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ORIGINAL RESEARCH ARTICLE

The association between placenta-associated circulating biomarkers and composite adverse delivery outcome of a likely placental cause in healthy post-date pregnancies

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Abstract

Introduction: Post-date pregnancies have an increased risk of adverse delivery outcome. Our aim was to explore the association between placenta-associated circulating biomarkers and composite adverse delivery outcome of a likely placental cause in clinically healthy post-date pregnancies.

Material and methods: Women with healthy singleton post-date pregnancies between 40^{+2} and 42^{+2} weeks of gestation were recruited to this prospective, observational study conducted at Oslo University Hospital, Norway (NCT03100084). Placental growth factor (PlGF) and soluble fms-like tyrosine kinase-1 (sFlt-1) were measured in the maternal serum samples closest to delivery. The composite adverse delivery outcome included fetal acidemia, low Apgar score (<4 at 1 min or <7 at 5 min), asphyxia, fetal death, assisted ventilation for more than 6 h, meconium aspiration, hypoxic–ischemic encephalopathy, therapeutic hypothermia, operative delivery due to fetal distress, or pathological placental histology findings. Two study-independent senior consultant obstetricians blinded to biomarker results concluded, based on clinical expert opinion, whether the adverse delivery outcomes were most likely associated with placental dysfunction ("likely placental cause") or not. Means were compared using one-way analysis of variance and Bonferroni corrected pairwise comparisons between groups. Receiver operating characteristic (ROC) curves assessed the predictive ability of PlGF, sFlt-1/PlGF ratio, and PlGF <10th centile after adjustment for gestational age at blood sampling.

Results: Of 501 pregnancies reviewed for predefined adverse delivery outcomes and for a likely placental cause, 468 were healthy pregnancies and subsequently assigned to either the "uncomplicated" (no adverse outcome, $n = 359$), "intermediate" (non-placental cause/undetermined, $n = 90$), or "complicated" (likely placental cause, $n = 19$) group.

Abbreviations: GA, gestational age; GW, gestational weeks; PlGF, placental growth factor; PREPPeD, PREdelivery Placental biomarkers—Pregnancy and Delivery outcome; ROC, receiver operating characteristic; sFlt-1, soluble fms-like tyrosine kinase-1; SGA, small for gestational age.

There was a significant difference in mean PlGF and sFlt-1/PlGF ratio between the "complicated", "intermediate", and "uncomplicated" groups (108, 185, and 179 pg/mL, $p = 0.001$; and 48.3, 23.4, and 24.6, $p = 0.002$, respectively). There was a higher proportion of PlGF concentration <10th centile in the "complicated" group compared with the "intermediate" and "uncomplicated" groups (42.1% vs. 11.1% and 9.5%, $p = 0.001$). The largest area under the ROC curve for predicting "complicated" outcome was achieved by PlGF concentration and gestational age at blood sampling (0.76; 95% CI 0.65–0.86).

Conclusions: In clinically healthy post-date pregnancies, an antiangiogenic pre-delivery profile (lower PlGF level and higher sFlt-1/PlGF ratio) was associated with composite adverse delivery outcome of a likely placental cause.

KEYWORDS
angiogenic factors, biomarkers, neonatal outcome, placental growth factor, post-date, post-term, pregnancy, soluble fms-like tyrosine kinase-1

1 | INTRODUCTION

Worldwide, pregnancies continuing past 42 gestational weeks (GW) are estimated to cause 14% of all stillbirths.[1] Placental dysfunction is important in the pathophysiology of post-term pregnancies,[2] but adverse perinatal outcome risk increases gradually already after 40 GW.[3–5] Imbalances in placenta-derived proteins, such as the angiogenic placental growth factor (PlGF) and antiangiogenic soluble fms-like tyrosine kinase-1 (sFlt-1), have been suggested as markers for syncytiotrophoblast stress, and so represent general "placental health markers".[2,6] We have recently shown that these biomarkers are altered at and beyond term, even in clinically healthy pregnancies without fetal or maternal labor or delivery complications.[7] These biomarkers have also been found to be promising in predicting intrapartum fetal distress in uncomplicated pregnancies beyond 36 GW.[8,9] A reliable tool for antenatal identification of fetuses at risk of compromise is needed, and maternal levels of PlGF may be useful.[7]

Optimal tools for the decision on the ideal time for delivery are currently lacking. The World Health Organization recommends induction of labor at 41 GW,[10] but some obstetric guidelines allow prolongation until 42 GW in clinically healthy pregnancies.[11,12] A recent randomized controlled trial (SWEPIS) compared induction of labor at 41 GW with expected management and induction at 42 GW.[5] The trial was stopped prematurely because of a higher rate of perinatal mortality in the expected management group, despite no significant difference in the primary composite adverse perinatal outcome.[5] In contrast, a multicenter randomized trial (INDEX)[13] found that induction at 41 GW was associated with lower, though not significant, composite adverse perinatal outcome when compared with expected management to 42 GW, but no difference in perinatal mortality.[13] These study results differ substantially and underline that better tools are warranted for fetal risk assessment in post-date pregnancies.

