**Thesis**

# Endothelial Dysfunction
## Overview and current perspectives in the development of pre-eclampsia


submitted by

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*Graz, 1.11.2022* *Michaela Spielhofer m.p*

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# Abbreviations

**ACE**..... Angiotensin converting enzyme

**ACR** ..... Albumin-Creatinine-Ratio

**ADMA**..... Asymmetric Dimethylarginine

**AGE** ..... Advanced Glycation End Products

**ATGL**..... Adipose Triglyceride Lipase

**ATP** ..... Adenosine Triphosphate

**$AT_1$** ..... Angiotensin II Receptor Type 1

**BMI** ..... Body-Mass-Index

**BP** ..... Blood Pressure

**BPM** ..... Beats per Minute

**CAM**..... Cell Adhesion Molecule

**$Ca^{2+}$** ..... Calcium Ions

**Cd4**..... Cluster of Differentiation 4

**CFH**..... Complement Factor H

**CGI-58** ..... Comparative Gene Identification-58

**cGMP** ..... Cyclic Guanosine Monophosphate

**cNK** ..... Cytolytic Natural Killer Cell

**CRP**..... C-reactive Protein

**CTG** ..... Cardiotocograph

**CVD** ..... Cardiovascular Disease

**Cx** ..... Connexin

**CXCL16**..... Chemokine Ligand 16

**DAMP** ..... Damage-associated Molecular Patterns

**DIC**..... Disseminated Intravascular Coagulation

**DNA** ..... Deoxyribonucleic Acid

**dNK**..... Decidual Natural Killer Cell

**EDHF** ..... Endothelium-Derived Hyperpolarizing Factor

**eNOS** ..... Endothelial Nitric Oxide Synthase

**E-selectin** ..... Endothelial Selectin

**ET-1** ..... Endothelin 1

**FFA** ..... Free Fatty Acid

**Gas 6** ..... Growth arrest-specific 6

**GTP**..... Guanosine-Triphosphate

**HDL** ..... High-density Lipoprotein

**HELLP**..... Haemolysis, Elevated Liver Enzyme Levels, Low Platelet Count

**HS**..... Heparan Sulphate

**HtrA4**..... High Temperature Requirement A4

**HUVEC**..... Human Umbilical Vein Endothelial Cells

**ICAM** ..... Intercellular Adhesion Molecule

**IL**..... Interleukin

**I/M ratio** ..... Intima/Media Ratio

**IVF** ..... In-vitro Fertilisation

**LCN** 2..... Lipocalin 2

**LDL**..... Low-density Lipoprotein

**LOX-1** ..... Oxidized Low-density Lipoprotein Receptor 1

**LPL** ..... Lipoproteinlipase

**LR**..... Likelihood Ratio

**L-selectin** ..... Leukocyte Selectin

**MAC**..... Membrane Attack Complex

**MAP** ..... Mean Arterial Pressure

**miRNA** ..... Micro Ribonucleic Acid

**MMP** ..... Matrix Metalloproteinase

**mRNA** ..... Messenger Ribonucleic Acid

**mtDNA**..... mitochondrial Deoxyribonucleic Acid

**NF-κB**..... Nuclear Factor kappa-light-chain-enhancer of activated B Cells

**NO** ..... Nitric Oxide

**NP**..... Normotensive pregnant women

**ox-LDL**..... Oxidised Low-density Lipoprotein

**PAPP-A**..... Pregnancy-associated Plasma Protein A

**PE** ..... Pre-eclampsia

**$PGI_2$**..... Prostacyclin/Prostaglandin 2

**PlGF** ..... Placental Growth Factor

**PON-1** ..... Serum Paraoxonase and Arylesterase 1

**$pO_2$**..... Partial Pressure of Oxygen

**P-selectin**..... Platelet Selectin

**PW**..... Pregnancy Week

**RAAS** ..... Renin-Angiotensin-Aldosterone-System

**RAGE**..... Receptor for Advanced Glycation End Products

**RNA** ..... Ribonucleic Acid

**ROS**..... Reactive Oxygen Species

**RUPP** ..... Reduced Uterine Perfusion Pressure

**sAxl**..... Soluble Axl

**sFlt-1** ..... Soluble fms-like Tyrosine Kinase 1

**STBEV** ..... Syncytiotrophoblast Extracellular Vesicles

**STBMV** ..... Syncytiotrophoblast Micro Vesicles

**sVCAM** ..... Soluble Vascular Cell Adhesion Molecule

**TAM**..... Tyro3, Axl, Mer

**TC**..... Total Cholesterol

**TG** ..... Triglyceride

**TNF-α**..... Tumor Necrosis Factor alpha

**$TXA_2$**..... Thromboxane A2

**VCAM**..... Vascular Cell Adhesion Molecule

**VEGF** ..... Vascular Endothelial Growth Factor

**VEGF-R**..... Vascular Endothelial Growth Factor Receptor

**25-(OH)D** ..... 25-Hydroxvitamin D

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# Zusammenfassung (German Abstract)

**Einleitung:** Präeklampsie ist eine multisystemische Erkrankung, die definiert wird durch erhöhten Blutdruck über 140/90 mmHg und Proteinurie oder andere Organmanifestationen. Obwohl Präeklampsie weltweit eine der häufigsten Ursachen für mütterliche und fetale Morbidität und Mortalität ist, ist die Entstehung noch weitgehend unklar. Plazentare Maladapdation und gestörtes Remodelling von den Spiralarterien verursachen reduzierte uteroplazentare Perfusion (RUPP). Daraus resultiert plazentare Ischämie, die wiederum das Freisetzen von bioaktiven Faktoren stimuliert. Dadurch entsteht ein Ungleichgewicht zwischen pro- und anti-angiogenen Faktoren. Zudem wird eine entzündliche Reaktion und oxidativer Stress getriggert, und folglich endotheliale Schädigung und Dysfunktion. Folglich entsteht eine generalisierte Endotheliose in systemischen, hepatischen, renalen und zerebralen Blutgefäßen, die das Gleichgewicht zwischen Vasokonstriktoren und Vasodiladatoren stört und mitbeteiligt ist, an der Entstehung von Hypertonie. Abschließend ist die Pathogenese von Präeklampsie ein multifaktorielles Geschehen, dessen Verständnis wichtig für Prävention, Diagnose und Therapieoptionen in der Zukunft ist, da diese zu diesem Zeitpunkt noch sehr limitiert sind.

**Die Ziele dieser Arbeit:** Das Ziel dieser Arbeit ist es, einen Überblick über die neuesten Erkenntnisse bezüglich der Pathogenese von Präeklampsie, in besonderer Hinsicht auf endotheliale Dysfunktion und Hypertonie, zu geben.

**Methoden:** Es wurde eine systematische Literaturrecherche bezüglich endothelialer Dysfunktion in prä-eklamptischen Patientinnen durchgeführt. Es wurden nur Publikationen der letzten fünf Jahre inkludiert, um den Fokus wirklich auf die neuesten Entwicklungen zu legen.

**Ergebnisse:** Die Literatursuche ergab 903 Artikel, von denen 40 die Einschlusskriterien erfüllten und ausgewählt wurden.

**Diskussion:** Die Ergebnisse zeigen ein Zusammenspiel aus verschiedenen Faktoren in der Entstehung von Präeklampsie: RUPP ändert die Freisetzung von bioaktiven Faktoren wie zum Beispiel sFlt-1, VEGF, PlGF und auch CAMs, wodurch ein Ungleichgewicht zwischen pro- und anti-angiogenen Substanzen führt. Zusätzlich werden proinflammatorische Cytokine, wie CRP und Interleukine, und ROS freigesetzt, die das Endothel, glatte Muskelzellen und die Glykokalix schädigen. Weiters haben Studien gezeigt, dass die

Bioverfügbarkeit von NO erniedrigt ist und die Vasodilatation beeinträchtigt. Erhöhtes intrazelluläres $Ca^{2+}$ stimuliert die Kontraktion von glatten Muskelzellen und erhöht die Vasokonstriktion somit weiter. Dyslipidämie und Veränderungen im Komplementsystem scheinen auch eine Rolle in der Pathophysiologie von Präeklampsie zu spielen. Neue Faktoren, die als mögliche Indikatoren für die Diagnose oder als therapeutische Targets dienen könnten, wurden entdeckt. Dazu gehören Lipocalin-2, sAxl, Galectin-3, microRNA und HtrA4. Die eingeschränkte Funktion von Matrixmetallproteinasen verursacht arterielle Verkalkung und inadäquates vaskuläres Remodelling, was zu Hypertonie führt. Ziele zukünftiger Forschungsarbeiten sollten eine Anpassung der Diagnosekriterien, neue therapeutische Targets, frühere Diagnose und bessere Prävention sein.

# Abstract

**Introduction:** Pre-eclampsia is a multisystem disorder that combines elevated blood pressure over 140/90 mmHg with proteinuria or any other manifestations. Although pre-eclampsia (PE) is a major threat to maternal and foetal well-being all over the world, its development has not been understood well yet. Placental maladaptation and a disturbed remodelling of uterine arteries cause reduced uteroplacental perfusion (RUPP). As a result, placental ischemia occurs and stimulates the expression of bioactive factors, producing an imbalance between pro- and anti-angiogenic factors. Furthermore, it triggers an inflammatory response and oxidative stress, and subsequent endothelial damage and dysfunction. Consequently, this leads to generalized endotheliosis, in systemic, hepatic, renal and cerebral blood vessels, leading to an imbalance of vasoconstrictors and vasodilators and causing hypertension. In conclusion, the pathogenesis of PE is a multifactorial event, whose understanding is from great importance for prevention, diagnosis and future therapeutical options, as they are still very limited for now.

**Objectives:** The aim of this work is to give an overview on the current literature of the pathogenesis of PE, regarding endothelial dysfunction and hypertension.

**Methodology:** A systematic literature research on endothelial dysfunction in pre-eclamptic patients was conducted. Only articles published in the past five years were included to really focus on the newest insights into this topic.

**Results:** The literature search resulted in 903 articles, of which 40 publications met all the inclusion criteria and were selected.

**Discussion:** Present findings demonstrate an interaction of several factors in the pathogenesis of PE : RUPP changes the expression of bioactive factors like sFlt-1, VEGF, PlGF, as well as CAMs, causing an imbalance in the pro- and anti-angiogenic metabolism. Additionally, proinflammatory cytokines, like CRP and interleukins and reactive oxygen species, are released, damaging the endothelium, smooth muscle cells and the glycocalyx. Furthermore, studies presented a decreased NO bioavailability, causing impaired vasodilatation. Enhanced intracellular $Ca^{2+}$ stimulates contraction in smooth muscle cells, increasing vasoconstriction further. Dyslipidaemia and changes in the complements system also seem to play a part in the pathophysiology. New indicators for diagnosis and/or possible therapeutical targets include lipocalin-2, sAxl, galectin-3 microRNA and HtrA4.

Compromised functions of matrix metalloproteinases cause arterial stiffness and inadequate vascular remodelling, leading to hypertension. Concluding this new found data, future directions could include an adjustment of diagnosis criteria, new therapeutical targets, earlier diagnosis and better prevention

# 1. Introduction

## 1.1 Hypertensive Disorders in Pregnancy

Hypertensive disorders are the main cause of medical complications during pregnancy, affecting 5-10% of women worldwide. The risk for maternal, foetal and neonatal mortality and morbidity is significantly increased. The main foetal risks include intrauterine growth retardation, prematurity and intrauterine death. The mother is at high risk for placental abruption, stroke, disseminated intravascular coagulation and multiple organ failure. (1) Hypertensive disorders in pregnancy are the leading cause of maternal death in Europe, especially pre-eclampsia, which is associated with 70.000 deaths worldwide each year, whereof up to 90% are potentially preventable. (2)

### 1.1.1 Definition and classification of hypertension in pregnancy

Hypertension in pregnancy is defined as blood pressure values over 140/90 mmHg (mild) and over 160/100 mmHg (severe). It is not a single entity, including different characteristics as follows: (1)

Chronic Hypertension comprises preconceptionel hypertension, as well as hypertension diagnosed in the first trimester. (2)

Gestational Hypertension is specified as hypertension occurring after 20 weeks of gestation, with a normotensive pregnancy until then and resolving itself within 12 weeks after delivery. (1-3)

Gestational proteinuria is classified as a newly developed proteinuria above 300 mg/d or an albumin-creatinine ratio over 30 mg/mmol, without any other criteria of pre-eclampsia or an existing renal disorder.

Pre-eclampsia, is defined as any blood pressure elevation over 140/90 mmHg, with at least one other organ manifestation, including proteinuria or any other pathological findings within the kidneys, liver, respiratory system, haematological system, placental function or central nervous system.

The HELLP Syndrome consists of haemolysis, elevated liver enzymes and thrombocytopenia. (2)

## 1.2 Pre-eclampsia

Pre-eclampsia is a multisystem disorder and combines an elevated blood pressure over 140/90 mmHg with proteinuria or any other organ manifestations, which include symptoms like headache, visual disturbances, abdominal pain or abnormal laboratory tests like low platelets or abnormal liver function. (1)

There are two types to differentiate, due to their dissimilar pathophysiology: early-onset PE (< 34. PW) and late-onset PE (>34. PW). (4)

As a special course, the superimposed gestosis combines pre-existing hypertension with newly developed proteinuria or other signs of pre-eclampsia. (1)

### 1.2.1 Diagnosis

The main diagnostical criteria consist of blood pressure measurement and urinalysis. The measurement should be taken in a sitting position, using an appropriately sized arm cuff at heart level and the Korotkoff V for diastolic pressure. (1) In addition, the comparison between both arms helps to objectify the measures and in case of any uncertainties, a 24-h-BP-Measurement should be intended, which also helps to cancel out white-coat hypertension.

