediction of Short-Term Outcome in Pregnant Women with Suspected Preeclampsia Study (PROGNOSIS), the PELICAN Study (Plasma Placental Growth Factor in the diagnosis of women with pre-eclampsia requiring delivery within 14 days) and more recently the randomised trial of aspirin versus placebo for preterm pre-eclampsia (ASPRE) have shown that the circulating levels of angiogenic and anti-angiogenic glycoproteins produced by placental trophoblasts could be used either to screen, predict and or monitor which women are at increased risk of developing pre-eclampsia (PE) across the three trimesters prior to overt signs and symptom being evident [1–3]. Women at risk of developing pre-eclampsia have been shown to have reduced levels of angiogenic factors such as vascular endothelial growth factor (VEGF), placental growth factor (PlGF) and higher levels of anti-angiogenic factors such as soluble FMS-like Tyrosine Kinase-1 (sFlt-1) [4–9]. Guidelines have now been published describing how PlGF or sFlt-1 levels relative to PlGF can be incorporated into clinical practise [10,11].

At present, it is assumed that PlGF and the angiogenic ratio are interchangeable with similar clinical efficacy. The United Kingdom National Institute Clinical Excellence (UKNICE) recommends that either PlGF-1-2-3 levels below 184 pg/ml or the sFlt-1:PlGF ratio above (> 85 or > 110) or below (< 33 or < 38) specific thresholds may be used rule

* Corresponding author at: Department of Obstetrics and Gynaecology, The Chinese University of Hong Kong, Prince of Wales Hospital, Hong Kong, China.
E-mail address: daljit@cuhk.edu.hk (D.S. Sahota).

https://doi.org/10.1016/j.preghy.2019.06.004
Received 4 February 2019; Received in revised form 10 May 2019; Accepted 18 June 2019
Available online 20 June 2019

in or out development of either short term or long term PE from the late second trimester [10]. More recently, the COMPARE study indicated that PlGF-1-2-3 thresholds for clinical management could be lowered from < 184 pg/ml to levels < 150 pg/ml, to maintain similar clinical utility [12].

PlGF, a homo-dimeric glycoprotein highly expressed in placental trophoblastic cells and placental villi however has four alternatively spliced forms: PlGF-1, PlGF-2, PlGF-3 and PlGF-4 composed of 131, 152, 203 and 224 amnio acids respectively. PlGF-1 and PlGF-3 isoforms are non-heparin diffusible which bind only with sFlt-1 whereas PlGF-2 and PlGF-4 isoforms have additional heparin binding domains. PlGF-2 and PlGF-4 isoforms therefore also bind to both the neuopilin-1 and PlGF receptors [13,14]. A number of automated analyser-assay combinations capable of measuring PlGF concentrations during pregnancy are now available for clinical use. The analyser-assay combinations however differ in regard to analytical methodology.

There is currently little information with regard to the extent to which these immunoassays differ in their analytical behaviour, isoform cross-reactivity and whether ratios are interchangeable or need to be standardised prior to assessment of clinical utility. The objective of the current study was to compare and characterize inter-manufacturer automated immunoassays for sFlt-1 and PlGF and its ratio.

2. Methods

2.1. Patient samples

Chinese pregnant women having a viable singleton spontaneously conceived pregnancy attending for their Hospital Authority 1st trimester Down's syndrome screening test between April 2015 and April 2016 were randomised to undergo phlebotomy between 20 and 39 weeks of gestation. A detailed description of the study cohort, inclusion and exclusion criteria, method of randomisation and patient hypertensive status at time of study blood taking and at delivery are published elsewhere [15–17]. Blood samples were collected using a butterfly and Vacuette polyethylene therephthalate Z serum separator clot activator tube with gel (Part no: 456018, Greiner Bio-One GmbH, Kremsmunster, Austria). All samples were inverted at least 5 times after the blood draw procedure to ensure proper mixing and then left to clot at room temperature for approximately 30 min before undergoing centrifugation at 2000*g* at 4 °C for 10 min. Post centrifugation, the serum was separated and stored at −80 °C pending analysis. One aliquot was analysed at the investigational site whilst a second aliquot was analysed by BRAHMS ThermoFisher (Nimes, France).

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

2.2. In-vitro samples

Human male serum (HMS, Scantibodies Laboratory Inc, USA) aliquots with pre-analytical concentrations ranging from 500 to 10,000 pg/ml were prepared using glycosylated human PlGF-1 (Product Id: 300-015, ReliaTech GmbH, Germany), PlGF-2 (Product Id: 300-019, ReliaTech GmbH, Germany) and, rhPlGF-3 (7758-PL, R&D Systems Inc, USA) isoforms, or sFlt-1 (Product Id: S01-010, ReliaTech GmbH, Germany) or human VEGF-165 (Product Id: 300-035, ReliaTech GmbH, Germany) were prepared and stored at −80 °C until analysis.

