

PERLA-Brazil: A Cohort Study Exploring Preeclampsia and Latent Cardiovascular Risk After Delivery

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Study protocol

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Ethics approval and consent to participate

This study was approved by the Research Ethics Committee at UFMG (CAAE: 4887721.0.0000.5149) and was conducted in accordance with the principles of the Declaration of Helsinki. All participants provided written informed consent prior to enrollment.

26

27 **Availability of data and materials**

28 The datasets used and/or analyzed during the current study are available from
29 the corresponding author on reasonable request.

30

31 **Competing interests**

32 The authors declare that they have no competing interests

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41 I declare that I participated in the conception and design of the work, analysis
42 and interpretation of data and that I have seen and approved the final version. I
43 have no conflicts of interest.

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91

92 **Abstract**

Background: Over the past few decades, many high-income countries have experienced significant reductions in the incidence of preeclampsia (PE). However, this trend has not been mirrored in many low- and middle-income countries, including Brazil. In light of this, it is essential to understand the long-term health outcomes of women who develop PE to better inform health policies and care strategies for these women.

Methods: The Brazilian Cohort Study: Preeclampsia and Latent Risk After Delivery (PERLA-Brazil) seeks to address this gap by recruiting women 6-15 years after a PE diagnosis to assess the development and progression of both clinical and subclinical cardiovascular diseases. In this report, the authors outline the study's primary objectives and key methodological features. The baseline assessment (conducted in 2022–2023) included comprehensive interviews, clinical and anthropometric evaluations, as well as blood and urine sample collection.

Discussion: Long-term storage of these biological samples will support future research into biomarkers predictive of cardiovascular diseases. Participants will be monitored through regular telephone follow-ups, and a second round of in-person assessments — including interviews, clinical exams, anthropometric measurements, and biospecimen collection — is planned for 2027, five years after the baseline evaluation.

Keywords: Preeclampsia; Hypertension; Cardiovascular risk; Maternal mortality; Pregnancy; Epidemiologic methods; Cohort studies.

1 BACKGROUND

Preeclampsia (PE) is a serious hypertensive disorder of pregnancy that can affect multiple organ systems. In the general population, the prevalence of PE is 2–6% [1], and it remains one of the leading causes of maternal mortality and morbidity in Brazil and worldwide [1]. In such cases, the diagnosis is based on the presence of hypertension accompanied by one or more severe clinical features [2].

Despite the growing recognition that a history of PE is a significant risk factor for CVD, no specific follow-up strategy has been established to prevent CVD in these women, and the optimal timing to initiate protective measures remains unclear [3]. Current postpartum clinical guidelines for women with a history of PE typically recommend lifestyle changes and regular monitoring of blood pressure, glucose, and cholesterol—strategies adapted from general CVD prevention [4].

Although these recommendations effectively reduce CVD risk in the general population and remain beneficial for women who had normotensive pregnancies [5], the high incidence of chronic hypertension and other cardiovascular conditions following PE underscores the need for personalized postpartum guidelines.

Identifying women at higher risk of CVD and developing effective prevention strategies requires a deeper understanding of the molecular mechanisms linking PE to cardiovascular dysfunction — a relationship that remains poorly defined [5–7]. Strong and growing evidence indicates that endothelial damage from PE often persists after delivery [8]. As Muijsers [8] states, “PE may be considered a first cardiovascular event that requires secondary prevention and appropriate follow-up”. Despite this, few studies have explored subclinical markers to detect ongoing endothelial dysfunction in women with a history of PE.

A major limitation in existing research is the inclusion of participants with pre-existing risk factors — such as chronic hypertension, diabetes, obesity, kidney disease, and metabolic syndrome — which overlap with both PE and CVD and may confound findings. Accurate participant selection is therefore crucial. In the present study, careful screening and strict adherence to exclusion criteria were applied to minimize confounding factors.

We hypothesize that women with a history of PE may exhibit subclinical changes, herein referred to as latent CVD, with altered biological markers preceding the onset of clinical disease. Accordingly, we are conducting a retrospective cohort study, named “Preeclampsia and Latent Risk After Delivery – PERLA-Brazil” (in Portuguese, “Pré-eclâmpsia e Riscos Latentes Após o Parto – PERLA-Brasil”), focused on the risk of CVD in Brazilian women with a history of PE. This study aims to contribute to the development of tools for identifying elevated cardiovascular risk, particularly in the subclinical phase, to optimize post-PE management strategies. Here, we present the study’s objectives and main methodological features.

2 MATERIALS AND METHODS

The main objectives of PERLA-Brazil study are to investigate blood and urine biomarkers associated with endothelial function, immune response, hemostasis, biochemical status, and angiogenesis, in women 6 to 15 years after experiencing PE.

2.1 Study design

PERLA-Brazil is a retrospective cohort study involving 205 women from Belo Horizonte (Minas Gerais, Brazil) and its metropolitan area, including 103 with a history of PE and 102 with a history of normotensive pregnancies (NPE).