For the detection of proteinuria, the albumin-creatinine ratio is used, which is classified as elevated with data over 30 mg/mmol. Another way is the 24-h-urine collection, where the limit of 300 mg/d defines proteinuria. (2) This test, however, is more inaccurate and often delays the diagnosis, therefore the dipstick test is recommended as the first step. Resulting >1+, an ACR should be quantified. (1)

Furthermore, other laboratory tests, like, blood count, haematocrit, liver enzymes, serum creatinine and serum uric acid should be compiled and in addition, a neurological status should be done regularly. (2)

**Table 1.** Diagnostic criteria for pre-eclampsia.

Reproduced from: Task force on Hypertension in Pregnancy (*Hypertension in Pregnancy* 2013) (5)

| | |
|---|---|
| **Blood pressure** | Greater than or equal to 140 mmHg systolic or greater than or equal to 90 mmHg diastolic on two occasions at least 4 hours apart after 20 weeks of gestation in women with a previously normal BP<br>OR<br>Greater than or equal to 160 mmHg systolic or greater than or equal to 100 mmHg diastolic, hypertension can be confirmed within a short interval (minutes) to facilitate timely antihypertensive therapy |
| and | |
| **Proteinuria** | Greater than or equal to 300 mg per 24-hour urine collection (or this amount extrapolated from a timed collection)<br>OR<br>Protein/creatinine ratio greater than or equal to 0,3 mg/dL<br>Dipstick reading of 1+ (used only if other quantitative methods are not available) |
| Or in the absence of proteinuria, new-onset hypertension with the new onset of any of the following: | |
| **Thrombocytopenia** | Platelet count less than 100,00/microliter |
| **Renal insufficiency** | Serum creatinine concentrations greater than 1,1 mg/dL or a doubling of the serum creatinine concentration in the absence of other renal diseases |
| **Impaired liver function** | Elevated blood concentrations of liver transaminases to twice normal concentration |
| **Pulmonary oedema** | |
| **Cerebral or visual symptoms** | |

There are two subdivisions of pre-eclampsia: a severe and a mild form. To distinguish the severe form from the mild form, one or more of the following criteria must be fulfilled as

well: BP over 160/110 mmHg, neurological symptoms, impaired liver function, pulmonary oedema, oliguria, thrombocytopenia or epigastric or right upper quadrant pain. (6,7)

### 1.2.2 Screening

Different screening methods are currently used, but none of them can detect pre-eclampsia efficiently. They are used for risk assessment only and to identify women who are at higher risk and therefore need more thorough supervision.

The screening in the first trimester includes maternal characteristics like age of the mother, risk factors, race and body mass index, as well as an examination of the uterine arteries via doppler ultrasound and the determination of biochemical risk markers, such as Pregnancy-associated Plasma Protein A (PAPP-A) and Placental Growth Factor (PlGF). This algorithm gives a pretty accurate risk evaluation for early-onset pre-eclampsia, however, the overall detection rate in every gestational age is significantly lower.

The screening in the second and third trimesters only comprises BP measurements and dipstick tests, hence there is no evidence showing a benefit for further testing. Nevertheless, for risk evaluation purposes, doppler sonography and the determination of angiogenic factors can be used in addition. The mean pulsatility index is known to be the best marker for pre-eclampsia in the second trimester, with a sensitivity up to 93%. However, in non-risk pregnancies, it only reaches 43%. Additionally, the depiction of the notching phenomenon in the uterine arteries is not recommended, due to highly subjective interpretations.

A screening of all women with the sFlt/PlGF quotient is not recommended either, because of the low prevalence of pre-eclampsia and the little predictive value. Studies have shown, that in low- and high-risk pregnancies, only 30% of cases are predicted. However, in high-risk pregnancies, the quotient can be of great value, because of its high negative predictive value. The quotient is able to cancel out the incidence of pre-eclampsia in the following week in 99,3% of the cases. (2)

### 1.2.3 Risk Factors

There are many different risk factors leading to pre-eclampsia, which can be divided into groups: anamnestic risk factors and pregnancy-associated risk factors. The following tables show an overview of those. LR describes the likelihood ratio. (2)

**Table 2.** Anamnestic risk factors for pre-eclampsia.

Modified from: Guideline of the German Society of Gynecology and Obstetrics (*Hypertensive Pregnancy Disorders* 2019) (2)

| | |
|---|---|
| **Antiphospholipid syndrome** | LR ~ 9 |
| Previous pregnancy with **pre-eclampsia** | LR ~ 7 |
| **BMI** > 30 | LR ~ 3-5 |
| Pre-existing **Diabetes mellitus** | LR 3,5 |
| **Familial predisposition** | LR ~ 3 |
| Pre-existing **renal disease** | LR ~ 3 |
| **First pregnancy** | LR ~ 2,5-3 |
| **Age** > 40 | LR ~ 2 |
| **Chronic hypertension** | LR ↑ |
| - With one other risk factor | LR ~ 1,55 |
| - With 2 other risk factors | LR ~ 3 |
| - BP diastolic > 110 mmHg (< 20 weeks) | LR ~ 3,2 |
| **Autoimmune diseases** | LR ~ 7-9,7 |
| **Ethnicity** (African American) | LR ~ 2 |

**Table 3.** Pregnancy associated risk factors for pre-eclampsia.

Modified from: Guideline of the German Society of Gynecology and Obstetrics (*Hypertensive Pregnancy Disorders* 2019) (2)

| | |
|---|---|
| **Bilateral notching/increased resistance** in Aa. Uterinae (> 90. Percentile, persisting > 20. PW) | LR ~ 3,4-6,5 |
| **Multiple pregnancies** | LR ~ 3 |
| **IVF/egg donation** | LR ↑↑↑ |

### 1.2.4 Risk of repetition

The risk of recurrence for pre-eclampsia after a previous diagnosis is around 11,5-27%, and after two previous events even 32%. The risk is also increased in pregnancies with a previous diagnosis of gestational hypertension to around 2-7% and after suffering eclampsia, the risk for pre-eclampsia in the following pregnancy is elevated to 22-35%. The risk of repetition and also of prognosis are dependent on the gestational age of the manifestation of pre-eclampsia, which is higher, the lower the PW and the severity of the disease. In addition to that, other factors like an elevated BMI or other comorbidities

influence recurrence and outcome. After suffering from pre-eclampsia or HELLP syndrome, the risk for other hypertensive disorders in pregnancy is increased. (2)

### 1.2.5 Prevention

Women at high risk should be taking 150 mg of Acetylsalicylic acid daily, preferably from the twelfth week of pregnancy, until the 37. week. This medication significantly reduces the risks for pre-eclampsia, as well as gestational hypertension. However, the general prescription of Aspirin is not advocated. Any other supplements, like vitamin D or calcium, are not recommended, due to no beneficial effect on the prevention of pre-eclampsia. (2) Vitamin C and E are even counterproductive, because of their association with low birth weight and adverse perinatal outcomes. In addition, a calcium supplement is not useful in populations with adequate intake. (1,5) Furthermore, salt restriction and restriction of physical activity should not be used as primary prevention. (5)

### 1.2.6 Management

The first fundamental step is the identification of the women at high risk for hypertensive disorders during pregnancy, best possible during their first examination. They need to be supervised more closely and also taught to do regular BP measurements at home by themselves. It is also of the utmost importance to sensitise the expectant mothers for symptoms like visual disturbances, upper abdominal pain, nausea, emesis or dyspnoea, which can be prodromes for pre-eclampsia. Stress reduction may be necessary, which can include mandatory medical leave and reduction of exercise. However, strict bedrest is not recommended in general and should be assessed individually. Ultrasound of the foetus should also be done more frequently, than in a non-risk pregnancy. For other laboratory tests see chapter 1.2.1 Diagnosis. (2,3,5)

In case of blood pressure over 140/90 mmHg and other maternal or foetal risk factors, like diabetes mellitus, antiphospholipid syndrome, multiple pregnancies or early gestational age, hospital admission is inevitable. This also applies for BP over 160/110 mmHg, as well as HELLP-syndrome, eclampsia or pre-eclampsia suspicion. (2)

Stationary management includes a thorough primary evaluation of the mother and foetus, to rule out an immediate life-threatening situation for either one of them. The basic evaluation consists of a CTG, BP measurements, and clinical status, including reflex status, especially the patellar tendon reflex, which can indicate hyperreflexia, as a symptom of pre-eclampsia. Additionally, a urine dipstick test, blood work, sonography, including biometry, amniotic

fluid quantity and placental assessment, as well as foetal and maternal doppler checks should be ordered. Continuative diagnostics involve BP monitoring, follow-up of clinical symptoms, like hyperreflexia, cephalea, visual disturbances, disturbance of consciousness, dyspnoea, bleeding tendency and especially upper abdominal pain, proteinuria quantification, monitoring of urine output, respiratory monitoring and laboratory checks. Biometry should be done on the foetus every 14 days, as well as doppler, CTG and Oxford CTG in case of IUGR. (2)

### 1.2.7 Pharmacological management

The goal of treating particularly high BP (over 160/110 mmHg), is to reduce maternal risks, especially for cerebro-/ and cardiovascular complications. (2) However, the agents administrated must offer efficiency, as well as safety for the foetus. (1) Interestingly, there is still no evidence, showing a benefit of reduction in BP for the prognosis or development of the foetus. Furthermore, there is still no worldwide consensus about the treatment of mild hypertension, meaning BP between 140-159/90-109 mmHg. The risk of a too low BP reduction, is possible reduced perfusion of the placenta, resulting in insufficient blood supply for the foetus. Therefore, the recommended diastolic BP is > 80 mmHg and the adjustment of BP should take place hospitalised, according to the 'rules', start low and go slow. (2)

The recommendation of the European Guidelines is an antihypertensive treatment for all women with BP constantly higher than 150/95 mmHg and BP higher than 140/90 mmHg if there is gestational hypertension, pre-existing hypertension with superimposed gestational hypertension or hypertension with organ damage co-diagnosed. (1)

The first choice drug is alpha-methyldopa. Other valid options are labetalol (beta blocker) or nifedipine (calcium antagonist). Strictly contraindicated are ACE inhibitors, angiotensin-renin-blocker and direct renin inhibitors because of possible foetal complications. Dihydralazin may be used in emergencies or when there is no sufficient BP adjustment possible, with the other agents. It is not recommended in general, due to its common maternal side effects like severe headaches and reflex tachycardia.

In emergencies, Urapidil may also be used, as well as the options mentioned above.

For prevention, as well as treatment, of eclampsia, magnesium sulphate is recommended. In particular in severe cases of pre-eclampsia, with central-nervous symptoms, the administration of magnesium sulphate has shown a significant reduction in seizures. (2)

### 1.2.8 Delivery

The delivery is indicated as soon as the 37. week of pregnancy is completed. (1) The prolongation only serves the prevention of premature birth and thus complications for mother and child. However, delivery is the only curative treatment for pre-eclampsia. If there are no other contraindications, vaginal birth is possible. (2) However, the mode of delivery should be decided considering foetal and maternal conditions, gestational age, cervical status and foetal presentation. (5)

From 34+0 PW, delivery is recommended in case of severe pre-eclampsia. Nevertheless, the risks for the mother in prolonging the pregnancy, as well as the risks for the foetus should be evaluated carefully, because there is still a higher rate of neonatal morbidity, as well as respiratory distress syndrome in premature delivery. One should also consider, the increased risk for intrauterine death in case of severe pre-eclampsia, as well as foetal growth restriction, which can occur.

From 24+0 to 33+0 weeks of pregnancy, a conservative procedure is recommended, if there are no severe complications in the mother's health expected. Continuous supervision is indicated, by CTG and doppler of the ductus venosus. With this combination, the right time for delivery is best to be evaluated. Additionally, it is of great importance to re-assess the situation each day and plan an administration of steroids for lung development. Nonetheless, the maternal indications for delivery must not be neglected. These can vary from untreatable chronic hypertension, renal insufficiency, newly onset central nervous symptoms, early placental abruption and other symptoms.

The delivery of a foetus under the gestational age of 24 weeks hast to be an interdisciplinary decision between neonatologists and obstetricians, as well as the parents, due to the very low chance of survival. Therefore, termination of pregnancy must be discussed. (2)

Figures 1 and 2 show an overview of the management of mild and severe pre-eclampsia in form of a flow chart for a clearer presentation of the preceding subsections.

