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## ORIGINAL ARTICLE



# Sphingosine-1-Phosphate, a Marker of Endothelial Injury and Disease Severity in Preeclampsia

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**BACKGROUND:** Preeclampsia is a hypertensive pregnancy disorder marked by endothelial damage. Healthy endothelium is covered by a protective glycocalyx layer, which, when degraded, releases detectable products into the blood. Sphingosine-1-phosphate (S1P) is a cardiovascular biomarker involved in glycocalyx preservation, linked to placentation and preeclampsia development. The study aimed to test the hypothesis that plasma S1P is altered alongside glycocalyx degradation products in severe preeclampsia compared with controls.

**METHODS:** We included 121 females: 41 with severe preeclampsia requiring treatment in the intensive care unit, 40 with preeclampsia but no need of intensive care unit treatment, and 40 with normotensive pregnancies. Plasma levels of S1P and glycocalyx degradation products—hyaluronic acid, SDC-1 (syndecan-1), and HSPG2 (heparan sulfate proteoglycan-2)—were analyzed from blood samples taken within 27 hours postpartum.

**RESULTS:** Postpartum plasma S1P was significantly lower in the intensive care unit cohort compared with both preeclampsia controls and normotensive controls ( $P < 0.001$ ). Hyaluronic acid and SDC-1 levels were elevated in the intensive care unit group versus normotensive controls ( $P = 0.009$  and  $P = 0.023$ ), while HSPG2 was lower ( $P < 0.001$ ). Plasma S1P correlated with hyaluronic acid and blood pressure.

**CONCLUSION:** Intensive care patients with severe preeclampsia have lower plasma S1P levels and higher concentrations of glycocalyx degradation products, indicating more pronounced endothelial damage. These findings suggest that S1P is associated with preeclampsia severity and may serve as a biomarker to assess vascular damage in this patient population. Further studies are needed to explore the potential role of S1P in long-term cardiovascular risk assessment for patients with preeclampsia. (*Hypertension*. 2025;82:914–925. DOI: 10.1161/HYPERTENSIONAHA.124.24118.) • **Supplement Material.**

**Key Words:** biomarkers ■ blood pressure ■ endothelium ■ preeclampsia ■ severity ■ sphingosine-1-phosphate

Preeclampsia is a pregnancy disorder characterized by endothelial damage, which leads to hypertension and heterogenous multiorgan complications.<sup>1,2</sup> Preeclampsia affects 2% to 5% of all pregnancies, with high mortality and morbidity, especially in low-income countries.<sup>3</sup> Several pathophysiological pathways and preeclampsia biomarkers have been identified, mostly

focusing on the preeclampsia-associated antiangiogenic imbalance.<sup>3–5</sup> Based on etiology, preeclampsia is generally divided into 2 subtypes.<sup>6</sup> Early-onset preeclampsia (EPE) with delivery before gestation week 34 is characterized by defective placentation, intrauterine growth restriction, and more severe manifestations.<sup>7</sup> Late-onset preeclampsia (LPE) is dictated by maternal constitutional

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## NOVELTY AND RELEVANCE

### What Is New?

Lower plasma sphingosine-1-phosphate (S1P) levels in severe preeclampsia requiring intensive care. Correlation of S1P with endothelial glycocalyx degradation markers. Evidence linking S1P levels to preeclampsia severity and vascular damage.

### What Is Relevant?

S1P as a potential biomarker for endothelial injury and preeclampsia severity. Association of reduced S1P with higher Sequential Organ Failure Assessment severity scores in intensive care unit patients. Importance of glycocalyx integrity in vascular health during hypertensive pregnancy disorders.

### Clinical/Pathophysiological Implications?

S1P as a unique biomarker for preeclampsia severity that may offer potential to differentiate mild and severe preeclampsia forms. Dynamic changes in S1P levels could enable real-time monitoring of endothelial stress to support early identification of females at risk for severe preeclampsia. The role of S1P in endothelial health may open pathways for new diagnostics and therapies.

## Nonstandard Abbreviations and Acronyms

<b>ASAT</b>	aspartate aminotransferase
<b>EPE</b>	early-onset preeclampsia
<b>GFR</b>	glomerular filtration rate
<b>HA</b>	hyaluronic acid
<b>HDL</b>	high-density lipoprotein
<b>Hpx</b>	hemopexin
<b>HSPG2</b>	heparan sulfate proteoglycan-2
<b>ICU</b>	intensive care unit
<b>LPE</b>	late-onset preeclampsia
<b>S1P</b>	sphingosine-1-phosphate
<b>SDC-1</b>	syndecan-1
<b>sFit-1</b>	soluble fms-like tyrosine kinase-1
<b>SOFA</b>	Sequential Organ Failure Assessment
<b>TM</b>	thrombomodulin
<b>VEGF</b>	vascular endothelial growth factor

factors and senescence of a normally formed placenta leading to dysfunction later in pregnancy. Syncytiotrophoblast stress is the common denominator between the 2 forms of preeclampsia.<sup>8</sup> The lack of mechanistic insights into preeclampsia pathophysiology currently leaves symptomatic treatment as the only available therapeutic option and makes delivery the only curative intervention. Identifying signaling pathways relevant to preeclampsia pathophysiology may lead to the implementation of new predictive markers and potential new therapeutic options.

