Circulating maternal placental growth factor responses to low-molecular-weight heparin in pregnant patients at risk of placental dysfunction

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BACKGROUND: Patients at high risk of severe preeclampsia and fetal growth restriction have low circulating levels of placental growth factor and features of maternal vascular malperfusion placental pathology at delivery. Multimodal screening and commencement of aspirin prophylaxis at 11 to 13 weeks’ gestation markedly reduces the risk of preterm delivery with preeclampsia. However, the additional role of low-molecular-weight heparin and mechanisms of action remain uncertain. Because low-molecular-weight heparin augments the production and release of placental growth factor 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 placental growth factor in the early second trimester.

OBJECTIVE: This study aimed to define a gestational age-specific reference range for placental growth factor and to test the hypothesis that prophylactic low-molecular-weight heparin administered in the early second trimester may restore deficient circulating placental growth factor levels and thereby prolong pregnancy.

STUDY DESIGN: Centile curves for circulating placental growth factor levels from 12 to 36 weeks’ gestation were derived using quantile regression of combined data from a published cohort of 4207 unselected nulliparous patients in Cambridge, United Kingdom, at 4 sampling time points (12, 20, 28, and 36 weeks’ gestation) and the White 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 7 patients with a circulating placental growth factor at the <10th centile in the early second trimester received daily prophylactic low-molecular-weight heparin (enoxaparin; 40 mg subcutaneously) and were followed up until delivery (group 1). Their baseline characteristics, delivery details, and placental pathologies were compared with 5 similar patients who did not receive low-molecular-weight heparin during the observation period (group 2) and further with 21 patients who delivered with severe preeclampsia (group 3) in the same institution.

RESULTS: A gestational age-specific reference range for placental growth factor levels at weekly intervals between 12 and 36 weeks was established for White women with singleton pregnancies. Within group 1, 5 of 7 patients demonstrated a sustained increase in circulating placental growth factor levels, whereas placental growth factor levels did not increase in group 2 or group 3 patients who did not receive low-molecular-weight heparin. Group 1 patients receiving low-molecular-weight heparin therapy exhibited a later gestation at delivery, relative to groups 2 and 3 (36 weeks [33–37] vs 23 weeks [22–26] and 28 weeks [27–31], respectively), and consequently had higher birthweights (1.93 kg [1.1–2.7] vs 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 of 7]), relative to groups 2 and 3 (80% [4 of 5] and 29% [6 of 21], respectively). Maternal vascular malperfusion was the most common placental pathology found in association with abnormal uterine artery Doppler.

CONCLUSION: In patients at high risk of a serious adverse pregnancy outcome owing to placental disease, the addition of low-molecular-weight heparin to aspirin prophylaxis in the early second trimester may restore deficient circulating placental growth factor to mediate an improved perinatal outcome. These data support the implementation of a multi-center pilot randomized control trial where patients are recruited primarily based on the assessment of placental function in the early second trimester.

Key words: biomarkers, fetal growth restriction, placental pathology, preeclampsia/eclampsia, treatment-management

Introduction
Placental growth factor (PlGF) is a proangiogenic protein produced by both the maternal vascular endothelium and the trophoblast layer covering the placental villi. Circulating PlGF levels rise steadily in maternal blood until the beginning of the third trimester, reflecting both the development of the utero-placental circulation and placental growth. Between 20 and 36 weeks’ gestation, a single cutoff value of 100 pg/mL has high diagnostic test precision for patients with suspected preeclampsia and placent-mediated fetal growth restriction. Consequently, real-time PlGF testing is an effective clinical tool for high-risk pregnancy management.

