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Technical note

Midpregnancy testing for soluble fms-like tyrosine kinase 1 (sFlt-1) and placental growth factor (PlGF): An inter-assay comparison of three automated immunoassay platforms

Carin Black[a,b,*], Ahmed Al-Amin[e,f], Daniel Lorber Rolnik[d,e], Stefan C. Kane[a,b,f], Caroline Stolarek[a], Adrienne White[a], Fabricio da Silva Costa[c,d], Shaun Brennecke[a,b]

[a] Pregnancy Research Centre, Department of Maternal-Fetal Medicine, Royal Women's Hospital, 20 Flemington Rd, Parkville, Victoria, 3052, Australia
[b] The University of Melbourne, Department of Obstetrics and Gynaecology, The Royal Women's Hospital, 20 Flemington Rd, Parkville, Victoria, 3052, Australia
[c] Department of Gynecology and Obstetrics, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, São Paulo, Brazil
[d] Perinatal Services, Monash Medical Centre, 246 Clayton Rd, Clayton, Victoria, 3168, Australia
[e] Department of Obstetrics and Gynaecology, Monash University, Wellington Rd, Clayton, Victoria, 3800, Australia
[f] Pauline Gandel Imaging Centre, Royal Women's Hospital, 20 Flemington Rd, Parkville, Victoria, 3052, Australia

A B S T R A C T

We performed an inter-assay comparison among three immunoassay platforms for midpregnancy testing of sFlt-1, PlGF and the sFlt-1/PlGF ratio, which are established markers for pre-eclampsia. Maternal blood was collected 19–22 weeks' gestation. Raw data values were converted to multiples of the median (MoM). PlGF and sFlt-1 values among platforms were highly correlated ($p < 0.001$). There was significant variation in raw data values for PlGF and sFlt-1 among platforms, eliminated following conversion to MoM. When directly comparing raw data values among platforms, platform-specific reference ranges values should be used. MoM values were equivalent among platforms, allowing direct inter-assay result comparison.

- Serum PlGF and sFlt-1 are effective biomarkers for the prediction of pre-eclampsia. There are several automated immunoassays available for testing these biomarkers.
- The performance of three platforms when tested at midpregnancy were compared. Raw data values between platforms were essentially equivalent using platform-specific reference ranges. MoM values between platforms were equivalent.
- These findings support the reliability of these platforms for testing sFlt-1 and PlGF at midpregnancy.

1. Introduction

Pre-eclampsia is a multisystem disorder complicating 2–8% of pregnancies [1]. In pregnancies that develop preeclampsia, maternal serum placental growth factor (PlGF) levels decrease significantly, while soluble fms-like tyrosine kinase 1 (sFlt-1) levels increase significantly prior to symptom onset [2]. Several automated immunoassays are currently available for clinical use in testing PlGF, sFlt-1 and the sFlt-1/PlGF ratio. Here we undertook an inter-assay comparison of the analytical performance of three different immunoassay platforms for midpregnancy testing of sFlt-1, PlGF and the sFlt-1/PlGF ratio in an asymptomatic patient population.

2. Methods

2.1. Blood sample collection and storage

512 participants were included in the analysis. Maternal blood was collected from singleton pregnancies 19–22 weeks gestation at The Royal Women's Hospital, Melbourne, Australia, between June 2012 and January 2015. 10 mL was drawn and divided between non-heparinised, silicone coated tubes for serum samples and ethylenediaminetetraacetic acid (EDTA) tubes for plasma samples. Samples were centrifuged and transferred into plain polypropylene tubes, then stored as serum or plasma at −80 °C until the time of analysis.

2.2. Immunoassay platforms

Single measurements for each patient were performed on each of the following three immunoassay platforms according to manufacturer's instructions:

1) **DELFIA® Xpress** (Delfia): PlGF 1-2-3™ only

* Corresponding author. Pregnancy Research Centre, Department of Maternal-Fetal Medicine, Royal Women's Hospital, Parkville, Victoria, 3052, Australia.
E-mail address: carinblack@gmail.com (C. Black).

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Table 1
Description of immunoassay platforms and reagents used in the study.

