OBSTETRICS

First-trimester preterm preeclampsia prediction with metabolite biomarkers: differential prediction according to maternal body mass index

Robin Tuytten, PhD; Argyro Syngelaki, PhD; Grégoire Thomas, PhD; Ana Panigassi, MD; Leslie W. Brown, BS; Paloma Ortea, PhD; Kypros H. Nicolaides, MD

BACKGROUND: Prediction of preeclampsia risk is key to informing effective maternal care. Current screening for preeclampsia at 11 to 13 weeks of gestation using maternal demographic characteristics and medical history with measurements of mean arterial pressure, uterine artery pulsatility index, and serum placental growth factor can identify approximately 75% of women who develop preterm preeclampsia with delivery at <37 weeks of gestation. Further improvements to preeclampsia screening tests will likely require integrating additional biomarkers. Recent research suggests the existence of distinct maternal risk profiles. Therefore, biomarker evaluation should account for the possibility that a biomarker only predicts preeclampsia in a specific maternal phenotype.

OBJECTIVE: This study aimed to verify metabolite biomarkers as preterm preeclampsia predictors early in pregnancy in all women and across body mass index groups.

STUDY DESIGN: Observational case-control study drawn from a large prospective study on the early prediction of pregnancy complications in women attending their routine first hospital visit at King's College Hospital, London, United Kingdom, in 2010 to 2015. Pregnant women underwent a complete first-trimester assessment, including the collection of blood samples for biobanking. In 11- to 13-week plasma samples of 2501 singleton pregnancies, the levels of preselected metabolites implicated in the prediction of pregnancy complications were analyzed using a targeted liquid chromatography-mass spectrometry method, yielding high-quality quantification data on 50 metabolites. The ratios of amino acid levels involved in arginine biosynthesis and nitric oxide synthase pathways were added to the list of biomarkers. Placental growth factor and pregnancy-associated plasma protein A were also available for all study subjects, serving as comparator risk predictors. Data on 1635 control and 106 pregnancies complicated by preterm preeclampsia were considered for this analysis, normalized using multiples of medians. Prediction analyses were performed across the following patient strata: all subjects and the body mass index classes of <25, 25 to <30, and ≥30 kg/m^2. Adjusted median levels were compared between cases and controls and between each body mass index class group. Odds ratios and 95% confidence intervals were calculated at the mean ±1 standard deviation to gauge clinical prediction merits.

RESULTS: The levels of 13 metabolites were associated with preterm preeclampsia in the entire study population ($P<.05$) with particularly significant ($P<.01$) associations found for 6 of them, namely, 2-hydroxy-(2/3)-methylbutyric acid, 25-hydroxyvitamin D3, 2-hydroxybutyric acid, alanine, dodecanoylcarnitine, and 1-(1Z-octadecenyl)-2-oleoyl-sn-glycero-3-phosphocholine. Fold changes in 7 amino acid ratios, all involving glutamine or ornithine, were also significantly different between cases and controls ($P<.01$). The predictive performance of some metabolites and ratios differed according to body mass index classification; for example, ornithine ($P<.001$) and several ornithine-related ratios ($P<.0001$ to $P<.01$) were only strongly associated with preterm preeclampsia in the body mass index of <25 kg/m^2 group, whereas dodecanoylcarnitine and 3 glutamine ratios were particularly predictive in the body mass index of ≥30 kg/m^2 group ($P<.01$).

CONCLUSION: Single metabolites and ratios of amino acids related to arginine bioavailability and nitric oxide synthase pathways were associated with preterm preeclampsia risk at 11 to 13 weeks of gestation. Differential prediction was observed according to body mass index classes, supporting the existence of distinct maternal risk profiles. Future studies in preeclampsia prediction should account for the possibility of different maternal risk profiles to improve etiologic and prognostic understanding and, ultimately, clinical utility of screening tests.

Key words: biomarkers, first-trimester screening, metabolites, metabolomics, prediction, preeclampsia, pregnancy, preterm

Introduction

Preeclampsia (PE) is not a single disorder but a syndrome with distinct etiologies.[1,2] However, the increased understanding of pathophysiology has not contributed significantly to bettering prediction or expanding prevention or treatment options.[3,4] To explain this lack of progress, it has been hypothesized that maternal syndrome develops through distinct pathophysiological pathways. If confirmed, there is a need to recognize different subtypes of PE to yield more clinical utility.[5] Recent data from Than et al[6] suggest that there are distinct maternal and placental disease pathways and that their interaction determines the clinical presentation of PE. With the activation of maternal disease pathways detected in advance of placental dysfunction, their data also pointed to preexisting, possibly subclinical, maternal risk profiles. The existence of different etiologic pathways and risk profiles is implicitly accounted for when determining a patient's specific risk of developing PE, either directly, by considering discrete patient phenotype-specific risks inferred from epidemiologic data,[7,8] or by combining maternal previous risk information into regression models.[9]

Cite this article as: Tuytten R, Syngelaki A, Thomas G, et al. First-trimester preterm preeclampsia prediction with metabolite biomarkers: differential prediction according to maternal body mass index. Am J Obstet Gynecol 2023;229:55.e1-10.

0002-9378/$36.00

https://doi.org/10.1016/j.ajog.2022.12.012

Click Supplemental Materials and Video under article title in Contents at ajog.org

AJOG at a Glance

Why was this study conducted?

This study aimed to verify metabolite biomarkers as first-trimester predictors for preterm preeclampsia (PE) and to investigate the interaction of biomarker prediction with maternal body mass index (BMI).

Key findings

Several single metabolites were confirmed to be associated with preterm PE. Improved prediction was found for ratios between amino acids related to arginine bioavailability and nitric oxide synthase pathways. The predictive value of some of the metabolites and metabolite ratios varied according to the BMI of the women.

What does this add to what is known?

The findings of differential biomarker prediction according to body mass index support the existence of different maternal risk profiles and associated biomarker profiles in women whereby PE develops via different pathophysiological pathways. Future research in PE risk should consider this complexity.

Current benchmark prediction models for PE risk combine maternal risk factors, Doppler velocimetry of the uterine arteries, mean arterial pressure, and the blood levels of the proteins placental growth factor (PlGF) and pregnancy-associated plasma protein A (PAPP-A),[10] whereby the biophysical and biochemical data are transformed into multiples of the median (MoM) values using population- and site-specific models.[11] The detection rate (DR) of these models for identifying patients at risk of preterm PE already enables preventive strategies.[12] The integration of additional biomarkers may further improve DRs; additional biomarkers may associate with specific PE risk profiles or patient phenotypes.

