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Predicting Placenta Accreta Spectrum Disorder Through Machine Learning Using Metabolomic and Lipidomic Profiling and Clinical Characteristics

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OBJECTIVE: To perform metabolomic and lipidomic profiling with plasma samples from patients with placenta accreta spectrum (PAS) to identify possible biomarkers for PAS and to predict PAS with machine learning methods that incorporated clinical characteristics with metabolomic and lipidomic profiles.

METHODS: This was a multicenter case-control study of patients with placenta previa with PAS (case group n=33) and previa alone (control group n=21). Maternal third-

trimester plasma samples were collected and stored at -80°C . Untargeted metabolomic and targeted lipidomic assays were measured with flow-injection mass spectrometry. Univariate analysis provided an association of each lipid or metabolite with the outcome. The Benjamini-Hochberg procedure was used to control for the false discovery rate. Elastic net machine learning models were trained on patient characteristics to predict risk, and an integrated elastic net model of lipidome or metabolome with nine clinical features was trained. Performance using the area under the receiver operating characteristic curve (AUC) was determined with Monte Carlo cross-validation. Statistical significance was defined at $P<.05$.

RESULTS: The mean gestational age at sample collection was 33 3/7 weeks (case group) and 35 5/7 weeks (control group) ($P<.01$). In total, 786 lipid species and 2,605 metabolite features were evaluated. Univariate analysis revealed 31 lipids and 214 metabolites associated with the outcome ($P<.05$). After false discovery rate adjustment, these associations no longer remained statistically significant. When the machine learning model was applied, prediction of PAS with only clinical characteristics (AUC 0.685, 95% CI, 0.65–0.72) performed similarly to prediction with the lipidome model (AUC 0.699, 95% CI, 0.60–0.80) and the metabolome model (AUC 0.71, 95% CI, 0.66–0.76). However, integration of metabolome and lipidome with clinical features did not improve the model.

CONCLUSION: Metabolomic and lipidomic profiling performed similarly to, and not better than, clinical risk factors using machine learning to predict PAS among patients with PAS with previa and previa alone.

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Placenta accreta spectrum (PAS) is a life-threatening condition of pregnancy in which there is a pathologic adherence of the placenta to myometrium.¹ The prevalence of PAS has continued to increase, from an estimated 0.03% in the 1990s to recent estimates of 0.01 to 1.1%.² This marked rise has been attributed to the increasing prevalence of cesarean delivery.³ Degrees of severity of PAS have also been described, from placenta accreta (placental trophoblast adherence to the myometrium) to placenta increta (trophoblast attachment into the myometrium) and placenta percreta (trophoblast through the myometrium), with the last two, which confer the most severe maternal morbidity, often referred to as severe PAS.^{4,5}

Although multiple risk factors for PAS have been described, the mechanism behind its development has yet to be elucidated. Proposed mechanisms have primarily included abhorrent trophoblast invasion into the myometrium, implantation in the uterine scar or niche, a deficiency or dehiscence of decidua enabling placental implantation more directly onto the myometrium, or a combination of these.^{5–7}

Ultrasonographic evaluation is an important step in detecting PAS, yet approximately one-half of cases go undetected before delivery.⁸ Antenatal detection is critically important to ensure that patients deliver in adequately resourced environments that include a multidisciplinary team with appropriate experience in deep pelvic surgery, resources such as interventional radiology, and availability of a large volume of blood products.^{9–11} Given this, the identification of antenatal biomarkers for early detection has the potential to appreciably improve patient outcomes.

Previous studies have investigated first-trimester screening analytes, proteomics, and genomics as possible biomarkers, but none have resulted in clinically relevant detection tools. First-trimester analytes of interest have included pregnancy-associated plasma protein A, maternal serum α -fetoprotein, and β -human chorionic gonadotropin.^{12–14} Previously described pathways of interest using proteomics and genomics include factors related to cell growth, angiogenesis, and invasion among others.^{15–23}

More recently, researchers have investigated biomarker discovery using metabolomics and lipidomics, which comprise a comprehensive set of both signaling and structural small molecules that reflect the final downstream products of gene transcription. As a result of the sensitivity of the technology to changes in external stimuli, metabolomics investigates the link between biochemical mechanisms and molecular phenotypes associated with clinical outcomes.²⁴ It is important to note that metabolomics allows the inves-

tigation of multiple molecules simultaneously, potentially leading to the identification of a “panel” of markers with the potential to be more sensitive and specific for clinical application.²⁵ Metabolomics has been investigated in various pregnancy conditions, including gestational diabetes, preterm birth, preeclampsia, and fetal growth restriction.^{26–29} Metabolic profiling has not previously been applied to the study of placenta accreta.

