Serum amyloid A1 and pregnancy zone protein in pregnancy complications and correlation with markers of placental dysfunction

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BACKGROUND: Hypertensive disorders of pregnancy (preeclampsia, gestational hypertension, and chronic hypertension), diabetes mellitus, and placental dysfunction confer an increased risk of long-term maternal cardiovascular disease. Preeclampsia is also associated with acute atherosis that involves lesions of uteroplacental spiral arteries, resembling early stages of atherosclerosis. Serum amyloid A1 is involved in hypercoagulability and atherosclerosis and may aggregate into amyloid—aggregations of misfolded proteins. Pregnancy zone protein may inhibit amyloid aggregation. Amyloid is involved in Alzheimer's disease and cardiovascular disease; it has been identified in preeclampsia, but its role in preeclampsia pathophysiology is unclear.

OBJECTIVE: We hypothesized that serum amyloid A1 would be increased and pregnancy zone protein decreased in hypertensive disorders of pregnancy and diabetic pregnancies and that serum amyloid A1 and pregnancy zone protein would correlate with placental dysfunction markers (fetal growth restriction and dysregulated angiogenic biomarkers) and acute atherosis.

STUDY DESIGN: Serum amyloid A1 is measurable in both the serum and plasma. In our study, plasma from 549 pregnancies (normotensive, euglycemic controls: 258; early-onset preeclampsia: 71; late-onset preeclampsia: 98; gestational hypertension: 30; chronic hypertension: 9; diabetes mellitus: 83) was assayed for serum amyloid A1 and pregnancy zone protein. The serum levels of angiogenic biomarkers soluble fms-like tyrosine kinase-1 and placental growth factor were available for 547 pregnancies, and the results of acute atherosis evaluation were available for 313 pregnancies. The clinical characteristics and circulating biomarkers were compared between the pregnancy groups using the Mann-Whitney *U*, chi-squared, or Fisher exact test as appropriate. Spearman's rho was calculated for assessing correlations.

RESULTS: In early-onset preeclampsia, serum amyloid A1 was increased compared with controls (17.1 vs 5.1 μg/mL, $P<.001$), whereas pregnancy zone protein was decreased (590 vs 892 μg/mL, $P=.002$). Pregnancy zone protein was also decreased in diabetes compared with controls (683 vs 892 μg/mL, $P=.01$). Serum amyloid A1 was associated with placental dysfunction (fetal growth restriction, elevated soluble fms-like tyrosine kinase-1 to placental growth factor ratio). Pregnancy zone protein correlated negatively with soluble fms-like tyrosine kinase-1 to placental growth factor ratio in all study groups. Acute atherosis was not associated with serum amyloid A1 or pregnancy zone protein.

CONCLUSION: Proteins involved in atherosclerosis, hypercoagulability, and protein misfolding are dysregulated in early-onset preeclampsia and placental dysfunction, which links them and potentially contributes to future maternal cardiovascular disease.

Key words: acute atherosis, biomarker analysis, chronic hypertension, diabetes mellitus, gestational diabetes, gestational hypertension, hypercoagulability, hypertensive disorders of pregnancy, preeclampsia, protein misfolding

Introduction

Preeclampsia is a potentially severe pregnancy complication, defined as new-onset hypertension and at least 1 other preeclampsia-associated sign of organ dysfunction (eg, proteinuria, fetal growth restriction, and elevated liver transaminases) in the second half of pregnancy.[1] Hypertensive disorders of pregnancy, including preeclampsia and other pregnancy complications associated with placental dysfunction (eg, fetal growth restriction, preterm birth, and gestational diabetes mellitus [GDM]) are associated with a 2−8-fold increased risk of future maternal cardiovascular disease.[2−4] Pregnancy can thus be regarded as a sex-specific stress test for predicting maternal risk of cardiovascular disease. Cardiovascular diseases remain a major cause of death for both men and women, but female-specific pathophysiological mechanisms have so far been understudied.[5]

The maternal features of preeclampsia result from excessive systemic vascular inflammation secondary to increased shedding of proinflammatory factors from a malperfused and dysfunctional placenta.[6,7] Placental malperfusion leads to oxidative and endoplasmic reticulum stress, eliciting the release of proinflammatory and antiangiogenic factors.[8] Placental dysfunction in early-onset preeclampsia is associated with insufficient physiological spiral artery transformation.[6,9] Both early- and late-onset preeclampsia are associated with excessive placental senescence and high rates of uteroplacental acute atherosis.[6,10,11] Acute atherosis is a spiral artery wall lesion with some morphologic similarities to the early stages of atherosclerosis.[12,13] It is characterized by subintimal foam cells, artery wall necrosis, and inflammation and is associated with thrombosis and a risk of downstream placental infarcts.[14]

Circulating proangiogenic placental growth factor (PlGF) and antiangiogenic soluble fms-like tyrosine kinase-1

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AJOG MFM at a Glance

Why was this study conducted?

