Hypertension

PREECLAMPSIA

PlGF (Placental Growth Factor) Testing in Clinical Practice

Evidence From a Canadian Tertiary Maternity Referral Center

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ABSTRACT: There is little evidence evaluating angiogenic growth factor testing in real-world obstetric settings. This investigation evaluated maternal and perinatal pregnancy outcomes associated with maternal PlGF (placental growth factor) levels in real-world clinical care of high-risk pregnancies. From March 2017 to December 2019, 979 pregnant women with suspected risk of placental dysfunction, hypertensive disorders of pregnancy, or fetal growth restriction completed PlGF testing between 20+0 and 35+6 weeks of gestation. Maternal, fetal, and delivery characteristics were extracted through the electronic medical record system. The primary outcome of preterm birth was assessed using Royston-Parmar survival models and summarized with Kaplan-Meier methods. Of the 979 pregnant women, 289 had low PlGF levels (29.5%), and 690 had normal PlGF levels (70.5%). The survival probability of ongoing pregnancy free from preterm birth within 2- and 4-weeks following PlGF testing was significantly reduced in women with low PlGF levels, relative to women with normal PlGF levels (0.57 versus 0.99, standardized survival difference, −0.43 [95% CI, −0.76 to −0.09], and 0.37 versus 0.99, standardized survival difference, −0.62 [95% CI −0.87 to −0.38], respectively). Women with low PlGF levels were more likely to develop early-onset preeclampsia (adjusted odds ratio, 58.2 [95% CI, 32.1−105.4]) and have a stillbirth (adjusted odds ratio, 15.9 [95% CI, 7.6–33.3]). PlGF status distinguished placental from fetal causes of stillbirth. Low PlGF levels in high-risk pregnant women are strongly associated with increased rates of imminent preterm birth, as well as related adverse outcomes, including early-onset preeclampsia and stillbirth. **(*Hypertension*. 2021;77:2057–2065. DOI: 10.1161/HYPERTENSIONAHA.121.17047.)** • **Data Supplement**

Key Words: hypertension ■ placental growth factor ■ preeclampsia ■ pregnancy outcomes ■ stillbirth

Preeclampsia represents the most clinically severe manifestation of hypertension in pregnancy and remains a major cause of maternal morbidity and mortality on a global scale.[1] The clinical management of women at risk of preeclampsia needs improvement to minimize the significant risks of maternal and perinatal adverse outcomes associated with preeclampsia and underlying placental disease, including iatrogenic preterm birth, fetal growth restriction, and stillbirth.[2]

Measurement of circulating angiogenic growth factor levels in the maternal circulation is an important advancement for the accurate prediction and timely diagnosis of preeclampsia.[3–9] The dysregulated trophoblast surface covering the abnormal placental villi, typically characterized as maternal vascular malperfusion disease, mediate elevations of antiangiogenic sFlt-1 (soluble fms-like tyrosine kinase-1) protein levels that result in suppression of circulating proangiogenic PlGF (placental growth factor) protein levels in women who develop preeclampsia.[10,11] The resultant imbalance in circulating maternal angiogenic growth factors is hypothesized to promote systemic maternal endothelial dysfunction, manifesting as vasoconstriction, hyperpermeability, and impaired perfusion of critical maternal organs and the placenta.[12,13]

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Novelty and Significance

What Is New?

- Low PlGF (placental growth factor) status in pregnant women was associated with markedly higher rates of imminent preterm birth, with 43% of this cohort delivering preterm 2 weeks following PlGF testing, relative to 1% of women with normal PlGF status.
- PlGF status distinguished placental from fetal causes of stillbirth.

What Is Relevant?

- The integration of PlGF testing into clinical care has the potential to provide clinicians with practical knowledge regarding risk of pregnancy progression and the opportunity to tailor clinical management.

Summary

This investigation provides real-world evidence that complements previous research to support the integration of PlGF testing in high-risk pregnancy care.

Nonstandard Abbreviation and Acronyms

AFP	alpha-fetoprotein
ALT	alanine aminotransferase
AST	aspartate aminotransferase
hCG	human chorionic gonadotropin
PAPP-A	pregnancy-associated plasma protein A
PlGF	placental growth factor
sFlt-1	soluble fms-like tyrosine kinase-1

The introduction of angiogenic protein testing in the antenatal setting has the potential to profoundly improve clinical outcomes in women at high risk of preeclampsia by providing obstetricians with an objective tool to inform effective management strategies.[5] Acquiring real-time insight into the underlying maternal phenotype provides an opportunity for a tailored-approach to stratify patients into well-established care pathways.[14–16]

Angiogenic testing has yet to be widely integrated into standard clinical care. Previous research has investigated PlGF testing as a screening tool for the prediction of disease in asymptomatic women and women presenting with suspected preeclampsia, as well as a diagnostic tool to accurately identify clinical disease in women presenting with suspected preeclampsia.[3–6,8] Importantly, the integration of PlGF testing into standard clinical care has been investigated in women with suspected preeclampsia or fetal growth restriction, as well as women with chronic disease.[7,9] However, there remains a lack of evidence to support the use of PlGF testing in a real-world clinical setting in an unselected high-risk obstetric population outside of a research protocol. High-risk clinicians require more real-world insight into the clinical significance of abnormal PlGF levels in high-risk pregnant women to justify the widespread use of PlGF testing.

