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Placental growth factor as a marker of fetal growth restriction caused by placental dysfunction

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ABSTRACT

Introduction: Discriminating between placentally-mediated fetal growth restriction and constitutionally-small fetuses is a challenge in obstetric practice. Placental growth factor (PlGF), measurable in the maternal circulation, may have this discriminatory capacity.
Methods: Plasma PlGF was measured in women presenting with suspected fetal growth restriction (FGR; ultrasound fetal abdominal circumference <10th percentile for gestational age) at sites in Canada, New Zealand and the United Kingdom. When available, placenta tissue underwent histopathological examination for lesions indicating placental dysfunction, blinded to PlGF and clinical outcome. Lesions were evaluated according to pre-specified severity criteria and an overall severity grade was assigned (0–3, absent to severe). Low PlGF (concentration <5th percentile for gestational age) to identify placental FGR (severity grade ≥ 2) was assessed and compared with routine parameters for fetal assessment. For all cases, the relationship between PlGF and the sampling-to-delivery interval was determined.
Results: Low PlGF identified placental FGR with an area under the receiver-operator characteristic curve of 0.96 [95% CI 0.93–0.98], 98.2% [95% CI 90.5–99.9] sensitivity and 75.1% [95% CI 67.6–81.7] specificity. Negative and positive predictive values were 99.2% [95% CI 95.4–99.9] and 58.5% [95% CI 47.9–68.6],

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respectively. Low PlGF outperformed gestational age, abdominal circumference and umbilical artery resistance index in predicting placental FGR. Very low PlGF (<12 pg/mL) was associated with shorter sampling-to-delivery intervals than normal PlGF (13 vs. 29.5 days, P < 0.0001).
Discussion: Low PlGF identifies small fetuses with significant underlying placental pathology and is a promising tool for antenatal discrimination of FGR from fetuses who are constitutionally-small.

1. Introduction

Placentally-mediated fetal growth restriction (FGR) is a pathological process that reduces the growth trajectory of a fetus and increases the risk of stillbirth, preterm delivery, serious neonatal complications and lifelong sequelae [1–3]. FGR is clinically suspected when the ultrasound estimated fetal weight or fetal abdominal circumference is below the 10th percentile for gestational age, or serial ultrasounds suggest decreasing growth velocity [4–6]. However, many fetuses with suspected FGR are small due to constitutional factors and are at low risk for adverse outcomes ("small but healthy" fetuses) [4].

Antenatal discrimination of fetuses that are small due to placental dysfunction, rather than constitutionally-small, would improve clinical management by focusing care on fetuses that are truly at-risk of adverse perinatal outcome, reducing surveillance fatigue and unnecessary intervention for pregnancies with constitutionally-small fetuses [7,8]. Placental biomarkers such as placental growth factor (PlGF), present in the maternal circulation, may provide an additional clinical tool for identifying placental FGR antenatally. Pilot work by our group suggests that low circulating levels of PlGF may characterize pregnancies complicated by FGR associated with significant placental pathology [9] but larger studies are required to elucidate its clinical utility. In this study, we assessed the ability of PlGF to antenatally identify placental FGR, histologically confirmed after birth by the presence of significant placental pathology. Additionally, we assessed the sampling-to-delivery to determine if low PlGF is an indication of clinically-important FGR, with earlier delivery reflecting the physician's decision to deliver in response to perceived perinatal risks.

2. Materials and methods

2.1. Study design

Through the Global Pregnancy Collaboration (http://pre-empt.cfri.ca/colaboratory), we complemented a prospectively-recruited cohort of antenatally-suspected FGR pregnancies in Canada with two extant cohorts from New Zealand and the United Kingdom. All women provided written informed consent to participate in the study.

Eligibility criteria was: antenatally-suspected FGR, defined as a fetal abdominal circumference (AC) < 10th percentile for gestational age (GA) on ultrasound by local criteria, maternal age 18–45 years with a singleton pregnancy between 20^{+0}–41^{+6} weeks of gestation. Women with chronic or gestational hypertension and/or preeclampsia [10] at enrolment, premature rupture of membranes at enrolment or a fetus with known chromosomal and/or congenital abnormalities at enrolment or confirmed after delivery were excluded. Blood samples were collected within 14 days of the ultrasound identification of FGR. The study was powered to estimate sensitivity and specificity within ±5% percentage points for the placental pathology-based analysis. Based on our pilot data [9], obtaining 95% sensitivity and 90% specificity (the lower 95% confidence interval limit of the point estimate in the pilot study) for PlGF to identify placental FGR required enrollment of 211 pregnancies with suspected FGR, assuming a conservative 35% rate of placental FGR.