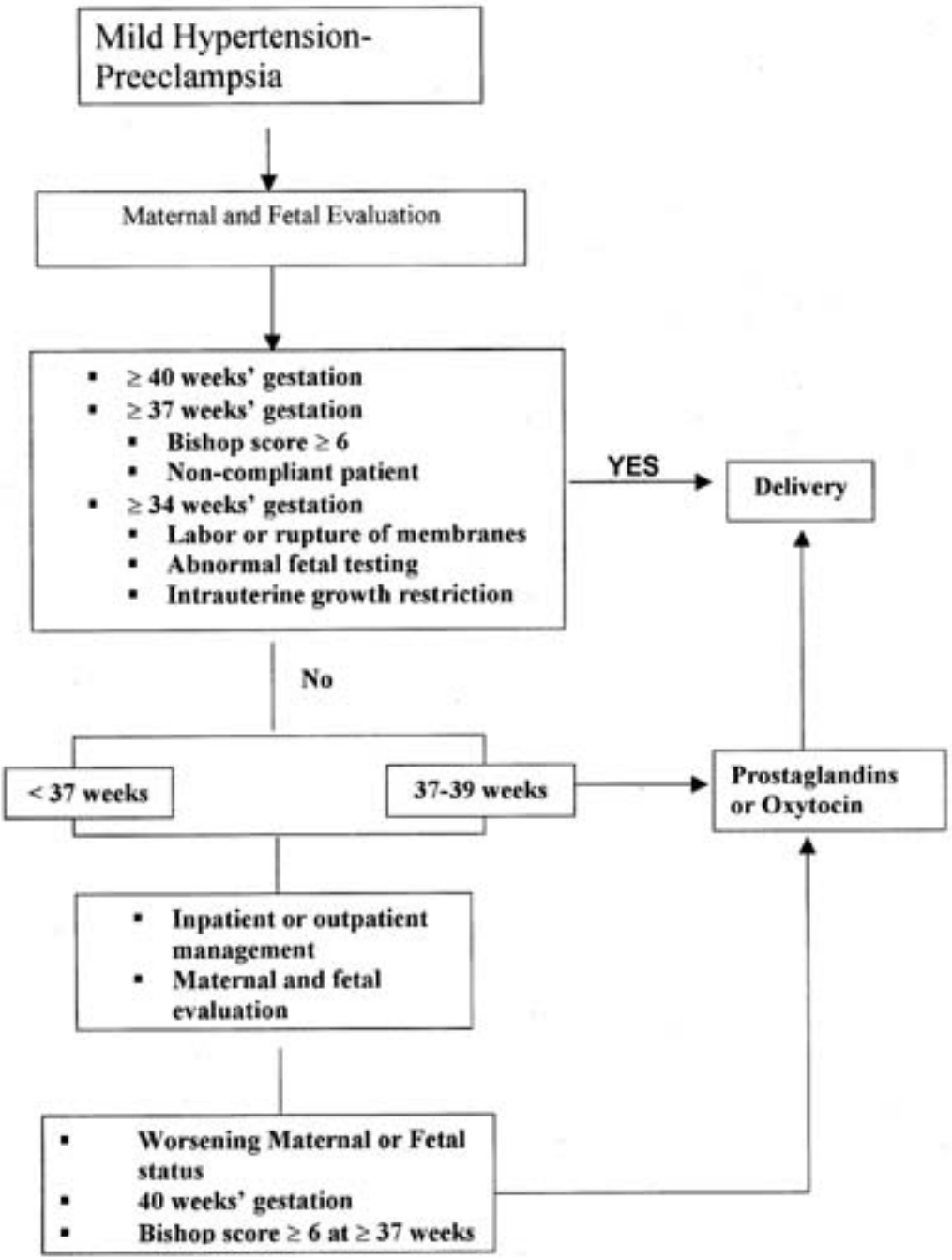


**Figure 1.** Recommended management of mild gestational hypertension or pre-eclampsia Obtained from Coppage et al. *(Preeclampsia and Eclampsia* 2008) (6)

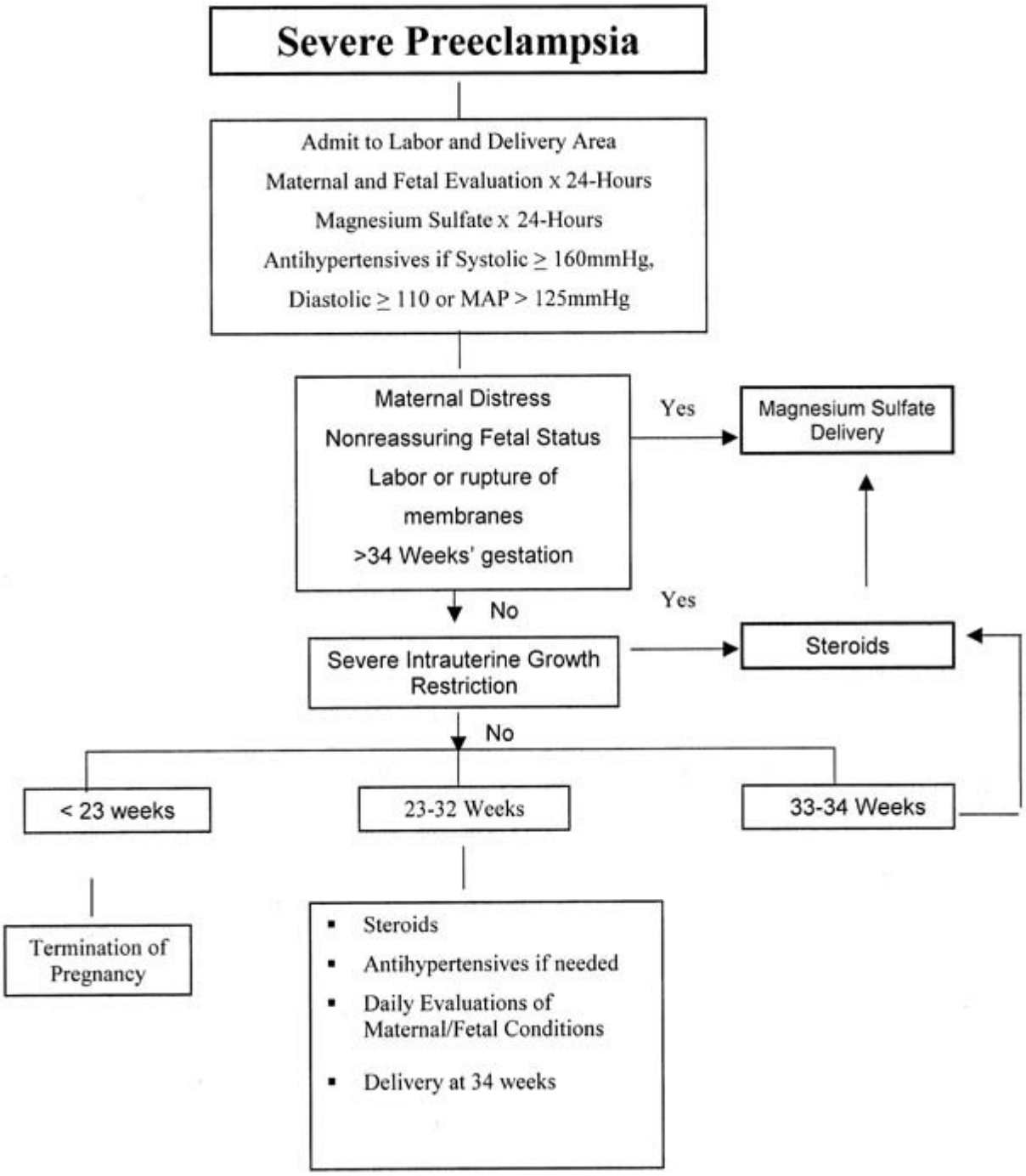


**Figure 2.** Recommended management of severe pre-eclampsia
Obtained from Coppage et al. *(Preeclampsia and Eclampsia* 2008) (6)

### 1.2.9 Prognosis after pregnancy

The blood pressure typically normalises within a week after delivery. However, there is also a risk for an exacerbation or a postnatal HELLP-syndrome or eclampsia, so especially those women with cases of severe pre-eclampsia need to be monitored closely. A thorough blood pressure monitoring is of the essence here and if the hypertension is persistent, the antihypertensive medication needs to be adjusted. (2)

Breastfeeding is possible, but one needs to be careful, considering that nifedipine and propranolol are present in the breast milk at the same level as in the mother's plasma. Other antihypertensive agents however are found in just a very low level in breast milk. (1)

The long-term risk for women, who experienced hypertension in their pregnancy, needs to be considered as well. Those women are at high risk for hypertension in a subsequent pregnancy and are also at increased risk for cardiovascular disease in their later lives. Therefore lifestyle modifications and yearly check-ups, including metabolic status, BP and BMI, are recommended. (1,2,5)

### 1.2.10 Pathophysiology

Pre-eclampsia is a multisystemic disorder, affecting not only the BP and renal function, but other organ systems as well. The exact pathogenesis is yet to be understood. However, the placenta seems to be the root cause behind the development of this disease and the first stage in this two-part theory. It is understood, that the trophoblast function becomes altered through the immune system, causing a reduction in trophoblast invasion into the spiral arteries. Due to this reduced invasion, the arteries are not remodelled properly, resulting in an insufficient blood supply to the placenta. As a result of the increased blood velocity in the intervillous room and the reduced perfusion of the placenta, the placental function is limited. This leads to placental oxidative and endoplasmic reticulum stress and releasing mediators, that are responsible for endothelial function and angiogenesis, which causes general endothelial dysfunction. (5,8) As a result, all organ systems may be affected, causing different kinds of complications. One consequence might be the activation of coagulation factors leading to haemostasis and therefore to infarcts or DIC. Because of endothelial dysfunction, vascular permeability is impaired and facilitates the development of oedema. (7)

In the first stage, cytotrophoblast cells physiologically invade maternal spiral arteries during pregnancy, transforming them from small capacity/ high resistance vessels to high-capacity / low-resistance vessels, ensuring the necessary blood supply for the foetus. This remodelling process begins at the end of the first trimester and ends approximately at 18.-20. PW. The cytotrophoblast cells transform from an epithelial to an endothelial phenotype, which is called 'vascular mimicry', because they imitate blood vessels. In pre-eclamptic patients however, this process is not completed. The invasion stays shallow and only reaches the

decidua and not the myometrium, so no proper vascular differentiation takes place and the vessels remain high-resistant with small capacities. (9,10)

In the second stage, endothelial dysfunction plays a key role. Studies have shown, that there is a reduction of angiogenic factors, like VEGF and PlGF, in mothers with pre-eclampsia. (9,10) PlGF is part of the growth and maturation of new vessels, as well as enhancing the viability, proliferation and migration of endothelial cells in existing vessels. It is also involved in inducing the dilation of resistance arteries in the uterus, the mesentery and the skin. sFlt-1 is the soluble receptor for PlGF, as well as VEGF. (11) Those factors seem to be antagonized by high levels of sFlt-1, occurring in PE, and therefore inducing general endothelial dysfunction. (8) It is also understood, that the amount of mRNA for VEGF and its receptor, is decreased in pre-eclamptic placental tissue, as shown through biopsies. These could all be signs of dysfunctional angiogenesis.

Another important factor is the disbalance between vasoconstrictive and vasodilative substances. $PGI_2$ (vasodilator and inhibitor of platelet aggregation) and $TXA_2$ (counterpart to $PGI_2$, vasoconstrictor and inductor of platelet aggregation) are from essence here. The ratio between those agents is disturbed, resulting in an increased level of $TXA_2$, which causes endothelial damage, activation of the coagulation cascade, as well as resulting in organ damage.

In addition to that, one could prove a higher familial incidence and therefore suggest a genetic component in the development of pre-eclampsia. It is yet to be determined, which genetic mutations are responsible for that, but one assumes that it is probably caused by an interaction of various mutations on different genes. (4,7) However, there seems to be a connection between single-nucleotide polymorphisms near the FLT1 gen on the foetal chromosome 13 and the occurrence of pre-eclampsia, as well as an association between trisomy 13 and higher levels of sFlt-1 in the mother. (8)

Another point being discussed is the role of the maternal immune system. Although there were signs of abnormal activation of maternal immune factors during the pregnancy, no proof could be found yet.

It seems that RAAS could also be part of the pathogenesis and an explanation of hypertension, occurring with PE. Different studies have shown that in pregnancies with PE, RAAS components were decreased (or even elevated), but the women also showed signs of increased sensitivity to vasopressors, leading to the conclusion, that this might cause or at

least have a part in the development of PE. Whereas in normal pregnancies, the opposite, vascular desensitisation, happens. (4,7) Furthermore, AT1 receptor autoantibodies were determined in the blood of pre-eclamptic women, triggering increased peripheral vasoconstriction and therefore hypertension. (12)

Figure 3. gives an overview of the multifactorial pathogenesis of PE.

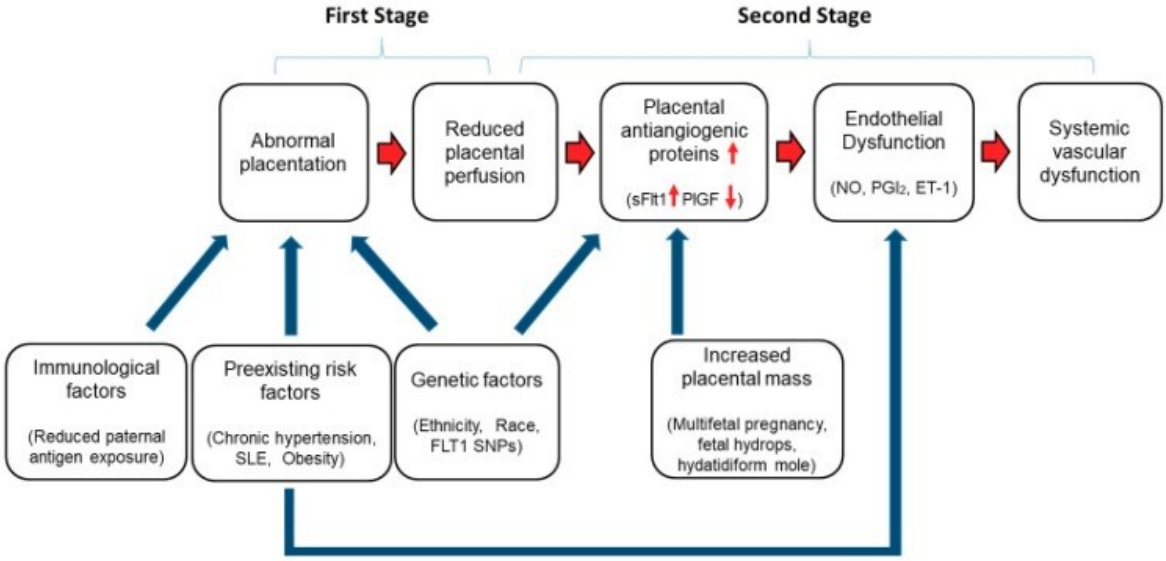


**Figure 3.** Two-stage theory of the pathophysiology of pre-eclampsia

Obtained from Tomimatsu et al. *(Preeclampsia: Maternal Systemic Vascular Disorder Caused by Generalized Endothelial Dysfunction Due to Placental Antiangiogenic Factors* 2019) (8)

*Note:* sFlt-1= soluble fms-like tyrosine kinase-1; PlGF= platelet growth factor; NO= nitric oxide; $PGI_2$= prostaglandin $I_2$, ET-1= endothelin-1; SLE= systemic lupus erythematosus; FLT1 SNP= FLT1 single-nucleotide polymorphism

## 1.3 Placenta

The placenta is the central organ that connects the foetus and the mother. Its main three functions are: Exchanging gas and waste products, transfer of immunoglobulins from the mother to the foetus and secretion of hormones for the health and development of the foetus.