The objective of this investigation was to evaluate maternal and perinatal pregnancy outcomes associated with maternal PlGF levels in a large tertiary perinatal institution, with testing available in real-world clinical care of high-risk pregnancies.

METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Integration of Real-Time PlGF Testing

In March 2017, Mount Sinai Hospital launched real-time PlGF testing within the central clinical laboratory (Elecsys platform, Roche Diagnostics, Penzberg, Germany). Test results were made available in the patient's hospital electronic medical record system (Cerner PowerChart) to the referring physician and clinical care team within 2 hours. Based on previous research, a single pragmatic cutoff value of 100 pg/mL was used as the clinical threshold to categorize women as having normal PlGF levels (≥100 pg/mL) or low PlGF levels (<100 pg/mL).[5,6] PlGF testing was made available to managing clinicians based at Mount Sinai Hospital in the following settings: general antenatal clinics, maternal-fetal medicine clinics, hospital triage unit, antenatal high-risk ward, and in the labor and delivery environment.

High-risk pregnancy clinicians were recommended to order PlGF testing between 20+0 and 35+6 weeks of gestation as part of standard assessment of pregnant women suspected to be at risk of placental dysfunction, hypertensive disorders of pregnancy, or fetal growth restriction. Patients were identified as high-risk based on a standard evaluation that would prompt referral to maternal-fetal medicine evaluation, including prepregnancy health (autoimmune disorders, obesity, chronic hypertension, advanced reproductive age, diabetes), obstetric history (history of placental dysfunction, hypertensive disorders of pregnancy, fetal growth restriction, stillbirth, unexplained loss >20 weeks of gestation), and assessments in the current pregnancy (abnormal first-trimester screening, multimarker test abnormalities found during screening for Down Syndrome, abnormal ultrasound). PlGF testing between 20+0 and 35+6 weeks of gestation was also recommended for patients presenting to clinic or triage with suspected placental dysfunction, hypertensive disorders of pregnancy, or fetal growth restriction, as part of the standard workup.

Abnormal PlGF levels prompted managing clinicians to undertake a comprehensive maternal-fetal evaluation, similar to other abnormal findings in standard clinical assessments of placental dysfunction, hypertensive disorders of pregnancy, or fetal growth restriction. No specific recommendations were provided to managing clinicians based on PlGF test results regarding clinical management, antenatal surveillance, preventative or treatment therapies, or timing of delivery. Patient management was not based on PlGF testing alone but taken into consideration with standard assessments as part of the overall clinical workup.

Participant Selection

Between March 2017 and December 2019, 979 high-risk pregnant women with a live, singleton fetus completed PlGF testing between 20+0 and 35+6 weeks of gestation had a PlGF test and subsequently delivered at Mount Sinai Hospital. This study was reviewed and approved by the Human Subjects Ethics Review Committee of Mount Sinai Hospital (REB 17-0120-E); informed consent was not required.

Extraction of Patient Information From Electronic Medical Records Audit Platform

A data query was constructed within our hospital's electronic patient record system (Cerner PowerChart) to evaluate the integration of PlGF testing. The main trigger to appropriately identify patients for the query was completion of a PlGF test; the query was then amplified to include maternal, fetal, and delivery data documented in the medical record system.

Information was collected regarding maternal age, ethnicity, and obstetric history. Data regarding biochemical characteristics were also collected, including first-trimester testing biomarkers (PAPP-A [pregnancy-associated plasma protein A], hCG [human chorionic gonadotropin], and AFP [alpha-fetoprotein]), recorded as multiple of median values. All maternal measurements of systolic blood pressure, diastolic blood pressure, platelet count, hemoglobin, renal insufficiency (serum creatinine), liver enzymes (ALT [alanine aminotransferase] and AST [aspartate aminotransferase]), uric acid, and proteinuria (urine protein:creatinine ratio) were collected.

Exposure

The primary exposure was low PlGF, defined as serum PlGF levels <100 pg/mL. The nonexposed group was categorized as the normal PlGF group, with serum PlGF levels ≥100 pg/mL. For women who had >1 PlGF test completed, the earliest PlGF test completed after 20+0 weeks of gestation was utilized to define exposure.

Outcomes

The primary outcome of this investigation was preterm birth <37 weeks of gestation. Secondary maternal and perinatal outcomes included hypertensive diagnosis, mode of delivery, birthweight <10th centile, stillbirth, and abnormal Appearance, Pulse, Grimace, Activity, and Respiration (APGAR) scores.