Preeclampsia and placental dysfunction place women at risk of future cardiovascular disease, but the mechanisms are largely unknown. This study aimed at assessing biomarkers that may contribute to this risk.

Key findings

Circulating serum amyloid A1 and pregnancy zone protein—markers of protein misfolding— are dysregulated in early-onset preeclampsia, and their levels correlate with antiangiogenic biomarkers and fetal growth restriction, which are proxies for placental dysfunction.

What does this add to what is known?

Our finding of a correlation between protein misfolding markers and proxies for placental dysfunction is novel. Markers of protein misfolding are implicated in early-onset preeclampsia and placental dysfunction and may provide a pathway linking these pregnancy complications to the epidemiologic increased risk of maternal cardiovascular disease.

(sFlt-1) are mainly of placental origin during pregnancy and are dysregulated in preeclampsia and other placental dysfunction syndromes such as fetal growth restriction.[15,16] Consequently, we have argued that dysregulated angiogenic biomarkers (eg, low PlGF, high sFlt-1/PlGF ratio) may be utilized as proxy markers for placental dysfunction and syncytiotrophoblast stress.[7]

Amyloid consists of fibrous aggregates of misfolded proteins, has slow spontaneous dissociation,[17] and is implicated in several chronic, progressive diseases, including Alzheimer's disease, type 2 diabetes mellitus (DM), and atherosclerosis.[18,19] Amyloid also accumulates in the urine and placenta of women with preeclampsia.[20,21] Circulating amyloid precursors transthyretin and amyloid precursor protein are dysregulated in preeclampsia.[20–22] Urine testing for amyloid has been suggested as a test for preeclampsia severity.[23] Serum amyloid A1 (SAA1) is an acute-phase protein mainly produced in the liver and is one of many proteins able to aggregate into amyloid.[17] Placental expression of SAA1 may play a role in initiating parturition, though its expression throughout pregnancy is uncertain.[24] SAA1 is also implicated in hypercoagulability by promoting amyloid formation in fibrin(ogen) and driving platelets into a prothrombotic state.[25]

Pregnancy zone protein (PZP) is a plasma protein that is highly upregulated during pregnancy,[26] and it may play a progestational role through immunomodulation.[27] Further, low circulating levels of the protein have been linked to pregnancy loss.[28] In vitro, PZP stabilizes and inhibits misfolded amyloid-β from aggregating into amyloid fibrils.[29] Whether PZP inhibits SAA1 aggregation into amyloid-A—the amyloid type derived from SAA1—is unknown.

Circulating SAA1 has been shown to be increased in GDM[30] and has also been studied in preeclampsia, though results from these predominantly small studies are conflicting.[31–36] Derived PZP levels were decreased in women with preeclampsia in 2 small studies.[37,38] These previous SAA1 and PZP studies from pregnancy have not assessed biomarker concentrations in relation to placental dysfunction.

As SAA1 and PZP have opposite roles in augmenting and attenuating amyloid aggregation, we hypothesized that circulating SAA1 is increased and PZP is decreased in preeclampsia, particularly in early-onset disease and in pregnancies complicated by diabetes. We further hypothesized that SAA1 and PZP levels correlate with proxies for placental dysfunction (eg, dysregulated angiogenic biomarkers, fetal growth restriction, or acute atherosis) as an indication of amyloid deposition in placental dysfunction.

Materials and Methods

Patient recruitment and sample collection

Data and biological samples from 549 singleton pregnant women recruited between 2001 and 2017 to the Oslo Pregnancy Biobank at Oslo University Hospital were included in this study. All had extensive clinical data from pregnancy and delivery and blood samples available for biomarker analyses. All women gave informed written consent. The study was approved by the Regional committee for Medical and Health Research Ethics in South-Eastern Norway and conducted in accordance with the principles of the Helsinki Declaration.

