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NOVEL MOLECULAR BIOLOGICAL MARKERS OF PREECLAMPSIA

PhD thesis

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List of abbreviations

ACD	acid citrate dextrose (blood sample tube)
AOPP	advanced oxidation protein products
APC	allophycocyanin
BMI	body mass index
CD	cluster of differentiation
ETT-TUKEB	Ethic Committee of Medical Research Council of Hungary (Egészségügyi Tudományos Tanács - Tudományos és Kutatásetikai Bizottság)
EV	extracellular vesicle
FITC	fluorescein isothiocyanate
FSC	forward scatter
gpIIb/IIIa	glycoprotein IIb/IIIa
HP	healthy pregnant (women)
IQR	interquartile range
ISSHP	International Society for the Study of Hypertension in Pregnancy
IUGR	intrauterine growth restriction
MDA	malondialdehyde
OR	odds ratio
PBS	phosphate buffered saline
PE	preeclampsia
Pe	phycoerythrin
P-EV	platelet-derived extracellular vesicle
PFP	platelet-free plasma
PlGF	placental growth factor
PRDX1	peroxiredoxin-1
ROS	reactive oxygen species
SD	standard deviation
SEM	standard error of mean
sEng	soluble endoglin
sFlt-1	soluble Fms-like tyrosine kinase 1

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SOD	superoxide dismutase
SSC	side scatter
TAC	total antioxidant capacity
TF	tissue factor
Th1	T-helper 1 cell
Th2	T-helper 2 cell
TRX1	thioredoxin-1

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1 Introduction

Preeclampsia is a common and serious complication of pregnancy, affecting approximately 2-8% of all pregnancies worldwide. It is one of the main contributors to fetal and maternal morbidity and mortality. According to the guideline issued by the International Society for the Study of Hypertension in Pregnancy (ISSHP) this condition can be diagnosed after the 20th gestational week by the presence of hypertension accompanied by proteinuria and/or evidence of maternal end organ dysfunction (acute kidney injury, liver dysfunction, neurological features, hemolysis or thrombocytopenia) or fetal growth restriction [1].

Despite extensive research, the exact etiology of preeclampsia remains largely unknown. Multiple factors, including placental dysfunction, antiangiogenic factors, oxidative stress, and maternal immune response have been proposed to play a role in the development of the condition [2-4].

Preeclampsia can be classified into two subtypes: early onset and late onset. The boundary between early and late-onset type is commonly defined as the 34th gestational week. Early onset preeclampsia is largely attributed to impaired placental function, while late onset preeclampsia is likely to be driven by complex interactions between the aging placenta and maternal predisposition to metabolic and cardiovascular disorders. However, the causative factors of preeclampsia may vary among individuals and across the gestational age spectrum [5].

There are numerous known risk factors for preeclampsia, including primiparity, advanced maternal age (>40 years), chronic hypertension, obesity (BMI >30), multiple gestation, a history of preeclampsia in a previous pregnancy or in the family, an interval of more than 10 years since the previous pregnancy, pregestational diabetes mellitus, renal disease, systemic autoimmune disease, antiphospholipid syndrome, thrombophilia (factor V Leiden mutation, protein S and C deficiency), fetal hydrops, molar pregnancy, short duration of sexual relationship (<6 months) and women who underwent assisted reproductive procedures particularly with donor eggs or sperm, or received donor embryo [6]. The risk factors of preeclampsia are summarized in Table I.

Table I. Risk factors of preeclampsia [7, 8] (OR: odds ratio)

Risk factor	**OR**
Antiphospholipid antibody syndrome	9.7
Renal disease	7.8
Prior preeclampsia	7.2
Systemic lupus erythematosus	5.7
Nulliparity	5.4
Chronic hypertension	3.8
Diabetes mellitus	3.6
Multiple gestation	3.5
Strong family history of cardiovascular disease	3.2
Obesity	2.5
Family history of preeclampsia	2.3
Advanced maternal age (>40) for multiparas	2.0
Advanced maternal age (>40) for nulliparas	1.7
Assisted reproductive procedures	1.7

While no single test or group of tests can accurately predict the future development of preeclampsia during the first or second trimester, a combination of maternal risk factors, systemic arterial blood pressure, serum level of placental growth factor (PlGF), and uterine artery blood flow evaluation by Doppler ultrasound may help identify such women who could benefit from aspirin therapy to prevent preterm preeclampsia. The ISSHP advocates for first trimester screening of preeclampsia risk, provided that it is feasible within local healthcare systems, but further research is needed to establish its cost-effectiveness. For women who exhibit strong clinical risk factors for preeclampsia, such as prior preeclampsia, chronic hypertension, antiphospholipid syndrome, pregestational diabetes, maternal BMI over 30 kg/m^2, or conceived by assisted reproduction, ISSHP recommends low-dose aspirin therapy [1].

In a recent systematic review Van Doorn et al. reported that the incidence of preterm preeclampsia was significantly reduced by the use of 150 mg/day aspirin prophylaxis

compared to lower dosages (82-100 mg/day) [9]. In addition, Rolnik et al. recommended that optimally the low-dose aspirin (150 mg/day) therapy should be administered from the 11 to 14th weeks until the 36th week of gestation [10].

The pathophysiology of preeclampsia is an ongoing topic of research and debate, as the processes underlying this disorder are complex and multifactorial, while novel technical advances are identifying additional mechanisms. In recent years, studies have focused on the role of angiogenic factors, immune dysregulation, and abnormal placental evolution in the development of preeclampsia, as well as the potential involvement of oxidative stress and extracellular vesicles [6, 11].

The course of the disease can be explained by two stages. The first stage occurs in the first half of the pregnancy, when the disease is localized to the placenta and no clinical symptoms are present. The earliest detectable alteration is the defective placentation. The placental invasion to the uterine wall is insufficient as the cytotrophoblast fails to properly remodel the distal myometrial segments of spiral arteries. Thus, the resistance arteries retain the contractile medial layer, the placenta remains chronically hypoperfused and hypoxic [12].

The placental malperfusion results in syncytiotrophoblast stress which is a key factor in the development of preeclampsia. When syncytiotrophoblasts become stressed, they release various substances to the maternal circulation. These factors are hypothesized to play a central role in the development of preeclampsia and may be recognized as linking elements between the two stages of the disease [5].

Several types of these linking elements connecting the two stages have been hypothesized, among the most important are the antiangiogenic factors soluble Fms-like thyrosine kinase-1 (sFlt-1) and soluble endoglin (sEng), type-1 T helper (Th1)-associated cytokines and oxidative stress [2, 3].

The second stage of preeclampsia, the feto-maternal clinical syndrome can develop in the second half of the pregnancy [13]. It seems that the key process of developing the clinical syndrome of preeclampsia is mainly the vascular endothelial dysfunction [14, 15]. The two-stage model of preeclampsia depicted in Figure 1**.** was proposed by Robert Taylor and Christopher Redman, and has been continuously adapted to incorporate new knowledge.

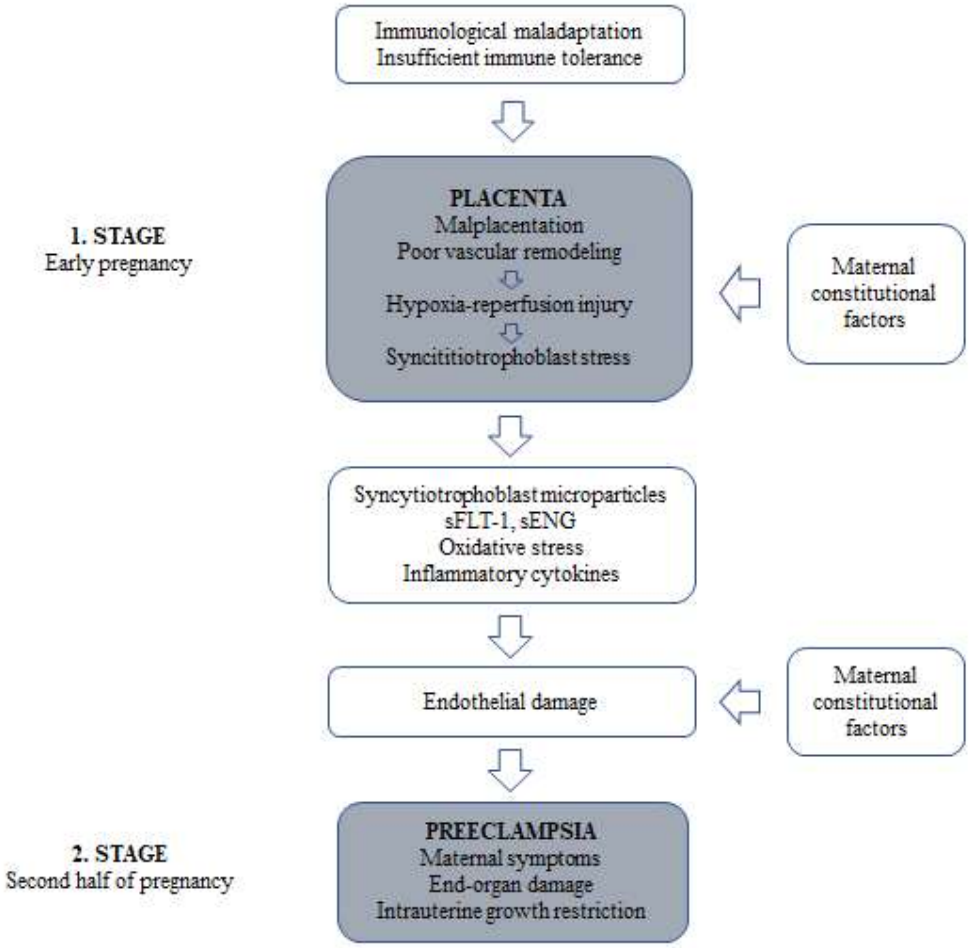

Figure 1. The present view of the two-stage model of preeclampsia depicting its progression over time, based on previous publications [16, 17]

The resulting endothelial dysfunction can cause activation of the coagulation system and lead to a prothrombotic state. In addition, platelet activation and aggregation are increased in preeclampsia, which further contributes to the hypercoagulable state [18]. These changes can increase the risk of thrombotic complications, such as deep vein thrombosis, pulmonary embolism, stroke, and placental thrombosis [19].

Platelet activation also leads to increased production of platelet-derived extracellular vesicles (EVs). The elevated level of platelet derived EVs is generally considered as a marker of platelet activation [20]. During pregnancy, the amount of circulating EVs significantly increases compared to the non-pregnant state. Notably, in preeclampsia, the count of EVs is further elevated, suggesting their potential involvement in the pathogenesis of preeclampsia [21].

The presence of oxidative stress has long been investigated in preeclampsia and it may play a central role in the development of the disease. It is a complex process that involves the generation of free radicals and other reactive oxygen species (ROS), which can cause cellular damage and dysfunction. In preeclampsia, oxidative stress is thought to be caused by a variety of factors, including placental ischemia and inflammation [22].

Several studies have investigated the relationship between oxidative stress and preeclampsia, and have demonstrated that oxidative stress markers are significantly elevated in women with the condition compared to healthy pregnant women. These markers include advanced oxidation protein products (AOPP), malondialdehyde (MDA), and total antioxidant capacity (TAC), among others [3]. However, there is evidence of little or lacking effect of antioxidant therapy in preventing or treating preeclampsia [23].