An individual's metabolome is considered to reflect, at any given time, the interaction between one's genetic makeup and external influences, such as diet or environmental factors.[13] Therefore, metabolite biomarkers have been posited as good candidates to capture maternal risk related to the environment and possibly to the interaction with the placental unit and/or fetus.[14] However, few metabolite candidates have been consistently confirmed as PE risk predictors to date,[15] warranting the need for a large-scale verification of metabolite biomarkers of interest. To account for the complexity of the disease, such a verification effort should ideally consider the specificity of metabolomic biomarkers for different maternal risk profiles. Without readily available biochemical signatures to accurately phenotype pregnant women early in pregnancy, we hypothesized that differences in disease risk as associated with maternal traits in epidemiologic studies reflect an enriched presence of one or more discrete risk profiles within the population exhibiting the particular trait. With obesity strongly associated with PE rates,[16,17] we used the World Health Organization body mass index (BMI) classification as the first maternal trait to create the patient strata.[18]

Here, we investigated whether metabolite biomarkers can predict preterm PE early in pregnancy in all patients and across different BMI groups. Furthermore, we considered metabolite ratios associated with arginine bioavailability and regulation of the nitric oxide synthase pathway as these biochemical pathways have been implicated in PE pathophysiology and cardiovascular risk.[19–25] To achieve this, we leveraged an extensive collection of first-trimester plasma samples and a dedicated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the targeted analysis of a panel of metabolites reported to associate with adverse pregnancy outcomes.[26] A comparison with well-characterized biomarkers (PlGF and PAPP-A) was also performed.

Materials and Methods

Study population

This was an observational case-control study drawn from a large prospective screening study on the early prediction of complications of pregnancy in women presenting for their routine first hospital visit (11 0/7 to 13 6/7 weeks of gestation) at King's College Hospital, London, United Kingdom, in 2010–2015. Pregnant women received a complete first-trimester assessment according to the Fetal Medicine Foundation (FMF)-described protocols,[9,10] including collecting blood samples for first-trimester biochemical screening (PAPP-A, free beta-human chorionic gonadotropin, PlGF) and biobanking. Data on pregnancy outcomes were collated for the study participants, and the criteria of the American College of Obstetricians and Gynecologists (2019) were used for the diagnosis of PE.[27] Written informed consent was obtained from all women; the study was approved by the National Research Ethics Committee (reference number 02-03-033). Within the study, all major pregnancy outcomes (ie, PE, fetal growth restriction, gestational diabetes mellitus, and spontaneous preterm birth) (n=866) and uncomplicated pregnancy outcomes (n=1635) were represented; the latter served as controls in biomarker analyses. Here, we reported on the nested data for preterm PE (n=106) vs controls.

Descriptive statistics were presented as mean (standard deviation, SD), median (interquartile range), and frequency of observations (percentage), as appropriate. Comparisons of patient characteristics and pregnancy outcomes between women with preterm PE and controls were performed using the chi-square test or Mann-Whitney *U* test, as appropriate (Table 1).

Biomarker analyses

First-trimester plasma samples of 2501 singleton pregnancies were analyzed with a targeted tandem LC-MS/MS method for metabolite biomarkers (Metabolomic Diagnostics, Cork, Ireland) using analytical

methodology as previously reported.[26] In brief, biobanked plasma samples received from FMF (London, United Kingdom) were thawed once on ice and subaliquoted in 40 μL aliquots (Agilent Bravo, Agilent Technologies, Santa Clara, CA). At that time patient aliquots were also combined in a pooled study quality control (QC), from which study-wide QC aliquots were created. Aliquots for the 2501 study participants and replicates for 349 randomly selected subjects were randomized in 38 analytical batches, with each batch featuring 75 clinical samples (inclusive replicates), 8 calibrator samples, 9 pooled study QC samples, and 2-by-2 ordinary QC samples (QC low and QC high). Metabolite analysis was performed in 38 consecutive days using 2 LC-MS/MS setups (Agilent Technologies) in parallel. Following mass spectrometric analysis, the mass spectrometric signals were quantified using a predefined quantification method (MassHunter Quant Software, Agilent Technologies, Santa Clara, CA). Data were reviewed by 2 independent analysts; all manual curations were recorded for data integrity purposes. Further technical information regarding the metabolite analyses are detailed in Supplementary File 1.

Data preprocessing and quality assurance

Laboratory personnel were blinded to sample status (pregnancy outcome) at all stages of the study. A structured review of all mass spectrometry was performed to confirm the quantification metric to use. Pairwise dependencies between metabolite quantification metrics and recorded experimental variables were computed using, as appropriate, the Spearman rank correlation, Mann-Whitney *U* test, or Kruskal-Wallis test; only minor interday batch effects in some of the quantifications were found. Hence, the relative concentrations were scaled per batch using the median concentration of the 9 pooled study QC samples for the given batch over the overall median concentration.[28] Data missingness and imprecision criteria were applied, except for cotinine a reporter metabolite for smoking status, with data missingness for a given metabolite quantification of <20% across all clinical samples and coefficient of variation (%) of ≤20% as calculated for the 349 pairs of replicate samples. Finally, metabolite quantifications from LC-MS/MS assay with poorly understood specificity were also purged. Additional details on data preprocessing and quality assurance are presented in Supplementary File 1.

Following quality control, quantification data for 50 metabolites across 15 chemical classes were available for prediction analysis with amino acids (n=17), fatty acids (n=8), acylcarnitines (n=6), and monoacylglycerophosphocholines (n=4), accounting for 60% of the metabolites. Of note, 2 pairs of structural isomers were not analytically resolved and were analyzed as single analytes, that is, 3-hydroxy(iso)valeric acid and 2-hydroxy-(2/3)-methylbutyric acid. The same applied to the 3 structural isomers and stereoisomers of dilinoleoyl glycerol.

TABLE 1
Baseline characteristics of the study population

Characteristic	Preterm PE (n=106)	Controls (n=1635)
Gestational age at sampling (wk)	12.6 (12.22–12.98)	12.7 (12.3–13.0)
Maternal age (y)	30.5 (27.5–35.4)	32.1 (28.4–35.5)
Race[a]		
White	48 (45.3)	1025 (62.7)
Black	51 (48.1)	433 (26.5)
South Asian	4 (3.8)	66 (4.0)
East Asian	1 (0.9)	52 (3.2)
Mixed	2 (1.9)	59 (3.6)
Height (cm)	164 (160–167)	165 (160–169)
Weight (kg)[a]	75.2 (65.8–87.0)	65.4 (59.0–75.7)
Body mass index class (kg/m^2)[a]		
<25	33 (31.1)	944 (57.7)
25 to <30	33 (31.1)	419 (25.6)
≥30	40 (37.7)	272 (16.6)
Conception		
In vitro fertilization	7 (6.6)	45 (2.8)
Ovulation drugs	1 (0.9)	13 (0.8)
Smoking	4 (3.8)	91 (5.6)
Diabetes mellitus		
Type 1	0 (0.0)	11 (0.7)
Type 2	4 (3.8)	15 (0.9)
SLE or APS	1 (0.9)	6 (0.4)
Chronic hypertension[a]	15 (14.2)	24 (1.5)
Family history of PE[a]	11 (10.4)	58 (3.5)
Gestational age at delivery (wk)[a]	34.2 (31.6–35.7)	39.2 (38.7–39.5)
Birthweight (g)[a]	1771 (1354–2093)	3295 (3100–3515)
Birthweight percentile[a]	0.48 (0.03–10.14)	47.13 (29.19–66.87)

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

APS, antiphospholipid syndrome; *PE*, preeclampsia; *SLE*, systemic lupus erythematosus.

