Circulating Maternal Placental Growth Factor Responses to Low Molecular Weight Heparin in Pregnant Patients at Risk of Placental Dysfunction

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The authors report no conflicts of interest.

No financial support was received for this study.

Abstract word count: 511

Main text word count: 3856

CONDENSATION

Prophylactic low molecular weight heparin (LMWH) may augment deficient circulating placental growth factor (PlGF) in second trimester high-risk pregnancies and may prolong pregnancy compared with untreated patients.

SHORT TITLE

Low molecular weight heparin in pregnancies at risk of placental dysfunction.

AJOG at a Glance

A. Why was this study conducted?

- Meta-analysis of randomized control trials suggests that a sub-group at highest risk of developing severe early-onset preeclampsia may benefit from LMWH in addition to low-dose aspirin but the underlying mechanism of action of LMWH in this context is unknown.
- Women that develop severe early-onset preeclampsia have low maternal circulating Placenta Growth factor (PlGF) levels at presentation and prior to disease onset.
- Low molecular weight heparin (LMWH) exerts several non-anticoagulant actions *in-vitro,* including an ability to enhance PlGF release by placental villi and the maternal vascular endothelium

B. What are the key findings?

- A reference range for maternal circulating PlGF was generated for unselected nulliparous Caucasian women at 12-36 week's gestation.
- In a small number of patients at high risk of placenta-mediated adverse pregnancy outcomes and low circulating PlGF in the early second trimester, the administration of LMWH induced sustained elevations in PlGF.

C. What does this study add to what is already known?

- Future trials evaluating the potential benefits of LMWH for the prevention of placenta-mediated complications should consider recruitment of women in the early second trimester with low circulating PlGF, so as to focus on those at highest risk .

ABSTRACT:

Background: Patients at high risk of severe preeclampsia and/or fetal growth restriction have low circulating levels of placental growth factor (PlGF) and features of maternal vascular malperfusion placental pathology at delivery. Multi-modal screening and commencement of aspirin prophylaxis at 11-13 weeks' gestation significantly reduces the risk of preterm delivery with preeclampsia. However, the additional role of low molecular weight heparin (LMWH) and mechanisms of action remain uncertain. Since LMWH augments the production and release of PlGF *in-vitro* by both placental villi and vascular endothelium, it may be effective to suppress the risk of severe preeclampsia in a niche group of high-risk patients with low circulating PlGF in the early second trimester.

Objectives: The purpose of the study was to define a gestational age-specific reference range for PlGF and to test the hypothesis that prophylactic LMWH administered in the early second trimester may restore deficient circulating PlGF levels and thereby prolong pregnancy.

Study Design: Centile curves for circulating PlGF levels from 12-36 weeks' gestation were derived using quantile regression of combined data from a published cohort of 4207 unselected nulliparous patients in Cambridge, U.K. at four sampling time points (12, 20, 28, 36 weeks' gestation) and the Caucasian majority (n= 531) of a healthy nulliparous cohort in Toronto, Canada at 16 weeks' gestation using the same test platform. Within

a specialty high-risk clinic in Toronto, a niche group of seven patients with a circulating PlGF <10th centile in the early second trimester received daily prophylactic LMWH (enoxaparin; 40mg subcutaneously) and were followed until delivery (Group 1). Their baseline characteristics, delivery details and placental pathologies were compared with five similar patients who did not receive LMWH during the observation period (Group 2), and with a further 21 patients delivered with severe preeclampsia (Group 3) in the same institution.

Results: A gestational age-specific reference range for PlGF levels at weekly intervals between 12-36 weeks was established for Caucasian women with singleton pregnancies. Within Group 1, five of seven patients demonstrated a sustained increase in circulating PlGF levels, while PlGF levels did not increase in Group 2 or Group 3 patients that did not receive LMWH. Group 1 patients receiving LMWH therapy exhibited a later gestation at delivery, relative to Groups 2 and 3 (36 weeks [33–37] versus 23 weeks [22–26] and 28 weeks [27-31], respectively), and consequently had higher birthweights (1.93 kg [1.1–2.7] versus 0.32 kg [0.19–0.39] and 0.73 kg [0.52-1.03], respectively). The incidence of stillbirth was lowest in Group 1 (14% (1/7)), relative to Groups 2 and 3 (80% (4/5) and 29% (6/21), respectively). Maternal vascular malperfusion was the most common placental pathology found in association with abnormal uterine artery Doppler.

Conclusions: In patients at high risk of a serious adverse pregnancy outcome due to placental disease, the addition of LMWH to aspirin prophylaxis in the early second

trimester may restore deficient circulating PlGF to mediate an improved perinatal outcome. These data support the implementation of a multicenter pilot randomized control trial where patients are recruited primarily based on the assessment of placental function in the early second trimester.

Key words: Preeclampsia/eclampsia; Growth restriction; Biomarkers; Treatment/management; Placental pathology

INTRODUCTION

Placental growth factor (PlGF) is a pro-angiogenic protein produced by both the maternal vascular endothelium and the trophoblast layer covering the placental villi[1, 2]. Circulating PlGF levels rise steadily in maternal blood until the beginning of the 3rd trimester, reflecting both the development of the uteroplacental circulation and placental growth[3, 4]. Between 20-36 weeks' gestation, a single cut-off value of 100 pg/mL has high diagnostic test precision for patients with suspected preeclampsia and placenta-mediated fetal growth restriction[5, 6]. Consequently, real-time PlGF testing is an effective clinical management tool for high-risk pregnancy management[7].

Prior to 20 weeks' gestation, PlGF measurements may be useful to screen pregnancies for significant placental dysfunction causing early-onset preeclampsia, for example, in combination with clinical risk factors and mean uterine artery Doppler at 16 weeks'[8, 9]. Multi-modal screening at an earlier gestation of 11-13 weeks' gestation, incorporating PlGF and pregnancy-associated placental protein-A [PAPP-A] with mean uterine artery Doppler, blood pressure and maternal characteristics, is an effective tool to prevent preterm delivery due to severe preeclampsia with the institution of low dose aspirin prophylaxis[10-12]. Despite this important advancement, aspirin is not universally effective and may be of no benefit in specific subgroups, such as patients with chronic hypertension[13]. Comprehensive assessment of more complex high-risk individuals on aspirin prophylaxis at 16 weeks may identify those destined to fail treatment and deliver preterm with placenta-mediated complications.

135 The dominant placental pathology underlying severe early-onset preeclampsia is
136 termed maternal vascular malperfusion (MVM)[14]. This complex disease is a
137 constellation of gross and microscopic pathological findings initiated by diseased spiral
138 arteries, whereby the injured placenta secretes reduced amounts of PlGF followed by
139 excessive amounts of the vascular endothelial growth factor (VEGF) antagonist sFlt-1[8, 15,
140 16]. Though aspirin is principally known to be an anti-platelet agent, the beneficial
141 actions of aspirin in the context of preeclampsia prevention are largely unknown.
142 Low molecular weight heparin (LMWH) is a complex macromolecule, frequently
143 prescribed safely in pregnancy for its anticoagulant properties. However, LMWH is also
144 capable of exerting a range of non-anticoagulant actions that are highly relevant to the
145 maternal circulatory and placental pathogenesis of severe preeclampsia summarized in
146 Figure 1. These actions include stimulation of PlGF production by explanted human
147 placental villi and the vascular endothelium[1, 17, 18]. Favorable acute vascular changes
148 accompany augmented PlGF levels following bolus administration of LMWH to pregnant
149 patients at risk of severe placental dysfunction[19], while pregnant patients chronically-
150 anticoagulated with higher dose LMWH exhibit significant elevations in circulating
151 PlGF[20]. Collectively, these data form a compelling argument in favor of exploring the
152 potential value of LMWH to reduce disease severity in a sub-group of patients
153 considered most at risk of early-onset preeclampsia, a conclusion that is supported by
154 the most recent meta-analysis of randomized controlled trials[21, 22].

155 Based on these observations, our goal was to explore the potential of daily
156 LMWH administration to restore deficient circulating levels of PlGF of clinically high-risk
157 patients, beginning in the second trimester.

MATERIALS AND METHODS

PlGF Reference Range

A reference range for placental growth factor (PlGF) was constructed by merging data from two studies of unselected nulliparous patients using the same assay method (Roche). Data at four sampling time points (12, 20, 28, 36 weeks' gestation) from a cohort of 4207 unselected nulliparous patients in Cambridge U.K. (92% Caucasian)[23] were combined with data at the single time point of 16 weeks' gestation from the Caucasian majority (531/773; 68.7%) within a healthy nulliparous cohort in Toronto, Canada[24]. The PlGF assay coefficient of variation was 2.7-4.1% in the Cambridge study[3] and 3.8-6.7% in the Toronto study[24].