2.3. Measurement of PlGF and sFlt-1

The stored aliquots were retrieved and PlGF and sFlt-1 were determined using three commercial fully automated immunoassays performed on the Roche Cobas® e411 electrochemiluminescence platform (Roche Diagnostics GmbH, Mannhein, Germany), the AutoDELFIA (PerkinElmer Inc, Wallac, Turku, Finland) and the Kryptor Compact Plus system (BRAHMS, ThermoFisher Scientific, Germany) analysers. PlGF assays required between 40 μL and 70 μL per assay and reported concentration levels in picograms per millilitre (pg/ml). Platform specific two level quality-controls were used to determine assay coefficients of variation. The Elecsys® electrochemiluminescence assay (Roche Diagnostics, GmbH, Mannheim, Germany) measurement range for PlGF and sFlt-1 are respectively 3–10,000 pg/ml and 10–85,000 pg/ml [18,19]. The Elecsys® PlGF immunoassay detects free PlGF isoforms as well as that part of PlGF isoforms complexed with sFlt-1. It has a limit of quantitation of 10 pg/ml and manufacturer quoted cross-reactivity with PlGF-2 of < 8% [18].

The PlGF-1-2-3 assay (Wallac Oy, PerkinElmer Inc, Turku, Finland) is a time-resolved fluoroimmunometric sandwich assay designed to measure the level of the free PlGF-1 isoform [20]. It has a measurement range from 1.9 to 4000 pg/ml, a limit of quantitation of 3.3 pg/ml and with manufacturer quoted PlGF-2 and PlGF-3 isoforms cross-reactivity of 28% and 20% (non-glycosylated) respectively [20].

The BRAHMS PlGF plus and sFlt-1 are homogeneous sandwich immunoassays based on Trace technology [21]. The BRAHMS PlGF plus assay is designed to measure the free PlGF-1 isoform with a range of 3.6–7000 pg/ml with a limit of quantitation of 6.9 pg/ml [11,22]. The BRAHMS PlGF plus assay has a manufacturer quoted cross-reactivity with PlGF-2 and PlGF-3 isoforms of 13% and 4% respectively [22,23]. PlGF-2 concentrations were determined using the BRAHMS PlGF-2 immunoassay previously reported by Nucci et al. [23]. The PlGF-2 assay has a range of 15–10,000 pg/ml, a limit of detection and quantitation of 15 pg/ml and 42 pg/ml respectively [23]. The BRAHMS PlGF-2 assay was calibrated with recombinant human PlGF-2 (Ref No 6837-PL, R&D Systems Europe Ltd). The BRAHMS Kryptor sFlt-1 assay measures total sFlt-1 levels, with a measuring range from 22 to 90,000 pg/ml and a limit of quantitation of 34 pg/ml [24].

All Elecsys® PlGF, Elecsys® sFlt-1 and PlGF-1-2-3 measurements were performed at the investigational laboratory. All BRAHMS PlGF plus and PlGF-2 measurements were performed at BRAHMS ThermoFisher, Nimes, France as the BRAHMS PlGF-2 is a manufacturer in-house research assay designed to measure total, both free and bound, PlGF-2 isoform concentration levels. All samples were transported in dry ice by overnight courier.

2.4. Statistical analyses

All data management and statistical analysis were performed using the Statistical Package for Social Sciences for Windows version 22 (SPSS, IBM, Armonk, NY, USA), MedCalc Statistical Software version 18.9 (MedCalc Software bvba, Ostend, Belgium) or 'R' statistical software package (version 3.3.2) [25]. A 'p' value of < 0.05 was considered statistically significant.

In-vitro HMS based samples were assessed to 1) determine each immunoassays recovery of PlGF-1 as well as their cross-reactivity to PlGF-2 and PlGF-3 isoforms; 2) recovery of PlGF-2 as well as its cross-reactivity to PlGF-1 and PlGF-3 isoforms; 3) recovery of sFlt-1 and 4) estimate the difference between predicted and reported concentrations based on expected isoform recovery and cross-reactivity.

Patient samples were assessed as follows:- 1) Bland–Altman Analysis charts were constructed and paired *t*-test were performed to determine the significance of any difference [26]; 2) concordance correlation coefficients (ρ_c) for continuous variables were determined and 3) Passing-Bablok regression [27]. Concordance correlation coefficient was determined using Lin's correlation coefficient assesses which represents the degree to which pairs of observations fall on the 45° line through the origin assuming one measurement is a gold standard. The concordance correlation coefficient reflects the precision (ρ) determined from Pearson correlation coefficient and accuracy (C_b) where C_b is a bias correction determined from the expression $\rho_c = \rho C_b$ [28]. Concordance correlation coefficient < 0.9 indicates poor agreement [29]. Passing-Bablok makes no assumptions regarding the distribution of the

Table 1
Measured concentrations (pg/ml) in spiked in-vitro samples determined by each immunoassay.

In vitro Sample Details	PlGF Level				sFlt-1 Level	
	PerkinElmer PlGF-1-2-3	Roche Elecsys	Brahms PlGF plus	Brahms PlGF-2	Roche Elecsys	Brahms
Human Male Serum	12	6	9	0	28	24
+500 pg/ml PlGF-1	298	196	280	185	29	23
+5000 pg/ml PlGF-1	2770	1795	2599	2068	30	30
+500 pg/ml PlGF-2	104	93	80	79	28	30
+5000 pg/ml PlGF-2	781	576	522	1050	35	33
+500 pg/ml PlGF-3	180	116	127	324	31	26
+5000 pg/ml PlGF-3	1270	776	792	2404	29	21
+5000 pg/ml PlGF-1 5000 pg/ml PlGF-2	3470	2324	2966	2903	29	32
+10000 pg/ml sFlt1	3	3	1	0	1341	619
+10000 pg/ml sFlt1 5000 pg/ml VEGF	11	5	11	0	1082	580

Abbreviations
HMS: Human male serum.
PlGF: Placental growth factor.
sFlt1: Soluble FMS-like tyrosine kinase-1.
VEGF: Vascular endothelial growth factor.

samples and measurement errors and assumes that all measurement have some degree of error. Lastly, the sFlt-1:PlGF ratio were derived for each patient sample measured using the BRAHMS and Elecsys® assays and compared using Passing-Bablok. Ratios were natural log transformed prior to comparison.

