Letter to the Editor

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Biological variation of two serum markers for preeclampsia prediction

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To the Editor,

Preeclampsia currently affects 2%–8% of pregnancies and is considered the leading cause of maternal and perinatal morbidity and mortality. Several algorithms including personal history, demographic factors, ultrasound and biochemical markers have been developed to screen pregnant women and stratify for the risk of this complication. Recent evidence supports the utility of the ratio of soluble fms-like tyrosine kinase 1 (sFlt-1) to placental growth factor (PlGF) in serum for ruling out short-term preeclampsia with a negative predictive value >99% in women with singleton pregnancies in whom the syndrome is suspected clinically [1]. Considering that the clinical value of the ratio is influenced by the biology of sFlt-1 and PlGF, the availability of biological variation (BV) information for these markers is central for optimizing their clinical use. However, these data are still lacking in the literature. Here, we report the assessment of analytical and biological components of variation of sFlt-1, PlGF and sFlt-1/PlGF ratio measurements in serum.

We recruited 15 apparently healthy fertile Caucasian women (ages 21–45 years) from the staff members of our University Hospital. In accordance with the Helsinki II Declaration, the design and execution of the experiment were explained thoroughly to the subjects and informed consent was obtained. All participants were interviewed at time of the study to confirm that they were not pregnant, were free of any evident disease, had no history of chronic disease and were not receiving any medication. Other criteria for inclusion were that they had regular menstrual cycles and did not use hormonal contraceptives. None of the subjects consumed substantial (>10 g of ethanol/day) quantities of alcohol or were smokers. We collected a total of four venous blood samples from each of study participants, on the same day, every week for 4 consecutive weeks. All blood samples were obtained between 07.30 and 10.00 a.m. from seated subjects, who had fasted overnight and had not exercised that morning. The blood draw was performed by the same skilled phlebotomist with minimal stasis directly into 4-mL vacuum collection tubes with no anticoagulant and polymer gel for serum separation (Becton Dickinson Vacutainer ref. no. 369032). Serum specimens for sFlt-1 and PlGF determinations were obtained by centrifugation and immediately aliquoted and stored at −80 °C until analysis. When all specimens were available, they were thawed, mixed, centrifuged and analyzed in a single run in duplicate in random order. sFlt-1 and PlGF were measured on the Cobas e801 platform (Roche Diagnostics) using the electrochemiluminescence immunoassay principle. The limit of detection (LOD) of sFlt-1 and PlGF assays is 10 ng/L and 3 ng/L, respectively. Biological and analytical components of variation, with corresponding 95% confidence intervals, were calculated using CV-ANOVA [2]. Outlier identification was performed as described before [3]. The steady-state situation was verified by a visual check. The Shapiro-Wilk test was applied separately to the set of normalized residuals for each individual to check data distribution and validate the normality hypothesis. The index of heterogeneity (IH) was evaluated to study the heterogeneity of within-subject variations [3].

The study involved a collection of 60 serum samples, each assayed in duplicate yielding 120 analytical results. All sFlt-1 and PlGF results were above the respective LOD. One within-subject variance for PlGF and the sFlt-1/PlGF ratio was detected as a statistical outlier and data from the corresponding individual were eliminated. In addition, one mean sFlt-1 value (101.1 ng/L) was also eliminated as it was an outlier. The Shapiro-Wilk test accepted the hypothesis of normality for the data distribution in all subjects

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Table 1: Mean values, estimated average analytical (CV_A), intra-individual (CV_I) and inter-individual (CV_G) variability components, indices and analytical performance specifications derived from biological variation data for serum sFlt-1, PlGF and sFlt-1/PlGF ratio.

Parameter	No. of subjects	Mean	CV_A[a]	CV_I, 95% CI	CV_G, 95% CI	II[b]	RCV[c]	n[d]	Analytical performance specifications					
									Standard measurement uncertainty[e]			Allowable bias[f]		
									M	D	O	M	D	O
sFlt-1	14	79.1 ng/L	1.6%	4.0% (1.9–5.2)	7.9% (5.5–13)	0.30	13%	1	≤3.0%	≤2.0%	≤1.0%	≤±3.3%	≤±2.2%	≤±1.1%
PlGF	14	13.0 ng/L	2.6%	7.9% (5.2–10.2)	12.9% (8.9–21.4)	0.38	21%	2	≤5.9%	≤3.9%	≤2.0%	≤±5.7%	≤±3.8%	≤±1.9%
sFlt-1/PlGF ratio	14	6.3	2.9%	8.9% (6.1–11.5)	16.1% (11.2–26.6)	0.36	29%	4	≤6.7%	≤4.4%	≤2.2%	≤±6.9%	≤±4.6%	≤±2.3%

CI, confidence intervals; II, index of individuality; RCV, reference change value; n, number of blood specimens that should be collected to estimate the homeostatic set point of an individual within ±10%; M, minimum, D, desirable, O, optimum quality level, respectively. [a]Obtained from duplicate measurements of subject samples. [b]Calculated as (S^2_{I+A}/S^2_G), where S^2_{I+A} is the average within-subject total variance and S^2_G is the between-subject biological variance. [c]Calculated by the non-parametric method according to Røraas et al. [2]. [d]Calculated as $[1.96^2 (CV_A^2 + CV_I^2)/100]$. [e]Calculated as 0.75 CV_I (minimum), 0.50 CV_I (desirable), and 0.25 CV_I (optimum). [f]Calculated as 0.375 $(CV_I^2 + CV_G^2)^{0.5}$ (minimum), 0.250 $(CV_I^2 + CV_G^2)^{0.5}$ (desirable), and 0.125 $(CV_I^2 + CV_G^2)^{0.5}$ (optimum).

for all parameters. Table 1 shows the results obtained. The sFlt-1/PlGF ratio had a low index of individuality showing that conventional population-based reference intervals have little use in its interpretation. For its clinical use, it is therefore better to rely on an optimal fixed cut-off as recommended by Zeisler et al. [1]. An alternative approach, however still not validated in a clinical setting, may be the longitudinal interpretation of the individual ratio variation by the reference change value (RCV). However, given that the calculated IH (0.9) did not fulfil the homogeneity condition (IH < 0.707), the RCV documented in this study does not appear to be ubiquitously valid. Finally, using the BV model [4], we derived the analytical performance specifications (APS) for measurements of the evaluated preeclampsia markers. APS for measurement uncertainty on clinical samples and tolerable bias to permit the interchangeable use of biomarker results regardless of laboratory performing measurements and of employed measuring system are reported in Table 1 [5].

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References

1. Zeisler H, Llurba E, Chantraine F, Vatish M, Staff AC, Sennström M, et al. Predictive value of the sFlt-1:PlGF ratio in women with suspected preeclampsia. N Engl J Med 2016;374:13–22.
2. Røraas T, Støve B, Petersen PH, Sandberg S. Biological variation: the effect of different distributions on estimated within-person variation and reference change values. Clin Chem 2016;62:725–36.
3. Braga F, Panteghini M. Generation of data on within-subject biological variation in laboratory medicine: an update. Crit Rev Clin Lab Sci 2016;53:313–25.
4. Sandberg S, Fraser FG, Horvath AR, Jansen R, Jones G, Oosterhuis W, et al. Defining analytical performance specifications: Consensus Statement from the 1st Strategic Conference of the European Federation of Clinical Chemistry and Laboratory Medicine. Clin Chem Lab Med 2015;53:833–5.
5. Infusino I, Panteghini M. Measurement uncertainty: friend or foe? Clin Biochem 2018;57:3–6.