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Elecsys® and Kryptor immunoassays for the measurement of sFlt-1 and PlGF to aid preeclampsia diagnosis: are they comparable?

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Abstract

Background: For pregnant women with suspected preeclampsia, the soluble fms-like tyrosine-kinase 1 (sFlt-1)/placental growth factor (PlGF) ratio is a biomarker to aid diagnosis. We performed method comparisons between Elecsys® and Kryptor sFlt-1 and PlGF immunoassays and assessed the diagnostic performance for preeclampsia.

Methods: Serum samples from a case-control study involving 113 pregnant women with preeclampsia/elevated liver enzymes and low platelet count (HELLP) and 270 controls were analyzed. sFlt-1 and PlGF were measured using Roche Elecsys® and BRAHMS Kryptor sFlt-1/PlGF immunoassays. The sFlt-1/PlGF ratios were calculated, and Passing-Bablok regression/Bland-Altman plots were performed. Gestation-specific cut-offs, ≤ 33 and $\geq 85/\geq 110$, were assessed.

Results: Mean (± 2 standard deviation [SD]) differences between the Elecsys® and Kryptor values were: sFlt-1, 173.13 pg/mL (6237.66, -5891.40); PlGF, -102.71 pg/mL (186.06, -391.48); and sFlt-1/PlGF, 151.74 (1085.11, -781.63). The Elecsys® and Kryptor immunoassays showed high correlation: Pearson's correlation coefficients were 0.913 (sFlt-1) and 0.945 (PlGF). Slopes were 1.06 (sFlt-1) and 0.79 (PlGF), resulting in ~20% lower values for Kryptor PlGF. Sensitivities and specificities using the sFlt-1/PlGF ≥ 85 cut-off for early-onset preeclampsia (20 + 0 to 33 + 6 weeks) were 88.1%/100.0% (Elecsys®) and 90.5%/96.2% (Kryptor), respectively, and using the ≥ 110 cut-off for late-onset preeclampsia (≥ 34 + 0 weeks) were 51.3%/96.5% (Elecsys®) and 78.9%/90.1% (Kryptor), respectively. Using

Elecsys® and Kryptor sFlt-1/PlGF, 0% and 3.8% of women, respectively, were falsely ruled-in for early-onset, and 3.5% and 9.9%, respectively, for late-onset preeclampsia.

Conclusions: Despite high correlation between the Elecsys® and Kryptor immunoassays, we observed significant differences between sFlt-1/PlGF and PlGF results. Therefore, sFlt-1/PlGF cut-offs validated for Elecsys® immunoassays are not transferable to Kryptor immunoassays.

Keywords: immunoassay; preeclampsia; sFlt-1/PlGF.

Introduction

Preeclampsia is a disorder of pregnancy characterized by the onset of hypertension and proteinuria after 20 weeks of gestation [1–3]. Preeclampsia occurs in 2%–8% of pregnancies worldwide, and diagnosis of the condition is often complicated by a heterogeneous disease course and a lack of specific, sensitive diagnostic methods [1, 4]. Placental dysfunction leads to altered angiogenic factors in maternal circulation, such as soluble fms-like tyrosine-kinase 1 (sFlt-1) and placental growth factor (PlGF), resulting in an elevated sFlt-1/PlGF ratio [5–8].

Clinical use of gestational age-specific sFlt-1/PlGF cut-offs to aid in the prediction and diagnosis of preeclampsia is increasing [9]. There are several commercially available electrochemiluminescence immunoassays for the determination of sFlt-1 and PlGF concentrations in serum, including the Roche Elecsys® (Roche Diagnostics, Mannheim, Germany) and BRAHMS Kryptor (Thermo Fisher Scientific, Hennigsdorf, Germany) sFlt-1 and PlGF assays. These are designed for use in conjunction with one another to facilitate the measurement of the sFlt-1/PlGF ratio in women with suspected preeclampsia.

Gestational age-specific cut-offs for early-onset (20 + 0 to 33 + 6 weeks) and late-onset (≥ 34 + 0 weeks) preeclampsia have previously been validated to aid in the diagnosis of preeclampsia using the Elecsys® sFlt-1/PlGF immunoassays. Verlohren et al. demonstrated high sensitivity and specificity of sFlt-1/PlGF ratio ≤ 33 for ruling out women with suspected preeclampsia, and sFlt-1/PlGF ratios ≥ 85 and ≥ 110 for ruling in women with suspected preeclampsia

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in the early and late gestational phase, respectively [10]. In a meta-analysis of six studies (including that of Verlohren et al. [10]), Cerdeira et al. further demonstrated high pooled sensitivity and specificity for these cut-offs [9]. For ruling out preeclampsia within 4 weeks in women with suspected preeclampsia, a sFlt-1/PlGF cut-off of ≤ 38 was validated [11, 12]. As the cut-off of ≤ 38 was evaluated for the short-term prediction of preeclampsia rather than to aid in the diagnosis of the condition, it was not evaluated in the present analyses.