[a] Chi-square test or Mann Whitney *U* test as appropriate ($P<.01$).

Tuytten. Metabolite biomarkers in preeclampsia. Am J Obstet Gynecol 2023.

Confounding associations

Biomarker data were normalized using MoM.[29] Log-normality was confirmed in control subjects using the Shapiro-Wilk test and quantile-quantile plots. Moreover, parameters for MoM normalization of a given biomarker were selected using multiway analysis of variance (ANOVA) on the control pregnancies ($P<.001$). Adjustments were warranted for storage age of the sample, gestational age at sampling, maternal age, weight and BMI, smoking, and racial origin, as summarized in Supplementary File 2. The normalization coefficients were computed and applied to all study participants. Of note, 2 associations were found for other maternal risk factors, that is, one with chronic hypertension (dilinoleoyl glycerol) and one with conception by in vitro fertilization (25-hydroxyvitamin D3); no adjustment was made for these. Cotinine was excluded from normalization. For biomarkers without confounding associations, readouts were transformed into simple medians on the controls.

Weak associations with storage time under −80°C conditions were corrected for 25-hydroxyvitamin D3 (25(OH)D3), arginine, symmetric dimethylarginine (SDMA), glutamine, and lactic acid. The levels of PlGF and PAPP-A increased significantly with gestational age at blood sampling. None of the metabolites showed this association except bilirubin, which levels trended downward. Confounding associations with maternal characteristics were found for 44 of 49 metabolites, with cotinine not considered. Adjustments were made for maternal age (16/49), BMI (25/49), smoking (7/49), and maternal race (26/49). The levels of 8 metabolites were associated with more than 3 parameters. The associations for 25(OH)D3, ergothioneine, and 1-(1Z-octadecenyl)-2-oleoyl-sn-glycero-3-phosphocholine (PC[P-18:0/18:1n9]) are plotted in Supplementary File 2.

Pairwise correlations

Pairwise correlations among all normalized biomarker readouts were calculated using the Spearman rank correlation test.[30] To elicit possible redundant predictors, biomarkers were grouped based on the Spearman correlation coefficients $r^2 \geq 0.5$; this resulted in 8 clusters. No correlation was observed between the proteins PlGF and PAPP-A and any of the metabolite biomarkers. Further details on pairwise correlations and biomarker clustering are available in Supplementary File 3.

Composite biomarkers

Informed by literature,[19–25] an additional set of composite biomarkers was created by taking the pairwise ratios of the amino acids alanine, glutamine, arginine, citrulline, SDMA, asymmetric dimethylarginine (ADMA), homoarginine, NG-monomethyl-L-arginine (L-NMMA), and ornithine. In the main, these ratios were less prone to confounding associations.

Prediction analysis

Prediction analyses were performed across the following subject strata: all subjects, BMI of <25 kg/m^2, BMI of 25 to <30 kg/m^2, and BMI of ≥ 30 kg/m^2. The discriminative performance of the normalized biomarker concentrations was estimated using the Mann-Whitney U test with the significance level set at $P<.05$, except for composite markers, for which $P<.01$ was applied. Of note, 2-way ANOVA was used to assess the significance ($P<.05$) of the interaction between clinical outcome and BMI.[31] The significance of pairwise differences in fold change among strata was estimated from marginal means using the Tukey test between cases and controls in and among BMI groups.[32] To gauge the clinical utility of the biomarkers, the odds ratios (ORs) and 95% confidence intervals (CIs) were calculated at the mean +1 SD based on the distribution of the normalized biomarker levels in controls[33,34]; for down-regulated markers (cases vs controls), mean −1 SD was used. Mean and SD were estimated using median and median absolute deviation, respectively. With the biomarker data normally distributed, the 1 SD cutoff corresponds to a false-positive rate (FPR) of approximately 16%. Therefore, the reported ORs allow for comparing the prediction performance of the biomarkers at fixed FPRs. P values were not adjusted for multiplicity and should be considered exploratory. Statistical analyses were performed in R.[35]

Results

Within the study population (Table 1), patients of Black race, with higher body weight or BMI, were more likely to develop preterm PE. The fraction of women with chronic hypertension and/or a family history of PE was significantly increased in the case population. The median gestation at delivery in the preterm PE group was 34.2 weeks, and the median birthweight was 1771 g, both outcome metrics significantly lower than observed in the control group with medians of 39.2 weeks and 3295 g, respectively.

Preterm preeclampsia prediction

Of note (Table 2), 5 biomarkers were identified with significantly lower MoM levels in the cases than in the controls, that is, PlGF and PAPP-A ($P<.0001$) and the metabolites PC(P-18:0/18:1n9) ($P<.01$), bilirubin, and glutamine ($P<.05$). The median levels of 10 metabolites were significantly higher in the cases, in particular 2-hydroxy-(2/3)-methylbutyric acid, 25(OH)D3, 2-hydroxybutyric acid ($P<.001$), alanine, and dodecanoylcarnitine ($P<.01$). The medians in 7 composite markers were also significantly different between cases and controls ($P<.01$). All ratios were either glutamine ratios or ornithine ratios. In terms of clinical risk prediction, other than for PlGF (OR, 7.04; 95% CI, 4.69–10.65), the ORs were typically modest for the biomarkers with only PAPP-A, 25(OH)D3, alanine-to-glutamine ratio, and arginine-to-glutamine ratio delivering ORs of >2.