Recently, the bioactive phospholipid sphingosine-1-phosphate (S1P) emerged as a potential marker and therapeutic target for hypertensive disease<sup>9,10</sup> as it is

involved in several processes important to hypertension pathophysiology, including regulation of vascular tone and heart rate, lymphocyte trafficking, chemotaxis, and inflammation.<sup>11,12</sup> S1P-dependent cardiovascular effects are mainly mediated by signaling through S1PRs (S1P receptors; S1PR1–3). Importantly, S1P is critically involved in placental development by promoting trophoblast invasion and migration.<sup>13</sup> Alterations in S1P have been associated with insufficient trophoblast differentiation<sup>14,15</sup> and hence a dysfunctional placenta, which may predispose to preeclampsia development. Nonetheless, conflicting results regarding plasma and placental S1P levels in preeclampsia are still a topic of discussion.<sup>16–18</sup> Interestingly, S1P stabilizes and preserves the endothelial glycocalyx,<sup>19–22</sup> a protective glycolipid layer covering the vascular endothelium. Disruption of the endothelial barrier and shedding of the glycocalyx are characteristic for several pathological conditions, including preeclampsia.<sup>23</sup> Previous studies have reported increased maternal blood levels of glycocalyx degradation products, such as SDC-1 (syndecan-1), hyaluronic acid (HA), thrombomodulin, soluble vascular adhesion molecule-1, and heparan sulphate proteoglycans (HSPG) in preeclampsia maternal blood.<sup>24–29</sup> Increased levels of degradation products also correlate with lower microvascular perfusion.<sup>28</sup>

This study hypothesizes that plasma S1P levels are altered in patients with severe preeclampsia requiring intensive care, indicating its potential as a biomarker for assessing preeclampsia severity. To further evaluate the potential of plasma S1P as a biomarker for preeclampsia-associated endothelial injury, this study analyzed plasma and placenta tissue S1P concentrations and plasma glycocalyx degradation products together

with clinical biomarkers in a cohort consisting of severe preeclampsia cases in need of intensive care after delivery, preeclampsia controls and normotensive controls.

## METHODS

### Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

### Ethical Permission

The study was approved by the Regional Ethical Review Board in Lund, Sweden, for studies in human subjects (Diarienummer 2019-0468, 2016/49 and for Swedish critical care biobank Swecrit 2015/267).

### Study Population

In this retrospective cohort study, a total of 121 participants were included. Of these, there were 41 cases with severe preeclampsia (EPE and LPE) with end-organ failure and need of postpartum intensive care (intensive care unit [ICU] cohort) taken from the Swecrit study, which during 2015 to 2018 collected samples from all intensive care patients in the Region of Skåne, Sweden (Blood Samples From Critically Ill Patients and Healthy Controls; <https://www.clinicaltrials.gov>; unique identifier: NCT04974775). As controls (preeclampsia and normotensive controls), we used matched samples from the biobank at the Department of Obstetrics and Gynecology, Lund University, Sweden. There were 40 cases with preeclampsia (severe and nonsevere EPE and LPE but with no need for ICU admission) managed at the delivery ward (preeclampsia controls) and 40 normotensive pregnancies (controls; Figure 1). No other selection criteria were applied to avoid selection bias. The study cohort included participants of diverse ethnic backgrounds. The ICU group consisted of 7.3% individuals of Middle Eastern descent, 4.9% Asian, 4.9% African, and the remainder of Northern European descent. The preeclampsia control and normotensive control groups each included 2.5% individuals of Middle Eastern descent, with the rest being of Northern European descent. The cohorts have previously been published

in 2 separate studies, evaluating biomarkers for oxidative stress in severe preeclampsia<sup>30</sup> and for the development of a prediction model for severe preeclampsia with intensive care need.<sup>31</sup>

Preeclampsia was defined according to the International Society for the Study of Hypertension in Pregnancy as de novo hypertension manifesting after 20 weeks of gestation accompanied by proteinuria or evidence of maternal end-organ dysfunction.<sup>32</sup> Severe preeclampsia was defined as blood pressure >160 mm Hg systolic or 110 mm Hg diastolic.<sup>7</sup> EPE was considered as severe preeclampsia with delivery before 34 weeks of gestation. The hemolysis, elevated liver enzymes, and low platelets syndrome, a serious manifestation of preeclampsia, was also defined as a severe form of preeclampsia.<sup>32</sup>

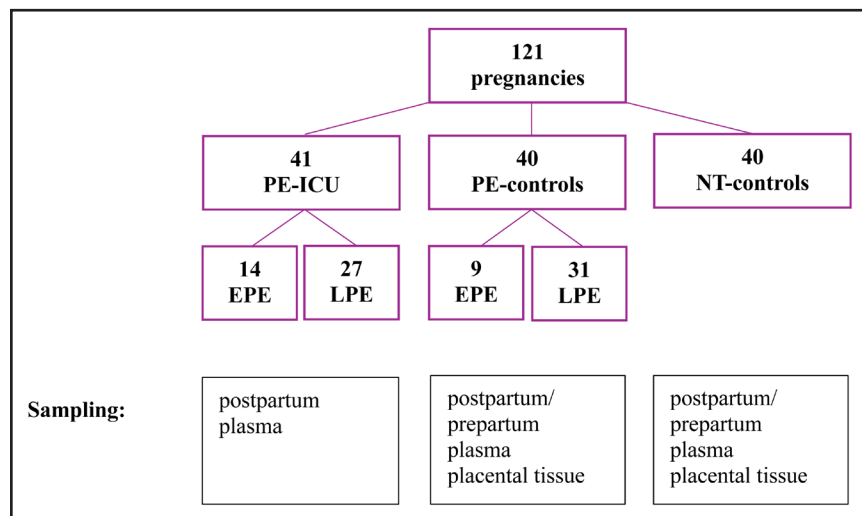
### Blood Pressure Measurement

Brachial systolic and diastolic blood pressure measurements were obtained in the supine position using an appropriately sized automated cuff around the right arm (Welch Allyn Connex Spot Monitor).

### Sample Collection

All blood samples were obtained by venipuncture or via arterial cannula (ICU cohort only) and collected in EDTA plasma tubes. Samples were centrifuged at 2000g to 2200g for 10 minutes, to separate the plasma, and thereafter stored at  $-80^{\circ}\text{C}$  until further analysis. Time from sampling to freezing was <2 hours.