The studies described earlier demonstrated the need for current diagnostic criteria in preeclampsia to be strengthened through the inclusion of biomarker-specific cut-offs. There are currently no validated cut-offs available specifically for Kryptor sFlt-1/PlGF, however.

Previous studies have evaluated the diagnostic performance of the Elecsys® and Kryptor sFlt-1 and PlGF assays independently, and suggested that they are largely comparable [13–15]. Andersen et al. found lower specificity for the Kryptor sFlt-1/PlGF ratio compared with Elecsys® for late-onset preeclampsia using the cut-off of 110 and the same specificity for early-onset preeclampsia using the cut-off of 85 [13]. Van Helden and Weiskirchen noted that sensitivity, specificity and area under the curve (AUC) values were comparable for a sFlt-1/PlGF cut-off of 85 and gestational age-specific cut-offs for early-onset and late-onset preeclampsia across both the assay platforms [14]. Dröge et al. investigated the diagnostic performance of Kryptor sFlt-1/PlGF only in their study and observed similar AUC values for the Kryptor assays compared with previously published data of Elecsys® sFlt-1/PlGF. However, they proposed a cut-off of 103 for Kryptor sFlt-1/PlGF to aid the diagnosis of preeclampsia [15].

The aim of the present study was to perform method comparisons between the Elecsys® and Kryptor sFlt-1 and PlGF assays, and to assess diagnostic performance to aid in the diagnosis of preeclampsia and hemolysis, elevated liver enzymes and low platelet count (HELLP) syndrome in a large cohort of pregnant women.

Materials and methods

Study design

This was an analysis of samples from a European multi-center case-control study, completed in 2012, in pregnant women presenting with preeclampsia/HELLP syndrome [10]. Our analysis included samples from eight of the nine original collection sites involved. Sample collection for this study was based on available sample volume and

matching cases/controls, the possibility to retest samples according to informed consent, and availability of results for Elecsys® sFlt-1 and PlGF. Serum samples were collected according to a standard operating procedure at each center and frozen at -80°C until measurement [10].

Pregnant women aged ≥ 16 years with preeclampsia/HELLP syndrome were enrolled. Preeclampsia was defined as the new onset of both hypertension (elevated systolic blood pressure [BP] ≥ 140 mmHg) and/or elevated diastolic BP ≥ 90 mmHg) on repeated measurements >6 h apart within 1 week) and proteinuria (>0.3 g protein/24 h [or in emergency cases only a dipstick $\geq 1+$ on more than one occasion] or ≥ 30 mg/dL protein in a spot urine test if a 24-h urine protein collection could not be obtained) after 20 weeks of gestation. Exclusion criteria were as follows: multi-fetal pregnancy, intra-uterine growth restriction (IUGR) defined as estimated fetal weight or abdominal circumference <5 th percentile (adjusted for gender and ethnicity according to charts routinely used by the study site) in the presence of a pathologic process that inhibits expression of normal intrinsic growth potential (≥ 1 occurrence after gestational week 22, defined by the following criteria: oligohydramnios [amniotic fluid index <10 th percentile] or pathologic flow in umbilical artery [pulsatility index >95 th percentile]), loss to follow-up (pregnancy outcome unknown), anti-phospholipid antibody syndrome, systemic lupus erythematosus or any other autoimmune disease, chronic corticosteroid, or non-steroidal anti-inflammatory drug use (except low-dosage aspirin ≤ 150 mg/day). Women with type 2 diabetes mellitus and renal disease were not excluded.

Women who were normotensive during pregnancy and had a normal pregnancy outcome were enrolled as gestational age-matched controls. Other inclusion criteria for control women were no diagnosis of any form of preeclampsia, no diagnosis of IUGR in the infant and no multi-fetal pregnancy.

The study protocol was approved by the local Ethics Committees and the Institutional Review Boards prior to study initiation. All participants provided signed, written informed consent. This study was conducted in accordance with the principles of the Declaration of Helsinki and the International Conference on Harmonization guidelines for Good Clinical Practice.