Key message

To identify an at-risk fetus within a group of "healthy" post-date pregnancies before the onset of labor is challenging. Our study shows that in healthy post-date pregnancies, placenta-associated biomarkers are associated with adverse delivery outcome of a likely placental cause.

In line with our hypothesis of increasing placental stress as a continuum towards the end of all pregnancies,[6] we hypothesized an association in healthy post-date pregnancies between altered circulating maternal antiangiogenic biomarker pattern and adverse delivery outcomes associated with placental dysfunction. Our study aim was to explore the association between placenta-associated circulating biomarkers and composite adverse delivery outcome of a likely placental cause in clinically healthy post-date pregnancies (≥40^{+2} GW).

2 | MATERIAL AND METHODS

2.1 | Recruitment and clinical assessment of the post-date pregnancy cohort

This prospective cohort study was conducted from September 2016 to December 2017 at the Department of Obstetrics, Oslo University Hospital, Ullevål, with approximately 7000 deliveries annually. The study is part of the PREPPeD study (PREdelivery Placental biomarkers—Pregnancy and Delivery outcome), where a study-independent clinical expert group composed of two senior consultant obstetricians, blinded to the maternal placental biomarkers, concluded whether predefined adverse delivery outcomes had

a likely placental cause or not.[7] A convenience sample from women with singleton pregnancies at ≥40^{+2} GW, referred for routine clinical post-date evaluation[7,14] and/or induction of labor, was included. Exclusion criteria were non-Norwegian or non-English language, communicable disease, and age <18 years. Recruited women gave informed written consent. Women and clinicians were blinded to biomarker results. Induction of labor, labor, and delivery were managed according to department protocol. Induction was offered to women with abnormal fetal or maternal findings, at 42^{+0} GW or within 41^{+2} GW for women ≥40 years of age. Gestational age (GA) and estimated date of delivery (40^{+2}) was calculated based on routine ultrasound screening at 17–20 GW according to Norwegian national guidelines or, when not available, from last menstrual period. Birthweight centile was calculated according to Norwegian population-based sex-adjusted reference ranges.[15] Small for gestational age (SGA) was defined as a birthweight below the 10th sex-adjusted centile. Diagnosis of fetal distress was made by the attending clinician based on abnormal fetal heart rate patterns and/or fetal scalp lactate >4.8 mmol/L.

2.2 | Blood and placental tissue sampling and laboratory analyses of the post-date pregnancy cohort

A venous maternal blood sample was taken at study inclusion and, if possible, daily until labor onset. Further details of storage and centrifugation of the blood samples are described previously.[7] In women with repeated post-date samples, maternal serum PlGF and sFlt-1 were analyzed post-partum from the blood sample closest to delivery. All samples were analyzed, blinded for clinical information, at the Department of Medical Biochemistry, Oslo University Hospital on a cobas e 801 analyzer, as previously described.[7] The PlGF and sFlt-1 concentrations were quantified using the fully automated Elecsys® system (Roche Diagnostics). The novel post-date reference ranges for PlGF, sFlt-1, and their ratio have been described previously (Table S1).[7]

The results from the routinely sampled arterial and venous umbilical cord blood gas analyses were included in the study if all the criteria for ensuring sample validity by Kro et al were fulfilled.[16] GA-dependent reference levels for arterial umbilical cord blood gas lactate[17] were used in the adverse delivery outcome evaluation.

The PREPPeD-study protocol included examination by a pathologist of every fifth placenta, and departmental guidelines for requisition of histomorphological placenta examination were followed (ie in cases of a clinical indication).

2.3 | Assignment of pregnancies to the different outcome groups

All fetal and delivery outcomes were externally reviewed by a "Diagnostic Advisory Group" (DAG), consisting of two senior consultant obstetricians not affiliated to the study and blinded for PlGF and sFlt-1 results. After a post-partum review of the mother's and neonate's medical journals (including placental histology, where available), the DAG concluded whether there was a predefined adverse delivery outcome (Table 1). Thereafter, the DAG judged, based on clinical expert opinion, whether this adverse delivery outcome most likely was associated with placental dysfunction ("likely placental cause") or not. In case of dissent, a third senior consultant obstetrician, equally independent and blinded for biomarker results, reviewed and adjudicated the case. Women that developed preeclampsia/gestational hypertension/chronic hypertension or (pre)gestational diabetes mellitus before collection of the blood sample closest to delivery, were excluded from the final post-date pregnancy cohort (Figure 1). If a pregnancy resulted in an adverse delivery outcome and this was judged to be likely placental dysfunction-associated by the DAG, it was included in the "complicated" group (Figure 1). If the adverse delivery outcome was most likely of non-placental cause or undetermined, the respective pregnancy was included in the "intermediate" group. The final "uncomplicated" group consisted of all pregnancies without adverse outcomes (Figure 1).