All postpartum plasma samples were taken within 27 hours after delivery. For the ICU cohort, plasma samples were taken within 2 hours of admission to intensive care to be stored in the regional hospital biobank for future analysis. The ICU cohort only had postpartum plasma samples. For the 2 control groups (preeclampsia and normotensive controls), plasma samples were taken before delivery and postpartum. Whole tissue placenta biopsies were taken at the time of delivery for preeclampsia and normotensive controls. Tissue was rinsed in saline solution to remove blood clots, portioned, snap-frozen on dry-ice, and stored at  $-80^{\circ}\text{C}$  until further use. Regarding the ICU patients, the Swecrit study was only focused on blood sampling, and, therefore, there were no available placenta biopsies for these patients. All samples for preeclampsia and



**Figure 1. Flowchart of included pregnancies.**

EPE indicates early-onset preeclampsia; ICU, intensive care unit; LPE, late-onset preeclampsia; NT, normotensive; and PE, preeclampsia.

normotensive controls were stored in the local biobank at the Department of Obstetrics and Gynecology, Lund University, Sweden. All preeclampsia cases had routine laboratory parameters such as ASAT (aspartate aminotransferase), creatinine, and uric acid documented in their medical records during their hospital stay (Table S1). As part of a previous study, plasma concentrations of Hpx (hemopexin) and sFlt-1 (soluble fms-like tyrosine kinase-1) were determined in the ICU cohort as markers for oxidative stress and angiogenic imbalance in severe preeclampsia.<sup>30,31</sup> Additionally, Sequential Organ Failure Assessment (SOFA) severity score (0–24)<sup>33</sup> at admission to ICU and plasma albumin levels were determined in the ICU cohort (Table S1). An initial SOFA score of >11 is associated with >90% mortality.<sup>34</sup>

To ensure the stability and integrity of the analytes, all samples were processed and stored following strict protocols. Immediately upon collection, plasma and tissue samples were stored at  $-80^{\circ}\text{C}$ , thus minimizing the risks of degradation during processing. All samples of all groups underwent the same numbers of freeze and thaw cycles at the time of the individual experiments. Univariate linear modeling was performed to assure that sample age does not affect the analyte levels ( $P=0.674$  for S1P,  $P=0.345$  for HA,  $P=0.877$  for SDC-1, and  $P=0.493$  for HSPG2 [heparan sulphate proteoglycan-2]).

### S1P Quantitation

Plasma sample preparation was performed as previously described.<sup>35</sup> Briefly, 10  $\mu\text{L}$  of plasma was mixed with 90  $\mu\text{L}$  ice-cold methanol containing 22 nmol/L S1P-D7 (Avanti Polar Lipids/Merck, Darmstadt, Germany) as internal standard in 1.5 mL tubes and put on ice. The tubes were vortexed vigorously for 10 s, which was repeated after 15 minutes on ice. After a total of 30-minute incubation on ice, the samples were centrifuged at 20 000g for 10 minutes at 4  $^{\circ}\text{C}$ . The supernatant was transferred to an autosampler vial and stored at  $-80^{\circ}\text{C}$  until analysis with mass spectrometry.

S1P extraction from the placental tissue was adapted from a previously described protocol.<sup>36</sup> Briefly, placental tissue samples were weighed (mg), mixed with 1 mL PBS, and homogenized using an Ultra-Turrax homogenizer. Homogenate volumes equivalent to 20 mg tissue were added to a glass extraction vial and filled up to 1 mL with PBS. Fifty picomoles per liter internal standard S1P-D7 (5  $\mu\text{mol/L}$ ; Avanti Polar Lipids/Merck) was added, and the suspension was mixed with 1 mL methanol, 200  $\mu\text{L}$  6 mol/L hydrochloric acid, and 2 mL chloroform. After 3 minutes of vigorous vortexing, the samples were centrifuged at 1900g for 3 minutes. The lower organic phase was transferred with a glass Pasteur pipette to a clean glass tube. Previous steps, including the addition of chloroform and centrifugation, were repeated with the remaining aqueous phase, and the 2 organic phases were then combined before chloroform was evaporated under nitrogen stream in a fume hood. Dried lipids were dissolved in 100 to 200  $\mu\text{L}$  methanol and transferred to an autosampler vial. A 6-point standard curve was generated from extracts of 5 to 80 pmol S1P in fatty acid-free BSA/PBS applying the same extraction procedure.

The samples were analyzed by liquid chromatography–coupled tandem mass spectrometry on a 6495 QQQ instrument (Agilent Technologies, Sweden) as previously described.<sup>9</sup>

### ELISA

Plasma levels of HA, SDC-1, and HSPG2 were analyzed using commercial ELISA kits (Hyaluronan Quantikine ELISA [Bio-Techne, R&D Systems], Human Syndecan-1 [Thermo Fisher Scientific], and Human HSPG2 ELISA [Aviva Systems Biology]) according to manufacturers' protocols. Standards and samples were run in duplicates. The optical density of each well was determined using a GloMax Discover Microplate Reader (Promega) at 450 and 540 nm to correct for optical imperfections in the plate. The concentration of the glyocalyx degradation products was calculated from the absorbance values using the standard curve and adjusting for the dilution to a final concentration in nanograms per milliliter.

### Statistics

Based on the sample size and the observed variability in S1P levels and glyocalyx degradation products in our cohort, we estimated the study's ability to detect minimal relevant differences. For plasma S1P and levels of circulating markers of endothelial dysfunction, we considered effect sizes of previous studies on blood pressure and vascular function assessment to estimate a detectable difference.<sup>9,37</sup> A minimum of 32 samples per group were calculated to be required using human reference values for plasma S1P concentrations (757.4 nmol/L) and assuming a 12% change in plasma S1P in patients with preeclampsia.

Statistical analyses were performed in R (version 4.2.2; R Foundation for Statistical Computing, Vienna, Austria), SPSS (version 30; IBM, Armonk, NY), and Prism (version 10.2.0.335; GraphPad Software, La Jolla, CA).

Data are expressed as mean (95% CI), median (interquartile range), correlation coefficient, or percentage. Data distribution was assessed by the Shapiro-Wilk normality test. Differences between groups were assessed using the Kruskal-Wallis test or 1-way ANOVA for overall  $P$  values followed by Mann-Whitney unpaired test or  $t$  test dependent on data distribution. When comparing categorical data, the Fisher exact test was used. Regression analyses were performed to evaluate the effect of confounding factors and changes throughout pregnancy (eg, gestational age, amount of bleeding at delivery, and kidney function). A  $P$  value of <0.05 was considered significant. Correlation analyses were performed by calculation of Spearman correlation coefficients and exact  $P$  value computation before Bonferroni correction for multiple comparisons.