In Canada, women were recruited from inpatient and outpatient services at BC Women's Hospital (Vancouver, H12-00504 C&W Research Ethics Board) and the Ottawa Hospital (Ottawa, 20120660 TOH Ethics Board) between April 2012–June 2014, extending the published pilot study [9]. Baseline and post-enrolment data about the women, their pregnancies and perinatal outcomes were abstracted from medical charts after delivery. Umbilical artery resistance index (RI) percentile was determined for GA at Doppler examination [11]. Birthweight percentile was determined using a Canadian national birthweight reference [12].

A cohort of FGR pregnancies from Auckland, New Zealand with banked maternal blood samples and wax-embedded placental tissue (NTX/11/056/02 Northern Regional Ethics Committee) was identified through the Global Pregnancy Collaboration. Eligible women, both inpatients and outpatients, were recruited from National Women's Hospital for a series of antenatal studies between 1993 and 1997 [13–16]. Detailed data pertaining to these women, their pregnancies and perinatal outcomes were collected by research midwives after delivery and stored in a study database. From this database, women meeting our eligibility criteria were selected for inclusion. Umbilical artery RI percentile for GA was determined [11]. Birthweight percentile was determined using a New Zealand reference [17].

A cohort of FGR pregnancies from the United Kingdom with banked maternal blood samples was identified through the Global Pregnancy Collaboration. The PELICAN-FGR Study (East London Research Ethics Committee, ref.10/H0701/117) [18] recruited women presenting with reduced symphysis-fundal height between 2011 and 2013. Fetal biometry was assessed by ultrasound and a maternal blood sample was collected during this antenatal visit. Detailed data pertaining to these women, their pregnancies and perinatal outcomes were collected by research midwives after delivery and stored in a study database. From this database, women meeting our eligibility criteria were selected for inclusion. Birthweight percentile was determined using the Canadian standard as a recent multiethnic standard [12].

2.2. PlGF analysis

In all cohorts, maternal venous blood was collected by venipuncture using 10 mL EDTA plasma tubes. Plasma was isolated by centrifugation at 3000 rpm for 10 min and stored at −80 °C at all centres. Samples were batch assayed for PlGF using an automated immunoassay (Triage®, Alere, San Diego, CA, USA) [9,19,20]. The detection range of the assay is 12–3000 pg/mL. Low PlGF was defined as a concentration <5th percentile for GA [20]. Very low PlGF was defined as a concentration <12 pg/mL. Laboratory staff were masked to clinical and pathology data and clinicians were masked to PlGF results. The integrity of the New Zealand samples after prolonged storage at −80 °C was confirmed in a subset of cases prior to this analysis (Supplemental Fig. S1).

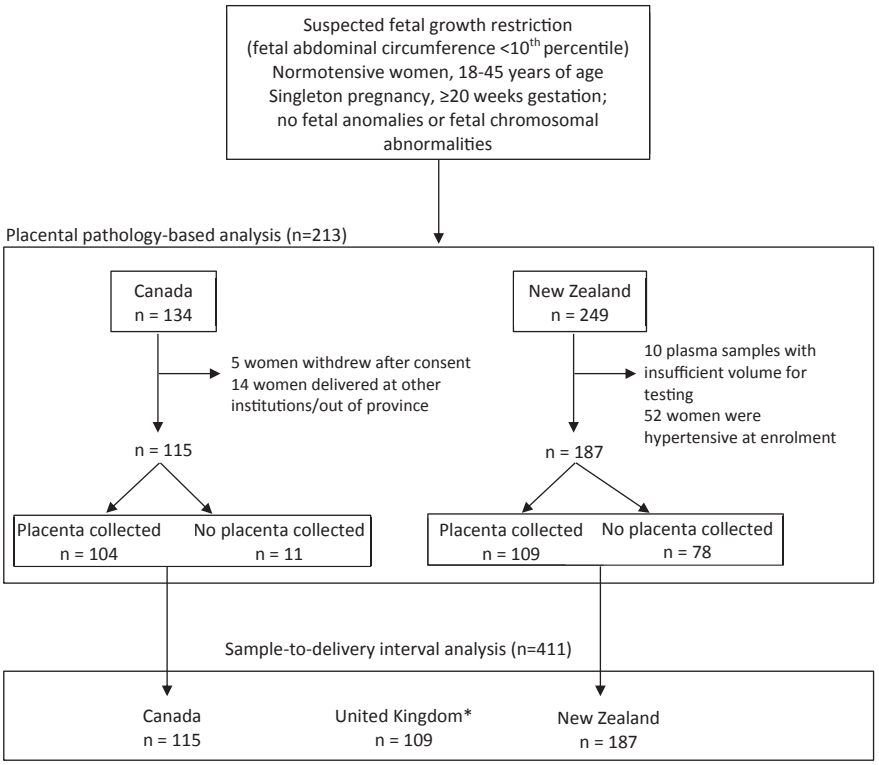

Fig. 1. Women with suspected FGR from study cohorts in Canada, New Zealand and the United Kingdom. In all cases, women were normotensive at enrolment with no documented signs or symptoms of preeclampsia, ruptured membranes, fetal anomalies, or fetal chromosomal abnormalities. Placentas were collected at delivery when possible Women were enrolled at study centres in Vancouver and Ottawa (Canada) from 2012 to 2014. Women from Auckland (New Zealand) were identified from among those enrolled in a previous study conducted from 1993 to 1997. Women were identified from the United Kingdom PELICAN-FGR Study conducted between 2011 and 2013. Placenta tissue collection at the time of delivery was not part of the original PELICAN-FGR Study design.