2.1.1 Study population: recruitment and sample size

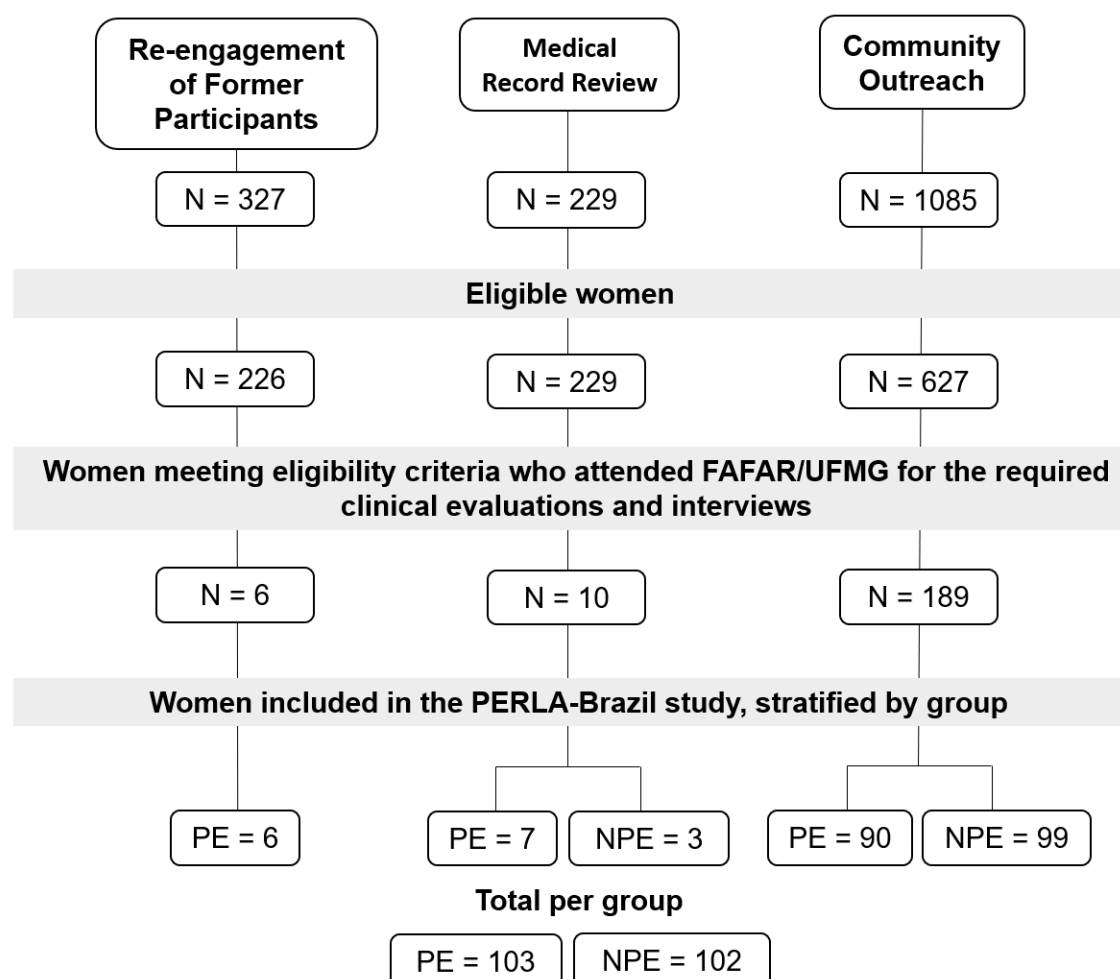
A total of 1,641 women were contacted. Among them, 1,082 fulfilled the inclusion criteria, however, only 205 consented to participate and attended the clinical assessments at the Faculty of Pharmacy (FAFAR), UFMG. The recruitment process was conducted in three main phases:

Phase 1 - Re-engagement of Former Participants: The first approach targeted women who had participated in previous studies conducted by the Preeclampsia Research Group at FAFAR/UFMG between 2008 and 2011 [9,10]. Of the 229 women contacted, only 10 attended clinical assessments, examinations, and interviews at FAFAR/UFMG and were subsequently considered eligible for inclusion in the new study.

Phase 2 – Medical Record Review: Due to challenges in locating former participants and low initial engagement, the recruitment strategy was expanded to include a review of 327 medical records from women diagnosed with PE during the same period (2008-2011) at Júlia Kubitschek Hospital in Belo Horizonte, Brazil (CAAE: 46792921.3.3002.5119). After applying the study's inclusion and exclusion criteria, 226 eligible cases were identified. However, despite multiple phone contact attempts, only six women agreed to participate and completed assessments at FAFAR/UFMG.