Preterm birth was defined as delivery <37+0 weeks of gestation. Normotensive pregnancy was defined by systolic blood pressure <140 mm Hg or diastolic blood pressure <90 mm Hg before delivery. Gestational hypertension was defined as systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg on 2 occasions at least 4 hours apart after 20+0 weeks of gestation.[17] Preeclampsia was defined as systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg on 2 occasions at least 4 hours apart after 20+0 weeks of gestation, with evidence of related organ injury: proteinuria (urine protein:creatinine ratio ≥30 mg/mmol), thrombocytopenia (platelet levels $<100\times10^9$/L), renal insufficiency (serum creatinine ≥1.1 mg/dL), or impaired liver function (AST ≥70 U/L or ALT ≥70 U/L).[17] Late-onset preeclampsia was defined as preeclampsia with delivery ≥34+0 weeks of gestation, whereas early-onset preeclampsia was defined as preeclampsia with delivery <34+0 weeks of gestation.

Delivery information collected included gestational age at delivery, mode of delivery, fetal sex, birthweight, APGAR scores, and neonatal outcome. Customized birthweight centiles were calculated utilizing Intergrowth-21st, based on fetal sex, gestational age at delivery, and birthweight.[18] Small for gestational age was defined as birthweight <10th centile for gestation.[19]

Placental pathology reports of stillbirth deliveries were evaluated to determine stillbirth pathogenesis and any major placental pathology findings, according to the Amsterdam Criteria of Standardized Placental Classification.[20]

Statistical Analysis

Maternal, fetal, and pregnancy outcomes were summarized as medians and interquartile ranges, or frequencies and percentages as appropriate.

All pregnancies were considered at risk of preterm birth starting at 20+0 weeks of gestation up to and including 36+6 weeks. Ongoing pregnancies were right-censored from 37+0 weeks onward, as they were no longer at risk of preterm birth after this gestational age. As PlGF testing was completed between 20+0 and 36+6 weeks of gestation, time-to-event data were structured by splitting pregnancy-time at risk in weeks based on exposure status: unexposed included time before the PlGF test, and time after a normal PlGF test result, and exposed included the time following a low PlGF test result. Time-to-event data were evaluated with the use of Kaplan-Meier estimates and Royston-Parmar flexible parametric hazards models.[21] The latter used restricted cubic splines to model a continuous hazard function, rather than a step function as in traditional Cox proportional hazards models. In addition, the use of splines allows for modeling of hazards with more complex functional forms.[21,22] We calculated the baseline hazard function (Figure S3 in the Data Supplement) and survival (the probability of ongoing pregnancy free from preterm birth) with 95% CI, and 2-sided *P* values, adjusting for maternal age, parity status, and gestational age at the time of PlGF testing. Standardized survival curves and standardized survival differences were calculated to compare the probability of ongoing pregnancy between low PlGF and normal PlGF exposure status at predetermined times: 1, 2, 4, 8, and 12 weeks following PlGF testing.[23] Models were adjusted for maternal age, parity, and the gestational age at the time of PlGF testing. We evaluated models with between 2 to 5 spline knots, spaced at equal centiles of uncensored log-survival time. Violation of the proportional hazards assumption was assessed with postestimation

tests of Schoenfeld residuals and graphically by comparing Kaplan-Meier observed survival curves versus Cox predicted curves and log-negative log plots for categorical variables.[24] Covariates that violated the proportional hazards assumption were included as a time-varying covariate. The interaction with time was also modeled flexibly using a second restricted cubic spline, again evaluating models with between 2 and 5 equally spaced internal knots. The final model was selected based on the lowest Akaike information criterion and Bayesian information criterion values. These results excluded women with impossible data, due to completion of their earliest PlGF test on the same day as delivery.

A mixed model was utilized for analysis of earliest PlGF levels across gestation between women with normal blood pressure and women with hypertensive disorders of pregnancy (gestational hypertension, late-onset preeclampsia, and early-onset preeclampsia) and estimated the least-squares mean differences between outcomes. Odds ratios for maternal pregnancy outcomes, mode of delivery, and perinatal outcomes with corresponding 95% CI were calculated to compare outcome risk between women with normal and low PlGF levels, adjusted by maternal age, ethnicity, and parity status. Fisher exact test was utilized to compare stillbirth pathogenesis between women with normal and low PlGF levels.

Analyses were completed with SAS software, version 9.4 (SAS Institute) and Stata software, version 13.1 (StataCorp).

RESULTS

Patients

Demographic characteristics are summarized in Table 1. Of the 979 high-risk pregnant women identified, 289 women were included in the low PlGF group (29.5%), and 690 women were included in the normal PlGF group (70.5%).

