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Basic performance assessment of reagents for measuring soluble FMS-like tyrosine kinase 1 (sFlt-1) and placental growth factor (PlGF) in Japanese women

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ABSTRACT

Among the factors associated with angiogenesis, soluble FMS-like tyrosine kinase-1 (sFlt-1) is antiangiogenic and placental growth factor (PlGF) is proangiogenic. The sFlt-1/PlGF ratio is considered useful for the short-term prediction of preeclampsia (PE) in high-risk pregnancies and has been used clinically in Japan since July 2021. Regarding the clinical use of the sFlt-1/PlGF ratio in Japan, there have been no published reports demonstrating that sFlt-1 and PlGF assay reagents have the same basic performance in Japanese and European women. To our knowledge, we conducted the first basic performance assessment of the sFlt-1 and PlGF assay reagents using sera from Japanese women. We obtained satisfactory results for repeatability, intermediate precision, linearity, effects of interferents, and the LoQ. The sFlt-1 and PlGF assay reagents performed well, and we believe that they are entirely adequate for use in routine clinical assays of Japanese patients, similar to those in European patients.

Key words: *sFlt-1*, *PlGF*, *hypertensive disorders of pregnancy*, *preeclampsia*

INTRODUCTION

Hypertensive disorders of pregnancy are conditions that present as hypertension, which arises during pregnancy, and preeclampsia (PE) is especially important because of the danger of serious complications to both the mother and fetus[5)]. Vascular endothelial dysfunction is involved in PE pathogenesis. Placental ischemia due to defective uterine spiral artery remodeling is thought to be the first step that leads to systemic vascular endothelial dysfunction by disrupting the balance of angiogenic factors released into the maternal vasculature[4)]. Soluble FMS-like tyrosine kinase-1 (sFlt-1) is antiangiogenic and a splice mutation of vascular endothelial growth factor receptor-1 (VEGFR-1), which is a placental growth factor (PlGF) receptor. PlGF is a proangiogenic factor expressed by trophoblast cells and placental villi. sFlt-1 and PlGF levels were measured to aid in the short-term prediction of PE onset in high-risk pregnancies, and this method became clinically available in Japan in July 2021. A prediction was made using the ratio of sFlt-1 to PlGF because it has been shown that pregnant women who develop PE have a high serum sFlt-1 to PlGF ratio prior to PE onset[7)]. In Europe, the sFlt-1/PlGF cutoff for short-term prediction of PE onset is 38, i.e., a ratio of ≤ 38 predicts no onset of PE within 1 week and a ratio of > 38 predicts the onset of preeclampsia within 4 weeks[1)].

To our knowledge, no results have been published thus far demonstrating that sFlt-1 and PlGF assay reagents have the same basic performance in Japanese and European women. Therefore, herein, we report an initial basic performance assessment of sFlt-1 and PlGF assay reagents using sera from Japanese women.

MATERIALS AND METHODS

Subjects

We used two concentrations of control sera Elecsys PreciControl® MM (hereinafter referred to as PC MM1 and PC MM2) to examine within-run performance for repeatability and between-day performance for intermediate precision. To examine the linearity of dilution, effects of interferents, and limit of quantitation (LoQ), we used serum samples collected in the collaborative study on the dynamics of sFlt-1/PlGF ratios in the physiology and pathology of pregnancies, conducted by the Department of Obstetrics and Gynecology, Hiroshima University Hospital, and the University of Oxford, after the serum samples were measured for the collaborative

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study. Serum samples were obtained between November 2019 and May 2021 from Japanese women who: (1) consented to the above collaborative study as well as the present study, and (2) aged between 19 to 44 years (median: 33 years) and between 18 weeks of gestation and approximately 1 month postpartum. Individual serum samples were used to study dilution linearity, whereas pooled sera were prepared and used to study the effects of interferents and the LoQ. This study was approved by the Ethics Committee for Epidemiological Research of Hiroshima University (no. E-1782).

Measurement instruments and reagents

The measurement instrument was a Roche Cobas® 8000 e 801 module, and the measurement reagents were Elecsys sFlt-1 and PlGF, which were the same as those used in Europe. Calibrations were performed using Elecsys sFlt-1 and Elecsys PlGF calibrators, with PC MM1 and PC MM2 used as control sera (all reagents were obtained from Roche Diagnostics Inc.).

