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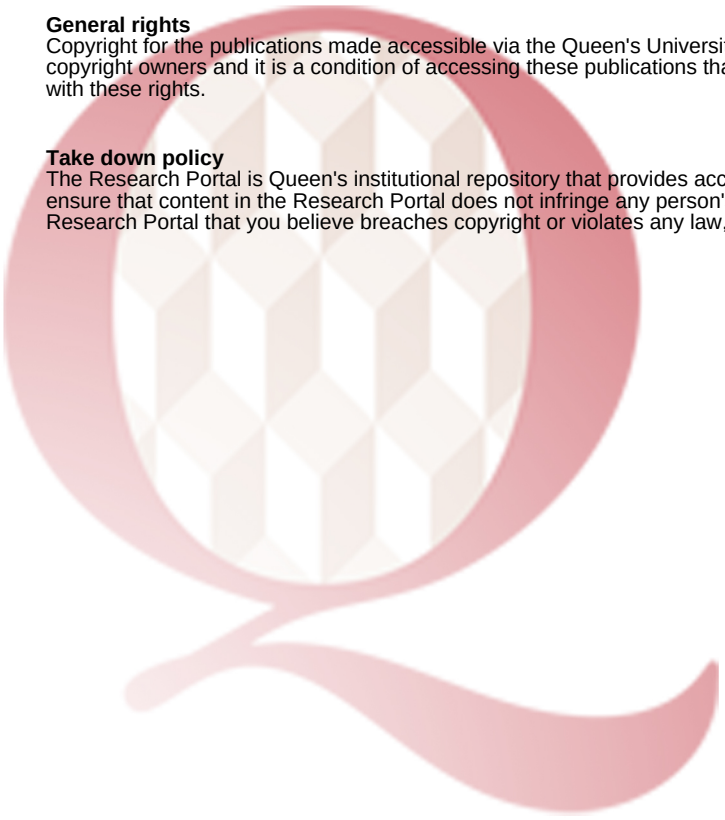

The Role of Angiogenic and Antiangiogenic Factors in the Second Trimester in the Prediction of Preeclampsia in Pregnant Women With Type 1 Diabetes

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OBJECTIVE—To assess the association between circulating angiogenic and antiangiogenic factors in the second trimester and risk of preeclampsia in women with type 1 diabetes.

RESEARCH DESIGN AND METHODS—Maternal plasma concentrations of placental growth factor (PlGF), soluble fms-like tyrosine kinase 1 (sFlt-1), and soluble endoglin (sEng) were available at 26 weeks of gestation in 540 women with type 1 diabetes enrolled in the Diabetes and Preeclampsia Intervention Trial.

RESULTS—Preeclampsia developed in 17% of pregnancies ($n = 94$). At 26 weeks of gestation, women in whom preeclampsia developed later had significantly lower PlGF (median [interquartile range]: 231 pg/mL [120–423] vs. 365 pg/mL [237–582]; $P < 0.001$), higher sFlt-1 (1,522 pg/mL [1,108–3,393] vs. 1,193 pg/mL [844–1,630] $P < 0.001$), and higher sEng (6.2 ng/mL [4.9–7.9] vs. 5.1 ng/mL[(4.3–6.2]; $P < 0.001$) compared with women who did not have preeclampsia. In addition, the ratio of PlGF to sEng was significantly lower (40 [17–71] vs. 71 [44–114]; $P < 0.001$) and the ratio of sFlt-1 to PlGF was significantly higher (6.3 [3.4–15.7] vs. 3.1 [1.8–5.8]; $P < 0.001$) in women who later developed preeclampsia. The addition of the ratio of PlGF to sEng or the ratio of sFlt-1 to PlGF to a logistic model containing established risk factors (area under the curve [AUC], 0.813) significantly improved the predictive value (AUC, 0.850 and 0.846, respectively; $P < 0.01$) and significantly improved reclassification according to the integrated discrimination improvement index (IDI) (IDI scores 0.086 and 0.065, respectively; $P < 0.001$).

CONCLUSIONS—These data suggest that angiogenic and antiangiogenic factors measured during the second trimester are predictive of preeclampsia in women with type 1 diabetes. The addition of the ratio of PlGF to sEng or the ratio of sFlt-1 to PlGF to established clinical risk factors significantly improves the prediction of preeclampsia in women with type 1 diabetes.