## RESULTS

### Characteristics of the Patient Cohort

Patient characteristics showed a significantly older age in the ICU cohort compared with controls (preeclampsia controls and normotensive controls), while all patients with preeclampsia (ICU cohort and preeclampsia controls) had a significantly higher body mass index compared with normotensive controls (Table). The ICU cohort had significantly higher maximal systolic and diastolic blood pressure, was delivered earlier, and showed lower fetal birthweights. There was a trend toward a greater proportion of male fetuses

**Table. Clinical Characteristics of the Study Groups**

Clinical characteristics	ICU cohort (n=41)	PE controls (n=40)	Controls (n=40)	P value
Age, y	33 (31–35)*	29 (28–31)	29 (28–31)	0.008
Parity, n	0 (0–1)	0 (0–1)	0 (0–0)	0.030
BMI, kg/m <sup>2</sup>	27 (25–29)	27 (25–29)†	24 (23–26)	0.030
Gestation days at delivery	250 (225–267)*	273 (256–278)†	282 (269–288)	<0.001
Highest systolic BP,‡ mm Hg	171 (164–178)*	160 (155–165)†	123 (121–126)	<0.001
Highest diastolic BP,§ mm Hg	107 (103–112)*	101 (98–103)†	76 (74–70)	<0.001
Birthweight, g	2155 (1551–3048)*	3131 (2346–3709)	3395 (3163–3775)	<0.0001
Fetal sex, n (%)				
Male	24 (58.5)	20 (50.0)	15 (37.5)	0.110
Female	15 (36.6)	18 (45.0)	25 (62.5)	
Female and male	2 (4.9)	2 (5.0)	0	
EPE/LPE, n	14/27	9/31	0	...

Values are shown as mean (95% CI) for normal distributed data and median (quartiles) for not normal distributed data. Exact *P* values are given for the Kruskal-Wallis test. BMI indicates body mass index; BP, blood pressure; EPE, early-onset preeclampsia; ICU, intensive care unit; LPE, late-onset preeclampsia; and PE, preeclampsia.

\*Significant differences between the ICU cohort and PE controls after Mann-Whitney *U* test or unpaired *t* test.

†Significant differences between PE controls and controls after Mann-Whitney *U* test or unpaired *t* test.

‡Highest systolic blood pressure recorded during hospitalization or in maternity care (1–30 d before delivery).

§Highest diastolic blood pressure recorded during hospitalization or in maternity care (1–30 d before delivery).

in the more severe cases, but this difference did not reach statistical significance. Among the preeclampsia controls, 9 patients (22.5%) were classified as EPE, while in the ICU cohort, 14 patients were classified as EPE (34%). The routine laboratory markers ASAT and uric acid were significantly higher in the ICU cohort compared with preeclampsia controls (Table S1). Hpx was significantly lower in the ICU cohort compared with preeclampsia and normotensive controls (Table S1).<sup>30</sup> The postpartum median levels of sFit-1 in the ICU cohort were 9502 (6201–16130) pg/mL,<sup>30</sup> and plasma albumin levels showed a mean value of 23.9 (22.4–25.3) g/L, thus well below normal levels (35–50 g/L).<sup>38</sup> The mean SOFA score for the ICU cohort was 3.3 (2.5–4.1), which is generally associated with <10% mortality.<sup>34</sup>

### Plasma S1P Levels Are Lower in Patients With Preeclampsia Needing Intensive Care

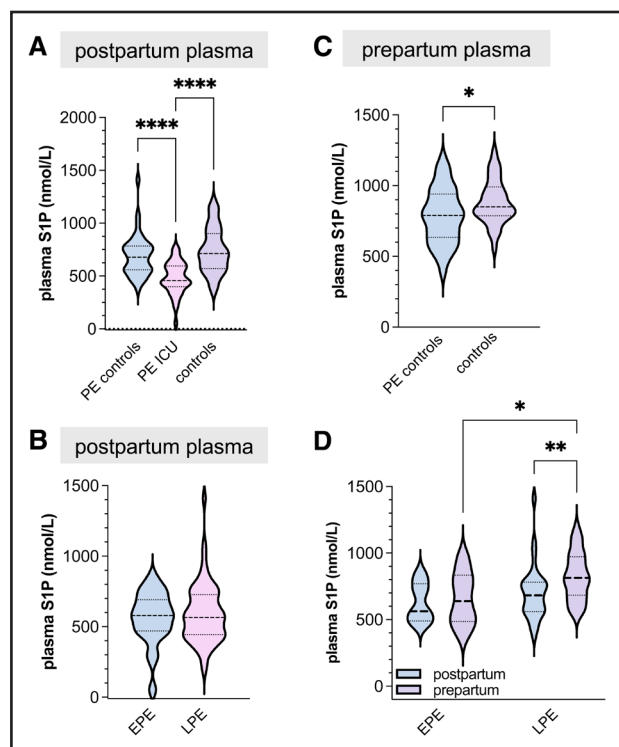
Plasma S1P concentrations in the ICU cohort, preeclampsia controls, and normotensive controls were determined in postpartum plasma samples and revealed significantly lower S1P plasma levels in the ICU cohort (458 [399–595] nmol/L) compared with preeclampsia controls (678 [559–785] nmol/L) and normotensive controls (712 [572–901] nmol/L; Figure 2A). This difference remained significant after adjustment for gestational age and amount of bleeding at delivery (Table S2). Moreover, the observed difference between the preeclampsia controls and the ICU cohort remained statistically significant after adjusting for kidney function (ie, creatinine levels;  $P < 0.001$ ). There was no significant

difference between preeclampsia controls and normotensive controls (Figure 2A; Table S2). Subgroup analysis of the patients with preeclampsia including the ICU cohort showed no significant difference regarding S1P plasma concentration in EPE compared with LPE (Figure 2B). This remained nonsignificant after adjustment for gestational age (Table S3).