Measurement principle

Elecsys sFlt-1 and PlGF reagents use electrochemiluminescence immunoassay (ECLIA) as the measurement principle, and the sandwich technique was used for immunoreactions[4]. For the first reaction, biotinylated and ruthenium-labeled antibodies were incubated with the samples at 37°C for 9 min. For the second reaction, streptavidin-coated magnetic microparticles (SA magnetic MPs) were added to the antigen-antibody complex formed in the first reaction and incubated for another 9 min. For bound-free (B/F) separation, the reaction mixture was subsequently transferred into the measurement cell, the SA magnetic MPs in the cell were magnetically drawn to the electrodes, and the unreacted material was removed with a tripropylamine solution. When the electrodes were charged, ruthenium was oxidized and tripropylamine was reduced, resulting in repeated excitation and emission, and the intensity (signal) of the resulting luminescence at a given time was measured using a photomultiplier tube. The concentrations of sFlt-1 and PlGF in the sample were calculated from the calibrator signals operated in the same manner.

Assessment of basic performance of reagents

The basic performance evaluation of reagents included within-run assessments for repeatability, between-day assessments for intermediate precision, dilution linearity, effects of interferents, and LoQ, consistent with the routing assay performance evaluation[6]. The number of specimens and examination methods followed the guidelines and protocols of the Japanese Society of Clinical Chemistry[2,3].

1. Within-run precision for repeatability

Ten consecutive measurements were performed using control sera PC MM1 and PC MM2 at two different concentrations.

2. Between-day assessments for intermediate precision

Duplicate measurements were performed for 10 days using control sera PC MM1 and PC MM2 at two different concentrations.

3. Linearity of dilution

Samples with high measured values in the residual sera collected in this study were used to create a five-step dilution series and duplicate measurements were performed. Elecsys Sample Diluent (S), Elecsys Sample Diluent MA (S), and Elecsys NSE Sample Diluent are dedicated diluents used in Cobas® 8000, and we used Elecsys NSE Sample Diluent, which had low signal values for both sFlt-1 and PlGF.

4. Effects of interferents

The effects of bilirubin F, bilirubin C, hemolytic hemoglobin, chyle, and RF factors were evaluated in pooled sera prepared from the residual serum samples collected for this study using Interference Check A Plus and Interference Check-RF Plus (Sysmex Corporation).

5. LoQ

Pooled sera samples at five different concentrations were prepared, and each was measured five times to develop an approximate equation from the average measured values and the coefficient of variation (CV). The limit of permissible error was determined to be 20% of the CV of the measured values, and an approximate equation was used to determine the LoQ.

Statistical analysis

We used the F-test to evaluate the linearity. The Validation-Support/Excel Ver3.5 program was used for validation calculations to analyze the results of measurements for which a linear relationship was observed.

RESULTS

1. Within-run assessments for repeatability

For sFlt-1, the CV was 0.68% with PC MM1 and 0.76% with PC MM2; for PlGF, the CV was 0.30% with PC MM1 and 0.69% with PC MM2. (Table 1)

2. Between-day assessments for intermediate precision

For sFlt-1, the CV was 0.89% with PC MM1 and 0.77% with PC MM2; for PlGF, the CV was 1.54% with PC MM1 and 0.88% with PC MM2. (Table 2)

3. Linearity of dilution

We could not obtain residual sera near the upper limits of reagent measurement; therefore, samples with the highest values among those collected were used in the study. Consequently, we verified linearity up to 24,000 pg/mL for sFlt-1 and 2,000 pg/mL for PlGF. (Figure 1 and 2)

4. Effects of interferents

All measured concentrations were within ± 5% for both sFlt-1 and PlGF; bilirubin F varied up to 18.8 mg/dL, bilirubin C up to 20.2 mg/dL, hemolytic hemoglobin up to 480 mg/dL, lactobacilli up to 15,900 FTU, and RF factor up to 550 IU/mL. (Figure 3 and 4)

5. LoQ

The LoQ was 3.57 pg/mL for sFlt-1 and 0.84 pg/mL for PlGF. (Figure 5 and 6)

Table 1 Within-run assessments (n = 10)

	sFlt-1		PlGF	
	PC MM1	PC MM2	PC MM1	PC MM2
Mean	103.4	987.7	107.1	976.5
Min	102	979	107	969
Max	104	1001	108	987
SD	0.70	7.54	0.32	6.77
CV(%)	0.68	0.76	0.30	0.69