Before 20 weeks’ gestation, PlGF measurements may be useful to screen pregnancies for substantial placental dysfunction causing early-onset preeclampsia, for example, in combination with clinical risk factors and mean uterine artery Doppler at 16 weeks. Multimodal screening at an earlier gestation of 11 to 13 weeks’ gestation, incorporating PlGF and pregnancy-associated plasma protein-A (PAPP-A) with mean uterine artery Doppler, blood pressure, and maternal characteristics, is an effective tool to prevent preterm delivery owing to severe preeclampsia with the institution of low-dose aspirin prophylaxis. Despite this important advancement, aspirin is not universally...
A meta-analysis of randomized control trials suggests that a subgroup at highest risk of developing severe early-onset preeclampsia may benefit from low-molecular-weight heparin (LMWH) in addition to low-dose aspirin but the underlying mechanism of action of LMWH in this context is unknown. Women who develop severe early-onset preeclampsia have low maternal circulating placental growth factor (PIGF) levels at presentation and before disease onset. LMWH exerts several non-anticoagulant actions in vitro, including an ability to enhance PIGF release by placental villi and the maternal vascular endothelium.

Why was this study conducted?
A reference range for maternal circulating PIGF was generated for unselected nulliparous White women at 12 to 36 weeks’ gestation. In a small number of patients at high risk of placenta-mediated adverse pregnancy outcomes with low circulating PIGF in the early second trimester, the administration of LMWH induced sustained elevations in PIGF.

Key findings
A reference range for maternal circulating PIGF was generated for unselected nulliparous White women at 12 to 36 weeks’ gestation. In a small number of patients at high risk of placenta-mediated adverse pregnancy outcomes with low circulating PIGF in the early second trimester, the administration of LMWH induced sustained elevations in PIGF.

What does this add to what is 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 PIGF, so as to focus on those at highest risk.

Materials and Methods
Placental growth factor reference range
A reference range for PIGF was constructed by merging data from 2 studies of unselected nulliparous patients using the same assay method (Roche Diagnostics, Germany). Data at 4 sampling time points (12, 20, 28, and 36 weeks’ gestation) from a cohort of 4207 unselected nulliparous patients (92% White) in Cambridge, United Kingdom, were combined with data at the single time point of 16 weeks’ gestation from the White majority (531 of 773; 68.7%) within a healthy nulliparous cohort in Toronto, Canada. The PIGF assay coefficient of variation was 2.7% to 4.1% in the Cambridge study and 3.8% to 6.7% in the Toronto study.

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. Eligible patients included those at the age of >18 years with a live singleton fetus and followed up in the period between July 2017 and March 2021. Patients were not eligible if they were already on LMWH therapy for a maternal indication or if previously diagnosed with a major thrombophilia disorder or antiphospholipid 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 prepregnancy health and obstetrical history, received low-dose (162 mg nightly) aspirin prophylaxis for preeclampsia from 12 weeks’ gestation as part of standard clinical management. 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 at the >95th centile were considered abnormal. Based on previous cohort data, we
included same-day PlGF testing, because this test became available for the real-time management of high-risk pregnant patients in 2017 using the Elecsys platform (Roche Diagnostics, Germany).7,8 PlGF values <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 at the <10th centile between 16 and 20 weeks' gestation.

An additional cohort of 21 high-risk pregnant patients were identified between 20 and 24 weeks' gestation with low circulating PlGF (<100 pg/mL) who were not on LMWH therapy and subsequently developed early-onset pre-eclampsia with delivery at  <34 weeks' gestation. All participants continued to receive maternal-fetal medicine obstetrical care at the placenta clinic, with appointments every 2 to 4 weeks and delivered at the same hospital.14 After 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, because LMWH therapy is approved for use in high-risk pregnant patients for the prevention of placentation complications, including preeclampsia.31