Assays

Serum sFlt-1 and PlGF concentrations were measured using the Elecsys® sFlt-1 and PlGF electrochemiluminescence immunoassays on cobas e 170 and cobas e 601 analyzers, and the PlGF plus Kryptor and sFlt-1 Kryptor immunofluorescent assays on the Kryptor compact plus analyzer. Kryptor measurements were performed at MVZ Labor Krone, Bad Salzungen, Germany; Elecsys® measurements were performed at Roche Diagnostics GmbH, Penzberg, Germany and Leipzig University Clinic, Leipzig, Germany.

The Elecsys® sFlt-1 and PlGF assays and Kryptor sFlt-1 and PlGF assays use the sandwich test principle and are intended for use in conjunction with one another to measure sFlt-1 and PlGF concentrations in serum, to facilitate the calculation of the sFlt-1/PlGF ratio. The Elecsys® sFlt-1 and PlGF assays have limits of quantitation (LoQ) and limits of detection (LoD) of 15 pg/mL and 10 pg/mL, and 10 pg/mL and 3 pg/mL, respectively (measuring range: sFlt-1: 10–85,000 pg/mL; PlGF: 3–10,000 pg/mL). The Elecsys® assays have an overall turnaround time of 18 min [16, 17]. The Kryptor sFlt-1 and PlGF assays have LoQs and LoDs of 34 pg/mL and 6.9 pg/mL,

and 22 pg/mL and 3.6 pg/mL, respectively (measuring range: sFlt-1: 22–90,000 pg/mL; PlGF: 3.6–7000 pg/mL) [18, 19]. The Kryptor sFlt-1 assay has a turnaround time of 9 min, and the Kryptor PlGF assay has a turnaround time of 29 min.

For Elecsys® and Kryptor assays, familiarization experiments and quality checks were performed in accordance with a standard operating procedure.

Method comparison

Inter-assay method comparisons were based on Passing-Bablok regression and Bland-Altman plots and were conducted in accordance with the Clinical Laboratory Standards Institute (CLSI) EP09-A3 guidelines [20].

Diagnostic performance

Women with preeclampsia were assigned to the early-onset preeclampsia group if the condition was diagnosed at 20+0 to 33+6 weeks of gestation and were assigned to the late-onset preeclampsia group if diagnosed at $\geq 34+0$ weeks of gestation. Pre-established gestational age-specific sFlt-1/PlGF cut-offs validated for Elecsys® sFlt-1/PlGF to aid in the diagnosis of preeclampsia were assessed [6, 10]; the cut-offs were ≤ 33 and ≥ 85 for early-onset preeclampsia, and ≤ 33 and ≥ 110 for late-onset preeclampsia.

Receiver operator characteristic (ROC) curves were used to evaluate AUC, sensitivity, specificity and false-positive rates for all sFlt-1/PlGF cut-offs at different gestational ages.

Statistical analyses

Basic statistics, including median and quartiles, were calculated for demographics from all evaluable women. Statistical significance was assigned where the p-value was ≤ 0.05 , and confidence intervals (CI) were set at 95%. All statistical analyses were performed using R version 3.4.0.

Results

Analysis population

A total of 383 participants were enrolled in this study (including 113 women with preeclampsia/HELLP syndrome and 270 controls; Table 1), from which 396 evaluable samples were collected (cohort with preeclampsia cases: 118; control cohort: 278). The median age and body mass index (BMI) for women with early-onset (33.0 years; 23.5 kg/m²) and late-onset (30.5 years; 24.1 kg/m²) preeclampsia were similar. The median gestational age at delivery was higher for women with late-onset preeclampsia

compared with early-onset preeclampsia (38.1 weeks vs. 32.0 weeks). The majority of women with early-onset preeclampsia (79%) and late-onset preeclampsia (73%) were White/Caucasian.