3. Results

The coefficients of variation (CV) 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. Corresponding CV's for the PerkinElmer PlGF-1-2-3 immunoassay controls were 4.99% and 4.90% whilst those for the BRAHMS Kryptor PlGF plus and sFlt-1 immunoassay controls assays were 5.93% and 3.08% and 3.08 and 5.38% respectively. BRAHMS PlGF-2 assay lower and upper quality control sample CV's were 6.44% and 2.34% respectively.

Observed concentrations of PlGF and sFlt-1 in-vitro HMS spiked samples are reported in Table 1. Table 2 summaries assays recovery, cross reactivity to glycosylated proteins levels present. PlGF-1 isoform recovery was similar (≈50–60%) for the BRAHMS PlGF plus and PerkinElmer PlGF-1-2-3 assays, both were much higher than the 35–40% recovery rate for the Elecsys® PlGF assay.

Table 2
Immunoassay specific recovery of PlGF-1 isomer and cross reactivity to potential glycosylated proteins.

Immunoassay characteristic	Perkin Elmer	Roche Elecsys®	BRAHMSKryptor
PlGF-1 recovery	55–60%	36–39%	52–56%
Assay Cross reactivity to PlGF-2	16–21%	12–19%	10–16%
Assay Cross reactivity to PlGF-3	25–36%	16–23%	16–25%
BRAHMS PlGF-2 recovery			16–21%
Assay Cross reactivity to PlGF-1			37–41%
Assay Cross reactivity to PlGF-3			48–65%
HMS + 5000 pg/mL PlGF-1 + 5000 pg/mL PlGF-2			
Predicted value	3550	2400	3100
Observed difference as % of predicted	−2.2%	−3.3%	−6.4%
sFlt-1 recovery		13%	6%

Abbreviations
HMS: Human male serum.
PlGF: Placental growth factor.
sFlt1: Soluble FMS-like tyrosine kinase-1.
VEGF: Vascular endothelial growth factor.

PlGF-1 and PlGF-2 isoform recovery and cross reactivity to other PlGF isoforms was concentration dependent and increased as sample concentration decreased from 5000 pg/ml to 500 pg/ml. PerkinElmer PlGF-1-2-3 cross-reactivity to PlGF-2 isoform at a concentration of 5000 pg/ml was 43% lower (16% vs 28%) and 25% higher for PlGF-3 isoform (25 vs 20%) compared to the manufacturer stated performance [19]. BRAHMS PlGF-2 assay had cross reactivity to both PlGF-1 and PlGF-3 isomers. sFlt-1 recovery using the Elecsys® assay was 13% and double that of the BRAHMS sFlt-1 assay.

965 patient samples were available for measurement. The patient characteristics, blood withdraw gestation and pregnancy outcome were previously reported [15–17]. The Roche Elecsys® was unable to report concentration levels in 6 (0.62%) after 2 attempts and were excluded from pairwise assessment despite concentration levels being reportable using both the BRAHMS PlGF plus and the PerkinElmer PlGF-1-2-3 immunoassays. Measured PlGF-1-2-3 levels in the 6 samples ranged from 49.9 to 692 pg/ml. Biotin concentration in these 6 samples ranged from 106 ng/L to 308 ng/L and were not significantly different from those determined in samples in which Roche Elecsys® was able to measure PlGF ($F = 0.19$, $p = 0.67$).

Table 3 reports the concordance and inter-manufacturer immunoassay comparison. Figs. 1 and 2 respectively report the Passing–Bablock and Bland-Altman plots for PlGF. Manufacturer PlGF immunoassay concordance correlation coefficients were ≥0.9, indicating agreement. 95% CI limits for intercept and slope determined using Passing-Bablok indicated both a systematic and proportional error. Bland-Altman inter-assay PlGF measurement differences as a percentage of the mean of two assays indicated biases ranging from 12.5 to 37.1% whilst that for sFlt-1 was 18.7%. In addition, a statistically significant negative correlation was noted between percentage sFlt-1assay difference and the mean. The correlation ($r^2 = 0.98$) between BRAHMS PlGF-2 specific assay and BRAHMS PlGF plus was high.

After log transformation, the Elecsys® versus BRAHMS sFlt-1:PlGF ratio concordance correlation coefficient increased from 0.85 (95% CI 0.845–0.861) to 0.98 (95% CI 0.976 and 0.981). Passing-Bablok 95% CI limits for intercept and slope indicated both a systematic and proportional error as shown in Fig. 3.

Ratios determined on one platform could be transformed into equivalent values on the alternative platform using the expressions:-

$$\text{Elecsys® sFlt-1:PlGF Ratio} = \exp^{(0.87*\ln(\text{BRAHMS ratio}) + 0.143)}$$
$$\text{BRAHMS sFlt-1:PlGF plus Ratio} = \exp^{(1.1488*\ln(\text{Elecsys® ratio}) - 0.1637)}$$

Table 3
Inter-manufacturer immunoassay concordance and agreement.