It is disc-shaped, weighs around 500g at term and has a diameter of around 15-20 cm. The structure consists of chorionic villi, which are vascular projections of foetal tissue encircled by chorion. The chorion has a two-layer structure, with the outer syncytiotrophoblast, which is in contact with the maternal blood through the intervillous space, and the inner cytotrophoblast. The intervillous space is a wide cavernous room, into which the villi go. Blood for the uterus is supplied through the uterine or rather ovarian

arteries, which form the arcuate arteries and further radial arteries, which invade the myometrium. The radial arteries further divide into spiral arteries, which are essential for the blood supply in the intervillous room. Two umbilical arteries drain deoxygenated blood from the foetus via chorionic arteries into capillaries within the villi. In a similar way substances pass through the intervillous space through the layers including the syncytiotrophoblast capillaries and then into chorionic veins, which join into one umbilical vein, supplying the foetus. Figure 4 depicts the anatomy of the uterine circulation.

The uterine blood flow from the mother at term is about 600 ml/min, of which more than two-thirds go to the placenta. As the uteroplacental blood flow is not autoregulated, it is dependent on the mean uterine perfusion pressure and the uterine vascular resistance. In conclusion, this means that uterine blood flow is decreased by maternal hypertension and high uterine pressure, like during contractions. (13)

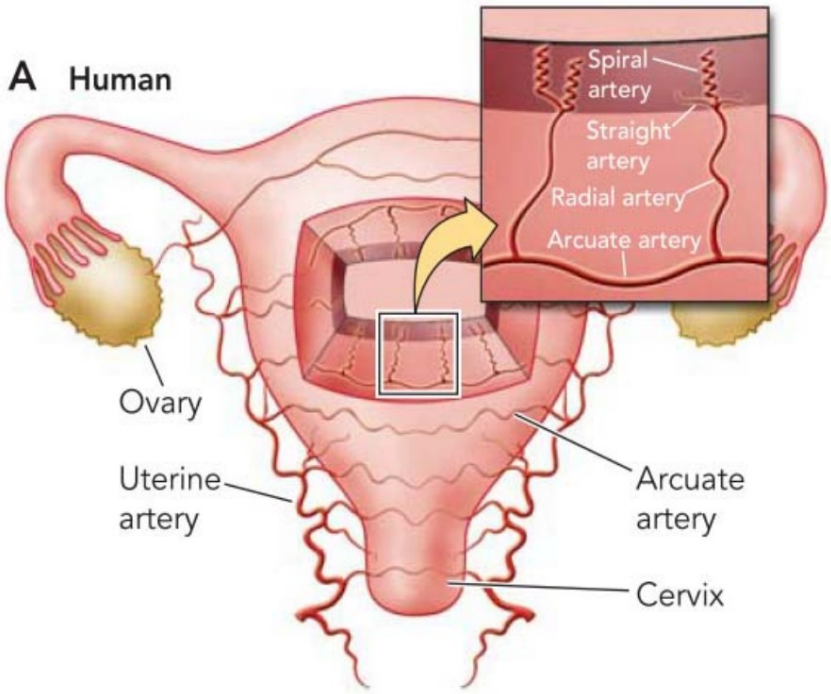


**Figure 4.** Anatomy of the uterine circulation in humans
Modified from: Osol et al. (*Maternal Uterine Vascular Remodeling During Pregnancy* 2009) (14)

## 1.4 Physiological cardiovascular adaption during pregnancy

Important changes in the maternal cardiovascular system happen during pregnancy, to provide the best environment for the foetus to grow. Three main changes occur: the circulating blood volume rises, the maternal heart increases in size and the central venous pressure elevates, as the peripheral venous pressure decreases. All of these changes improve uteroplacental circulation, resulting in an improved blood supply for the foetus.

Due to a local rise in prostacyclin (PG I) and NO in endothelial cells through the increased levels of oestrogen, the vascular walls are less sensitive to vasoconstrictive stimuli. This effect results in a BP decrease of 5-11 mmHg systolic and 10-15 mmHg diastolic in the second trimester, despite the increase in blood volume of around 1-1,5 l, which again secures the foetal oxygen and energy supply.

As a result of the enlarged capacity of the maternal vessels, the effective circulating blood volume decreases, leading to the activation of RAS, which results in an enhanced sodium and water retention. Progesterone boosts that mechanism further, increasing the blood volume of around 30-40%. Characteristically, the plasma volume progresses hereby over 50%, whereas volume of the erythrocytes only reaches around 15-20%, resulting in a decreased haemoglobin and albumin concentration, which causes a physiological pregnancy-associated anaemia and results in a drop in the oncotic pressure. Furthermore, the augmented blood volume causes secondarily an amplified heart rate, with a plus of 10-15 bpm and an increase of 40% in cardiac output. These factors combined explain the hypertrophy of the maternal heart, affecting especially the left ventricle.

As pregnancy progresses, the pressure on the Vena cava increases, causing a rise in venous pressure, particularly in the bottom body half. In combination with the decreased oncotic pressure, this results in ankle oedema and possibly varicosis. Furthermore, due to an augmented production of coagulation factors and a slightly reduced fibrinolysis, the risk for thrombophlebitis and thrombosis is significantly higher. (15-17)

## 1.5 Endothelial Dysfunction

The endothelium is a single layer of squamous cells, lining the lumina of blood and lymphatic vessels, with a surface of more than 1000m^2. It has numerous different functions, including vascular permeability and tone, thrombogenicity, inflammation, and angiogenesis. Together with the basal layer, they form the tunica intima, as depicted in figure 5. Arteries have a three-layer construction, due to the higher pressure, they are exposed to, in contrast to other blood vessels. The tunica intima, as already prescribed, is the innermost layer, next to the tunica media, which consists of smooth muscle cells and which is responsible for the regulation of the vasotone. The outermost layer, the tunica adventitia, contains nerve endings, perivascular adipose tissue, and connective tissue. The glycocalyx is the luminal layer of the endothelium, consisting of negatively charged glycoproteins, proteoglycans and glycosaminoglycans. It is part of the permeability regulation, as well as the protection against pathogens. The negative charge functions as a 'filter' and repulses cells like platelets, erythrocytes, and leukocytes. In addition to that, protein binding complexes (tight junctions, adherens junctions and gap junctions) are localised in the endothelium and support the barrier function, as visualised in figure 5. Furthermore, its anticoagulant and antiplatelet function is mainly caused by regulating the expression of binding sites for anticoagulant and procoagulant factors on the cell surface. The regulation of vascular tone is stimulated by mechanical (e.g. shear stress) or chemical (e.g. ATP, acetylcholine, bradykinin) triggers, which cause the release of vasoactive substances. The endothelium produces thromboxane A2 ($TXA_2$) and endothelin-1 (ET-1) as the main vasoconstrictors, and NO, prostacyclin ($PGI_2$), and endothelium-derived hyperpolarization factor (EDHF) as vasodilators. There are different types of the endothelium, dependent on organ localisation and functions. Endothelial dysfunction increasingly plays part in various diseases (18-20), with characteristics like reduced vasodilatation, inflammation and prothrombotic qualities. It is involved in the pathophysiology of diseases like hypertension, coronary artery disease, chronic heart failure, peripheral artery disease, diabetes, and chronic renal failure. (21) Endothelial dysfunction was defined as an impaired vasodilative capacity. Today, however, the term includes every change in vasoprotective homeostatic function of the endothelium. (22)

The following sections assess pathogenic mechanisms, that contribute to endothelial damage more precisely.

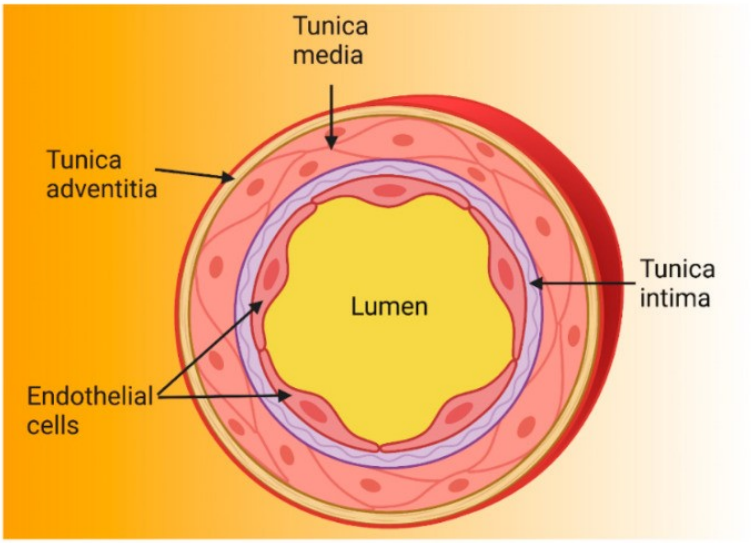


**Figure 5.** Structures of the arterial wall

Obtained from Medina-Leyte et al. *(Endothelial Dysfunction, Inflammation and Coronary Artery Disease: Potential Biomarkers and Promising Therapeutical Approaches* 2021) (20)

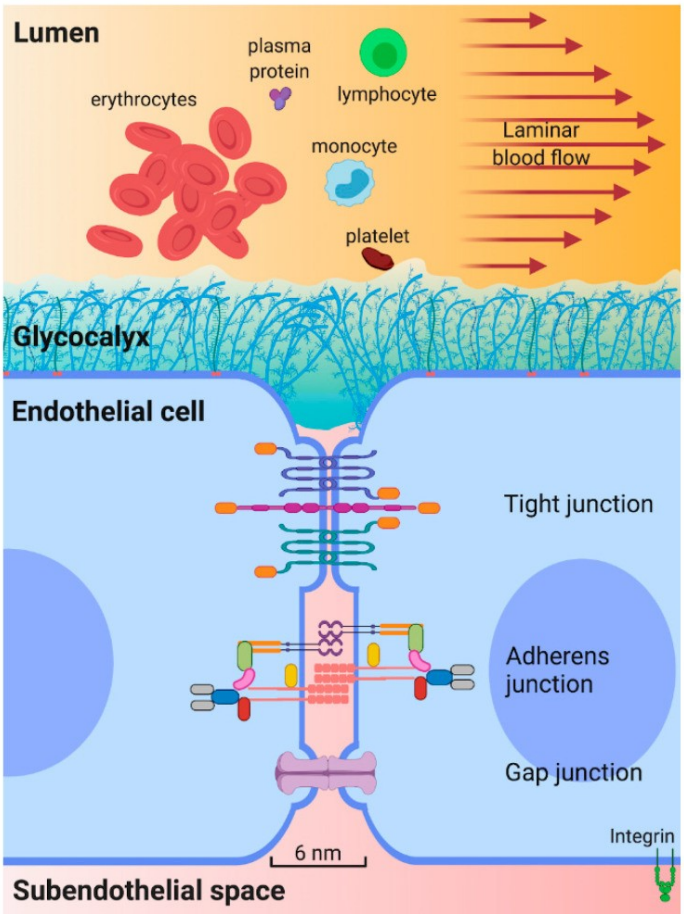


**Figure 6.** Key structures for epithelium permeability

Modified from Medina-Leyte et al. *(Endothelial Dysfunction, Inflammation and Coronary Artery Disease: Potential Biomarkers and Promising Therapeutical Approaches* 2021) (20)

### 1.5.1 NO

Nitric oxide is one of the most potent vasodilators expressed by the endothelium. It is produced from L-arginine with NO synthase as a catalysator. After the synthesisation, it is dispersed from endothelial cells to smooth muscle cells, where it activates guanylate cyclase, which then transforms GTP to cGMP, removing $Ca^{2+}$ and finally resulting in relaxation. In addition to that, it reduces inflammation, inhibits the proliferation of smooth muscle cells, regulates adhesion of leucocytes and has anti-aggregant effects on platelets, as depicted in figure 7. Shear stress is the most potent stimulus for the release of NO, which is caused by increased blood velocity. This explains as well the importance of the haemodynamic factor in the development of atherosclerosis: In places where the blood flow is oscillating or retrograde, shear stress is low and so are NO levels, resulting in reduced vasodilation and a pro-inflammatory environment. On the other hand, extremly high levels of shear stress can cause endothelial erosion and provoke platelet aggregation this way. Decreased levels of NO are associated with endothelial dysfunction, as it is also maintaining endothelial integrity and permeability and acts as a potent oxygen radical scavenger. There are two possible ways for decreased NO levels: Either the synthetisation is impaired, due to an inhibition of eNOS or a reduction of the substrate L-arginine, or its bioavailability is diminished. This can happen for example through ROS or increased levels of CRP in an inflammatory state. Another potential mechanism is by inhibiting eNOS through ADMA (asymmetric dimethylarginine). ADMA is a product of protein turnover, whose production is stimulated by stress. It is competing with L-arginine for the binding site of NOS. (19,21,23)