2.4 | Statistical analyses

Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 25.0 (IBM Corp.) and STATA Special Edition, version 16.1 (StataCorp). Categorical variables were compared using Fisher's exact test and medians were compared by Kruskal–Wallis test. Biomarker measurements were right skewed, and therefore log-transformed. Means were then compared using one-way analysis of variance, and subsequent pairwise comparisons between groups were Bonferroni corrected. Means presented in the Results section (and Table S2) were obtained by back transformation using the exponential function. Values of p less than 0.05 were considered statistically significant.

We performed logistic regression analyses with maternal circulating PlGF and sFlt-1, and their ratio, together with GA at blood sampling as independent variables and "complicated" outcome as dependent variable (Table 3). Due to a limited number of pregnancies with a "complicated" outcome, only GA at blood sampling was included in addition to the biomarkers. Receiver operating characteristic (ROC) curves were used to assess the predictive ability of PlGF, SFlt-1/PlGF ratio, and PlGF <10th centile for the "complicated" outcome after adjustment for GA at blood sampling.

2.5 | Ethical approval

National research ethical and institutional bodies have approved the PREPPeD study, of which the present study is part (The Regional Committee for Medical and Health Research Ethics in South-Eastern

TABLE 1 Primary (A: 1–9) and secondary (B: 1–2) adverse pregnancy and delivery outcomes as defined for the PREPPeD study

A: Primary adverse outcomes (Either of the composite adverse outcomes 1–9):	
1	Fetal acidemia, evaluated by: A. Arterial umbilical cord blood gases I In neonates delivered by cesarean section (CS) without labor (defined as absence of regular uterine contractions): umbilical artery blood (transporting blood from the fetus to the placenta) pH <7.13 and arterial base deficit (BD) >10.0 II In neonates from labored delivery (regardless of subsequent method, vaginal or CS): Umbilical artery blood pH <7.05 and arterial BD >14 OR B. Umbilical artery blood lactate above reference level for respective gestational age[17]
2	Newborn low Apgar score A. <4 at 1 min OR B. <7 at 5 min (any newborn intubated at this time point will be registered as low Apgar at 5 min, as Apgar cannot be assessed in assisted ventilation)
3	Newborn asphyxia: defines as fetal acidemia (#1 above) AND newborn low Apgar (#2 above)
4	Intrauterine fetal demise/intra-/post-partum fetal death
5	Neonatal intubation/mechanical ventilation >6 h
6	Meconium aspiration syndrome
7	Neonatal hypoxic-ischemic encephalopathy
8	Therapeutic hypothermia of the neonate
9	Acute cesarean section (due to suspected fetal distress)
B: Secondary adverse outcomes	
1	Operative vaginal deliveries (forceps/vacuum/combined; due to suspected fetal distress)
2	Pathological placenta histology findings

Norway ref 2016/652, approval date 20 May 2016). The PREPPeD biobank is coordinated as a thematic biobank within the Oslo Pregnancy Biobank (The Regional Committee for Medical and Health Research Ethics in Eastern Norway, ref 529-02162, approval date 13 December 2002). The PREPPeD study is registered at ClinicalTrials.gov, NCT0310008.

3 | RESULTS

In total, 501 women were recruited pre-delivery. Of these, the following were excluded: 10 (2%) because the blood sample closest to delivery was taken at $<40^{+2}$ GW, 20 (4%) because of developing preeclampsia/gestational hypertension/gestational diabetes mellitus before collection of the blood sample closest to delivery and three (0.6%) because of an obstetric catastrophe (two with cord prolapse, one with uterine rupture) (Figure 1). Table 2 shows the clinical characteristics of the "uncomplicated" group of 359 women without adverse outcome, the "intermediate" group of 90 women with an adverse delivery outcome without a likely placental cause, and the "complicated" group of 19 women with an adverse delivery outcome of likely placental cause (Figure 1). All the women in the "complicated" group had an operative delivery (either cesarean section or operative vaginal delivery) and 95% were due to suspected fetal distress (Table S3). In the "complicated" group, 79% had undergone induction of labor compared with 54% of the "intermediate" group and 46% in the "uncomplicated" group ($p = 0.004$). Although none of the included women had a hypertensive disorder at inclusion, the median diastolic blood pressure at inclusion was significantly ($p = 0.029$) higher (2–3 mmHg) in the "complicated" group compared with the two other groups (Table 2). Only one woman in the "uncomplicated" group developed preeclampsia after donation of the blood sample included in the study. The "complicated" group had a significantly lower median neonatal birthweight and placental weight as well as a higher proportion of pregnancies with SGA infants, compared with the "intermediate" and "uncomplicated" groups (all $p \leq 0.001$) (Table 2).

3.1 | Maternal circulating biomarkers

The logarithmic values of the mean maternal PlGF concentration from blood samples closest to delivery are shown in Figure 2 across the three outcome groups. The median time interval from the last blood sample draw to delivery was the same for all three groups (2 days, ranging from 0 to 11 days, $p = 0.371$).