Study Population

We conducted a single-center pilot observational study within a Maternal-Fetal Medicine Placenta Clinic program of care focused on placental dysfunction disorders[14]. Eligible patients included those over 18 years of age with a live singleton fetus and followed in the period between July 2017 to March 2021. Patients were not eligible if already on LMWH therapy for a maternal indication or if previously diagnosed with a major thrombophilia disorder or anti-phospholipid syndrome. Approval by the Research Ethics Board of Mount Sinai Hospital was obtained (REB 20-0067-E). Patients at high risk of severe placental dysfunction in the current pregnancy, based on pre-pregnancy health and obstetric history received low dose (162 mg nightly) aspirin prophylaxis for preeclampsia from 12 weeks' gestation as part of standard clinical management[10]. At

the 16-week assessment, each patient completed an early placental health assessment comprising fetal biometry, amniotic fluid and mean uterine artery Doppler ultrasound. Mean uterine artery pulsatility index (PI) values >95th centile were considered abnormal[25]. Based on prior cohort data, we included same-day placental growth factor (PlGF) testing, as this test became available for the real-time management of high-risk pregnant patients in 2017 using the Elecsys platform (Roche Diagnostics, Penzberg, Germany)[7, 8]. PlGF values below the 10th centile at 16 weeks' gestation were considered abnormal. Between July 2017 and March 2021, 12 pregnant patients were identified for study inclusion with PlGF results <10th centile between 16 – 20 weeks' gestation.

An additional cohort of 21 high-risk pregnant patients were identified between 20 – 24 weeks' gestation with low circulating PlGF (< 100 pg/mL) who were not on LMWH therapy and subsequently developed early-onset preeclampsia with delivery <34 weeks' gestation. All patients received standardized high-risk obstetrical care in Mount Sinai Hospital by a group practice of three Maternal-Fetal Medicine physicians with appointments every 2-4 weeks and delivered at the same hospital[14]. Following delivery, the placenta was sent for histopathology testing by a dedicated perinatal pathologist blinded to treatment during pregnancy. This observational study was not registered as a clinical trial, as LMWH therapy is approved for use in high-risk pregnant patients for the prevention of placental complications, including preeclampsia[26].

Study Intervention

Based on prior experimental data supporting the therapeutic potential of LMWH to restore circulating PlGF[19, 20], the option of adjunctive prophylactic LMWH (enoxaparin 40 mg/day subcutaneously) for the prevention of preeclampsia was reviewed with 12 patients that had low circulating PlGF before 20 weeks' gestation. Of these, seven received prophylactic LMWH therapy in addition to aspirin and five continued aspirin alone. Five patients who initiated LMWH therapy had two PlGF tests between 16 – 20 weeks' gestation <10th centile, while two had a single abnormal PlGF test. An additional 21 high-risk pregnant patients with low PlGF levels between 20 – 24 weeks' gestation who did not initiate LMWH therapy and subsequently developed early-onset preeclampsia with delivery <34 weeks' gestation were identified. All participants continued to receive Maternal-Fetal Medicine obstetric care at the Placenta Clinic, with appointments every 2-4 weeks, and delivered at Mount Sinai Hospital.

Outcomes

The primary outcome of this pilot observational study was change in circulating maternal PlGF levels in response to LMWH, measured every 2-4 weeks in the Placenta Clinic appointments. Maternal PlGF levels after 16 weeks' gestation were overlaid on the newly derived reference range for comparative purposes. Secondary outcomes included change in mean uterine artery Doppler PI, gestational age at delivery, maternal and perinatal outcomes, and placental pathology diagnosis. Preeclampsia was defined as systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg on 2 occasions at least 4 hours apart after 20+0 weeks' gestation, with evidence of related

223 organ injury: proteinuria (urine protein:creatinine ratio ≥30 mg/mmol),
224 thrombocytopenia (platelets <100×109/L), renal compromise (serum creatinine ≥1.1
225 mg/dL), or impaired liver function (AST ≥70 U/L or ALT ≥70 U/L)[27]. Placental pathology
226 diagnoses were established according to the Amsterdam Criteria of Standardized
227 Placental Classification[28].
228
229 Statistical Analysis
230 The reference range of PlGF levels across gestation in healthy pregnant patients
231 generated by the Cambridge, United Kingdom cohort and the Caucasian majority of the
232 Toronto, Canada cohort was described by quantile regression curves using the LMS
233 method described by Cole and Green[29], and implemented in the gamlss package[30]
234 within the R statistical computing system (Version 4.0.2.)[31]. The flexibility of the quantile
235 curves was controlled by 5 degrees of freedom, to reflect the five broad groupings of
236 gestational ages across the Cambridge and Toronto datasets and so to ensure smooth
237 transition across areas where the data are sparse. Quantile regression curves are shown
238 on the plot for the median (blue, dashed), lower 10% (green, dotted) and 5% (red, full).
239 PlGF derived from non-Caucasian patients in the Toronto, Canada cohort were excluded
240 due to published variation in circulating PlGF at 16 weeks' gestation across ethnic
241 groups and to align with the ethnic composition of the Cambridge, United Kingdom
242 cohort[32]. No comparative statistics of the three study groups are presented due to the
243 limited sample size.
244

RESULTS

PlGF Reference Range

The gestational age-specific reference range for PlGF levels together with the 50th, 10th, 5th and 2.5th centile are shown in Figure 2. Specific cut-off values for each week of gestational age between 12-36 weeks are shown in Table 1.

Pregnant Patients at Risk of Placental Dysfunction

Table 2 summarizes demographic characteristics and obstetric history, with patients stratified into three groups: patients with low PlGF levels in the early second trimester who initiated LMWH therapy (Group 1), patients with low PlGF levels in the early second trimester who did not initiate LMWH therapy (Group 2) and patients with low PlGF levels in the late second trimester who did not initiate LMWH therapy and developed early-onset preeclampsia (Group 3). Group 3 patients were initially assessed and identified at a later gestational age than patients in Groups 1 and 2.

Maternal Circulating PlGF following LMWH Administration

Figure 3 displays individual circulating PlGF results as gestation advanced in patients with severe placental dysfunction. Five of seven patients in Group 1 exhibited increases in circulating PlGF following initiation of LMWH, which ultimately decreased as gestational age advanced. In contrast, no patients in Group 2 demonstrated a notable rise in circulating PlGF. Patients in Group 3, with an initial PlGF test completed ≥20 weeks' gestation, exhibited a similar trend as Group 2. Longitudinal PlGF values across

gestation are shown in Figure 4 by patient group with the superimposed 2.5th, 5th and 10th centiles of the newly derived PlGF reference curve from Figure 2.

Pregnancy Outcomes

Table 3 shows maternal, fetal and delivery outcomes together with placental pathology findings. Patients in Group 1 exhibited a later gestational age at delivery, relative to Groups 2 and 3 (36 weeks [33–37] versus 23 weeks [22–26] and 28 weeks [27-31], respectively). Birthweights were higher in Group 1 pregnancies, relative to Groups 2 and 3 (1.93 kg [1.1–2.7] versus 0.32 kg [0.19–0.39] and 0.73 kg [0.52-1.03], respectively). The incidence of stillbirth was lowest in Group 1, relative to Groups 2 and 3 (14% versus 80% and 29%, respectively).

Maternal vascular malperfusion was the predominant principal placental pathology in all patients, with highest incidence in Group 3, relative to Groups 1 and 2 (90% versus 43% and 40%, respectively). In Group 1, both patients with normal mean uterine artery Doppler were found to each have a rare placental pathology diagnosis (peri-villous fibrin deposition; chronic histiocytic inter-villositis) while the remaining five with abnormal mean uterine artery Doppler expressed features of MVM pathology. Mean uterine artery pulsatility index across gestation stratified by placental pathology and LMWH therapy is presented in Supplementary Figure 1.

COMMENT

Principal Findings

The principal findings of the study are threefold. First, we merged data from two cohort studies of healthy nulliparous patients to create a reference curve for clinicians to interpret circulating placental growth factor (PlGF) on a weekly basis between 12-36 weeks' gestation. Second, our approach to the assessment of placental function at 16 weeks' gestation through PlGF testing identified patients at high risk of placental dysfunction destined to have adverse placenta-mediated pregnancy outcomes despite aspirin prophylaxis. Finally, the administration of prophylactic daily LMWH to a small group of women with low circulating PlGF in the early second trimester resulted in sustained elevations in circulating PlGF.

