

Maternal Serum sFlt1 Concentration Is an Early and Reliable Predictive Marker of Preeclampsia

To the Editor:

Preeclampsia is a leading cause of maternal and fetal morbidity and mortality worldwide. It occurs in two phases: abnormal implantation of the placenta leads to impaired placental blood flow, which in turn induces the release of a critical placental substance into the maternal circulation (1). Clinical onset usually occurs in the third trimester of pregnancy, long after initiation of the underlying process.

Recently, Maynard et al. (2) compared the gene expression profile in placental tissue from women with and without preeclampsia and identified soluble Flt1 (sFlt1), a vascular endothelial growth factor receptor, as a molecule of particular pathophysiological interest. It is now suspected that trophoblastic injury markedly enhances placental sFlt1 production, antagonizing the endothelial protective role of vascular endothelial growth factor and/or placental growth factor and eventually leading to clinical preeclampsia (2, 3). A recent study pointed out that, compared with women with a retrospective diagnosis of normal pregnancy (i.e., without hypertension), preeclamptic women had increased serum sFlt1 several weeks before the onset of clinical disease, suggesting that this protein might be used as a predictive marker for preeclampsia (4).

We measured sFlt1 concentrations in serial serum samples from pregnant women with normal or pathologic pregnancy outcomes, including patients with gestational or chronic hypertension but without preeclampsia. The study population consisted of 23 pregnant women followed prospectively in our Obstetric Gynecology Department between 1996 and 2001. The study was approved by our Institutional Review Board. Inclusion criteria were as follows: available serial serum samples collected through pregnancy (stored at -20°C) and a final diagnosis of

preeclampsia, isolated hypertension, or normal pregnancy. Preeclampsia was defined as the onset, after 20 weeks of gestation, of both hypertension ($>140/90$ mmHg) and proteinuria (>300 mg/L), or proteinuria (>300 mg/L) in a patient with pre-conceptual hypertension. Gestational hypertension was defined as hypertension occurring after 20 weeks of gestation, without proteinuria. Chronic hypertension was defined as preconceptual treated hypertension. Patients with gestational and chronic hypertension were pooled for analysis.

Serum sFlt1 concentrations were measured by an immunoassay (Quantikine; R&D Systems); the person (A.H.) who performed the assay was blinded to the clinical diagnoses of the patients. All measurements were made in duplicate on 1:10 dilutions of the sera. Comparisons between multiple groups were made with the nonparametric Mann-Whitney test, and correlations between data were analyzed by the Spearman test. A P value <0.05 was considered statistically significant.

We analyzed 93 serum samples, from 23 patients, collected at various stages of pregnancy. Eight women had preeclampsia (one of whom had chronic hypertension), either mild ($n = 7$) or severe ($n = 1$). Intrauterine growth retardation was present in one case. Six women had isolated

hypertension (four with chronic hypertension and two with gestational hypertension), and nine women had normal pregnancies.

Mean (SD) serum sFlt1 concentrations at delivery were higher in women with preeclampsia [5332 (3187) ng/L] than in those with normal pregnancies [1483 (1148) ng/L; $P = 0.027$] or isolated hypertension [1607 (1059) ng/L; $P = 0.039$ vs preeclampsia; $P = 0.724$ vs normal pregnancy]. As shown in Fig. 1, sFlt1 concentrations increased gradually throughout pregnancy in women with preeclampsia. No significant difference in sFlt1 concentrations was observed among the three groups before 20 weeks of gestation. In contrast, mean (SD) serum sFlt1 was significantly higher between 25 and 28 weeks of gestation in women with preeclampsia [2779 (1837) ng/L] than in women with normal pregnancies [552 (246) ng/L; $P = 0.028$] or isolated hypertension [544 (359) ng/L; $P = 0.0526$ vs preeclampsia; $P = 0.732$ vs normal pregnancy]. No specific pattern was observed in the group with isolated hypertension.

A sFlt1 cutoff value of 957 ng/L between 25 and 28 weeks of gestation yielded a specificity of 100% and sensitivity of 80% (95% confidence interval, 28.8–96.7%), respectively, for subsequent clinical onset of preeclampsia. Only one of the eight

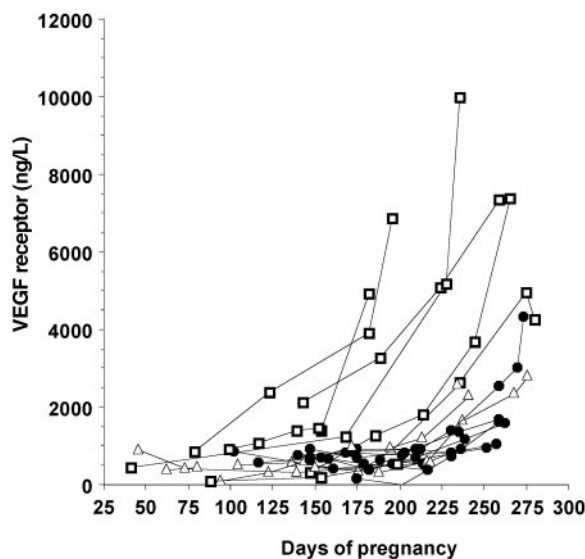


Fig. 1. sFlt1 concentrations (ng/L) in 93 serial serum samples from 23 women with a final diagnosis of normal pregnancy (●), isolated hypertension (△), or preeclampsia (□).