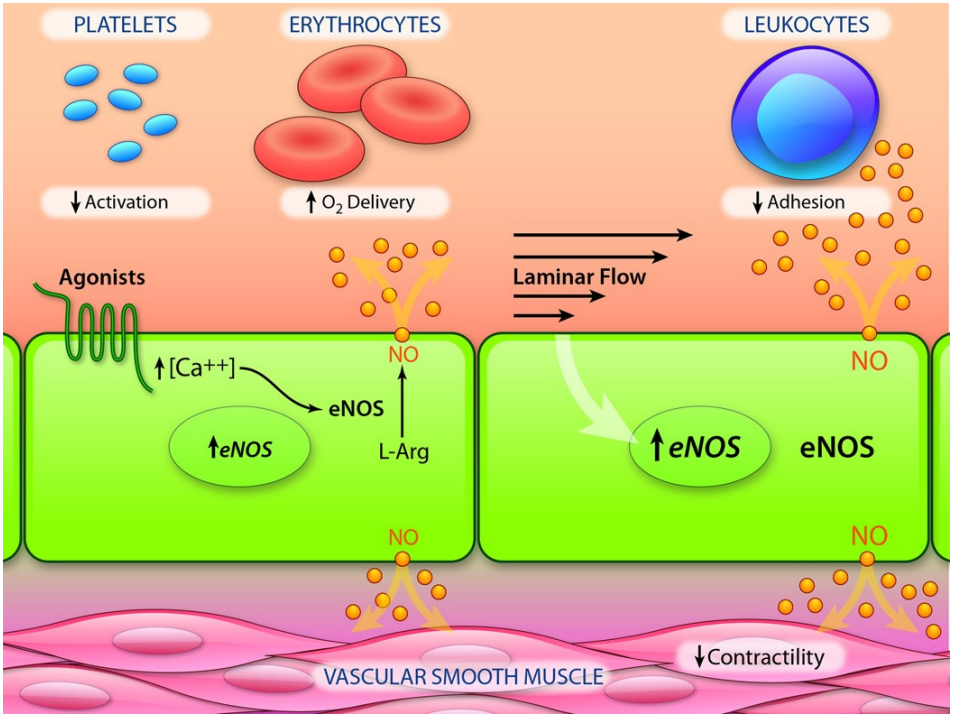


**Figure 7.** Endothelial-derived Nitric Oxide: Production and Biological Actions

Obtained from: Gimbrone et al. *(Endothelial Cell Dysfunction and the Pathobiology of Atherosclerosis* 2017) (24)

### 1.5.2 Angiotensin II

Endothelial cells release angiotensin II as well, which functions as an antagonist of NO. By binding to the $AT_1$-receptor, angiotensin II triggers vasocontraction, leukocyte adhesion, adhesion molecule expression, moreover prothrombogenic effects and pro-inflammatory factors. In addition to that, endothelin-1, a major vasoconstrictor, gets stimulated. (23) By triggering cell adhesion, monocytes are able to enter the endothelium and transform into macrophages and later on foam cells. Foam cells release cytokines and inflammatory markers, causing an inflammatory response along with alterations in arterial walls. The first step in this pathological remodelling is a disruption of the elastic blades in the intima media. Smooth muscle cells migrate to the subendothelial space and convert into (myo-) fibrocytary cells. (25)

### 1.5.3 Oxidative Stress

ROS and oxidative stress play key roles in the development of endothelial dysfunction. ROS (reactive oxygen species) physiologically act as second messengers in cells and are secreted by mitochondria, peroxisomes and the endoplasmic reticulum. An imbalance of reactive ROS and the antioxidant system however, leads to endothelial damage, by influencing

MMPs function, vascular remodelling, smooth muscle hypertrophy and cell apoptosis. Furthermore, ROS leads to an increased expression and release of VCAM-1, ICAM-1, IL-6 and TNF-α, through activating a pathway that results in the release of NF-κB. (20,26) Furthermore, in situations of increased oxidative stress, co-factors, that are needed for the production of NO, are supplied insufficiently, due to oxidation through ROS. As a result, superoxide is synthesized, instead of NO, a process known as NO uncoupling. (19)

### 1.5.4 Mitochondrial Dysfunction

As already mentioned, mitochondria play a major part in the production of ROS. However, there are also other pathways, which are important in the development of endothelial dysfunction. For instance, in states of high oxidative stress, the mitochondria themselves can be damaged, resulting in an increased permeability for mitochondrial DNA molecules (mtDNA), leading to further inflammation and ROS production. Moreover, mtDNA fragments function as damage-associated molecular patterns (DAMPs), which trigger an innate immune response. (26)

### 1.5.5 CRP

C-reactive protein is a marker for systemic inflammation and acute phase reactant, mainly produced by the liver. Its release is stimulated by cytokines like IL-1, IL-6 and TNF-α and it functions as a down-regulator for eNOS in endothelial cells, resulting in decreased NO levels. Additionally, CRP seems to upregulate the expression of ICAM-1, VCAM-1, and E-selectin, adhesion molecules. (20)

### 1.5.6 Cytokines

Cytokines are important inflammatory mediators, that exacerbate inflammation and activate the expression of adhesion molecules (E- and P-selectin), in endothelial cells and leucocytes, resulting in further endothelial damage. TNF-α is an important cytokine, that stimulates endothelial cells to further release cytokines, chemokines and adhesion molecules, that allow monocytes to migrate into the vessel intima. In addition to that, it activates monocytes and triggers foam cell formation, hence its association with atherosclerosis, coronary artery disease and hypertension. Another cytokine, interleukin 6 (IL-6), is linked to the acute phase reaction and other immunological processes. IL-8 on the other hand is a strong attractor for neutrophils and T-lymphocytes. Moreover, macrophages produce IL-18, which stimulates the release of other cytokines and is strongly associated with the pathogenesis of atherosclerosis. (20)

Adipose tissue is known to secrete numerous humoral factors, also called adipokines, which have pro- and anti-inflammatory properties. However, some have been linked to vascular inflammation and atherogenesis. Similarly, adipose pregnant women show increased levels of cytokines and placental macrophages. (26)

### 1.5.7 Cell adhesion molecules

Endothelial cells and leukocytes produce CAMs in case of inflammation, which then cause endothelial dysfunction and the migration of leukocytes. These include E-selectin, P-selectin, ICAM-1, and VCAM-1, and their soluble forms. VCAM-1 binds monocytes and T-lymphocytes, initiating the invasion of inflammatory cells into the vessel wall. However, due to the fact that they are not only produced by the endothelium, but other tissue as well, their diagnostic value regarding endothelial dysfunction is limited. (20,21)

### 1.5.8 Insulin resistance and dysregulated lipid signalling

In patients suffering from diabetes, insulin signalling is altered, resulting in a downregulation of eNOS and therefore NO. Likewise, insulin resistance is associated with plaque formation and atherogenesis. Furthermore, insulin resistance-induced dyslipidaemia is linked with an increased cardiovascular risk, due to enhanced triglycerides, decreased HDL and the formation of small-dense LDL, which is promoted by impaired adipocyte function. These mechanisms form a circulus vitiosus: Insulin resistance and dyslipidaemia are triggered by a NO deficiency and ROS. Insulin resistance itself provokes ROS secretion and inhibits NO, resulting in endothelial damage which reduces insulin motility, due to damaged capillaries. Lipide deposition and oxidative stress lead to vascular wall damage, which triggers an inflammatory response and the release of cytokines, which then furthers insulin resistance and endothelial damage. (21,26)

Oxidised LDL (ox-LDL) develops due to oxidative stress, stimulating the expression of chemokines and CAMs. As a result, monocytes and t-lymphocytes are recruited and invade the subendothelial space. Monocytes are able to differentiate into macrophages and form scavenger receptors, ready to uptake ox-LDL and transform into foam cells. LOX-1 is a transmembrane glycoprotein, which binds ox-LDL and is found on endothelial cells, macrophages, platelets, fibroblasts and smooth muscle cells. Via binding to his receptor, ox-LDL causes leucocyte adhesion, increased release of ROS, apoptosis and ultimately endothelial dysfunction. (27)

### 1.5.9 Advanced Glycation End Products

AGEs are a group of heterogeneous compounds, that persists out of glycated proteins, nucleic acids or lipids. They are generated in states of high blood glucose levels, e.g. in the case of insulin resistance. There is also an exogenous source for AGEs, for example in food or tobacco products. AGEs accumulate in aging tissue and by binding to their main receptor RAGE, they induce oxidative stress and inflammatory responses, leading to vascular damage. The AGE/RAGE axis is associated with various diseases like diabetes, cardiovascular disorders, arthritis, cancers and neurological disorders (26,28)

# 2. Aims and Objectives

Hypertensive pregnancy disorders affect 6-8% of pregnancies worldwide, increasing foetal and maternal morbidity and mortality drastically, with pre-eclampsia being the main cause of death. This disease is connected with 10-15% of all maternal deaths and is responsible for more than 70.000 deaths per year worldwide. Furthermore, 90% of these deaths in Europe are preventable. Placental abruption, stroke and multi-organ failure are just a few of the possible risks for the mother. In addition to that, the foetus is at high risk for premature labour, intrauterine growth retardation or even intrauterine death. (1,2)

Taking all of these aspects together, there is more than enough reason for researching pre-eclampsia deeper. The main focus lies on understanding the pathophysiology behind this crucial disease, in order to find better ways of prevention, diagnosis and treatment, as options in these areas are still very limited.

This literature review aims to examine the role of endothelial dysfunction in the pathophysiology of pre-eclampsia, by providing the current state-of-the-art scientific knowledge and updating it with recent study results. Furthermore, those results will be put into context, to evaluate possible connections. The following sections examine the results of several studies from the past five years, regarding the development of pre-eclampsia, with a particular focus on endothelial dysfunction and hypertension.

# 3. Methods

In order to get a general overview of pre-eclampsia and endothelial dysfunction, my first step was to search those two terms in “Google”, as well as “AMBOSS” and “Doccheck”. In addition to that, I used my lecture notes to get further insight into the topic and find useful references. Furthermore, I used the online library of the Medical University of Graz, to find useful textbooks for secondary literature and to familiarize myself deeper.

For the introduction of this diploma thesis, mainly the current medical guidelines from the European Heart Association and Deutsche/Schweizerische/Österreichische Gesellschaft für Gynäkologie und Geburtshilfe, as well as textbooks on physiology, pathophysiology and gynaecology and obstetrics and other issued articles were used. For the main part, as primary literature, published journal articles and research papers were obtained, primarily via PubMed. The Web of Science database was utilized additionally to get further information on the impact of said articles and to see how often they were cited. Furthermore, lists of references were collated and Google Scholar functioned as an extra source of information. Chosen articles were downloaded into the literature program “RefWorks” and sorted according to subject and importance.

By using free text search, I was able to combine various search terms. My first approach was to search for “pre-eclampsia” AND “endothelial dysfunction”, using the Boolean operator “AND”, as well as quotes, to specify my search. This way, over 900 listed entries were shown, so the following filters were applied, to limit the results:

- The availability of the full article for free.
- The publication date was set to five years, to focus on the latest findings and further limit the search.
-The article was written in English.

Via using those filters, 123 results were shown. Those articles were seen through and the most relevant ones were chosen for download, via their titles and abstracts. After that, they were read thoroughly in order to compare and structure the articles. Based on this search, I was able to identify the most relevant issues and divide them into small subgroups for a better overview. For more precise descriptions, and to add further literature, terms like “pre-eclampsia” AND “nitric oxide” OR “sFlt-1” were searched, if needed. Ultimately, articles that only dealt with the pathophysiology, and not specifically or just marginally with

endothelial dysfunction and/or hypertension, were excluded. Furthermore, publications about molecular genetics were not included in order to focus on the pathogenesis of endothelial dysfunction itself. This led to a total of 40 articles contributing to this review.

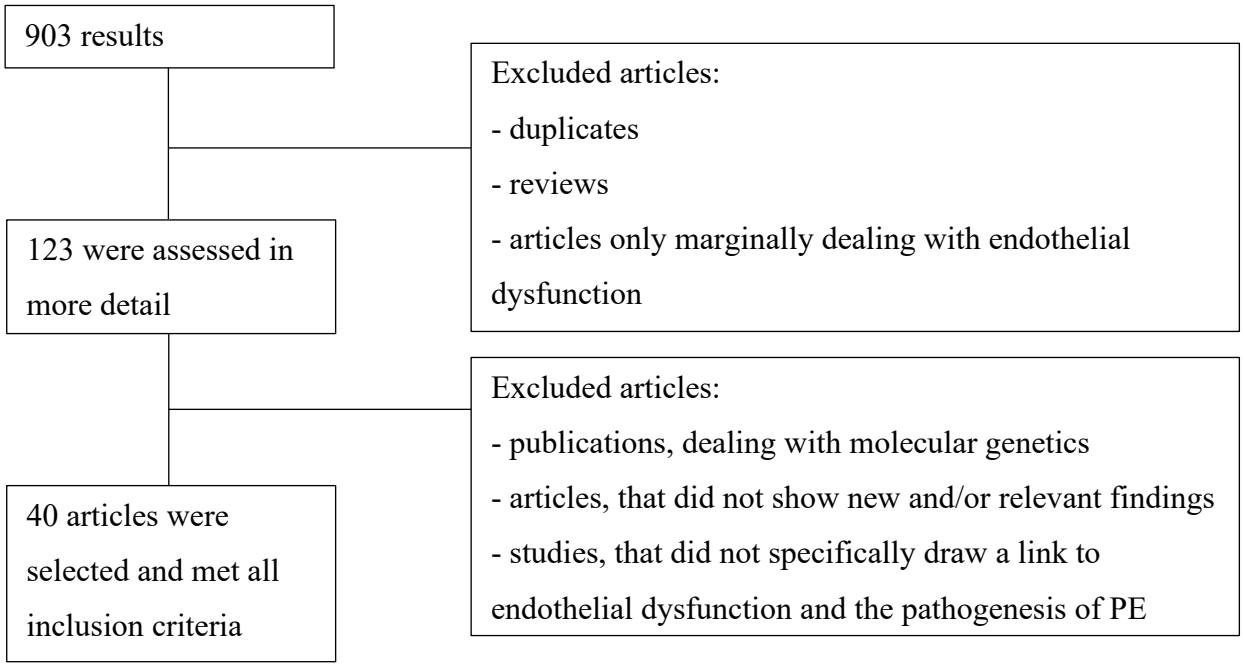


**Figure 8.** Flowchart Illustrating the Selection Process of the PubMed Research

# 4. Update of the current literature

This section will demonstrate the findings of a systematic literature review, concerning the pathogenesis of pre-eclampsia. The aim is to summarize and update the current literature in regard to endothelial dysfunction, a hallmark of PE. The following tables show the relevant studies, that were used for this review, arranged by content-related groups.