Additionally, S1P concentrations in the prepartum plasma were lower in preeclampsia controls (787 [722–851] nmol/L) compared with normotensive controls (880 [828–934] nmol/L; Figure 2C). The difference was no longer significant after adjusting for gestational age ( $P = 0.105$ ). Subgroup analysis for the preeclampsia controls showed a significant difference in plasma S1P levels between LPE and EPE, with lower prepartum S1P in EPE. This difference remained significant after adjustment for gestational age (Table S3). Comparing prepartum and postpartum S1P plasma levels in preeclampsia controls revealed lower postpartum S1P levels (665 [558–780] versus 790 [635–940] nmol/L; Figure S1). This difference was significant in patients with LPE but not in those with EPE (Figure 2D).

### Placental S1P Concentrations Are Higher in Patients With EPE

In addition to circulating S1P levels, placental tissue S1P concentrations were determined for 35 preeclampsia controls and 35 normotensive controls. There was no significant difference between preeclampsia controls (0.60 [0.48–1.1] pmol/mg) and the normotensive group (0.65 [0.49–0.79] pmol/mg; Figure 3A). However, when comparing EPE and LPE cases, significantly



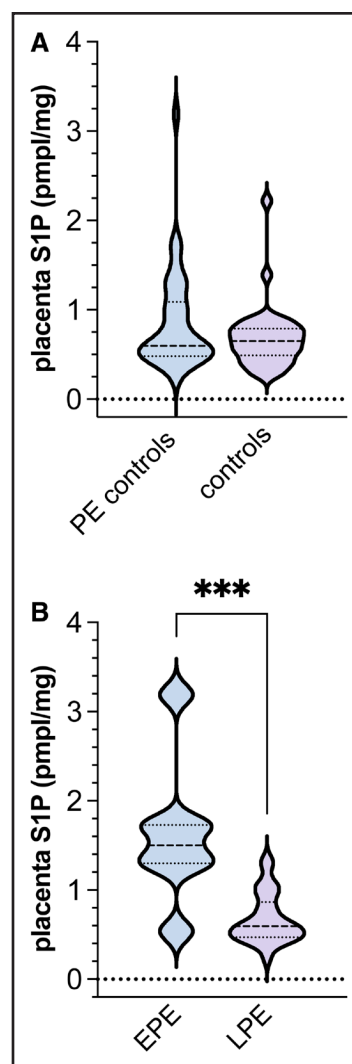
**Figure 2. Plasma concentration of sphingosine-1-phosphate (S1P).**

**A**, S1P concentration in postpartum plasma for all groups (intensive care unit [ICU] cohort,  $n=41$ ; preeclampsia [PE] controls,  $n=40$ ; controls,  $n=40$ ). **B**, S1P concentration in postpartum plasma in all patients with PE (ICU cohort and PE controls) divided in early-onset PE (EPE;  $n=14$  and  $n=9$ ) and late-onset PE (LPE;  $n=27$  and  $n=31$ ). **C**, S1P concentration in prepartum plasma for PE controls ( $n=39$ ) and controls ( $n=35$ ). **D**, Comparison of prepartum and postpartum plasma concentrations of S1P in PE controls divided in EPE ( $n=9$ ) and LPE ( $n=30$  for prepartum plasma and  $n=31$  for postpartum plasma). \* $P<0.05$ , \*\* $P<0.01$ , \*\*\*\* $P<0.0001$ .

higher placental S1P concentrations were observed in the EPE group (Figure 3B). The difference did not hold significance after adjustment for gestational age ( $P=0.376$ ).

### Glycocalyx Degradation Products Are Increased in Plasma of Patients With Preeclampsia

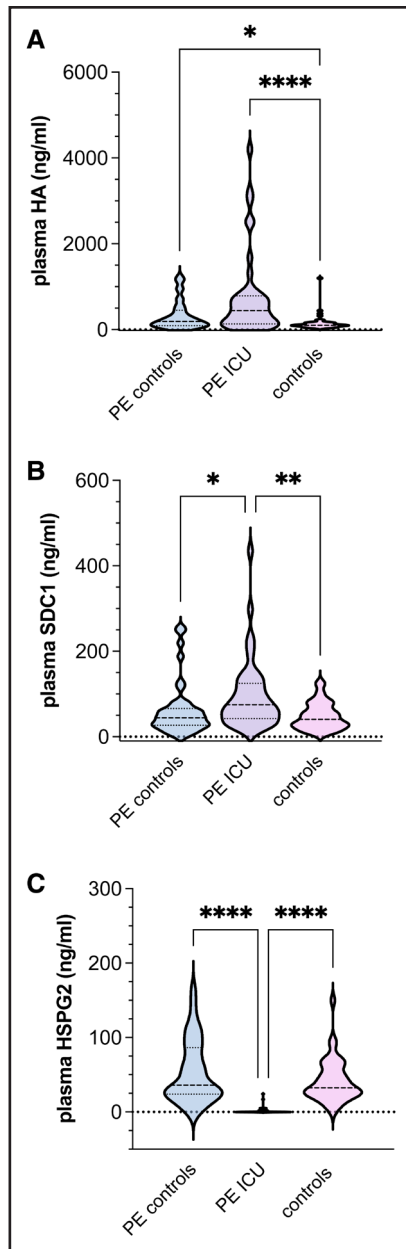
Plasma levels of glycocalyx degradation products were determined in postpartum plasma from all groups. Postpartum plasma HA concentrations were significantly higher in the ICU cohort (442 [132–788] ng/mL) and in the preeclampsia controls (191 [91–454] ng/mL) compared with normotensive controls (102 [77–159] ng/mL; Figure 4A). After adjustment for gestational age and amount of bleeding at delivery, the difference between the ICU cohort and normotensive controls remained significant, whereas the difference between preeclampsia and normotensive controls was no longer significant (Table S4). Similarly, adjustment for creatinine levels in preeclampsia controls and the ICU cohort diminished the



**Figure 3. Placental concentration of sphingosine-1-phosphate (S1P).**

**A**, Comparison of placental concentrations of S1P in preeclampsia (PE) controls ( $n=35$ ) compared with controls ( $n=35$ ). **B**, Comparison of placental concentrations of S1P in PE controls divided in early-onset PE (EPE;  $n=7$ ) and late-onset PE (LPE;  $n=28$ ). \*\*\* $P<0.001$ .