Untargeted metabolomic and targeted lipidomic assays typically generate high-dimensional, high-content data characterized by the number of measurements being larger than the size of the cohort. Such data necessitate the use of machine learning methods to develop prediction models and to identify the most predictive biomarkers. Machine learning has also been used to improve the detection of diseases with clinical characteristics, including obstetric conditions such as preeclampsia, preterm birth, and adverse perinatal outcomes.^{30–32}

High-throughput omics measurements produce a large number of measured features, typically measured on a much smaller number of samples. In our data, these include metabolomic and lipidomics features. Such high-dimensional data in which the number of features is much larger than the number of samples cannot be efficiently analyzed with univariate analysis because of insufficient statistical power; it does not take into account the correlation structure of the data and cannot capture complex interaction in the data. Instead, high-dimensional data can be efficiently analyzed with sparsity-promoting machine learning methods that can perform multivariate analysis and identify the most informative features to be included in more easily interpreted and computable prediction models for disease.³³

In the present study, we aimed to identify metabolomic and lipidomic species that are differentially expressed in plasma samples of patients with severe PAS (placenta increta and placenta percreta) compared with a control group with placenta previa. This study had two primary objectives: to identify molecular features to build a possible clinical classifier and to explore the underlying biology to gain insight into possible novel pathophysiology. Using these data and prospectively collected patient clinical characteristics, we then used machine learning to predict PAS.

METHODS

Maternal plasma samples were selected from an established tissue bank from Stanford University (IRB protocol identifier 18683) and the University of Utah (IRB protocol identifier 00069530). Samples



were collected between 2010 and 2020. Inclusion criteria for the study were pregnant patients aged 18 years or older, ultrasonographic findings of placenta previa without evidence of PAS on ultrasonography or placental pathology (control group), and, for the accreta samples, placenta previa with pathologic confirmation of either placenta increta or percreta (case group). Available control (previa without PAS) samples were limited compared with case samples in the biobanks at both institutions, and samples that were collected at earlier gestational ages from patients who had received betamethasone were prioritized to better match the characteristics of patients with PAS. To minimize any batch effect between institutions in addition to using a shared protocol for sample collection, we balanced the number of patients included in the case group from each of the two institutions, and the two cohorts of patients with PAS were matched as closely as possible on factors such as degree of PAS invasion, betamethasone administration, and gestational age on the basis of biobank availability. Race was included in our demographic data collection and analysis given the exploratory nature of the study to evaluate whether it contributes meaningful information to our prediction models and in the event that it is found to be relevant in future studies. We acknowledge that race is a social construct that may reflect other outcomes attributable to structural and social factors rather than inherent racial differences. Intravenous blood samples were collected during the third trimester with EDTA-coated tubes, and plasma was prepared by centrifugation at 1,200g for 10 minutes at 4°C. The plasma was then removed and placed into four Eppendorf tubes, spun for an additional 10 minutes at 1,200g at 4°C with a microcentrifuge, and transferred into cryovials. The aliquots were then stored at −80°C.

Comprehensive profiling of metabolites and complex lipids was performed on 54 maternal plasma samples (33 patients in the case group with PAS, 21 patients in the control group with previa). Metabolites and lipids were extracted from 40 microliters plasma using a biphasic separation protocol with ice-cold methanol (260 microliters), methyl tert-butyl ether (1,000 microliters), and water (250 microliters).³⁴ After centrifugation, the upper phase containing lipids and lower phase containing metabolites were collected. Lipids were dried down under nitrogen, reconstituted with 200 microliters methanol, and stored at −20°C. On the day of the experiment, lipids were dried down under nitrogen and reconstituted with 300 microliters of 10 mM ammonium acetate in 9:1 methanol:toluene. Proteins were further precipitated from

the metabolite phase with the addition of 500 microliters ice-cold methanol:acetone:acetonitrile (1:1:1 vol/vol) and overnight incubation at −20°C. Metabolites were then dried down and reconstituted with 100 microliters methanol:water (1:1, vol/vol). For quality control, three reference plasma samples (NIST SRM 1950, 40 microliters plasma) and controls lacking samples (blanks) were processed in parallel.