**Table 4.** Studies investigating different factors in the pathogenesis of pre-eclampsia, with a focus on endothelial dysfunction

| Authors/Year | Study cohort | Study design | Objective | Results |
|---|---|---|---|---|
| | | **Serum lipids** | | |
| Al-Maihahy et al. 2021 (29) | 37 PE<br>24 NP | Case-control study | dyslipidaemia | ↑ TC, TG, LDL<br>↓ HDL |
| Ebogo-Belobo et al. 2021 (30) | 48 PE<br>96 NP | Case-control study | dyslipidaemia | ↑ TC, LDL |
| Dong et al. 2021 (31) | 37 PE<br>40 NP | Case-control study | Abnormal lipid metabolism | ↓ CGI-58, LPL |
| | | **Nitric oxide** | | |
| Tashie et al. 2020 (32) | 88 PE<br>92 NP | Case-control study | Arginine-nitric oxide pathway | ↓ NO<br>↑ ADMA, L-arginine |
| Hodžić et al. 2017 (33) | 20 PE<br>40 NP<br>20 non-pregnant | Case-control study | NO biosynthesis | ↓ NO |
| Darkwa et al. 2018 (34) | 30 PE<br>30 NP | Case-control study | Plasma NO levels | = NO |
| Guerby et al. 2019 (35) | 13 PE<br>9 NP | Case-control study | S-glutathionylation of eNOS | ↑ |

| | | | | |
|---|---|---|---|---|
| Motta-Mejia et al.<br>2017 (36) | 8 PE<br>11 NP | Case-control study | Placental vesicles carrying eNOS | ↓ STBEX |
| Kim et al.<br>2018 (37) | 17 PE<br>17 NP | Case-control study | NF-κB-dependent biogenesis of microRNA (miR)-31-5p | ↓ eNOS |
| **PlGF and sFlt-1** | | | | |
| Jammalamadaga et al.<br>2016 (38) | 100 PE<br>100 NP<br>100 E | Case-control study | Factors influencing endothelial dysfunction | ↑ sFlt-1<br>↓ VEGF, PlGF |
| Ali et al.<br>2019 (39) | 18 PE<br>18 NP | Case-control study | mRNA expression of VEGF and receptors | ↓ mRNA VEGF, -R1, -R2<br>↑ sFlt-1 |
| Pant et al.<br>2019 (40) | 44 PE<br>44 NP | Observational cross-sectional study | sFlt-1:PlGF ratio | ↑ ratio in PE |
| Akhter et al.<br>2017 (41) | 55 PE<br>64 NP | Case-control study | Angiogenic factors and arterial ageing | ↑ sFlt-1<br>↓ PlGF<br>↑ I/M ratio |
| Sánchez-Aranguren et al.<br>2018 (42) | 23 PE<br>23 NP<br>10 non-pregnant | Case-control study | Influence of sFlt-1 on mitochondrial activity | sFlt-1 causes mitochondrial dysfunction |
| Eddy et al.<br>2019 (43) | Human placental trophoblast cells | In-vitro study | Heparanase regulation of sFlt-1 release | ↑ heparanase |

| | | | | |
|---|---|---|---|---|
| Lahsinoui et al. 2021 (44) | 20 PE<br>14 NP | Case-control study | Glycocalyx components | ↑ dermatan sulphate |
| **VEGF** | | | | |
| Adu-Bonsaffoh et al. 2017 (45) | 100 PE<br>102 NP<br>75 non-pregnant | Cross-sectional study | VEGF serum levels | ↓ |
| Agarwal et al. 2019 (46) | 42 PE<br>42 NP | Case-control study | VEGFA mRNA expression | ↓ |
| Istrate et al. 2018 (47) | 13 PE<br>22 NP | Case-control study | VEGF receptors | ↑ VEGF-R1 |
| Wheeler et al. 2018 (48) | 19 PE<br>24 NP | Case-control study | VEGF and macrophages recruitment | ↑ M1<br>↓ M2 |
| Dong et al. 2020 (49) | 20 PE<br>20 NP | Case-control study | Influence of MicroRNA-646 on vasculogenesis | ↓ VEGFA |
| Witvrouwen et al. 2021 (50) | 24 PE<br>30 NP | Case-control study | MicroRNA targeting VEGF | miR16/200c correlates with endothelial dysfunction |
| **Inflammatory compounds** | | | | |
| Raio et al. 2019 (51) | 15 PE<br>16 NP | Case-control study | Inflammatory compounds | ↑ CRP, IL-6 |

| | | | | |
|---|---|---|---|---|
| Arlier et al.<br>2017 (52) | 7 PE<br>12 NP | Case-control study | Influence of leptin on inflammation | ↑ Leptin, IL-8 |
| Collier et al.<br>2019 (53) | 25 PE (6 HELLP)<br>18 NP | Case-control study | Complement activation | ↑ MAC, C4d |
| Matsuyama et al.<br>2021 (54) | 10 NP HUVEC | In-vitro study | Complement activation | ↓ CFH |
| Ampey et al.<br>2019 (55) | Non-pregnant sheep,<br>pregnant ewes | In-vitro study | Influence of TNF-α on $Ca^{2+}$<br>signalling | ↑ TNF-α<br>↓ $Ca^{2+}$ bursts |
| Travis et al.<br>2020 (56) | 8–12 NP rats<br>7–12 RUPP rats 7-12<br>RUPP + IL-17RC rats | Case-control study | Influence of IL-17 on cNK | ↑ IL-17, cNK |
| **Endocan** | | | | |
| Lan et Liu<br>2020 (57) | 8 matched case-control<br>studies<br>451 PE<br>442 NP | Meta-analysis | Circulating endocan level | ↑ endocan |
| **Galectin 3** | | | | |
| Pankiewicz et al.<br>2020 (58) | 39 PE<br>38 NP | Case-control study | Serum galectin-3 level | ↑ serum and placenta |

| | | | | |
|---|---|---|---|---|
| **Vitamin D** | | | | |
| Chen et al.<br>2019 (59) | 100 PE<br>100 NP | Case-control study | Association of 25[OH]D and sFlt-1 | ↓ 25[OH]D<br>↑ sFlt-1 |
| **Glycocalix** | | | | |
| Weissgerber et al.<br>2019 (60) | 14 early PE<br>29 late PE<br>73 NP | Case-control study | Glycocalyx degradation | Found in PE |
| **CAMS** | | | | |
| Mistry et al.<br>2020 (61) | 17 PE<br>17 NP | Case-control study | Maternal, foetal, and placental selectins | ↑ maternal, placental E-selectin<br>↓ foetal E-selectin; P-/L-selectin |
| Kornacki et al.<br>2020 (62) | 20 early PE<br>20 late PE<br>20 NP | Case-control study | Level of endothelial damage in late VS. early-onset PE | Similar levels |
| **Oxidative stress** | | | | |
| Ahmad et al.<br>2019 (63) | 23 PE<br>91 NP | Case-control study | Oxidative stress | ↓ catalase |

| | | | | |
|---|---|---|---|---|
| Al-Kuraishy et al. 2018 (64) | 40 PE<br>28 NP | Case-control study | Oxidative stress | ↓ PON-1 |
| **MMP** | | | | |
| Timokhina et al. 2020 (65) | 61 PE<br>31 NP | Case-control study | Role of MMP-2 and MMP-9 | ↑ MMP-2<br>↓ MMP-9 |
| **Soluble factors** | | | | |
| Li et al. 2021 (66) | 186 PE (99 mild, 87 severe)<br>72 NP | Case-control study | Serum levels of CXCL 16, LCN-2 and sFlt-1 | ↑ CXCL 16, LCN-2, sFlt-1 |
| Wang et al. 2016 (67) | HUVECs | In-vitro study | Influence of HtrA4 on the expression of endothelial genes | ↑ HtrA4, gene expression altered |
| Gui et al. 2021 (68) | 30 PE<br>30 NP | Case-control study | Role of sAxl in vascular injury | ↑ sAxl |

## 4.1 Serum lipids

Pre-eclampsia is associated with a dyslipidemic status and a higher atherogenic profile, as well as an increased LDL/HDL ratio, as Al-Maiahy et al. showed. Physiological hyperlipidaemia is not associated with atherogenesis, however hyperlipidaemia is correlated with the severity of hypertension in PE, which mostly goes along with an elevated BMI and obesity in pregnant women. Moreover, PE and hyperlipidaemia seem to be connected to changes in platelet indices. In this study, women with PE also had higher serum levels of TC, TG and LDL, compared to the controls, as well as decreased levels of HDL. The current belief is, that due to high levels of TG and FFA, endothelial prostacyclin and NO are suppressed, inducing oxidative stress and therefore causing endothelial dysfunction. (29) Ebogo-Belobo et al. found in their study significant increase in TC and LDL serum levels in pre-eclamptic women as well. Their analysis suggests a significant positive correlation between those parameters and blood pressure. They believe that endothelial dysfunction, mainly LDL oxidation, causes glomerular lesions, resulting in proteinuria, which is part of the symptoms complex of pre-eclampsia. Furthermore, Ebogo-Belobo and his colleagues showed that the cardiovascular risk of pre-eclamptic women is five to seven times higher than in normal pregnancies. (30)

Another study from Dong et al. investigated the role of comparative gene identification-58 (CGI-58) and lipoprotein lipase (LPL) in the pathogenesis of pre-eclampsia. These two factors play an important role in the hydrolysis of TG, which is a necessary energy source for the foetus. Maternal serum lipid levels increase physiologically with progressive gestational age. Maternal triglycerides get split into fatty acids by the placental adipose TG lipase (ATGL), before going into the foetus. LPL, as part of the ATGL family, is strongly expressed in adipocytes, macrophages and myocardial and skeletal muscle cells and hydrolyses TGs in chylomicrons and low-density lipoprotein granules. A distorted placental lipid transport however, can cause lipid accumulation and an increase in lipid peroxidation. As a result, inflammation, enhanced vascular permeability and an inward flow of extravascular lipids occur, causing endothelial damage. An upregulation of LPL, therefore results in hydrolysis of intracellular TG, moreover the accumulation of lipids in macrophages. CGI-58 is a glycoprotein, which is found among others in fat, testis, muscles and the liver. It functions as an agonist in the hydrolysis of ATGL, meaning LPL is able to enhance its activity by binding with CGI-58, as a co-activating protein. Macrophages, in which CGI-58 is not working properly, show similarities to foam cells, which hold high

quantities of TG and cholesteryl esters. Accumulation of those in the endothelium causes oxidative stress and results in oxLDL, which then further stimulates atherogenesis. This study showed decreased expression levels of CGI-58 and LPL, resulting in disturbed lipid transport, which therefore can cause lipid accumulation and increased lipid peroxide formation. All of this may contribute to endothelial cell damage and dysfunction. Furthermore, the resulting oxidative stress affects the invasion and apoptosis of trophoblast cells, which then cause impaired placental lipid transport. (31)

## 4.2 NO

NO plays an important role in normal endothelial function. It regulates vasodilation, platelet aggregation and leukocyte adhesion in blood vessels. The enzyme NOS synthesizes NO through L-arginine. As a counterpart, the methylated form ADMA inhibits eNOS. Therefore the L-arginine/ADMA ratio can be used as an indicator of eNOS function. Tashie and his colleagues demonstrated in their study a significantly lower level of NO, and corresponding higher levels of L-arginine and ADMA in pre-eclamptic women, than in normotensive pregnancies. Consequently, they found a decreased L-arginine/ADMA ratio. All of these findings suggest a reduced NO bioavailability, contributing to the endothelial dysfunction, as part of the pathogenesis of pre-eclampsia. Furthermore, it could be demonstrated that those factors are correlated with disease severity. In addition, in severe PE cases, this study could show a significant correlation between ADMA levels and systolic blood pressure, indicating its possible value as an early marker for PE. It is noted, that external L-arginine supplementation could reestablish a normal L-arginine/ADMA ratio and therefore further eNOS activity. Moreover, it has been proven, that levels of cyclic GMP are consistently decreased in pre-eclamptic women, suggesting a said reduced NO bioavailability. (32)