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Preeclampsia is characterized by the development of hypertension and new-onset proteinuria during the second half of pregnancy (1,2), leading to increased maternal morbidity and mortality (3). Women with type 1 diabetes are at increased risk for development of preeclampsia during pregnancy, with rates being two-times to four-times higher than that of the background maternity population (4,5). Small advances have come from preventive measures, such as low-dose aspirin in women at high risk (6); however, delivery remains the only effective intervention, and preeclampsia is responsible for up to 15% of preterm births and a consequent increase in infant mortality and morbidity (7).

Although the etiology of preeclampsia remains unclear, abnormal placental vascular remodeling and placental ischemia, together with maternal endothelial dysfunction, hemodynamic changes, and renal pathology, contribute to its pathogenesis (8). In addition, over the past decade accumulating evidence has suggested that an imbalance between angiogenic factors, such as placental growth factor (PlGF), and antiangiogenic factors, such as soluble fms-like tyrosine kinase 1 (sFlt-1) and soluble endoglin (sEng), plays a key role in the pathogenesis of preeclampsia (8,9). In women at low risk (10–13) and women at high risk (14,15), concentrations of angiogenic and antiangiogenic factors are significantly different between women who later develop preeclampsia (lower PlGF, higher sFlt-1, and higher sEng levels) compared with women who do not.

Few studies have specifically focused on circulating angiogenic factors and risk of preeclampsia in women with diabetes, and the results have been conflicting. In a small study, higher sFlt-1 and lower PlGF were reported at the time of delivery in women with diabetes who developed preeclampsia (16). In a longitudinal prospective cohort of pregnant women with diabetes, Yu et al. (17) reported increased sFlt-1 and reduced PlGF in the early third trimester as potential predictors of preeclampsia in women with type 1 diabetes, but they did not show any difference in sEng levels in women with preeclampsia compared with women without preeclampsia. By contrast, Powers et al. (18) reported only increased sEng in the second trimester in women with pregestational diabetes who developed preeclampsia.

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The aim of this study, which was significantly larger than the previous studies highlighted, was to assess the association between circulating angiogenic (PlGF) and antiangiogenic (sFlt-1 and sEng) factors and the risk of preeclampsia in women with type 1 diabetes. A further aim was to evaluate the added predictive ability and clinical usefulness of angiogenic factors and established risk factors for preeclampsia risk prediction in women with type 1 diabetes.

RESEARCH DESIGN AND METHODS

The study population comprised a subset of women with type 1 diabetes who were recruited into the Diabetes and Preeclampsia Intervention Trial (DAPIT) (19) from 25 joint antenatal–metabolic clinics across Northern Ireland, Scotland, and Northwest England between April 2003 and June 2008. DAPIT was a multicenter, randomized, placebo-controlled intervention trial of vitamin C and vitamin E supplementation during pregnancy to prevent preeclampsia in women with type 1 diabetes. Eligibility criteria included type 1 diabetes preceding pregnancy, singleton pregnancy, and age 16 years or older. Women were enrolled in the study between 8 and 22 weeks of gestation, with a mean gestational age at randomization of 14 weeks of gestation (19). Written informed consent was obtained from all women. The West Midlands Multicentre Research Ethics Committee provided ethical approval (MREC 02/7/016).

Preeclampsia was the primary outcome in DAPIT and was defined as gestational hypertension with proteinuria for previously normotensive women according to the International Society for the Study of Hypertension in Pregnancy (ISSHP) guidelines (1) and according to the National High Blood Pressure Education Program Working Groups guidelines for women with preexisting hypertension or proteinuria (2), as previously described (19). Each case of hypertensive pregnancy was confirmed by three senior clinicians acting independently.

Study-specific peripheral venous blood samples were collected from women enrolled in DAPIT at 26 weeks of gestation (±2 weeks) and after separation were stored immediately at −70°C until analysis. Plasma samples were batch-analyzed centrally and concurrently in the Nutrition and Metabolism Laboratories, Centre for Public Health, Queen's University Belfast. Commercially available immunoassay kits from R&D Systems (Minneapolis, MN) were used to measure circulating angiogenic (PlGF) and antiangiogenic (sFlt-1 and sEng) factors. All kits for each analyte were from the same manufacturing batch and laboratory staff members performing the analyses were blind to the pregnancy outcome (preeclampsia or no preeclampsia). Mean interassay coefficients of variation were 8.3% for PlGF, 16.6% for sFlt-1, and 8.6% for sEng.