Hypertensive disorders of pregnancy (HDP) were defined according to the 2018 Guidelines of the International Society for the Study of Hypertension in Pregnancy.[1] Gestational hypertension (GH) was defined as new-onset hypertension (blood pressure ≥140 mm Hg systolic and/or ≥90 mm Hg diastolic on ≥2 occasions ≥6 hours apart) at ≥20 weeks' gestation. Preeclampsia was defined as GH accompanied by ≥1 other new-onset sign(s) of maternal organ dysfunction[1] (eg, proteinuria, acute kidney injury, liver involvement, eclampsia, hemolysis, and fetal growth restriction) at ≥20 weeks' gestation. Early-onset preeclampsia (EO-PE) and late-onset preeclampsia (LO-PE) were defined as preeclampsia delivered <34 vs ≥34 weeks' gestation, respectively.[6] Chronic hypertension (CHT) was defined as hypertension occurring prepregnancy or diagnosed <20 weeks' gestation. Superimposed preeclampsia was defined as CHT and ≥1 new-onset preeclampsia-associated feature(s) at ≥20 weeks' gestation. DM diagnoses (type 1 DM [DM1], type 2 DM [DM2], gestational DM [GDM]) were based on diagnoses identified in the clinical setting by endocrinologists according to current guidelines.[39,40] Women with both an HDP and DM diagnosis were classified as HDP+DM. Controls were women who remained normotensive and euglycemic throughout pregnancy.

None of the included women had regular uterine contractions, ruptured

fetal membranes, or signs of infection at the time of blood sampling. None had known chronic diseases apart from women with CHT (n=25), pregestational DM (n=79), or hypothyroidism (n=33). No controls had CHT or DM. Gestational age at inclusion was determined by routine ultrasound screening at gestational week 17 to 20, except in 25 women, where gestational age was determined by embryo transfer date (in vitro fertilization) and in 7 women by first day of the last menstrual period before pregnancy. Sex-specific newborn weight percentiles were calculated according to Norwegian fetal growth curves.[41] Fetal growth restriction was defined as sex-specific newborn weight ≤third percentile. No newborns had structural or apparent chromosomal abnormalities.

The presence or absence of uteroplacental acute atherosis was assessed in decidua basalis vacuum suction biopsies collected after delivery of the placenta and evaluated as described previously.[42] Acute atherosis was defined as spiral arteries with ≥2 adjacent CD68-positive vacuolated subintimal cells.[43]

For most women, blood samples were obtained immediately before elective cesarean delivery and centrifuged within 120 minutes of sampling at 4°C for 10 minutes at 1800 g. Ethylenediaminetetraacetic acid (EDTA) plasma was stored at −80°C until immunoassay. For some of the included women (n=81), a blood sample was collected 1 −31 days before delivery instead of at delivery. Blood was sampled from an antecubital vein or intravenous cannula without ongoing infusion or from an arterial catheter (n=20). Maternal serum samples were also collected at the same time point as EDTA plasma as described previously.[44]

Biochemistry and immunoassays

The SAA1 and PZP concentrations were measured by enzyme-linked immunosorbent assay (ELISA) in maternal EDTA plasma in duplicates. The mean value of the duplicates was used for analysis. All reagents were obtained from R&D Systems (Minneapolis, MN) catalog numbers DY3019-05 (SAA1) and DY8280-05 (PZP). Assays were performed in accordance with the manufacturer's instructions. Optical density was determined at 450 nm and corrected at 540 nm. The coefficients of variation were 6.4% for SAA1 and 2.4% for PZP.

Serum levels of sFlt-1 and PlGF were determined for 547 pregnancies as previously described[44] using Elecsys immunoassays (Roche Diagnostics, Switzerland) with a fully automated electrochemiluminescence immunoassay platform (Cobas E analyzer, Roche Diagnostics).

Serum levels of acute-phase reactant high-sensitivity C-reactive protein (hsCRP) in either fresh (n=219) or thawed (n=161) samples were analyzed as described previously[45] using a particle-enhanced turbidimetric method (Cobas 8000 c702; Roche Diagnostics, Rotkreuz, Switzerland). hsCRP was included in this biomarker study owing to its known association to cardiovascular diseases[46] and preeclampsia.[47] CRP correlates with SAA outside pregnancy,[48] whereas a correlation in pregnancy has been less studied.[33]

Statistics

The data were analyzed using SPSS Statistics 26.0 (IBM). The Mann-Whitney *U* test was used for continuous variables. The chi-squared or Fisher exact test was used for categorical variables, as appropriate. Spearman rho (r_S) was calculated for analyzing correlations between continuous variables, as data were skewed. We did not correct for multiple comparisons. A *P* value <.05 was considered significant.