Phase 3 – Community Outreach: To further improve recruitment, a broad outreach campaign was launched, utilizing social media, radio announcements, UFMG-wide email distribution, and strategically placed posters. These efforts generated interest from 1085 women, of whom 627 met the inclusion criteria. Ultimately, 189 participants attended FAFAR/UFMG to undergo the required examinations and interviews (Figure 1).

Figure 1 – Flowchart illustrating the recruitment and enrollment process for participants in the PERLA-Brazil study (2022-2023)



FAFAR: Faculty of Pharmacy; PE: Women with preeclampsia history; NPE: Women with normotensive pregnancy history; UFMG: Federal University of Minas Gerais.

Sample size estimation was performed to achieve high statistical reliability, targeting a power of 99% and adopting a significance level of 5%. This estimation was based on the observed variability in low-density lipoprotein cholesterol (LDL-c) values derived from the PERLA-Brazil database, given the established role of LDL-c as a major indicator of cardiovascular risk, as recognized by the American Heart Association and the Brazilian Society of Cardiology [11,12]. Based on these assumptions, a total of 158 participants was considered sufficient, with equal allocation between groups (n = 79 per group).

2.1.2 Inclusion and exclusion criteria

Medical records, biochemical test results from pregnancy, and prenatal and childbirth follow-up records were reviewed for women with a history of PE with severe features, as well as for those who had previous normotensive pregnancies (NPE). Participants were selected based on pre-established inclusion and exclusion criteria. All data were obtained exclusively from medical records. Participants without a prenatal care record and/or hospital discharge summary were excluded from the study.

2.1.2.1 Inclusion criteria

Women aged 18 years or older who experienced a pregnancy occurring between January 1, 2008, and December 31, 2017, were eligible for inclusion in the study.

2.1.2.1.1 Group with history of PE

This group consisted of women with a history of PE meeting severity criteria. Classification followed the criteria established by the American College of Obstetricians and Gynecologists (ACOG) [13], characterizing PE as hypertension arising after 20 weeks of gestation and up to 12 weeks postpartum, with systolic and/or diastolic blood pressure values reaching $\geq 160/110$ mmHg on repeated measurements obtained at rest and separated by at least four hours, in conjunction with signs of target-organ dysfunction.

Evidence of organ involvement included renal, hepatic, hematologic, or neurological abnormalities, as well as persistent or severe clinical manifestations such as proteinuria, thrombocytopenia, elevated liver enzyme levels, impaired kidney function, refractory headache, visual symptoms, epigastric or right upper quadrant pain, nausea, and vomiting. Proteinuria was identified by urinary protein excretion ≥ 300 mg over 24 hours, a urine dipstick result of 2+ or higher, and/or a protein-to-creatinine ratio ≥ 0.3 . Only women who met the severity criteria for PE were included in this study.

2.1.2.1.2 NPE group

The NPE group consisted of women who maintained BP levels below 120/80mmHg throughout their previous pregnancy or pregnancies and had no diagnosis of PE, gestational hypertension, chronic hypertension, or any other high-risk prenatal condition.

2.1.2.2 Exclusion criteria for both groups

Women with a history of autoimmune diseases, coagulation disorders, cancer, diabetes, or chronic hypertension either during or prior to pregnancy were excluded from the study.

2.2 Data collection

The initial phase of participant enrollment, interviews, and clinical assessments was conducted from February 2022 through August 2023. Five years after this initial phase, participants will be invited to take part in a follow-up assessment to evaluate longitudinal changes and outcomes.

Eligible women who agreed to participate provided written informed consent, underwent an interview, and had blood and urine samples collected at FAFAR/UFGM. Telephone follow-up and additional examinations (BP, body, height, body fat percentage, body mass index (BMI) waist, abdominal, and hip circumferences (centimeters) and biochemical markers – Table 2) may be scheduled as the study progresses.

During the visit to FAFAR/UFGM, data were collected on sociodemographic characteristics, obstetric history, family history of disease, lifestyle behaviors, current medication use, and existing health conditions. Chronic disease diagnoses at the time of the study recruitment was established by the continuous use of prescribed medications for these conditions. The key components of the interviews are summarized in Table 1.

Table 1 – Overview of Interview components in the PERLA-Brazil study (2022-2023)

Domain	Variables assessed
Sociodemographic characteristics	Age and race/ethnicity Civil status Nationality Place of birth Educational and occupational history Residential address Telephone number and e-mail address
Medical history	History of cardiovascular disease, diabetes, kidney and liver disease, coagulation disorders, cancer, chronic hypertension, and other relevant chronic illnesses, and medical procedures. Also included: Blood type.
Obstetric history	Number of pregnancies Previous multiple pregnancy Pregnancy years Pregnancy-related complications Medications during pregnancy Delivery method Birth intervals Number of fathers Number of living children Newborn weight and sex Preterm births