statistical difference in HA concentration between these groups ( $P=0.184$ ). Subgroup analysis of the HA plasma concentration in EPE compared with LPE showed no significant difference (Table S3). As for HA, plasma SDC-1 levels were the highest in the ICU cohort (75 [42–125] ng/mL) compared with preeclampsia controls (45 [27–66] ng/mL) and normotensive controls (41 [25–69] ng/mL; Figure 4B). The difference between the ICU cohort and normotensive controls remained significant after adjustment for gestational age and the amount of bleeding at delivery, while the difference between preeclampsia controls and the ICU cohort was not detected after adjustment for gestational age at delivery (Table S4). Similar results were obtained after adjustment for creatinine levels in preeclampsia controls and the ICU cohort ( $P=0.111$ ). Here, subgroup analysis of patients



**Figure 4. Postpartum plasma concentrations of glyocalyx degradation products.**

**A**, Comparison of postpartum plasma concentrations of hyaluronic acid (HA) in all groups (intensive care unit [ICU] cohort,  $n=41$ ; preeclampsia [PE] controls,  $n=40$ ; controls,  $n=40$ ). **B**, Comparison of postpartum plasma concentrations of SDC-1 (syndecan-1) in all groups (ICU cohort,  $n=41$ ; PE controls,  $n=40$ ; controls,  $n=40$ ). **C**, Comparison of postpartum plasma concentrations of HSPG2 (heparan sulphate proteoglycan-2) in all groups (ICU cohort,  $n=41$ ; PE controls,  $n=40$ ; controls,  $n=40$ ). \* $P\leq 0.05$ , \*\* $P\leq 0.01$ , \*\*\*\* $P\leq 0.0001$ .

with preeclampsia including the ICU cohort showed no statistically significant difference (Table S3).

In contrast, HSPG2 plasma concentration was lower in the ICU cohort (1.76 [0–3.2] ng/mL) compared with preeclampsia controls (53.6 [40.2–70] ng/mL;  $P<0.001$ ) and normotensive controls (41.7

[32.5–50.8] ng/mL;  $P<0.001$ ; Figure 4C). There was no significant difference between preeclampsia controls and normotensive controls. Like for HA and SDC-1, no differences were observed between EPE and LPE (Table S3).

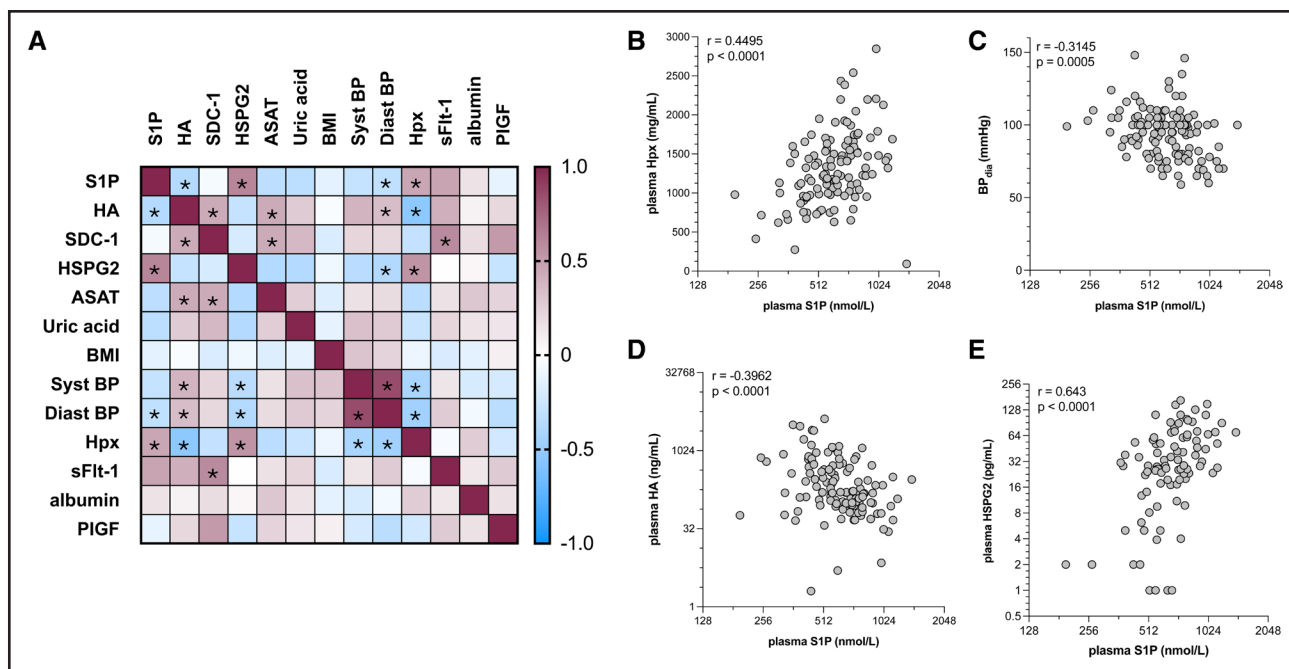
### S1P Levels Correlate With Markers for Endothelial Injury and Preeclampsia Severity

To understand the potential relationships between S1P, endothelial injury, and clinical preeclampsia markers at admission to ICU, correlation analyses were performed. The correlation matrix depicted in Figure 5A shows the associations between all variables, with significant associations highlighted after correction for multiple comparisons. Hpx, a protective heme scavenger rapidly consumed during preeclampsia and verified as a marker for preeclampsia severity,<sup>39</sup> revealed a positive correlation with plasma S1P (Figure 5B) and plasma HSPG2. Hpx inversely correlated with systolic and diastolic blood pressure, as well as plasma HA (Figure 5A). An inverse relationship for plasma S1P was observed for diastolic blood pressure (Figure 5C) and plasma HA (Figure 5D), while plasma HSPG2 was positively correlated with plasma S1P (Figure 5E). Plasma S1P did not significantly correlate with ASAT or sFlt-1 after correction for multiple comparisons (Table S6). However, sFlt-1 presented with a positive correlation with SDC-1.

SOFA score was documented in each patient in the ICU cohort at admission to ICU (Table S1). The maximum assigned SOFA score was 7. Both plasma Hpx and S1P were the lowest in patients with higher SOFA scores (Table S5).