Peroxiredoxin-1 (PRDX1) and thioredoxin-1 (TRX1) are antioxidant enzymes that are involved in the regulation of oxidative stress [24]. These coupled enzymes carry out the enzymatic reduction of peroxides thus protecting the organism against the toxic effects of peroxides [24]. The schematic representation of their enzymatic activity can be seen in Figure 2. So far they have not been extensively studied in preeclampsia, and their potential role in this disease is not well understood.

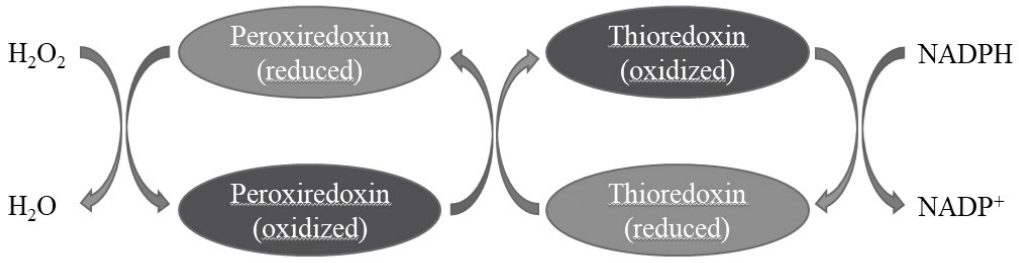

Figure 2. The redox enzymatic activity of peroxiredoxin-thioredoxin enzymes. Modified figure from: [25]

In summary, peroxiredoxins are a family of thiol-dependent peroxidases that play important roles in regulating cellular redox balance and protecting against oxidative stress. Their dysregulation has been implicated in numerous diseases including cancer and neurodegeneration. Overall, peroxiredoxins are essential enzymes in cellular defense mechanisms against oxidative stress and represent promising targets for therapeutic interventions [26].

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2 Objectives

Despite extensive research, the pathogenesis of preeclampsia is still not fully understood. However, it is clear that oxidative stress, endothelial dysfunction, and altered hemostasis significantly contribute to the development and progression of the disease.

Thus we aimed to identify novel biomarkers of preeclampsia by analyzing platelet derived extracellular vesicles (P-EVs) and by investigating the antioxidant systems of circulating lymphocytes and monocytes

Specifically, the objectives of our studies were:

1. To confirm increased platelet activation in preeclamptic women compared to healthy pregnant controls.
2. To determine the levels of platelet-derived extracellular vesicles (EVs) and tissue factor-bearing procoagulant EVs in the plasma of pregnant women with preeclampsia and to compare these levels to those of healthy pregnant women.
3. To confirm the presence of increased oxidative stress by measuring the level of the oxidative stress biomarker AOPP in pregnant women with preeclampsia and to compare its level to those of healthy pregnant women.
4. To investigate the exofacial expression of peroxiredoxin-1 (PRDX1) and thioredoxin-1 (TRX1) regulatory enzymes in lymphocytes and monocytes of pregnant women with preeclampsia and to compare these levels to those of healthy pregnant women.
5. To examine the relationship between oxidative stress and adverse pregnancy outcomes, such as intrauterine growth restriction (IUGR).

Overall, this thesis aims to provide a better understanding of the underlying mechanisms involved in the development of preeclampsia and to identify novel biomarkers for the diagnosis and management of this serious pregnancy complication and thereby giving an insight into the state of art knowledge of the pathogenesis of preeclampsia.

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3 Methods

3.1 Study group

Fifteen preeclamptic patients (PE) and fifteen third trimester healthy pregnant women (HP) were enrolled into the investigation of platelet markers. Third trimester healthy pregnant women (HP) were accepted as control group. Twelve patients with de novo preeclampsia (PE) and seven healthy pregnant controls (HP) were enrolled into the study analyzing the biomarkers of oxidative stress [27].

De novo preeclampsia was defined as a new onset high blood pressure (more than 140 mmHg systolic or 90 mmHg diastolic) in the presence of significant proteinuria according to the ISSHP guideline published in 2014 [28]. Prenatal care (routine obstetrical examination) was provided at the Department of Obstetrics and Gynecology, Semmelweis University in Budapest, Hungary. None of the patients took aspirin nor applied heparin [29].

Intrauterine growth restriction (IUGR) was defined as the birth weight of the newborn being below the 10th percentile corresponding to the gestational age at delivery. Birth weight percentile chart published by the Fetal Medicine Foundation was used to determine the presence of IUGR [30].

The studies were approved by the Ethical Community of the Semmelweis University according to the Helsinki Declaration and were also authorized by the Ethic Committee of Medical Research Council of Hungary (ETT-TUKEB: 10147-4/2015/EKU (93/2014). An informed consent form was signed by every participant before the sample collection.

The clinical data of the patients participating in the study of platelet biomarkers shows significant ($p<0.05$) difference between the control and preeclamptic groups in terms of maternal age, gestational age at birth and birth weight of the neonate, but the gestational age at the time of sample collection was not different [27]. (Table II.)

Table II. Patient data of the study analyzing platelet markers [27]. (n/a = not applicable, ns = not significant)

	Preeclamptic third trimester pregnant women (n=15)	**Healthy third trimester pregnant women (n=15)**	**Significance**
Maternal age (years, mean ± SD)	30.4 ± 5.3	34.4± 3.7	p<0.05
Primiparity (%, n)	53% (8/15)	27% (4/15)	p<0.05
Systolic blood pressure (mmHg, mean ± SD)	158 ± 20.5	114.3 ± 8.6	p<0.05
Diastolic blood pressure (mmHg, mean ± SD)	94.7 ± 14.6	71.1 ± 5.9	p<0.05
Urine protein (mean ± SD)	3490± 1886 mg/24 h	not detectable	-
Early onset PE (%, n)	53,3% (8/15)	n/a	-
HELLP syndrome (%, n)	20% (3/15)	n/a	-
Gestational age at sampling (weeks, mean ± SD)	33.0 ± 4.4	34.1 ± 3.4	ns
Gestational age at birth (weeks, mean ± SD)	33.0 ± 4.2	38.5 ± 1.2	p<0.05
Birth weight (grams, mean ± SD)	1750 ± 891	3584 ± 309	p<0.05
Pre-pregnancy BMI (kg/m2, mean ± SD)	25.3 ± 7.8	22.9 ± 3.2	ns

The clinical data of patients participating in the study of oxidative stress biomarkers show significant (p<0.05) difference between the preeclamptic and control groups in terms of systolic and diastolic blood pressure, gestational age at birth and birth weight of the neonate, whereas maternal age and gestational age at the time of sample collection was not statistically different. (Table III.) None of the patients had clinical signs or laboratory results indicating inflammation [31].

Table III. Patient data of the study analyzing oxidative stress markers [29]. (n/a = not applicable, ns = not significant)

	Preeclamptic third trimester pregnant women (n= 12)	**Healthy third trimester pregnant women (n=7)**	**Significance**
Maternal age (years, mean ± SD)	30.6 ± 3.3	35 ± 5.6	ns
Primiparity (%)	58% (7/12)	43% (3/7)	ns
Systolic blood pressure (mmHg, mean ± SD)	155.0 ± 17.9	114.9 ± 6.3	$p<0.05$
Diastolic blood pressure (mmHg, mean ± SD)	94.7 ± 13.9	71.7 ± 2.9	$p<0.05$
Early onset PE (%)	58.3% (7/12)	n/a	–
HELLP syndrome (%)	25 % (3/12)	n/a	–
Gestational age at sampling (weeks, mean ± SD)	33.7 ± 3.8	35.3 ± 3.9	ns
Gestational age at birth (weeks, mean ± SD)	33.9 ± 3.8	39.3 ± 0.76	$p<0.05$
Birth weight (grams, mean ± SD)	1888 ± 872	3639 ± 371	$p<0.05$
Pre-pregnancy BMI (kg/m2, mean ± SD)	24.9 ± 7.6	21.8 ± 1.56	ns

3.2 Plasma sample collection

The samples were obtained in the third trimester, but always before the onset of labor. Peripheral venous blood samples were collected from the median cubital vein. Vacutainer® Brand Plus Acid Citrate Dextrose (ACD) Tubes of Greiner Bio One International GmbH (Germany) were used for preventing the in vitro platelet activation and EV production [32]. Samples were transferred at room temperature into the flow cytometric laboratory immediately after blood collection. Platelet count was measured by using an automated blood cell counter at the Department of Laboratory Medicine, Semmelweis University [27, 29].

3.3 Measurement of platelet markers

3.3.1 Separation of platelet free plasma for EV measurements

Platelet-free plasma (PFP) was prepared using a 3-step centrifugation procedure: 1) 2000 rpm centrifugation for 5 minutes at room temperature for depletion of peripheral blood cells; 2) repeated centrifugation at 2500 g for 5 minutes at 20°C for preparation of PFP. PFP samples were stored at -80°C for future experiments [27].

3.3.2 Flow cytometric analysis of platelets and P-EVs

Unseparated plasma samples (1:500 dilution in sterile filtered phosphate buffered saline - PBS) were stained by phycoerythrin (Pe) conjugated anti-CD42b (anti-von Willebrand factor receptor antibody) and fluorescein isothiocyanate (FITC) labelled Annexin V for the determination of resting and activated thrombocytes. The gating strategy is depicted in Figure 3.