	Pregnancy outcomes
Signs and symptoms during pregnancy	High blood pressure ($\geq 160/110$ mmHg, at least two episodes)
	Pulmonary edema
	Medication-resistant headache
	Epigastric pain
	Visual disturbances (scotoma)
	Proteinuria
	Thrombocytopenia
	Elevated serum creatinine (> 1.1 mg/dL)
	Elevated liver enzymes ($\geq 2x$ normal)
Risk factors for PE presented during pregnancy	Preeclampsia in a previous pregnancy
	Family history of preeclampsia
	Primigravida
	Primipaternity
	Chronic kidney disease
	Chronic hypertension
	Maternal age ≤ 18 or ≥ 35
	Long interpregnancy interval (> 10 years)
	Multiple pregnancy
	AB blood type
	Diabetes mellitus
	BMI $\geq 35\text{kg/m}^2$
Reproductive health	Menopausal status
	Contraceptive use

Psychosocial factors during pregnancy	Pregnancy planning Social support Employment Relationship with the baby's father Caregiving support for other children (if applicable) Emotional response to the pregnancy
Dietary and gastrointestinal health	Food consumption patterns Use of dietary supplements or vitamins Food allergies Bowel function
Substance use	Cigarette smoking (past and current), secondhand smoke exposure, consumption of alcohol and other types of recreational drugs (type and frequency)
Medication use	Use of prescription and nonprescription medications in the past month

269

270 Trained investigators conducted a comprehensive set of examinations and
271 measurements using standardized equipment and techniques. The following
272 parameters were collected: BP (millimeters of mercury), body weight (kilograms),
273 height (centimeters), body fat percentage, body mass index (kilograms per
274 square meter), and waist, abdominal, and hip circumferences (centimeters). BP
275 was assessed a single time with the participant seated, following a 10-minute rest

period after arrival, using the Smartpro Q-400 TechLine® automated wrist device [14]. Body mass and body fat composition were measured using a calibrated electronic bioimpedance device (Cadence Smart Care BAL200®), with participants dressed in light clothing and no shoes [15]. BMI was calculated using the formula “BMI = weight / height²”. Waist, abdominal, and hip circumferences were measured with a tape measure following standardized anthropometric [15].

PERLA-Brazil also aims to investigate various biochemical markers. Blood samples (12 mL) were collected by venipuncture without fasting. Both serum samples and plasma samples (collected using heparin, ethylenediaminetetraacetic acid - EDTA, and citrate anticoagulants) were obtained from the participants. EDTA-anticoagulated blood was used immediately after collection for complete blood count, glucose, and glycated hemoglobin analyses. Serum samples were analyzed for glucose, total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides, very low-density lipoprotein (VLDL), non-HDL cholesterol, creatinine, gamma-glutamyl transferase, aspartate aminotransferase, alanine aminotransferase, and uric acid. Citrate plasma was reserved for thrombin generation assays, while heparin plasma will be used for research on novel biomarkers.

A 4-hour urine sample was collected to assess urobilinogen, glucose, ketones, bilirubin, protein, nitrite, pH, blood, density, and leukocytes. Sediment analysis was performed immediately after collection when indicated Table 2 shows the laboratory parameters assessed in all PERLA-Brazil participants. All analyses were performed at UFMG.

300 **Table 2 – Baseline Laboratory Measurements and Methods Used in the PERLA-Brazil**
 301 **Study (2022-2023)**

Analyte			Method/Kit supplier
Complete blood count (CBC)			Automated method (Yumizen H500®)
Glucose			Reagent strips (Glicoo Easyfy®); Enzymatic colorimetric assay (Bioclin®)
Total cholesterol			Enzymatic colorimetric assay (Bioclin®)
High-density lipoprotein (HDL)	cholesterol		Enzymatic colorimetric assay (Bioclin®)
Low-density lipoprotein (LDL)	cholesterol		Enzymatic colorimetric assay (Bioclin®)
Triglycerides			Enzymatic colorimetric assay (Bioclin®)
Very low-density lipoprotein (VLDL)			Calculated using the Martin equation [28]
Non-HDL cholesterol			Calculated using the Martin equation [28]
Creatinine			Enzymatic colorimetric assay (Jaffé method, Bioclin®)
Gamma-glutamyltransferase (GGT)			Enzymatic colorimetric assay (Bioclin®)
Aspartate aminotransferase (AST) and alanine aminotransferase (ALT)			Enzymatic colorimetric assay (Bioclin®)
Uric acid			Enzymatic colorimetric assay (Bioclin®)
Glycated hemoglobin (hemoglobin A1c)			Ion-exchange chromatography (Gold Analisa®)
Urine chemical examination			Reagent strips

302

303 **2.3 Sample processing**304 **2.3.1 Plasma and serum samples**

305 Peripheral blood samples collected in tubes with anticoagulants (EDTA or
306 heparin) and without anticoagulants were centrifuged at 2250 x g for 10 minutes
307 at 4°C using a Heraeus Multifuge X1R centrifuge (ThermoFisher Scientific®)
308 within two hours of collection to obtain the plasma and serum, respectively.