The original DAPIT trial of vitamin C and vitamin E for the prevention of preeclampsia indicated that antioxidants did not significantly reduce risk of preeclampsia in women with type 1 diabetes (15% vs. 19%; risk ratio, 0.81; 95% CI, 0.59–1.12; $P = 0.20$) (19). There was no significant difference in the concentrations of PlGF, sFlt-1, and sEng between the antioxidant and placebo groups. In addition, there was no significant interaction between treatment group and the angiogenic and antiangiogenic markers in the logistic regression models for preeclampsia risk. Therefore, the treatment group and placebo group were combined for these analyses.

Statistical analysis

Analysis of sEng, sFlt-1, PlGF, ratio of PlGF to sEng, and ratio of sFlt-1 to PlGF was performed after logarithmic transformation and values are reported as median (interquartile range). Comparisons between those who later developed preeclampsia and those who did not were performed using independent sample *t* tests and χ^2 tests. Logistic regression analysis was used to assess the association of the angiogenic and antiangiogenic factors with preeclampsia and was adjusted for established risk factors, including age, BMI, diabetes duration, parity, history of preeclampsia, current smoking, and clinical parameters measured at the time of blood sampling, such as second trimester systolic blood pressure, diastolic blood pressure, HbA_{1c}, and renal status (normoalbuminuria, microalbuminuria, or macroalbuminuria). Analyses also were adjusted for gestation at blood sampling and treatment group assignment in the original DAPIT trial.

To assess the performance of angiogenic and antiangiogenic markers measured in the second trimester for the prediction of preeclampsia in women with type 1 diabetes, receiver operating characteristic analysis was derived from the logistic model, both with and without covariates. In addition, to quantify the added clinical value of these biomarkers in comparison with established clinical risk factors, the integrated discrimination improvement

Table 1—*Maternal characteristics and second trimester clinical profiles in women with and without preeclampsia*

	Preeclampsia, $n = 94$	No preeclampsia, $n = 446$	P
Age, years	29.9 (5.5)	30.1 (5.5)	0.74
Gestational age at second trimester visit	26.1 (1.6)	26.4 (1.5)	0.05
BMI			
BMI, kg/m^2†	27.7 (4.0)	27.6 (5.2)	0.87
Overweight or obese (BMI >25 kg/m^2)	72 (79%)	280 (64%)	0.006
Primiparous	58 (62%)	196 (44%)	0.002
History of preeclampsia	18 (19%)	43 (10%)	0.008
Diabetes duration, years	16.1 (7.3)	14.1 (8.4)	0.04
Current smoker	12 (13%)	84 (19%)	0.16
Systolic BP at second trimester visit, mmHg‡	126.4 (13.1)	118.4 (11.9)	<0.001
Diastolic BP at second trimester visit, mmHg‡	80.6 (8.4)	74.3 (8.2)	<0.001
HbA_{1c} at second trimester visit, %§	6.9 (0.9)	6.7 (0.8)	0.01
HbA_{1c} at second trimester visit, mmol/mol§	52 (10)	49 (9)	0.01
Renal status at second trimester visit‖¶			<0.001
Normoalbuminuria	50 (60%)	349 (87%)	
Microalbuminuria	15 (18%)	33 (8%)	
Macroalbuminuria	18 (22%)	19 (5%)	

Data are mean (SD) or *n* (%) unless otherwise stated. BP, blood pressure. †BMI calculated at a mean age of 14 weeks of gestation. Data available for 91 women with preeclampsia and 437 women without preeclampsia. ‡Data available for 93 women with preeclampsia and 445 women without preeclampsia. §Data available for 91 women with preeclampsia and 431 women without preeclampsia. ‖Data available for 83 women with preeclampsia and 401 women without preeclampsia. ¶Ratio of urine albumin to creatinine (ACR): normoalbuminuria, ACR <3.5 mg/mmol; microalbuminuria, ACR ≥3.5 mg/mmol and <30 mg/mmol; macroalbuminuria, ACR ≥30 mg/mmol.