2.3. Placental pathology evaluation

Placentas were collected at delivery (Canada and New Zealand). Trimmed placental weight was recorded and 4–5 biopsies of villous parenchyma (1 cm^3 each) were randomly excised from the central and marginal regions of the placental disc. Tissue were fixed in 4% neutral buffered formalin (Canada) or 10% neutral buffered formalin (New Zealand), paraffin-embedded, sectioned (5 μm) and stained with hematoxylin and eosin using standard protocol [21]. High-resolution digital images were taken (Aperio® ScanScope [Canada], Metasystems® VSlide [Auckland]). Images were stored on external hard drives for evaluation in Ottawa (Canada).

A single, experienced placental pathologist (D.G.), masked to PlGF and clinical outcomes, examined the digital images of placental tissue using a Placental Examination Rubric (Supplemental Table S1) that evaluated and graded pathological lesions of maternal malperfusion, fetal villous stromal maldevelopment, villitis, perivillous fibrin deposition, fetal thrombotic vasculopathy, abruption, intraplacental hematoma and chorioamnionitis according to pre-specified definitions based on published guidelines [22–24]. A placental pathology grade (0–3) was assigned to each placenta as defined *a priori* in the Examination Rubric. A severity grade of 2 or 3 was considered to reflect placental FGR and a grade of 0 or 1 to reflect a constitutionally-small fetus.

For 53 of the 109 Auckland women, tissue blocks were unavailable. For these cases, the placental pathology severity grade was assigned with the Examination Rubric (by D.G.) using placental pathology variables from the study database. These pathology variables were collected prospectively by direct microscopy by a single Perinatal Pathologist (by Y.C.) using pre-specified criteria during the original study period and included placental infarction, ischaemic changes, calcification, syncytial knots, villous maturity, fibrin deposition, villitis, intraplacental hematoma and placental abruption ranked mild, moderate or severe.

2.4. Statistics

Analyses were performed using Prism 5.0 (GraphPad, CA, USA). P-values <0.05 were considered to indicate statistical significance. Normally-distributed data were reported using means with standard deviations and non-normally distributed data using medians with interquartile ranges. Categorical variables were reported using counts and proportions.

For the placental pathology-based analysis, the ability of low PlGF (concentration <5th percentile for GA) to predict placental FGR was assessed by calculating sensitivity, specificity, positive and negative predictive values, and positive and negative likelihood ratios with 95% confidence intervals. These analyses were repeated to include only women with blood samples collected prior to 35 weeks of gestation to account for the physiological decline of PlGF towards term.[20] Additionally, performance was assessed according to gestational age at onset (early vs late defined as <32 weeks and ≥32 weeks, respectively). Area under the receiver-operator characteristic (AUROC) curves were calculated for prediction of placental FGR by PlGF (as a percentile for GA at sampling) and for comparison, GA at the time of suspected FGR, ultrasound AC percentile, and umbilical artery RI percentile.

In all cases (with and without placental histology), the sampling-to-delivery intervals between women with very low PlGF (<12 pg/mL), low PlGF (<5th percentile, ≥12 pg/mL) and normal PlGF concentrations were compared. The percentage of ongoing pregnancies for each gestational age day after sampling was plotted

Table 1
Characteristics of pregnancies with antenatally suspected growth restriction by PlGF concentration at enrolment from Canada, New Zealand and the United Kingdom included in the placenta pathology-based analysis (n = 213, placental histology at delivery) and the sample-to-delivery interval analysis (n = 411).