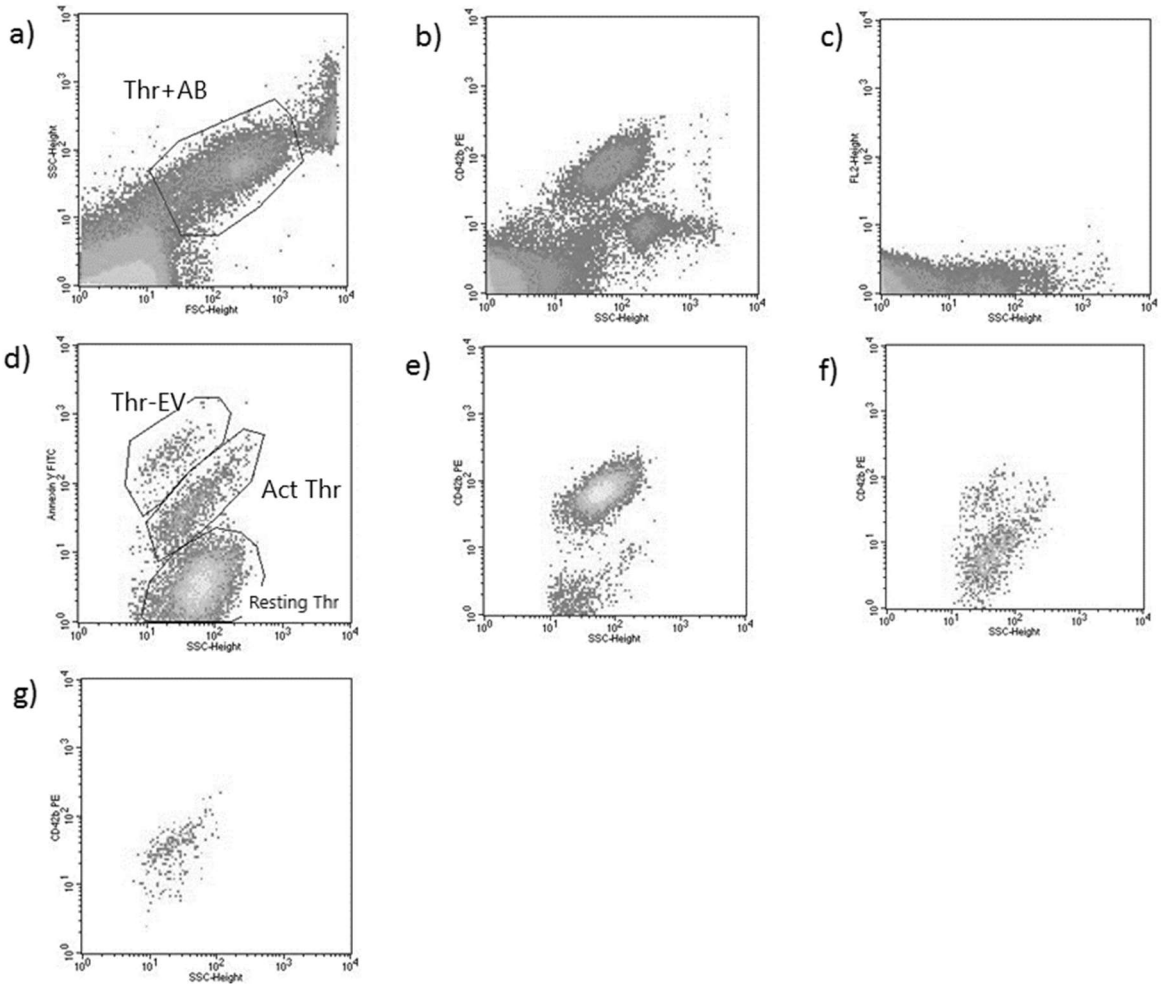

Figure 3. Gating strategy

Logarithmic scale was used for the detection of both forward and side scatter parameters (FS/SS). a) Platelets and circulating apoptotic bodies were gated out on FS/SS dot plot (thr-AB gate). b) Exofacial CD42b expression was compared to FL2 autofluorescence (c) and was used for the identification of platelets. d) Activated platelets were identified by their exofacial phosphatidylserine expression (Annexin V positivity). e-f-g) Annexin V positive platelets and platelet derived vesicles were confirmed by the documentation of their CD42b expression. (Thr-EV = platelet-derived EVs, Act Thr = activated platelets; Resting Thr = resting platelets)

PBS-diluted PFP samples (1:500 dilution in sterile filtered PBS) were used for the identification of platelet derived EVs. Anti-CD41a-FITC (anti-gpIIb/IIIa complex antibody, fluorescein isothiocyanate conjugated), anti-CD42b-Pe (anti-von Willebrand factor receptor antibody phycoerythrin conjugated) were used for the determination of

platelet derived EVs. Activation state of circulating platelet derived EVs were analyzed by anti-CD62P APC (anti-P-selectin antibody, allophycocyanin conjugated), anti-CD142-Pe (anti-tissue factor antibody, phycoerythrin conjugated), anti-CD63-Pe (anti tetraspanin antibody, phycoerythrin conjugated) and Annexin V FITC staining. Anti-CD41a, anti-CD42b, anti-CD62P and anti-CD63 antibodies were manufactured by BD Biosciences Pharmingen (San Jose, CA, USA). Annexin V and anti-CD142 antibody were produced by Sony Biotechnology Inc. (Tokyo, Japan). The „Direct Immunofluorescence Staining of Cells Using a Lyse/No-Wash Procedure" protocol of BD Biosciences was adapted for the staining of exofacial molecules of EVs. The presence of EVs was confirmed by differential detergent lysis [33]. Those events that did not disappear in the presence of 0.1% Triton-X 100 were rejected from analysis. Count Check Beads (Sysmex Partec GmbH) were used as an internal standard for the calculation of absolute number of circulating platelet-derived EVs [27].

Measurements were carried out by using a FACSCalibur flow cytometer (BD, San Jose, CA, USA) on the day of the staining. Forward (FSC) and side scatter parameters were set in log scale, and threshold was set at the SSC parameter. EV gating was accomplished by preliminary standardization experiments using Megamix-Plus SSC beads (Biocytex, France) and was optimized with 1 μm Silica Beads Fluo-Green Green (Kisker Biotech GmbH & Co; Steinfurt, Germany). CellQuestPro software (BD, San Jose, CA, USA) was used for both the acquisition and analysis [27].

Circulating platelets were defined on the basis of their CD42b expression, while activated and resting thrombocytes were distinguished by the exofacial presence or absence of phosphatidylserine (Annexin-V positivity) [27].

The procoagulant activity of platelet derived EVs was examined by the presence of tissue factor (TF) on their surface. Circulating platelet derived EVs were demonstrated by exofacial labelling of gpIIb/IIIa complex (CD41a) and von Willebrand factor receptor (CD42b). Exofacial CD62P and CD63 were used for the isolation of platelet derived EVs produced by activated thrombocytes [27].

3.4 Measurement of oxidative stress biomarkers

3.4.1 Isolation of lymphocytes and monocytes from the blood samples

Histopaque-1077 (Sigma, St. Louis, USA) cell separation medium was used for the separation of peripheral blood mononuclear cells (lymphocytes and monocytes) under sterile conditions, as recommended by the supplier. Samples were frozen in the presence of 10% dimethyl sulfoxide containing fetal bovine serum at a final concentration of 2×10^6 cells/mL and were stored at -80°C degrees [29].

3.4.2 Plasma advanced oxidation protein products (AOPP)

Plasma AOPP were determined by spectrophotometry (Thermo/LabSystems Multiscan MS, Artisan Technology Group ®, Champaign, Illinois, United States) as described by V. Witko-Sarsat et al. [34] and modified by E. L. Taylor et al. [35] . Chloramine-T (Sigma, St. Louis, MO) solution which absorbs at 340 nm in the presence of potassium iodide was used for calibration [29].

3.4.3 Flow cytometry of the cells:

Measurements were carried out using a FACSCalibur flow cytometer (BD, San Jose, CA, USA) on the day of the staining, collecting $3x10^4$ cells/tube. Lymphocytes and monocytes were defined on the basis of their size and granularity on forward scatter – side scatter (FSC/SSC) dot plots. CellQuestPro software (BD, San Jose, CA, USA) was used for both the acquisition and analysis. Only those samples have been investigated, in which the viability of peripheral blood mononuclear cells exceeded 95% [29].

3.4.4 Peroxiredoxin-1 (PRDX1) and thioredoxin-1 (TRX1) in lymphocytes and monocytes

Exofacial presence of TRX1 and PRDX1 were detected by flow cytometry using fluorescein-isothiocyanate and phycoerythrine conjugated monoclonal antibodies purchased from Cloud Clone Corporation (USA, Texas). The „Direct Immunofluorescence Staining of Cells Using a Lyse/Wash Procedure” protocol of BD Biosciences was adapted for the staining of exofacial proteins on lymphocytes and monocytes. Cells were fixed with 4% paraformaldehyde solution for 15 min at room

temperature. Cells were incubated with appropriate amounts of fluorochrome conjugated monoclonal antibodies for 15 minutes at room temperature. Unbound antibodies were removed by washing. During the analysis the percentage of exofacial PRDX1 and TRX1 was calculated [29].

3.5 Statistical analysis

Microsoft Excel (2010) software was used for data processing. Statistical differences between the studied groups were tested using “GraphPad Prism 8” software (San Diego, CA). If datasets have passed the test of normality, statistical significances were analyzed by two-tailed Student's t-test, and descriptive statistics have been shown as mean ± standard error of mean (SEM). If datasets have not passed the test of normality, statistical significances were analyzed by the non-parametric Mann-Whitney test, and descriptive statistics have been shown as median and interquartile range (IQR). Regression analysis was performed and correlational coefficients were calculated to explore potential relationships between variables. Statistical significance was defined as $p < 0.05$ [27, 29].

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4 Results

4.1 Measurement of platelet markers

4.1.1 Platelet count in normal pregnancy and preeclampsia

The mean value of platelet count was significantly lower in the preeclamptic group (168.9 G/l ± 19.08, mean ± SEM) compared to healthy pregnant group (242.1 G/l ± 9.90) ($p<0.05$, n=15 in both groups) [27]. (Figure 4.)

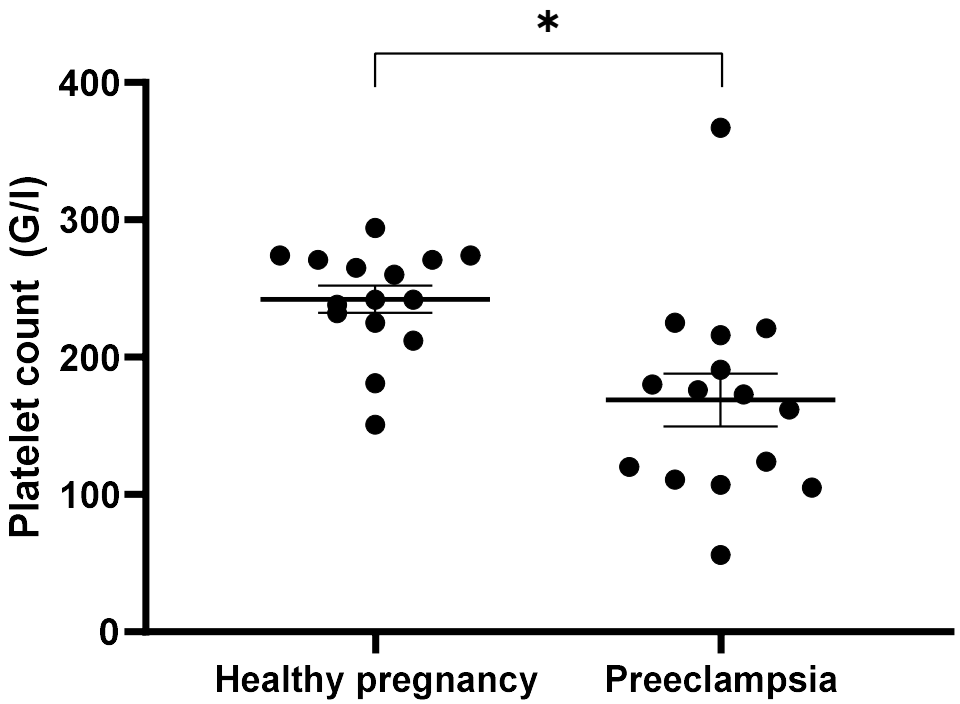

Figure 4. Platelet count in preeclamptic patients and healthy controls (n=15, mean ± SEM) * $p<0.05$

4.1.2 Flow cytometric analysis of activation state of circulating platelets

Significantly higher ratio of activated (CD42b+/AnnexinV+) platelets was detected in the plasma of preeclamptic patients (median: 4.05%, IQR: 1.72-6.48%, n=15) compared to third trimester healthy pregnant women (median: 15.23%, IQR: 2.50-21.19%, n=15)($p<0.05$) [27]. (Figure 5.)