index (IDI) and net reclassification improvement index (NRI) were calculated for each measure as described by Pencina et al. (20). The NRI requires that preeclampsia risks predicted by the logistic model are assigned to categories. We used the following three categories: <10%; 10–19.9%; and ≥20%. The categorization obtained from a logistic model containing only established risk factors was compared with the categorization obtained after adding the angiogenic factor to the model. The proportion of patients with preeclampsia moved to a higher risk category (net of any moved to a lower risk category) was calculated. Likewise, the proportion of patients without preeclampsia moved to a lower risk category (net of any moved to a higher risk category) was calculated. The sum of these two proportions provided the NRI. The IDI was calculated using the predicted risks without the need for categorization and therefore was not affected by the choice of cutoff values for the categories. It was defined as the average increase in predicted risk among patients with preeclampsia added to the average decrease in predicted risk among patients without preeclampsia. Established risk factors included age, BMI, diabetes duration, parity, history of preeclampsia, smoking status, and second trimester clinical measures such as systolic blood pressure, diastolic blood pressure, HbA_{1c}, and ratio of urine albumin to creatinine. Analyses also were adjusted for gestation at the time of blood sampling and treatment group assignment in the original DAPIT trial. Statistical analyses were performed using SPSS version 17 (SPSS, Chicago, IL) and Stata release 11 (StataCorp, College Station, TX).

RESULTS—A total of 762 women were enrolled in the Diabetes and Preeclampsia Intervention Trial. Blood samples were available for analysis of angiogenic and antiangiogenic factors in the second trimester for 540 women, of whom 94 women (17%) developed preeclampsia, concordant with the overall rate of preeclampsia in the full DAPIT cohort (19). In addition, the preterm delivery rate of 37% in those subjects with available samples was in agreement with the overall rate in the full DAPIT cohort. Of the women delivering before 37 weeks of gestation, 31% had preeclampsia compared with 10% of those delivering after 37 weeks of gestation.

Maternal characteristics and second trimester clinical profiles for women with and without preeclampsia are shown in Table 1. Although there was no significant difference in BMI between the groups, more women with preeclampsia were overweight or obese (BMI >25 kg/m^2). Women with preeclampsia were more likely to be primiparous, to have a history of preeclampsia, and to have a longer duration of diabetes. Women who developed preeclampsia had significantly higher second trimester measurements of systolic blood pressure, diastolic blood pressure, and HbA_{1c} concentrations, and they were more likely to have microalbuminuria or macroalbuminuria. Blood sampling was performed slightly earlier in women who later developed preeclampsia when compared with those who did not (mean [SD] gestational age 26.1 weeks [1.6] vs. 26.4 weeks [1.5]; $P = 0.05$).

Angiogenic and antiangiogenic markers measured during the second trimester were significantly different in women with subsequent development of preeclampsia compared with those who did not develop preeclampsia (Table 2). At 26 weeks of gestation, women who later developed preeclampsia had significantly lower PlGF, significantly higher sFlt-1, and significantly higher sEng compared with women who did not develop preeclampsia. In addition, the ratio of PlGF to sEng was significantly lower and the ratio of sFlt-1 to PlGF was significantly higher in women who later developed preeclampsia. A similar pattern of angiogenic and antiangiogenic factors was evident in women who delivered before and after 37 weeks of gestation, with the exception of sEng, which was more comparable in women with and without preeclampsia who delivered at term (after 37 weeks of gestation) (Table 2).

To further explore the role of angiogenic and antiangiogenic factors, the risk of preeclampsia was examined in relation to quarters of the distribution of each biomarker, both unadjusted and adjusted for established risk factors (Table 3). After adjustment, a significant association remained between the three higher risk quarters and the reference lowest risk quarter for sFlt-1. For sEng, PlGF, ratio of PlGF to sEng, and ratio of sFlt-1 to PlGF, a significant association was evident between the two highest risk quarters compared with the reference lowest risk quarter.