Although the majority of women identified as White, their fraction in our cohort (32.9%) illustrated the ethnically diverse population of Toronto. The median gestational age at earliest PlGF test was 29.9 weeks (interquartile range, 24.5–33.2). Women with low PlGF levels were characterized by a classic preeclampsia phenotype, with increased blood pressure and aberrant renal and liver function (Table 1). The median gestational age at delivery of women with low PlGF levels was six weeks earlier in women than women with normal PlGF levels (31 weeks [28–34] versus 37 weeks [36–38]). Birthweight and customized birthweight centile were also lower in women with low PlGF levels, relative to women with normal PlGF levels (1.2 kg [0.8–1.7] versus 2.8 kg [2.3–3.3], and 7% [2–19] versus 47% [16–72], respectively).

Of the 979 pregnant women in this investigation, 374 (38.2%) had a normotensive pregnancy outcome, 244 (24.9%) women developed gestational hypertension, 189 (19.3%) women developed late-onset preeclampsia, and 172 (17.6%) women developed early-onset preeclampsia. A total of 58 women (5.9%) subsequently had a stillbirth (Table 2).

Table 1. Characteristics of High-Risk Pregnant Women With Normal PlGF Levels (≥100 pg/mL) and Low PlGF Levels (<100 pg/mL) Between 20+0 and 35+6 wk of Gestation

Maternal characteristics	Normal PlGF levels N=690	Low PlGF levels N=289
Demographic and clinical characteristics		
Age, y	35 [32–38]	35 [31–39]
Ethnicity, n (%)		
White	246 (36)	76 (26)
Black	69 (10)	16 (6)
Asian	28 (4)	3 (1)
South Asian	80 (11)	38 (13)
Other	76 (11)	16 (6)
Not reported	191 (28)	140 (48)
Nulliparous, n (%)	308 (45)	154 (53)
Biochemical characteristics		
First-trimester screening		
PAPP-A, MoM	0.82 [0.6–1.2]	0.67 [0.5–1.0]
Highest systolic blood pressure, mm Hg	146 [132–161]	164 [152–177]
Highest diastolic blood pressure, mm Hg	94 [85–103]	103 [96–110]
Highest protein:creatinine ratio	20 [13–38]	100 [25–380]
Highest ALT, U/L	20 [13–35]	32 [19–82]
Highest AST, U/L	22 [17–32]	34 [23–67]
Lowest platelet, $\times 10^9$/L	188 [153–223]	167 [115–202]
Highest creatinine, mg/dL	0.66 [0.6–0.8]	0.76 [0.6–0.9]
Lowest hemoglobin, g/L	102 [91–112]	103 [95–114]
Highest uric acid, µmol/L	296 [238–369]	389 [315–464]

Data are presented as median [interquartile range], or n (% of column). ALT indicates alanine aminotransferase; AST, aspartate aminotransferase; MoM, multiple of median; PAPP-A, pregnancy-associated plasma protein A; and PlGF, placental growth factor.

Preterm Birth Survival Analysis

The probability of ongoing pregnancy free from preterm birth is shown in Figure 1. A total of 529 women (54.0%) had a preterm birth; 92.4% of women with low PlGF levels; and 38.0% of women with normal PlGF levels had a preterm birth.

The probability of ongoing pregnancy 2 weeks following PlGF testing was significantly reduced in women with low PlGF levels, relative to women with normal PlGF levels (0.57 versus 0.99, standardized survival difference, −0.43 [95% CI, −0.76 to −0.09]); Figure 1 and Table S1). Survival continued to diminish in low PlGF exposed pregnancies, such that by 12 weeks following PlGF testing, the survival probability was 0.05 versus 0.91 in pregnancies with normal PlGF levels (standardized survival difference, −0.86 [95% CI, −0.91 to −0.82]). Preterm birth hazard ratios were increased with younger maternal age and increasing parity; there was no association with gestational age at time of PlGF testing (Table S1). The

Table 2. Adjusted Odds Ratios for Maternal Pregnancy Outcomes, Mode of Delivery, and Perinatal Outcomes of High-Risk Pregnant Women With Low PlGF Levels (<100 pg/mL) Between 20+0 and 35+6 wk of Gestation, Relative to High-Risk Pregnant Women With Normal PlGF Levels (≥100 pg/mL)