Metabolite extracts were analyzed with a broad-spectrum untargeted liquid chromatography mass spectrometry platform³⁵; complex lipids were quantified with a targeted mass spectrometry-based approach.³⁶

- 1) Liquid chromatography mass spectrometry. Metabolic extracts were analyzed four times with hydrophilic interaction and reverse-phase liquid chromatography separation in both positive and negative ionization modes. Data were acquired on a Thermo Q Exactive HF mass spectrometer for hydrophilic interaction and a Thermo Q Exactive mass spectrometer for reverse-phase liquid chromatography.
- 2) Targeted lipidomics using the Lipidyzer platform. A targeted lipidomic approach was used given that it has previously been demonstrated to provide comprehensive profiling of the most prevalent lipids in plasma (with rates similar to an untargeted approach) with the Lipidyzer platform, as described by Contrepois et al³⁶ in 2018. Lipid extracts were analyzed with the Lipidyzer platform that comprises a 5500 QTRAP system equipped with a SelexION differential mobility spectrometry interface (SCIEX) and a high-flow LC-30AD solvent delivery unit. Lipid molecular species were identified and quantified with multiple reaction monitoring and positive-negative ionization switching.

Data from each mode were independently analyzed with Progenesis QI software 2.3. Metabolic features from blanks and those that did not show sufficient linearity on dilution in quality control samples ($r < 0.6$) were discarded. Batch effect was corrected by application of median normalization, and only metabolic features present in more than two-thirds of the samples were kept for further analysis, as described by Contrepois et al³⁴ in 2021. Missing values were imputed by drawing from a random distribution of low values in the corresponding sample. Intensity drift was corrected with locally estimated scatterplot smoothing.³⁷ Data from each mode were merged, and metabolites were formally identified by matching fragmentation spectra and retention time to



analytic-grade standards when possible or matching experimental mass spectrometry–mass spectrometry to fragmentation spectra in publicly available databases. We used the Metabolomics Standards Initiative level of confidence to grade metabolite annotation confidence (levels 1–4, Appendix 1, available online at <http://links.lww.com/AOG/E77>).³⁴

Lipidizer data were reported by the Lipidomics Workflow Manager 1.0.5.0 software, which calculates concentrations for each detected lipid as average intensity of the analyte multiple reaction monitoring/average intensity of the most structurally similar internal standard multiple reaction monitoring multiplied by its concentration. Lipids detected in fewer than two-thirds of the samples were discarded, and missing values were imputed by drawing from a random distribution of low values class-wise in the corresponding sample. We detected lipid species belonging to 13 classes (eg, cholesteryl esters, ceramides, diacylglycerol, free fatty acids, hydroxyceramide, lactosylceramide, dihydroceramides, lysophosphatidylethanolamine, lysophosphatidylcholine, phosphatidylcholines, phosphatidylethanolamine, sphingomyeline, triacylglycerides), and their abundance was reported as concentrations in nanomoles per gram.

The Welch *t* test was used to calculate significance between patients in the case group with severe PAS and those in the control group with previa. Analytes with a value of $P < .05$ were considered significant. Demographic data were analyzed with Stata 17.0. Categorical variables were analyzed by the Fisher exact test. Continuous variables were analyzed by two-sided *t* test or Wilcoxon rank-sum test.

Volcano plots were generated to analyze associations between each metabolite or lipid with the outcome. For the plots, the Wilcoxon rank-sum test was used to calculate *P*-values (y-axis), and log-transformed fold change quantified the amount of change between the case and control groups (x-axis). The Benjamini–Hochberg procedure was used to adjust the *P*-values for the False Discovery Rate control.

Elastic net regression method was used for training the prediction model for metabolome, lipidome, and clinical data.³⁸ Because of the relatively small cohort size, cross-validation was applied to evaluate the prediction accuracy. Each cross-validation step was repeated 100 times. In each cross-validation step, data were randomly split into train (85%) and test (15%) sets. The machine learning model was fitted on the train data. The shrinkage parameter λ that controls the amount of penalization in the algorithm was

obtained on the training data only with the *cv.glmnet* function in the *glmnet* R package. The predictive performance was quantified with the area under the receiver operating curve (AUC). R 4.1.3 was used to perform analyses. A summary of the study design is outlined in Figure 1.