Characteristic	Placental pathology-based analyses Mean ± SD, median [IQR] or n (%)		Sample-to-delivery interval analysis Mean ± SD, median [IQR] or n (%)	
At enrolment	Low PlGF (n = 94/213)	Normal PlGF (n = 119/213)	Low PlGF (n = 157/411)	Normal PlGF (n = 254/411)
Maternal age (years)	30.4 ± 5.8	28.5 ± 6.1[a]	29.5 ± 6.0	28.3 ± 5.9
Nulliparity	53 (56.4)	64 (53.8)	98 (62.4)	131 (51.6)[a]
Smoking during pregnancy	12 (12.8)	33 (27.7)[a]	33 (21.0)	34 (26.8)
Pre-pregnancy weight (kg)	61.5 ± 11.5	60.7 ± 14.0	60.9 ± 13.4	60.3 ± 12.3
Maternal BP (mmHg)				
Systolic	113.3 ± 12.5	108.0 ± 13.0[a]	116.9 ± 16.2	111.2 ± 12.9[b]
Diastolic	68.4 ± 8.7	65.7 ± 8.6[a]	73.6 ± 13.5	69.9 ± 10.6[b]
Gestational age (weeks)	33.0 [29.8–35.0]	34.3 [32.3–36.0][a]	33.3 [30.2–35.4]	34.4 [32.4–36.0][b]
After 32 weeks	54 (57.4)	93 (78.2)[a]	96 (61.1)	195 (76.8)[b]
Fetal ultrasound measurements (percentile for gestational age)				
Abdominal circumference	3.0 [1.0–5.0]	4.0 [2.5–4.0][a]	2.2 [1.0–5.0]	3.0 [1.5–6.4][a]
Estimated fetal weight	8.0 [3.0–16.0]	11.0 [4.5–26.0][a]	4.0 [1.0–7.0]	6.0 [3.0–9.0][a]
Umbilical artery RI[c]	88.2 [68.0–95.0] (n = 65)	72.2 [45.0–92.3][a] (n = 93)	86.9 [75.1–94.9] (n = 46)	67.8 [39.5–90.2][b] (n = 179)
Ultrasound to blood sampling interval (days)	1 [0–5]	0 [0–3][a]	0 [0–5]	0 [0–3][a]
Pregnancy Outcomes				
HDP developed after enrollment	25 (26.6)	7 (5.9)[b]	39 (24.8)	16 (6.3)[b]
Pre-eclampsia[d]	8 (8.5)	0 (0)[a]	11 (7.0)	2 (0.8)[a]
Gestational hypertension[e]	17 (18.1)	7 (5.9)[a]	28 (17.8)	14 (5.5)[b]
Last ultrasound assessment prior to delivery (percentile for gestational age)				
Abdominal circumference	3.0 [1.0–4.0]	6.5 [2.3–11.0][a]	–	–
Estimated fetal weight	3.5 [2.0–8.3]	7.0 [3.0–19.0][a]	–	–
Umbilical artery RI[c]	85.8 [54.2–96.3]	76.0 [45.3–90.3][a]	–	–
Gestational age at delivery (weeks)	36.1 [33.6–37.6]	38.3 [37.3–39.1][b]	36.9 [34.5–38.1]	38.7 [37.7–40.0][b]
Pre-term delivery <37 weeks	58 (61.7)	22 (18.5)[b]	79 (50.3)	33 (13.0)[b]
Birthweight (g)	1855 ± 721	2529 ± 402[b]	1993 ± 724	2629 ± 411[b]
Birthweight percentile[f]	2.0 [1.0–4.6]	5.0 [1.9–10.0][a]	2.0 [1.0–6.1]	5.0 [2.0–11.4][b]
Birthweight <3rd percentile	55 (58.5)	35 (29.4)[b]	87 (55.4)	72 (28.3)[b]
Trimmed placental weight (g)	293 ± 112	365 ± 78[b]	–	–
Induction of labour for fetal indication	35 (37.2)	57 (47.9)	54 (34.4)	99 (40.0)
Caesarean delivery for fetal indication[g]	28 (29.8)	10 (8.4)[b]	41 (26.1)	19 (7.5)[b]
Stillbirth	6 (6.4)	0 (0)[a]	6 (3.8)	1 (0.4)[a]
Neonatal death	0 (0)	0 (0)	1 (0.6)	0 (0)
Neonatal outcome				
APGAR <7 at 5 min	12 (12.8)	1 (0.8)[b]	14 (8.9)	2 (0.8)[b]
NICU admission >48 h	26 (27.7)	12 (10.1)[a]	31 (19.7)	15 (5.9)[b]
Placental IUGR				
Pathology grade 2 or 3	55 (58.5)	1 (0.8)[b]	–	–

FGR: fetal growth restriction; HDP: hypertensive disorders of pregnancy; IQR: interquartile range; NICU: neonatal intensive care unit; PlGF: placental growth factor; RI: resistance index.
Comparisons were performed between columns with women with low PlGF at the time of enrolment as the comparator group to women with normal PlGF concentration at enrolment for each study analysis.
[a] P<0.05.
[b] P < 0.001.
[c] Umbilical artery resistance index percentile was determined for gestational age week at the time of Doppler examination [11].
[d] Preeclampsia was defined as hypertension (blood pressure ≥ 140/90 mm Hg, on at least two occasions >4 h apart after 20 weeks gestation) and new onset proteinuria (≥2 + dipstick reading, ≥0.3 g/day by 24 h urine collection, or ≥30 mg/mmol by protein:creatinine ratio) [10].
[e] Gestational hypertension was defined as non-proteinuric hypertension developing at $\geq 20^{+0}$ weeks gestation [10].
[f] Birthweight percentiles were determined based on Canadian [12] (population-based) and New Zealand [17] (customized) birthweight references.
[g] Defined as absent or reversed end diastolic flow and/or an abnormal/non-reassuring heart rate trace during intrapartum monitoring.

using Kaplan-Meier survival curves and the median interval (in days) from sampling-to-delivery was compared between the groups using the log-rank test.