Method comparison

Bland-Altman plots are presented for the Elecsys® and Kryptor sFlt-1, PlGF and sFlt-1/PlGF assays in Figure 1. The limits of agreement (LoA) were calculated as the interval defined by ± 2 standard deviation (SD) of the mean difference between comparator assays, within which 95% of the differences lie. The mean (± 2 SD) difference in sFlt-1 measured by Elecsys® vs. Kryptor assays was 173.13 pg/mL (LoA: 6237.66, –5891.40; Figure 1A) and for PlGF was –102.71 pg/mL (LoA: 186.06, –391.48; Figure 1B). For sFlt-1, most values were clustered within the LoA, with less variation around the mean. For PlGF, the Elecsys® assay measured higher, which is reflected by a negative mean bias, with values distributed toward the lower LoA. The mean (± 2 SD) difference in the sFlt-1/PlGF ratio measured by Elecsys® vs. Kryptor assays was 151.74 (LoA: 1085.11, –781.63; Figure 1C). The Elecsys® assays determined lower values for the sFlt-1/PlGF ratio, which is reflected by a strong positive mean bias. A wide LoA was also observed for sFlt-1/PlGF, caused by higher variation in individuals with high sFlt-1/PlGF ratios, i.e. above the cut-off (≥ 85), compared with individuals with lower sFlt-1/PlGF ratios, i.e. below the cut-off (< 85 ; Figure 1C, D). The mean (± 2 SD) difference in sFlt-1/PlGF ratios ≥ 85 measured by Elecsys® vs. Kryptor assays was 524.46 (LoA: 2092.69, –1043.76); for sFlt-1/PlGF ratios < 85 , the mean (± 2 SD) difference was 34.1 (LoA: 414.84, –346.63). Substantial inter-assay differences were observed in the sFlt-1/PlGF ratio measurements between the Elecsys® and Kryptor assays. The median sFlt-1/PlGF ratio measurements were higher for Kryptor vs. Elecsys® in both pregnant women with early-onset and late-onset preeclampsia/HELLP syndrome (early-onset: 710.4 vs. 286.2; late-onset: 231.7 vs. 113.2; Table 1).

A high correlation was observed between the Elecsys® and Kryptor assays for sFlt-1 and PlGF measurements according to Passing-Bablok analyses. Pearson's correlation coefficients (r) were 0.913 for sFlt-1 (Figure 2A), 0.945 for PlGF (Figure 2B) and 0.894 for sFlt-1/PlGF (Figure 2C). The resulting equations for the Passing-Bablok regression fit were: Kryptor sFlt-1 = $1.06 \times \text{Elecsys}^{\circledast} \text{ sFlt-1} - 358.19$; Kryptor PlGF = $0.79 \times \text{Elecsys}^{\circledast} \text{ PlGF} - 17.89$; and Kryptor sFlt-1/PlGF = $1.89 \times \text{Elecsys}^{\circledast} \text{ sFlt-1/PlGF} - 3.08$ (Figure 2A–C). PlGF measurements were substantially lower using Kryptor PlGF plus compared with Elecsys®

Table 1: Participant demographics.

	Evaluable women (n=383)			
	Early-onset preeclampsia (20+0 to 33+6 weeks; n=142)		Late-onset preeclampsia (≥34+0 weeks; n=241)	
	Preeclampsia/HELLP (n=39)	Controls (n=103)	Preeclampsia/HELLP (n=74)	Controls (n=167)
Age, years	33.0 (28.0–35.0)	30.0 (25.0–33.0)	30.5 (25.0–34.0)	31.0 (26.0–35.0)
Height, cm	165.0 (160.0–170.0)	166.0 (160.0–169.0)	167.0 (161.0–171.0)	165.0 (160.0–170.0)
Weight, kg	68.0 (56.0–75.0)	60.0 (53.0–69.0)	69.0 (61.5–76.0)	64.0 (57.0–74.0)
BMI, kg/m ²	23.5 (21.6–29.4)	21.9 (19.7–26.0)	24.1 (22.4–27.2)	23.3 (20.9–26.3)
Gestational age at delivery, weeks	32.0 (28.4–33.7)	38.6 (37.0–40.0)	38.1 (37.0–39.4)	39.7 (38.4–40.9)
Birth weight, g	1534 (1075–1850)	3100 (2620–3500)	3125 (2680–3400)	3390 (2970–3780)
Blood pressure, mmHg				
Max. systolic–median (Q1–Q3)	159 (145–174)	112 (105–120)	150 (140–160)	120 (110–130)
Max. diastolic–median (Q1–Q3)	95 (90–110)	67 (60–71)	95 (90–100)	70 (63–80)
Smoking status, n (%)				
Never	29 (74)	72 (70)	58 (78)	105 (63)
Past	5 (13)	16 (16)	11 (15)	32 (19)
Current	3 (8)	12 (12)	4 (5)	22 (13)
Unknown/NA	2 (2)	3 (3)	1 (1)	8 (8)
Race, n (%)				
White/Caucasian	31 (79)	78 (76)	54 (73)	136 (81)
Other	6 (15)	8 (8)	8 (11)	8 (5)
Asian	0 (0)	11 (11)	3 (4)	5 (3)
Black	2 (5)	4 (4)	5 (7)	3 (2)
Unknown/NA	0 (0)	2 (2)	4 (4)	15 (15)
Family history of preeclampsia, n (%)				
Yes	3 (8)	2 (2)	6 (8)	4 (2)
No	31 (79)	81 (79)	56 (76)	141 (84)
Unknown/NA	5 (5)	20 (20)	12 (12)	22 (22)
Angiogenic markers, pg/mL				
sFlt-1 (Elecsys®)	13,442 (9354–17,132)	1716 (1157–2674)	8844 (6168–11,690)	2950 (2220–4441)
sFlt-1 (Kryptor)	12,875 (8497–17,730)	1415 (989–2463)	8911 (5970–12,765)	2668 (1880–4385)
PlGF (Elecsys®)	44.2 (27.9–70.4)	475.4 (321.7–694.0)	84.0 (54.8–113.8)	233.9 (133.4–484.5)
PlGF (Kryptor)	17.2 (9.3–34.4)	351.4 (231.1–576.8)	39.7 (24.9–59.7)	157.1 (74.9–331.2)
sFlt-1/PlGF (Elecsys®)	286.2 (172.4–490.8)	3.2 (1.9–6.2)	113.2 (58.7–168.0)	12.0 (5.4–32.1)
sFlt-1/PlGF (Kryptor)	710.4 (371.4–1700.8)	3.8 (2.1–7.6)	231.7 (115.7–384.9)	16.0 (7.0–56.0)