RESULTS

Characteristics of patients in the case group with severe PAS and those in the control group with previa are depicted in Table 1 and were collected prospectively at each site. Thirty-three plasma samples were collected from patients with severe PAS (17 from Stanford University and 16 from the University of Utah; 21 samples were included as controls from patients with placenta previa without PAS (three from Stanford University and 18 from the University of Utah). Gestational age differed between groups (33.4 weeks vs 35.7 weeks, $P < .01$). As expected, the number of prior cesarean deliveries, gravidity, and parity also differed between groups (Table 1). In addition, the receipt of betamethasone for fetal lung maturity also differed between groups (84.8% in the case group, 47.6% in the control group, $P < .01$). The remaining baseline characteristics were not statistically different between groups (Table 1).

Metabolomic profiling was then performed with a broad-spectrum mass spectrometry metabolomics platform; 2,605 individual features could be annotated from the in-house library and public databases (data not shown). Of the identified and annotated species, 214 metabolites were differentially expressed ($P < .05$) in control plasma samples compared with patients with severe PAS in univariate analysis (Fig. 2A). A full list of differentially expressed metabolites can be found in Appendix 1, <http://links.lww.com/AOG/E77>.

These species can be grouped into metabolic classes and pathways, and the most frequently represented clusters of species that differed between patients in the case group with PAS and those in the control group with previa were amino acid metabolism, which accounted for 33 of the 214 metabolites (15.4%), and phospholipid and sphingolipid metabolism, which accounted for 26 of the 214 metabolites (12.1%) (Fig. 2B). The “other” category, which comprised 49.5% of all species, included 16 other pathways comprising fewer than 10 metabolites each and 68 metabolite features that were unassigned to either a class or pathway. After the false discovery rate was controlled for, statistical significance was lost in all species.



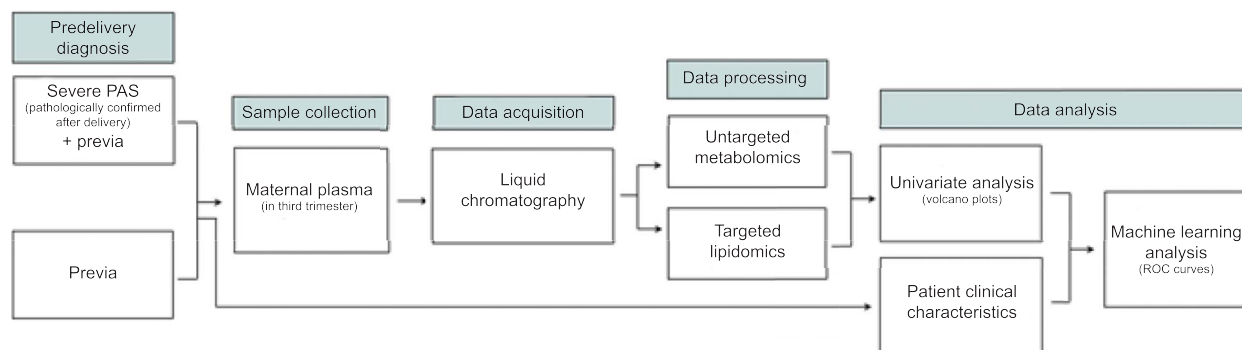


Fig. 1. Summary of the study design. PAS, placenta accreta spectrum disorder; ROC, receiver operating characteristic. Miller. Predicting PAS Through Machine Learning. *Obstet Gynecol* 2025.

Of the 786 species identified in plasma samples, 31 were found to be differentially expressed ($P < .05$) in the case group compared with the control group in univariate analysis (Fig. 3A). Of these 31 lipids, ceramides (including hexosylceramides and lactosylceramides) made up 35.3%, with cholesteryl esters being the next most frequently represented group of lipids (20.6%) (Fig. 3B). A full list of differentially expressed lipid species can be found in Appendix 2, available online at <http://links.lww.com/AOG/E78>. As in the case of the metabolome, after false discovery rate correction, no associations remained statistically significant.

Receiver operating characteristic curves were generated with the machine learning elastic net regression method to evaluate the predictability of

PAS incorporating metabolomic and lipidomic profiles and eight clinical characteristics. These included number of prior cesarean deliveries, age, race, smoking status in pregnancy, ethnicity, body mass index (BMI, calculated as weight in kilograms divided by height in meters squared), gravidity, and parity (distribution between groups listed in Table 1). The AUCs performed similarly with clinical characteristics alone (AUC 0.685, 95% CI, 0.65–0.72, Fig. 4A), the lipidome (AUC 0.699, 95% CI, 0.603–0.795, Fig. 4B), and the metabolome (AUC 0.711, 95% CI, 0.663–0.758, Fig. 4C). The performance did not improve when these three factors were combined (AUC 0.69, 95% CI, 0.62–0.79) (data not shown).