There was a significant difference in mean PlGF concentration and sFlt-1/PlGF ratio between the "complicated", the "intermediate", and the "uncomplicated" group (108, 185, and 179 pg/mL, $p = 0.001$; and 48.3, 23.4, and 24.6, $p = 0.002$). The mean PlGF concentration was significantly lower and sFlt-1/PlGF ratio was significantly higher in the "complicated" compared with the "intermediate" group ($p = 0.001$) and in the "complicated" compared with the "uncomplicated" group ($p = 0.001$). Between the "intermediate" and "uncomplicated" groups there was no significant difference ($p > 0.999$) (Figure 2A,C and Table S2). Mean sFlt-1 concentration was highest in the "complicated" group, although no significant difference was found between the "complicated", "intermediate", and "uncomplicated" groups (5191, 4329, and 4397 pg/mL, $p = 0.295$).

The "complicated" group had a higher proportion of SGA compared with the "intermediate" and "uncomplicated" groups (47% vs. 4% and 5%, $p < 0.001$) (Table 2). When we excluded the pregnancies with SGA babies from the "complicated" group, the group still had lower levels of PlGF compared with the "intermediate" and "uncomplicated" groups (110, 187, and 181 pg/mL, $p = 0.026$).

A post-date pre-delivery maternal PlGF concentration below the 10th centile (91 pg/mL, according to our predefined reference)[7] was present at a higher proportion in the "complicated" group with an adverse delivery outcome of a likely placental cause, compared with the "intermediate" and "uncomplicated" groups (42.1% vs. 11.1% and 9.5%, $p = 0.001$) (Table 2). Proportions of sFlt-1 >90th centile