All data are presented as median (Q1–Q3) unless otherwise stated. BMI, body mass index; HELLP, hemolysis, elevated liver enzymes and low platelet count; NA, not available; PlGF, placental growth factor; Q1, first quartile; Q3, third quartile; sFlt-1, soluble fms-like tyrosine-kinase 1.

PlGF, with an approximate difference of 20% observed between measurements. Overall, Kryptor sFlt-1/PlGF ratios were approximately 90% higher than Elecsys sFlt-1/PlGF ratios.

Diagnostic performance

The ROC resulted in similar AUC values for Elecsys® vs. Kryptor sFlt-1/PlGF across all gestational ages (0.95 vs. 0.94; Figure 3A) and early gestational ages (1.0 vs. 0.98; Figure 3B). AUC values were the same for Elecsys® and Kryptor for late gestational ages (0.91 vs. 0.91; Figure 3C).

Significant inter-assay differences in sensitivity and specificity of the Elecsys® and Kryptor assays were observed at each cut-off value and for each gestational age (Table 2). For an sFlt-1/PlGF cut-off of ≥85, across all gestational ages, the specificity was higher for Elecsys® vs. Kryptor (96.8% vs. 88.5%; $p < 0.001$), and the sensitivity was lower for Elecsys® vs. Kryptor (72.9% vs. 87.3%; $p < 0.001$).

Across all sFlt-1/PlGF cut-offs for all gestational ages, fewer women had false-positive preeclampsia diagnoses with Elecsys® compared with Kryptor. Using an sFlt-1/PlGF cut-off of ≥85, 0% and 3.8% of women were falsely ruled-in for early-onset preeclampsia, while

Figure 1: Bland-Altman plots for sFlt-1, PlGF and sFlt-1/PlGF ratio measured using Elecsys® and Kryptor assays.

Bland-Altman plots for the Elecsys® and Kryptor assays: (A) sFlt-1, (B) PlGF, (C) sFlt-1/PlGF (cut-offs combined) and (D) sFlt-1/PlGF (<85 [left] and ≥85 [right]). The red dashed lines represent the LoA, calculated as the interval between which 95% of the differences between comparator assays lie. LoA, limits of agreement; PlGF, placental growth factor; SD, standard deviation; sFlt-1, soluble fms-like tyrosine-kinase 1.

Figure 2: Passing-Bablok regression fit for sFlt-1, PlGF and sFlt-1/PlGF ratio measured using Elecsys® and Kryptor assays. Passing-Bablok regression fit for the Elecsys® and Kryptor assays (A) sFlt-1, (B) PlGF and (C) sFlt-1/PlGF. The grey line represents $x = y$, the blue shading represents 95% confidence interval. PlGF, placental growth factor; sFlt-1, soluble fms-like tyrosine-kinase 1.