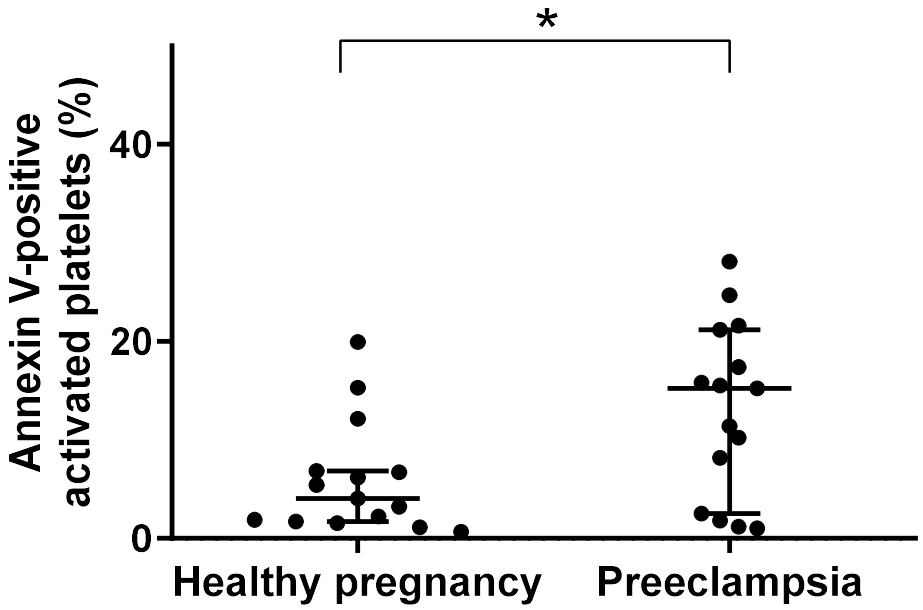

Figure 5. Activation state of circulating platelets in preeclamptic patients (n=15) and healthy controls (n=15) (median and IQR) * $p<0.05$

4.2 Flow cytometric analysis of platelet-derived extracellular vesicles (P-EVs)

4.2.1 Plasma level of tissue factor (TF, CD142) positive P-EVs

Significantly higher amount of procoagulant TF bearing (CD142+) circulating P-EVs was detected in the plasma of preeclamptic patients (20638 events/μL ±7143, mean ± SEM, n=10) than in healthy pregnancy (5283 events/μL ± 788, mean ± SEM, n=13) ($p < 0.03$) [27]. (Figure 6.)

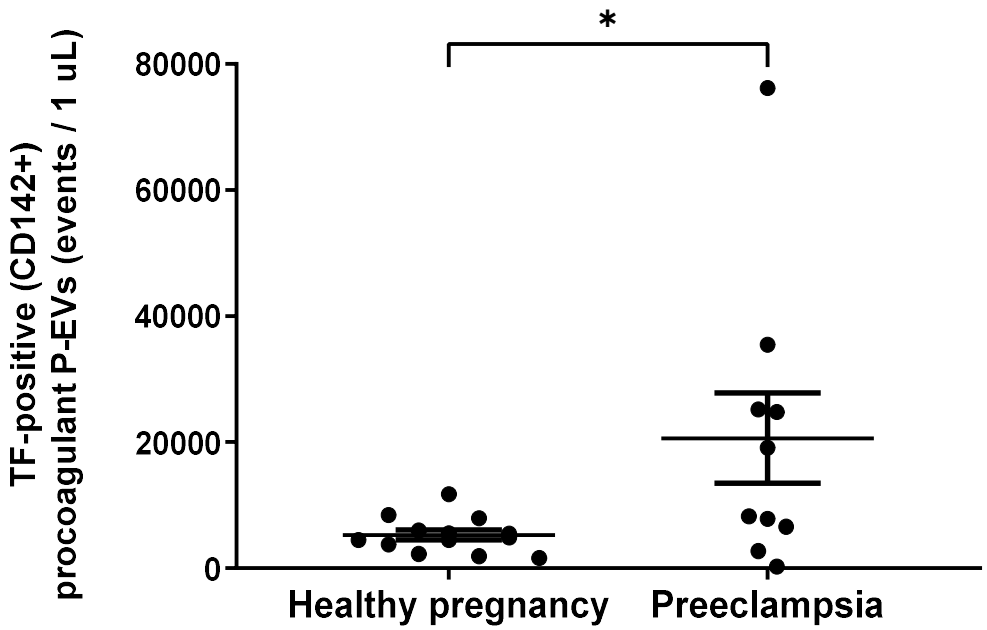

Figure 6. Tissue factor (TF) bearing (CD142+) circulating procoagulant EVs in preeclamptic patients (n=10) and healthy controls (n=13) (mean ± SEM). * $p<0.05$

4.2.2 Plasma level of GpIIb/IIIa complex (CD41a) positive P-EVs

The median value of circulating CD41a+ P-EVs produced by activated thrombocytes was significantly lower in the plasma of preeclamptic patients (median: 11903 events/μL IQR: 8041-19533, n=15) compared to third trimester healthy women (median: 54297 events/μL, IQR: 30395-116438, n=15) ($p<0.05$) [27]. (Figure 7.)

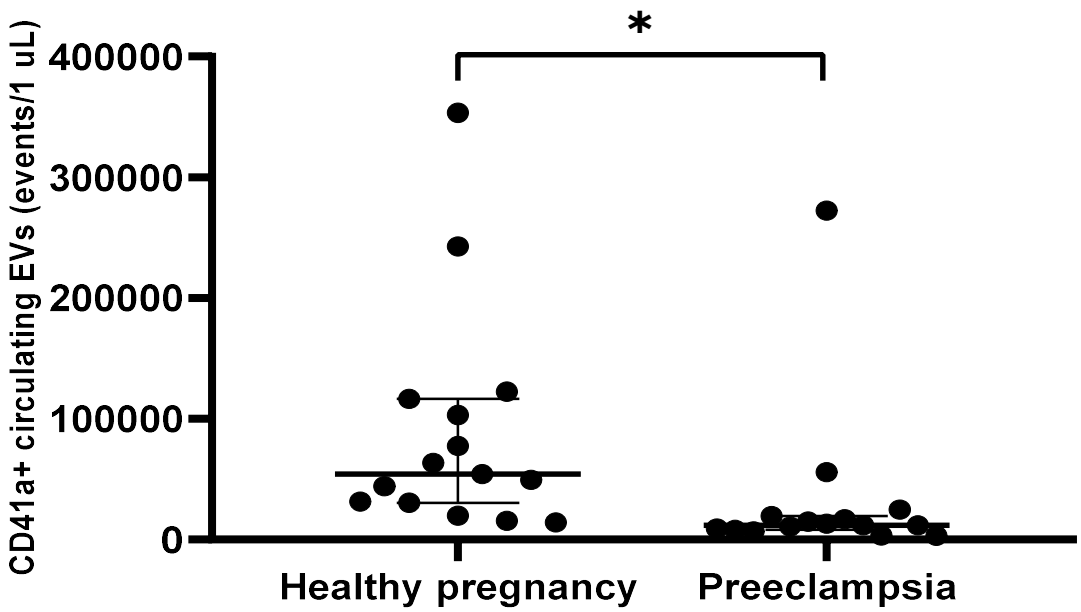

Figure 7. Circulating CD41a+ platelet derived EVs
Significantly lower amount of CD41a+ platelet derived EVs could be detected in the plasma of preeclamptic patients (n=15) compared to third trimester pregnant-matched group (n=15). (median and IQR; *$p<0.05$)

4.2.3 Plasma level of P-Selectin (CD62) positive P-EVs

The absolute amount of circulating CD62P/CD41a double positive P-EVs produced by activated thrombocytes was significantly lower in the plasma of preeclamptic patients (median: 191 events/μL IQR: 62-677, n=15) compared to third trimester healthy women (median: 2717 events/μL, IQR: 997-5445, n=14) ($p<0.05$) [27]. (Figure 8.)

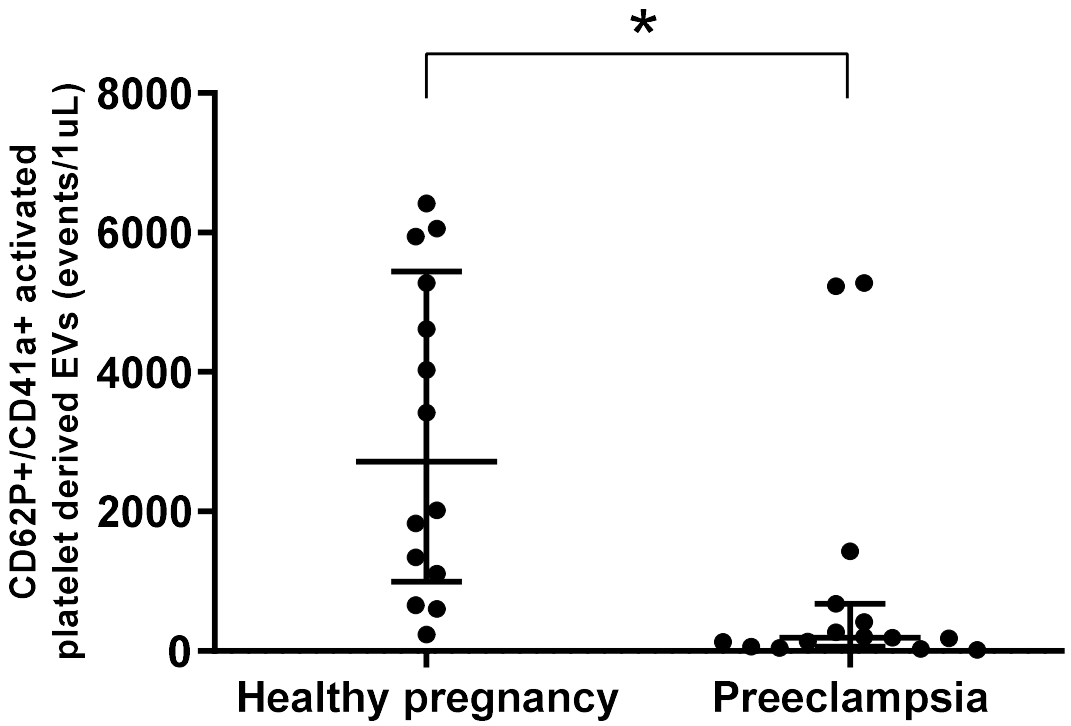

Figure 8. Circulating CD62P+/CD41a+ platelet derived EVs
Significantly lower amount of CD62P+/CD41a+ activated platelet derived EVs could be detected in the plasma of preeclamptic patients (n=15) compared to third trimester pregnant-matched group (n=14). (median and IQR; * $p<0.05$)

4.2.4 Plasma level of tetraspanin (CD63) positive P-EVs

We could observe higher amount of circulating tetraspanin positive (CD63+) P-EVs in preeclamptic patients (median = 4107 events/µL IQR: 2095-19247, n=13) compared to third trimester healthy pregnant women (median: 3352 events/µL, IQR: 1110-5716, n=13), but the difference was not statistically significant p=0.15) [27]. (Figure 9.)