Results

Table lists the clinical characteristics of the main pregnancy groups (controls, n=258; HDP, n=163; DM, n=83; HDP +DM, n=45), acute atherosis rate, and circulating biomarker concentrations. Prepregnancy body mass index (BMI), mean blood pressures in the first half of pregnancy and at inclusion, rates of acute atherosis, hsCRP, and sFlt-1/PlGF ratio were increased, and gestational age and newborn weight percentiles were lower in HDP and HDP+DM than in controls. Supplemental Tables 1 and 2 list the number of women in each HDP and DM subgroup, respectively, and their clinical characteristics.

There were no significant differences in SAA1 or PZP levels or clinical characteristics when comparing the group with samples collected 1 to 31 days before delivery with the group with samples collected at cesarean delivery (data not shown).

Serum amyloid A1 and pregnancy zone protein concentrations in hypertensive and diabetic pregnancies

As shown in Table 1, the plasma SAA1 levels were markedly higher in HDP than in controls (normotensive and euglycemic, *P*<.001), whereas SAA1 did not differ between DM or HDP+DM compared with controls. Of the HDP subgroups, the EO-PE group had increased levels of SAA1 than the controls (*P*<.001, Supplemental Table 1) and than all remaining subgroups of HDP (Figure 1, A). SAA1 did not differ between any DM subgroup and controls (normotensive and euglycemic, Supplemental Table 2).

The PZP concentration was lower in both DM and in HDP+DM than in controls (both *P*=.01). In contrast, PZP was similar in HDP and controls. The levels of PZP were decreased in EO-PE than in controls (*P*=.002, Supplemental Table 1) and CHT (Figure 1, B). PZP was decreased in women with DM1 and DM2 than in controls (*P*=.02 and.003, respectively, Supplemental Table 2).

Correlation between serum amyloid A1 and glycated hemoglobin in diabetic pregnancies

In DM1, SAA1 (at sampling in late pregnancy) correlated negatively with glycated hemoglobin (HbA1c) measured in the first (r_S=−0.43; *P*=.002), second (r_S=−0.27; *P*=.04), and third (r_S=−0.33; *P*=.01) trimesters. First trimester HbA1c ≥53 mmol/mol was associated with lower SAA1 (2.9 vs 5.0 μg/mL; *P*=.03). PZP did not correlate with HbA1c (data not shown).

TABLE 1
Clinical characteristics and circulating biomarkers by pregnancy inclusion groups (total cohort, n=549)

Variable	Controls (n=258)	HDP (n=163)	*P* value	DM (n=83)	*P* value	HDP+DM (n=45)	*P* value
Maternal age at inclusion (y)	33.5 (20.1–45.5)	33.1 (19.0–52.9)	.25	33.4 (24.1–45.9)	.64	33.0 (21.1–44.0)	.22
Prepregnancy body mass index (kg/m^2)	22.4 (17.6–39.5)	24.0 (18.3–41.1)	<.001	24.5 (19.1–39.4)	<.001	27.3 (19.6–41.1)	<.001
Mean SBP <20 wk (mm Hg)	110 (85–140)	120 (90–158)	<.001	114 (92–138)	<.001	120 (95–164)	<.001
Mean DBP <20 wk (mm Hg)	68 (40–88)	75 (55–103)	<.001	69 (50–84)	.21	73 (50–90)	<.001
First pregnancy	24%	45%	<.001	22%	.61	42%	.01
First delivery	39%	62%	<.001	41%	.77	53%	.07
Previous preeclampsia, n (%)[a]	12 (8%)	26 (43%)	<.001	7 (14%)	.17	9 (43%)	<.001
SBP at inclusion (mm Hg)	121 (86–156)	151 (117–220)	<.001	120 (90–155)	.77	150 (120–189)	<.001
DBP at inclusion (mm Hg)	75 (48–110)	95 (63–130)	<.001	74 (50–90)	.94	92 (66–110)	<.001
Gestational age at inclusion (wk)	39.0 (33.7–42.1)	34.4 (24.3–41.1)	<.001	38.0 (28.7–40.4)	<.001	36.9 (29.0–40.0)	<.001
Preterm delivery (<37 wk gestation)	3%	65%	<.001	10%	.01	40%	<.001
Early-onset preeclampsia (delivery <34 wk gestation)	—	48%		—		16%	
Newborn weight (g)	3483 (1325–5130)	2087 (500–5040)	<.001	3802 (988–5857)	<.001	3574 (620–5424)	.70
Newborn weight percentile	62.6 (0–100)	5.0 (0–99.9)	<.001	86.1 (0–100)	<.001	90.9 (0–100)	.01
Fetal growth restriction	2%	45%	<.001	5%	.10	4%	.22
Acute atherosis[b]	9% (14/160)	39% (37/96)	<.001	7% (2/31)	1.00	27% (7/26)	.01
Serum-sFlt-1 (pg/mL)[c]	3683 (1081–17,185)	11,204 (1429–61,348)	<.001	4629 (1260–18,857)	.003	7597 (2003–21,430)	<.001
Serum-PlGF (pg/mL)[c]	172 (23–2074)	58 (6–624)	<.001	166 (35–1027)	.82	104 (29–1852)	<.001
sFlt-1/PlGF ratio[c]	22.3 (1.1–615.1)	208.6 (2.3–3264.3)	<.001	24.9 (2.7–209.0)	.24	78.9 (1.4–470.8)	<.001
Serum-hsCRP (mg/L) (ref.: 0.0–4.0)[d]	3.1 (0.6–76.6), n=201	4.4 (0.6–107.0), n=117	.002	3.9 (0.6–32.7), n=36	.26	4.8 (0.8–15.9), n=26	.04
Plasma-SAA1 (μg/mL)	5.08 (0.41–120)	9.90 (0.80–120)	<.001	4.06 (0.37–120)	.06	6.07 (0.22–115.95)	.22
Plasma-PZP (μg/mL)	892 (32–3240)	818 (12–2992)	.15	683 (40–3406)	.01	548 (25–3280)	.01