Limitations

We acknowledge that these are preliminary proof of principal findings, limited by sample size and study design. Our data cannot be interpreted as a recommendation for the use of LMWH to improve placental function and therefore clinical outcomes in high-risk pregnancy care. Although all high-risk pregnant patients in our study were identified through serial PlGF testing and ultrasound imaging, we acknowledge that there may have been inherent differences between patients who did and did not consent to initiate LMWH therapy; for example, baseline PlGF levels appear to be higher in patients in Group 1, relative to patients in Group 2, while PAPP-A multiple of median values were lowest in Group 2.

Results in the Context of What is Known

By applying quantile regression analysis to PlGF data merged from two prospective cohorts of nulliparous Caucasian women, we established a continuous reference range between 12-36 weeks' gestation. We restricted the construction of the reference range to these two cohorts due to their adequate size and uniformity of assay manufacturer, since assay type is a source of data variability.[33] Previous studies have grouped reference PlGF values into blocks of time[4, 34-36], though one study used quantile regression to derive a reference range for PlGF using serial samples from 180 uncomplicated pregnancies beginning at 12 weeks' gestation[37]. Burke et al. merged data derived from 4 test platforms across 22 studies into a continuous reference range, however this analysis commenced from 20 weeks' gestation, and is therefore of no value in the context of assessing placental function in the early second trimester[38]. In contrast to the use of a pragmatic single cut-off diagnostic value of 100pg/ml for PlGF at 20-36 weeks, when circulating PlGF has achieved a plateau, a continuous reference range resource during the rising phase of PlGF in the early second trimester is a necessity in order to use serial PlGF to screen high-risk women on aspirin for severe placenta-mediated adverse outcomes[4]. This resource is, however, limited by the use of one test platform and being predominantly derived from Caucasian patients. A recent investigation determined that circulating PlGF levels at 16 weeks of gestation varied significantly by ethnicity[32]; further studies involving adequate numbers of specific ethnic

groups are warranted, especially since some non-Caucasian groups are especially vulnerable to developing severe preeclampsia.

Several investigators have demonstrated an association between the abnormal expression of placenta-derived angiogenic factors and placental disease, principally MVM, which is the most common pathologic finding in this context[16, 39, 40]. Recently, the hypoxic-ischemic features of this disease have been demonstrated *in-vivo* in patients with early-onset preeclampsia and low circulating PlGF using advanced magnetic resonance imaging methods which measure regional tissue oxygenation by T2* oximetry[41]. These observations are consistent with *in-vitro* data in human placental explants demonstrating hypoxia-mediated repression of PlGF synthesis[42]. Data from the SCOPE consortium, demonstrating that low circulating placenta growth factor combined with abnormal uterine artery Doppler at 16 weeks' gestation is highly predictive of early-onset preeclampsia[9], suggests that a strategy of early serial PlGF testing may identify women with significantly impaired early placental development. Up to 15% of women with severe placental dysfunction exhibit diseases other than MVM, especially massive peri-villous fibrin deposition and chronic histiocytic inter-villositis, in which uteroplacental blood flow is not typically restricted[7, 14, 43]. Such patients are therefore likely to exhibit normal uterine artery Doppler waveforms, yet may be identified in tandem with a majority that are developing MVM placental disease in the early second trimester by their common deficiency in a failure of PlGF to rise in the 16-20 week period. Interestingly, 2 of the 7 patients in Group 1 followed this pattern, with

353 normal uterine artery Doppler, a failure to elevate PlGF and subsequent demonstration
354 of non-MVM placental pathologies.

355 The extremely high rates of placental pathology in our subjects, together with
356 their near universal risk of preterm delivery, validated our strategy to identify a niche of
357 patients who remain vulnerable to the effects of severe placental dysfunction despite
358 taking aspirin. The concept of dual screening with circulating angiogenic growth factors
359 and uterine artery Doppler to identify the most common type of underlying placental
360 MVM disease was originally developed by Espinosa et al., who, in a screening study of
361 3348 unselected patients in a 22-26 week window, noted that a PlGF cut-off value of
362 280 pg/mL in patients with abnormal uterine artery Doppler predicted 84% of early-
363 onset and 89% of severe preeclampsia[8]. These findings were largely replicated at an
364 earlier gestational age around 16 weeks' gestation in 3529 healthy nulliparous patients
365 by the SCOPE consortium, which demonstrated a positive likelihood ratio of 9.4 for the
366 prediction of preterm preeclampsia when both uterine artery Doppler and circulating
367 PlGF were normal[9]. Common to both studies, patients were not exposed to aspirin, and
368 neither reported placental pathology findings. With the increased use of first trimester
369 screening methods for the prevention of early-onset preeclampsia with aspirin, the
370 high-risk patients in our study were already on aspirin by 12 weeks' gestation due to
371 their significant prior risk factors[10-12]. As such, any inference from our findings that
372 LMWH may be of benefit cannot be extended beyond this niche group of vulnerable
373 patients on aspirin therapy, identified in the early second trimester.

The negative findings from two large high-quality trials[44, 45] testing the hypothesis that prophylactic low molecular weight heparin (LMWH) can further reduce the risk of preterm delivery in addition to aspirin coupled with evidence that aspirin alone is an effective agent has led clinicians to avoid recommending LMWH in this context[11, 12]. Both trials recruited women based on their prior obstetric history, and had low rates of serious adverse perinatal outcomes (perinatal death or iatrogenic delivery <32 weeks) in the control arms, suggesting adequate placental function in a majority of participants. A previous cohort study, involving 212 women of similar risk characteristics, found that a majority (59%) had normal measures of placental function in the early second trimester and had significantly more favorable outcomes than the remainder with one or more placental test abnormalities, for example 19/23 women developing HELLP syndrome and requiring delivery <34 weeks had one or more test abnormalities[46]. These observations are relevant in the context of the most updated systematic review on the adjunct role of LMWH which evaluated 15 studies with variable entry criteria, demonstrating that LMWH in addition to low-dose aspirin before 16 weeks' gestation significantly lowered the risk of early-onset disease (odds 0.62, 95% CI 0.41-0.95)[22]. Concentrating future trial recruitment on a subgroup of clinically high-risk women with abnormal measures of placental function in early pregnancy may provide definitive answers on the role of LMWH in this niche group of women.

The potential mechanisms of action of LMWH in the context of prevention of early-onset preeclampsia are varied, and do not necessarily involve anticoagulation of maternal blood within the placenta[47]. Several non-anticoagulant actions of LMWH may

396 be pertinent in the context of preventing early-onset preeclampsia associated with
397 MVM of the placenta[21]. These include suppression of leukocyte activation and
398 complement activity alongside promotion of angiogenesis[17]. In *in-vivo* studies of
399 pregnant subjects, LMWH acutely elevated suboptimal circulating PlGF by 1.5-fold, and
400 larger increases in circulating PlGF levels have been found in patients receiving
401 therapeutic levels of LMWH[19, 20]. A subsequent publication from the Heparin-
402 Preeclampsia (HEPEPE) trial group focused on circulating angiogenic growth factor
403 profiles in high-risk pregnant patients, demonstrating no significant differences in
404 circulation PlGF across gestation between patients randomized to receive aspirin alone
405 or aspirin with LMWH therapy[48]. Very few women in this randomized trial had low
406 circulating PlGF levels prior to 18 week's gestation, few had severe adverse perinatal
407 outcomes, and the trajectory of PlGF in both arms of the trial are similar to the
408 reference range data reported in this study. In contrast, the current observational study
409 recruited high-risk patients (Groups 1 and 2) based on low PlGF levels early in the
410 second trimester, and was confirmed by both their clinical outcomes and placental
411 pathology testing. Our interpretation is that LMWH therapy could exert beneficial
412 effects in high-risk pregnant patients with significant placental dysfunction, by reversing
413 a low PlGF phenotype found in the early second trimester. The Enoxaparin for the
414 Prevention of Preeclampsia and Intrauterine Growth Restriction (EPPI) trial had a similar
415 design and overall findings[44], and neither the HEPEPE or EPPI trial has reported placental
416 pathology data, which would be of significant interest to elucidating any potential
417 beneficial impacts of LMWH therapy.