309 Citrate plasma was obtained by centrifugation at 3000 x g for 15 minutes at 4°C
310 using the same Heraeus X1R Multifuge centrifuge within two hours of collection.

311 For each participant, approximately four plasma and serum aliquots of 500 µL
312 were prepared and stored at -80°C in the Biochemistry Laboratory at
313 FAFAR/UFGM.

314

315 **2.3.2 Urine samples**

316 Citrate plasma and urine supernatant were obtained by centrifugation at 3000 x
317 g for 15 minutes at 4°C using the same Heraeus X1R Multifuge centrifuge within
318 two hours after collection. For each participant, approximately four plasma
319 aliquots of 500 µL and two urine supernatant aliquots of with 2000 µL were
320 prepared and stored at -80°C in the Biochemistry Laboratory at FAFAR/UFGM.

321 An additional citrate tube was centrifuged at 5720 x g for 15 minutes at 4°C using
322 a Sigma 2K15 centrifuge®. Approximately one aliquot of 1200 µL plasma with per

participant was prepared and stored at -80°C in the Biochemistry Laboratory (FAFAR/UFMG).

2.4 Analysis of laboratory markers

2.4.1 Hematological assessment

Hematological measure is already performed in PERLA-BRAZIL, in the first round of examinations, using blood collected in EDTA tubes for complete blood count (CBC), conducted immediately after sample collection. Furthermore, we will assess circulating platelet counts, along with advanced platelet parameters, including mean platelet volume (MPV), platelet distribution width (PDW), plateletcrit (PCT), platelet-monocyte aggregates (PMA), and platelet-neutrophil aggregates (PNA). In addition, we will evaluate hematologically derived inflammatory indices such as the Neutrophil-Lymphocyte Ratio (NLR), Derived Neutrophil-Lymphocyte Ratio (dNLR), Lymphocyte-Monocyte Ratio (LMR), Systemic Inflammation Response Index (SIRI), Aggregate Index of Systemic Inflammation (AISI), and Systemic Immune-Inflammation Index (SII).

2.4.2 Immune markers

For immunological profiling, serum samples will be used to quantify a broad panel of chemokines, pro-inflammatory and regulatory cytokines, and growth factors — including CXCL8, CCL11, CCL3, CCL4, CCL2, CCL5, CXCL10, IL-1 β , IL-6, TNF, IL-12, IFN- γ , IL-17A, IL-1Ra, IL-4, IL-9, IL-10, IL-13, PDGF, VEGF, G-CSF and IL-7 using high-throughput microbeads array (Bio-Plex Pro™ Human Cytokine

27-plex Assay, Bio-Rad Laboratories, Hercules, CA, USA) according to the manufacturer's instructions. Understanding the immune and inflammatory landscape is essential in the context of CVD, as these mediators are closely involved in the development and progression of the disease [16,17]. Additionally, the quantification of pro-resolving mediators, such as annexin A1, may offer further insight into the individual's capacity to control and resolve inflammatory processes [18]. The analysis of these immune biomarkers could inform early identification of individuals at increased risk for CVD and may ultimately support the development of targeted preventive strategies.

2.4.3 Extracellular vesicle characterization

Extracellular vesicles (EVs) have been implicated in the pathogenesis of CVD, with elevated circulating levels frequently reported in affected individuals [19–21]. EVs, often referred to as “liquid biopsies”, offer a promising platform for non-invasive diagnostics [22,23]. Beyond their diagnostic potential, EVs may also serve as therapeutic targets or delivery vehicles for interventions aimed at modulating pathological processes underlying CVD [22].

Given their potential clinical relevance, we aim to characterize circulating EVs using flow cytometry (CytoFLEX S, Bechman Coulter, Brea, CA, USA). Experimental design will follow the guidelines outlined in the Minimal Information for Studies of Extracellular vesicles (MISEV2023) [24]. EVs will be labeled using a lipid-binding fluorescent dye (vFRed, Cellarcus Biosciences, San Diego, CA, USA) to enable their identification by flow cytometry. EVs will be first gated on vFRed fluorescence to distinguish them from background noise and non-

vesicular particles. From this vFRed population, we will analyze the surface expression of selected markers to characterize the phenotype and potential cellular origin of the EVs [25]. Specifically, we will assess the expression of the following markers on the membrane of EVs: CD49e, VCAM-1, ICAM-1, VWF, CD62P, CD41b, CD42a, CD51/CD61, CD69, CD142, and CD14. These markers were selected because they play key roles in endothelial dysfunction, inflammation, and coagulation [17] – processes central to the pathophysiology of CVD.