(pg/mL)

Table 2 Between-day precision (n = 20)

	sFlt-1		PlGF	
	PC MM1	PC MM2	PC MM1	PC MM2
Mean	105.9	966.8	104.7	988.8
Min	105	958	102	976
Max	108	984	107	1001
SD	0.94	7.41	1.62	8.70
CV(%)	0.89	0.77	1.54	0.88

(pg/mL)

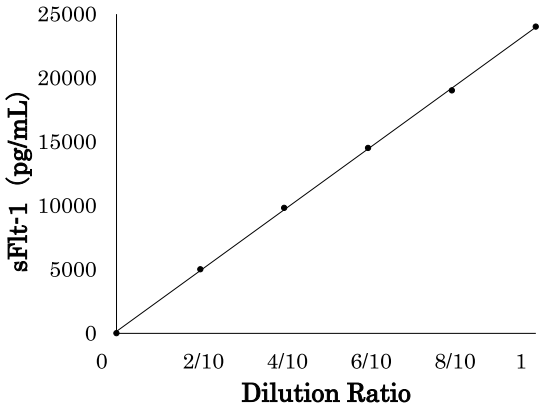

Figure 1 Linearity of dilution (sFlt-1) The sample was diluted to ~24,000 pg/mL with NSE sample diluent in five steps and measured twice.

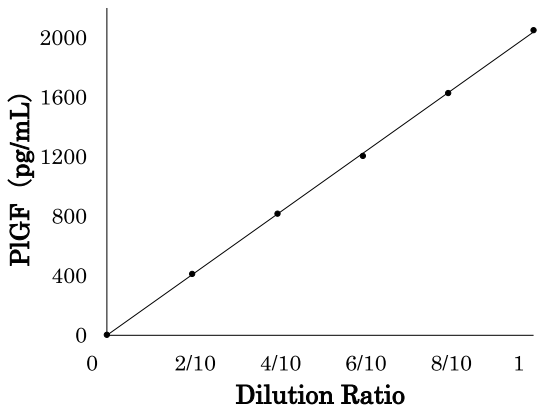

Figure 2 Linearity of dilution (PlGF) The sample was diluted to ~2,000 pg/mL with NSE sample diluent in five steps and measured twice.

DISCUSSION

In this study, we performed an initial basic performance assessment of the Elecsys sFlt-1 and PlGF reagents using sera from Japanese women. We obtained satisfactory results for both reagents with a repeatability of ≤ 1% CV and an intermediate precision of ≤ 2% CV. These findings were comparable to those reported by Schiettecatte et al.[6] for the performance assessment of these reagents in Europe.

The effects of coexisting substances showed no large fluctuations that were either directly proportional or inversely proportional to the added concentrations of bilirubin F, bilirubin C, hemolytic hemoglobin, lactobacilli, or rheumatoid factor. This finding confirms that the coexisting substances found in the conventional measurements do not affect the prediction. We verified linearity up to 24,000 pg/mL for sFlt-1 and 2,000 pg/mL for PlGF and found considerable linearity near the highest values measured in the samples collected in this study. However, the measurement ranges in the reagent package inserts were 10–85,000 pg/mL for sFlt-1 and 3–10,000 pg/mL for PlGF; therefore, linearity must be further verified in the future when measuring samples with higher values. For dilutions used in the linearity investigation, we chose the Elecsys NSE Sample Diluent in which the sFlt-1 and PlGF concentrations were thought to contain the lowest values compared to those in the other dedicated diluents. According to the manufacturer's instructions, sample dilution is not required for normal use because of the wide measurement range. Therefore, dilution was not necessary for routine clinical use.

We could not determine the limit of blank (LoB) or limit of detection (LoD) in this study because it was impossible to obtain a blank sample that did not contain the measured components. However, the LoQ values were sufficiently low for both reagents, and we believe that they can be measured with high accuracy at low concentration ranges.

Our findings confirm that the performance of Elecsys sFlt-1 and PlGF reagents is similar to those in Europe[6]. We believe that these reagents can be used for routine clinical measurements in the Japanese and European populations. To our knowledge, this is the first study to demonstrate that these reagents can be used in Japanese and European women.