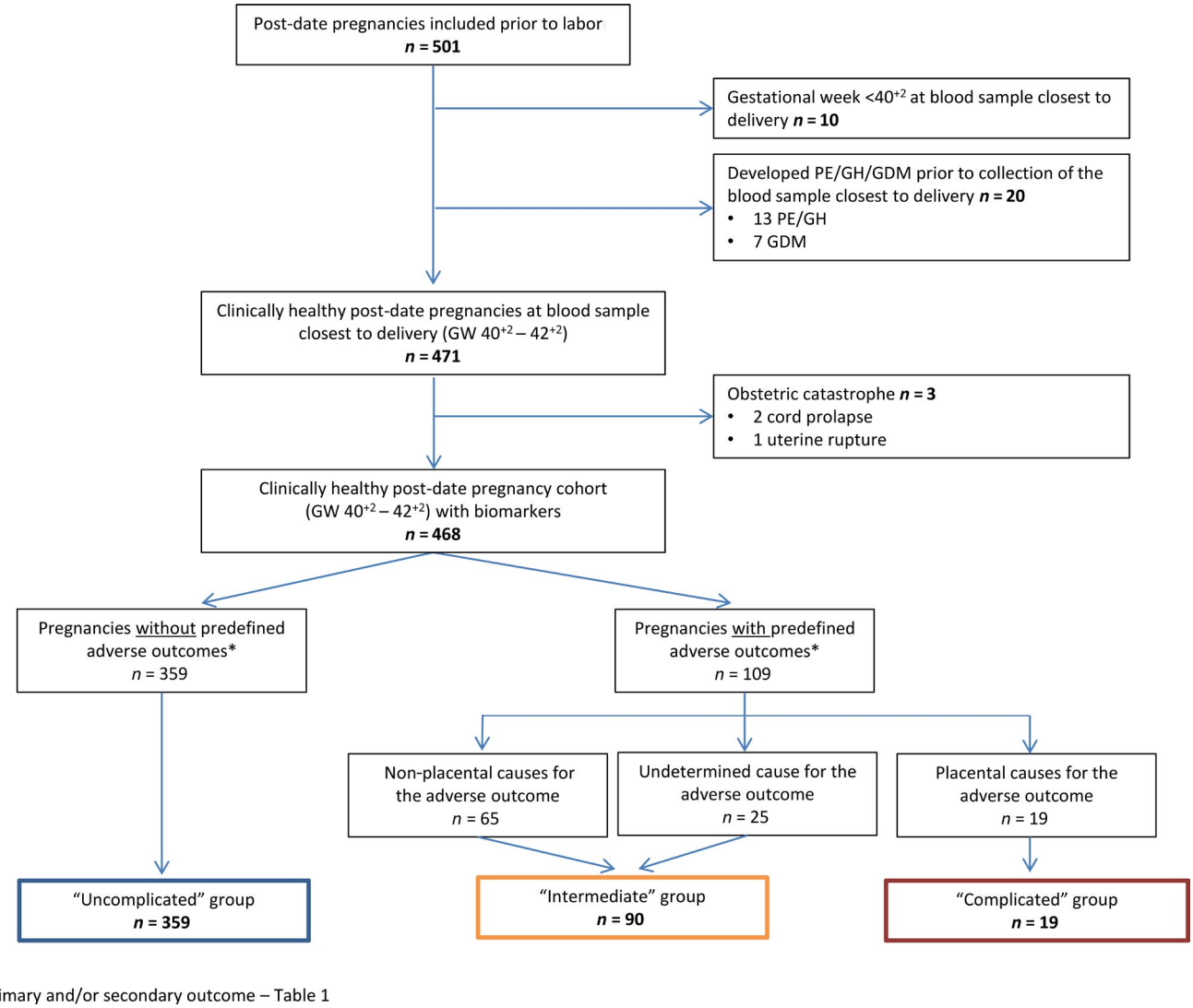

FIGURE 1 Participant flow chart of the final post-date pregnancy cohort ($n = 468$), with the "uncomplicated" group of clinical healthy pregnancies without adverse outcome, the "intermediate" group with adverse delivery outcome without a likely placental cause, and the "complicated" group with adverse outcomes of likely placental cause. GDM, gestational diabetes mellitus; GH, gestational hypertension; GW, gestational week; PE, preeclampsia; SGA, small for gestational age. *Primary and/or secondary outcome: Table 1

(7955.3 pg/mL)[7] did not differ between the "complicated", "intermediate", and "uncomplicated" groups (Table 2). An sFlt-1/PlGF ratio >90th centile (63.6),[7] however, was present in 31.6% of the "complicated" group, compared with only 11.1% in the "intermediate" group and 10.3% in the "uncomplicated" group ($p = 0.031$) (Table 2).

3.2 | Logistic regression and ROC curves

Results from logistic regression with respect to the risk of having a "complicated" outcome are shown in Table 3. Univariate analysis demonstrated a potential association with the "complicated" group and GA at blood sampling, PlGF, sFlt-1/PlGF ratio, PlGF <10th centile, and sFlt-1/PlGF ratio >90th centile, but no potential associations for the variables sFlt-1 and sFlt-1 >90th centile. The largest area under the ROC (AUROC) curve for the prediction of the "complicated" outcome was achieved by maternal levels of PlGF and GA at blood sampling (AUROC 0.76; 95% CI 0.65–0.86; Figure 3).

4 | DISCUSSION

We are the first to report an association between dysregulated maternal angiogenic biomarkers from clinically healthy post-date pregnancies and composite adverse delivery outcome of a likely placental cause. First, we observed a lower circulating pro-angiogenic biomarker pattern in in the "complicated" group that had an adverse delivery outcome of likely placental cause, compared with the "intermediate" and "uncomplicated" groups. Second, the proportion of low pre-delivery PlGF (below the 10th centile of our previously

TABLE 2 Clinical characteristics of the post-date pregnancy cohort (40^{+2}–42^{+2} weeks of gestation) (n = 468) according to clinical outcome groups

Characteristics	"Uncomplicated" $n = 359$	"Intermediate" $n = 90$	"Complicated" $n = 19$	P value[c]
Nulliparous, *n* (%)	192 (53.5)	67 (74.4)	17 (89.5)	<0.001
Maternal age in years at inclusion, median (IQR)	33.0 (30.0–36.0)	33.5 (30.8–36.3)	30.0 (30.0–34.0)	0.231
Body mass index in first trimester[a], median (IQR)	22.6 (21.0–24.5)	23.5 (21.2–26.2)	22.5 (21.0–24.9)	0.121
Systemic blood pressure at inclusion, median (IQR)	121 (116–126)	119 (113–127)	124 (119–128)	0.185
Diastolic blood pressure at inclusion, median (IQR)	77 (70–82)	78 (71–82)	80 (77–87)	0.029
HbA1c at inclusion, median (IQR)[a]	5.1 (5.0–5.3)	5.1 (4.9–5.4)	5.2 (5.0–5.4)	0.642
Serum creatinine at inclusion (μmol/L), median (IQR)[a]	54.0 (48.8–59.0)	56.0 (49.5–60.5)	55.0 (50.0–60.0)	0.261
Preeclampsia after blood sample closest to delivery, *n* (%)	1 (0.3)	0	0	>0.999
Maternal smoking/snus (moist tobacco), *n* (%)	54 (15.0)	17 (18.9)	3 (15.8)	0.652
Ethnicity, *n* (%)				0.182
White	344 (95.8)	83 (92.2)	17 (89.5)	
Black or Afro-American	7 (1.9)	4 (4.4)	2 (10.5)	
Asian	6 (1.7)	3 (3.3)	0	
Other	2 (0.6)	0	0	
Gestational age at blood sampling closest to delivery in days, median (IQR)	290.0 (288.0–291.0)	289.5 (288.0–291.0)	289.0 (284.0–291.0)	0.243
Time from blood sampling to delivery in days, median (IQR)	2.0 (1.0–3.0)	2.0 (1.0–4.0)	2.0 (1.0–3.0)	0.371
Child male sex, *n* (%)	215 (59.9)	53 (58.9)	9 (47.4)	0.543
Birthweight, median (IQR)	3876 (3585–4160)	3860 (3566–4110)	3200 (2924–3455)	<0.001
Small for gestational age, *n* (%)	17 (4.7)	4 (4.4)	9 (47.4)	<0.001
Deliveries (total), *n* (%)				<0.001
Vaginal (non-operative)	291 (81.0)	16 (17.8)	0	
Vacuum/forceps	20 (5.6)	46 (51.1)	10 (52.6)	
Cesarean section	48 (13.4)	28 (31.1)	9 (47.4)	
Induction of labor, *n* (%)	165 (46.0)	49 (54.4)	15 (78.9)	0.004
Placenta histology available, *n* (%)	55 (15.3)	29 (32.2)	7 (36.8)	0.001
Placenta and umbilical cord weighed after blood withdrawn (before biopsies) in g, median (IQR)[a]	730 (634–827)	706 (620–800)	608 (520–733)	0.001
PlGF level <10th centile[b], *n* (%)	34 (9.5)	10 (11.1)	8 (42.1)	0.001
sFlt-1 level >90th centile[b], *n* (%)	36 (10.0)	10 (11.1)	4 (21.1)	0.263
sFlt-1/PlGF level >90th centile[b], *n* (%)	37 (10.3)	10 (11.1)	6 (31.6)	0.031