2.5 Machine learning

As a final component of the study, we aim to develop a Machine Learning (ML) model incorporating sociodemographic and clinical data, family history, laboratory results, and vital signs to identify patterns associated with a history of PE and CVD risk. ML techniques have shown great promise in medical research by uncovering complex, non-linear relationships between variables and revealing diagnostic or prognostic patterns that may not be readily apparent through conventional analysis [26]. Using parameters from the PERLA study, we will construct a high-performing ML model for CVD detection. To address limitations in dataset size and variability, we will implement a novel synthetic data generation method (Data Augmentation Strategy – DAS) to enhance data quality and model robustness [27]. The final model will employ a simplified and interpretable ridge regression algorithm, allowing for the identification of meaningful associations between laboratorial parameters and PE history.

2.6 Statistical analysis

The distribution of the data will be evaluated using the Shapiro–Wilk test. Differences between women with previous PE and those with previous normotensive pregnancies will be examined for continuous variables by applying Student's *t* test when normality assumptions are met, or the Mann–Whitney *U* test when distributions deviate from normality. Associations involving categorical variables will be assessed using Pearson's chi-square (χ^2) test. All analyses will be conducted in the R statistical environment (version 4.3.2), adopting a significance threshold of $p \leq 0.05$.

2.7 Study management

As a principal investigator (PI), Professor Patrícia Nessralla Alpoim oversees the overall conduct of the study. The study team includes early-career researchers, senior scientists, and external scientific consultants. The investigators developed detailed operations manuals and led the training of study personnel. A dedicated database was established for data entry and management, and a centralized biorepository was created for the long-term storage of biologic samples.

2.8 Quality assurance

Ongoing training is provided to personnel responsible for the clinical interviews and laboratory examinations, in accordance with the study protocol. All standardized procedures are outlined in detail in the PERLA-Brazil study Operations Manuals.

417

418 2.9 Data management

419 All data are stored on a secure project drive managed by the PI. The data are
420 encoded and protected with password-restricted access, ensuring that only
421 authorized study personnel can access the information. These measures are
422 implemented to safeguard participant confidentiality and ensure full compliance
423 with the Brazilian General Data Protection Law.

424

425 2.10 Ethical considerations

426 Ethical approval for this research was granted by the Research Ethics Committee
427 of UFMG (CAAE: 4887721.0.0000.5149), and all procedures were performed in
428 compliance with the Declaration of Helsinki. Beyond consenting to participate in
429 the study, participants also provided specific authorization for the long-term
430 storage and future use of their biological specimens.

431

432 2.11 Notification of study results and participant referrals

433 At the conclusion of the clinic visit, participants receive summary report containing
434 key health metrics, including height, weight, blood pressure, body fat percentage,
435 waist and hip circumference, as well as urinalysis, complete blood count, and
436 other routine test results. Any findings that raise clinical concern (“alert” results)
437 are promptly communicated to participants and referred to appropriate medical
438 follow-up.

439

3 DISCUSSION

The PERLA-Brazil study holds substantial promise for advancing our understanding of the long-term cardiovascular consequences of PE. This study is uniquely positioned to address the integrate clinical assessments, laboratory data, and sociodemographic information to identify predictive biomarkers and patterns of early cardiovascular changes.

The availability of a wide range of stored biologic specimens — including plasma (collected in citrate, heparin, and EDTA tubes), serum, and urine — greatly enhances the capacity to investigate both established and emerging risk factors for CVD. The insights will be especially relevant given Brazil's shifting demographic and epidemiological landscape, where chronic diseases, particularly CVD, are becoming increasingly prevalent [29].

Despite its many strengths, the study faces certain operational challenges that warrant acknowledgement. Some assessments (e.g., Doppler Ultrasonography) were strongly considered for inclusion in the baseline examination but could not be implemented due to cost and logistic constraints. While the absence of these measures may limit specific analyses, it does not diminish the overall scientific value of the study. In the future, 5 years after the initial recruitment, may incorporate these and other measures, further strengthening the study's ability to evaluate cardiovascular outcomes comprehensively.

4 CONCLUSIONS

In summary, the PERLA-Brazil study will offer valuable insight into the long-term cardiovascular health of women with a history of PE. Its findings will support the

464 development of targeted prevention and control strategies aimed at reducing the
465 burden of CVD in Brazil and other low and middle-income countries.