418 In *in-vitro* studies with human tissues, LMWH augmented the synthesis and
419 release of PlGF by placental villous tissue explants and a similar stimulatory effect by
420 LMWH has recently been demonstrated in endothelial cells[1, 18]. The possibility of
421 inducing an endogenous rise in circulating PlGF via LMWH is preferable to the
422 administration of parenteral exogenous PlGF to achieve the same effect[49]. Collectively
423 these studies support a plausible mechanism whereby LMWH, administered to a small
424 subset of patients at greatest risk of placenta-mediated early-onset preeclampsia, may
425 be capable of inducing of a clinically meaningful sustained rise in their deficient PlGF
426 levels.
427
428 Clinical Implications
429 For clinicians managing pregnancies at high-risk of placental dysfunction, especially in a
430 Maternal-Fetal Medicine setting, the ability to identify an asymptomatic vulnerable
431 subset in the early second trimester, despite receiving aspirin prophylaxis, is a key
432 strategic goal. The availability of PlGF testing in real-time (within 90 minutes) combined
433 with uterine artery Doppler assessment, may be one such strategy for clinicians to
434 adopt, a we have done. This approach can focus clinical resources on women that need
435 higher levels of care, and it in addition may facilitate further research into the adjunct
436 role of additional medical therapies to prevent stillbirth and extreme preterm delivery[7,
437 8, 50].
438
439

Research Implications

Our findings do not support the clinical use of LMWH to prevent placenta-mediated complications, but they may inform the design of new pilot randomized control trials confined to a sub-group of high-risk patients already on aspirin prophylaxis. This approach was taken in a small 2011 trial where LMWH and aspirin were used concurrently and was conducted prior to the advent of PlGF screening[51]. At present, we do not know if any of the observed *in-vitro* effects of LMWH could exert clinically meaningful actions on the placenta such as restoring PlGF release into the maternal circulation, directly acting on the systemic maternal endothelium or on improving the typically restricted uteroplacental circulation that is found in women at highest risk of severe preeclampsia[8, 9, 52]. Therefore, future baseline trial data could incorporate serial urine analysis of the stable C5b fragment that reflects complement activation[53], together with serial maternal blood angiogenic growth factors, and incorporate specialist placental pathology blinded to trial allocation.

Conclusions

A continuous reference range for maternal circulating PlGF was established between 12-36 weeks' gestation for high-risk pregnancy clinicians to assess the ongoing risk of preterm delivery due to severe placental dysfunction. Amongst a small subgroup of pregnancies considered high-risk in this context with low maternal circulating PlGF at 16-20 weeks' gestation, the addition of prophylactic LMWH to aspirin prophylaxis induced a rise in circulating PlGF that was sustained in a majority of patients over

several weeks prior to delivery. Since the most recent evidence obtained from systematic reviews of relevant trials support limited use of LMWH in this context, our data may inform future efforts to conduct a pilot randomized controlled trial to further explore the relevant biologic actions of LMWH in this context, which if favourable could inform a subsequent definitive randomized trial with entry criteria that focus on the assessment of placental function in-vivo.

REFERENCES

1. McLaughlin K, Nadeem L, Wat J, Baczyk D, Lye SJ and Kingdom JC. Low molecular weight heparin promotes transcription and release of placental growth factor from endothelial cells. *American journal of physiology Heart and circulatory physiology*. 2020;318:H1008-h1017.
2. Clark DE, Smith SK, Licence D, Evans AL and Charnock-Jones DS. Comparison of expression patterns for placenta growth factor, vascular endothelial growth factor (VEGF), VEGF-B and VEGF-C in the human placenta throughout gestation. *J Endocrinol*. 1998;159:459-67.
3. Sovio U, Gaccioli F, Cook E, Hund M, Charnock-Jones DS and Smith GC. Prediction of Preeclampsia Using the Soluble fms-Like Tyrosine Kinase 1 to Placental Growth Factor Ratio: A Prospective Cohort Study of Unselected Nulliparous Women. *Hypertension (Dallas, Tex : 1979)*. 2017;69:731-738.
4. Agrawal S, Shinar S, Cerdeira AS, Redman C and Vatish M. Predictive Performance of PlGF (Placental Growth Factor) for Screening Preeclampsia in Asymptomatic Women: A Systematic Review and Meta-Analysis. *Hypertension (Dallas, Tex : 1979)*. 2019;74:1124-1135.
5. Chappell LC, Duckworth S, Seed PT, Griffin M, Myers J, Mackillop L, Simpson N, Waugh J, Anumba D, Kenny LC, Redman CW and Shennan AH. Diagnostic accuracy of placental growth factor in women with suspected preeclampsia: a prospective multicenter study. *Circulation*. 2013;128:2121-31.
6. Benton SJ, McCowan LM, Heazell AE, Grynspan D, Hutcheon JA, Senger C, Burke O, Chan Y, Harding JE, Yockell-Lelièvre J, Hu Y, Chappell LC, Griffin MJ, Shennan

AH, Magee LA, Gruslin A and von Dadelszen P. Placental growth factor as a marker of fetal growth restriction caused by placental dysfunction. *Placenta*. 2016;42:1-8.

7. McLaughlin K, Snelgrove JW, Audette MC, Syed A, Hobson SR, Windrim RC, Melamed N, Carmona S and Kingdom JC. PlGF (Placental Growth Factor) Testing in Clinical Practice: Evidence From a Canadian Tertiary Maternity Referral Center. *Hypertension (Dallas, Tex : 1979)*. 2021:Hypertensionaha12117047.

8. Espinoza J, Romero R, Nien JK, Gomez R, Kusanovic JP, Gonçalves LF, Medina L, Edwin S, Hassan S, Carstens M and Gonzalez R. Identification of patients at risk for early onset and/or severe preeclampsia with the use of uterine artery Doppler velocimetry and placental growth factor. *American journal of obstetrics and gynecology*. 2007;196:326.e1-13.

9. Myers JE, Kenny LC, McCowan LM, Chan EH, Dekker GA, Poston L, Simpson NA and North RA. Angiogenic factors combined with clinical risk factors to predict preterm pre-eclampsia in nulliparous women: a predictive test accuracy study. *BJOG : an international journal of obstetrics and gynaecology*. 2013;120:1215-23.

10. Rolnik DL, Wright D, Poon LC, O'Gorman N, Syngelaki A, de Paco Matallana C, Akolekar R, Cicero S, Janga D, Singh M, Molina FS, Persico N, Jani JC, Plasencia W, Papaioannou G, Tenenbaum-Gavish K, Meiri H, Gizurarson S, Maclagan K and Nicolaides KH. Aspirin versus Placebo in Pregnancies at High Risk for

Preterm Preeclampsia. *The New England journal of medicine*. 2017;377:613-622.

11. Wright A, Wright D, Syngelaki A, Georgantis A and Nicolaides KH. Two-stage screening for preterm preeclampsia at 11-13 weeks' gestation. *American journal of obstetrics and gynecology*. 2019;220:197.e1-197.e11.

12. Guy GP, Leslie K, Diaz Gomez D, Forenc K, Buck E, Khalil A and Thilaganathan B. Implementation of routine first trimester combined screening for pre-eclampsia: a clinical effectiveness study. *BJOG : an international journal of obstetrics and gynaecology*. 2020.

13. Poon LC, Wright D, Rolnik DL, Syngelaki A, Delgado JL, Tsokaki T, Leipold G, Akolekar R, Shearing S, De Stefani L, Jani JC, Plasencia W, Evangelinakis N, Gonzalez-Vanegas O, Persico N and Nicolaides KH. Aspirin for Evidence-Based Preeclampsia Prevention trial: effect of aspirin in prevention of preterm preeclampsia in subgroups of women according to their characteristics and medical and obstetrical history. *American journal of obstetrics and gynecology*. 2017;217:585.e1-585.e5.

14. Kingdom JC, Audette MC, Hobson SR, Windrim RC and Morgen E. A placenta clinic approach to the diagnosis and management of fetal growth restriction. *American journal of obstetrics and gynecology*. 2018;218:S803-s817.

15. O'Brien M, Baczyk D and Kingdom JC. Endothelial Dysfunction in Severe Preeclampsia is Mediated by Soluble Factors, Rather than Extracellular Vesicles. *Sci Rep*. 2017;7:5887.

16. Zur RL, Kingdom JC, Parks WT and Hobson SR. The Placental Basis of Fetal Growth Restriction. *Obstetrics and gynecology clinics of North America*. 2020;47:81-98.

17. Wat JM, Hawrylyshyn K, Baczyk D, Greig IR and Kingdom JC. Effects of glycol-split low molecular weight heparin on placental, endothelial, and anti-inflammatory pathways relevant to preeclampsia. *Biology of reproduction*. 2018;99:1082-1090.

18. Drewlo S, Levytska K, Sobel M, Baczyk D, Lye SJ and Kingdom JC. Heparin promotes soluble VEGF receptor expression in human placental villi to impair endothelial VEGF signaling. *J Thromb Haemost*. 2011;9:2486-97.

19. McLaughlin K, Baczyk D, Potts A, Hladunewich M, Parker JD and Kingdom JC. Low Molecular Weight Heparin Improves Endothelial Function in Pregnant Women at High Risk of Preeclampsia. *Hypertension (Dallas, Tex : 1979)*. 2017;69:180-188.