	Immunoassay Concordance			Passing-Bablok		Bland–Altman	
	Coefficient (95% CI) (ρ_c)	Pearson (ρ)	Correction Factor (C_b)	Slope (95% CI)	Intercept (95% CI)	Bias (%) (95% CI)	LoA (%)
BRAHMS PlGF plus vs PerkinElmer PlGF-1-2-3	0.90 (0.89–0.91)	0.92	0.98	0.88 (0.86–0.90)	37.18 (31.27–45.16)	12.5 (10.0–15.0)	−63.3 to 88.3
Elecsys® PlGF vs PerkinElmer PlGF-1-2-3	0.89 (0.88–0.90)	0.93	0.96	1.051 (1.03–1.08)	71.92 (64.34–77.57)	37.1 (34.9–39.4)	−33.6 to 107.9
Elecsys® PlGF vs BRAHMS PlGF plus	0.91 (0.90–0.92)	0.98	0.93	1.20 (1.18–1.21)	21.42 (16.87–25.60)	26.0 (25.1–27.0)	−3.0 to 55.0
BRAHMS sFlt-1 vs Elecsys® sFlt-1	0.97 (0.97–0.97)	0.99	0.97	1.04 (1.04–1.05)	234.3 (220.8 –247.2)	18.7 (18.2–19.3)	2.3–35.2

There was a statistically significant inverse correlation ($r^2 = 0.52$, $p < 0.001$) between log PerkinElmer PlGF-1-2-3 and log Elecsys® sFlt-1:PlGF ratio as shown in Fig. 4. The PerkinElmer PlGF-1-2-3 equivalent level to Elecsys® sFlt-1:PlGF rule-out level of 38 is 56 pg/ml.

Table 4 reports the manufacture equivalent values sFlt-1:PlGF ratio decision levels to rule-out or rule–in pre-eclampsia recommended by UKNICE [11]. Though not having symptoms of suspected pre-eclampsia, 1.4% and 0.5% of women still had BRAHMS and Elecsys® sFlt-1:PlGF ratios > 110 respectively. Corresponding rates for the rule-out decision level were 93.8% and 96.7% respectively. Nearly one quarter (n = 229) of women had a PerkinElmer PlGF-1-2-3 < 150 pg/ml, the decision level reported in the COMPARE Study, and 29.9% (n = 287) had a PerkinElmer PlGF-1-2-3 < 184 pg/ml using the current UKNICE PlGF-1-2-3 decision criteria.

4. Discussion

Little or no information exists with regard to inter-manufacturer PlGF and sFlt-1 immunoassays differences, isoform cross-reactivity and whether the ratio of the two proteins are interchangeable or if they could be standardised prior to assessment of clinical utility. Our data and analysis indicated significant inter-manufacture PlGF and sFlt-1 differences, with HMS spiked samples indicating that PlGF-1 isoform sensitivity was lowest for the Elecsys® PlGF and highest for PerkinElmer PlGF-123 assays whilst the Elecsys® sFlt-1 immunoassay recovery rate was double that of the equivalent BRAHMS assay. All assays showed