466

467 **5 LIST OF ABBREVIATIONS**

A1c	Glycated Hemoglobin (Hemoglobin A1c)
AI SI	Aggregate Index of Systemic Inflammation
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
BAL200	Cadence Smart Care BAL200 (Bioimpedance Scale Model)
BMI	Body Mass Index
BP	Blood Pressure
CAAE	Certificate of Presentation for Ethical Consideration
CBC	Complete Blood Count
CCL2	C-C Motif Chemokine Ligand 2
CCL3	C-C Motif Chemokine Ligand 3
CCL4	C-C Motif Chemokine Ligand 4
CCL5	C-C Motif Chemokine Ligand 5
CCL11	C-C Motif Chemokine Ligand 11

CD	Cluster of Differentiation
CVD	Cardiovascular Disease
CXCL8	C-X-C Motif Chemokine Ligand 8
CXCL10	C-X-C Motif Chemokine Ligand 10
DAS	Data Augmentation Strategy
dNLR	Derived Neutrophil-to-Lymphocyte Ratio
EDTA	Ethylenediaminetetraacetic Acid
EVs	Extracellular Vesicles
FAFAR	Faculty of Pharmacy
G-CSF	Granulocyte Colony-Stimulating Factor
GGT	Gamma-Glutamyl Transferase
HDL	High-Density Lipoprotein
HDL-C	High-Density Lipoprotein Cholesterol
ICAM-1	Intercellular Adhesion Molecule 1
IFN- γ	Interferon Gamma
IL	Interleukin
IL-1 β	Interleukin 1 Beta
IL-1Ra	Interleukin 1 Receptor Antagonist

LDL	Low-Density Lipoprotein
LDL-C	Low-Density Lipoprotein Cholesterol
LMR	Lymphocyte-to-Monocyte Ratio
MISEV2023	Minimal Information for Studies of Extracellular Vesicles 2023
ML	Machine Learning
MPV	Mean Platelet Volume
NLR	Neutrophil-to-Lymphocyte Ratio
NPE	Normotensive Pregnancy
PCT	Plateletcrit
PDGF	Platelet-Derived Growth Factor
PDW	Platelet Distribution Width
PE	Preeclampsia
PERLA-Brazil	Preeclampsia and Latent Risk After Delivery – Brazil
PI	Principal Investigator
PMA	Platelet-Monocyte Aggregates
PNA	Platelet-Neutrophil Aggregates
SBC	Brazilian Society of Cardiology
SII	Systemic Immune-Inflammation Index

SIRI	Systemic Inflammation Response Index
TNF	Tumor Necrosis Factor
UFMG	Federal University of Minas Gerais
VCAM-1	Vascular Cell Adhesion Molecule 1
VEGF	Vascular Endothelial Growth Factor
VLDL	Very Low-Density Lipoprotein
VWF	Von Willebrand Factor

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469 **REFERENCES**

- 470 1. Stuart JJ, Gray KJ, Rich-Edwards JW *et al.* Epidemiology of Hypertensive
471 Disorders in Pregnancy. *Chesley's Hypertensive Disorders in Pregnancy*.
472 Elsevier, 2022, 21–43.
- 473 2. ACOG. Gestational Hypertension and Preeclampsia: ACOG Practice Bulletin,
474 Number 222. *Obstetrics and gynecology* 2020;135:e237–60.
- 475 3. Thakkar A, Agarwala A, Michos ED. Secondary Prevention of Cardiovascular
476 Disease in Women: Closing the Gap. *European Cardiology Review* 2021;16,
477 DOI: 10.15420/ecr.2021.24.
- 478 4. Atkinson J, Simpson G, Walker SP *et al.* The long-term risk of cardiovascular
479 disease among women with a history of hypertensive disorders of pregnancy: a
480 systematic review of clinical practice guidelines. *BMC Cardiovasc Disord*
481 2023;23:443.

- 482 5. Pinckard K, Baskin KK, Stanford KI. Effects of Exercise to Improve
483 Cardiovascular Health. *Front Cardiovasc Med* 2019;6, DOI:
484 10.3389/fcvm.2019.00069.
- 485 6. Benshop L, Duvekot JJ, Roeters JE. Future risk of cardiovascular disease risk
486 factors and events in women after a hypertensive disorder of pregnancy. *Heart*
487 2019;105:1273–8.
- 488 7. Virani SS, Alonso A, Aparicio HJ *et al.* Heart Disease and Stroke Statistics—
489 2021 Update. *Circulation* 2021;143, DOI: 10.1161/CIR.0000000000000950.
- 490 8. Muijsers HEC, Roeleveld N, van der Heijden OWH *et al.* Consider
491 Preeclampsia as a First Cardiovascular Event. *Curr Cardiovasc Risk Rep*
492 2019;13:21.
- 493 9. Alpoim PN, Perucci LO, Godoi LC *et al.* Oxidative stress markers and
494 thrombomodulin plasma levels in women with early and late severe
495 preeclampsia. *Clinica Chimica Acta* 2018;483:234–8.
- 496 10. Alpoim PN, Gomes KB, Pinheiro M de B *et al.* Polymorphisms in endothelial
497 nitric oxide synthase gene in early and late severe preeclampsia. *Nitric Oxide*
498 2014;42:19–23.
- 499 11. Arnett DK, Blumenthal RS, Albert MA *et al.* 2019 ACC/AHA Guideline on the
500 Primary Prevention of Cardiovascular Disease: Executive Summary: A Report of
501 the American College of Cardiology/American Heart Association Task Force on
502 Clinical Practice Guidelines. *Circulation* 2019;140, DOI:
503 10.1161/CIR.0000000000000677.