**Study intervention**

Based on previous experimental data supporting the therapeutic potential of LMWH to restore circulating PlGF,18,19 the option of adjunctive prophylactic LMWH (enoxaparin 40 mg/d subcutaneously) for the prevention of preeclampsia was reviewed with 12 patients who had low circulating PlGF before 20 weeks' gestation. Of these, 7 received prophylactic LMWH therapy in addition to aspirin and 5 continued aspirin alone. Notably, 5 patients who initiated LMWH therapy had 2 PlGF tests between 16 and 20 weeks' gestation at the <10th centile, whereas 2 had a single abnormal PlGF test. An additional 21 high-risk pregnant patients with low PlGF levels between 20 and 24 weeks' gestation who did not initiate LMWH therapy and subsequently developed early-onset preeclampsia with delivery <34 weeks' gestation were identified.
every 2 to 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 to 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. Pre-eclampsia was defined as systolic blood pressure of ≥140 mm Hg or diastolic blood pressure of ≥90 mm Hg on 2 occasions at least 4 hours apart after 20+0 weeks’ gestation, with evidence of related organ injury: proteinuria (urine protein to creatinine ratio of ≥30 mg/mmol), thrombocytopenia (platelets of <100×10⁹/L), renal compromise (serum creatinine of ≥1.1 mg/dL), or impaired liver function (aspartate aminotransferase of ≥70 U/L or alanine aminotransferase of ≥70 U/L).³² Placental pathology diagnoses were established according to the Amsterdam Criteria of Standardized Placental Classification.³³

**Statistical analysis**
The reference range of PlGF levels across gestation in healthy pregnant patients generated by the Cambridge, United Kingdom, cohort and the White majority of the Toronto, Canada, cohort was described by quantile regression curves using the LMS method described by Cole and Green³⁴ and implemented in the gamlss package³⁵ within the R statistical computing system (version 4.0.2, R Foundation for Statistical Computing, Vienna, Austria). The flexibility of the quantile curves was controlled by 5 degrees of freedom, to reflect the 5 broad groupings of gestational ages across the Cambridge and Toronto data sets and to ensure smooth transition across areas where the data are sparse. Quantile regression curves are shown on the plot for the median (blue, dashed) and lower 10% (green, dotted) and 5% (red, full). PlGF derived from non-White patients in the Toronto, Canada, cohort were excluded owing to published variation in circulating PlGF at 16 weeks’ gestation across ethnic groups and to align with the ethnic composition of the Cambridge, United Kingdom, cohort.³⁶ No comparative statistics of the 3 study groups are presented owing to the limited sample size.

**Results**

**Placental growth factor reference range**
The gestational age-specific reference range for PlGF levels together with the 50th, 10th, 5th, and 2.5th centiles are presented in Figure 2. Specific cutoff values for each week of gestational age between 12 and 36 weeks are presented in Table 1.

**Pregnant patients at risk of placental dysfunction**
The demographic characteristics and obstetrical history are presented in Table 2, with patients stratified into the following 3 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 pre-eclampsia (group 3). Group 3 patients were initially assessed and identified at a later gestational age than patients in groups 1 and 2.

**Maternal circulating placental growth factor after low-molecular-weight heparin administration**
Individual circulating PlGF results as gestation advanced in patients with severe placental dysfunction are presented.
in Figure 3. Notably, 5 of 7 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 at ≥20 weeks’ gestation, exhibited a similar trend as group 2. Longitudinal PlGF values across gestation are presented 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
Maternal, fetal, and delivery outcomes together with placental pathology findings are presented in Table 3. Patients in group 1 exhibited a later gestational age at delivery, relative to groups 2 and 3 (36 weeks [33–37] vs 23 weeks [22–26] and 28 weeks [27–31], respectively). Birthweights were higher in group 1 pregnancies than in groups 2 and 3 (1.93 kg [1.1–2.7] vs 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% vs 80% and 29%, respectively).

MVM was the predominant principal placental pathology in all patients, with the highest incidence in group 3, relative to groups 1 and 2 (90% vs 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 (perivillous fibrin deposition and chronic histiocytic intervillitis), whereas the remaining 5 with abnormal mean uterine artery Doppler expressed features of MVM pathology. Mean uterine artery PI across gestation stratified by placental pathology and LMWH therapy is presented in the Supplemental Figure.