20. Yinon Y, Ben Meir E, Margolis L, Lipitz S, Schiff E, Mazaki-Tovi S and Simchen MJ. Low molecular weight heparin therapy during pregnancy is associated with elevated circulatory levels of placental growth factor. *Placenta*. 2015;36:121-4.

21. McLaughlin K, Scholten RR, Parker JD, Ferrazzi E and Kingdom JCP. Low molecular weight heparin for the prevention of severe preeclampsia: where next? *British journal of clinical pharmacology*. 2018;84:673-678.

22. Cruz-Lemini M, Vázquez JC, Ullmo J and Llurba E. Low-molecular-weight heparin for prevention of preeclampsia and other placenta-mediated

558 complications: a systematic review and meta-analysis. *American journal of*
559 *obstetrics and gynecology*. 2021.
560 23. Gaccioli F, Lager S, Sovio U, Charnock-Jones DS and Smith GCS. The pregnancy
561 outcome prediction (POP) study: Investigating the relationship between serial
562 prenatal ultrasonography, biomarkers, placental phenotype and adverse
563 pregnancy outcomes. *Placenta*. 2017;59:S17-S25.
564 24. Wright E, Audette MC, Ye XY, Keating S, Hoffman B, Lye SJ, Shah PS and
565 Kingdom JC. Maternal Vascular Malperfusion and Adverse Perinatal Outcomes
566 in Low-Risk Nulliparous Women. *Obstetrics and gynecology*. 2017;130:1112-
567 1120.
568 25. Gómez O, Figueras F, Fernández S, Bennasar M, Martínez JM, Puerto B and
569 Gratacós E. Reference ranges for uterine artery mean pulsatility index at 11-41
570 weeks of gestation. *Ultrasound in obstetrics & gynecology : the official journal of*
571 *the International Society of Ultrasound in Obstetrics and Gynecology*.
572 2008;32:128-32.
573 26. Magee LA, Pels A, Helewa M, Rey E and von Dadelszen P. Diagnosis, evaluation,
574 and management of the hypertensive disorders of pregnancy: executive
575 summary. *Journal of obstetrics and gynaecology Canada : JOGC = Journal*
576 *d'obstetrique et gynecologie du Canada : JOGC*. 2014;36:416-41.
577 27. Hypertension in pregnancy. Report of the American College of Obstetricians
578 and Gynecologists' Task Force on Hypertension in Pregnancy. *Obstetrics and*
579 *gynecology*. 2013;122:1122-1131.

28. Khong TY, Mooney EE, Ariel I, Balmus NC, Boyd TK, Brundler MA, Derricott H, Evans MJ, Faye-Petersen OM, Gillan JE, Heazell AE, Heller DS, Jacques SM, Keating S, Kelehan P, Maes A, McKay EM, Morgan TK, Nikkels PG, Parks WT, Redline RW, Scheimberg I, Schoots MH, Sebire NJ, Timmer A, Turowski G, van der Voorn JP, van Lijnschoten I and Gordijn SJ. Sampling and Definitions of Placental Lesions: Amsterdam Placental Workshop Group Consensus Statement. *Arch Pathol Lab Med*. 2016;140:698-713.

29. Cole TJ and Green PJ. Smoothing reference centile curves: the LMS method and penalized likelihood. *Statistics in medicine*. 1992;11:1305-19.

30. Rigby RA and Stasinopoulos DM. Generalized additive models for location, scale and shape. *Journal of the Royal Statistical Society: Series C (Applied Statistics)*. 2005;54:507-554.

31. Team RC. R: A Language and Environment for Statistical Computing. . *Vienna, Austria: R Foundation for Statistical Computing* 2020.

32. Audette MC, Levytska K, Lye SJ, Melamed N and Kingdom JC. Parental ethnicity and placental maternal vascular malperfusion pathology in healthy nulliparous women. *Placenta*. 2018;66:40-46.

33. McCarthy FP, Gill C, Seed PT, Bramham K, Chappell LC and Shennan AH. Comparison of three commercially available placental growth factor-based tests in women with suspected preterm pre-eclampsia: the COMPARE study. *Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology*. 2019;53:62-67.

34. Levine RJ, Maynard SE, Qian C, Lim KH, England LJ, Yu KF, Schisterman EF, Thadhani R, Sachs BP, Epstein FH, Sibai BM, Sukhatme VP and Karumanchi SA. Circulating angiogenic factors and the risk of preeclampsia. *The New England journal of medicine*. 2004;350:672-83.

35. Knudsen UB, Kronborg CS, von Dadelszen P, Kupfer K, Lee SW, Vittinghus E, Allen JG and Redman CW. A single rapid point-of-care placental growth factor determination as an aid in the diagnosis of preeclampsia. *Pregnancy hypertension*. 2012;2:8-15.

36. Verlohren S, Herraiz I, Lapaire O, Schlembach D, Zeisler H, Calda P, Sabria J, Markfeld-Erol F, Galindo A, Schoofs K, Denk B and Stepan H. New gestational phase-specific cutoff values for the use of the soluble fms-like tyrosine kinase-1/placental growth factor ratio as a diagnostic test for preeclampsia. *Hypertension (Dallas, Tex : 1979)*. 2014;63:346-52.

37. Chaiworapongsa T, Romero R, Savasan ZA, Kusanovic JP, Ogge G, Soto E, Dong Z, Tarca A, Gaurav B and Hassan SS. Maternal plasma concentrations of angiogenic/anti-angiogenic factors are of prognostic value in patients presenting to the obstetrical triage area with the suspicion of preeclampsia. *The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet*. 2011;24:1187-207.

38. Burke Ó, Benton S, Szafranski P, von Dadelszen P, Buhimschi SC, Cetin I, Chappell L, Figueras F, Galindo A, Herraiz I, Holzman C, Hubel C, Knudsen U,

Kronborg C, Laivuori H, Lapaire O, McElrath T, Moertl M, Myers J, Ness RB, Oliveira L, Olson G, Poston L, Ris-Stalpers C, Roberts JM, Schalekamp-Timmermans S, Schlembach D, Steegers E, Stepan H, Tsatsaris V, van der Post JA, Verlohren S, Villa PM, Williams D, Zeisler H, Redman CW and Staff AC. Extending the scope of pooled analyses of individual patient biomarker data from heterogeneous laboratory platforms and cohorts using merging algorithms. *Pregnancy hypertension*. 2016;6:53-9.

39. Benton SJ, Hu Y, Xie F, Kupfer K, Lee SW, Magee LA and von Dadelszen P. Can placental growth factor in maternal circulation identify fetuses with placental intrauterine growth restriction? *American journal of obstetrics and gynecology*. 2012;206:163.e1-7.

40. Korzeniewski SJ, Romero R, Chaiworapongsa T, Chaemsaithong P, Kim CJ, Kim YM, Kim JS, Yoon BH, Hassan SS and Yeo L. Maternal plasma angiogenic index-1 (placental growth factor/soluble vascular endothelial growth factor receptor-1) is a biomarker for the burden of placental lesions consistent with uteroplacental underperfusion: a longitudinal case-cohort study. *American journal of obstetrics and gynecology*. 2016;214:629.e1-629.e17.

41. Ho AEP, Hutter J, Jackson LH, Seed PT, McCabe L, Al-Adnani M, Marnerides A, George S, Story L, Hajnal JV, Rutherford MA and Chappell LC. T2* Placental Magnetic Resonance Imaging in Preterm Preeclampsia: An Observational Cohort Study. *Hypertension (Dallas, Tex : 1979)*. 2020;75:1523-1531.

42. Khaliq A, Dunk C, Jiang J, Shams M, Li XF, Acevedo C, Weich H, Whittle M and Ahmed A. Hypoxia down-regulates placenta growth factor, whereas fetal

growth restriction up-regulates placenta growth factor expression: molecular evidence for "placental hyperoxia" in intrauterine growth restriction. *Lab Invest*. 1999;79:151-70.

43. Levytska K, Higgins M, Keating S, Melamed N, Walker M, Sebire NJ and Kingdom JC. Placental Pathology in Relation to Uterine Artery Doppler Findings in Pregnancies with Severe Intrauterine Growth Restriction and Abnormal Umbilical Artery Doppler Changes. *American journal of perinatology*. 2017;34:451-457.

44. Groom KM, McCowan LM, Mackay LK, Lee AC, Said JM, Kane SC, Walker SP, van Mens TE, Hannan NJ, Tong S, Chamley LW, Stone PR and McLintock C. Enoxaparin for the prevention of preeclampsia and intrauterine growth restriction in women with a history: a randomized trial. *American journal of obstetrics and gynecology*. 2017;216:296.e1-296.e14.