Figure 3: Receiver-operator characteristic curves for sFlt-1/PlGF at different gestational ages measured using Elecsys® and Kryptor assays, including cut-off marks. Receiver-operator characteristic curves for the Elecsys® and Kryptor assays: (A) all preeclampsia groups (sFlt-1/PlGF: ≥ 85), (B) early preeclampsia group (sFlt-1/PlGF: ≤ 33 and ≥ 85) and (C) late preeclampsia group (sFlt-1/PlGF: ≤ 33 and ≥ 110). AUC, area under the curve; CI, confidence interval; PlGF, placental growth factor; sFlt-1, soluble fms-like tyrosine-kinase 1.

Table 2: Sensitivity and specificity across the sFlt-1/PlGF ratio cut-offs for the Elecsys® and Kryptor assays.

sFlt-1/PlGF ratio cut-off	Preeclampsia group (n)	Assay	Number of samples analyzed (n/N)	Sensitivity, % (95% CI)	p-Value	Number of samples analyzed (n/N)	Specificity, % (95% CI)	p-Value
Uniform cut-off value								
≥85	All (118)	Roche Elecsys®	86/118	72.9 (64.2–80.1)	<0.001	269/278	96.8 (94.0–98.3)	<0.001
		BRAHMS Kryptor	103/118	87.3 (80.1–92.1)		246/278	88.5 (84.2–91.7)	
	Early (42)	Roche Elecsys®	37/42	88.1 (75.0–94.8)	1	106/106	100.0 (96.5–100.0)	0.134
		BRAHMS Kryptor	38/42	90.5 (77.9–96.2)		102/106	96.2 (90.7–98.5)	
	Late (76)	Roche Elecsys®	49/76	64.5 (53.3–74.3)	<0.001	163/172	94.8 (90.4–97.2)	<0.001
		BRAHMS Kryptor	65/76	85.5 (75.9–91.7)		144/172	83.7 (77.5–88.5)	
Gestational age-specific cut-off values								
≤33	Early (42)	Roche Elecsys®	39/42	92.9 (81.0–97.5)	1	104/106	98.1 (93.4–99.5)	0.023
		BRAHMS Kryptor	40/42	95.2 (84.2–98.7)		97/106	91.5 (84.6–95.5)	
	Late (76)	Roche Elecsys®	71/76	93.4 (85.5–97.2)	1	131/172	76.2 (69.3–81.9)	<0.001
		BRAHMS Kryptor	71/76	93.4 (85.5–97.2)		109/172	63.4 (56.0–70.2)	
≥85	Early (42)	Roche Elecsys®	37/42	88.1 (75.0–94.8)	1	106/106	100.0 (96.5–100.0)	0.134
		BRAHMS Kryptor	38/42	90.5 (77.9–96.2)		102/106	96.2 (90.7–98.5)	
≥110	Late (76)	Roche Elecsys®	39/76	51.3 (40.3–62.2)	<0.001	166/172	96.5 (92.6–98.4)	0.003
		BRAHMS Kryptor	60/76	78.9 (68.5–86.6)		155/172	90.1 (84.7–93.7)	

CI, confidence interval; PlGF, placental growth factor; sFlt-1, soluble fms-like tyrosine-kinase 1.

5.2% and 16.3% of women were falsely ruled-in for late-onset preeclampsia, for Elecsys® and Kryptor sFlt-1/PlGF, respectively. Using an sFlt-1/PlGF cut-off of ≤33, 7.1% and 4.8% of women were falsely ruled-out for early-onset preeclampsia for Elecsys® and Kryptor sFlt-1/PlGF, respectively. The rate of false negative ruled-out results with the two assays was the same for late-onset preeclampsia at a cut-off of ≤33 (6.6% vs. 6.6%). Using a cut-off of ≥110, 3.5% and 9.9% of women were falsely ruled-in for late-onset preeclampsia using Elecsys® and Kryptor sFlt-1/PlGF, respectively (Table 2).