Outcomes	Normal PlGF levels PlGF≥100 N=690	Low PlGF levels PlGF<100 N=289	Odds ratio (95% CI)
Maternal pregnancy diagnosis			
Normotensive pregnancy, n (%)	327 (87)	47 (13)	…
Gestational hypertension, n (%)	197 (81)	47 (19)	1.6 (1.0–2.5)
Late-onset preeclampsia (≥34 wk of gestation), n (%)	147 (78)	42 (22)	2.0 (1.2–3.1)
Early-onset preeclampsia (<34 wk of gestation), n (%)	19 (11)	153 (89)	58.2 (32.1–105.4)
Mode of delivery*			
Spontaneous vaginal delivery, n (%)	234 (73)	86 (27)	…
Operative vaginal delivery, n (%)	46 (90)	5 (10)	0.2 (0.1–0.6)
Elective cesarean, n (%)	171 (85)	29 (15)	0.5 (0.3–0.8)
Unplanned/emergency cesarean, no (%)	184 (54)	155 (46)	2.1 (1.5–3.0)
Perinatal outcomes			
Birthweight centile, n (%)†			
≥10th centile	565 (82)	124 (18)	…
<10th centile	125 (43)	165 (57)	6.4 (4.6–8.8)
Fetal outcome*			
Live birth, n (%)	670 (74)	239 (26)	…
Stillbirth, n (%)	10 (17)	48 (83)	15.9 (7.6–33.3)
APGAR score*			
APGAR 5 min ≥6	645 (75)	211 (25)	…
APGAR 5 min <6	18 (37)	30 (63)	5.8 (3.0–11.0)

Outcomes were adjusted by maternal age, ethnicity, and parity status. Data are presented as n (% of row) and odds ratio (95% CI). PlGF indicates placental growth factor.

*Mode of delivery data were missing from 55 women with normal PlGF levels and 14 women with low PlGF levels. Fetal outcome data were missing from 10 women with normal PlGF levels and 2 women with low PlGF levels. APGAR score data were missing from 27 women with normal PlGF levels and 48 women with low PlGF levels.

†Customized birthweight centiles were calculated utilizing Intergrowth-21st, based on fetal sex, gestational age at delivery, and birthweight.[18]

probability of ongoing pregnancy free from preterm birth stratified into 4-week blocks by gestational age testing window is shown in Figure S1.

Gestational Changes in PlGF Levels

Figure 2 shows longitudinal PlGF levels across gestation, stratified by hypertensive diagnosis. PlGF levels of women with any hypertensive disorder of pregnancy differed significantly across gestation, relative to normotensive pregnant women (Figure 2 and Figure S2). Women who developed early-onset preeclampsia exhibited significant suppression of PlGF levels across gestation, relative to women with gestational hypertension and late-onset preeclampsia (Figure 2 and Figure S2).

Maternal, Fetal, and Delivery Outcomes

The risks of maternal, fetal, and pregnancy outcomes by maternal PlGF level status are presented in Table 2. Relative to normotensive pregnant women, women with low PlGF levels were more likely to develop gestational hypertension (adjusted odds ratio, 1.6; [95% CI, 1.0–2.5]), late-onset preeclampsia (adjusted odds ratio, 2.0 [95% CI, 1.2–3.1]), and, most strikingly, early-onset preeclampsia (adjusted odds ratio, 58.2 [95% CI, 32.1–105.4]). Low PlGF levels were associated with an increased risk of unplanned or emergency cesarean delivery, and birthweight <10th centile, relative to women with normal PlGF levels (Table 2).

Stillbirths

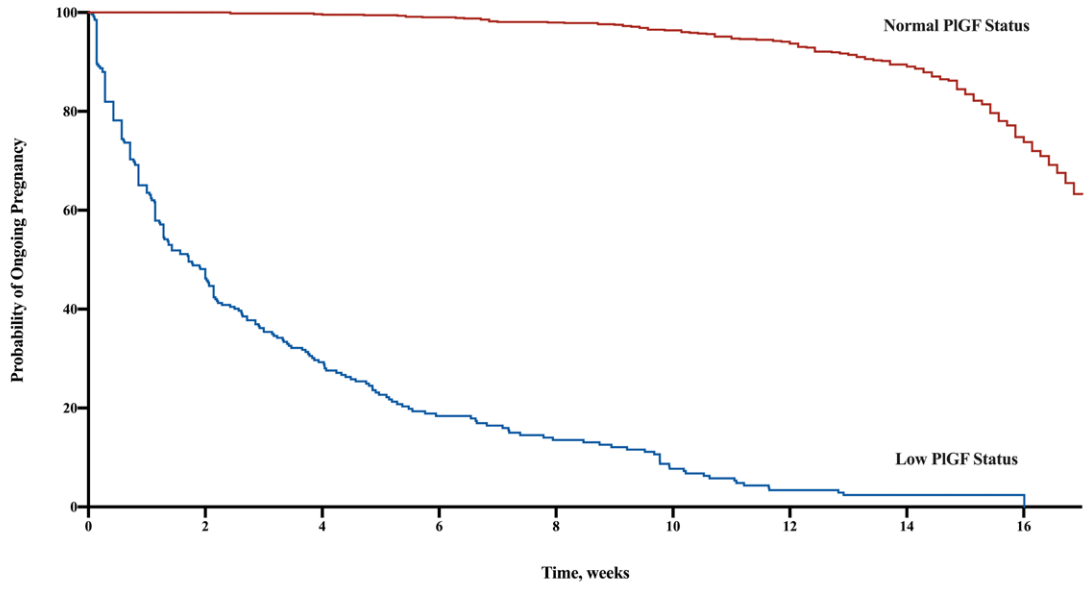
Stillbirth occurred in 58 (5.9%) pregnant women of the total population. Low PlGF levels were associated with an increased risk of stillbirth, relative to women with normal PlGF levels (adjusted odds ratio, 15.9 [95% CI, 7.6–33.3]; Table 2). The majority of stillbirths were anticipated based on clinical context (Table 3). The pathogenesis of stillbirth differed significantly between women with normal and low PlGF levels ($P<0.0001$; Table 3). The cause of stillbirth in women with low PlGF levels was primarily characterized by severe underlying placental disease with evidence of maternal vascular malperfusion pathology, whereas the majority of stillbirths in women with normal PlGF levels were mediated by an underlying fetal diagnosis with minimal placental pathology findings (Table 3).