For continuous variables, the median value and range are shown; for categorical variables, percentages are shown. Statistical differences were calculated by comparing each pregnancy group with the control group (normotensive and euglycemic pregnant women).

DBP, diastolic blood pressure; *DM*, diabetes mellitus (pregestational or gestational); *HDP*, hypertensive disorders of pregnancy; *HDP+DM*, women with both HDP and DM; *hsCRP*, high-sensitivity C-reactive protein; *PlGF*, placental growth factor; *PZP*, pregnancy zone protein; *SAA1*, serum amyloid A1; *SBP*, systolic blood pressure; *sFlt-1*, soluble fms-like tyrosine kinase-1.

[a]Nulliparous women were excluded when analyzing the rate of previous preeclampsia

[b]Decidua basalis acute atherosis was assessed in 313 women (all had spiral arteries present)

[c]sFlt-1 and PlGF concentrations were available for all except 2 women with HDP

[d]hsCRP concentration was available for 380 women.

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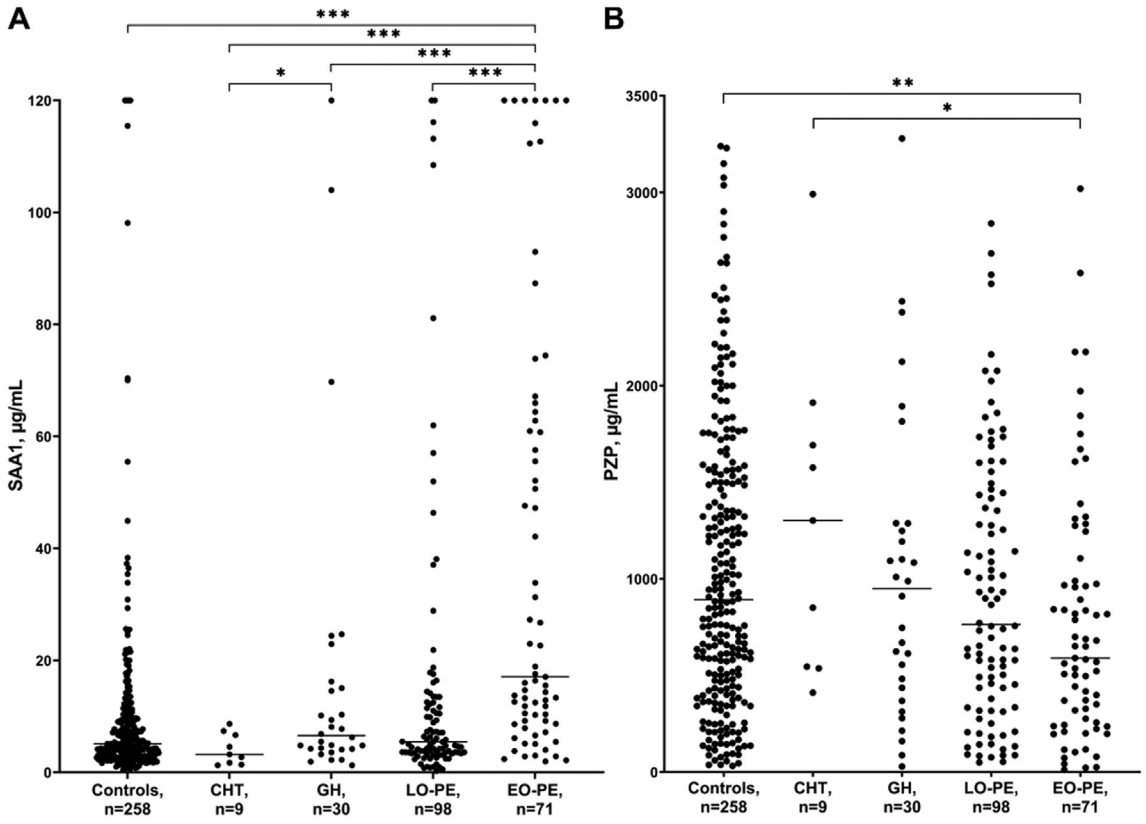
Serum amyloid A1 and pregnancy zone protein concentrations and their relationship with proxies for placental dysfunction