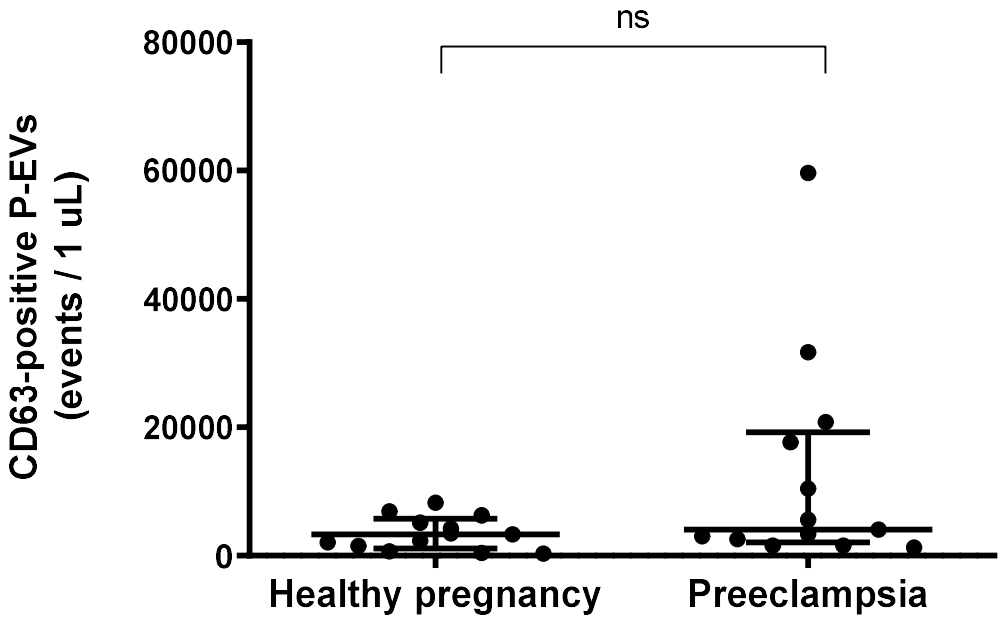

Figure 9. The median amount of CD63 (tetraspanin) positive activated platelet derived extracellular vesicles in preeclampsia (n=13) and healthy controls (n=13). (median and IQR, p=0.15). ns = not significant

4.3 Measurement of oxidative stress biomarkers

4.3.1 Plasma advanced oxidation protein products (AOPP) level

A significantly higher plasma AOPP level was detected in preeclamptic patients compared to healthy pregnant women (mean ± SEM: PE group = 27.97 μM chloramine T equivalents ± 3.6; HP group = 11.75 μM chloramine T equivalents ± 3.7, $p<0.05$) [29]. (Figure 10.)

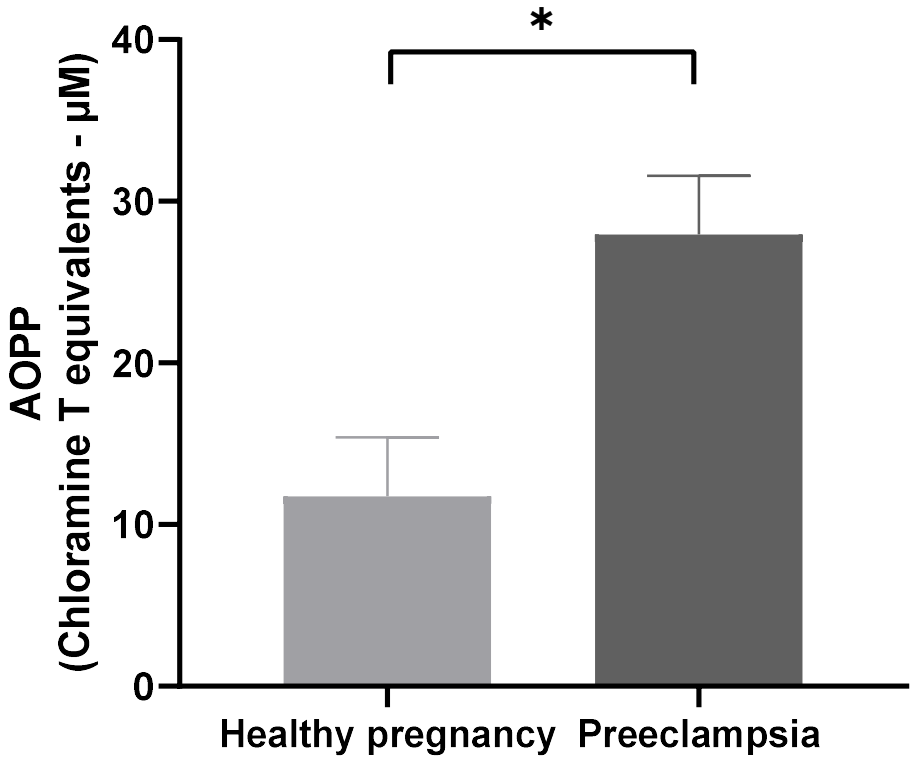

Figure 10. The serum level of advanced oxidation protein products (AOPP)
The serum level of advanced oxidation protein products in healthy (n=7) and preeclamptic (n=11) pregnant women. * $p<0.05$

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4.3.2 The ratio of peroxiredoxin-1 positive lymphocytes and monocytes

The ratio of PRDX1 positive circulating lymphocytes in the third trimester was significantly higher in patients with preeclampsia (mean ± SEM: 73.64% ± 4.9) compared to healthy pregnant women (mean ± SEM: 29.97% ± 6.1, $p<0.05$) [29]. (Figure 11.)

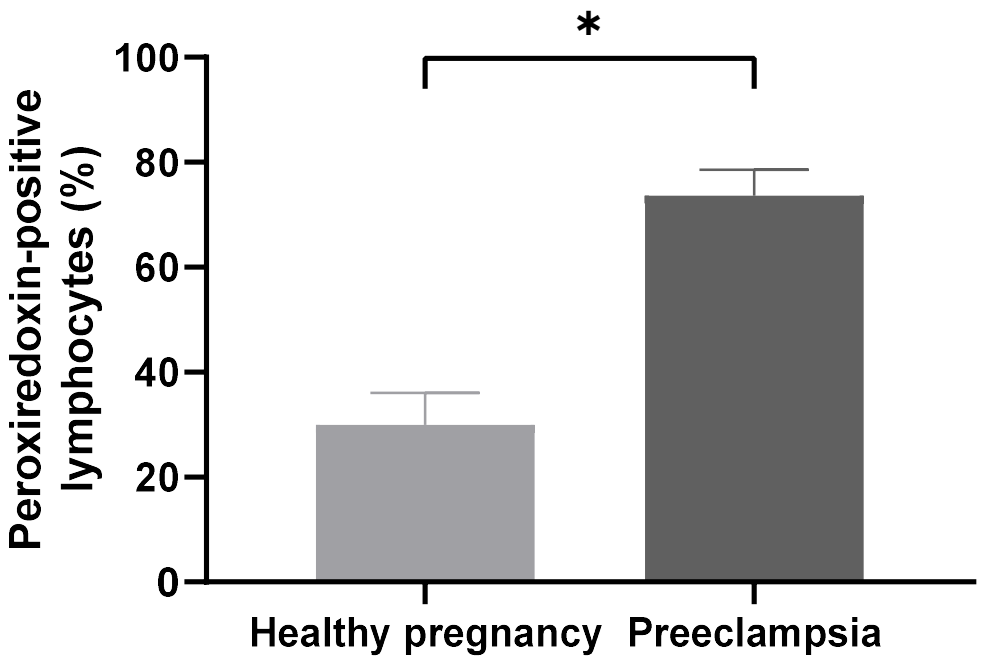

Figure 11. The ratio of PRDX1-positive lymphocytes in the blood samples of healthy (n=7) and preeclamptic (n=12) pregnant women. * $p<0.05$

Similarly to lymphocytes, a significantly higher ratio of circulating monocytes expressed cell surface PRDX1 in patients with preeclampsia (mean ± SEM: 81.69% ± 5.0) compared to the control group (mean ± SEM: 36.81% ± 8.0, $p<0.05$) [29]. (Figure 12.)

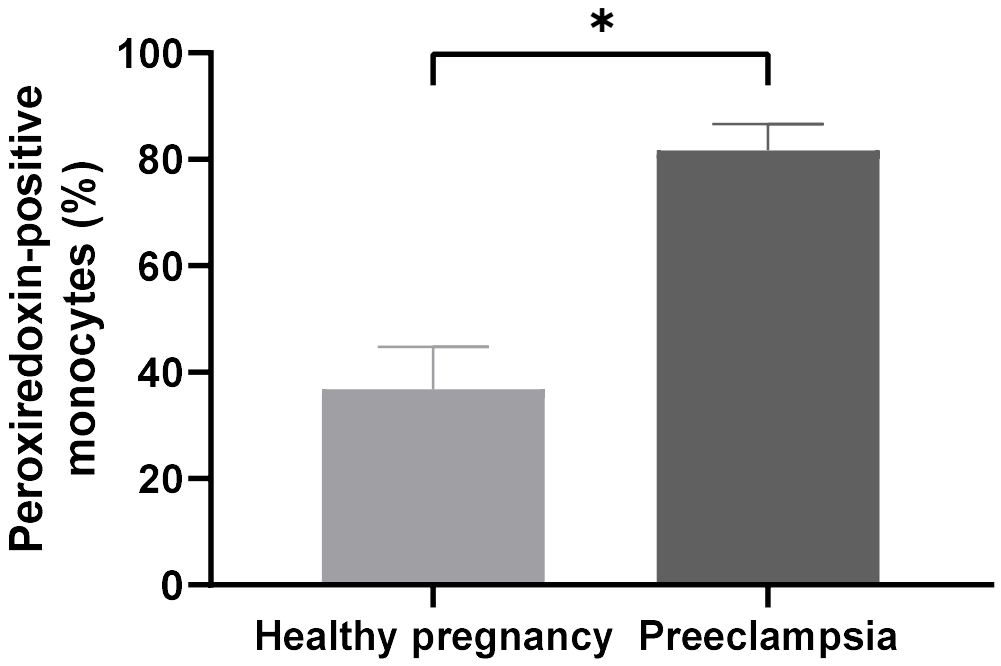

Figure 12. The ratio of PRDX1-positive monocytes in the blood samples of healthy (n=7) and preeclamptic (n=12) pregnant women. * $p<0.05$

4.3.3 The ratio of thioredoxin-1 positive lymphocytes and monocytes

The ratio of circulating lymphocytes that carried cell surface TRX1 was significantly higher in preeclamptic patients (mean ± SEM: 80.72% ± 3.9) compared to the third trimester healthy pregnant women (mean ± SEM: 16.67% ± 4.0, $p<0.05$) [29]. (Figure 13)

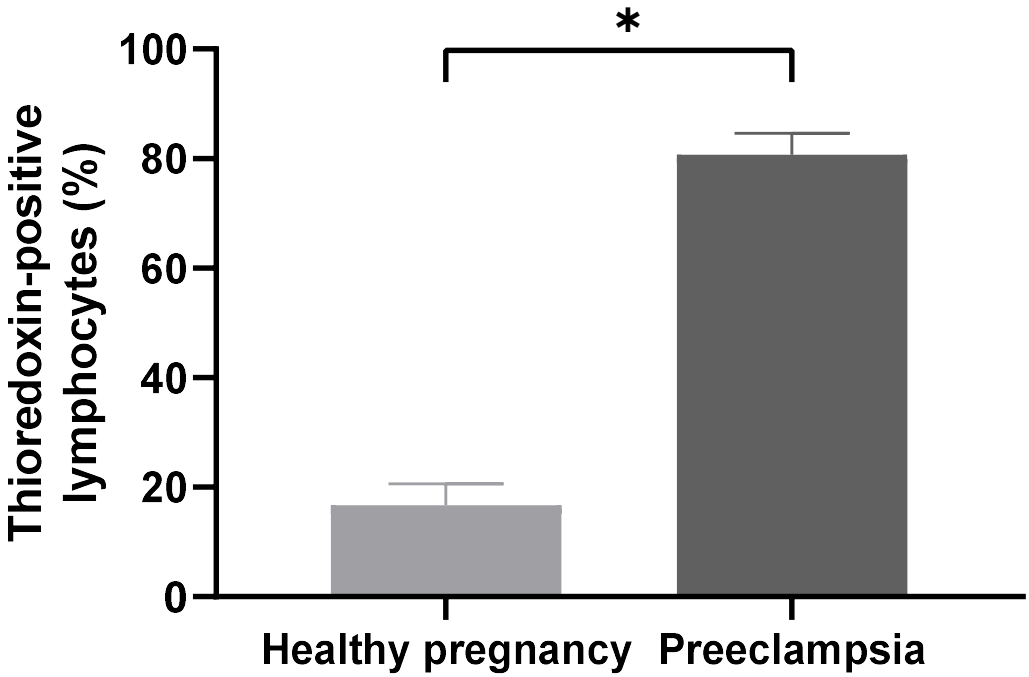

Figure 13. The ratio of TRX-positive lymphocytes in the blood samples of healthy (n=7) and preeclamptic (n=12) pregnant women. * $p<0.05$

We have also found that a significantly higher ratio of circulating monocytes carried cell surface TRX1 in preeclamptic patients (mean ± SEM: 89.10% ± 2.8) compared to the control group (mean ± SEM: 31.51% ± 7.0, $p<0.05$) [29]. (Figure 14.)