Abbreviations: IQR, interquartile range; PlGF, placental growth factor; SD, standard deviation; sFlt-1, soluble fms-like tyrosine kinase.

[a]*Missing data*: 1 Body mass index 1.trimester in "Uncomplicated" group and 1 in "Intermediate" group, 3 HbA1c in "Intermediate" group and 11 in "Uncomplicated" group, 1 Serum creatinine in "Intermediate" group and 5 in "Uncomplicated" group, 1 Placenta + umbilical cord weighed after blood withdrawn but before biopsies in "Complicated" group and 5 in "Intermediate" group and 13 in "Uncomplicated" group.

[b]Centiles based on the novel reference ranges.[7]

[c]Comparison between all three groups ("Uncomplicated", "Intermediate", and "Complicated"). Categorical variables were compared using Fisher's exact test. For continuous variables medians were compared by Kruskal–Wallis test.

published healthy post-date reference group)[7] was significantly higher in the "complicated" group when compared with the "intermediate" and "uncomplicated" groups.

The findings of a significantly lower mean pre-delivery maternal PlGF level in the "complicated" group with an intrapartum intervention due to fetal distress compared with the "uncomplicated" group correspond with findings described by others for pregnancies from 36 GW onwards. Those studies included substantially fewer post-date pregnancies.[8,9,18] In addition, we also found a significantly higher mean sFlt-1/PlGF ratio in the "complicated" group compared with the "intermediate" and "uncomplicated" groups. Interestingly, the "intermediate" group of pregnancies, ie the group with an adverse delivery outcome of a non-placental cause or undetermined cause, had similar levels of biomarkers as the "uncomplicated" group,