- 504 12. SOCIEDADE BRASILEIRA DE CARDIOLOGIA. *Atualização Da Diretriz*
505 *Brasileira de Dislipidemias e Prevenção Da Aterosclerose*. Moreira LF, Barreto-
506 Filho J, Evora P, et al. (eds.). Catasonho, 2017.
- 507 13. ACOG. ACOG Practice Bulletin No. 202: Gestational Hypertension and
508 Preeclampsia. *Obstetrics & Gynecology* 2019;133:1–1.
- 509 14. Muntner P, Shimbo D, Carey RM *et al.* Measurement of Blood Pressure in
510 Humans: A Scientific Statement From the American Heart Association.
511 *Hypertension* 2019;73, DOI: 10.1161/HYP.0000000000000087.
- 512 15. Priscila Milene Angelo Sanches, Deise Bresan, Patrícia Vieira Del Ré. *Guia*
513 *Prático de Antropometria Para Adultos: Técnicas, Índices e Indicadores.*, 2020.
- 514 16. Dusi V, Ghidoni A, Ravera A *et al.* Chemokines and Heart Disease: A Network
515 Connecting Cardiovascular Biology to Immune and Autonomic Nervous Systems.
516 *Mediators Inflamm* 2016;2016:1–16.
- 517 17. Bhol NK, Bhanjadeo MM, Singh AK *et al.* The interplay between cytokines,
518 inflammation, and antioxidants: mechanistic insights and therapeutic potentials
519 of various antioxidants and anti-cytokine compounds. *Biomedicine &*
520 *Pharmacotherapy* 2024;178:117177.
- 521 18. Vago JP, Tavares LP, Riccardi C *et al.* Exploiting the pro-resolving actions of
522 glucocorticoid-induced proteins Annexin A1 and GILZ in infectious diseases.
523 *Biomedicine & Pharmacotherapy* 2021;133:111033.
- 524 19. Lugo-Gavidia LM, Nolde JM, Carnagarin R *et al.* Association of Circulating
525 Platelet Extracellular Vesicles and Pulse Wave Velocity with Cardiovascular Risk

- 526 Estimation. *Int J Mol Sci* 2022;23:10524.
- 527 20. Gąsecka A, Siniarski A, Duchnowski P *et al.* Leukocyte Extracellular Vesicles
 528 Predict Progression of Systolic Dysfunction in Heart Failure with Mildly Reduced
 529 Ejection Fraction (LYCHEE) - A Prospective, Multicentre Cohort Study. *J*
 530 *Cardiovasc Transl Res* 2025;18:17–27.
- 531 21. Koga H, Sugiyama S, Kugiyama K *et al.* Elevated Levels of VE-Cadherin-
 532 Positive Endothelial Microparticles in Patients With Type 2 Diabetes Mellitus and
 533 Coronary Artery Disease. *J Am Coll Cardiol* 2005;45:1622–30.
- 534 22. Chong SY, Lee CK, Huang C *et al.* Extracellular Vesicles in Cardiovascular
 535 Diseases: Alternative Biomarker Sources, Therapeutic Agents, and Drug Delivery
 536 Carriers. *Int J Mol Sci* 2019;20, DOI: 10.3390/ijms20133272.
- 537 23. Zhou B, Xu K, Zheng X *et al.* Application of exosomes as liquid biopsy in
 538 clinical diagnosis. *Signal Transduct Target Ther* 2020;5:144.
- 539 24. Welsh JA, Goberdhan DCI, O'Driscoll L *et al.* Minimal information for studies
 540 of extracellular vesicles (MISEV2023): From basic to advanced approaches. *J*
 541 *Extracell Vesicles* 2024;13, DOI: 10.1002/jev2.12404.
- 542 25. Sandau US, Duggan E, Shi X *et al.* Methamphetamine use alters human
 543 plasma extracellular vesicles and their microRNA cargo: An exploratory study. *J*
 544 *Extracell Vesicles* 2020;10:e12028.
- 545 26. Chen P-HC, Liu Y, Peng L. How to develop machine learning models for
 546 healthcare. *Nat Mater* 2019;18:410–4.
- 547 27. Pezoulas VC, Zaridis DI, Mylona E *et al.* Synthetic data generation methods

548 in healthcare: A review on open-source tools and methods. *Comput Struct*
549 *Biotechnol J* 2024;23:2892–910.

550 28. Martin SS, Blaha MJ, Elshazly MB *et al.* Comparison of a Novel Method vs
551 the Friedewald Equation for Estimating Low-Density Lipoprotein Cholesterol
552 Levels From the Standard Lipid Profile. *JAMA* 2013;310:2061.