3. Results

3.1. Placental pathology-based analysis (N = 213)

In total, 213 pregnancies with suspected FGR and placental tissue collected at delivery for placental pathology were included in the study (Canada: n = 104, New Zealand: n = 109; Fig. 1). Of these 213 women, 94 (44.1%) had low PlGF at enrolment. Women with low PlGF were enrolled at earlier GAs, had fetuses with smaller AC percentiles, delivered earlier and preterm, had babies with lower birthweight and birthweight percentile and were more likely to develop pregnancy hypertension after enrolment compared with women with normal PlGF (Table 1). All stillbirths occurred among women with low PlGF. The median interval between ultrasound identification of fetal AC <10th percentile and blood sample collection was 1 day [interquartile range (IQR) [0–5] for women with low PlGF and 0 days [0–3] for women with normal PlGF (P = 0.003).

PlGF concentration by GA and placental pathology grade is shown in Fig. 2. Of the 94 women with low PlGF at enrolment, 55

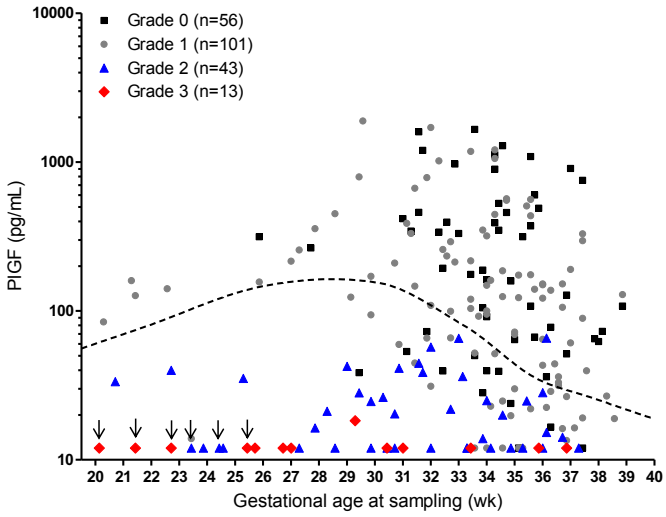

Fig. 2. Maternal PlGF concentrations at the time of enrolment and placental pathology grading in 213 pregnancies with suspected GR. PlGF concentration shown for normotensive women with no pathology (Grade 0, n = 56; black squares), mild pathology (Grade 1, n = 101; gray circles), moderate pathology (Grade 2, n = 43; blue triangles) and severe pathology (Grade 3, n = 13; red squares). PlGF was below the 5th percentile cut-off for gestational age at sampling in all cases of severe pathology. The black arrows represent the cases that ended in stillbirth. The black dashed black line represents the 5th percentile PlGF concentration for gestational age at sampling [20].

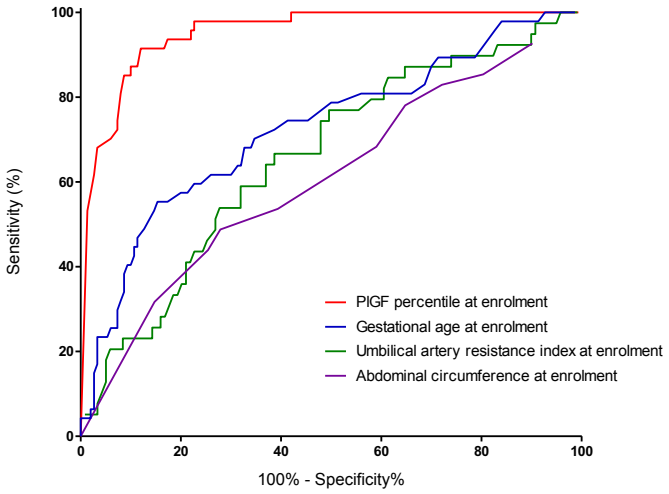

Fig. 3. Area under the receiver operating characteristic curves for the prediction of placental FGR by PlGF and other clinical parameters of fetal assessment in 213 pregnancies with antenatally suspected FGR and placental histology. PlGF (percentile for gestational age at the time of enrolment) had an area under the curve of 0.96 (0.93–0.98) for predicting placental FGR (red line), outperforming gestational age at enrolment [0.73 (0.65–0.81); blue line], umbilical artery resistance index percentile at enrolment [(0.66 (0.60–0.76); green line] and abdominal circumference percentile at enrolment [0.64 (0.54–0.73); purple line].