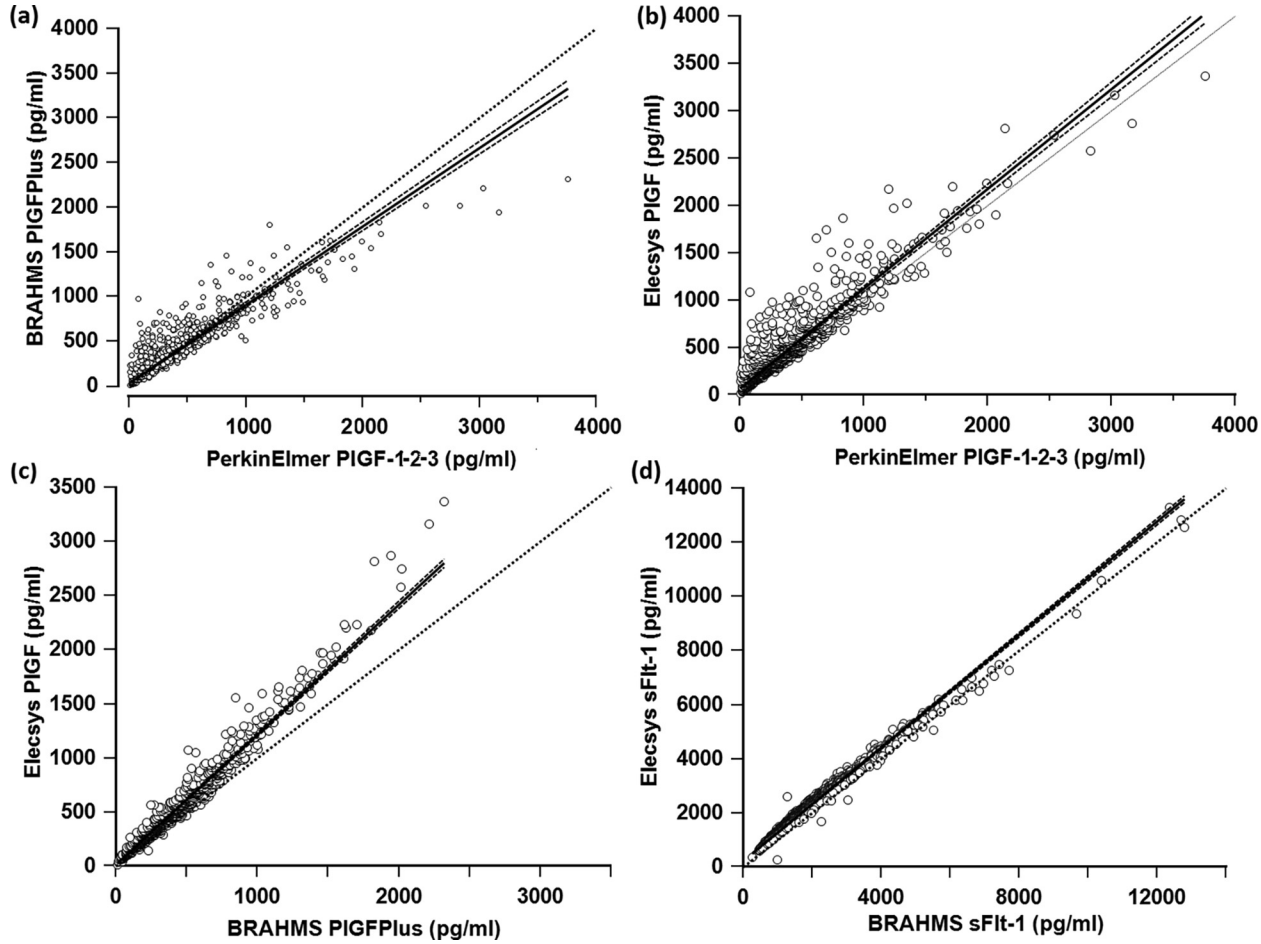

Fig. 1. Passing-Bablok charts of placental growth factor and soluble FMS-like tyrosine kinase-1 immunoassays comparisons showing the identity line (….), regression line (solid line) and 95% confidence bands for the regression line (——).

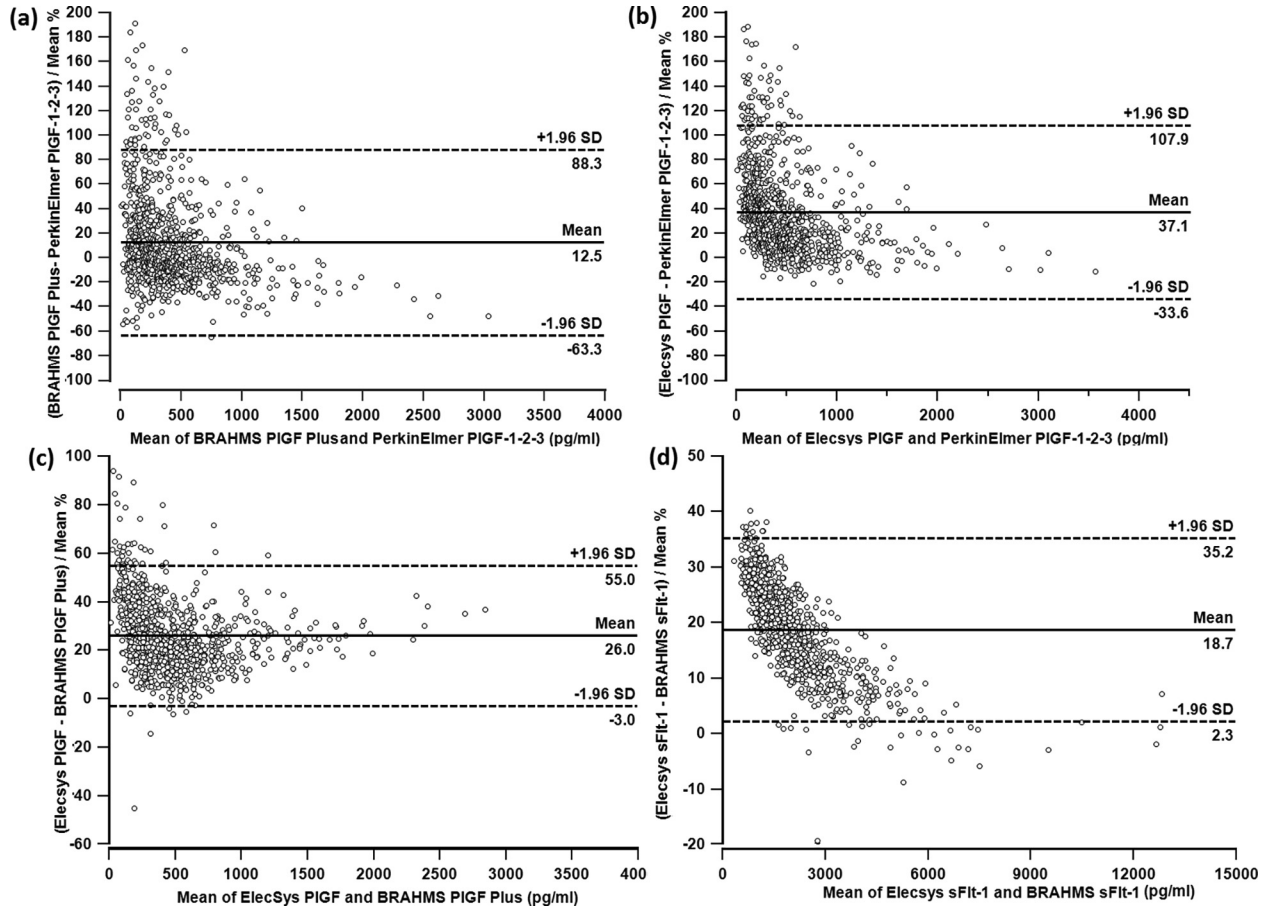

Fig. 2. Bland-Altman concentration comparison charts between placental growth factor and soluble FMS-like tyrosine kinase-1 immunoassay comparisons showing bias and 95% limits of agreement.