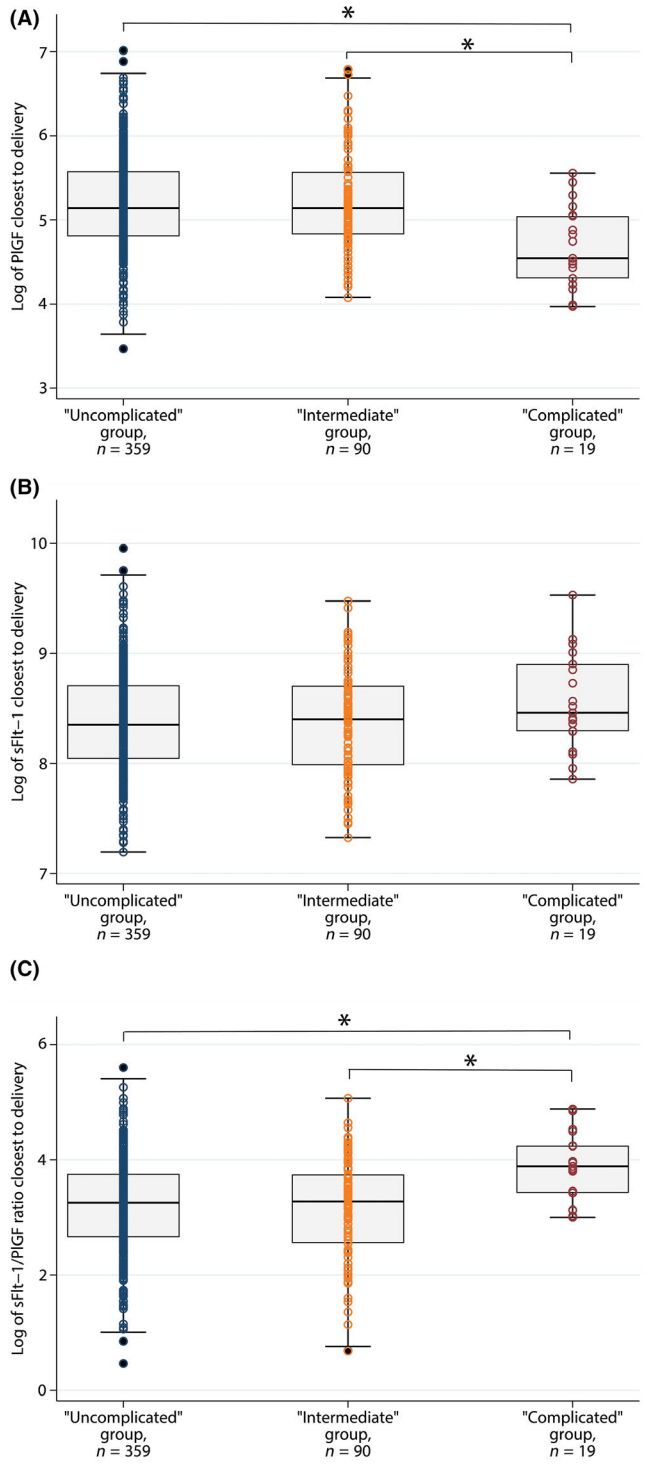

FIGURE 2 Individual logarithmic scale values of maternal serum placental growth factor (PlGF) level (A), serum soluble fms-like tyrosine kinase-1 (sFlt-1) level (B), and sFlt-1/PlGF ratio (C) for the final post-date pregnancy cohort (40^{+2}–42^{+2} weeks of gestation, $n = 468$). The large horizontal bar shows the median value for the three pregnancy outcome groups, and the smaller bars show the interquartile ranges (25th–75th). *Statistically significant if $p < 0.05$

and both mean PlGF level and sFlt-1/PlGF ratio differed significantly from the "complicated" group (Figure 2A,C and Table S2). We suggest that these findings highlight the importance of classifying likely placental cause or no placental cause for the adverse delivery outcome when investigating the usefulness in predicting adverse delivery outcome with placenta-associated biomarkers. Placental biomarkers cannot be expected to have a significant predictive value when assessing all complications that may occur in labor, but are likely to play a role in predicting adverse delivery outcomes related to preceding placental dysfunction with syncytiotrophoblast stress.[2]

In our post-date study we found significantly lower levels of PlGF and higher antiangiogenic ratio in the "complicated" group, despite including only normotensive women. SGA may serve as a surrogate marker of placental function and lower levels of PlGF have been shown to identify fetal growth restriction of placental cause.[19] However, after excluding the pregnancies with SGA babies from the "complicated" group, we still found lower levels of PlGF compared with the "intermediate" and "uncomplicated" group. These results are in line with our hypothesis that PlGF represents a general "placental health marker".[2,6] PlGF performed better as a predictive test for composite adverse delivery outcome in our study compared with previous findings by Dunn et al.[20] In contrast to Dunn et al,[20] we had predefined an adverse outcome to be of a likely placental cause or not. Dunn et al.[20] tested their reference centiles for PlGF on the same population that they used to define the centiles. In this study we have also used the same cohort as that from which the novel post-date reference ranges for PlGF, sFlt-1, and their ratio were derived.[7] However, when developing the reference ranges we included only pregnancies without predefined complications (SGA, preeclampsia/gestational hypertension, gestational diabetes mellitus/diabetes mellitus and obstetric catastrophes) and without adverse delivery outcomes with a likely placental cause.[7]

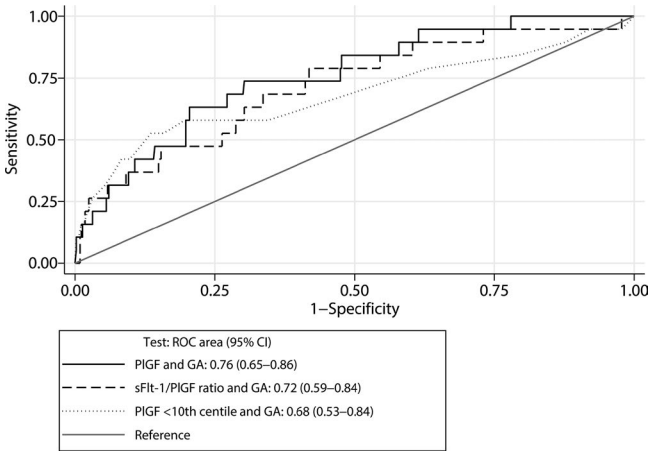

FIGURE 3 Receiver operating characteristic curves for prediction of "complicated" outcome ($n = 19$) in the post-date pregnancy cohort ($n = 468$) using logistic regression model with maternal circulating PlGF level (solid black line), sFlt-1/PlGF ratio (dashed black line) and PlGF <10th centile (dotted black line) adjusted for gestational age of the blood sample taken closest to delivery. GA, gestational age; PlGF, placental growth factor; sFlt-1, soluble fms-like tyrosine kinase-1

TABLE 3 Univariable and multivariable regression analysis with adverse outcome of likely placental cause (assignment to "complicated" group) as outcome in the post-date pregnancy cohort (n = 468)