45. Haddad B, Winer N, Chitrit Y, Houfflin-Debarge V, Chauleur C, Bages K, Tsatsaris V, Benachi A, Bretelle F, Gris JC and Bastuji-Garin S. Enoxaparin and Aspirin Compared With Aspirin Alone to Prevent Placenta-Mediated Pregnancy Complications: A Randomized Controlled Trial. *Obstetrics and gynecology*. 2016;128:1053-1063.

46. Toal M, Chan C, Fallah S, Alkazaleh F, Chaddha V, Windrim RC and Kingdom JC. Usefulness of a placental profile in high-risk pregnancies. *American journal of obstetrics and gynecology*. 2007;196:363.e1-7.

47. Kingdom JC and Drewlo S. Is heparin a placental anticoagulant in high-risk pregnancies? *Blood*. 2011;118:4780-8.

48. Lecarpentier E, Gris JC, Cochery-Nouvellon E, Mercier E, Touboul C, Thadhani R, Karumanchi SA and Haddad B. Angiogenic Factor Profiles in Pregnant Women With a History of Early-Onset Severe Preeclampsia Receiving Low-Molecular-Weight Heparin Prophylaxis. *Obstetrics and gynecology*. 2018;131:63-69.

49. Spradley FT, Tan AY, Joo WS, Daniels G, Kussie P, Karumanchi SA and Granger JP. Placental Growth Factor Administration Abolishes Placental Ischemia-Induced Hypertension. *Hypertension (Dallas, Tex : 1979)*. 2016;67:740-7.

50. Dröge LA, Perschel FH, Stütz N, Gafron A, Frank L, Busjahn A, Henrich W and Verlohren S. Prediction of Preeclampsia-Related Adverse Outcomes With the sFlt-1 (Soluble fms-Like Tyrosine Kinase 1)/PlGF (Placental Growth Factor)-Ratio in the Clinical Routine: A Real-World Study. *Hypertension (Dallas, Tex : 1979)*. 2021;77:461-471.

51. Kingdom JC, Walker M, Proctor LK, Keating S, Shah PS, McLeod A, Keunen J, Windrim RC and Dodd JM. Unfractionated heparin for second trimester placental insufficiency: a pilot randomized trial. *J Thromb Haemost*. 2011;9:1483-92.

52. Mello G, Parretti E, Fatini C, Riviello C, Gensini F, Marchionni M, Scarselli GF, Gensini GF and Abbate R. Low-molecular-weight heparin lowers the recurrence rate of preeclampsia and restores the physiological vascular changes in angiotensin-converting enzyme DD women. *Hypertension (Dallas, Tex : 1979)*. 2005;45:86-91.

693 53. Burwick RM, Fichorova RN, Dawood HY, Yamamoto HS and Feinberg BB.
694 Urinary excretion of C5b-9 in severe preeclampsia: tipping the balance of
695 complement activation in pregnancy. *Hypertension (Dallas, Tex : 1979)*.
696 2013;62:1040-5.
697 54. Ellison J, Thomson AJ, Conkie JA, McCall F, Walker D and Greer A.
698 Thromboprophylaxis following caesarean section--a comparison of the
699 antithrombotic properties of three low molecular weight heparins--dalteparin,
700 enoxaparin and tinzaparin. *Thromb Haemost*. 2001;86:1374-8.
701 55. Quenby S, Mountfield S, Cartwright JE, Whitley GS and Vince G. Effects of low-
702 molecular-weight and unfractionated heparin on trophoblast function.
703 *Obstetrics and gynecology*. 2004;104:354-61.
704 56. Manduteanu I, Dragomir E, Voinea M, Capraru M and Simionescu M.
705 Enoxaparin reduces H2O2-induced activation of human endothelial cells by a
706 mechanism involving cell adhesion molecules and nuclear transcription
707 factors. *Pharmacology*. 2007;79:154-62.
708 57. Torricelli M, Reis FM, Florio P, Severi FM, Bocchi C, Picciolini E, Guidoni CG and
709 Petraglia F. Low-molecular-weight heparin improves the performance of
710 uterine artery Doppler velocimetry to predict preeclampsia and small-for-
711 gestational age infant in women with gestational hypertension. *Ultrasound*
712 *Med Biol*. 2006;32:1431-5.
713

714 TABLES

Gestational Age	2.5th Centile	5th Centile	10th Centile	50th Centile
12	19	21	25	40
13	23	26	30	49
14	28	32	38	63
15	35	42	49	84
16	45	53	64	110
17	57	67	80	139
18	69	81	96	166
19	80	94	111	191
20	91	106	126	217
21	101	118	141	246
22	110	131	157	280
23	120	144	175	321
24	131	159	196	368
25	140	173	216	420
26	148	186	234	471
27	153	194	248	513
28	153	196	252	539
29	147	189	246	542
30	135	175	229	523
31	119	155	206	489
32	103	134	179	444
33	89	116	155	396
34	78	101	134	349
35	71	90	118	305
36	67	83	106	267

715

716

717 Table 1. Gestation age-specific values for circulating maternal placental growth factor (pg/mL) at the 5th, 10th and 50th centiles

718 derived using quantile regression of merged data from comparable Cambridge[3, 23] and Toronto[24] cohorts of nulliparous patients. All

719 7 subjects receiving low molecular weight heparin had a PlGF value <10th centile prior to administration at <20 weeks' gestation.

720

Maternal Characteristics	**Early Second Trimester Low PlGF, LMWH**	**Early Second Trimester Low PlGF, No LMWH**	**Late Second Trimester Low PlGF, Early-Onset Preeclampsia No LMWH**
	N=7	**N=5**	**N=21**
Demographic characteristics			
Age, years	35 [34-38]	39 [35-40]	33 [29-37]
Ethnicity			
White	5 (71)	1 (20)	13 (62)
Black	0 (0)	0 (0)	2 (10)
East Asian	1 (14)	1 (20)	2 (10)
South Asian	1 (14)	3 (60)	4 (19)
BMI, kg/m^2	27 [24-31]	26 [21-29]	32 [23-39]

Chronic hypertension	0 (0)	1 (20)	3 (14)
Pre-existing diabetes	0 (0)	0 (0)	2 (10)
Initial assessment, weeks' gestation	12 [11-13]	16 [16-18]	22 [20-24]
Systolic blood pressure at initial assessment, mmHg*	118 [92-120]	112 [105-143]	120 [113-132]
Diastolic blood pressure at initial assessment, mmHg*	66 [59-72]	80 [63-89]	71 [66-82]
Obstetrical history			
History of placental complications	5 (71)	2 (40)	6 (29)
Previous preeclampsia	2 (29)	2 (40)	4 (19)
Early-onset preeclampsia <34 weeks' gestation	2	2	3

Late-onset preeclampsia ≥34 weeks' gestation	0	0	1
Previous fetal death ≥20 weeks' gestation	3 (43)	2 (40)	1 (5)
Obstetrical characteristics			
Nulliparous	2 (29)	2 (40)	10 (48)
First trimester aneuploidy screening			
Not performed	1 (14)	1 (20)	7 (33)
PAPP-A, MoM	0.63 [0.39-0.91]	0.16 [0.12-0.39]	0.70 [0.51-1.48]
hCG, MoM	1.00 [0.98-1.32]	2.17 [1.28-3.01]	1.62 [1.28-1.86]
Mean uterine artery PI			
14 – 20 weeks' gestation	1.99 [1.38-2.49]	1.54 [1.42-1.66]	.
20 – 24 weeks' gestation	1.57 [1.36-1.82]	1.53 [1.45-1.60]	2.09 [1.73-2.54]
24 – 28 weeks' gestation	1.54 [1.16-1.78]	.	1.98 [1.80-2.50]

28 – 36 weeks' gestation	1.21 [0.90-1.38]	.	1.56 [1.35-2.02]

721
722 Table 2. Characteristics of Pregnant Patients at Risk of Placental Dysfunction. Data are presented as median [interquartile range], or
723 n (% of column). *Blood pressure data missing from 1 patient in Group 1.
724 PlGF = placental growth factor (pg/mL). LMWH = low molecular weight heparin. BMI = body mass index. PAPP-A = pregnancy
725 associated plasma protein-A. hCG = human chorionic gonadotropin. MoM = multiples of the median. PI=pulsatility index.