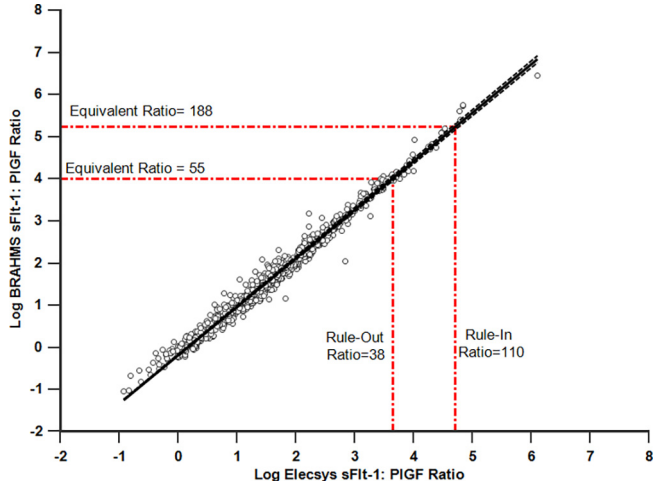

Fig. 3. Relationship and decision level equivalent levels between Elecsys® sFlt-1:PlGF ratio and BRAHMS sFlt-1:PlGF plus ratio in women who did not have pre-eclampsia within 4 weeks.

cross-reactivity to PlGF-2 with cross-reactivity increasing as spiked concentration decreased.

Whilst PlGF-1 and -2 are the two most abundant forms of PlGF, it has previously been reported that PlGF-2 levels were 3.7 times higher than that of PlGF-1 [23]. This would potentially be significant as development of pre-eclampsia is associated with reduced levels of circulating PlGF-1 [4]. Assuming assay sensitivity to PlGF-1 of 55% and PlGF-2 cross-reactivity of 15% then approximately 40–50% of reported immunoassay concentrations of PlGF could be due to presence of the PlGF-2 isoform. Further studies would thus be needed to clarify whether concentrations of free-PlGF-2 isoform are higher, lower or similar to that of the free-PlGF-1 isoform especially given the degree of cross-reactivity of the existing immunoassays to PlGF-2. All assays had cross-reactivity to the PlGF-3 isoform, ranging from 16 to 36% for PlGF-3. Cross–reactivity of the BRAHMS PlGF plus to PlGF-3 isoform was 3 times higher than that quoted in the manufacturer's assay insert [22]. We postulate that reasons for the higher cross-reactivity in our study are firstly, the use of a glycosylated isoform, the addition of glycosyl radicals to proteins, and secondly the spiked concentration level used. Ideally, manufacturers should determine cross-reactivity using glycosylated spiked samples, as PlGF is secreted as a glycosylated homodimer [30], at standard concentrations across physiologically range of expected levels. Inter-assay comparison would be simplified further if all immunoassays were assessed using a standard protocol.

Whilst our study has demonstrated inter-manufacturer differences in determined PlGF measured concentrations, it could be argued that these differences are not clinically relevant and that ultimately clinical performance and whether pregnancies with and without significant hypertensive disease are distinguishable from each other. The recently published COMPARE study indicated that AUROC in prediction of delivery within 14 days of PerkinElmer PlGF-1-2-3 assay was similar and not statistically significant from that of the Elecsys® sFlt-1:PlGF ratio (0.84 vs 0.86). However when assessed independent of sample type, sensitivity (86% vs 74%) and false positive rate (21.2% vs 8%) of the PerkinElmer PlGF-1-2-3 using a decision level of < 150 pg/ml were both higher than that of the Elecsys® sFlt-1:PlGF ratio decision level of > 38 [11]. Had pregnancies in the COMPARE study been managed according to the PerkinElmer PlGF-1-2-3 level or Elecsys® ratio then the

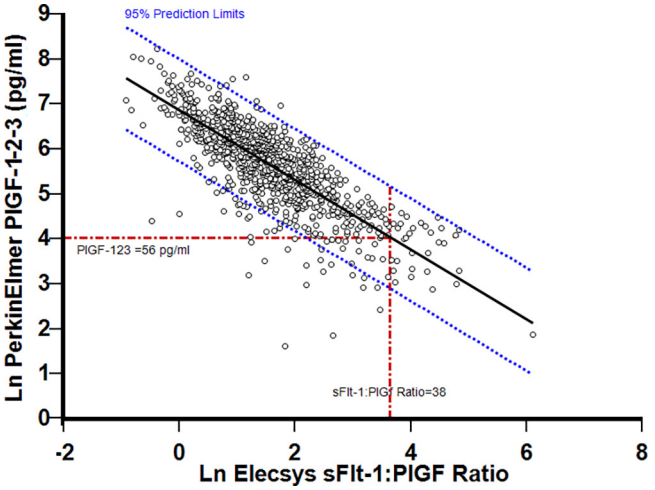

Fig. 4. Relationship between PerkinElmer PlGF-1-2-3 immunoassay and Elecsys® sFlt-1:PlGF ratio.

Table 4
Equivalent levels of sFlt-1:PlGF ratios between the Elecsys and BRAHMS immunoassays based on UK NICE recommended decision levels for the ratio.