DISCUSSION

This investigation of PlGF testing in the real-world clinical care of high-risk pregnancies provides evidence that low PlGF levels are strikingly associated with increased rates of imminent preterm birth, regardless of maternal clinical diagnosis. Women with low PlGF levels also exhibited significantly higher risk of early-onset preeclampsia and stillbirth, primarily associated with placental maternal vascular malperfusion disease.

Iatrogenic preterm birth is a necessary intervention if the risk for serious maternal morbidities arising from critical organ damage due to preeclampsia becomes unacceptably high, regardless of gestational age and chances of fetal survival. In this investigation, low PlGF status in high-risk pregnant women was associated with markedly higher rates of preterm birth, with 43% of these women delivering preterm 2 weeks following PlGF testing and 63% of these women

A



No. at risk									
Normal PlGF Status	978	965	919	877	838	778	714	637	506
Low PlGF Status	266	127	70	39	29	17	8	5	2

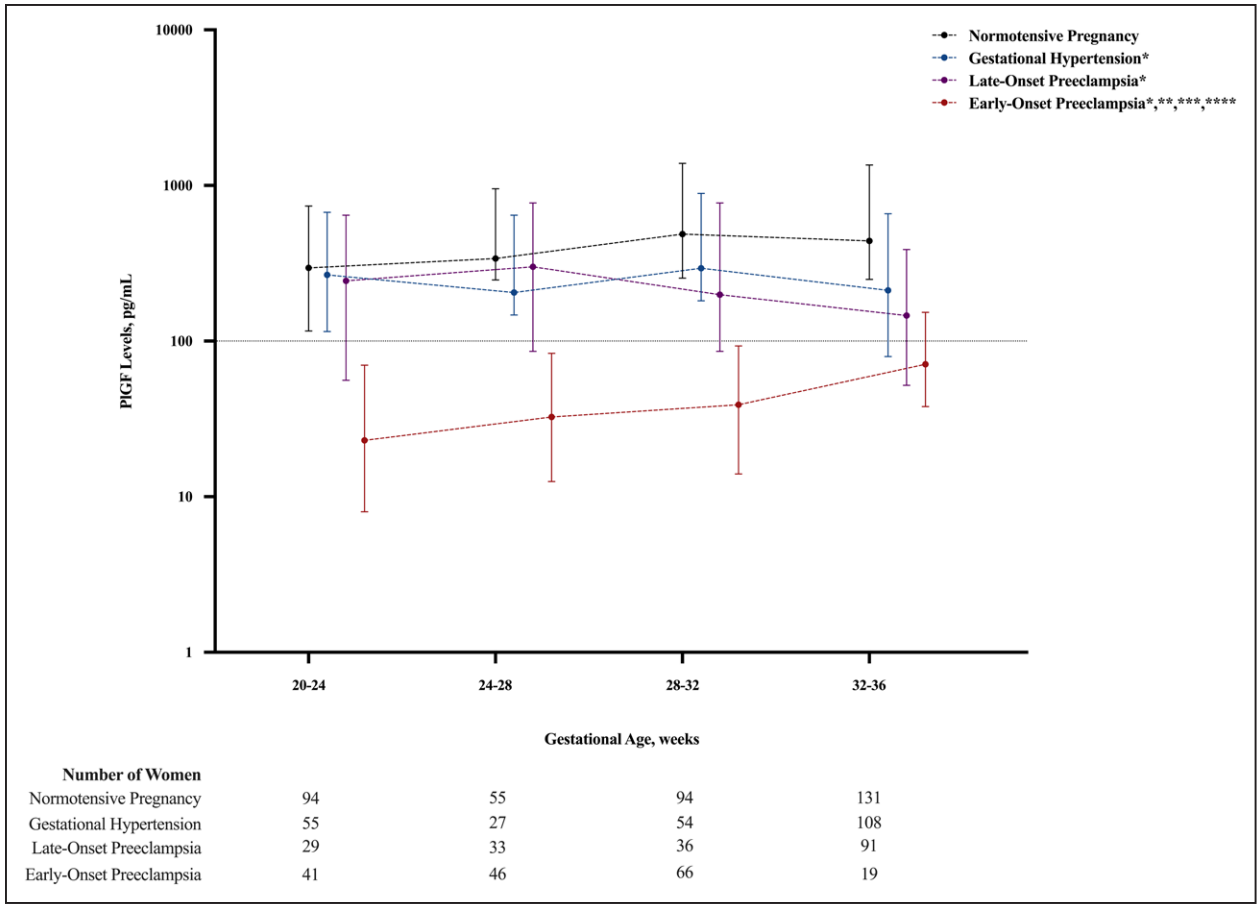
B

Weeks since PlGF Test	Survival from Preterm Birth		Standardized Survival Difference	95% Confidence Interval	
	Normal PlGF Status	Low PlGF Status		Lower	Upper
1	0.99	0.72	-0.28	-0.63	0.07
2	0.99	0.57	-0.43	-0.76	-0.09
4	0.99	0.37	-0.62	-0.87	-0.38
8	0.97	0.15	-0.82	-0.93	-0.71
12	0.91	0.05	-0.86	-0.91	-0.82

Figure 1. Survival from preterm birth <37 wk of gestation and PlGF (placental growth factor) status.
A, Kaplan-Meier graph showing survival from preterm birth <37 wk of gestation in high-risk pregnant women with normal PlGF levels (≥100 pg/mL) and low PlGF levels (<100 pg/mL). **B**, Standardized survival curve estimates of the probability of preterm birth <37 wk of gestation in high-risk pregnant women with normal PlGF levels (≥100 pg/mL) and low PlGF levels (<100 pg/mL).

delivering 4 weeks following testing. By contrast, only 1% of high-risk pregnant women with normal PlGF status delivered preterm within this time period. This investigation was unable to differentiate spontaneous and iatrogenic preterm birth. Although specific recommendations were not made to managing clinicians to guide clinical management based on the results of PlGF testing, including timing of delivery, it is possible that knowledge of PlGF levels may have influenced the decision on timing for iatrogenic preterm birth. Regardless of diagnosis, it is profoundly relevant for managing clinicians to recognize the high probability of imminent preterm birth in high-risk pregnant women with low PlGF status, which informs a range of clinical decisions that include fetal lung maturation with steroids and neonatal pediatric counseling. PlGF testing exhibits the potential to increase the proportion of women achieving optimized perinatal care before iatrogenic preterm birth.