## DISCUSSION

Plasma S1P level alterations have previously been linked to cardiometabolic disease, vascular deregulation, and inflammation.<sup>9,12</sup> Specifically, increments in plasma S1P associate with systolic blood pressure elevation and several markers of cardiometabolic disease and inflammation.<sup>9</sup> Interestingly, our findings show an inverse relationship between plasma S1P and blood pressure in severe preeclampsia. Lower postpartum S1P plasma levels in severe preeclampsia may result from albumin leakage mediated through tubular and glomerular damage; however, only a small proportion of circulating S1P is bound to albumin in plasma.<sup>40</sup> In conditions like severe sepsis, lower plasma S1P levels were linked to decreases in HDL (high-density lipoprotein)-bound S1P.<sup>41</sup> Similar mechanisms may apply in severe preeclampsia given the dysregulated HDL profile in this condition.<sup>42–44</sup> The current study did not test S1P binding partner profiles in preeclampsia, but considering the importance of chaperones for S1P signaling, this is warranted for future studies. The observed association between plasma S1P



**Figure 5. Correlations between plasma biomarkers.**

**A**, Correlation matrix showing the associations between all variables, with significant associations highlighted after correction for multiple comparisons. **B**, Correlation between postpartum plasma concentration of sphingosine -1-phosphate (S1P) and Hpx (hemopexin; all groups,  $n=121$ ). **C**, Correlation between postpartum plasma concentration of S1P and the diastolic blood pressure ( $BP_{dia}$ ; all groups,  $n=121$ ). **D**, Correlation between postpartum plasma concentration of S1P and postpartum plasma concentration of hyaluronic acid (HA; all groups,  $n=121$ ). **E**, Correlations between postpartum plasma concentration of S1P and postpartum plasma concentration of HSPG2 (heparan sulphate proteoglycan-2; all groups,  $n=121$ ). ASAT indicates aspartate aminotransferase; BMI, body mass index; Diast BP, diastolic blood pressure; PIGF, placental growth factor; SDC-1, syndecan-1; sFlt-1, soluble fms-like tyrosine kinase; and Syst BP, systolic blood pressure.

levels and SOFA scores at ICU admission highlights the potential of S1P as a severity marker,<sup>41,45</sup> a notion supported by detected S1P relationships with ASAT and uric acid, previously identified as predictors of severe disease.<sup>31</sup> Positive associations between plasma S1P and Hpx also emphasize the relationship with oxidative stress, as Hpx consumption has been reported in severe preeclampsia.<sup>30,39</sup> Collectively, these findings suggest that reduced S1P levels may reflect endothelial injury severity in preeclampsia and provide insights into mechanisms underpinning vascular dysfunction.

Endothelial glycocalyx degradation, reflected by increased HA and SDC-1 in the ICU cohort, supports the concept of severe endothelial injury in preeclampsia.<sup>46,47</sup> The association of SDC-1 with sFlt-1 aligns with evidence of sFlt-1-mediated glycocalyx collapse<sup>48</sup> and impaired endothelial barrier function.<sup>49</sup> The low plasma levels of HSPG2 in the ICU cohort further indicate advanced endothelial damage, as HSPG2 is essential for vascular integrity and its reduction may disrupt maternal hemodynamics.<sup>50</sup> Elevated HA and SDC-1 likely represent compensatory or pathological responses to glycocalyx damage, with HA fragments known to activate signaling pathways that compromise barrier function.<sup>51,52</sup> These alterations suggest a complex interplay between endothelial dysfunction and glycocalyx degradation. Lowering of HSPG2 as a critical component of the

extracellular matrix and glycocalyx<sup>53</sup> may be indicative of a disruption in the structural integrity of the endothelial barrier,<sup>54,55</sup> while the elevation of HA and SDC-1 levels may reflect an adaptive or pathological response to injury as HA acts as a signaling molecule promoting repair but may also contribute to increased vascular permeability.<sup>56</sup> The increase in SDC-1 may be indicative of heightened endothelial injury and instability.<sup>57</sup>

S1P is well established in promoting endothelial stability, glycocalyx regeneration, and barrier integrity through S1PR1 activation.<sup>19–22,58–61</sup> Several landmark studies have provided first insight into how plasma S1P may affect endothelial barrier integrity by showing that S1P interacts with S1PR1 on the luminal surface of endothelial cells, providing a consistent stimulus that preserves the integrity of the endothelial barrier, while the leakage of S1P from plasma across the endothelial barrier activates S1PR1, prompting endothelial cells to reinforce and tighten the barrier.<sup>62</sup> Moreover, endogenous S1P production by endothelial cells has been demonstrated to enhance barrier integrity through activation of the S1PR1 receptor.<sup>63</sup> Given the interplay between S1P and the glycocalyx, lower S1P levels alongside increased HA and SDC-1 in severe preeclampsia may critically impact endothelial homeostasis. Further studies should explore the mechanistic links between plasma S1P levels, glycocalyx degradation,

and their implications for endothelial dysfunction in preeclampsia, particularly in relation to cardiovascular risk.

Endothelial dysfunction in preeclampsia involves not only structural damage to the glycocalyx but also impaired signaling pathways that regulate vascular tone. A key aspect of this dysfunction is the reduction in NO bioavailability and elevated endothelin-1<sup>64</sup> production,<sup>65,66</sup> influenced by the antiangiogenic factor sFlt-1, which antagonizes VEGF (vascular endothelial growth factor) signaling.<sup>67</sup> In a healthy endothelium, VEGF promotes NO synthesis, supporting vasodilation and vascular stability.<sup>68</sup> However, elevated sFlt-1 levels in preeclampsia reduce NO production and augment endothelin-1 generation,<sup>69</sup> contributing to vasoconstriction and heightened vascular resistance by hindering NO to promote cGMP-mediated relaxation in vascular smooth muscle cells.<sup>70</sup> Assessing NO surrogates in patients with preeclampsia may help delineate the role of NO signaling disruption in disease progression and may offer a more comprehensive understanding of endothelial health in this high-risk cohort. Future studies including NO surrogates or cGMP are warranted to further explore these mechanisms alongside glycocalyx integrity markers.