Comment
Principal findings
The principal findings of the study are 3-fold. First, we merged data from 2 cohort studies of healthy nulliparous patients to create a reference curve for clinicians to interpret circulating PlGF on a weekly basis between 12 and 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

<table>
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<th>Gestational age (wk)</th>
<th>2.5th centile</th>
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<td>118</td>
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<tr>
<td>36</td>
<td>67</td>
<td>83</td>
<td>106</td>
<td>267</td>
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</table>

All 7 subjects receiving low-molecular-weight heparin had a PlGF value at the <10th centile before administration at <20 weeks’ gestation.

PlGF, placental growth factor.
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 seem to be higher in patients in group 1 than in patients in group 2, whereas PAPP-A multiple of median values were lowest in group 2.

### TABLE 2
Characteristics of pregnant patients at risk of placental dysfunction

<table>
<thead>
<tr>
<th>Maternal characteristics</th>
<th>Early-second-trimester low PlGF, LMWH n=7</th>
<th>Early-second-trimester low PlGF, no LMWH n=5</th>
<th>Late-second-trimester low PlGF, early-onset preeclampsia, no LMWH n=21</th>
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</thead>
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<td>Demographic characteristics</td>
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<tr>
<td>Age, y</td>
<td>35 (34–38)</td>
<td>39 (35–40)</td>
<td>33 (29–37)</td>
</tr>
<tr>
<td>Ethnicity</td>
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<tr>
<td>White</td>
<td>5 (71)</td>
<td>1 (20)</td>
<td>13 (62)</td>
</tr>
<tr>
<td>Black</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>2 (10)</td>
</tr>
<tr>
<td>East Asian</td>
<td>1 (14)</td>
<td>1 (20)</td>
<td>2 (10)</td>
</tr>
<tr>
<td>South Asian</td>
<td>1 (14)</td>
<td>3 (60)</td>
<td>4 (19)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>27 (24–31)</td>
<td>26 (21–29)</td>
<td>32 (23–39)</td>
</tr>
<tr>
<td>Chronic hypertension</td>
<td>0 (0)</td>
<td>1 (20)</td>
<td>3 (14)</td>
</tr>
<tr>
<td>Preexisting diabetes</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>2 (10)</td>
</tr>
<tr>
<td>Initial assessment, wk gestation</td>
<td>12 (11–13)</td>
<td>16 (16–18)</td>
<td>22 (20–24)</td>
</tr>
<tr>
<td>Systolic blood pressure at initial assessment, mm Hg(^{a})</td>
<td>118 (92–120)</td>
<td>112 (105–143)</td>
<td>120 (113–132)</td>
</tr>
<tr>
<td>Diastolic blood pressure at initial assessment, mm Hg(^{a})</td>
<td>66 (59–72)</td>
<td>80 (63–89)</td>
<td>71 (66–82)</td>
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<tr>
<td>Obstetrical history</td>
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<tr>
<td>History of placental complications</td>
<td>5 (71)</td>
<td>2 (40)</td>
<td>6 (29)</td>
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<tr>
<td>Previous preeclampsia</td>
<td>2 (29)</td>
<td>2 (40)</td>
<td>4 (19)</td>
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<tr>
<td>Early-onset preeclampsia &lt; 34 wk gestation</td>
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<td>2</td>
<td>3</td>
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<td>Late-onset preeclampsia ≥ 34 wk gestation</td>
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<td>1</td>
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<tr>
<td>Previous fetal death at ≥ 20 wk gestation</td>
<td>3 (43)</td>
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<td>Obstetrical characteristics</td>
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<td>First trimester aneuploidy screening</td>
<td>1 (14)</td>
<td>1 (20)</td>
<td>7 (33)</td>
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<tr>
<td>Not performed</td>
<td>0.63 (0.39–0.91)</td>
<td>0.16 (0.12–0.39)</td>
<td>0.70 (0.51–1.48)</td>
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<tr>
<td>PAPP-A, MoM</td>
<td>1.00 (0.98–1.32)</td>
<td>2.17 (1.28–3.01)</td>
<td>1.62 (1.28–1.86)</td>
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<td>Mean uterine artery PI</td>
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<tr>
<td>14–20 wk gestation</td>
<td>1.99 (1.38–2.49)</td>
<td>1.54 (1.42–1.66)</td>
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<tr>
<td>20–24 wk gestation</td>
<td>1.57 (1.36–1.82)</td>
<td>1.53 (1.45–1.60)</td>
<td>2.09 (1.73–2.54)</td>
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<td>24–28 wk gestation</td>
<td>1.54 (1.16–1.78)</td>
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<td>1.98 (1.80–2.50)</td>
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<tr>
<td>28–36 wk gestation</td>
<td>1.21 (0.90–1.38)</td>
<td>—</td>
<td>1.56 (1.35–2.02)</td>
</tr>
</tbody>
</table>

