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Research Article

Diagnosis of preeclampsia with soluble Fms–like tyrosine kinase 1/placental growth factor ratio: an inter–assay comparison

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Abstract

The angiogenic factor ratio soluble Fms–kinase 1 (sFlt–1)/placental growth factor (PlGF) is a novel diagnostic tool for preeclampsia. We compared the efficacy of the KRYPTOR (BRAHMS) automated assays for sFlt–1 and PlGF with the Elecsys (Roche) assays in a routine clinical setting. Preeclamptic women (n = 39) were included shortly after the time of diagnosis. Normotensive control pregnancies were matched by gestational age (n = 76). The KRYPTOR assays performed comparably or superior to Elecsys (sFlt–1/PlGF area under the curve 0.746 versus 0.735; $P = .09$; for non–obese 0.820 versus 0.805, $P = .047$). For early–onset preeclampsia, KRYPTOR area under the curve increased to 0.929 with a 100% specificity for preeclampsia at cut–off 85 and an 88.9% sensitivity for preeclampsia at cut–off 33. For women with preeclampsia and preterm delivery or Hemolysis, Elevated Liver enzymes, Low Platelet count (HELLP) syndrome, the KRYPTOR sFlt–1/PlGF ratio was manifold increased ($P < .01$). The sFlt–1/PlGF ratio proved especially useful in early–onset preeclampsia, preeclampsia with preterm delivery or HELLP, and among non–obese women. J Am Soc Hypertens 2015;9(2):86–96.

Keywords: Hypertension; obesity; pregnancy; HELLP syndrome.

Introduction

Preeclampsia is a leading cause of maternal and perinatal morbidity and mortality worldwide, with a global incidence of 3%–5% of all pregnancies annually.[1] The etiology is complex, and, in spite of decades of research, the exact mechanisms behind the disease remain unclear. Preeclampsia is currently defined as de novo hypertension (>140/90 mm Hg) in pregnancy and proteinuria (>0.3 g/24h) after 20 weeks of gestation.[2] However, the clinical presentation of preeclampsia, eclampsia, or Hemolysis, Elevated Liver enzymes, Low Platelet count (HELLP) syndrome is very

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diverse,[3–5] and it is not uncommon that women debut with eclampsia or elevated liver enzymes without prior blood pressure elevation or proteinuria. In the last decade, new biomarkers have emerged that potentially could have diagnostic or even predictive properties in preeclampsia, including the placental growth factor (PlGF) and soluble Fms–like tyrosine kinase 1 (sFlt–1),[6,7] both released from the placenta and the maternal endothelium.

sFlt–1 is a scavenger receptor for vascular endothelial growth factor (VEGF)–A, preventing the binding of VEGF and PlGF to their other receptors. A high serum concentration of sFlt–1 is thought to reduce the effect of VEGF–A and PlGF in the maternal circulation, thus exerting an anti–angiogenic function, lowering systemic NO, and increasing endothelin. Indeed, sFlt–1 infusion in pregnant rats leads to preeclampsia–like symptoms,[8] and use of tyrosine kinase VEGF receptor blockers in oncology settings precipitates preeclampsia–like syndrome with proteinuria and hypertension. PlGF is a member of the VEGF family and is released from the placenta and the maternal endothelium. Experimental evidence shows that, although PlGF has only little mitogenic effect alone, it potentiates the effects of VEGF,[9] and studies in PlGF–null mice suggest that PlGF may be important for vasculogenesis in a pathological setting.[10] Contrary to sFlt–1, PlGF is thought to exert a direct proangiogenic function in the maternal circulation, and the levels of PlGF are decreased early in pregnancies later complicated by preeclampsia.[11]

The ratio of sFlt–1/PlGF predicts the development of preeclampsia in some,[12–15] but not all studies.[16]

Failure to detect a correlation between an elevated sFlt–1/PlGF ratio and preeclampsia may reflect an imprecise diagnosis of preeclampsia or a separate disease entity; for example, failure of detecting the differential diagnosis of glomerulonephritis.[17] Moreover, the sFlt–1/PlGF ratio performs less well in multiparas,[18] late gestation,[15] and in women with high body mass index (BMI).[19,20] In addition, manual enzyme-linked immunosorbent assays (ELISAs) show high inter–assay coefficients of variation (10%–20%), in contrast to the <5% seen in the modern automated assays now available in the clinical setting.[21] We aimed to test the performance of novel automated assays for sFlt–1 and PlGF (KRYPTOR, BRAHMS) in diagnosing preeclampsia in an inter–assay comparison with established assays (Elecsys, Roche). Analyses were matched for gestational age between women developing preeclampsia and healthy pregnant controls from a routine clinical setting, and sub–analyses were undertaken for performance in different preeclampsia subgroups.

Methods

We retrospectively included women from two cohorts of pregnant women in Odense, Denmark. Preeclampsia cases were recruited from the Southern Danish Hypertension and Oedema in Pregnancy (SYDHOP) cohort of women with preeclampsia at Odense University Hospital, and from the Odense Child Cohort.[22] Non–hypertensive pregnant controls were recruited from Odense Child Cohort. All women gave informed consent to participate. The study complied with the Helsinki declaration and was approved by the Regional Scientific Ethical Committee for Southern Denmark, no. S–20110146 (SYDHOP) and S–20090130 (Odense Child Cohort).

Both preeclampsia cases and controls were recruited in the time period between January 1, 2010 and December 1, 2013. Inclusion criteria for participation in the SYDHOP cohort as a preeclampsia patient was preeclampsia as defined by repeated blood pressure measurements above 90 mm Hg diastolic and/or 140 mm Hg systolic, accompanied by values of +1 or more for protein in urine on dipstick. No differentiation was made between women with gestational hypertension and superimposed preeclampsia, and women with de novo onset hypertension and preeclampsia. Women with multifold pregnancy and women without serum samples drawn for sFlt–1 and PlGF analysis were excluded. Inclusion criteria for pregnant women from the Odense Child Cohort were residence in Odense municipality and donation of a blood sample either at mid–pregnancy (20–30 weeks of gestation) or at time of delivery (30–42 weeks of gestation).[22] For women with preeclampsia recruited from Odense Child Cohort, hospital files were retrospectively evaluated for verification of the preeclampsia diagnosis according to the same criteria as used in the SYDHOP cohort.

Early–onset preeclampsia was defined as diagnosis given prior to 34 + 0 weeks of gestation; and late–onset if the diagnosis was given from 34 + 0 weeks onwards. Enrolment in study by blood sampling was done after the diagnosis of preeclampsia was given. For controls, women with a diagnosis of preeclampsia, proteinuria before rupture of membranes, hypertension, coagulation defects, multifold pregnancy, and women without sFlt–1 and PlGF analysis were excluded. No exclusions were made for other illnesses such as Crohn's disease, thyroid disease, or diabetes in neither case nor control groups. Cases were matched 1:2 to controls if possible, otherwise 1:1, on the basis of gestational age at blood sampling. The women were matched in the following groups (weeks+days): Group 1, 24+0 to 26+6; group 2, 27+0 to 29+6; group 3, 30+0 to 32+6; group 4, 33+0 to 35+6; group 5, 36+0 to 39+0; and group 6, 39+1 to 41+6.

Blood was collected by antecubital venous puncture in EDTA tubes; serum was isolated after centrifugation and stored at minimum −20°C. No samples had undergone more than one freeze–thaw cycle before analysis.

Description of Assay Methods

Measurements for BRAHMS sFlt–1 and PlGF KRYPTOR assays were performed on the fully automated