VEGF, vascular endothelial growth factor.

women who went on to develop pre-eclampsia (at 34 weeks of gestation) had sFlt1 values within the reference interval. This woman had abruptio placenta at delivery (36 weeks of gestation). In the remaining seven patients, the sFlt1 assay would have predicted preeclampsia a mean of 11.2 weeks (range, 6.5–16.5 weeks) before clinical onset. No significant difference was observed in mean (SD) serum creatinine concentrations ($\mu\text{mol/L}$) among the three groups [normal pregnancy, 52.5 (8.9); preeclampsia, 57.0 (14.9); isolated hypertension, 54 (16.6)].

Our findings confirm that the maternal serum sFlt1 concentration is markedly increased at delivery in women with preeclampsia and is measurably increased long before clinical onset (minimum of 6.5 weeks before onset). We emphasize the surprising lack of negative predictive value of a sFlt1 concentration within reference values in pregnancies complicated by abruptio placenta. We believe that measurement of sFlt1 could permit early management of at-risk women and could also help to identify women at risk of developing preeclampsia among patients presenting with gestational or chronic hypertension.

References

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3-Deazaadenosine, a Stabilizer of Whole-Blood Homocysteine Content, Does Not Interfere with the Single-Enzyme Homocysteine Assay while Totally Inhibiting the Enzyme Conversion Homocysteine Immunoassay

To the Editor:

Plasma total homocysteine (tHcy) is a risk factor for cardiovascular disease and possibly other diseases (1). Release of homocysteine (Hcy) from erythrocytes into the plasma before measurement remains a problem. The erythrocyte continues to carry out methylation reactions at room temperature in whole blood, producing and exporting Hcy as an end

product while the blood is waiting for processing or during delivery. Export of Hcy from erythrocytes into the plasma is time- and temperature-dependent (2). At room temperature, the increase in plasma tHcy is $\sim 1.0 \mu\text{mol} \cdot \text{L}^{-1} \cdot \text{h}^{-1}$ (2). This corresponds to an $\sim 10\%$ increase per hour in a typical sample containing $10 \mu\text{mol/L}$ tHcy. Therefore, at present, the accuracy of Hcy measurements is compromised even when the plasma is separated within 1 h of sample collection (2). Hill et al. (3) studied the effect of temperature on the stability of plasma tHcy over a 72-h time course in blood collected into evacuated tubes containing either EDTA or 3-deazaadenosine (3-DA) and found that 3-DA is an effective stabilizer of plasma Hcy content. However, because 3-DA prevents Hcy production through competitive inhibition of the enzyme S-adenosylhomocysteine hydrolase (SAHH), 3-DA interferes with popular assays of tHcy that are enzyme-conversion immunoassays based on SAHH (1, 2, 4).

We have previously developed a simple assay method for tHcy (5, 6) that uses a single and specific recombinant homocysteine α,γ -lyase (rHCYase), which produces the analyte H_2S from Hcy. The single-enzyme tHcy assay has received 510(K) clearance. The purpose of this study is to compare the interference of 3-DA on SAHH and rHCYase.

To determine the interference of

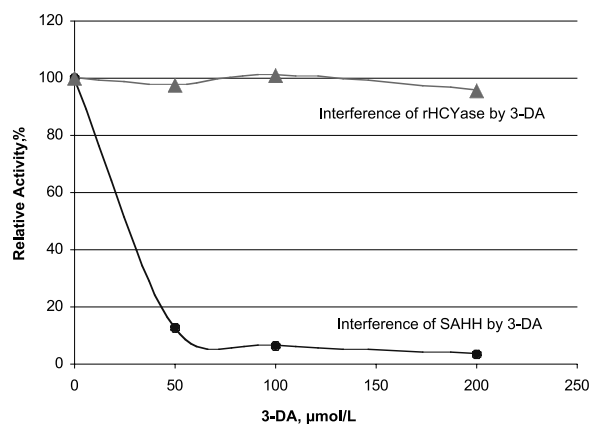


Fig. 1. Interference of 3-DA on SAHH and rHCYase.

The SAHH reaction (●) was carried out with $50 \mu\text{mol/L}$ SAH and 1.3×10^{-3} U of SAHH in assay buffer containing 0, 50, 100, or $200 \mu\text{mol/L}$ 3-DA, as described in the text. Enzyme activity was measured by determining the Hcy produced in the reaction by use of rHCYase, which in turn produced H_2S , which was measured at 675 nm with *N,N*-dibutylphenylenediamine hydrochloride as the chromophore, as described previously (5, 6). The rHCYase reaction (▲) was carried out with $50 \mu\text{mol/L}$ L-Hcy; 0, 50, 100, or $200 \mu\text{mol/L}$ 3-DA; and 0.05 U of rHCYase as described. The resulting H_2S was measured as described above.