Table 2
Sensitivity and specificity of a low PlGF (concentration < 5th percentile for gestational age) to identify placental FGR in 213 pregnancies with antenatally suspected FGR.

	Sensitivity [95% CI]	Specificity [95% CI]	PPV [95% CI]	NPV [95% CI]	+LR [95% CI]	-LR [95% CI]
Enrolment at any gestational age (n = 213)	98.2 [90.5–99.9]	75.1 [67.6–81.7]	58.5 [47.9–68.6]	99.2 [95.4–99.9]	3.95 [3.01–5.20]	0.024 [0.0030–0.17]
Enrolment <35 weeks gestation (n = 141)	100.0 [92.3–100.0]	74.7 [64.8–83.1]	65.7 [53.4–76.7]	100.0 [95.0–100.0]	3.92 [2.77–5.54]	0.014 [0.0010–0.23][a]
Enrolment <32 weeks gestation (n = 66)	100.0 [89.1–100.0]	76.5 [58.9–89.3]	80.0 [64.4–91.0]	100.0 [86.8–100.0]	4.25 [2.31–7.77]	0.004 [00.10–0.32][a]
Enrolment ≥32 weeks gestation (n = 147)	95.8 [78.9–98.9]	74.8 [66.2–82.2]	42.6 [29.2–56.8]	98.9 [94.2–100.0]	3.80 [2.77–5.21]	0.056 [0.0080–0.38]
New Zealand database cases excluded (n = 160)	98.0 [89.2–100.0]	71.2 [61.8–79.4]	60.0 [48.4–70.8]	98.8 [93.2–100.0]	3.40 [2.53–4.56]	0.029 [0.004–0.20]

+LR: positive likelihood ratio; -LR: negative likelihood ratio; CI: 95% confidence interval; NPV: negative predictive value; PPV: positive predictive value.
[a] Due to lack of false positive cases, a value of 0.5 was input into the 2 × 2 contingency table in order to calculate the negative likelihood ratio.

(58.5%) met the criteria of placental FGR whereas only 1 (0.8%) woman with normal PlGF had placental FGR (P < 0.0001). All six stillbirths showed moderate or severe placental pathology (Grade 2, n = 2; Grade 3, n = 4).

Low PlGF had 98.2% [95% confidence interval 90.5–99.9] sensitivity and 75.1% [67.6–81.7] specificity in identifying pregnancies with placental FGR as determined by placenta pathology grade. Negative and positive predictive values were 99.2% [95.4–99.9] and 58.5% [47.9–68.6], respectively (Table 2). Sensitivity analyses excluding women enrolled ≥35 weeks of pregnancy, or excluding the 53 New Zealand women who had pathology graded based on the New Zealand study database, did not alter the results (Table 2).

Low PlGF had an AUROC of 0.96 [0.93–0.98] to predict placental FGR. PlGF outperformed other readily-available clinical parameters (GA, AC percentile, umbilical artery RI percentile) in predicting placental FGR (Fig. 3).

3.2. Sampling-to-delivery interval (N = 411)

In total, 411 women with suspected FGR (with and without placental pathology; Table 1) were included in this analysis (Canada: n = 115, New Zealand: n = 187, United Kingdom: n = 109; Fig. 1). Of these, 157 (38.2%) women had low PlGF at enrolment. Women with low PlGF were enrolled approximately one week earlier than women with normal PlGF and had fetuses with smaller ACs, higher umbilical artery RI percentiles, delivered earlier, had babies with lower birthweight and birthweight percentiles and were more likely to develop pregnancy hypertension after enrolment (Table 1). The median interval between ultrasound identification of fetal AC <10th percentile and blood sampling for PlGF did not differ among women with low PlGF compared with those with normal PlGF (0 [0–2] versus 0 [0–0], P = 0.59).

Very low PlGF was associated with a shorter sampling-to-delivery interval compared with normal PlGF (13.0 days versus 29.5 days, P < 0.0001). Sample-to-delivery intervals were significantly shorter for women with very low and low PlGF when sampling occurred before 35 weeks of gestation: 14.0 days versus 33.5 versus 41.0 days, P < 0.0001 (Fig. 4).

In total, there were 7 stillbirths and one neonatal death; 6 stillbirths and the neonatal death occurred in women with low PlGF and one stillbirth with normal PlGF. Low PlGF had 87.5% [47.4–99.7] sensitivity and a specificity of 62.8% [57.9–67.5] to predict pregnancies that end in stillbirth with negative and positive predictive values of 99.6% [97.8–100.0] and 4.7% [1.8–9.0], respectively. The positive likelihood ratio is 2.35 [1.8–3.1] and the negative likelihood ratio is 0.2 [0.03–1.2]. Thus, in this cohort, the post-test odds of perinatal death after a low PlGF result were 4.7% and the post-test odds after a normal PlGF were 0.4%.