Data are presented as median (interquartile range) or number percentage of column.

| BMI, body mass index; hCG, human chorionic gonadotropin; LMWH, low-molecular-weight heparin; MoM, multiples of the median; PAPP-A, pregnancy-associated plasma protein-A; PI, pulsatility index; PlGF, placental growth factor (pg/mL). |

\(^{a}\) Blood pressure data missing from 1 patient in group 1.

Results in the context of what is known

By applying quantile regression analysis to PlGF data merged from 2 prospective cohorts of nulliparous White women, we established a continuous reference range between 12 and 36 weeks’ gestation. We restricted the construction of the reference range to these 2 cohorts owing to their adequate size and uniformity of assay manufacturer, because assay type is a source of data variability.37 Previous studies have grouped reference PlGF values into blocks of time,4,38 although one study used quantile regression to derive a reference range for PlGF using serial samples from 180 uncomplicated pregnancies beginning at 12 weeks’ gestation.41 Burke et al42 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 placentation function in the early second trimester. In contrast to the use of a pragmatic single cutoff diagnostic value of 100 pg/mL for PlGF at 20 to 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 to use serial PlGF to screen high-risk women on aspirin for severe placenta-mediated adverse outcomes.4 However, this resource is limited by the use of one test platform and being predominantly derived from White patients. A recent investigation determined that circulating PlGF levels at 16 weeks’ gestation varied considerably by ethnicity;6 further studies involving adequate numbers of specific ethnic groups are warranted, especially because some non-White 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.16,43,44 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 that measure regional tissue oxygenation by

![FIGURE 3](image-url)

**FIGURE 3**
PlGF levels 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, and 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.

LMWH, low-molecular-weight heparin; PlGF, placental growth factor.

Group 1 (patients with low PlGF levels in the early second trimester who initiated LMWH therapy) are presented in red. Group 2 (patients with low PlGF levels in the early second trimester who did not initiate LMWH therapy) are presented in green. Group 3 (patients with low PlGF levels in the late second trimester who did not initiate LMWH therapy and developed early-onset preeclampsia) are presented in blue.

LMWH, low-molecular-weight heparin; PlGF, placental growth factor.

These observations are relevant in the context of the most updated systematic review on the adjunct role of LMWH that 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 ratio, 0.62; 95% confidence interval, 0.41–0.95). These include suppression of leukocyte activation and complement activity alongside promotion of angiogenesis. In vivo studies of pregnant subjects, LMWH acutely elevated suboptimal circulating PlGF by 1.5-fold, and larger increases in circulating PlGF levels have been found in patients receiving therapeutic levels of LMWH. A subsequent publication from the Heparin-Preeclampsia (HEPEPE) trial group focused on circulating angiogenic growth factor