For ruling-in preeclampsia, the sensitivity was higher at an sFlt-1/PlGF cut-off of ≥85 (early-onset) vs. ≥110 (late-onset), with both Elecsys® and Kryptor (Elecsys®: 88.1% vs. 51.3%; Kryptor: 90.5% vs. 78.9%; Table 2). The specificity was higher at a cut-off of ≥85 (early-onset) vs. ≥110 (late-onset) with Elecsys® and Kryptor (Elecsys®: 100% vs. 96.5%; Kryptor: 96.2% vs. 90.1%). For ruling-out preeclampsia, the sensitivity was higher at an sFlt-1/PlGF cut-off of ≤33 (late-onset) vs. a cut-off of ≤33 (early-onset) with Elecsys® (93.4% vs. 92.9%), and lower at a cut-off of ≤33 (late-onset) vs. ≤33 (early-onset) with Kryptor (93.4% vs. 95.2%). The specificity was higher at a cut-off of ≤33 (early-onset) vs. ≤33 (late-onset) with both Elecsys® and Kryptor (Elecsys®: 98.1% vs. 76.2%; Kryptor: 91.5% vs. 63.4%). Across all cut-offs, for all gestational ages, the specificity was higher for Elecsys® vs. Kryptor.

Discussion

In this analysis of a case-control study in pregnant women with preeclampsia/HELLP, we showed that the sFlt-1/PlGF cut-offs validated using the Elecsys® immunoassays are not transferable to Kryptor assays. Despite high correlation between Elecsys® and Kryptor immunoassays, there were substantial inter-assay differences for sFlt-1/PlGF and PlGF measurements between the immunoassays of the two manufacturers. Using Elecsys®-validated sFlt-1/PlGF cut-offs of 85 and 110, Kryptor showed lower specificity than Elecsys®, resulting in a higher rate of false-positive preeclampsia diagnoses. Overall, PlGF measurements were markedly lower using PlGF plus Kryptor, with approximately 20% lower serum values compared with Elecsys® PlGF.

Implications for clinical practice

Increased accuracy of the sFlt-1 and PlGF assays is pivotal to inform clinical decision-making for pregnant women with suspected early-onset or late-onset preeclampsia, as an accurate and timely diagnosis could prevent unnecessary hospitalization and extended monitoring for preeclampsia [4, 10]. We found that the Elecsys® assays had higher specificity across all cut-offs for all gestational

ages compared with the Kryptor assays. The sFlt-1/PlGF ratio cut-offs of 85 and 110 were selected for high optimal specificity (fewer false positives) and validated for use on the Elecsys® assays; their use with other assays such as Kryptor may therefore result in suboptimal clinical performance. Accordingly, a higher number of women were falsely ruled-in for early-onset and late-onset preeclampsia using the Kryptor assays compared with Elecsys® assays at an sFlt-1/PlGF cut-off of ≥ 85 across all gestational ages. Therefore, use of the Elecsys® assays in the clinic could potentially decrease the number of women receiving extended, unnecessary monitoring for suspected preeclampsia [21].

We found that the Elecsys® assays had lower sensitivity across all cut-offs for all gestational ages compared with the Kryptor assays, except for rule-out of late-onset preeclampsia at a cut-off of ≤ 33 (93.4% for both). For Kryptor sFlt-1/PlGF, the sensitivity (87.3%) and specificity (88.5%) for early- and late-onset preeclampsia were within the range reported in previous studies (69.2%–94.1% and 64.5%–88.6%, respectively) [13–15].

The high correlation observed between the Elecsys® and Kryptor assays for sFlt-1 and PlGF measurements according to Passing-Bablok analyses was also found in the study of van Helden and Weiskirchen, with Pearson's r of 0.996 for sFlt-1, 0.990 for PlGF and 0.947 for sFlt-1/PlGF [14]. The Passing-Bablok regression fits reported by van Helden and Weiskirchen were similar to those found in the present study: Kryptor sFlt-1 = $1.108 \times \text{Elecsys}^\circledast \text{ sFlt-1} - 315$ compared with Kryptor sFlt-1 = $1.06 \times \text{Elecsys}^\circledast \text{ sFlt-1} - 358.19$ in this study; Kryptor PlGF plus = $0.814 \times \text{Elecsys}^\circledast \text{ PlGF} - 2.3$ compared with Kryptor PlGF plus = $0.79 \times \text{Elecsys}^\circledast \text{ PlGF} - 17.89$ in this study; and Kryptor sFlt-1/PlGF = $1.1451 \times \text{Elecsys}^\circledast \text{ sFlt-1/PlGF} - 0.85$ compared with Kryptor sFlt-1/PlGF = $1.89 \times \text{Elecsys}^\circledast \text{ sFlt-1/PlGF} - 3.08$ in this study [14]. As a consequence, PlGF measurements were substantially lower using Kryptor PlGF plus compared with Elecsys® PlGF, with an approximately 20% lower serum values observed for Kryptor PlGF plus, comparable with the difference found in the study by van Helden and Weiskirchen [14].