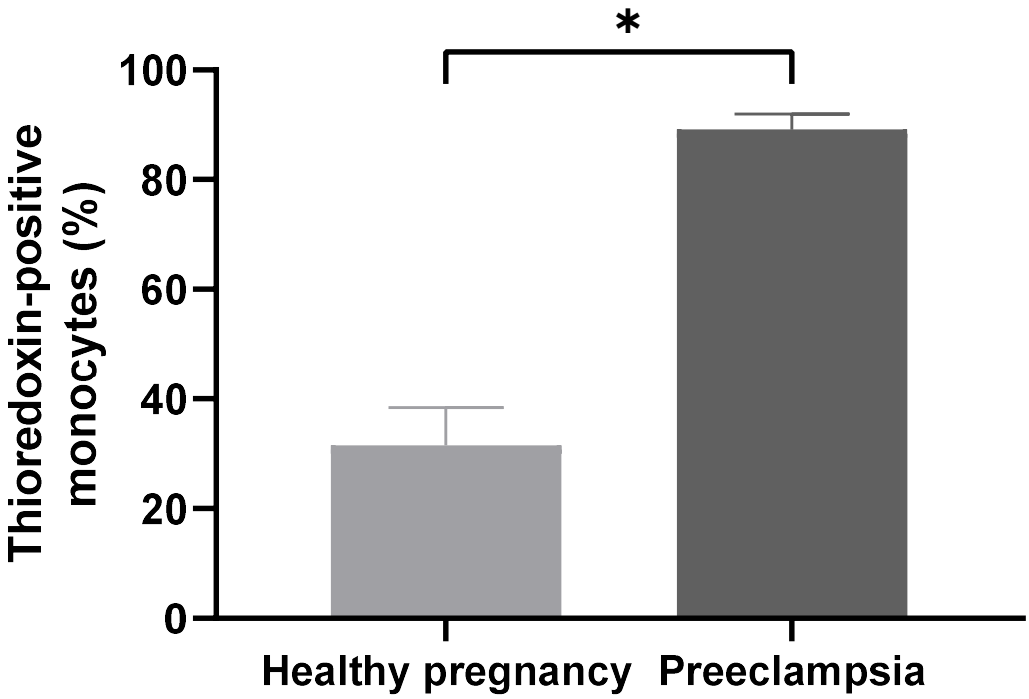

Figure 14. The ratio of TRX-positive monocytes in the blood samples of healthy (n=7) and preeclamptic (n=12) pregnant women. * $p<0.05$

4.3.4 Correlation of the ratio of peroxiredoxin-1 positive lymphocytes and monocytes with the birth weight of newborns

The ratio of PRDX1-positive lymphocytes showed a significant inverse correlation with the birth weight of newborns. (r: -0.88, $p<0.05$) (Figure 15.)

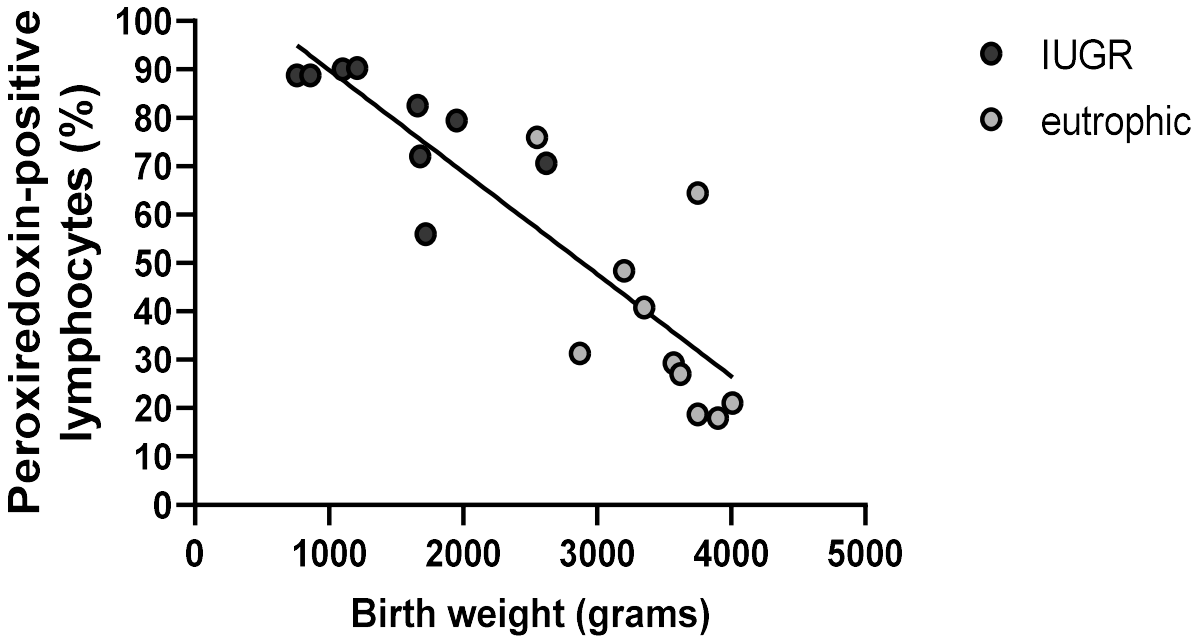

Figure 15. The correlation of the ratio of PRDX1-positive lymphocytes and the birth weight of eutrophic newborns (n=10) and newborns with IUGR (n=9). $p<0.05$, r=-0.88

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Similarly, a significant inverse correlation was observed between the ratio of peroxiredoxin-positive monocytes and the birth weight of newborns [29]. (r: -0.86, $p<0.05$) (Figure 16.)

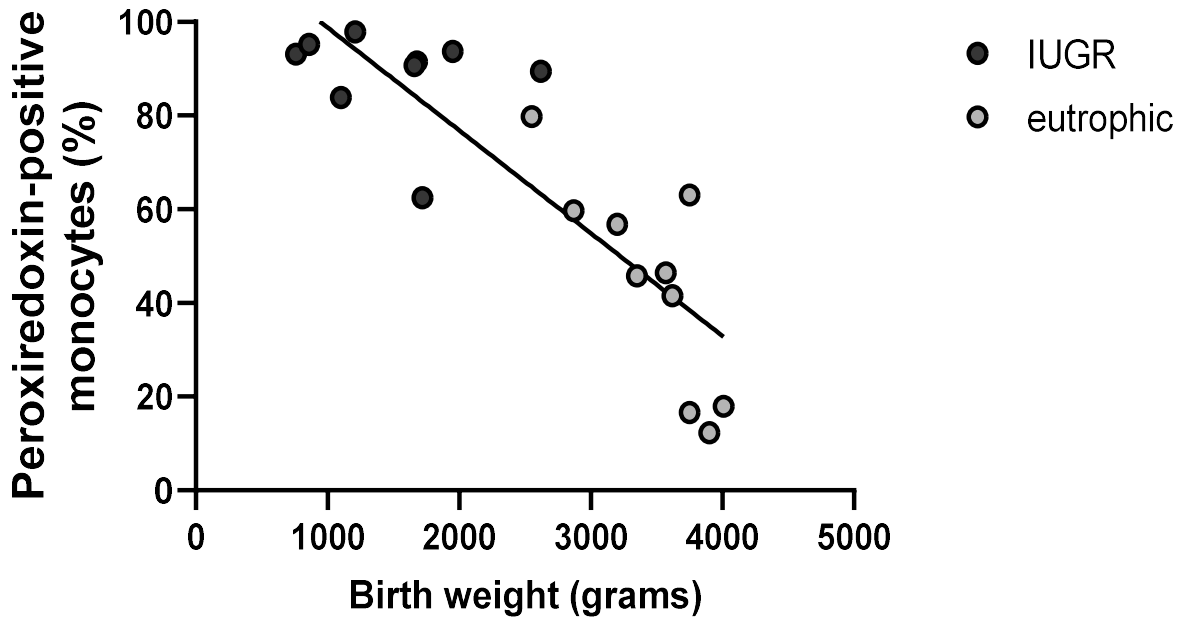

Figure 16. The correlation of the ratio of PRDX1-positive monocytes and the birth weight of eutrophic newborns (n=10) and newborns with IUGR (n=9). $p<0.05$, $r=-0.86$

4.3.5 Correlation of the ratio of thioredoxin-1 positive lymphocytes and monocytes with the birth weight of newborns

We have found that the ratio of TRX1-positive lymphocytes had a significant inverse correlation with the birth weight of newborns [29]. (r: -0.80, $p<0.05$) (Figure 17.)

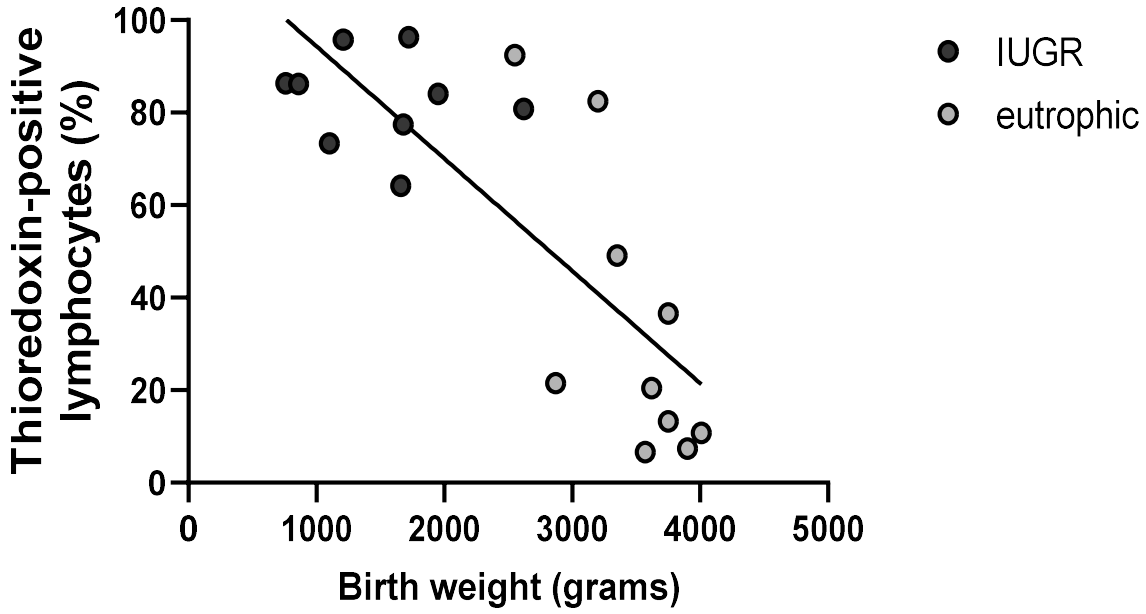

Figure 17. The correlation of the ratio of TRX-positive lymphocytes and the birth weight of eutrophic newborns (n=10) and newborns with IUGR (n=9). $p<0.05$, r=-0.80

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In concert with the above findings, the ratio of TRX1-positive monocytes has shown a significant inverse correlation with the birth weight of newborns [29]. (r: -0.75, $p<0.05$) (Figure 18.)