	Early Second Trimester **Low PlGF** **LMWH**	**Early Second Trimester** **Low PlGF** **No LMWH**	**Late Second Trimester** **Early severe preeclampsia** **No LMWH**
	N=7	**N=5**	**N=21**
Pregnancy outcome characteristics			
Gestational age at delivery, weeks	36 [33-37]	23 [22-26]	28 [27-31]
Maternal outcome			
Preeclampsia	4 (57)	0 (0)	21 (100)
Fetal outcome			
Live birth	6 (86)	1 (20)	15 (71)
Stillbirth	1 (14)	4 (80)	6 (29)
Antihypertensive medication use	3 (43)	2 (40)	19 (90)
Birthweight, kg	1.93 [1.1-2.7]	0.32 [0.19-0.39]	0.73 [0.52-1.03]

Placental Pathology*			
Principal placental pathology			
Maternal vascular malperfusion	3 (43)	2 (40)	19 (90)
Fetal thrombotic vasculopathy	0 (0)	2 (40)	1 (5)
Fetal vascular malperfusion	1 (14)	0 (0)	0 (0)
Chronic histiocytic intervillositis	1 (14)	0 (0)	1 (5)
Perivillous fibrin deposition	1 (14)	0 (0)	0 (0)
Villitis of unknown etiology	1 (14)	0 (0)	0 (0)
Massive perivillous fibrin deposition	1 (14)	0 (0)	0 (0)
Additional pathology features			
Maternal vascular malperfusion	2 (29)	1 (20)	1 (5)
Chronic histiocytic intervillositis	0 (0)	1 (20)	1 (5)
Fetal thrombotic vasculopathy	1 (14)	0 (0)	1 (5)

Weight <3rd centile	1 (14)	0 (0)	0 (0)
Fetal thrombotic vasculopathy	0 (0)	0 (0)	2 (10)

726
727

728 Table 3. Pregnancy Outcomes and Placental Pathology of Pregnant Patients at Risk of Severe Placental Dysfunction. Data are

729 presented as median [interquartile range], or n (% of column). *Placental pathology data missing from 1 patient in Group 2.

730 PlGF = placental growth factor (pg/mL). LMWH = low molecular weight heparin.

FIGURE LEGENDS

FIGURE 1. Systemic effects of low molecular weight heparin in pregnancy[1, 17, 19, 20, 52, 54-57].

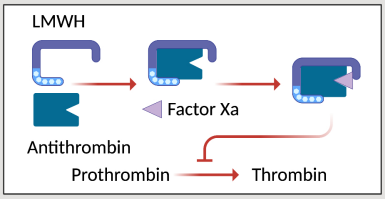
FIGURE 2. Gestation age-specific distribution of circulating maternal placental growth factor clustered at 4 time points with superimposed 2.5th, 5th, 10th and 50th centile curves derived using quantile regression. Data are from comparable Cambridge (individual data points at 12, 20, 28 and 36 weeks' gestation)[3, 23] and Toronto (box plot of median and interquartile range at 16 weeks' gestation)[24] cohorts of nulliparous patients. Gestation age-specific values at the 2.5th, 5th, 10th and 50th centiles from 12-26 weeks' gestation are shown in Table 1.

FIGURE 3. Individual Circulating PlGF Levels across Gestation in Pregnant Patients at Risk of Placental Dysfunction. Group 1 (patients with low PlGF levels in the early second trimester who initiated LMWH therapy, n=7) are presented in dark blue; dark blue open circles indicate pre-LMWH therapy, dark blue closed circles indicate post-LMWH therapy. Group 2 (patients with low PlGF levels in the early second trimester who did not initiate LMWH therapy, n=5) are presented in red. Group 3 (patients with low PlGF levels in the late second trimester who did not initiate LMWH therapy and developed early-onset preeclampsia, n=21) are presented in light blue. PlGF = placental growth factor, LMWH=low molecular weight heparin.

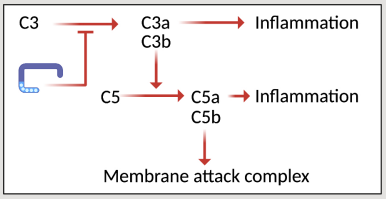
754 FIGURE 4. Individual Circulating PlGF Levels across Gestation in High-Risk Patients with
755 Placental Dysfunction Relative to 2.5th, 5th, and 10th Centile of the PlGF Reference Range.
756 Group 1 (patients with low PlGF levels in the early second trimester who initiated LMWH
757 therapy) are presented in red. Group 2 (patients with low PlGF levels in the early second
758 trimester who did not initiate LMWH therapy) are presented in green. Group 3 (patients
759 with low PlGF levels in the late second trimester who did not initiate LMWH therapy and
760 developed early-onset preeclampsia) are presented in blue. PlGF = placental growth
761 factor, LMWH=low molecular weight heparin.
762
763 Supplementary Figure 1. Maternal PlGF Levels Across Gestation in Pregnant Patients at
764 Risk of Severe Placental Dysfunction, Stratified by Placental Pathology Diagnosis and
765 LMWH Therapy. PlGF=placental growth factor, LMWH=low molecular weight heparin,
766 MVM=maternal vascular malperfusion.