Figure 5 shows the box plots of the difference between the case and control groups for the most

Table 1. Clinical Characteristics of Patients With Placenta Accreta Spectrum and Those With Previa

Characteristic	Patients With PAS (Case Group) (n=33)	Patients With Previa (Control Group) (n=21)	P*
Gestational age at sample collection (wk)	33.4±16.5	35.7±12.7	<.01
Maternal age (y)	35.1±5.4	31.7±6.6	.05
BMI (kg/m ²)	33.2±7.1	30.8±6.2	.21
Gravidity	5 (4–5)	3 (3–4)	.03
Parity	3 (2–3)	2 (1–3)	.03
Prior cesarean deliveries	2 (2–3)	1 (0–2)	<.01
Prior uterine surgery	33.3	23.8	.5
Ethnicity (Hispanic)	42.4	47.6	.8
Race			.18
Asian	3.0	9.5	
Black	0	4.8	
Native American	9.1	0	
White	57.6	71.4	
None of the above	30.3	14.3	
Received betamethasone	84.8	47.6	<.01

PAS, placenta accreta spectrum; BMI, body mass index.

Data are mean±SD, median (interquartile range), or % unless otherwise specified.

* P-values were calculated using Wilcoxon rank-sum test (gravidity, parity, number of prior cesarean deliveries), *t* test (gestational age, maternal age, BMI), or Fisher exact test (prior uterine surgery, race, ethnicity).



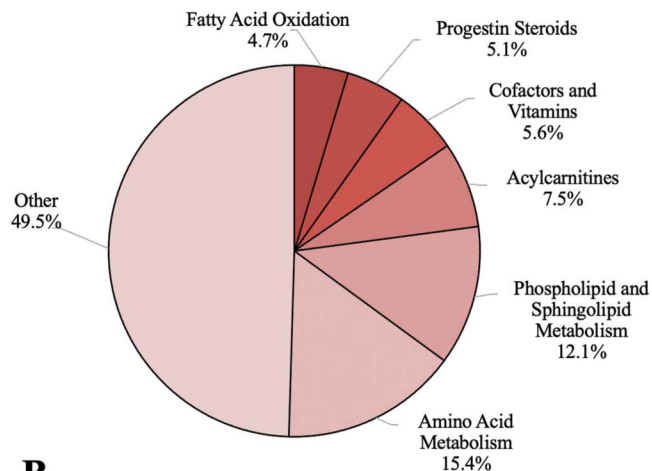
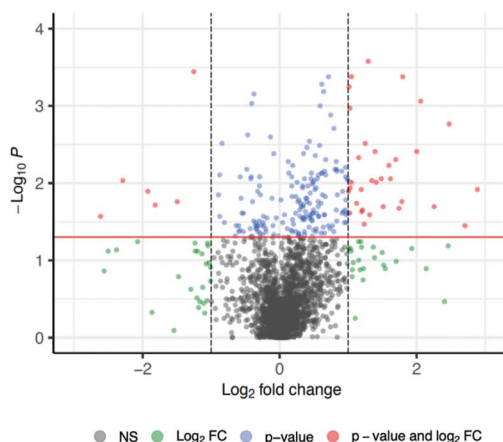


Fig. 2. A. Volcano plots of most differentially expressed individual metabolite features between patients with placenta accreta spectrum (case group) and those with previa (control group) using univariate analysis (*x*-axis, log-transformed fold change [FC] between the case and control groups; *y*-axis, *P*-value with the Wilcoxon rank-sum test). **B.** Pie chart representing associated pathway classes of differentially expressed metabolites.

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predictive clinical characteristics (Fig. 5A), lipids (Fig. 5B), and metabolites (Fig. 5C), as identified by the machine learning algorithm. The most predictive clinical characteristic was the number of prior cesarean deliveries ($P < .01$). Maternal age also correlated but was not statistically significant (Fig. 5A). The most predictive lipid features were cholesteryl esters 20.4

($P < .001$) and lactosylceramide d18.1.18.1 ($P < .05$) (Fig. 5B), and the most predictive metabolite species were androsterone sulfate ($P < .001$), C27H44O8 ($P < .001$), and pentadecatrienoic acid ($P < .01$). The most predictive features for each model are shown in Appendix 3, available online at <http://links.lww.com/AOG/E79>, which shows the frequency with