Variables	Unadjusted OR (95% CI)	p-value	Adjusted OR (95% CI)[a]	p-value[a]
sFlt-1	1.00 (1.00–1.00)	0.170	1.00 (1.00–1.00)	0.120
PlGF	0.99 (0.98–0.99)	0.003*	0.99 (0.98–0.99)	0.003*
sFlt-1/PlGF ratio	1.02 (1.01–1.03)	0.003*	1.02 (1.01–1.03)	0.002*
PlGF <10th centile	6.69 (2.56–17.53)	<0.001*	6.94 (2.62–18.36)	<0.001*
sFlt-1 >90th centile	2.34 (0.74–7.34)	0.146	2.41 (0.76–7.62)	0.135
sFlt-1/PlGF ratio >90th centile	3.95 (1.43–10.88)	0.008*	4.18 (1.50–11.65)	0.006*
Gestational age at blood sampling	0.89 (0.78–1.02)	0.082	—	—

Abbreviations: OR, odds ratio; PlGF, placental growth factor; sFlt-1, soluble fms-like tyrosine kinase-1.

[a]Adjusted for gestational age at blood sampling.

*Statistically significant if $p < 0.05$.

The risk of perinatal mortality and morbidity has been shown to increase gradually after 40 GW,[3–5] and our total post-date pregnancy cohort could therefore be considered a "high risk" group. We found, however, no difference in the mean GA at blood sampling for biomarker analysis between the "complicated", "intermediate", and "uncomplicated" groups, allowing us to conclude that differences in GA did not influence group differences. The diagnostic and predictive role of maternal circulating PlGF and sFlt-1 show promising results in predicting composite adverse delivery outcome of a likely placental cause, but external validation is needed on a larger population with a higher number of adverse outcomes.

This prospective, observational study is the first to use novel reference ranges developed for placenta-associated biomarkers in post-date pregnancies (≥40^{+2} GW).[7] Our post-date group was larger than in the study by Dunn et al,[20] and clinical characteristics were extensively phenotyped. Our results may therefore serve as reference for other post-date pregnant populations. All clinical decisions were made according to department protocol, blinded for the biomarker results. The broadly predefined adverse delivery outcomes reflect real-life clinical heterogeneity. All fetal and delivery outcomes were reviewed by an independent clinical expert group that concluded if there was a likely placental cause for the adverse delivery outcome or not. The distinction between likely placental cause or not was predefined and made blinded for biomarker results. By this manner of categorization we were able to analyze the value of maternal placental biomarkers in predicting adverse delivery outcome related to placental dysfunction.

Study limitations include a low ethnical heterogeneity for external validity and a high percentage of highly educated women, partly explained by the inclusion criteria (Norwegian and English language). Placenta histology and valid umbilical cord blood gas was lacking for some pregnancies. As part of the PREPPeD study, every fifth placenta was sent for histomorphological examination, and 300 pregnancies had umbilical cord blood gas results fulfilling all the strict criteria for validity by Kro et al.[16] As clinically healthy singleton post-date pregnancies (≥40^{+2} GW), ie pregnancies considered "fit" for prolongation beyond due date, were included in our study, the resulting "complicated" group with adverse delivery outcome is relatively small. The predictive accuracy of the biomarkers would most likely have improved with a larger sample size and larger "complicated" group. Another limitation is the reliance on clinical expert opinion with regard to judging whether the adverse delivery outcome was likely due to placental insufficiency and so was defined as having had a likely placental cause. However, we assured that the clinical expert opinions by two senior consultant obstetricians were independent, and blinded to biomarker results, and based on a thorough review of the womens' and neonates' medical journals. Further, there is no international consensus on the definition of placental dysfunction, neither clinically nor in histomorphological placenta criteria.

5 | CONCLUSION

Our study is first to report that in healthy post-date pregnancies, an antioangiogenic biomarker pre-delivery pattern associates with adverse delivery outcome of a likely placental cause, measured as lower maternal PlGF concentration and higher sFlt-1/PlGF ratio. A major clinical challenge in everyday obstetric care is to identify an at-risk fetus within a group of "low-risk" post-date women before the onset of labor. Placenta-associated biomarkers can potentially contribute to target post-date pregnancies with increased risk of adverse delivery outcome, and so in assessing the ideal timing and method for delivery. External validation studies are warranted, however, before translation into clinical practice.

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CONFLICT OF INTEREST

ACS has received recompensation from Roche Diagnostics for advisory board service in 2018 regarding preeclampsia screening. Roche Diagnostics provided ACS and MS with in-kind reagents for the

sFlt-1 and PlGF biomarker analyses. The remaining authors report no conflicts of interest.

AUTHOR CONTRIBUTIONS

MS, ACS, and CWR conceived the project. MS, ACS, SB, BMM, and CWR planned the study. SB, BMM, and MS collected the data and carried out the study. SB, JMG, AG, ACS, and MS were involved in the data analyses. SB, AG, CWR, ACS, and MS primarily wrote the manuscript with substantial contributions from all authors.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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