Receiver operating characteristic analysis of angiogenic and antiangiogenic factors was used to assess the predictive value of angiogenic factor and clinical factor measures for preeclampsia (Table 4). The area

Table 2—*Angiogenic and antiangiogenic factors at 26 weeks of gestation in women with type 1 diabetes with subsequent development of preeclampsia*

All women (n = 540)	Preeclampsia (n = 94)	No preeclampsia (n = 446)	P
sEng, ng/mL	6.2 (4.9–7.9)	5.1 (4.3–6.2)	<0.001
sFlt-1, pg/mL	1,522 (1,108–3,393)	1,193 (844–1,630)	<0.001
PlGF, pg/mL	231 (120–423)	365 (237–582)	<0.001
PlGF:sEng ratio	40 (17–71)	71 (44–114)	<0.001
sFlt-1:PlGF ratio	6.3 (3.4–15.7)	3.1 (1.8–5.8)	<0.001
Delivered at <37 weeks of gestation (n = 198)	**Preeclampsia (n = 61)**	**No preeclampsia (n = 137)**	**P**
sEng, ng/mL	6.4 (5.3–8.5)	5.3 (4.5–6.8)	<0.001
sFlt-1, pg/mL	1,583 (1,126–4,260)	1,235 (911–1,698)	<0.001
PlGF, pg/mL	221 (113–465)	371 (227–650)	<0.001
PlGF:sEng ratio	36 (15–69)	70 (39–130)	<0.001
sFlt-1:PlGF ratio	7.1 (3.5–17.6)	3.1 (1.7–7.7)	<0.001
Delivered at ≥37 weeks of gestation (n = 342)	**Preeclampsia (n = 33)**	**No preeclampsia (n = 309)**	**P**
sEng, ng/mL	5.5 (4.3–6.8)	5.0 (4.2–6.1)	0.07
sFlt-1, pg/mL	1,503 (1,050–1,826)	1,183 (818–1,583)	0.02
PlGF, pg/mL	253 (178–369)	364 (240–559)	<0.001
PlGF:sEng ratio	48 (25–83)	72 (47–110)	<0.001
sFlt-1:PlGF ratio	5.8 (3.4 – 8.6)	3.1 (1.9–5.5)	<0.001

Data presented as median (interquartile range).

Table 3—*Odds ratios for preeclampsia according to quarter for sEng, sFlt-1, PlGF, PlGF: sEng ratio, and sFlt-1:PlGF ratio*

Quarter	*n*	Preeclampsia	Unadjusted odds ratio (95% CI)	Adjusted odds ratio* (95% CI)	*P* trend unadjusted/ adjusted
sEng concentration, ng/mL					<0.001/<0.001
Q1: ≤4.37	134	12 (9%)	1.0 (reference)	1.0 (reference)	
Q2: 4.38–5.24	136	18 (13%)	1.6 (0.7–3.7)	1.9 (0.8–4.5)	
Q3: 5.25–6.54	135	26 (19%)	2.4 (1.1–5.5)	2.6 (1.1–6.1)	
Q4: ≥6.55	135	38 (28%)	4.0 (1.9–8.8)	5.7 (2.4–13.3)	
sFlt-1 concentration, pg/mL					<0.001/0.02
Q1: ≤873	135	9 (7%)	1.0 (reference)	1.0 (reference)	
Q2: 874–1,232	135	25 (19%)	3.2 (1.4–8.1)	2.5 (1.1–5.9)	
Q3: 1,233–1,688	135	23 (17%)	2.9 (1.2–7.3)	2.3 (1.0–5.6)	
Q4: ≥1,689	135	37 (27%)	5.3 (2.4–13.0)	3.3 (1.4–7.9)	
PlGF concentration, pg/mL					<0.001/0.001
Q1: <220	135	39 (29%)	3.8 (1.9–8.2)	3.7 (1.6–8.1)	
Q2: 220–346	135	25 (19%)	2.1 (1.0–4.8)	2.4 (1.1–5.3)	
Q3: 347–546	135	17 (13%)	1.4 (0.6–3.2)	1.3 (0.5–3.1)	
Q4: ≥547	135	13 (10%)	1.0 (reference)	1.0 (reference)	
PlGF:sEng ratio					<0.001/<0.001
Q1: ≤39.1	135	43 (32%)	5.8 (2.7–13.7)	7.3 (3.0–17.6)	
Q2: 39.2–65.2	135	24 (18%)	2.7 (1.2–6.6)	3.7 (1.5–9.3)	
Q3: 65.3–108.0	135	17 (13%)	1.8 (0.7–4.6)	2.2 (0.8–5.5)	
Q4: ≥108.1	135	10 (7%)	1.0 (reference)	1.0 (reference)	
sFlt-1:PlGF ratio					<0.001/<0.001
Q1: ≤2.00	135	8 (6%)	1.0 (reference)	1.0 (reference)	
Q2: 2.01–3.50	135	17 (13%)	2.3 (0.9–6.3)	2.1 (0.8–5.6)	
Q3: 3.51–6.55	135	27 (20%)	4.0 (1.7–10.5)	3.8 (1.5–9.5)	
Q4: ≥6.56	135	42 (31%)	7.2 (3.1–18.4)	5.9 (2.4–14.6)	