Preterm preeclampsia prediction according to body mass index class

No significant difference in median levels was found for PlGF and PAPP-A across the BMI strata (Table 3). Among the single metabolite predictors, preterm PE prediction specific to the group with a BMI of <25 kg/m^2 was found for ornithine ($P<.001$), and preterm PE prediction specific to the group with a BMI of

TABLE 2
Preterm preeclampsia prediction in the entire study population

Biomarker	Ratio of median levels Cases-to-controls (95% CI)	OR per 1 SD increase (95% CI)[a]
Placental growth factor	0.63 (0.59–0.70)[b]	7.04 (4.69–10.65)
Pregnancy-associated plasma protein A	0.85 (0.71–0.89)[b]	2.04 (1.29–3.14)
2-Hydroxy-(2/3)-methylbutyric acid	1.17 (1.06–1.22)[c]	1.71 (1.06–2.68)
25-Hydroxyvitamin D3	1.16 (1.07–1.26)[c]	2.54 (1.57–4.00)
2-Hydroxybutyric acid	1.12 (1.06–1.26)[c]	1.69 (1.02–2.70)
Alanine	1.07 (1.02–1.10)[d]	1.62 (0.99–2.54)
Dodecanoylcarnitine[e]	1.22 (1.08–1.43)[d]	1.61 (0.98–2.56)
Decanoylcarnitine[e]	1.18 (1.02–1.43)	1.66 (1.02–2.62)
Octanoylcarnitine[e]	1.18 (1.01–1.37)	1.48 (0.89–2.36)
1-(1Z-octadecenyl)-2-oleoyl-sn-glycero-3-phosphocholine	0.93 (0.89–0.98)[d]	1.73 (1.08–2.70)
3-Hydroxy(iso)valeric acid	1.12 (1.02–1.24)	1.66 (0.99–2.66)
Ergothioneine	1.16 (1.02–1.33)	1.56 (0.94–2.49)
Bilirubin	0.89 (0.81–0.99)	1.35 (0.82–2.18)
Threonine	1.05 (1.00–1.11)	1.47 (0.88–2.36)
Glutamine	0.95 (0.93–1.00)	1.38 (0.83–2.19)
Alanine-to-glutamine ratio	1.09 (1.05–1.15)[b]	1.98 (1.24–3.09)
Alanine-to-ornithine ratio	1.09 (1.04–1.16)[d]	2.14 (1.36–3.29)
Asymmetric dimethylarginine–to–ornithine ratio	1.08 (1.03–1.14)[d]	1.58 (0.97–2.49)
Symmetric dimethylarginine–to–ornithine ratio	1.10 (1.03–1.15)[d]	1.26 (0.75–2.02)
Symmetric dimethylarginine–to–glutamine ratio	1.06 (1.03–1.13)[d]	1.55 (0.95–2.46)
Arginine-to-glutamine ratio	1.07 (1.02–1.15)[d]	2.09 (1.34–3.20)
Arginine-to-ornithine	1.06 (1.02–1.14)[d]	1.51 (0.93–2.38)

Effect size of the case-to-control ratio are presented as estimate (95% CI). Difference between estimated case and control levels evaluated using Mann-Whitney *U* test.

Selection: Mann-Whitney *U* test $P<.05$ for single markers; $P<0.01$ for composite markers.

CI, confidence interval; *OR*, odds ratio; *SD*, standard deviation.

[a] Down-regulated markers inverted for OR; [b] $P<.0001$; [c] $P<.001$; [d] $P<.01$.; [e] Member of the "medium-chain carnitine" cluster.

Tuytten. Metabolite biomarkers in preeclampsia. Am J Obstet Gynecol 2023.

≥30 kg/m^2 was found for decanoylcarnitine and dodecanoylcarnitine and SDMA ($P<.05$). Within the respective BMI strata, the effect sizes in median level ratios were larger for these biomarkers than those observed in the entire study population. Clinical risk prediction was also accentuated, with all ORs >2. For SDMA, this was driven by a subset BMI of ≥30 kg/m^2 with markedly up-regulated levels. Some differential preterm PE prediction was found in the group with a BMI of 25 to <30 kg/m^2 (bilirubin, biliverdin, PC[P-18:0/18:1n9]), yet data review suggested that these were likely false discoveries, as expected in the absence of multiple testing correction. A marked delineation of preterm PE prediction between the groups with a BMI of <25 kg/m^2 and a BMI of ≥30 kg/m^2 was found for the composite markers. Of note, 7 ornithine-based ratios were specifically predicted in women with a BMI of <25 kg/m^2 ($P<.0001$ to $P<.01$), with accentuated ratios of median levels between cases and controls and good clinical prediction (ie, ORs >2.0). In addition, the alanine-to-ornithine ratio (Figure) has an OR of 6.03 (95% CI, 2.96–12.44), equivalent to PlGF in this BMI stratum. The homo-arginine–to–citrulline ratio was characterized by distinct up-regulation in the group with a BMI of <25 kg/m^2 and down-regulation in the group with a BMI of 25 to <30 kg/m^2. Of note, 3 glutamine-based ratios, that is, arginine-to-glutamine ratio (Figure), alanine-to-glutamine, and SDMA-to-glutamine ratio, but not glutamine-to-ornithine ratio, gave rise to specific preterm PE prediction in the group with a BMI of ≥30 kg/m^2 ($P<.01$).