S1P has further been linked to healthy trophoblast development and normal placentation,<sup>14</sup> although the effect of placental S1P concentrations on preeclampsia development is still a matter of debate. Contrasting findings suggest that elevated placenta S1P levels inhibit the differentiation of cytotrophoblast and thereby promote preeclampsia,<sup>14</sup> while on the other hand, lower placental S1P levels, sphingosine kinase 1 deficiency, and accompanying S1PR2 reduction have been associated with preeclampsia development.<sup>15</sup> Higher sphingolipid biosynthesis in chorionic arteries in preeclampsia has been reported to be mitigated by an impaired production and a simultaneous increase of S1P degradation by S1P lyase.<sup>71</sup> Our findings underscore the distinct pathophysiological mechanisms in EPE versus LPE. While placental S1P concentrations were higher in EPE compared with LPE, this difference did not persist after adjustment for gestational age. Given the divergent origins of EPE and LPE,<sup>72</sup> further studies are needed to elucidate the role of sphingolipid signaling and S1PR dynamics in placental development and preeclampsia progression.

## Summary and Conclusions

Patients with severe preeclampsia admitted to the ICU after delivery exhibited diminished plasma S1P levels and augmented levels of glycocalyx degradation markers, HA and SDC-1, suggestive of increased endothelial injury and glycocalyx shedding. Significant correlations were observed between postpartum plasma S1P and HA, Hpx, HSPG2, and diastolic blood pressure. Additionally, lower plasma S1P levels were associated with higher SOFA severity scores at ICU admission. These

findings indicate that plasma S1P may serve as a marker for the severity of preeclampsia, reflecting vascular dysfunction and the potential impact of glycocalyx integrity loss on endothelial health. Although several biomarkers exist for endothelial injury and glycocalyx degradation,<sup>57</sup> our study proposes that S1P has unique potential in this context for several reasons. Unlike many markers that indirectly indicate endothelial damage, S1P plays a direct role in maintaining endothelial barrier integrity.<sup>73</sup> Given its role in endothelial stabilization, S1P levels may provide a more accurate reflection of the dynamic changes in barrier integrity that are specific to preeclampsia. Moreover, S1P exerts its influence on both the vascular and immune systems, affecting inflammatory pathways that contribute to the progression of preeclampsia.<sup>74</sup> Its dual action may, therefore, provide insights into both vascular integrity and inflammatory states, potentially offering a more comprehensive biomarker profile. Due to its role as a signaling molecule, S1P levels may change more dynamically in response to endothelial stress, offering the potential to detect acute shifts in disease status or response to therapeutic interventions. This could make S1P useful in monitoring preeclampsia severity in real time and help identifying females at risk of developing severe preeclampsia earlier in the disease process, particularly in distinguishing between mild and severe forms.

## Study Limitations

Although this study uses well-characterized patient cohorts with a larger number of patients compared with previous studies, the sample size for severe cases is relatively low. The prevalence of EPE compared with LPE limits the strengths of subgroup analyses. Moreover, this study was conducted as a post hoc analysis, and as such, pre hoc power calculations specific to the current analyses were not included in the initial study design. We estimated the study's ability to detect minimal relevant differences based on our sample size and the variability observed in S1P levels and glycocalyx degradation products. While our estimations provide context for interpreting the findings, we acknowledge that the study may be underpowered for detecting smaller differences.

The study cohort is demographically representative to Sweden in terms of ethnic diversity, as the majority of participants across all groups were of Northern European descent and minority groups include individuals from Middle Eastern, Asian, and African descent. In a global setting, however, the results may not be generalizable. Although Sweden's universal health care system ensures broad and equitable access to care, minimizing disparities in access to care, the readiness of different ethnic groups to participate in clinical studies can vary due to cultural, social, and historical factors, including trust in the health care system and prior research experiences. Future research should incorporate more

diverse populations and analyze how social determinants of health and structural barriers influence the results. Integrating analyses of social determinants of health and genetic ancestry could provide a more comprehensive understanding of how S1P is influenced in diverse populations and its broader applicability as a biomarker.

The use of low-molecular-weight heparin has been shown to stabilize glycocalyx and reduce shedding in patients with COVID-19,<sup>75</sup> but only 2 patients in the ICU cohort were treated with low-molecular-weight heparin before delivery. In our study, plasma creatinine was measured only in patients with preeclampsia (both ICU and preeclampsia control groups), revealing a significant difference between these groups ( $P=0.006$ ). Although all preeclampsia control patients and the majority of ICU cohort patients had proteinuria, it remains unclear whether albumin-bound S1P leakage due to proteinuria would correlate with creatinine levels or glomerular filtration rate (GFR). This uncertainty arises because S1P is primarily metabolized through enzymatic degradation and hepatic uptake rather than renal clearance. While proteinuria might theoretically impact S1P leakage, it is uncertain whether individuals with lower GFR would exhibit greater S1P excretion than those with higher renal function. Nonetheless, we adjusted for creatinine levels, which did not change the observed results. Furthermore, urinary albumin could serve as an informative marker of filtration barrier integrity and glycocalyx injury, given its relevance to endothelial permeability. Thus, an inverse correlation may exist between urinary albumin levels and plasma S1P, with higher urinary albumin potentially reflecting greater glycocalyx degradation and hence reduced S1P stabilization effects on the endothelial barrier. Future studies could incorporate this relationship by prospectively collecting urinary albumin data alongside plasma S1P levels to test this hypothesis more directly.

## Perspectives

This study highlights the potential of S1P as a biomarker for vascular health in severe preeclampsia, offering insights into its relationship with endothelial glycocalyx degradation and disease severity. By uncovering distinct plasma profiles of S1P and glycocalyx components in patients with preeclampsia in need of intensive care, our findings open avenues for advancing diagnostic tools and therapeutic strategies. Future research could explore the role of S1P in predicting long-term cardiovascular outcomes and guiding interventions to mitigate vascular damage in populations with high-risk preeclampsia including different ethnic groups.

## ARTICLE INFORMATION

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## Disclosures

None.

## Supplemental Material

Tables S1–S6  
Figure S1

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