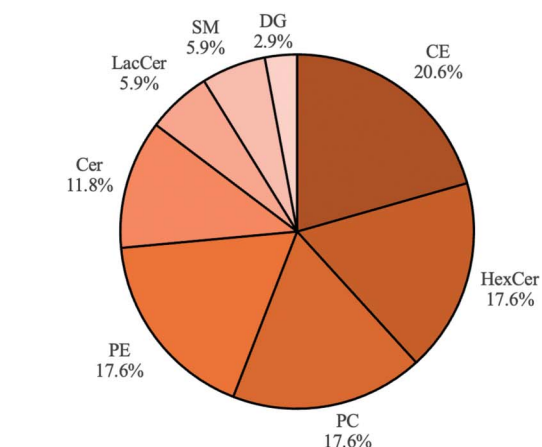
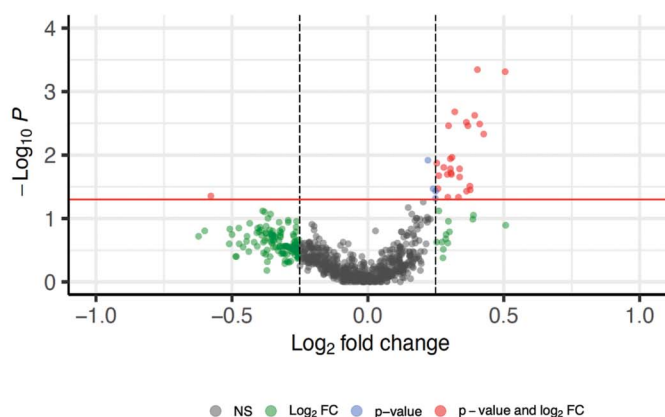
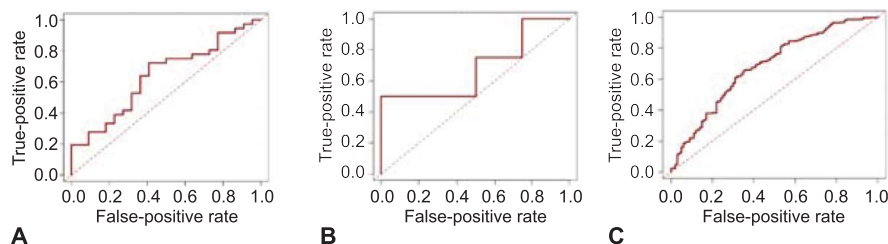


Fig. 3. A. Volcano plots of most differentially expressed individual lipid species between patients with placenta accreta spectrum (case group) and those with previa (control group) using univariate analysis (*x*-axis, log-transformed fold change [FC] between the case and control groups; *y*-axis, *P*-value with the Wilcoxon rank-sum test). **B.** Pie chart representing associated classes of differentially expressed lipids. CE, cholesteryl esters; HexCer, hexosylceramides; PC, phosphatidylcholines; PE, phosphatidylethanolamines; Cer, ceramides; LacCer, lactosylceramides; SM, sphingomyelins; DG, diglycerides.

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Fig. 4. Receiver operating characteristic curves generated with elastic net regression to predict placenta accreta spectrum disorder from clinical characteristics (area under the curve [AUC] 0.685 [95% CI, 0.652–0.718]) (A); lipid species (AUC 0.699 [95% CI, 0.603–0.795]) (B); and metabolite features (AUC 0.711 [95% CI, 0.663–0.758]) (C).



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which each of the biomarkers was chosen in the elastic net in repeated runs of the cross-validation.

DISCUSSION

Metabolomic and lipidomic profiles of patients with PAS performed comparably to the use of clinical characteristics alone when machine learning methods were used to predict PAS among patients with PAS with placenta previa compared with patients with previa alone.^{5,39} Although ultrasonography and clinical factors remain the primary means of antenatal diagnosis, many cases are undiagnosed before delivery, missing the opportunity to triage these patients to centers of excellence that have been shown to improve outcomes.^{11,40} Therefore, improving the methods of detection has been of particular research interest, including biomarker identification and machine learning models. Previous studies have elucidated the application of metabolomics to uncover molecular mechanisms and biomarkers and even to identify potential therapeutic targets in various diseases.