TABLE 3
Preterm preeclampsia prediction according to body mass index

Biomarker	ANOVA interaction Outcome × stratum (P<.05)	BMI of <25 kg/m^2 Cases, n=33 Controls, n=944		BMI of 25 to <30 kg/m^2 Cases, n=33 Controls, n=419		BMI of ≥30 kg/m^2 Cases, n=40 Controls, n=272	
		Ratio of median levels Cases-to-controls (95% CI)	OR per 1 SD increase (95% CI)[a]	Ratio of median levels Cases-to-controls (95% CI)	OR per 1 SD increase (95% CI)[a]	Ratio of median levels Cases-to-controls (95% CI)	OR per 1 SD increase (95% CI)[a]
PlGF	.162	0.61 (0.58–0.76)[bc]	6.13 (3.01–12.66)[b]	0.59 (0.52–0.74)[b,c]	8.62 (4.09–18.96)[b]	0.68 (0.59–0.76)[b,c]	6.25 (3.11–12.81)[b]
PAPP-A	.731	0.89 (0.74–1.07)[b]	0.95 (0.31–2.32)[b]	0.74 (0.59–0.89)[b,e]	3.06 (1.39–6.47)[b]	0.90 (0.63–0.98)[b,f]	2.25 (1.05–4.63)[b]
Ornithine	<.001	0.87 (0.76–0.92)[d]	3.01 (1.40–6.19)	1.03 (0.94–1.15)	0.65 (0.19–1.73)	1.03 (0.92–1.11)	0.86 (0.24–2.35)
Biliverdin[h]	.002	1.05 (0.91–1.21)	1.11 (0.36–2.70)	0.87 (0.75–0.99)[f]	1.89 (0.79–4.12)	0.96 (0.89–1.14)	1.22 (0.43–2.95)
Bilirubin[h]	.005	0.91 (0.80–1.13)	1.21 (0.44–2.81)	0.80 (0.66–0.93)[e]	2.23 (1.00–4.73)	0.93 (0.82–1.11)	1.00 (0.28–2.75)
1-(1Z-octadecenyl)-2-oleoyl-sn-glycero-3-phosphocholine	.011	0.92 (0.84–0.99)[f]	2.11 (0.94–4.43)	0.95 (0.87–1.03)	1.36 (0.52–3.12)	0.90 (0.82–0.98)[f]	2.06 (0.89–4.47)
Decanoylcarnitine[g]	.014	1.14 (0.83–1.54)	1.48 (0.58–3.32)	0.85 (0.74–1.33)	1.12 (0.36–2.80)	1.36 (1.03–1.77)[f]	2.09 (0.98–4.29)
Dodecanoylcarnitine[g]	.016	1.30 (0.90–1.52)	1.02 (0.34–2.48)	0.99 (0.80–1.32)	1.23 (0.44–2.94)	1.39 (1.11–1.73)[e]	2.57 (1.19–5.32)
Symmetric dimethylarginine	.043	0.97 (0.93–1.03)	0.85 (0.28–2.07)	1.01 (0.93–1.06)	1.54 (0.59–3.55)	1.04 (1.01–1.13)[f]	2.64 (1.20–5.58)
Arginine-to-glutamine ratio	<.001	0.96 (0.88–1.09)	1.54 (0.60–3.46)	1.04 (0.96–1.18)	1.60 (0.68–3.49)	1.10 (1.04–1.27)[e]	2.40 (1.18–4.81)
Alanine-to-glutamine ratio	<.001	1.04 (0.92–1.12)	1.74 (0.68–3.91)	1.07 (1.00–1.16)[f]	1.27 (0.49–2.92)	1.11 (1.05–1.21)[e]	2.45 (1.16–5.00)
Symmetric dimethylarginine–to–glutamine ratio	<.001	1.00 (0.93–1.09)	1.12 (0.37–2.72)	1.03 (0.94–1.15)	2.18 (0.95–4.69)	1.16 (1.05–1.23)[e]	1.11 (0.47–2.38)
Alanine-to-ornithine ratio	.001	1.32 (1.11–1.37)[d]	6.03 (2.96–12.44)	1.05 (0.92–1.14)	1.02 (0.37–2.42)	1.09 (0.99–1.19)	1.56 (0.59–3.65)
Symmetric dimethylarginine–to–ornithine ratio	.002	1.20 (1.10–1.32)[d]	2.26 (1.00–4.75)	1.00 (0.90–1.12)	0.68 (0.19–1.79)	1.10 (0.99–1.23)	1.12 (0.43–2.57)
NG-monomethyl-L-arginine–to-ornithine ratio	.004	1.19 (1.06–1.29)[e]	2.52 (1.11–5.29)	0.96 (0.86–1.08)	0.51 (0.12–1.50)	1.08 (0.96–1.20)	1.15 (0.44–2.64)
Glutamine–to–ornithine ratio	.008	1.22 (1.10–1.33)[d]	2.58 (1.14–5.43)	0.97 (0.86–1.06)	0.61 (0.17–1.62)	1.03 (0.89–1.09)	0.64 (0.21–1.58)

Tuytten. Metabolite biomarkers in preeclampsia. Am J Obstet Gynecol 2023.

(continued)

TABLE 3
Preterm preeclampsia prediction according to body mass index *(continued)*

Biomarker	ANOVA interaction Outcome × stratum ($P<.05$)	BMI of <25 kg/m^2 Cases, n=33 Controls, n=944		BMI of 25 to <30 kg/m^2 Cases, n=33 Controls, n=419		BMI of ≥30 kg/m^2 Cases, n=40 Controls, n=272	
		Ratio of median levels Cases-to-controls (95% CI)	OR per 1 SD increase (95% CI)[a]	Ratio of median levels Cases-to-controls (95% CI)	OR per 1 SD increase (95% CI)[a]	Ratio of median levels Cases-to-controls (95% CI)	OR per 1 SD increase (95% CI)[a]
Homo-arginine—to—ornithine ratio	.009	1.25 (1.11—1.57)[e]	2.16 (0.92—4.61)	0.79 (0.75—1.05)	0.41 (0.06—1.42)	1.09 (0.90—1.25)	1.01 (0.38—2.30)
Homo-arginine—to—citrulline ratio	.007	1.26 (1.06—1.49)[f]	2.25 (1.00—4.72)	0.75 (0.70—1.01)	1.00 (0.32—2.49)	1.02 (0.86—1.20)	1.29 (0.49—2.98)
Asymmetric dimethylarginine —to—ornithine ratio	.034	1.18 (1.11—1.31)[c]	3.27 (1.52—6.72)	1.04 (0.92—1.11)	0.93 (0.33—2.20)	1.04 (0.97—1.15)	1.09 (0.41—2.50)
Arginine-to-ornithine	.039	1.19 (1.05—1.29)[e]	2.46 (1.12—5.11)	1.03 (0.91—1.12)	1.01 (0.36—2.38)	1.09 (1.00—1.20)	1.42 (0.57—3.20)

Effect size of the case-to-control ratio (fold change cases vs controls) are presented as estimate (95% CI). Difference between estimated case and control levels evaluated using Mann-Whitney *U* test.

Selection: Mann-Whitney *U* test $P<.05$ for single markers and $P<.01$ for composite markers. ANOVA interaction "outcome" × "stratum" selection: comparison of marginal means $P<.05$.

ANOVA, analysis of variance; *BMI*, body mass index; *CI*, confidence interval; *OR*, odds ratio; *PAPP-A*, pregnancy associated plasma protein-A; *PlGF*, Placental growth factor; *SD*, standard deviation.

[a] Down-regulated markers inverted for OR; [b] $P\geq.05$; [c] $P<.0001$; [d] $P<.001$; [e] $P<.01$; [f] $P<.05$.

[g] Member of the "medium-chain carnitine" cluster.

[h] Member of the "bilirubin" cluster.

Tuytten. Metabolite biomarkers in preeclampsia. Am J Obstet Gynecol 2023.

Comment

Principal findings

We used targeted LC-MS/MS technology to perform a large-scale verification of preterm PE prediction utility at 11 0/7 to 13 6/7 weeks of gestation within a panel of metabolite biomarkers. With biomarker quantification data available for 1635 control pregnancies, we were able to elicit confounding associations and adjust for these. On a technical level, we confirmed that well-controlled, long-term storage at −80°C had minimal effect on metabolite levels in blood plasma. With the metabolome reflective of the interaction between genotype and environmental factors, confounding associations were expected. Without exception, adjustments for one or more maternal characteristics were required. Among the metabolites, only bilirubin levels were associated with gestational age at sampling.

We confirmed that several metabolite biomarkers and amino acid ratios were associated with preterm PE risk. The differences between the median metabolite levels for cases and controls were typically modest, which are also reflected in the corresponding ORs.