Company	Platform	Method	Measuring Range of Assay (pg/mL)	Limit of Quantification (pg/mL)	Sample volume (uL)	Assay Duration (mins)		Mean (pg/mL)	CV's by Manufacturer (%)		CV's in our laboratory (%)	
									Intra CV	Inter CV	Intra CV	Inter CV
Perkin Elmer	DELFIA® Xpress	DELFIA Xpress PlGF 1-2-3™ assay kits	1.9–4000	3.3	40	30						
							Low Level	25.7	4.7	2.2	4.68	4.54
							High Level	89.7	2.7	3.5	4.39	4.91
Roche	cobas® e 411	Roche Elecsys® PlGF assay kits*	3 - 10,000	10	50	18	Low Level	104	0.9	2.7	1.93	4.54
							High Level	1010	0.9	2.7	3.09	4.91
		Roche Elecsys® sFlt-1 assay kits*	10 - 85,000	15	20	18	Low Level	107	1.5	3.8	2.64	2.45
							High Level	1080	1.4	3.9	2.79	2.37
Thermo Scientific	B.R.A.H.M.S KRYPTOR compact PLUS	B·R·A·H·M·S PlGF PLUS assay kits^	3.6–7,000	6.9	70	29	Low Level	15–40	≤ 8	≤ 10	4.63	4.89
							Medium Level	40–150	≤ 5	7	3.96	2.82
							High Level	> 150	≤ 3	≤ 5	2.02	3.65
		B·R·A·H·M·S sFlt-1 KRYPTOR assay kit^	22 - 90,000	34	8	9	Low Level	500-2,000	≤ 1	≤ 5	1.63	2.03
							Medium Level	2,000–20,000	≤ 1	≤ 4	0.23	1.08
							High Level	2,000–20,000	≤ 1	≤ 4	1.39	3.42

2) **cobas® e 411** (Cobas): PlGF and sFlt-1, and

3) **B.R.A.H.M.S KRYPTOR compact PLUS (Kryptor):** PlGF and sFlt-1 (Table 1).

2.3. Statistical analysis

Results for PlGF, sFlt-1 and the sFlt-1/PlGF ratio from each immunoassay platform were expressed as multiples of the expected median (MoM).

Inter-assay comparison was performed using the Intraclass Correlation Coefficient (ICC), to measure reproducibility and reliability of results obtained among platforms. Bland-Altman plots were used to assess agreement of results among platforms (not shown). Pearson correlation was used to assess equivalence among plasma and serum results for each platform, as some patients had plasma samples only available. Data analysis was performed using Microsoft ® Excel ™ 2016 (Redmond, Washington, USA) and IBM Statistical Package for the Social Sciences (SPSS) Version 24 (Armonk, New York, USA).

3. Results

3.1. Correlation of biomarker results between immunoassay platforms

PlGF levels for the three platforms gave an alpha coefficient of 0.917 ($p < 0.001$) for raw data values and 0.896 ($p < 0.001$) for MoM values (Table 2). sFlt-1 levels for the Cobas and Kryptor platforms gave an alpha coefficient of 0.938 ($p < 0.001$) for raw data values and 0.934 ($p < 0.001$) for MoM values (Table 2). sFlt-1/PlGF ratios were also highly correlated between the Cobas and Kryptor platforms, with an alpha coefficient 0.915 ($p < 0.001$) for raw data values and 0.949 for MoM values (Table 2).

There was significant variation in raw data values for PlGF and sFlt-1 among platforms. Mean differences and limits of agreement for raw data and MoM values for both sFlt-1 and PlGF, as represented in Bland-Altman plots, are displayed in Table 2. The difference in MoM values for PlGF, sFlt-1 and the sFlt-1/PlGF ratio between platforms was minimal (Table 2).

3.2. Inter- and intra-assay variability results

Commercial quality control material was provided for each immunoassay platform and intra- and inter-assay coefficients of variation (CVs) were monitored. CVs obtained within our laboratory fell within an acceptable range to demonstrate minimal variation between samples tested [3].

3.3. Correlation between serum and plasma results for PlGF and sFlt-1 values

Serum samples were used in preference to plasma for testing, if available. Comparison of serum and plasma results showed a high correlation between serum and plasma samples for each platform. Pearson's correlation coefficient for PlGF plasma and serum samples for Delfia, Cobas and Kryptor platforms were 0.975 ($p < 0.0001$), 0.995 ($p < 0.0001$) and 0.977 ($p < 0.0001$), respectively. Pearson's correlation coefficient for sFlt-1 plasma and serum samples for Cobas and Kryptor platforms were 0.963 ($p < 0.0001$) and 0.968 ($p < 0.0001$), respectively, and for the sFlt-1/PlGF ratio for plasma and serum samples were 0.997 ($p < 0.0001$) and 0.996 ($p < 0.0001$), respectively.

Values obtained for serum and plasma were directly compared using Bland-Altman plots (not shown).

4. Discussion

Fully automated assays have become commercially available within the last decade [4–8]. These have replaced manual enzyme-linked

Table 2
Correlation and mean difference between raw data and MoM values for three different platforms.