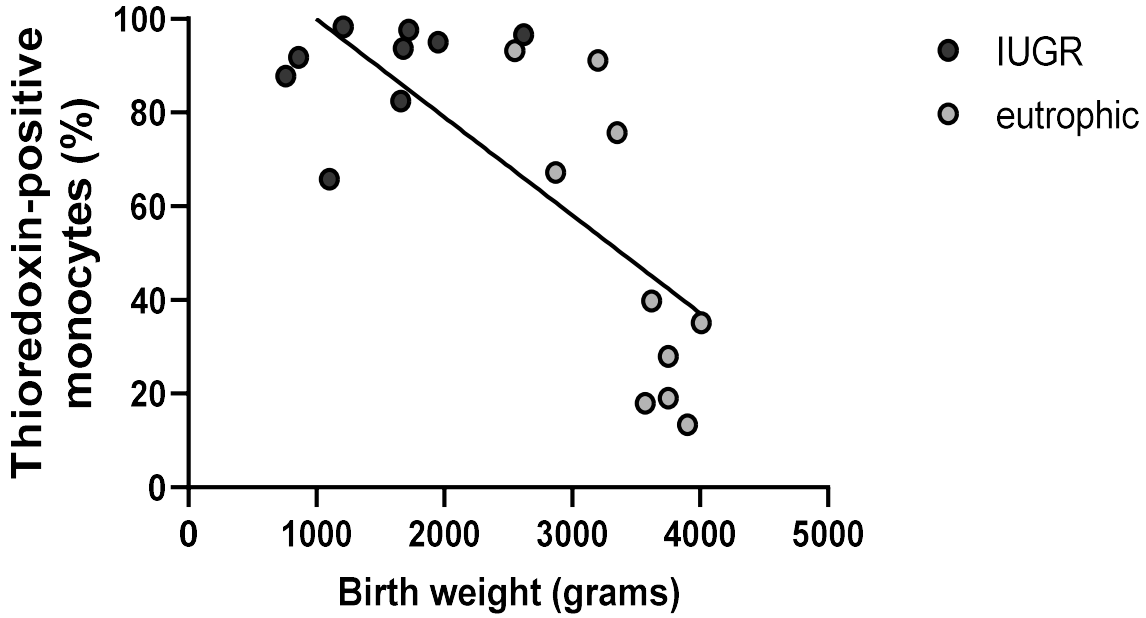

Figure 18. The correlation of the ratio of TRX-positive monocytes and the birth weight of eutrophic newborns (n=10) and newborns with IUGR (n=9). $p<0.05$, $r=-0.75$

5 Discussion

One of the novel discoveries of the study analyzing platelet-derived extracellular vesicles, is that pregnant women with preeclampsia had a significantly higher quantity of tissue factor-positive, thrombogenic platelet-derived extracellular vesicles in their plasma when compared to healthy pregnant women.

The study was conducted on pregnant women with preeclampsia, as determined by their parameters, which were compared to those of pregnancy-matched healthy women and presented in Table I.

Maintaining hemostatic balance is crucial for healthy blood circulation, which can be particularly challenging during pregnancy. Pregnancy is associated with a shift towards a procoagulant state, which can be viewed as a protective mechanism against significant blood loss during and after childbirth [19]. As a consequence healthy pregnant women can develop thromboembolic complications four to five times more often during the pregnancy compared to non-pregnant women [36]. About eighty percent of these thromboembolic events are located on the venous side of circulation and about twenty percent occur in the arteries [19].

Unfortunately, the incidence of preeclampsia is on a steady rise in developed countries due to an increase in maternal age, body-mass index, and associated comorbidities, resulting in an elevated risk of maternal and fetal morbidity and mortality [37]. Women with preeclampsia are at significantly higher risk of experiencing pregnancy-associated venous thromboembolism, with the risk being five times greater than that of the general pregnant population. This increased risk is particularly prominent during the postpartum period [38].

Generalized activation of the coagulation system can complicate severe preeclampsia or HELLP (Hemolysis, Elevated Liver enzymes and Low Platelets) syndrome [39]. Disseminated intravascular coagulation (DIC) is a secondary disorder caused by the over-activation of coagulation system that causes the consumption of coagulation factors, platelets and fibrinolytic proteins leading to uncontrollable,

multifocal bleeding. DIC is mostly associated with adverse maternal outcome, including massive transfusions, hysterectomy, and even death [40].

More than four decades ago, Redman and colleagues reported increased platelet consumption as an early characteristic of preeclampsia [41]. Consistent with this early discovery, our study also showed a significant reduction in platelet count within the preeclamptic group. Furthermore, platelet lifespan in preeclampsia is significantly shorter compared to uncomplicated pregnancy [42]. Several platelet properties, including mean platelet volume (MPV) and platelet distribution width (PDW), are regularly assessed using blood count analyzers and are significantly elevated in preeclampsia when compared to normal pregnancy [43]. There is an inverse correlation between platelet age and MPV, with younger platelets having higher MPV values [44]. The increase in MPV is attributed to rapid turnover and activation of platelets, making larger platelets more susceptible to aggregation[45]. It is also noteworthy that younger platelets exhibit stronger adhesive and thrombogenic functions compared to their older counterparts [46]. A recent meta-analysis provided compelling evidence supporting increased platelet activation in preeclampsia, with MPV found to be significantly elevated in preeclamptic women compared to healthy pregnant women [47].

Platelet-derived extracellular vesicles are generally recognized as markers of platelet activation [20], which triggers several changes in cell surface markers. Normally, phosphatidylserine (PS) is located on the cytoplasmic surface of the platelet membrane; however, platelet activation leads to its externalization. The exposed PS subsequently activates blood coagulation by enhancing the binding of factor X_a to factor V_a [48]. Our study revealed a significantly higher ratio of PS-expressing platelets and tissue factor-expressing platelet-derived EVs in the plasma of preeclamptic women compared to healthy controls, which may explain the hypercoagulable state observed in preeclampsia. Nevertheless, the exact role of EVs in preeclampsia has not yet been entirely elucidated.

In a study by Yi et al., the expression levels of CD41a, CD62P, and CD63 on platelets were investigated, and it was found that their levels were significantly higher in preeclamptic women compared to healthy pregnant women [49]. In our study, we observed a higher expression of CD63 in preeclamptic patients' platelet-derived

extracellular vesicles, although not significantly elevated, which could be due to the relatively low patient number. Interestingly, we found markedly lower amounts of CD41a and CD62P-positive platelet-derived extracellular vesicles in preeclamptic patients compared to healthy pregnant women. These findings may highlight differences in the glycoprotein expression patterns between platelets and platelet-derived extracellular vesicles.

Tissue factor is an integral membrane receptor for coagulation Factor VII that is constitutively expressed by cells surrounding blood vessels, however, it can also be detected on the surface of circulating extracellular vesicles [50]. Both tissue factor-bearing cells and extracellular vesicles are potent activators of the extrinsic coagulation cascade [51]. Previous studies have already detected elevated levels of tissue factor-expressing extracellular vesicles (EVs) in hypertensive disorders of pregnancy, including a higher amount of TF-positive syncytiotrophoblast microvesicles that enhance thrombin formation [52, 53]. It is hypothesized that extracellular vesicles exert their procoagulant effect through their tissue factor content [54]. One of the novel findings of our study is that we detected significantly higher amount of TF-positive platelet derived EVs in preeclampsia for the first time. According to our data, the absolute amount of circulating CD41a+ platelet derived EVs and CD41a+/CD62P+ EVs produced by activated thrombocytes were significantly lower in the plasma of preeclamptic women than in healthy pregnant women.

CD62P is a cell adhesion molecule also known as P-selectin, which plays a crucial role in the initial recruitment of leukocytes, platelet aggregation, platelet-leukocyte interactions, and adhesion to the endothelium as it translocates from cytoplasmic granules to the external cell membrane (40). Previous studies have reported an increase in circulating platelet-monocyte aggregates during preeclampsia [18, 55]. As platelets bind to monocytes predominantly via P-selectin–P-selectin glycoprotein ligand-1 (CD62P-PSGL-1) pathway [56], this may explain the discrepancy between the increased ratio of circulating activated platelets and the decreased CD62P+ P-EV count. The lower count of activated platelet derived EVs may probably be due to the P-selectin mediated aggregate formation in the preeclamptic group and these EVs may be more rapidly cleared

from circulation due to increased consumption and/or clearance by the reticuloendothelial system. Finally, it is possible that there may be differences in the mechanisms of EV release and clearance between preeclamptic and healthy pregnant women that contribute to the observed differences in EV levels.

CD63 is a glycoprotein found in the membrane of lysosomes, belonging to the tetraspanin family. In platelets, CD63 is involved in regulating the function of gpIIb/IIIa, helping to stabilize the newly formed clots (43). Our study found a trend towards an increased amount of CD63-positive platelet-derived extracellular vesicles in preeclamptic samples, which further supports the presence of increased platelet activation in this condition.

These findings support the hypothesis that preeclampsia modifies the procoagulant properties of blood and the quantity of circulating platelet-derived extracellular vesicles through increased platelet activation and tissue factor expression. These alterations are thought to play a role in the hemostatic imbalances observed in preeclampsia, which can result in thrombotic events in both the arterial and venous circulations. In addition, the obstruction of cerebral microvessels may contribute to further increase in systemic blood pressure and seizures seen in eclampsia. Previous studies have also proposed that platelet-derived microparticles could contribute to the development of hypertension by suggesting the existence of a positive feedback mechanism in preeclampsia [50]. In addition, maternal extracellular vesicles and platelets may promote preeclampsia via inflammasome activation in trophoblasts [57].

Recent evidence suggests that extracellular vesicles have the ability to transfer information not only to adjacent areas but also to remote areas of the body by eliciting surface receptor activation and unloading the vesicular cargo into recipient cells through internalization. This has been observed in various pathological conditions associated with tissue hypoxia, acidosis, and oxidative stress, such as cardiac infarction, stroke, and abnormal cell proliferation, including tumor formation. Interestingly, the degree of vesiculation has been shown to increase in a HIF-1α dependent manner in these conditions [58].

The increased release of thrombogenic extracellular vesicles in preeclampsia can lead to a hypercoagulable state as shown in this study. Indeed, the main features of preeclampsia are uteroplacental hypoxia and oxidative stress both of which are present in tumor development pointing to the common mechanism of vesiculation [58]. Our findings may promote further research in this field and will support clinical decision-making regarding diagnosis and treatment and may lead to revision of clinical guidelines.

The second salient finding of this research is related to the investigation of the potential involvement of the peroxiredoxin-thioredoxin system in the development of preeclampsia. On the basis of the present data we propose that the upregulation of this system in lymphocytes and monocytes plays an important protective role against oxidative stress in preeclampsia [29].

Human arterial hypertension has consistently been linked to increased oxidative stress across multiple studies [59, 60]. While causation remains unclear, hypertensive pregnancy-related conditions like preeclampsia or gestational hypertension are known to be associated with an imbalance of redox homeostasis [22, 61] and inflammation [62]. Despite the well-established association between oxidative stress and chronic inflammatory diseases, the underlying mechanism of this relationship remains poorly characterized.