Biomarkers have long been explored for PAS detection with methods such as noninvasive prenatal screening, aneuploidy serum analysis, proteomics, transcriptomics, and circulating microparticles. Serum markers of interest that have been identified include pathways involved in oxidative stress, including NRF2⁴¹; proteins involved in processes including invasion, angiogenesis, and vascularization such as DLK1, vascular endothelial growth factor, and placental growth factor^{23,42–45}; or proteins identified through noninvasive prenatal screening or transcriptomic analysis, including pregnancy-associated plasma protein A, α -fetoprotein, and pregnancy-specific β -1 glycoprotein.^{13,14,46} Despite these investigations, no biomarkers are used in routine clinical practice to detect PAS.

In addition to the search for predictive serum markers, studies have applied machine learning methods to PAS ranging from antenatal prediction

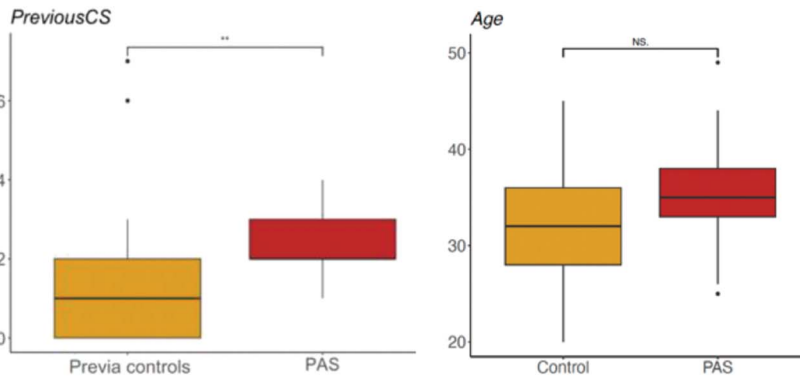
with imaging and clinical features to prediction of clinical outcomes in patients with PAS.^{47–49} However, the sensitivity of antenatal prediction models remains poor.

Although the predictive ability of PAS using metabolites, lipids, and clinical characteristics both separately and combined was modest, the present study adds to the body of literature using biomarkers and clinical characteristics in the prediction of PAS. Interestingly in our study, the predictive ability did not improve with the addition of clinical characteristics, which may suggest collinearity between the identified species and the clinical factors or that the species somehow encompass the included clinical information. The most predictive features may have mechanistic implications but also may highlight species or pathways of interest that could be investigated to improve prediction accuracy. As shown in prior translational studies, predictability of PAS may be improved, for example, at earlier gestational ages or with the addition of other clinical variables such as ultrasonography.^{44,50}

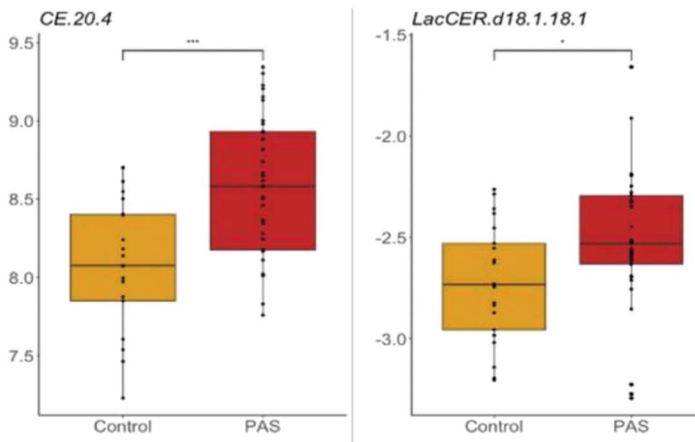
In the present study, the lipid species most upregulated in patients within the case group compared with those in the control group include ceramides, phosphatidylcholines, and cholesterol esters, although their statistical significance did not remain after controlling for the false discovery rate. Ceramides have been shown in prior studies to modulate cell migration and adhesion through their influence on membrane lipid composition.^{51–53} Moreover, ceramide biosynthesis and metabolism have been implicated in the process of trophoblast differentiation and invasion and uterine angiogenesis, which could disrupt the normal vascularity and placental adherence, and some ceramide species are downregulated in preeclampsia, which is thought to be a disease characterized by impaired angiogenesis.^{51,54,55}

Phosphatidylcholines were also upregulated in patients in the case group with PAS relative to those