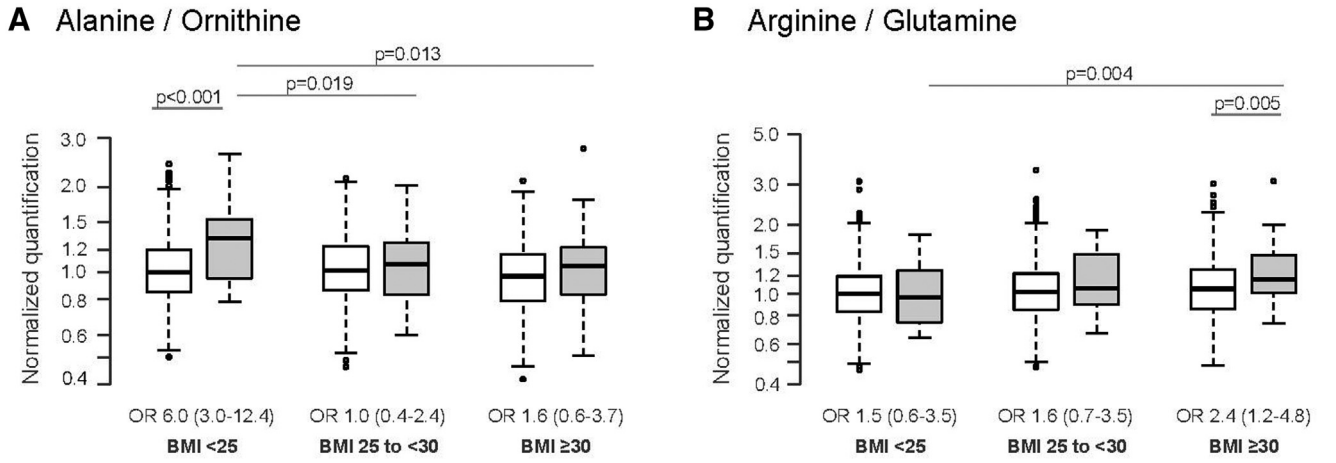
Supporting the hypothesis that different biomarkers may have relevance for different patient profiles, we showed several biomarkers that had predictive value in specific BMI classes. Strikingly, preterm PE prediction in the group with a BMI of <25 kg/m^2 was largely centered around decreased ornithine levels, either on its own or as part of ratios. In contrast, medium-chain carnitines and some glutamine ratios exhibited specific preterm PE prediction in the group with a BMI of ≥30 kg/m^2.

We note that in the various prediction analyses performed here, none of the fatty acids assayed reached significance.

Results in the context of what is known

The observed increases in early pregnancy in the levels of 2-hydroxybutyric acid, 3-hydroxy(iso)valeric acid, and medium-chain carnitines corroborate earlier metabolomics literature.[26,36–40] Our results have added precision to previous

FIGURE
Differential prediction of preterm preeclampsia according to BMI classes



A, Alanine-to-ornithine ratio. **B,** Arginine-to-glutamine ratio.

BMI, body mass index; *OR*, odds ratio.

Tuytten. Metabolite biomarkers in preeclampsia. Am J Obstet Gynecol 2023.

carnitine findings by indicating that prediction merits largely to the group with a BMI of $\geq$30 kg/m^2 only. PE prediction as reported by Koster et al[40] for the long-chain carnitine stearoylcarnitine was not confirmed. Similarly, we were not able to confirm dilinoleoyl glycerol as a stand-alone preterm PE predictor in this cohort.[26] Up-regulation of 2-hydroxy-3-methylbutyric acid was reported previously in women with confirmed PE.[41]

Conflicting literature exist on whether decreased or increased levels of 25(OH) D3 are associated with PE risk early in pregnancy.[15] Within the context of this study, 25(OH)D3 exhibited strong negative correlations with the confounding factors BMI and Black race, both well known to confer significant PE risk. After adjusting, we found that increased levels of 25(OH)D3 were associated with preterm PE risk.

Recently, ergothioneine has generated interest as a possible PE therapeutic following its mitochondrial-targeted antioxidant properties.[42,43] Contrary to expectations, we found that preterm PE risk was associated with increased levels of ergothioneine. For 1-(1Z-octadecenyl)-2-oleoyl-sn-glycero-3-phosphocholine (PC [P-18:0/18:1n9]), we reported decreasing MoM conferred preterm preeclampsia risk; Sovio et al[44] reported that the same metabolite that increases in MoMs are associated with FGR throughout pregnancy.

Although bilirubin is well established as a marker for liver compromise in the diagnosis of PE, we demonstrated again that lower levels of bilirubin are associated with PE risk in early pregnancy.[45] Hypobilirubinemia has been associated with increased risk of several cardiovascular diseases,[46] which may put it on PE pathophysiological pathways.[47]

The key finding of this study concerns the use of amino acids to predict preterm PE. First, it is clear from our large-scale investigation that normalized levels of single amino acids only yield limited fold changes between case and control populations, which may explain the many conflicting results recently summarized by Yao et al[48] and earlier for arginine and ADMA.[21] Second, we found that combining amino acids into ratios resulted in significant prediction. Surprisingly, the differential ratios found were typically not made up of 2 amino acids directly involved in nitric oxide formation by nitric oxide synthetase. The ratios involve ornithine or glutamine. Ornithine is the product of arginase activity, which also uses arginine as substrate; thus, ornithine reflects arginine usage in a competing pathway.[23] For the numerators, the ornithine ratios involve amino acids indirectly affecting arginine bioavailability (alanine, glutamine, SDMA) or direct actors in the nitric oxide synthetase pathway (arginine, homo-arginine, L-NMMA, ADMA). Based on our data, the existence of a specific group with a BMI of $<$25 kg/m^2 risk profile can be speculated. In the same way, we found glutamine-based ratios to be most predictive for preterm PE in the group with a BMI of $\geq$30 kg/m^2, with glutamine upstream in the arginine biosynthesis pathway.[49] Interestingly, the inhibition of arginine synthesis by glutamine has been reported for nitric oxide–producing endothelial cells.[50] The glutamine ratios found are reflecting the relation among 3 main metabolic pathways. Incidentally, Youssef et al[51] highlighted such interplay when comparing the metabolome profiles of women with early-onset severe PE (n=14) with that of control pregnancies (n=6).