Hodzic et al. came to similar findings, showing a slightly decreased level of NO in women with PE, but not significantly. Still, they found positive correlations between NO serum levels and systolic and diastolic blood pressure, as well as uric acid and creatinine clearance and a negative correlation with platelet count. In conclusion, all of these results suggest, that NO plays a part in the development of hypertension in pre-eclamptic women. (33)

However, the exact role of NO in the pathophysiology of pre-eclampsia is yet to be determined. Other studies showed different results than Tashie et al. For example, Darkwa et al. could not show a significant reduction of NO plasma levels in women with pre-eclampsia. (34) But these results or others of elevated NO levels may be due to a

compensatory reaction to endothelial dysfunction. Divergent findings may also be the result of different measuring methods, the disease severity, as well as the gestational age at which NO was measured. (32)

Guerby and his colleagues investigated the importance of eNOS more precisely. Their theory states that due to S-glutathionylation, a post-translational modification by oxidized glutathione of cysteine residues, induced by oxidative stress, eNOS gets uncoupled and NO production is reduced. In addition to its other functions, NO also promotes the invasion of spiral arteries by cytotrophoblast cells in an early pregnancy stage. Their theory suggests, that by the invasion of extravillous cytotrophoblasts to the maternal uterine spiral arteries, these arteries get plugged and show reduced blood flow and therefore $pO_2$. Due to this relative hypoxia, trophoblast migration and remodelling of spiral arteries get stimulated. Furthermore, low production of ROS and low levels of oxidative stress are the results, which is important, since the antioxidant system is yet to be developed fully in the early stages of pregnancy. In the course of pregnancy, the plugged arteries resolve gradually, and in combination with the remodelled arteries, an increased blood flow to the intervillous space is possible. This is important, as in PE, this mechanism is not fully evolved, due to the defected remodelling of the spiral arteries, which causes intermittent contractility and unsteady blood flow. As a result, local oxidative stress conditions develop and may be the reason behind the high level of eNOS glutathionylation. The study presented data, that the placentas of women suffering from PE have way higher levels of S-glutathionylated eNOS than the placentas of normal pregnancies. (45% VS. more than 80%) This mechanism may contribute to the reduced NO bioavailability and as a result the development of PE. However, low to moderate oxidative stress levels seem to be physiological in pregnancy, which would explain the 45% of glutathionylated eNOS in non-PE pregnancies. This rate of glutathionylation allows producing 70% of the maximum NO production, which is compatible with normal placentation, normal remodelling of uterine spiral arteries and normal foetal development. (35)

In another study, by Motta-Mejia and her colleagues, the importance of syncytiotrophoblast extracellular vesicles (STBEV), comprising microvesicles (STBMV) and exosomes (STBEX), carrying eNOS, was investigated. These vesicles function as a communication tool between the syncytiotrophoblast and the mother. STBEV can be found in maternal circulation from PW 10 onwards in increasing quantity. The two subgroups show different origins: microvesicles, which are shed from the plasma membrane in case of cell activation

or death, and exosomes, which develop through exocytosis from the endosome. In this study, they found out that both types of vesicles carry functionally active eNOS and that STBEV-eNOS activity is reduced in women with PE, significantly in STBEX-bound eNOS. This mechanism may play an important part in the diminished NO bioavailability. However, there are still a few uncertainties, that need to be further examined in the future. It is still to be determined, whether eNOS is donated to endothelial cells or produced nearby. In addition to that, they could show reduced NO production by STBEV-eNOS, but other influencing factors also need to be considered. These include alterations in gene NOS expression and activity, low substrate levels, elevated inhibitor levels and increased decomposition of NO itself. (36)

Another study demonstrated that the inflammatory cytokine tumor necrosis factor-α (TNF-α) downregulates eNOS, via the activation of NF-κB, which itself induces the biogenesis of microRNA (miR)-31-5p. These are small noncoding RNAs of ∼22 nucleotides, which are able to bind to target genes and induce post-transcriptional silencing. Kim et al. showed that miR-31-5p targets eNOS RNA and thereby suppresses its expression, contributing to pre-eclamptic hypertension through negative regulation of the eNOS/NO pathway. (37)

## 4.3 PlGF and sFlt-1

Different factors influence endothelial dysfunction in PE. In particular of importance are the Vascular Endothelial Growth Factor (VEGF) and one of its subfamily members Placenta Growth Factor (PlGF), both involved in vasculogenesis and angiogenesis. Increased levels of VEGF allow vasodilatation, by stimulating the production of NO and PlGF2, for a sufficient uterine blood flow, which is essential for a sufficient nutrients supply for the foetus. PlGF is highly expressed in placental tissue and takes part in angiogenesis and vasculogenesis, trophoblast differentiation, dilatation of uterine arteries and placental development. In PE, soluble fms-like Tyrosine Kinase (sFlt-1, the soluble form of VEGFR-1, a receptor for VEGF and PlGF) production increases, antagonizing VEGF and/or PlGF and their protective effects on the endothelium. Jammalamadaga and Abraham demonstrated in their study, that increased sFlt-1 levels and decreased levels of VEGF and PlGF, result in an imbalance of pro-angiogenic and anti-angiogenic factors, and so partly causing endothelial dysfunction. However, it is important to point out that studies have shown contradictory results on circulating levels of VEGF in PE. But this incongruity may be explained by the different detection methods used in these studies. VEGF-protein complexes cannot be identified by sandwich-type ELISA, due to a rise in VEGF binding proteins in

pregnant women. Studies that demonstrated a decrease in VEGF levels used an ELISA kit, which only calculates unbound VEGF, whereas studies showing an increase in VEGF levels utilized a radioimmunoassay or an ELISA kit, which measures both, bound and unbound VEGF. Decreased VEGF levels may be the cause of the found hypertension and proteinuria, accompanied by endothelial and podocyte damage in PE, as its playing a role in regulating blood pressure and glomerular healing. This could also explain the disorder of the endothelial cells preserving the blood-brain barrier, as well as the ones covering the choroid plexus, explaining the neurological symptoms and seizures in eclampsia. (38)

Ali et al. came to similar results in their study, showing a decrease in mRNA expression of VEGF, VEGFR-1, and VEGFR-2 (which are important for migration, proliferation, and differentiation of endothelial cells) and an increase in sFlt-1in peripheral blood mononuclear cells. VEGFR-1, which is mainly expressed in cytotrophoblasts, and shows lower kinase activity than VEGFR-2, which is essential for signalling cascades in endothelial cells. sFlt-1is a spliced variant of the VEGFR-1, with the difference of lacking the transmembrane and intracellular domain. Most prior studies analysed mRNA expression in placental tissue. In this study, peripheral blood samples were taken, in order to demonstrate that its expression is not only altered in the placenta, but also in peripheral blood mononuclear cells. The researchers could also show a negative correlation between sFlt-1 and VEGFR-2. The reason behind this correlation might be the decreased levels of VEGF itself, as sFlt-1 binds it and lowers its availability, which then ceases its stimulating effects of VEGFR-2 synthesis. (39)

A Nepalese study came to similar findings: Pant et al. assessed the sFlt-1/PlGF ratio in women diagnosed with PE. The results showed an increased level of sFlt-1 and a decreased level of PlGF as well, resulting in a higher sFlt-1/PlGF ratio in pre-eclamptic women than in normotensive ones. Additionally, this study could demonstrate a significant correlation between sFlt-1 serum levels and the severity of PE. Severe courses of PE showed significantly higher levels of sFlt-1 than cases with mild PE. A rise in sFlt-1 reduces PlGF levels in severe PE, which can be detected even before clinical symptoms occur. They could also determine similar findings for sFlt-1 concentration in maternal urine, at the time they were already showing symptoms. This could make periodic monitoring of sFlt-1 concentration in pre-eclamptic women a helpful tool for determining the severity in these cases. Likewise, the researchers were able to find a significant correlation between early-onset PE and higher levels of sFlt-1 and PlGF, than in late-onset PE, which may be because of its non-placental source. Furthermore, they found a significant positive association

between sFlt-1:PlGF ratio and diastolic blood pressure. A possible mechanism behind these findings might be the anti-vasodilative effect of sFlt-1 on vessels, promoting the development of hypertension. (40)

Akther et al. came to the same results: higher levels of sFlt-1, lower levels of PlGF, and a higher ratio between those two factors. In addition to that, they investigated the correlation of these factors to arterial ageing, since elevated sFlt-1 levels are also found in patients with acute myocardial infarction. Likewise, it can be used as an efficient biomarker for progressive heart failure in cardiovascular patients. Endothelial dysfunction also plays a key role in atherogenesis and the development of cardiovascular diseases as well, so PE is not surprisingly a risk factor for future cardiovascular diseases. They found out that women with PE show a thicker intima, a thinner media and a higher I/M ratio of the common carotid artery, which they estimated by high-frequency ultrasonography. Furthermore, there was a positive correlation between sFlt-1 and sFlt-1/PlGF ratio and intima thickness and I/M ratio as well. These findings apply also to one year postpartum. Similar results were found regarding two modifiable risk factors for CVD: BMI and high blood pressure. This study found that those two factors were higher in the group of PE, than in the normotensive pregnancy group. Postpartum, BMI and high blood pressure decreased in both groups, however in the PE group a positive correlation between BMI/ hypertension and arterial wall thickness was found, indicating longstanding effects of PE on the cardiovascular system. (41)

In addition to that, Sánchez-Aranguren and her colleagues could show that sFlt-1 acts as a mitochondrial disruptor. Mitochondrial activity is important in pregnancy to maintain placental metabolic activity. Upregulated sFlt-1 levels however, cause mitochondrial ROS formation in endothelial cells, leading to mitochondrial dysfunction, oxidative stress and endothelial dysfunction, furthermore, a metabolic switch to glycolysis in endothelial cells. This leads to the conclusion, that sFLT-1 plays a role in metabolic adjustment and reprogramming in endothelial cells during pregnancy. (42)

Eddy and his colleagues found in their in-vitro study a link between elevated heparanase levels and the increased release of sFlt-1. Heparanase is an enzyme that is able to cleave heparan sulphate (HS) chains out of extracellular matrix and is upregulated in pre-eclamptic women. The basement membrane of placentas consists of a large portion of HS-rich proteoglycans, which are able to bind sFlt-1. In case of higher levels of heparanase, like in

PE, more HS chains are cleaved and more sFlt-1 is released. Moreover, they researched the role of hypoxia on mRNA sFLt-1 concentration. Their findings showed, that those levels remain unchanged during hypoxic conditions, however the release of sFLT-1 into the media was elevated in hypoxia. This data needs to be further researched in future in-vivo studies. (43)

Another new hypothesis connected dermatan sulphate and syndecan-1 to sFlt-1. Syndecan-1, a proteoglycan, found in placental tissue, regulates VEGF signalling together with heparan sulphate, both as part of the endothelial glycocalyx. The glycocalyx is lining the vascular wall and consists of membrane-bound proteoglycans and negatively-charged glycosaminoglycans. Those are responsible for several vascular functions, including vascular permeability, adhesion of leukocytes, coagulation and vasodilatation, by facilitating NO release and VEGF signalling. Syndecan-1 forms a complex with VEGFR-2 and so alters VEGF-induced motility and migration of endothelial cells. Heparan sulphate acts as a co-receptor for VEGF. Decreased levels of heparan sulphate cause reduced phosphorylation of VEGFR-2. It also possesses a receptor for sFLT-1, and acts as a reservoir and prevents excessive release of it into the blood stream and is elevated in PE and normotensive pregnancies as well. Lahsinoui et al. demonstrated that dermatan sulphate, another glycosaminoglycan, was increased in women with PE in contrast to the control group, moreover negatively correlated with blood pressure and positively with syndecan-1. Therefore they studied syndecan-1 deficient mice, in which a sFlt1 induced rise in blood pressure was absent, compared to normal ones. This data suggests a role of dermatan sulphate and syndecan-1 in the sFlt-1 mediated hypertension. (44)

## 4.4 VEGF

Vascular endothelial growth factor (VEGF) is responsible for physiological vasculogenesis and endothelial permeability. It increases NO production, which is a potent vasodilator and is important for normal endothelial function. Adu-Bonsaffoh and his colleagues did a study in order to examine VEGF serum levels in normal pregnancies, pre-eclamptic women and non-pregnant women. Their results showed a significant decrease during pregnancy in comparison to non-pregnant women in both pregnancy groups. However, the decrease in the PE group was far greater than in the normotensive pregnancy group, suggesting that VEGF plays an important role in the pathogenesis of pre-eclampsia. Furthermore, VEGF serum levels were significantly lower in early-onset PE, than in late-onset PE. (45)

Another study, by Agarwal et al., investigated mRNA expression of VEGF-A (member of the VEGF group) in pre-eclamptic placentae in contrast to uncomplicated ones. Their findings demonstrated a decrease in VEGF-A mRNA in the PE group VS. the controls. Moreover, the levels were even lower in HELLP and severe PE patients. In contrast to that, they found an increased level of VEGF-A mRNA in non-severe cases, supporting the theory that there is an initial compensatory increase of VEGF expression in order to restore normal blood flow. As the disease progresses, more placental tissue gets damaged, resulting in a decreased VEGF-A expression. (46)

In addition to that, Istrate et al. researched the receptors, VEGF binds to, R1 and R2. VEGFR-1 functions as a barrier between maternal and foetal circulation and are responsible for suppressing angiogenesis and vascular permeability. On the other hand, VEGFR-2 enhances the proliferation, migration and permeability of endothelial cells. This study shows an increased expression of VEGFR-1 in PE, suggesting a key role for this receptor in the development of PE. (47)