Criteria	Elecsys® sFlt-1:PlGF	BRAHMS sFlt-1:PlGF plus Ratio
Rule Out	38	55
Rule In	110	188
Rule Out	27	38
Rule In	69	110

Abbreviations
Placental growth factor;
Flt1: Soluble FMS-like tyrosine kinase-1.

former would have identified 12% (6/50) additional pregnancies but would have required monitoring or inpatient admission of 19.1% (96/503) versus 7.1% (36/503) women, a nearly 3 fold increase [11]. McCarthy and colleagues did not compare sensitivity at a fixed false positive rate or compare false positive rates for fixed sensitivity. This approach is routinely used when comparing screening tests for down syndrome or pre-eclampsia prediction in the 1st trimester, and may become appropriate as more clinical data becomes available in management of hypertensive disorders in later pregnancy. The performance of both the PerkinElmer PlGF-1-2-3 and Elecsys® in our own patient cohort with regard to false positive rate would have been similar to that reported by McCarthy and colleagues as 23.8% of women had a PerkinElmer PlGF-1-2-3 level < 150 pg/ml whilst 3.4% had an Elecsys® ratio which exceeded 38. We were unable to determine from McCarthy and colleagues study to what extent the respective tests identified the same women or different women within the group who required delivery within 14 days and to what extent the additional six pregnancies identified using the PerkinElmer PlGF-1-2-3 were early preterm pregnancies (< 32 weeks) at initial presentation. An examination of Elecsys® sFlt-1:PlGF ratio in the PROGNOSIS Validation cohort would indicate that only 12 of 98 women who had PE within 7 days had a ratio > 38 at presentation, whilst 23 of 68 women who developed PE within 4 weeks of presentation had a ratio ≤38 [1]. Future studies using angiogenic markers should continue to interrogate optimal thresholds for management as the clinical data becomes clearer, and not assume similarities across different assays.

The strength of our study was that retrieved serum had been kept frozen for less than 2 years and had not previously been defrosted. Our previous study indicated that PlGF and sFlt-1 levels are stable for at least three years when stored at −80 °C [31]. Stability of PlGF and sFlt-1 following longer storage periods such as samples used in the COMPARE study has not been reported [12]. A limitation of our study was that we were unable to assess the Triage assay (Quidel, Inc., formerly Alere, Inc.) as it was not commercially available when the samples were analysed. Further studies may thus be needed to directly compare the levels of PlGF measured using the Alere test with that of current commercial assays now that the test has been made available again.

In conclusion inter-manufacture immunoassay are statistically significantly different in regard to assay sensitivities/recovery, cross-reactivity and thus the determined ratios. The sFlt-1:PlGF rule in /rule out criteria are manufacturer specific and should be validated to clinical outcomes but could be standardised to allow both uniform clinical management as well as a future individual patient data meta–analysis.

Declaration of Competing Interest

All authors contributed to the design of the study, data analysis and the writing of the manuscript. All authors declare that they have no conflicts of interest.

Acknowledgements

We wish to thank the members of the Fetal Medicine team, midwives and research assistants at the Prince of Wales Hospital in facilitating the performance of this study. We wish to also thank Dr Pascaline Caruhel and Celine De Vos of BRAHMS ThermoFisher, France and Dr Mikko Sairanen of Wallac Oy PerkinElmer Inc, Finland for the technical description of their respective assays and providing the immunoassays used in this study.

Sources of Funding

This study was supported by a grant from the Health and Medical Research Fund, Hong Kong (HMRF-02130156).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.preghy.2019.06.004.

References

[1] H. Zeisler, E. Llurba, F. Chantraine, M. Vatish, A.C. Staff, et al., Predictive value of the sFlt-1:PlGF ratio in women with suspected preeclampsia, N. Engl. J. Med. 374 (2016) 13–22.
[2] L.C. Chappell, S. Duckworth, P.T. Seed, M. Griffin, J. Myers, et al., Diagnostic accuracy of placental growth factor in women with suspected preeclampsia: a prospective multicenter study, Circulation 128 (2013) 2121–2131.
[3] D.L. Rolnik, D. Wright, L.C. Poon, N. O'Gorman, A. Syngelaki, et al., Aspirin versus placebo in pregnancies at high risk for preterm preeclampsia, N. Engl. J. Med. 377 (2017) 613–622.
[4] R.J. Levine, S.E. Maynard, C. Qian, K.H. Lim, L.J. England, et al., Circulating angiogenic factors and the risk of preeclampsia, N. Engl. J. Med. 350 (2004) 672–683.
[5] R. Thadhani, W.P. Mutter, M. Wolf, R.J. Levine, R.N. Taylor, et al., First trimester placental growth factor and soluble FMS-like tyrosine kinase 1 and risk for preeclampsia, J. Clin. Endocrinol. Metab. 89 (2004) 770–775.
[6] H. Masuyama, N. Suwaki, H. Nakatsukasa, A. Masumoto, Y. Tateishi, et al., Circulating angiogenic factors in preeclampsia, gestational proteinuria, and superimposed preeclampsia on chronic glomerulonephritis, Am. J. Obstet. Gynecol. 194 (2006) 551–556.
[7] S. Verlohren, I. Herraiz, O. Lapaire, D. Schlembach, M. Moertl, et al., The sFlt-1/PlGF ratio in different types of hypertensive pregnancy disorders and its prognostic potential in preeclamptic patients, Am. J. Obstet. Gynecol. 206 (58) (2012) e1–e8.
[8] T. Chaiworapongsa, R. Romero, S.J. Korzeniewski, J.M. Cortez, A. Pappas, et al., Plasma concentrations of angiogenic/anti-angiogenic factors have prognostic value in women presenting with suspected preeclampsia to the obstetrical triage area: a prospective study, J. Matern. Fetal. Neonatal. Med. 27 (2014) 132–144.
[9] S. Verlohren, H. Stepan, R. Dechend, Angiogenic growth factors in the diagnosis and prediction of pre-eclampsia, Clin. Sci. (Lond). 122 (2012) 43–52.
[10] H. Stepan, I. Herraiz, D. Schlembach, S. Verlohren, S. Brennecke, et al., Implementation of the sFlt-1/PlGF ratio for prediction and diagnosis of pre-eclampsia in singleton pregnancy: implications for clinical practice, Ultrasound Obstet. Gynecol. 45 (2015) 241–246.