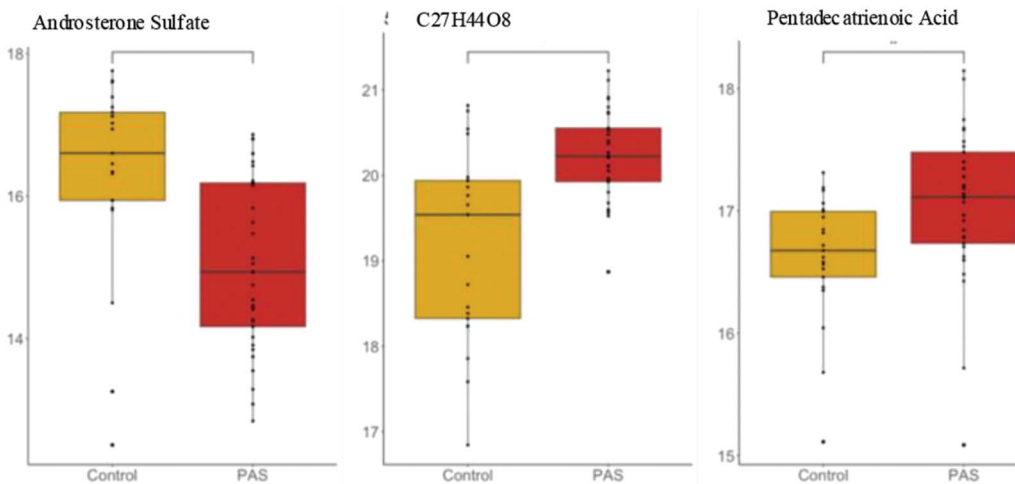




A



B



C

Fig. 5. Box plots indicating most predictive clinical characteristics (A); lipid species (B); and metabolites (C) identified with the machine learning algorithm. * $P < .05$, ** $P < .01$, *** $P < .001$, NS= $P \geq .05$. CS, cesarean section; PAS, placenta accreta spectrum disorder; CE, cholesterol esters; LacCER, lactosylceramides.

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in the control group with previa, with this species being one of the predominant phospholipids in the placenta, and are essential in supporting the rapidly expanding microvillous membrane surface area throughout gestation, allowing nutrient transfer between the fetal and maternal circulation.⁵⁶ Prior studies have suggested an increase in lipolytic enzymes that hydrolyze phosphatidylcholine and may therefore decrease fatty acid supply from the placenta to the fetus in the pathogenesis of fetal growth restriction.⁵⁷

The metabolomic pathways that contained the largest number of differentially expressed metabolites included phospholipid and sphingolipid metabolism (upregulated in PAS) and amino acid metabolism (variably upregulated or downregulated in PAS). Fourteen differentially expressed metabolites were involved in phospholipid metabolism, and 12 of them were upregulated in PAS. These findings are consistent with the lipidomic findings listed previously; phospholipid metabolism is implicated in cell membrane expansion, cell signaling related to cell survival and proliferation, inflammatory responses, and angiogenesis.⁵⁸ Sphingolipids also help to regulate cell survival, proliferation, and apoptosis in conjunction with ceramides (as mentioned).⁵² Similar to the lipid species discussed previously, after the false discovery rate was accounted for, these metabolites were no longer statistically significant between groups.

Although sparsity-promoting machine learning methods are well suited for high-dimensional data, having a small number of samples can pose challenges. Specifically, the selected features are often not stable and can change drastically with small variations in the data.⁵⁹ One way to address this is to consider frequency selection of the identified features, shown in Appendix 3, <http://links.lww.com/AOG/E79>.⁶⁰ A small number of samples can also result in overfitting of the data, which could be improved with larger cohort sizes in the future.

In addition, there were some statistically significant differences in the demographics between groups, including gestational age, gravidity and parity, number of prior uterine surgeries, and rates of betamethasone administration. Despite attempting to control for them during patient selection with the availability of biobank samples, we cannot account for all observed differences in the profiles of these characteristics. Although the patients with PAS were well balanced between the two institutions, the number of patients in the control group provided by Stanford University was limited by the availability of the biobank. It is important to acknowledge that we did not use an

independent cohort to validate the identified species, thereby hindering the external validity of the findings.

In addition, the PAS cohort was limited only to patients with placenta increta or percreta and did not include patients with lower-grade PAS to maximize the likelihood of identifying differential expression attributable to maximum disruption of the maternal-fetal interface. Future studies should include patients with lower-grade cases of PAS given that these are often the cases that go undetected before delivery. Future directions include metabolomic and lipidomic profiling on larger cohorts given that the current study may have been underpowered.

We identified potential metabolic and lipidomic pathways that might provide further information at the molecular level of PAS dysregulation. We observed that clinical characteristics continue to have high predictability in diagnosing PAS. Future studies with larger sample sizes are needed to better elucidate the differential expression of metabolic and lipidomic species in PAS.

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