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 anti-coagulation of maternal blood within the placenta. Several non-anticoagulant actions of LMWH may be pertinent in the context of preventing early-onset preeclampsia associated with MVM of the placenta. These include suppression of leukocyte activation and complement activity alongside promotion of angiogenesis.
profiles in high-risk pregnant patients, demonstrating no differences in circulating PlGF across gestation between patients randomized to receive aspirin alone and aspirin with LMWH therapy. Very few women in this randomized trial had low circulating PlGF levels before 18 weeks' gestation, few had severe adverse perinatal outcomes, and the trajectory of PlGF in both arms of the trial is similar to the reference range data reported in this study. In contrast, the current observational study recruited high-risk patients (groups 1 and 2) based on low PlGF levels early in the second trimester and was confirmed by both their clinical outcomes and placental pathology testing. Our interpretation is that LMWH therapy could exert beneficial effects in high-risk pregnant patients with considerable placental dysfunction, by reversing a low PlGF phenotype found in the early second trimester. The Enoxaparin for the Prevention of Preeclampsia and Intrauterine Growth Restriction (EPII) trial had a similar design and overall findings, and the HEPEPE and EPII trials have not reported placental pathology data, which would be of interest to elucidating any potential beneficial impacts of LMWH therapy.

In in vitro studies with human tissues, LMWH augmented the synthesis and release of PlGF by placental villous tissue explants and a similar stimulatory effect by LMWH has recently been demonstrated in endothelial cells. The possibility of inducing an endogenous rise in circulating PlGF via LMWH is preferable to the administration of parenteral exogenous PlGF to achieve the same effect. Collectively, these studies support a plausible mechanism whereby LMWH, administered to a small subset of patients at greatest risk of placenta-mediated early-onset preeclampsia, may be capable of inducing a clinically meaningful sustained rise in their deficient PlGF levels.

Clinical implications

For clinicians managing pregnancies at high-risk of placental dysfunction, especially in a maternal-fetal medicine setting, the ability to identify an asymptomatic vulnerable subset in the early second trimester, despite receiving aspirin prophylaxis, is a key strategic goal. The availability of PlGF testing in real time (within 90 minutes) combined with uterine artery Doppler assessment may be one such strategy for clinicians to adopt, as we have done. This approach can focus clinical resources on women who need higher levels of care, and in addition, it may facilitate further research into the adjunct role of additional medical therapies to prevent stillbirth and extreme preterm delivery.

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 subgroup of high-risk patients already on aspirin prophylaxis. This approach was taken in a small 2011 trial where LMWH and aspirin were used concurrently that was conducted before the advent of PlGF screening. At present, we do not know whether 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. Therefore, future baseline trial data could incorporate serial urine analysis of the stable C5b fragment that reflects complement activation, 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 and 36 weeks’ gestation for high-risk pregnancy clinicians to assess the ongoing risk of preterm delivery owing to severe placental dysfunction. Among a small subgroup of pregnancies considered high-risk in this context with low maternal circulating PlGF at 16 to 20 weeks’ gestation, the addition of prophylactic LMWH to aspirin prophylaxis induced a rise in circulating PlGF that was sustained in most patients over several weeks before delivery. Given that the most recent evidence obtained from systematic reviews of relevant trials support the 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 favorable could inform a subsequent definitive randomized trial with entry criteria that focus on the assessment of placental function in vivo.

References


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J.C. Kingdom has given talks on PlGF (placental growth factor) testing to high-risk pregnancy groups across Canada on behalf of Roche Diagnostics. J.C. Kingdom is in receipt of a pilot grant from Roche Diagnostics to evaluate the role of PlGF screening to deliver virtual antenatal care.

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Maternal PlGF levels across gestation in pregnant patients at risk of severe placental dysfunction, stratified by placental pathology diagnosis and LMWH therapy.

LMWH, low-molecular-weight heparin; MVM, maternal vascular malperfusion; PlGF, placental growth factor.