We compared SAA1 and PZP levels with proxies for placental dysfunction. Women with acute atherosis did not differ in SAA1 or PZP levels compared with women without acute atherosis within any of the pregnancy groups (Supplemental Table 3). However, increased SAA1 levels were seen in pregnancies with fetal growth restriction, and they correlated positively with the antiangiogenic sFlt-1/PlGF ratio in the total cohort (13.3 vs 5.1 μg/mL, r_S=0.24; both P<.001) and in HDP (13.7 vs 6.1 μg/mL, r_S=0.40; both P<.001). PZP was not associated with fetal growth restriction but correlated negatively with sFlt-1/PlGF in the total cohort (r_S=−0.13, P=.002), HDP (r_S=−0.19; P=.015), and HDP+DM (r_S=−0.30; P=.049).

Correlations between serum amyloid A1, pregnancy zone protein, and high-sensitivity C-reactive protein

Finally, we compared SAA1 and PZP with the general inflammation marker hsCRP. SAA1 correlated positively with hsCRP in the total cohort (r_S=0.46; P<.001) and in all pregnancy groups:

FIGURE 1
Scatter plots of circulating biomarkers



Concentrations of circulating biomarkers by subgroup of hypertensive disorder of pregnancy (irrespective of diabetes mellitus status) and controls (normotensive and euglycemic) (n=466). **A,** serum amyloid A1 (SAA1) concentrations; **B,** PZP concentrations. The Mann-Whitney *U* test was used to calculate statistical differences between groups. Line at median. Triple asterisks denote *P*<.001. *Double asterisks* denote *P*<.01. *Single asterisk* denotes *P*<.05

CHT, chronic hypertension; *EO-PE*, early-onset preeclampsia (delivery <34 weeks gestation); *GH*, gestational hypertension; *LO-PE*, late-onset preeclampsia (delivery ≥34 weeks gestation); *PZP*, pregnancy zone protein.

Fosheim. Markers of protein misfolding in pregnancy complications. Am J Obstet Gynecol MFM 2022.

controls (r_S=0.35; *P*<.001), HDP (r_S=0.58; *P*<.001), DM (r_S=0.35; *P*=.04), and HDP+DM (r_S=0.62; *P*=.001). Moreover, SAA1 was increased in women with hsCRP above an upper reference limit of 4 mg/L (9.4 vs 4.3 µg/mL; *P*<.001). We found no correlation between PZP and hsCRP (data not shown). We found no correlation between SAA1 and PZP for the total cohort or for any pregnancy groups except for HDP+DM (r_S=-0.37; *P*=.01).

SAA1 and PZP concentrations did not consistently correlate with prepregnancy BMI, early pregnancy blood pressure, gestational age at sampling, or fetal sex in the total cohort or in any subgroups; the few exceptions are listed in Supplemental Results.