Systemic Effects of Low Molecular Weight Heparin in Pregnancy

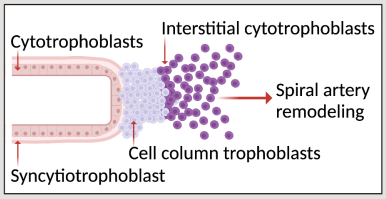
Inhibit thrombin formation

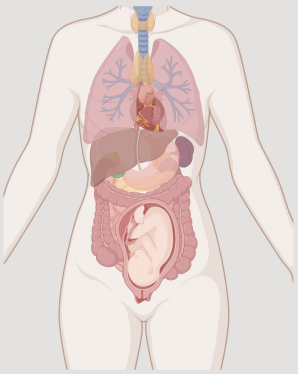


Suppress complement activation

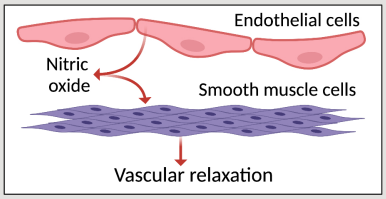


Promote differentiation and invasion of trophoblasts

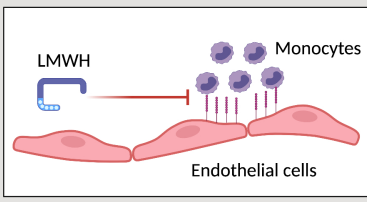




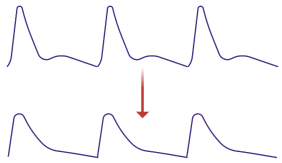
Improve systemic vascular function



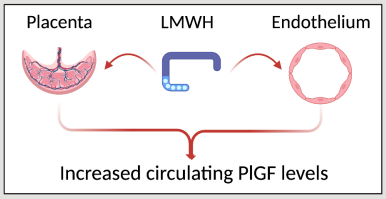
Prevent monocyte adhesion

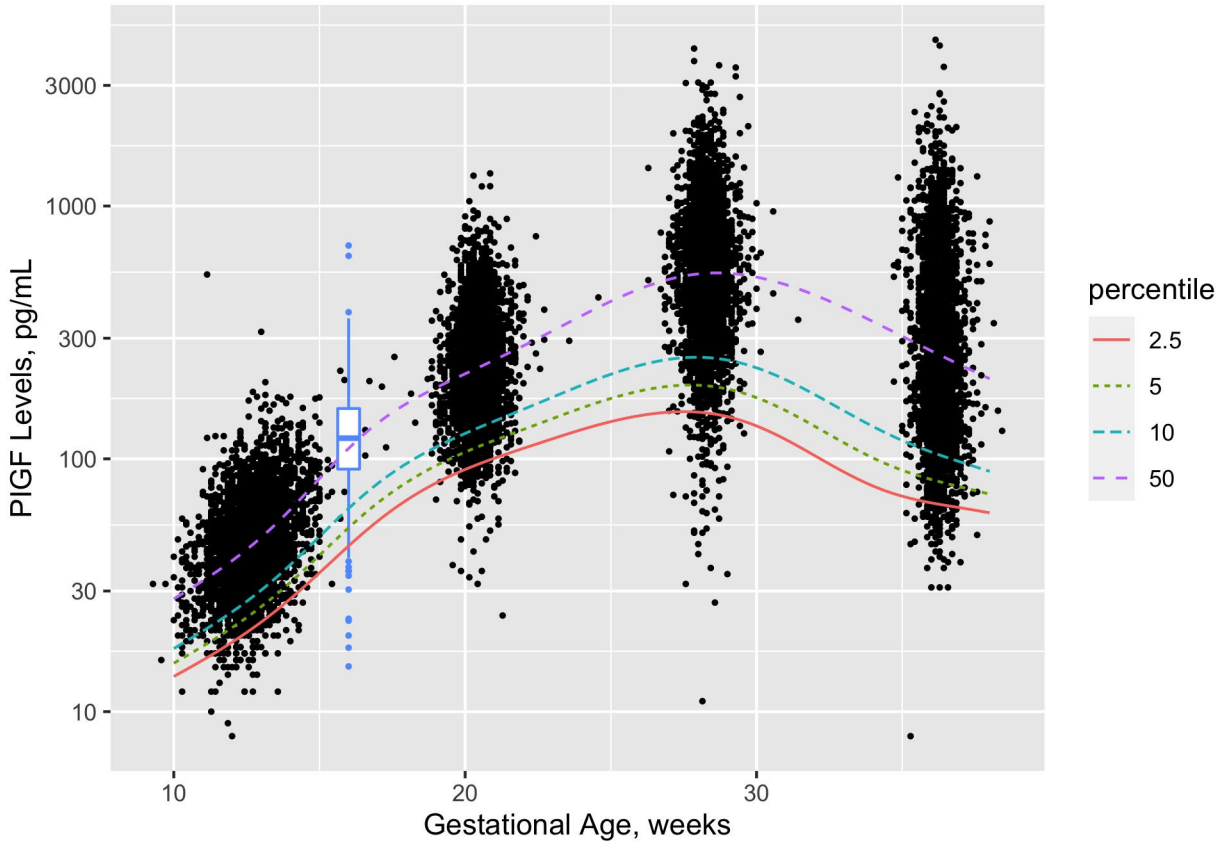


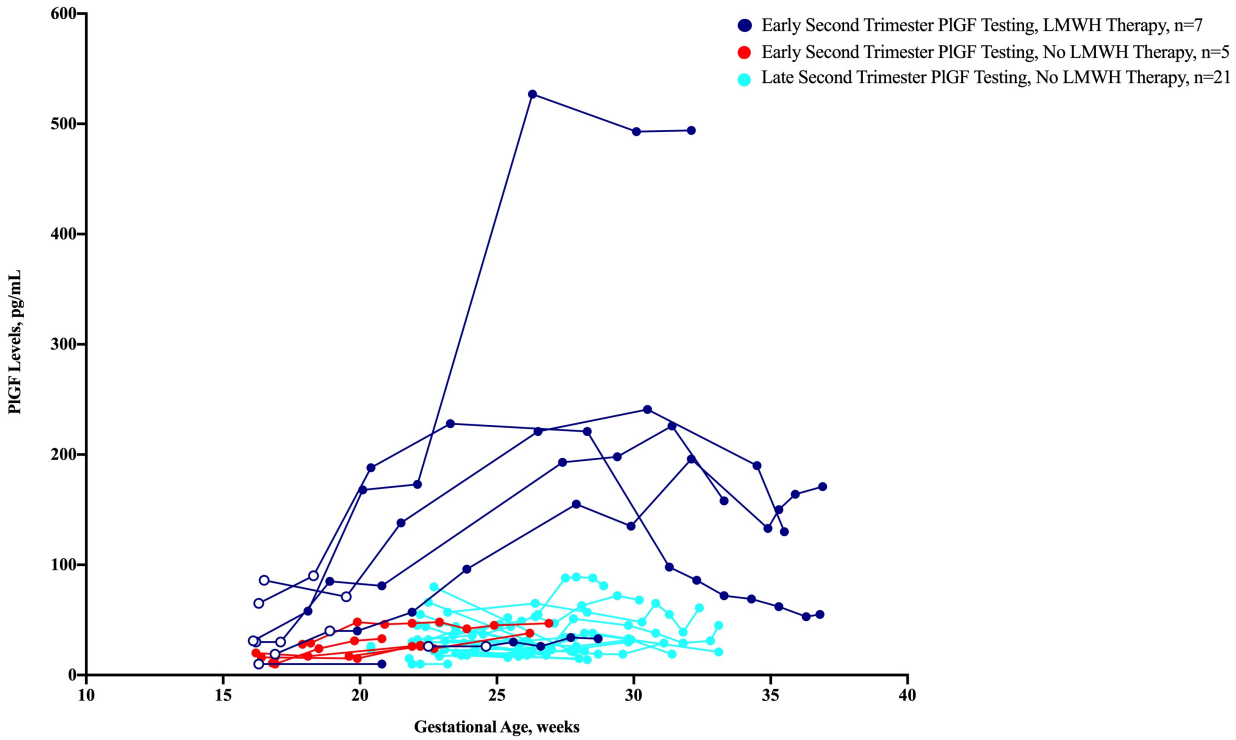
Decrease uterine artery pulsatility index

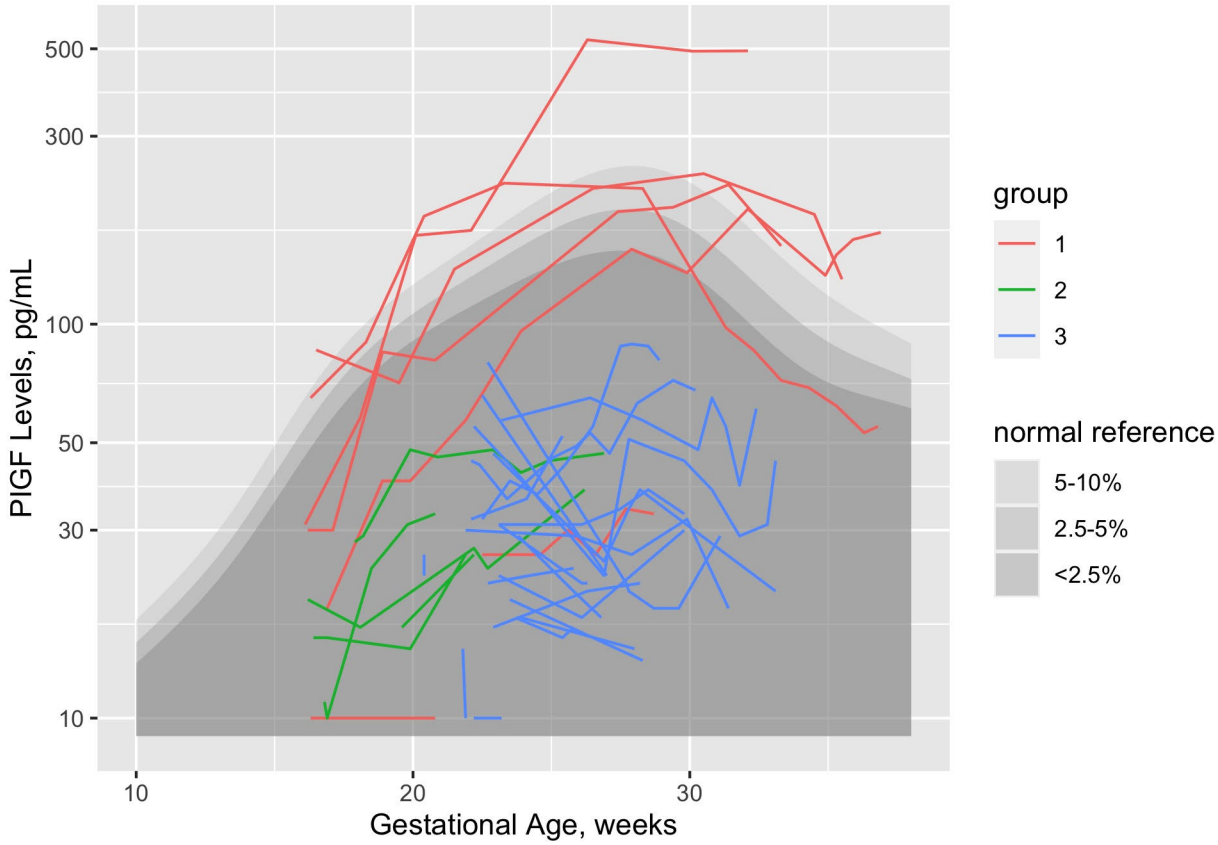


Increase circulating PlGF levels




3000
1000
300
100
30
10
PIGF Levels, pg/mL
10
20
30
Gestational Age, weeks
percentile
2.5
5
10
50


Early Second Trimester PlGF Testing, LMWH Therapy, n=7
Early Second Trimester PlGF Testing, No LMWH Therapy, n=5
Late Second Trimester PlGF Testing, No LMWH Therapy, n=21
PlGF Levels, pg/mL
600
500
400
300
200
100
0
10
15
20
25
30
35
40
Gestational Age, weeks


500
300
100
50
30
10
PIGF Levels, pg/mL
10
20
30
Gestational Age, weeks
group
1
2
3
normal reference
5-10%
2.5-5%
<2.5%