CONCLUSIONS

The results of the assessment of the basic performance of the Elecsys sFlt-1 and PlGF reagents in Japanese women were satisfactory. To the best of our knowledge, we have shown for the first time that the reagents can be used in both Japanese and European women. International harmonization is currently underway for several test parameters. Therefore, it is important to assess the basic performance of these reagents for clinical use in Japan and confirm whether they can be used in Japan because sometimes there are differences between races.

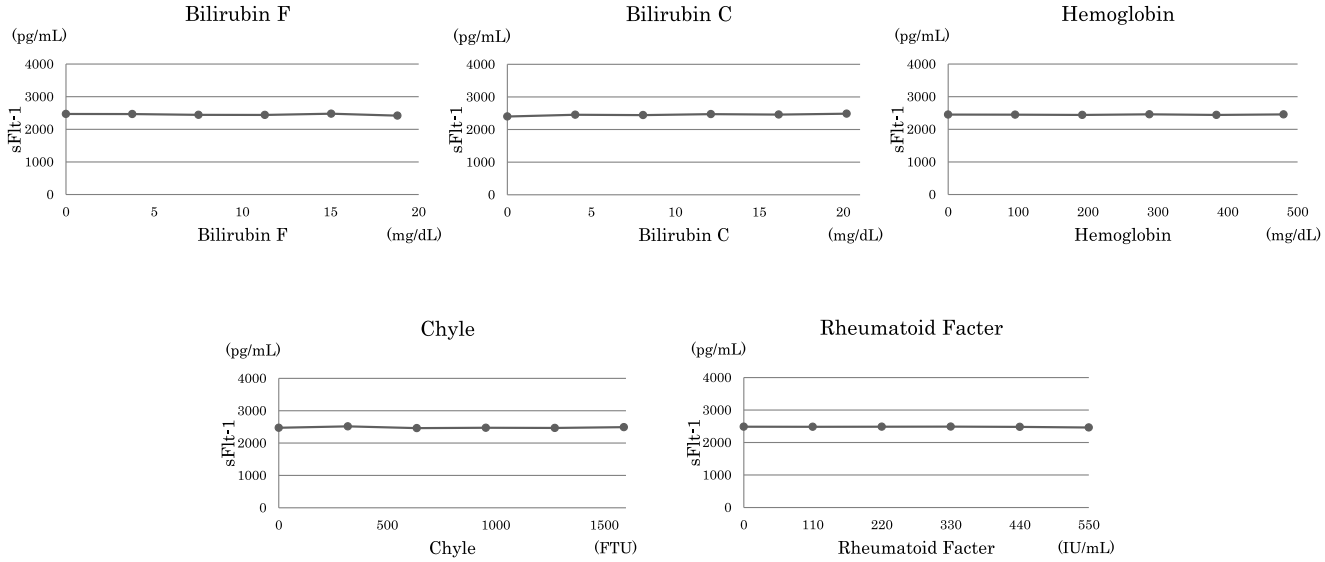

Figure 3 Effects of interferents (sFlt-1) Pooled sera comprising 20 samples at a concentration of ~2,400 pg/mL were prepared, and the effects of bilirubin F, bilirubin C, hemolytic hemoglobin, chyle, and RF factors were evaluated.