Discussion

Principal findings

In line with our hypothesis, we found increased SAA1 and decreased PZP in pregnancies complicated by EO-PE. SAA1 and PZP correlated significantly with signs of placental dysfunction, as determined by fetal growth restriction and an antiangiogenic biomarker pattern. In contrast to our hypothesis, we did not find an association between acute atherosis and SAA1 or PZP.

Results

Our finding of increased circulating SAA1 in EO-PE may indicate the presence of amyloid-A in these women. Amyloid-A deposits are typically found in chronic inflammatory diseases,[49] as prolonged inflammation (demonstrated by CRP elevation) causing persistently elevated SAA1 may lead to amyloid-A deposits.[17] Other types of amyloid (amyloid-β and transthyretin) have been identified by immunohistochemistry in placentae from preeclamptic pregnancies.[20,21] Whether amyloid-A is deposited in the placenta in response to increased SAA1 is unknown. We

speculate that this is the case and that placental amyloid-A might exacerbate existing placental dysfunction, particularly for women with EO-PE, by disturbing cell-cell interactions and tissue structure.[17]

Furthermore, our findings support our hypothesis that poor placentation and placental dysfunction, as evidenced by an antiangiogenic shift in the sFlt-1/PlGF ratio and fetal growth restriction, correlates with increasing SAA1 and decreasing PZP. PZP has been shown in vitro to form stable compounds with amyloid-β-peptide, thus inhibiting amyloid-β aggregation.[29] We hypothesize that PZP may similarly inhibit SAA1 misfolding. Although we did not explore any mechanistic interaction between them in this study, we hypothesize that low PZP in the setting of increased SAA1 may facilitate the formation of amyloid-A and other forms of amyloid associated with preeclampsia, such as amyloid-β and transthyretin.

Previous studies have found increased circulating levels of acute-phase proteins in preeclampsia,[33,36,47] in line with our findings of increased hsCRP in EO-PE (Supplemental Table 1). Also in line with previous observations,[33] SAA1 correlated positively with hsCRP levels in our cohort, confirming a role for excessive inflammation in SAA1 dysregulation.

Preeclampsia is characterized by hypercoagulability and a risk of thromboembolism.[1,50] SAA1 increases the thrombotic ability of platelets,[25] and as SAA1 is increased in EO-PE, we speculate that SAA1 participates in mediating hypercoagulability in EO-PE. Further studies are needed to explore this hypothesis.

Unexpectedly, SAA1 correlated negatively with HbA1c—a marker of blood glucose control—in women with DM1. Poor glucose control is associated with other inflammatory markers such as hsCRP and with increased cardiovascular risk.[30,51,52] A previous study found a positive correlation between SAA and HbA1c in women with GDM.[30] Assays detecting SAA measure both SAA1 and SAA2—nearly identical SAA isoforms. Hence, we expected a positive correlation between SAA1 and HbA1c in our DM1 group also. BMI likely did not confound our finding, as it was not associated with SAA1 in DM1 or other study groups apart from GDM (Supplemental Results). As seen from Supplemental Table 2, the median prepregnancy BMI of women with DM1 was within the normal range (24.2 kg/m^2). To the best of our knowledge, we are among the first to address SAA1 and glycemic control in DM1 during pregnancy. The negative correlation between SAA1 and HbA1c in DM1 may reflect the complex regulation of inflammation in pregnancy and merits further investigation.

Clinical and research implications

Low circulating PZP levels have been linked to pregnancy loss.[28] Women with pregestational DM and those who develop EO-PE may harbor an unfavorable preconception endometrial environment, resulting in inadequate placentation and clinical manifestations of preeclampsia during pregnancy.[53] We suggest that low circulating PZP levels in the second half of pregnancy may reflect such placentation issues and that low PZP may identify women with particularly poor placentation and subsequent high risk of early-onset placental dysfunction.

Amyloid has been identified in atherosclerotic plaques.[54] SAA1 and SAA2—nearly identical isoforms—may play a role in atherosclerosis, both locally and systemically. In a murine atherosclerosis model, SAA expression was increased in both early and late atherosclerosis lesions.[55] SAA possibly acts as a chemoattractant for leukocytes in lesions.[56] Increased circulating SAA levels have been associated with recurrent coronary events and stroke, as have elevated levels of hsCRP.[46] In a mouse model, even a short period of elevated SAA1 increased atherosclerosis lesion size.[57] We speculate that elevated SAA1 in women with preeclampsia may promote accelerated atherosclerosis progression even if the SAA1 elevation were temporary and restricted to some months during pregnancy. Although acute atherosis and early atherosclerosis share morphologic features,[58] we found no association between SAA1 and acute atherosis. This may be owing to different mechanisms for the development of acute atherosis, affecting small-caliber spiral arteries, and atherosclerosis, affecting larger arteries and also owing to large time differences for their development.