Q, quarter. *Adjusted for age, treatment group, gestation, BMI, diabetes duration, parity, history of preeclampsia, current smoking, systolic blood pressure, diastolic blood pressure, HbA_{1c}, and ratio of urine albumin to creatinine at second trimester visit.

under the curve (AUC) ranged from 0.652 to 0.711. When established risk factors for preeclampsia were entered into the logistic model, the AUC increased for each measure, ranging from 0.826 to 0.850. The addition of PlGF, ratio of PlGF to sEng, or ratio of sFlt-1 to PlGF to the logistic model containing only established risk factors (AUC, 0.813) significantly improved the predictive value (respectively: AUC, 0.838 [$P = 0.04$]; AUC, 0.850 [$P = 0.008$]; and AUC, 0.846 [$P = 0.008$]).

Finally, the addition of any of the angiogenic and antiangiogenic factors and both angiogenic and antiangiogenic ratios improved the ability of established risk factors to predict preeclampsia using the IDI (Table 4). Using the NRI, although PlGF and PlGF-to-sEng ratio improved the ability of established risk factors to predict risk of preeclampsia, this was not statistically significant ($P = 0.08$ and $P = 0.07$, respectively). The improvement in predicted risk of preeclampsia (as measured by the IDI) and in the reclassification of preeclampsia risk (as measured by the NRI) for the DAPIT cohort for PlGF-to-sEng ratio are shown in Fig. 1.

CONCLUSIONS—This is the largest study to date that has examined the role of angiogenic and antiangiogenic factors in the prediction of preeclampsia among pregnant women with type 1 diabetes. We observed higher sFlt-1, higher sEng, lower PlGF, and significantly altered angiogenic and antiangiogenic ratios during the second trimester in women who later developed preeclampsia compared with those who did not. Furthermore, we have shown for the first time that the addition of angiogenic and antiangiogenic markers, particularly PlGF, ratio of PlGF to sEng, and ratio of sFlt-1 to PlGF, to established clinical risk factors improves the prediction of preeclampsia within this population. Our findings build on previously published data within the DAPIT cohort in relation to glycemic control and risk of preeclampsia (21) and suggest that it may be feasible, in the future, to devise a risk assessment model for preeclampsia in women with type 1 diabetes.

Concordant with other studies of the general obstetric population involving women at low risk (10–13) and women at high risk (14,15), we found significantly different concentrations of angiogenic and antiangiogenic markers in women with type 1 diabetes who later developed preeclampsia (lower PlGF, higher sFlt-1, and higher sEng levels) compared with those who did not. Previous reports of angiogenic markers for the prediction of preeclampsia in women with diabetes have been conflicting (17,18) and inconsistent compared those using general obstetric populations (10–15). Powers et al. (18) reported significantly higher sEng at 26–30 weeks of gestation but no difference in PlGF or sFlt-1. However, Yu et al. (17) reported a significant decrease in PlGF and a significant increase in sFlt-1 at 32 weeks of gestation but no difference in sEng in women with diabetes who later developed preeclampsia compared with those who did not. It is unclear why these results (17,18) differ from those of DAPIT; however, it may be that numbers in these studies were too small to detect significant differences compared with the much larger DAPIT cohort. Furthermore, there are subtle differences in subject characteristics in these other studies that may affect biomarker status. For example, whereas women in the study by Yu et al. (17) had type 1 diabetes, those with microalbuminuria or other complications at baseline were excluded. In the other study (18), women were reported to have pregestational insulin-treated diabetes and, as such, may have included women with both type 1 and type 2 diabetes, between whom the pathogenesis of preeclampsia may differ. In our study, women who developed preeclampsia, irrespective of gestational age at delivery, had significantly higher sEng at 26 weeks of gestation compared with women who did not. Although sEng was significantly higher only in those women with preeclampsia who delivered before 37 weeks of gestation, a similar trend was observed in women delivering with preeclampsia after 37 weeks of gestation, although this did not reach statistical significance ($P = 0.07$).