[11] National Institute for health and care Excellence. PlGF-based testing to help diagnose suspected pre-eclampsia (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). Diagnostics guidance Published: 11 May 2016 nice.org.uk/guidance/dg23.
[12] F.P. McCarthy, C. Gill, P.T. Seed, K. Bramham, L.C. Chappell, et al., Performance of commercially available placental growth factor tests in women with suspected preterm pre-eclampsia; the COMPARE study, Ultrasound Obstet. Gynecol. (2018), https://doi.org/10.1002/uog.19051.
[13] S. Hauser, H.A. Weich, A heparin-binding form of placenta growth factor (PlGF-2) is expressed in human umbilical vein endothelial cells and in placenta, Growth Factors (Chur, Switzerland) 9 (1993) 259–268.
[14] W. Yang, H. Ahn, M. Hinrichs, R.J. Torry, D.S. Torry, Evidence of a novel isoform of placenta growth factor (PlGF-4) expressed in human trophoblast and endothelial cells, J. Reprod. Immunol. 60 (2003) 53–60.
[15] Y.K.Y. Cheng, J. Lu, T.Y. Leung, Y.M. Chan, D.S. Sahota, Prospective assessment of INTERGROWTH-21st and World Health Organization estimated fetal weight reference curves, Ultrasound Obstet. Gynecol. 51 (6) (2018 Jun) 792–798, https://doi.org/10.1002/uog.17514.
[16] Y.K.Y. Cheng, L.W. Law, T.Y. Leung, O.K. Chan, D.S. Sahota, Soluble FMS-like tyrosine kinase-1, placental growth factor and their ratio as a predictor for pre-eclampsia in East Asians, Pregnancy Hypertens. (2018), https://doi.org/10.1016/j.preghy.2017.12.002.
[17] Y.K. Cheng, L.W. Law, T.Y. Leung, O.K. Chan, D.S. Sahota, Soluble FMS-like tyrosine kinase-1 and placental growth factor in Chinese pregnant women during second and third trimesters, Hong Kong Med. J. 24 (Suppl 3(3)) (2018) 45–47.
[18] Roche Diagnostics GmbH. Elecsys® PlGF immunoassay. Method Sheet. 2018–02, V 9.0.
[19] Roche Diagnostics GmbH. Elecsys® sFlt-1 immunoassay. Method Sheet. 2017–03, V 7.0.
[20] Wallac Oy ,PerkinElmer Inc. DELFIA®/AutoDELFIA® PlGF 1-2-3 Time-resolved fluoroimmunoassay B055-301. Instructions for use. 13908271-1 (en).
[21] G. Mathis, Rare earth cryptates and homogeneous fluoroimmunoassays with human sera, Clin. Chem. 39 (1993) 1953–1959.
[22] BRAHMS GmbH, ThermoScientific. BRAHMS PlGF plus Kryptor. Instruction for use. 2018, V04en.
[23] M. Nucci, L.C. Poon, G. Demirdjian, B. Darbouret, K.H. Nicolaides, Maternal serum placental growth factor (PlGF) isoforms 1 and 2 at 11–13 weeks' gestation in normal and pathological pregnancies, Fetal Diagn. Ther. 36 (2014) 106–116.
[24] BRAHMS GmbH, ThermoScientific. BRAHMS sFlt-1 Kryptor. Instruction for use. 2018, V04en.
[25] R Development Core Team, R: a language and environment for statistical computing. R Foundation for Statistical Computing V, 2008. http://www.R-project.org (accessed 22 Mar, 2017).
[26] J.M. Bland, D.G. Altman, Statistical methods for assessing agreement between two methods of clinical measurement, Lancet. 327 (8476) (1986) 307–310.
[27] H. Passing, W. Bablok, R. Bender, B. Schneider, A general regression procedure for method transformation, J. Clin. Chem. Clin. Biochem. 26 (11) (1988) 783–790.
[28] L.K. Lin, A concordance correlation coefficient to evaluate reproducibility, Biometrics 45 (1989) 255–268.
[29] G.B. McBride, A proposal for strength-of-agreement criteria for Lin's Concordance Correlation Coefficient, NIWA Client Report: 2005; HAM2005-062.
[30] S. De Falco, The discovery of placenta growth factor and its biological activity, Exp. Mol. Med 44 (2012) 1–9.
[31] L.W. Law, D.S. Sahota, L.W. Chan, M. Chen, T.K. Lau, et al., Effect of long-term storage on placental growth factor and FMS-like tyrosine kinase 1 measurements in samples from pregnant women, J. Matern. Fetal Neonatal. Med. 23 (2010) 1475–1480.