Preeclampsia is a heterogenous hypertensive disorder of pregnancy with a broad clinical definition. This investigation determined that low maternal PlGF levels were strongly associated with early-onset preeclampsia, with far weaker associations to both late-onset preeclampsia and gestational hypertension. These findings support the hypothesis that hypertensive disorders of pregnancy represent distinct diseases with unique pathogenesis, underlying placental function, clinical phenotypes, and associated risks for serious maternal and perinatal complications.[2,15,25–27] Current standards of care for women at high risk of preeclampsia fundamentally focus on a review of clinical risk factors and symptoms, with interventions focusing on control of maternal blood pressure and ultrasound assessment of fetal growth and well-being. The ability to distinguish early-onset preeclampsia from other hypertensive disorders of pregnancy provides



Number of Women				
Normotensive Pregnancy	94	55	94	131
Gestational Hypertension	55	27	54	108
Late-Onset Preeclampsia	29	33	36	91
Early-Onset Preeclampsia	41	46	66	19

Figure 2. Maternal concentrations of PlGF (placental growth factor) across pregnancy according to hypertensive disorder of pregnancy diagnosis at delivery.
Data are presented as median±interquartile range. *P<0.0001, compared with normal pregnancy; **P<0.0001, compared with gestational hypertension; ***P<0.0001, compared with late-onset preeclampsia.

clinicians with a powerful tool to optimize decision-making regarding management strategies.[28,29]

In this investigation, pregnant women with low PlGF levels were determined to be at increased risk of stillbirth, the most serious perinatal adverse outcome associated with placental dysfunction, hypertensive disorders of pregnancy, and fetal growth restriction. Importantly, there was a clear distinction in stillbirth pathogenesis based on PlGF status. Stillbirth that occurred in women with low circulating PlGF was predominantly mediated by severe placental disease, mainly characterized by maternal vascular malperfusion pathology. By contrast, a range of rare underlying fetal causes of stillbirth was observed in women with normal PlGF levels. Clinical knowledge of PlGF levels could foster sensitive and informed discussions regarding the prognosis for growth-restricted fetuses with a periviable estimated fetal weight.[30] In pregnant women with normal PlGF levels, PlGF testing has the capacity to redirect clinical investigations towards underlying fetal causes and focus counseling on the specific fetal diagnosis, as opposed to a prognosis that is defined principally by gestational age, estimated fetal weight, and fetal Doppler data.[31]