Platforms Compared	Pearson's correlation coefficient (P-value)	Mean Difference (pg/mL)	Limit of agreement (± 2 SD)
RAW DATA VALUES			
cobas® e 411 PlGF and DELFIA® Xpress PlGF	0.917 (< 0.001)	84.25	−41.56 to 210.05
cobas® e 411 PlGF and B.R.A.H.M.S KRYPTOR compact PLUS PlGF	0.917 (< 0.001)	52.31	−69.73 to 174.36
B.R.A.H.M.S KRYPTOR compact PLUS PlGF and DELFIA® Xpress PlGF	0.917 (< 0.001)	31.94	−66.64 to 130.51
cobas® e 411 sFlt-1 and B.R.A.H.M.S KRYPTOR compact PLUS sFlt-1	0.938 (< 0.001)	137.33	−492.45 to 767.12
cobas® e 411 sFlt-1/PlGF and B.R.A.H.M.S KRYPTOR compact PLUS sFlt-1/PlGF	0.915 (< 0.001)	0.99	−3.24 to 5.22
MoM VALUES			
cobas® e 411 PlGF and DELFIA® Xpress PlGF	0.896 (< 0.001)	−0.00414	−0.198 to 0.1899
cobas® e 411 PlGF and B.R.A.H.M.S KRYPTOR compact PLUS PlGF	0.896 (< 0.001)	0.00133	−0.167 to 0.169
B.R.A.H.M.S KRYPTOR compact PLUS PlGF and DELFIA® Xpress PlGF	0.896 (< 0.001)	−0.0055	−0.213 to 0.202
cobas® e 411 sFlt-1 and B.R.A.H.M.S KRYPTOR compact PLUS sFlt-1	0.934 (< 0.001)	0.0039	−0.0157 to 0.165
cobas® e 411 sFlt-1/PlGF and B.R.A.H.M.S KRYPTOR compact PLUS sFlt-1/PlGF	0.949 (< 0.001)	0.009	−0.166 to 0.184

immunosorbent assay (ELISA) kits used in earlier clinical studies [2,9–11]. Automated assays allow standardised, inexpensive measurements with high turnover, minimal handling of samples and fast results.

Within this patient cohort, when investigating raw data values for PlGF, sFlt-1 and the sFlt-1/PlGF ratio, although there was an appreciable difference in results obtained among the three platforms, results were well correlated based on the Intraclass Correlation Coefficient. The average difference, or bias, assessed using Bland-Altman plots, among raw data values for PlGF, sFlt-1 and the sFlt-1/PlGF ratio was considerable, and the limits of agreement for these plots were relatively wide (Table 2) and likely to be clinically significant when comparing values directly among platforms. Hence raw data values obtained on the three different platforms should not be used interchangeably, and platform-specific specific reference ranges for each platform should be used. These findings concur with a recent inter-assay comparison performed by another group [12].

Conversion of raw data values to MoM essentially eliminated the variation in results among platforms (Table 2). Using MoM values, results obtained from each platform were essentially equivalent, and can be used interchangeably, negating the need for reference ranges specific to each platform. This could simplify patient management should specific cut-off values for PlGF or the sFlt-1/PlGF ratio become established.

The average difference in mean concentrations of PlGF, sFlt-1 and the sFlt-1/PlGF ratio between serum and plasma (data not shown) was 5.9% (2.7–9.2%), demonstrating an acceptable degree of agreement between plasma and serum sample values and indicating that serum and plasma may be considered equivalent for sFlt-1 and PlGF testing when using these three immunoassay platforms.

Four different PlGF isoforms (PlGF 1–4) [13−16] have been identified, each derived from different splicing of the primary gene transcripts [17] and each detected to varying extents by different immunoassay platform. Uncertainty surrounds which of these isoforms, if any, is most clinically relevant[17]. Cross reactivity, which is the non-specific influence of substances in a sample that structurally resemble the analyte and compete for a binding site on the antibody, could potentially result in over- or under-estimation of analyte concentration. This is a common cause of interference in immunoassays [18], particularly when comparing results between immunoassays, as whilst circulating PlGF in humans is predominantly PlGF 1, and the immunoassays are mainly specific to PlGF 1, Delfia and Cobas platforms both show some cross reactivity between PlGF isoforms 1 and 2, while the Kryptor platform shows some cross reactivity between isoforms 1, 2 and 3.

The results of this interassay comparison are primarily applicable to midpregnancy samples. First trimester assays require optimal precision at low values, as the difference between results for affected and non-affected samples is smaller during the first trimester. Hence the results from this study are not applicable to first trimester samples.

In summary, our findings have confirmed that both raw data and MoM values for PlGF, sFlt-1 and the sFlt-1/PlGF ratio from three platforms are well correlated. Raw data and MoM values for PlGF, sFlt-1 and the sFlt-1/PlGF ratio showed good agreement among platforms, however the average difference between raw data values were considerable, and likely to be clinically significant when comparing values directly between platforms. Hence when reporting raw data values, reference ranges for sFlt-1 and PlGF specific to each platform should be used in clinical practice. MoM values were directly comparable between platforms, and conversion of raw data values to MoM allows direct comparison of results between different commercial platforms.

Conflicts of interest

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The cobas* and Kryptor^ platforms, the sFlt-1/PlGF ratio measures the amounts of PlGF relative to sFlt-1 by combining the results from the PlGF assay and the sFlt-1 assays. Both the individual assay values and the sFlt-1/PlGF ratio are reported. Intra CV = Intra-assay CV. Inter CV = inter-assay CV.

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