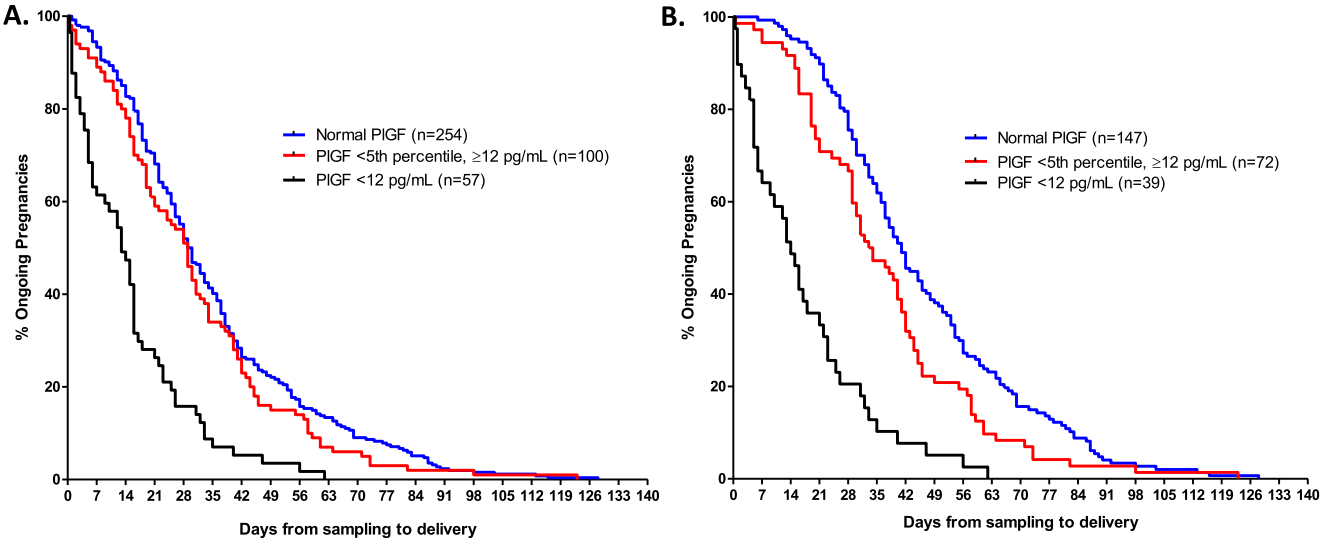

Fig. 4. Percentage of ongoing pregnancies from sampling to delivery in all 411 pregnancies with antenatally suspected FGR from Vancouver and Ottawa (Canada), Auckland (New Zealand) and the United Kingdom. Kaplan-Meier survival curves were used to plot the number of ongoing pregnancies for each day from sampling to delivery for each group. **(A)** Women with very low PlGF (PlGF <12 pg/mL, n = 57; black line) had shorter intervals from sampling to delivery (median: 13.0 days) than women with normal PlGF concentrations (median: 29.5 days, P-value < 0.0001; n = 254; blue line). Women with PlGF concentration <5th percentile but ≥12 pg/mL (n = 100; red line) had a median of 29 days from sampling to delivery. **(B)** The interval from sampling to delivery when sampling occurred before 35 weeks gestation in women with PlGF <12 pg/mL (n = 39), PlGF <5th percentile, ≥12 pg/mL (n = 72) and normal PlGF (n = 147), respectively (14.0 days versus 33.5 days versus 41.0 days, P-value<0.0001).

4. Discussion

Low maternal plasma PlGF (<5th percentile for GA) identified fetuses with placental FGR with high sensitivity (98.2%), reasonable specificity (75.1%), high negative predictive value (99.2%) and modest positive predictive value (58.5%). The high sensitivity, high negative predictive value and low negative likelihood ratio (0.02) imply that a normal PlGF concentration (≥5th percentile for GA) may be a useful 'rule-out' test for placental FGR, particularly among women presenting before 35 weeks' gestation. Also, the AUC of 0.96 indicates that low PlGF appears to be more useful than available clinical parameters, such as GA, AC or umbilical artery RI for identifying placental FGR antenatally. Moreover, low PlGF identified women destined to deliver within a shorter period of time, so the reassurance of a normal PlGF may support expectant management to improve neonatal outcomes.