Oxidative stress is defined as an imbalance between the production of reactive oxygen species (ROS) or reactive nitrogen species (RNS) and the antioxidant defenses of the organism [63]. ROS and RNS, due to their high reactivity, have the potential to cause damage to various components of the cell, including DNA, lipids, and proteins resulting in DNA damage, lipid peroxidation, and protein oxidation. As a result, molecules that have been modified by oxidative stress, such as 8-hydroxydeoxyguanosine (8-OHdG), malondialdehyde (MDA), oxidized LDL (oxLDL), and advanced oxidation protein products (AOPP), are often used as markers of oxidative stress [64].

Our study confirmed previous findings by demonstrating a significantly elevated serum AOPP level in preeclamptic women, indicating substantial oxidative stress. In addition to the antioxidant mechanisms previously described, such as SOD and

glutathione, we hypothesized that the peroxiredoxin-thioredoxin system is also affected in preeclampsia, and aimed to investigate this in our study [29].

PRDX1 is an important antioxidant enzyme containing a conserved cysteine in the NH2-terminal region of the protein, which enables the enzymatic conversion of peroxides and protects the organism against the toxic effects of peroxides [24]. Peroxiredoxins regulate various physiological processes by influencing the H_2O_2 mediated signal transduction involved in cell proliferation, maintenance of genome stability, apoptosis, immune response and metabolism [65, 66].

The upregulation of peroxiredoxin by wall shear stress in the luminal surface of endothelium implies that PRDX1 functions as a mechanosensitive antioxidant enzyme, which is critical in regulating the levels of reactive oxygen species in the vasculature [67]. As a result PRDX1 plays a crucial role in reducing endothelial activation and preventing the development of early atherosclerosis [68].

Upon considering these findings, we hypothesized that peroxiredoxin and the coupled enzyme thioredoxin, which have not been extensively studied in the context of preeclampsia, may be affected in this pregnancy complication. As a result, we conducted an analysis of PRDX1 and TRX1 expression in lymphocytes and monocytes of preeclamptic women.

Our results showed an increased ratio of exofacial PRDX1 expressing lymphocytes and monocytes in preeclampsia, which may indicate a compensatory role of PRDX1 in neutralizing reactive oxygen species [29]. Indeed, it has previously been demonstrated that oxidative stress induces the expression of regulatory molecules of redox homeostasis including PRDX1 [24, 69]. Similarly to PRDX1, a higher ratio of circulating exofacial TRX1-expressing lymphocytes and monocytes were detected in patients with preeclampsia [29], indicating its upregulation by micro-environmental factors including enhanced ROS production and inflammatory mediators.

Based on our findings, there is a significant negative correlation between the percentage of lymphocytes and monocytes expressing PRDX1 and TRX1 and the birth

weight of newborns across all the examined groups. These results suggest that there is higher burden of oxidative stress in pregnancies complicated with IUGR and also there is higher compensatory effect of these antioxidant regulatory enzymes.

We have also observed that the lowest ratio of PRDX1 expressing lymphocytes in IUGR was 56%. Therefore, we suggest that a ratio of PRDX1 expressing lymphocytes below 50% could potentially exclude IUGR. However, further studies are necessary to confirm this idea. Our findings are consistent with those of Nakatsukasa et al., who demonstrated a negative correlation between thioredoxin-1 and oxidative stress index, and a positive correlation between redox potential and neonatal birth weight [70].

To the best of our knowledge, our studies were the first to examine platelet-derived extracellular vesicles and the peroxiredoxin-thioredoxin system of peripheral blood mononuclear cells in preeclampsia. Our results are consistent with previous clinical observations and literature data, which provides further validation for our findings.

Our research has some limitations, such as the origin of AOPP, PRDX1 and TRX1 positive peripheral blood mononuclear cells was not investigated, however one can hypothesize that they arise from the pathological placenta. The interval from the onset of disease to diagnosis could not be accurately determined, and due to the cross-sectional nature of our study causation cannot be implied. Also, the low number of cases in some of the experiments limits the power of our findings. Nevertheless, we believe, that these data generated novel hypotheses, which will initiate further studies aimed at alleviating the burden of preeclampsia.

The strengths of this thesis lie in several key areas, which contribute to its overall significance and value. Firstly, the thesis presents novel findings that have not been previously reported. Secondly, these findings hold significant translational potential as they offer opportunities for early detection, monitoring, and management strategies for preeclampsia. These discoveries have the potential to ultimately improve outcomes for both mothers and infants affected by the condition, addressing a critical healthcare need.

Moreover, this research may open new fields for further investigation. The identification of these novel biomarkers and their correlation with adverse pregnancy outcomes, such as thromboembolism and intrauterine growth restriction (IUGR), has the

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potential to inspire future research aimed at exploring the underlying molecular mechanisms and developing targeted interventions. Overall, the strengths of this thesis lie in its novel findings, potential clinical impact, opening of new research fields, and contribution to obstetrics knowledge.

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6 Conclusions

6.1 Novel platelet biomarkers in preeclampsia

In conclusion, the study analyzing platelet biomarkers in preeclamptic and healthy pregnant women confirmed a reduction in platelet count and a higher ratio of activated (phosphatidylserine-expressing) platelets within the preeclamptic group. The findings indicate that pregnant women with preeclampsia have a significantly higher quantity of tissue factor-positive, thrombogenic platelet-derived extracellular vesicles in their plasma when compared to healthy pregnant women. Preeclampsia is associated with a shift towards a procoagulant state, which can lead to thromboembolic complications, and is particularly prominent during the postpartum period. The higher ratio of activated platelets and tissue factor-expressing platelet-derived extracellular vesicles in the plasma of preeclamptic women may explain the hypercoagulable state observed in preeclampsia.

Furthermore, the study found that the amount of CD41a and CD62P-positive platelet-derived extracellular vesicles was lower in preeclamptic patients than healthy pregnant women, which may indicate increased aggregate formation and highlight differences in the exofacial expression patterns between platelets and platelet-derived extracellular vesicles. Finally, the study found a higher expression of CD63 in preeclamptic patients' platelet-derived extracellular vesicles, although not significantly elevated.

These findings provide a better understanding of the pathophysiology of preeclampsia and can be utilized to develop diagnostic and therapeutic interventions to prevent maternal and fetal morbidity and mortality associated with this disease.

6.2 Novel oxidative stress biomarkers in preeclampsia

The study analyzing oxidative stress biomarkers aimed to investigate the involvement of the peroxiredoxin-thioredoxin system in the development of preeclampsia, a pregnancy-related condition associated with oxidative stress and inflammation. The study confirmed the elevated levels of oxidative stress in preeclamptic women and proposed that the upregulation of the peroxiredoxin-thioredoxin system in

lymphocytes and monocytes may play an important protective role against oxidative stress in preeclampsia.

The study found an increased ratio of exofacial PRDX1 and TRX1 expressing lymphocytes and monocytes in preeclamptic women, assuming their upregulation by micro-environmental factors including enhanced ROS production and inflammatory mediators.

The study also found a significant negative correlation between the percentage of lymphocytes and monocytes expressing PRDX1 and TRX1 and the birth weight of newborns across all the examined groups, suggesting a higher burden of oxidative stress in pregnancies complicated with intrauterine growth restriction (IUGR) and a higher compensatory effect of these antioxidant regulatory enzymes. The study suggests that a ratio of PRDX1 expressing lymphocytes below 50% could potentially exclude IUGR.

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7 Summary

This doctoral thesis is based on two case-control studies, which aimed to investigate novel platelet biomarkers and oxidative stress biomarkers of preeclampsia.

We confirmed the reduction in platelet count and the increased ratio of activated platelets in the preeclamptic group, consistent with previous reports. Notably, our findings revealed significantly elevated quantities of tissue factor-positive, thrombogenic platelet-derived extracellular vesicles in the plasma of preeclamptic women compared to healthy controls. These observations suggest a shift towards a procoagulant state in preeclampsia, potentially leading to thromboembolic complications. Additionally, we observed lower levels of CD41a and CD62P-positive platelet-derived extracellular vesicles in preeclamptic patients, indicating increased aggregate formation. Although not significantly elevated, our study also noted higher expression of CD63 in preeclamptic patients' platelet-derived extracellular vesicles.

In relation to oxidative stress biomarkers, our investigation focused on the peroxiredoxin-thioredoxin system. We confirmed elevated levels of advanced oxidation protein products (AOPP) in plasma samples of preeclamptic women, indicating a higher level of oxidative stress. Furthermore, we observed an increased ratio of exofacially expressed PRDX1 and TRX1 in lymphocytes and monocytes of preeclamptic women, suggesting that the upregulation of the peroxiredoxin-thioredoxin system in these immune cells may represent a protective response against oxidative stress in preeclampsia.

In addition, our study revealed a negative correlation between the percentage of lymphocytes and monocytes expressing PRDX1 and TRX1 and the birth weight of newborns, particularly in pregnancies complicated by intrauterine growth restriction (IUGR). This finding suggests a higher burden of oxidative stress in cases of IUGR and highlights a compensatory effect of these antioxidant regulatory enzymes. Moreover, we propose that a ratio of PRDX1-expressing lymphocytes below 50% could potentially serve as a useful indicator for ruling out IUGR. These findings not only enhance our present understanding of the condition but also hold the potential to open new fields for research in the realm of maternal-fetal medicine.

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9 Bibliography of the candidate's publications

9.1 Journal articles related to the dissertation

- **Alasztics B**, Kovács ÁF, Pállinger É, Szabó-Taylor K, Szabó G, Molvarec A, Koller A, Rigó J. (2022) Upregulation of exofacial peroxiredoxin-thioredoxin system of lymphocytes and monocytes in preeclampsia. Pregnancy Hypertens, 31: 54-59.
- **Alasztics B**, Kovács ÁF, Molvarec A, Koller Á, Szabó G, Fekete N, Buzás EI, Pállinger É, Rigó J. (2021) Platelet-derived extracellular vesicles may contribute to the hypercoagulable state in preeclampsia. J Reprod Immunol, 148: 103380.

9.2 Journal articles independent of the dissertation

- Csomó KB, **Alasztics B**, Sándor AP, Belik AA, Varga G, Hrabák A, Kukor Z. (2022) Characterization of oxidation of glutathione by cytochrome c. J Bioenerg Biomembr, 54: 1-8.
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9.3 Poster presentations related to the dissertation

- **Alasztics B**, Kovács ÁF, Koller A, Pállinger É, Rigó J, Jr. Exofacial expression of peroxiredoxin-1 and thioredoxin-1 on peripheral blood mononuclear cells in preeclampsia In: 23rd World Congress of the International Society for the Study of Hypertension in Pregnancy (ISSHP), Montpellier, France, 2022.
- **Alasztics B**, Kovács ÁF, Koller A, Pállinger É, Rigó J, Jr. Increased platelet activation and platelet-derived extracellular vesicles may contribute to intravascular thrombus formation and thus mini-strokes in preeclampsia. In: Frontiers in CardioVascular Biomedicine, Budapest, 2022.
- **Alasztics B**, Kovács ÁF, Joó JG, Fekete N, Buzás EI, Pállinger É, Rigó J, Jr. Platelet derived extracellular vesicles in preeclampsia. In: EuroISSHP Conference for the International Society of Hypertension in Pregnancy, Lund, Sweden, 2019.
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9.4 Poster presentations independent of the dissertation

- **Alasztics B**, Bíró O, Szabó G. A case of partial mole causing early-onset preeclampsia. In: Magyar Szülészeti-Nőgyógyászati Ultrahang Társaság, Szeged, 2019.
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