Clinical implications

Our findings provided support to the contemporary concept that different maternal risk profiles exist, considering that PE may develop via different pathophysiological pathways.[5,6] Accounting for different maternal risk profiles may allow for the formulation of improved,

yet more complex, risk prediction models. The prospect that different pathophysiological pathways for PE can be delineated through metabolomics opens the alluring possibility for more targeted pharmaceutical interventions, such as the stratification to aspirin or metformin prophylaxis.[12,52]

Research implications

First, this study highlighted that large-scale evaluations are a prerequisite to properly verify the prediction potential of candidate biomarkers to avoid the selection for confounding associations rather than disease. In this context, we caution about our results for 25(OH)D3, as no adjustment was attempted for the observed association with conception by in vitro fertilization or seasonal effects.[53] Evidently, our findings on differential prediction according to maternal phenotypic traits have significant implications for both biomarker discovery and their translation into clinically meaningful solutions. Here, we used a pragmatic approach rooted in epidemiologic observations to create patient strata and elicit differential prediction. More sophisticated stratifications (eg, the use of metabolic syndrome instead of BMI-based grouping) may prove even more informative. In future research we will investigate whether metabolite biomarkers can complement PlGF and/or PAPP-A to improve preterm PE prediction in a phenotypic way. Given the absence of clear associations with gestational age at sampling for the metabolites evaluated, their levels in early pregnancy may reflect prepregnancy maternal risks rather than pregnancy-induced risks.[6] Longitudinal studies are required to gauge whether the predictive merits of these metabolites will change throughout pregnancy.

Strengths and limitations

The strengths of this study included the size of the study population, enabling stratification of cases in phenotypic groups and robust estimations of confounding associations, and the number of biomarkers that were evaluated simultaneously. An implicit limitation of this study was the fact that the metabolite biomarkers available for evaluation were selected from biomarker studies for which BMI distributions were centered around population averages.

Conclusions

This study confirmed several metabolites and metabolite ratios as predictors for preterm PE early in pregnancy. In addition, we found clear indications of differential prediction according to maternal BMI. Lastly, our study highlighted that the existence of different maternal risk profiles should be considered when investigating PE risk. ■

Acknowledgments

We would like to thank the following personnel working at Metabolomic Diagnostics: Isabel Vega-Carrascal, PhD, and Stephen Heaphy, PhD, for their assistance in the practical planning and execution of the study design and Anna Suhr, PhD; Claire O' Connell; Ciara Sandquest; and Keith Oshaughnessy for their assistance in the preparation and analysis of the samples.

References

1. Myatt L, Roberts JM. Preeclampsia: syndrome or disease? Curr Hypertens Rep 2015;17:83.
2. Erez O, Romero R, Maymon E, et al. The prediction of late-onset preeclampsia: results from a longitudinal proteomics study. PLoS One 2017;12:e0181468.
3. Roberts JM, Bell MJ. If we know so much about preeclampsia, why haven't we cured the disease? J Reprod Immunol 2013;99:1–9.
4. Poon LC, Shennan A, Hyett JA, et al. The International Federation of Gynecology and Obstetrics (FIGO) initiative on pre-eclampsia: a pragmatic guide for first-trimester screening and prevention. Int J Gynaecol Obstet 2019;145(Suppl1):1–33.
5. Roberts JM, Rich-Edwards JW, McElrath TF, Garmire L, Myatt L; Global Pregnancy Collaboration. Subtypes of preeclampsia: recognition and determining clinical usefulness. Hypertension 2021;77:1430–41.
6. Than NG, Romero R, Tarca AL, et al. Integrated systems biology approach identifies novel maternal and placental pathways of preeclampsia. Front Immunol 2018;9:1661.
7. US Preventive Services Task Force, Davidson KW, Barry MJ, et al. Aspirin use to prevent preeclampsia and related morbidity and mortality: US Preventive Services Task Force Recommendation Statement. JAMA 2021;326:1186–91.
8. Hypertension in pregnancy: diagnosis and management. London, United Kingdom: National Institute of Health and Care Excellence (NICE); 2019.
9. Wright D, Syngelaki A, Akolekar R, Poon LC, Nicolaides KH. Competing risks model in screening for preeclampsia by maternal characteristics and medical history. Am J Obstet Gynecol 2015;213:62.e1–10.
10. Tan MY, Syngelaki A, Poon LC, et al. Screening for pre-eclampsia by maternal factors and biomarkers at 11-13 weeks' gestation. Ultrasound Obstet Gynecol 2018;52:186–95.
11. Cuckle H. Local validation and calibration of pre-eclampsia screening algorithms. Ultrasound Obstet Gynecol 2019;53:724–8.
12. Rolnik DL, Wright D, Poon LC, et al. Aspirin versus placebo in pregnancies at high risk for preterm preeclampsia. N Engl J Med 2017;377:613–22.
13. Beger RD, Dunn W, Schmidt MA, et al. Metabolomics enables precision medicine: "A White Paper, Community Perspective". Metabolomics 2016;12:149.
14. Fanos V, Atzori L, Makarenko K, Melis GB, Ferrazzi E. Metabolomics application in maternal-fetal medicine. BioMed Res Int 2013;2013:720514.
15. Mayrink J, Leite DF, Nobrega GM, Costa ML, Cecatti JG. Prediction of pregnancy-related hypertensive disorders using metabolomics: a systematic review. BMJ Open 2022;12:e054697.
16. Mbah AK, Kornosky JL, Kristensen S, et al. Super-obesity and risk for early and late preeclampsia. BJOG 2010;117:997–1004.
17. Bartsch E, Medcalf KE, Park AL, Ray JG. High Risk of Pre-eclampsia Identification Group. Clinical risk factors for pre-eclampsia determined in early pregnancy: systematic review and meta-analysis of large cohort studies. BMJ 2016;353:i1753.
18. Obesity: preventing and managing the global epidemic. Report of a WHO consultation. World Health Organ Tech Rep Ser 2000;894:1–253.
19. Savvidou MD, Hingorani AD, Tsikas D, Frölich JC, Vallance P, Nicolaides KH. Endothelial dysfunction and raised plasma concentrations of asymmetric dimethylarginine in pregnant women who subsequently develop pre-eclampsia. Lancet 2003;361:1511–7.
20. Khalil AA, Tsikas D, Akolekar R, Jordan J, Nicolaides KH. Asymmetric dimethylarginine, arginine and homoarginine at 11-13 weeks' gestation and preeclampsia: a case-control study. J Hum Hypertens 2013;27:38–43.
21. Khalil A, Hardman L, O Brien P. The role of arginine, homoarginine and nitric oxide in pregnancy. Amino Acids 2015;47:1715–27.
22. Johal T, Lees CC, Everett TR, Wilkinson IB. The nitric oxide pathway and possible therapeutic options in pre-eclampsia. Br J Clin Pharmacol 2014;78:244–57.
23. Tang WH, Wang Z, Cho L, Brennan DM, Hazen SL. Diminished global arginine bioavailability and increased arginine catabolism as metabolic profile of increased cardiovascular risk. J Am Coll Cardiol 2009;53:2061–7.
24. Wang Z, Tang WH, Cho L, Brennan DM, Hazen SL. Targeted metabolomic evaluation of