Findings presented here support the growing body of evidence that PlGF is a marker for placentally-mediated pregnancy complications [9,19,25–29]. PlGF is decreased in the circulation of women with preeclampsia, with the most significant decreases occurring in cases of early-onset disease (<34 weeks gestation) and those requiring delivery within 14 days of their clinical diagnosis [19,25–29]. Studies evaluating PlGF and normotensive FGR pregnancies are more limited with previous reports largely using infant birthweight percentile to define FGR [30–33]. These studies have reported decreased PlGF in women who delivered small-for-gestational age (SGA) neonates but poor diagnostic and/or predictive performance, probably related to study populations that included truly growth-restricted as well as constitutionally-small fetuses [4]. Defining placental FGR based on placental pathology (confirming the presence of lesions of placental dysfunction) as opposed to size alone allows for these populations of fetuses to be distinguished. Recently, decreased maternal PlGF was associated with late-onset SGA fetuses with histological lesions of placental underperfusion [34], suggesting an association between PlGF and placental dysfunction in these pregnancies as indicated by the presence of significant placental pathology. Our results support these findings as low PlGF characterised pregnancies with an outcome of placental FGR in our study. Additionally, low PlGF may indicate the severity of fetal compromise in placental disease. Our sampling-to-delivery interval findings support previous studies that suggest that low PlGF may be associated with need for early delivery due to fetal decompensation in preeclampsia and FGR [18,29,35]. Finally, our findings also suggest that PlGF significantly alters the likelihood of subsequent perinatal death, which is consistent with the observation that stillbirths associated with FGR exhibit a greater degree of placental abnormality than live births with FGR [36]. Taken together, these data suggest that PlGF may be a useful test to identify fetuses at increased risk of stillbirth following identification that they are small.

Major strengths of this study include the definition of pathologically-small babies according to placental pathological examination by a single perinatal pathologist, masked to PlGF results and clinical outcomes, as well as the use of graded placental pathology to identify the most significant cases. Accurate identification of placental FGR among fetuses suspected to be small is fundamental to identifying novel tools for antenatal identification of fetuses at-risk. To reduce observer subjectivity, a well-documented phenomenon associated with placental pathology [37], we developed a Placental Examination Rubric with pre-specified severity criteria for lesions of interest. Incorporating lesion severity into outcome definitions allows better definition of growth restriction of placental origin and the opportunity to better understand the association between placental pathology and biomarkers such as PlGF.

Limitations of our study include the temporal differences between the Canadian and New Zealand cohorts included in the placental pathology-based analysis. The use of slightly different criteria to define placental pathology grades in 53 pregnancies from New Zealand may have resulted in some misclassification. However, we believe that all significant lesions would have been noted by the reporting pathologist, as our sensitivity analysis excluding the New Zealand cases without tissue available for histological examination by the Canadian pathologist, did not alter our findings. Additionally, our study included cases of suspected FGR presenting over a wide range of gestational ages with a larger proportion of

cases in the low PlGF group enrolled at earlier gestational ages. The inclusion of more early-onset cases in the low PlGF group may have influenced our findings as it is more often associated with placental dysfunction. However, our sub-analysis including only late-onset FGR cases shows that PlGF performs well as a rule-out test for placental dysfunction (high sensitivity and NPV).

Future studies should determine how best to incorporate PlGF testing into clinical practice in the identification of these high-risk pregnancies and explore the relationship with existing antenatal surveillance tools. With an AUROC of 0.96, PlGF outperforms other biochemical markers of placental dysfunction such as human placental lactogen and progesterone in detecting FGR [38]. Inclusion of PlGF in a predictive model for fetal compromise after maternal perception of reduced fetal movements improved the AUROC from 0.75 (ultrasound only) to 0.88 (ultrasound + PlGF) [39]. Combining PlGF with umbilical artery Doppler did not improve the prediction of placental FGR in our study (data not shown), suggesting that PlGF may be a more powerful marker for placental FGR. Future studies should investigate the value of PlGF testing with other Doppler parameters such as uterine artery, middle cerebral artery and cerebral placental ratio indices for the prediction of adverse outcomes in FGR pregnancies [40]. We speculate that PlGF might be best as a rule-out test for placental dysfunction, offering reassurance that a fetus may be constitutionally-small and enable identification of cases with at-risk fetuses who warrant closer clinical surveillance (i.e. more detailed Doppler studies). Our data advance knowledge in this field by suggesting that PlGF is able to discriminate fetuses with placental disease from those who are constitutionally-small would improve clinical management of the truly at-risk fetus and reduce unnecessary intervention for women with pregnancies with constitutionally-small, healthy fetuses. In our view PlGF may be a promising tool for antenatal discrimination of growth restricted fetuses secondary to placental disease from those who are constitutionally-small.

Conflict of interest statement

The authors have no conflicts of interests to declare.

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This manuscript is dedicated to the memory of our dear friend and colleague, Dr. Andrée Gruslin.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.placenta.2016.03.010.

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