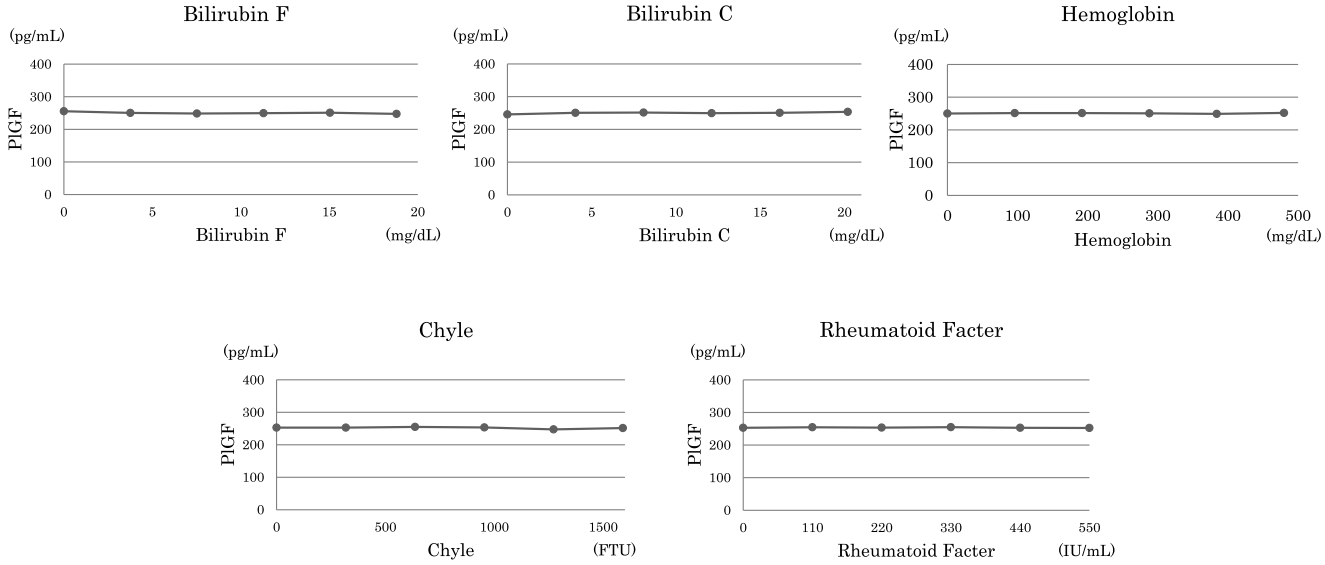

Figure 4 Effects of interferents (PlGF) Pooled sera comprising 20 samples at a concentration of ~250 pg/mL were prepared, and the effects of bilirubin F, bilirubin C, hemolytic hemoglobin, chyle, and RF factors were evaluated.

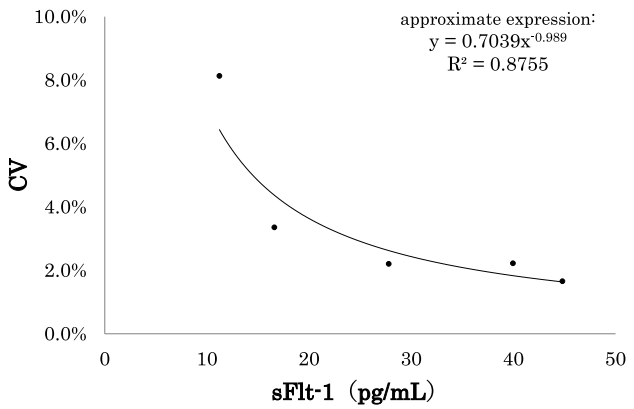

Figure 5 Limit of quantitation (LoQ; sFlt-1) A coefficient of variation (CV) value of 20% was defined as the LoQ from the approximate formula created by five measurements of five different concentrations of pooled sera. Each concentration of the pooled sera comprised two samples and was measured five times.

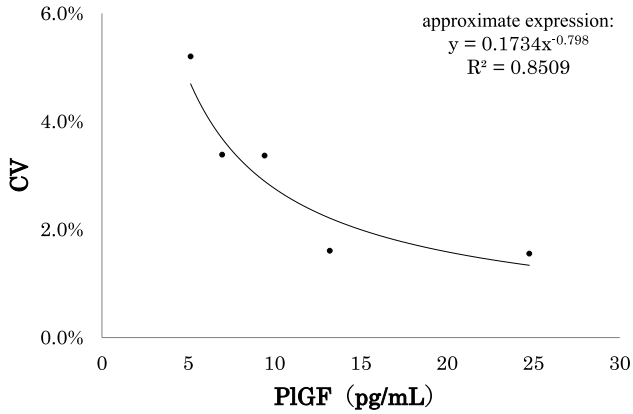

Figure 6 LoQ (PlGF) A CV value of 20% was defined as the LoQ from the approximate formula created by five measurements of five different concentrations of pooled sera. Each concentration of pooled sera comprised five samples and was measured five times.

Hopefully, clinical trials in Japan will confirm that the sFlt-1 to PlGF ratio is useful for the short-term prediction of PE onset.

Conflicts of Interest (COI)

Elecsys sFlt-1 and PlGF reagents used in this study were provided by Roche Diagnostics, Inc.

Ethics Material

This study was approved by the Ethics Committee for Epidemiological Research of Hiroshima University (No. E-1782).

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