In the present analyses, we used sFlt-1/PlGF cut-offs validated using the Elecsys® assays; this could account for the inter-assay difference in the sFlt-1/PlGF ratio measurements. Dröge et al. previously proposed a Kryptor-specific sFlt-1/PlGF cut-off of 103, demonstrating high sensitivity (88%) and specificity (92%) for rule-in of preeclampsia [15]. Van Helden and Weiskirchen proposed a similar Kryptor-specific cut-off of 99, with comparable sensitivity (92%) and specificity (100%) [14].

Both cut-offs are substantially higher than the validated Elecsys® cut-off of 85. Further studies are required to validate a Kryptor-specific cut-off, as it may prove optimal in the measurement of sFlt-1/PlGF using Kryptor assays [14]. In the method sheets of BRAHMS Kryptor PlGF plus and BRAHMS Kryptor sFlt-1, there are currently no optimal cut-offs for aid in diagnosis of preeclampsia validated for sFlt-1/PlGF Kryptor assays.

High comparability has previously been observed between the Elecsys® and Kryptor assays, both in terms of analytical performance and in general functionality. The present results were comparable with previous studies using the Elecsys® and Kryptor assays in terms of sensitivity and specificity. At an sFlt-1/PlGF cut-off of ≥ 85 , the sensitivity and specificity for the Elecsys® assays were 72.9% and 96.8% vs. previously reported values of 51.3%–96.1% and 88.24%–95.5%, respectively [10, 14, 15, 21].

Our results showed a trend toward higher sensitivity and specificity for both assays in ruling-in women for early-onset preeclampsia at an sFlt-1/PlGF cut-off of ≥ 85 (Elecsys®: sensitivity 88.1%, specificity 100%; Kryptor: sensitivity 90.5%, specificity 96.2%) compared with ruling-in late-onset preeclampsia at a cut-off of ≥ 110 (Elecsys®: sensitivity 51.3%, specificity 96.5%; Kryptor: sensitivity 78.9%, specificity 90.1%). Our findings are comparable to those of Verlohren et al. and Andersen et al.; for an sFlt-1/PlGF cut-off of ≥ 85 for early-onset preeclampsia, the sensitivity and specificity were 77.8%–88.0% and 99.5%–100% for Elecsys®, and 77.8% and 100% for Kryptor, respectively [10, 13]. For a cut-off of ≥ 110 for late-onset preeclampsia, the sensitivity and specificity were 85.5% for Elecsys® and 67.7% for Kryptor. Hence, the specificity for Elecsys® sFlt-1/PlGF for late-onset preeclampsia using an Elecsys®-validated sFlt-1/PlGF cut-off of 110 was significantly higher compared with Kryptor sFlt-1/PlGF [13]. These findings are consistent with those of the present study.

Study strengths and limitations

A key strength of this analysis is the recruitment setting for participants. Women were recruited from a routine clinical setting, and thus the results are likely reflective of everyday clinical practice. In addition, the present analyses involved a large study population and are therefore likely applicable to an international population of women. However, the case-control design of this study is not entirely reflective of the prospective use of the assays as a diagnostic tool. Nevertheless, our findings demonstrate the diagnostic utility of these assays to aid in the

diagnosis of preeclampsia. New aliquots were used for the present analyses to prevent any interference from multiple freeze/thaw cycles. Manufacturers' instructions were followed for both the assays and method comparisons were conducted in accordance with the CLSI EP09-A3 guidelines [20].

This study has some limitations. The samples analyzed with Kryptor assays had been stored for longer than those analyzed using the Elecsys® assays (measurements performed in November 2017 vs. pre-2014 for Elecsys®) [10]. Storage procedures may affect the levels of sFlt-1 and PlGF in serum; however, previously published data do not suggest a significant difference in biomarker concentrations caused by storage time [22]. Sample collection for this study was based on available new unused samples with gestational age-matched cases/controls, the possibility to retest samples according to informed consent and availability of results for Elecsys® sFlt-1 and PlGF.

Conclusions

Despite the high correlation observed between the Elecsys® and Kryptor immunoassays, there were significant inter-assay differences between Elecsys® and Kryptor sFlt-1/PlGF and PlGF. This analysis demonstrates that the sFlt-1/PlGF cut-offs validated with the Elecsys® immunoassays are not transferable to the Kryptor assays.

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