arginine methylation and cardiovascular risks: potential mechanisms beyond nitric oxide synthase inhibition. Arterioscler Thromb Vasc Biol 2009;29:1383–91.
25. Lowe DT. Nitric oxide dysfunction in the pathophysiology of preeclampsia. Nitric Oxide 2000;4:441–58.
26. Kenny LC, Thomas G, Poston L, et al. Prediction of preeclampsia risk in first time pregnant women: metabolite biomarkers for a clinical test. PLoS One 2020;15:e0244369.
27. American College of Obstetricians and Gynecologists, ACOG Practice Bulletin No. 203: Chronic Hypertension in Pregnancy. Obstet Gynecol; 2019;133:e26–50.
28. Broadhurst D, Goodacre R, Reinke SN, et al. Guidelines and considerations for the use of system suitability and quality control samples in mass spectrometry assays applied in untargeted clinical metabolomic studies. Metabolomics 2018;14:72.
29. Bishop JC, Dunstan FD, Nix BJ, Reynolds TM, Swift A. All MoMs are not equal: some statistical properties associated with reporting results in the form of multiples of the median. Am J Hum Genet 1993;52:425–30.
30. Schober P, Boer C, Schwarte LA. Correlation coefficients: appropriate use and interpretation. Anesth Analg 2018;126:1763–8.
31. Altman DG, Bland JM. Comparing several groups using analysis of variance. BMJ 1996;312:1472–3.
32. Tukey JW. Comparing individual means in the analysis of variance. Biometrics 1949;5:99–114.
33. Bland JM, Altman DG. Statistics notes. The odds ratio. BMJ 2000;320:1468.
34. Yang J, Pearl M, DeLorenze GN, et al. Racial-ethnic differences in midtrimester maternal serum levels of angiogenic and antiangiogenic factors. Am J Obstet Gynecol 2016;215:359.e1–9.
35. R Core Team. R: A language and environment for statistical computing. 2018: Available at: www R-project org.
36. Kenny LC, Broadhurst DI, Dunn W, et al. Robust early pregnancy prediction of later preeclampsia using metabolomic biomarkers. Hypertension 2010;56:741–9.
37. Bahado-Singh RO, Akolekar R, Mandal R, et al. Metabolomics and first-trimester prediction of early-onset preeclampsia. J Matern Fetal Neonatal Med 2012;25:1840–7.
38. Bahado-Singh RO, Syngelaki A, Akolekar R, et al. Validation of metabolomic models for prediction of early-onset preeclampsia. Am J Obstet Gynecol 2015;213:530.e1–10.
39. Bahado-Singh RO, Akolekar R, Mandal R, et al. First-trimester metabolomic detection of late-onset preeclampsia. Am J Obstet Gynecol 2013;208:58.e1–7.
40. Koster MP, Vreeken RJ, Harms AC, et al. First-trimester serum acylcarnitine levels to predict preeclampsia: a metabolomics approach. Dis Markers 2015;2015:857108.
41. Kenny LC, Broadhurst D, Brown M, et al. Detection and identification of novel metabolomic biomarkers in preeclampsia. Reprod Sci 2008;15:591–7.
42. Kerley RN, McCarthy C, Kell DB, Kenny LC. The potential therapeutic effects of ergothioneine in pre-eclampsia. Free Radic Biol Med 2018;117:145–57.
43. Morillon AC, Williamson RD, Baker PN, et al. Effect of L-Ergothioneine on the metabolic plasma profile of the RUPP rat model of preeclampsia. PLoS One 2020;15:e0230977.
44. Sovio U, Goulding N, McBride N, et al. A maternal serum metabolite ratio predicts fetal growth restriction at term. Nat Med 2020;26:348–53.
45. Vieira MC, Thomas G, Poston L, Tuytten R, Pasupathy D. Metabolite biomarkers for early prediction of pre-eclampsia in women with obesity. Reprod Sci 2019;26:275A.
46. Creeden JF, Gordon DM, Stec DE, Hinds TD Jr. Bilirubin as a metabolic hormone: the physiological relevance of low levels. Am J Physiol Endocrinol Metab 2021;320:E191–207.
47. Thilaganathan B, Kalafat E. Cardiovascular system in preeclampsia and beyond. Hypertension 2019;73:522–31.
48. Yao M, Xiao Y, Yang Z, et al. Identification of biomarkers for preeclampsia based on metabolomics. Clin Epidemiol 2022;14:337–60.
49. Wu G, Morris SM Jr. Arginine metabolism: nitric oxide and beyond. Biochem J 1998;336:1–17.
50. Sessa WC, Hecker M, Mitchell JA, Vane JR. The metabolism of L-arginine and its significance for the biosynthesis of endothelium-derived relaxing factor: L-glutamine inhibits the generation of L-arginine by cultured endothelial cells. Proc Natl Acad Sci U S A 1990;87:8607–11.
51. Youssef L, Crovetto F, Simoes RV, et al. The interplay between pathophysiological pathways in early-onset severe preeclampsia unveiled by metabolomics. Life (Basel) 2022;12:86.
52. Syngelaki A, Nicolaides KH, Balani J, et al. Metformin versus placebo in obese pregnant women without diabetes mellitus. N Engl J Med 2016;374:434–43.
53. Kiely ME, Zhang JY, Kinsella M, Khashan AS, Kenny LC. Vitamin D status is associated with uteroplacental dysfunction indicated by preeclampsia and small-for-gestational-age birth in a large prospective pregnancy cohort in Ireland with low vitamin D status. Am J Clin Nutr 2016;104:354–61.

Author and article information

From the Metabolomic Diagnostics, Cork, Ireland (Drs Tuytten and Panigassi, Mr Brown, and Dr Ortea); Harris Birthright Research Centre for Fetal Medicine, King's College Hospital, London, United Kingdom (Drs Syngelaki and Nicolaides); and SQU4RE, Lokeren, Belgium (Dr Thomas).

Received Aug. 19, 2022; revised Dec. 8, 2022; accepted Dec. 11, 2022.

A.P., L.W.B., P.O., and R.T. are employees of Metabolomic Diagnostics. G.T. is the owner of SQU4RE, an independent statistics and data mining provider. L.W.B., G.T., and R.T. are named inventors on several patent applications regarding the use of metabolites to predict preeclampsia risk. The associated rights are assigned to Metabolomic Diagnostics. A.S. and K.H.N. report no conflict of interest.

Metabolomic Diagnostics has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement number 822842 for parts of this research. Collection and storage of the maternal samples was funded by the Fetal Medicine Foundation (United Kingdom charity number 1037116). The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Corresponding author: Kypros H. Nicolaides, MD. kypros@fetalmedicine.com