Although this investigation did not assess the cost-effectiveness of PlGF testing, endorsement of this test by the National Institute for Clinical Excellence in the United Kingdom, combined with robust economic analyses both in the United States and the United Kingdom, provides a strong incentive to justify the integration of PlGF testing in high-risk pregnancy settings.[32–34]

Strengths of our analysis include the structuring of survival time by exposure status during pregnancy, which accounts for time spent in different exposure states and more accurately characterizes the risk associated with low PlGF. Using a flexible parametric approach that accounted for nonproportionality of hazards over time, we were able to directly model the baseline hazards and estimate the time-varying effects of low PlGF status.[21,22] We acknowledge that our investigation has several limitations. With the integration of PlGF testing into clinical care at our center, clinicians were recommended to undertake a comprehensive maternal-fetal evaluation in the case of an abnormal PlGF test. Although it is possible that revealing PlGF results to clinicians could have impacted the clinical management of these patients or pregnancy outcomes, these patients were already

Table 3. Stillbirth Pregnancy Characteristics of High-Risk Pregnant Women With Normal PlGF Levels (≥100 pg/mL) and Low PlGF Levels (<100 pg/mL) Between 20+0 and 35+6 wk of Gestation

Stillbirth characteristics	Stillbirth in women with normal PlGF levels N=10	Stillbirth in women with low PlGF levels N=48
Gestational age at delivery, wks	28 [24–34]	25 [23–28]
PlGF level, pg/mL	303 [199–499]	22 [12–32]
Clinical presentation		
Anticipated stillbirth, n (%)	7 (70)	43 (90)
Unanticipated stillbirth, n (%)	1 (10)	4 (8)
Clinically indicated termination of pregnancy, n (%)	2 (20)	1 (2)
Pathogenesis		
Severe previable placental disease, n (%)	0 (0)	41 (85)
Fetal abnormality, n (%)	8 (80)	3 (6)
Massive abruption, n (%)	1 (10)	1 (2)
Preterm premature rupture of the membranes, n (%)	1 (10)	0 (0)
Inadequate fetal growth surveillance, n (%)	0 (0)	2 (4)
Cord obstruction, n (%)	0 (0)	1 (2)
Major placental pathology findings		
Maternal vascular malperfusion, n (%)	0 (0)	37 (77)
Fetal thrombotic vasculopathy, n (%)	1 (10)	12 (25)
Abruption, n (%)	2 (20)	2 (4)
Chronic histiocytic intervillositis, n (%)	0 (0)	4 (8)
Villitis of unknown etiology, n (%)	0 (0)	3 (6)
Massive perivillous fibrin deposition, n (%)	0 (0)	1 (2)

Data are presented as median [interquartile range], or n (% of column). PlGF indicates placental growth factor.

identified as high-risk and under increased surveillance offered at our center. Next, as patient information was retrieved in a standard manner through an audit of the hospital's electronic medical record system, additional information recorded in additional electronic systems or patient charts was not captured. For example, the characterization of prepregnancy health and inclusion of further neonatal outcomes would have been of great interest. Next, pregnant women with normal PlGF levels were our control cohort for analysis purposes; however, these women do not necessarily represent a true low-risk population. Lastly, our data were derived from a single tertiary center, and although our study population was ethnically diverse, our results cannot be generalized to the wide range of centers that provide obstetric care. A stepped-wedge cluster-randomized controlled trial that integrates PlGF testing incrementally over time across a network of comparable large-volume perinatal centers would provide compelling evidence to justify the costs of real-time PlGF testing.[5]

PERSPECTIVES

This is a real-world investigation evaluating pregnancy outcomes associated with PlGF levels in a clinical setting, providing evidence that complements recent elegant research supporting angiogenic protein testing for preeclampsia screening and diagnosis.[3,5] In high-resource perinatal settings such as ours, the incidence of serious maternal morbidity associated with preeclampsia, such as eclampsia, stroke, myocardial infarction, and maternal mortality are fortunately rare.[5] However, the integration of PlGF testing into clinical care has the potential to provide clinicians with practical knowledge regarding risk of pregnancy progression and the opportunity to tailor clinical management.[12,14,16] Normal PlGF levels facilitate the avoidance of unnecessary surveillance and medical interventions, whereas low PlGF levels justify the deployment of higher-level maternal-fetal care and additional clinical interventions, such as optimally timed steroid administration for fetal lung maturation, admission for intensive monitoring, and iatrogenic preterm birth.[30,35] In addition to supporting the integration of PlGF testing into tertiary centers, the current findings support a role for PlGF testing as a contingency screening tool integrated into remote communities, or low-income settings. The associated risks of imminent preterm birth, early-onset preeclampsia, and stillbirth may warrant referral of high-risk women with low PlGF levels to higher-level centers. In this context, PlGF testing has the potential to overcome some of the very real challenges health care systems in Canada face in providing effective obstetric care to women in remote or low-income settings.

ARTICLE INFORMATION

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