Table 4—*Areas under the ROC curve, IDI, and NRI for logarithmically transformed angiogenic and antiangiogenic variables in a logistic regression analysis with other established risk factors

	Area under the ROC curve without covariates	Area under the ROC curve with covariates in logistic model	IDI	NRI
	AUC	AUC_1 (P†)	Score (P)	Score (P)
sEng	0.665	0.839 (0.07)	0.071 (<0.001)	0.048 (0.36)
sFlt-1	0.652	0.826 (0.13)	0.025 (0.007)	−0.003 (0.95)
PlGF	0.675	0.838 (0.04)	0.057 (<0.001)	0.103 (0.08)
PlGF:sEng ratio	0.703	0.850 (0.008)	0.086 (<0.001)	0.103 (0.07)
sFlt-1:PlGF ratio	0.711	0.846 (0.008)	0.065 (<0.001)	0.072 (0.20)

ROC, receiver operating characteristic; IDI, integrated discrimination improvement; NRI, net reclassification improvement. *Established risk factors (covariates) were age, BMI, diabetes duration, parity, history of preeclampsia, smoking status, and second trimester clinical measures such as systolic blood pressure, diastolic blood pressure, HbA_{1c}, and ratio of urine albumin to creatinine. Covariates also included treatment group and gestation. †Relative to AUC of 0.813 for a logistic model containing only the covariates.

Previous studies have reported the predictive performance of the sFlt-1-to-PlGF ratio for early-onset preeclampsia in a heterogeneous group of high-risk pregnancies (15) and among women with chronic hypertension (22). The PlGF-1-to-sEng ratio in the second trimester also was recently reported as a novel and promising diagnostic tool for prediction of early-onset preeclampsia in singleton pregnancies (23), although numbers were small. In the current study, we have been able to extrapolate these findings to a well-characterized population of women with type 1 diabetes. Our findings also demonstrate that in women with type 1 diabetes, ratios have better diagnostic accuracy than any of the individual factors alone. In the current study, the predictive AUC values for angiogenic and antiangiogenic factors among all women in the DAPIT cohort are similar to those reported in high-risk pregnancies in which preeclampsia developed at less than 37 weeks of gestation (14).

In clinical practice, traditional factors such as age, BMI, diabetes duration, parity, history of preeclampsia, blood pressure, HbA_{1c}, and ratio of urine albumin to creatinine are often considered to identify women with diabetes at high risk for development of preeclampsia, although no specific preeclampsia risk function is yet available. In our DAPIT cohort, the AUC was 0.813 for these established risk factors, endorsing their clinical utility and the importance of careful monitoring of women with diabetes throughout pregnancy. Perhaps most importantly, we observed that measures of angiogenic and antiangiogenic activity (PlGF, ratio of PlGF to sEng, and ratio of sFlt-1 to PlGF) significantly improved the AUC above and beyond traditional risk factors. Clinically, the question is whether any of these potential biomarkers, when added to current established risk factors, will offer a better estimate of an individual woman's risk, thus leading to more effective monitoring, early intervention, and possible selection of subjects for future clinical trials. In the current study, the IDI and NRI were used to examine whether the addition of the biomarker of interest to established risk factors would reclassify individuals into higher and lower risk categories for future preeclampsia (20). Each of the angiogenic and antiangiogenic factors and their ratios significantly improved discrimination as measured by the IDI, whereas PlGF and PlGF-to-sEng ratio improved overall reclassification of women into the correct risk category, albeit not significantly. However, in the absence of established risk thresholds for treatment of preeclampsia, the NRI categorization is arbitrary and, thus, the IDI is considered the more relevant index. Whereas these data suggest that PlGF, ratio of PlGF to sEng, and ratio of sFlt-1 to PlGF may be useful biomarkers of future preeclampsia risk in women with type 1 diabetes, this needs to be validated in another prospective cohort (24).

The DAPIT study population is one of the largest contemporary prospective datasets of women with type 1 diabetes (19), comprising a carefully characterized population of women with type 1 diabetes. A major strength of this study is the extent to which the ISSHP definition of preeclampsia criteria has been applied, with each hypertensive pregnancy being rigorously reviewed and each diagnosis of preeclampsia being confirmed by three senior clinicians. Another strength of the study is the inclusion of all women with type 1 diabetes, including those with essential hypertension, microalbuminuria, or macroalbuminuria. This allows us to extrapolate the findings to the majority of women with type 1 diabetes attending for joint antenatal–metabolic care.