Further studies are required to elucidate protein-protein interactions between SAA1 and PZP, their possible role in placental (dys)function, and in turn, their impact on circulating angiogenic proteins. Future longitudinal pregnancy studies may reveal whether circulating SAA1 and PZP could serve as predictors for placental dysfunction. Placenta tissue studies may reveal whether increased circulating SAA1, as in EO-PE, leads to placental amyloid-A deposits. Identifying predictors of future maternal cardiovascular health during pregnancy merits further investigation. Longitudinal studies are needed to identify whether SAA1 shows prolonged elevation following preeclampsia as hsCRP does,[59,60] placing these women at risk of postpartum amyloid-A deposition and subsequent organ damage.

Strengths and limitations

This study is the first to examine circulating SAA1 and PZP—potential markers of protein misfolding—in phenotypically well-characterized subgroups of women with hypertensive disorders of pregnancy and diabetes mellitus in relation to different proxies for placental function and uteroplacental acute atherosis. The population size (549 pregnancies) and well-described pregnancy groups are strengths of our study, which establishes that circulating SAA1 is increased in EO-PE and placental dysfunction. The distinction between EO-PE and LO-PE as shown in our study (delivery <34 or ≥34 weeks' gestation, respectively) could explain why some previous studies report increased SAA1 in preeclampsia[31,33,36] whereas others do not,[32,34,35] as none have made a similar differentiation. One study quantified circulating SAA1, sFlt-1, and PlGF in preeclampsia but

did not study correlations between SAA1 and sFlt-1/PlGF.[34]

PZP has been indirectly studied (by immunodiffusion gel) in 2 smaller studies of preeclampsia[37,38] and directly studied (by ELISA) in 1 very small study where plasma from 9 women with preeclampsia was pooled before assay.[29] We are, to the best of our knowledge, among the first to study circulating levels of PZP by ELISA in individual pregnancy samples from uncomplicated, hypertensive, and diabetic pregnancies.

We chose to overrepresent HDP and DM pregnancies in this study to obtain as much data on these pregnancy complications as possible while maintaining a reasonably large (n=258) control group. Most of the included women (n=468) were delivered by cesarean, either owing to medically complicated pregnancies (HDP, DM), owing to maternal request (including previous psychologically traumatic vaginal delivery or tocophobia), or because of breech presentation (the control group).

Prepregnancy BMI, early pregnancy blood pressure, gestational age at sampling, and fetal sex did not consistently correlate with SAA1 or PZP levels. Thus, we did not perform regression analyses. Gestational age at sampling correlated with SAA1 and PZP, but only in EO-PE, likely reflecting a more severe placental dysfunction with a clinical phenotype requiring earlier delivery. Our results are likely not confounded by our lack of gestational age matching, as gestational age did not correlate with SAA1 or PZP in any other groups.

A limitation to our study is the lack of longitudinal samples to establish whether SAA1 and/or PZP show prolonged dysregulation during and/or after pregnancy complications and lack of placental tissue samples for amyloid staining.

Conclusions

Understanding and identifying early risk markers for cardiovascular disease in women is essential for achieving targeted primary prevention, optimized health outcomes, and healthier societies. Our finding of increased SAA1 and hsCRP in women with EO-PE, fetal growth restriction, and other signs of placental dysfunction may provide further explanation as to the known epidemiologic link between these pregnancy complications and maternal cardiovascular disease in later life. Dysregulated SAA1 and PZP in EO-PE may point to amyloid-A as a mediator of the observed risk of cardiovascular disease in women with a history of preeclampsia or other forms of placental dysfunction, though longitudinal and mechanistic studies are needed to test this hypothesis.

Glossary

Acute atherosis: pregnancy-specific foam cell lesions in spiral arteries; may affect any pregnancy, but is most frequent in preeclampsia.

Protein misfolding: process by which proteins lose or fail to maintain normal structure, or fail to fold correctly during protein synthesis, thus losing physiological properties.

Amyloid: fibrillar aggregates of misfolded proteins; very stable compounds because of their cross-β sheet structure, involved in numerous human diseases. ■

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ajogmf.2022.100794.

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