Nevertheless, our study has a number of limitations. Although the inclusion criteria, as noted, were intended to facilitate recruitment, the subjects were a self-selecting population who agreed to participate and, thus, may not be totally representative of the entire population of women with type 1 diabetes. Because the DAPIT intervention of vitamin C and vitamin E did not significantly reduce the incidence of preeclampsia, both treatment groups were combined for analysis, albeit with adjustment for treatment group in the logistic regression model. The study only analyzed and reported angiogenic and antiangiogenic factors measured at one time-point during pregnancy, late in the second trimester. Longitudinal measurement of angiogenic factors would allow analysis of serial changes during pregnancy and may significantly add to the predictive values of some of these biomarkers. Likewise, uterine artery Doppler measurements were not available for these women and inclusion of such studies would benefit future exploration and validation of a clinically useful predictive model. Finally, it was not possible to date the onset of preeclampsia accurately; therefore, the data were analyzed by subgroups of women with preeclampsia according to gestational age at delivery. Nonetheless, regardless of gestational age when preeclampsia was diagnosed, we have demonstrated the clinical usefulness of PlGF, ratio of PlGF to sEng, and ratio of sFlt-1 to PlGF in the entire DAPIT cohort and not just in those who delivered at early gestation ages, as observed in other studies (15,22,23).

Analysis of this large cohort of women with type 1 diabetes confirms that those at risk for preeclampsia have lower PlGF, higher sFlt-1, and higher sEng levels during the second trimester as previously observed in women at low risk (10–13) and women at high risk (14,15). Furthermore, this study would suggest that these markers may have additional predictive risk above and beyond traditional clinical risk factors. Validation of the potential usefulness of PlGF, ratio of PlGF to sEng, and

A IDI for PlGF:sEng ratio

Average predicted probability of preeclampsia for 94 cases before including PlGF:sEng ratio in the model = 0.343

Average predicted probability of preeclampsia for 94 cases after including PlGF:sEng ratio in the model = 0.414

Improvement in predicted probability in 94 cases = 0.414 – 0.343 = 0.071

Average predicted probability of preeclampsia for 446 non-cases before including PlGF:sEng ratio in the model = 0.138

Average predicted probability of preeclampsia for 446 non-cases after including PlGF:sEng ratio in the model = 0.123

Improvement in predicted probability in 446 non-cases =0.138 – 0.123 = 0.015

Integrated discrimination improvement index (IDI) = 0.071 + 0.015 = 0.086

B NRI for PlGF:sEng ratio

		Preeclampsia risk predicted using both established risk factors and PlGF:sEng ratio		
Preeclampsia risk predicted using only established risk factors		Low	Intermediate	High
Cases (n=94)				
	Low	6	1	1
	Intermediate	6	6	10
	High	1	4	59
Non-cases (n=446)				
	Low	219	19	6
	Intermediate	50	40	14
	High	10	20	68

Proportion of cases reclassified in correct direction (toward higher risk) = 12/94

Proportion of cases reclassified in wrong direction (toward lower risk) = 11/94

Difference in proportion of cases reclassified = 1/94 = 0.011

Proportion of non-cases reclassified in correct direction (toward lower risk) = 80/446

Proportion of non-cases reclassified in wrong direction (toward higher risk) = 39/446

Difference in proportion of non-cases reclassified =41/446 = 0.092

Net reclassification improvement index (NRI) = 0.011 + 0.092 = 0.103

Figure 1—*IDI* (A) *and NRI* (B) *scores obtained by adding the PlGF-to-sEng ratio to a logistic regression model for preeclampsia containing established risk factors. For the NRI, risk categories were low <10%, intermediate 10–20%, and high >20%. Light gray boxes represent an improvement in reclassification (reclassified in the correct direction) and dark gray boxes represent the opposite. Unshaded boxes show no change in classification.*

ratio of sFlt-1 to PlGF in other diabetes cohorts is now indicated with the aim of deriving a predictive risk score for women at risk for preeclampsia.

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V.A.H. wrote the manuscript, researched data, and reviewed and edited the manuscript. I.S.Y., C.C.P, M.J.A.M., D.W.M.P., J.D.W., and D.R.M. researched data, contributed to discussion, and reviewed and edited the manuscript. V.A.H. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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