Wheeler et al. studied the role of VEGF in the pathogenesis of PE. They suggested a potential link between this factor and macrophages. During embryonal placentation, a massive inflammatory process takes place, in which macrophages have an important part in. There are two ways macrophages can be polarized: M1 macrophages produce pro-inflammatory cytokines among other things, whereas M2 macrophages are part of immune tolerance and tissue remodelling. In normal pregnancies, M1 is dominant at first, for the initial inflammatory response, but they switch to M2 for the rest of the pregnancy. This study shows a significant increase in M1 and a decrease in M2, as well as VEGF. Moreover, VEGF treatment enhanced macrophage recruiting and polarization shifts, suggesting that VEGF is important for normal placental development, hence creating a potential link to the pathogenesis of PE. (48) There are several other hypothesises on how the VEGF signalling pathway gets disrupted in PE. There are two studies on microRNAs, suggesting their role in the development of PE. Dong et al. showed that miR-646 reduces VEGF-A and therefore inhibits vasculogenesis. (49) The second study identified associated miR-16 with dysfunctional endothelium, as well as miR-200c with increased arterial stiffness. Nevertheless, the levels of miR-16 are not in-line with placental findings. Therefore those theories still need more investigation in the future. (50)

## 4.5 Inflammatory compounds

Pregnancy is known to be a low-grade state of chronic inflammation. Raio and his colleagues showed in their study, that CRP, an acute phase protein, which is highly expressed in case of infection or tissue injury, increases along with gestational age. The same applies to IL-6 (a pro-inflammatory cytokine). However, their findings demonstrated that in case of PE, both markers are elevated way higher, correlating with sFlt-1. In conclusion, there seems to be a connection between pro-inflammatory and anti-angiogenic markers in PE. (51)

Arlier studied the importance of leptin and the inflammatory response to it in the pathogenesis of PE. In this study, higher levels of leptin in the umbilical cord, correlating with increased secretion of IL-8 from endothelial cells, were found. The hormone leptin is responsible for energy balance, reproductive functions and immune reactions, linking the immune and inflammatory systems. Moreover, it has been recently discovered, that leptin takes also part in angiogenesis and regulating blood pressure. IL-8 is a pro-inflammatory cytokine, that regulates pathologic angiogenesis, among other things. This study suggests a connection between leptin and IL-8, in the manner of high leptin concentrations causing IL-8 secretion, leading to an enhanced neutrophil recruitment and leucocytes activation. As a consequence, this leads to endothelial destruction, an increased cytokine release and may be part of the development of PE. (52)

Recently, the scientific focus is more and more on the complement system being part of the pathogenesis of PE. The complement system is designed to protect the body from pathogens through activating the immune system and killing the pathogen with the membrane attack complex (MAC). Excessive activation of the complement system leads to different diseases in the human body, for example, haemolytic uremic syndrome. A disease, that strongly resembles PE and HELLP. Collier et al. studied if there is a correlation between Cd4 (a complement system activator), MAC and sFlt-1. Their findings showed a significantly increased complement activation in PE, associated with a rise of sFlt-1, resulting in placental damage. However, the scientists could not identify, if sFlt-1 is causing the complement activation or if it's the other way round, but suggested, in accordance with other studies, that early complement activation in pregnancy may be the reason for the increase in sFlt-1 and the development of PE. (53)

Matsuyama et al. researched the role of inhibitory complement factor H (CFH) in complement activation and vascular dysfunction. This study showed that an increased level

of sFlt-1 and a decrease of PlGF suppresses CFH secretion. As a result, CFH does not inhibit complement activation, causing endothelial dysfunction. (54)

In pregnancy, the vascular system needs to make major adaptions to provide adequate circulation for the growing foetus. Vasodilation of uterine arteries is of the essence or this adaption, mediated through vasodilators, like prostacyclin and nitric oxide. The biosynthesis of those factors is dependent on connexin (Cx) 43 and gap junction communication through $Ca^{2+}$ signalling bursts. Ampey and her colleagues showed in their study that increased levels of TNF-α, a pro-inflammatory cytokine that is commonly increased in women suffering from PE, inhibits Cx43 function and therefore $Ca^{2+}$ bursts, via phosphorylation. As a consequence, women with PE lose vasodilatory function in their uterine vessels, as a part of endothelial dysfunction in PE. (55)

Travis et al. investigated the role of interleukin-17 (IL-17) in the pathophysiology of pre-eclampsia. Their research showed that IL-17 mediates the activation of cytolytic natural killer cells (cNKs). NK cells belong to the innate immune system and have the ability to kill targeted cells without prior sensitization. In pregnancy, decidual NK cells are physiologically elevated. These cells however have little cytotoxic qualities and instead release chemokines, proangiogenic factors, and immunomodulatory cytokines. In PE, a shift from dNKs to cNKs seems to take place. This study identified IL-17 as a key stimulus for this shift, which makes it a possible target for future therapeutical strategies. (56)

## 4.6 Endocan

Lan and Liu identified endocan as a potential contributor to the pathogenesis of pre-eclampsia. Their meta-analysis showed, that pre-eclamptic women have higher levels of endocan, compared to normotensive pregnancies. Endocan is associated with different processes like cell adhesion, angiogenesis, inflammation and endothelial dysfunction and could therefore play a role in PE. Its exact function in this scenario however, is yet to be determined. (57)

## 4.7 Galectin

Pankiewicz et al. investigated in their study the role of Galectin-3 in the pathogenesis of PE. Galectin-3 belongs to the family of b-galactoside-binding lectins and is part of many physiological processes, like an inflammatory response, intercellular adhesion, fibrosis and angiogenesis. More importantly, its overexpression is associated with heart failure and it is therefore used as a cardiological biomarker. This study showed, that serum galectin-3 levels,

as well as the placental galectin-3 expression is increased in pre-eclamptic women. Their theory suggests that galectin-3 is expressed as part of a compensatory mechanism, due to impaired placentation and decreased placental perfusion. There are a few arguments supporting this theory: The expression of galectin-3 is dependent on the imbalance of anti-/angiogenic factors, which occurs in PE. Furthermore, its expression is triggered by hypoxia, hence it is counted as a positive stress response. Additionally, galectin-3 is a binding partner for endoglin, which is elevated in PE and functions as an anti-angiogenic factor. In PE, the biological functions, stimulating angiogenesis and inhibiting apoptosis, of galectin-3 seem to play an important role. The scientists believe that galectin-3 stimulates angiogenesis through the VEGF pathway, preventing VEGFR-2 from internalisation and increasing its sensitivity to VEGF. This process might be very important in PE, because of the increased levels of VEGF. (58)

## 4.8 Vitamin D

Vitamin D deficiency counts as one of the risk factors for pre-eclampsia. Vitamin D is part of the transcription and regulation of genes involved in placentation and normal angiogenesis. 25-hydroxyvitamin D [25-(OH)D], a metabolite of Vitamin D, is important for calcium and phosphate metabolism, stimulating calcium absorption and utilization. The active form, 1, 25-dihydroxyvitamin D prevents VEGF-derived cell activation and vascularisation. Chen et al. demonstrated in their study, that sFlt-1 level negatively correlates with the 25-(OH)D level in women suffering from PE. Furthermore, both are associated with disease severity and blood pressure indices. They therefore may be used as potential biomarkers for diagnosis, as well as prognosis of PE. (59)

## 4.9 Glycocalix

Another aspect of endothelial dysfunction in pre-eclampsia is the glycocalyx. The glycocalyx is a layer of glycoproteins and proteoglycans, lining the vascular endothelial cells. It has protective functions against endothelial activation, including cell adhesion and against the attack of proinflammatory and procoagulant molecules. Weissgerber and her colleagues found out, that patients with early-onset PE have signs of glycocalyx degradation and reduced microvascular perfusion, but not late-onset ones. (60)

## 4.10 Adhesion molecule

Adhesion molecules play an important part in endothelial activation and vascular injury, these include selectins [endothelial (E), platelet (P), and leucocytes (L)]. Selectins are

involved in leucocyte trafficking for example during inflammatory responses. Moreover, they participate in implantation, immune recognition, the adhesion of the blastocyst and trophoblast migration. Mistry et al. demonstrated the connections between selectins and PE. Their study showed elevated levels of maternal and placental E-selectin, suggesting an involvement in endothelial dysfunction. Interestingly, they found reduced levels of foetal E-selectin, which may be a protective adaption or the inability of producing it. In addition to that, they found lowered levels of P- and L-selectins. This study revealed a link between endothelial cells, platelets, and leucocytes and the development of PE, which definitely needs further investigation in the future. (61)

Another study from Kornacki et al. assessed, if there is a difference in the endothelial injury of early and late-onset PE. For this evaluation, they measured the concentration of hyaluronan, as an indicator of the endothelial glycocalyx, and the serum level of soluble vascular cell adhesion molecule-1 (sVCAM-1). The results showed similar levels of endothelial damage in both groups, which indicates the possibility of fatal outcomes in early and late-onset PE. (62)

## 4.11 Oxidative stress

Pre-eclampsia is associated with an elevated level of oxidative stress, after diagnosis. Ahmad et al. however, investigated the correlation of oxidative stress before the diagnosis of PE, meaning at a gestational age of 12-20 weeks of pregnancy. They analysed various antioxidants in their study, finding that catalase is the only one seemingly related to the severity of the disease. Nevertheless, this study suggests a link between oxidative stress and initiation, as well as progression, of PE. (63)

Al-Kuraishy et al. came to the same conclusion, finding in their study that PE is associated with an increased level of oxidative stress and a reduced level of the antioxidant paraoxonase (PON-1), which protects HDL and LDL from oxidation by free radicals. (64)

## 4.12 MMP

Matrix metalloproteinases (MMP) are extracellular proteinases, that are involved in numerous physiological and pathological mechanisms, for example embryogenesis, implantation, placental formation, neo-angiogenesis and tumour transformation. They also have anti-angiogenic qualities, causing endothelial dysfunction and also being part of the development of hypertension. Timokhina and her colleagues found in their study elevated levels of MMP-2, causing endothelial damage and hypertension, as well as decreased levels

of MMP-9, which is important for correct angiogenesis in spiral arteries. Both factors reflect the two stage theory of PE, on the one hand, MMP-9 being part of the first stage and a disrupted angiogenesis, and on the other hand, MMP-2 playing a role in the second stage with endothelial dysfunction. (65)

## 4.13 Other soluble factors

There are also other indicators for the diagnosis of PE. Besides sFlt-1, chemokine ligand 16 (CXCL 16) and lipocalin 2 (LCN-2) were assessed in a study by Li et al.. CXCL 16 is a protein with various functions, expressed in T-cells, B-cells and endothelial cells among others. It is part of the inflammatory response to endothelial damage. LCN-2 is part of the lipocalin family and has numerous different functions like mediating inflammation, inhibiting cell apoptosis and promoting tumour metastasis. It can also bind MMP9, causing reduced activation of MMP9, resulting in vascular remodelling, which can aggravate endothelial dysfunction. In addition to that, LCN-2 is an indicator of renal injury, a possible consequence of PE. Furthermore, it is correlated with insulin resistance, which is a risk factor for PE. Li et al. showed that all three of these factors are elevated in patients with PE, with LCN-2 having the highest association with diagnosis and grade of PE. (66)

High-temperature requirement A4 (HtrA4) is a placenta-specific serine protease, which is elevated in early-onset PE. Wang and Nie examined in their study, how HtrA4 is connected to endothelial dysfunction in PE. HtrA4 is involved in various cellular processes like apoptosis, proliferation and stress responses. In this study, they used human umbilical vein endothelial cells (HUVECs) to assess the effect of HtrA4 on the expression of endothelial genes. Knowing that at high levels, it disturbs tube formation of HUVEC, increases cellular permeability and disrupts cellular integrity, they found out, that HtrA4 alters gene expression, important for normal endothelial function and inflammatory responses. Furthermore, it stimulates the release of the pro-inflammatory cytokine IL6. This may be one of the underlying causes of developing PE and definitely requires further investigation in the future. (67)

Soluble Axl (sAxl), part of the TAM family of receptor tyrosine kinases, is an indicator of vascular dysfunction. It is involved in angiogenesis and a receptor for Growth arrest-specific 6 (Gas6), a factor that regulates migration and anti-apoptosis of vascular smooth muscle cells. Gui et al. demonstrated that sAxl is elevated in patients with PE. Their theory states that these high levels inhibit Gas6, resulting in impaired angiogenesis and endothelial cell

damage. Furthermore, the upregulated sAxl was positively correlated with plasma levels of sFlt-1 and negatively correlated with eNOS, supporting their theory of its involvement in the pathogenesis of PE. (68)

# 5. Conclusion and Future Directions

Pre-eclampsia is affecting 5-7% of pregnancies worldwide and is therefore a major cause of foetal and maternal morbidity and mortality. An understanding of the development of this disease is necessary to change that. Due to the poor understanding of its pathogenesis, prevention, diagnosis and treatment options have been limited. (69) Current studies have shown various new and different approaches. One new hypothesis concentrates on the mother's cardiovascular system and suggests that the women's predisposition and ability to adapt to pregnancy-induced vascular changes determines, whether PE occurs and its severeness. This theory is supported by findings of normal placentas in symptomatic mothers. Furthermore, there is still to be discussed, whether early- and late-onset PE develops through the same mechanisms. (11)

However, the present findings demonstrate an interaction of several factors: The current valid theory describes a two-stage process in the development of PE. First, an impaired placental formation, occurring in the first few weeks of pregnancy, takes place. Due to an incomplete trophoblast invasion and remodelling of the spiral arteries, the placentas reperfusion is diminished, damaging the tissue and causing ischemia and oxidative stress. The second stage includes endothelial dysfunction, with the development of hypertension and multiorgan damage. (65)

Different mechanisms play part in those two stages, concentrating on the second stage as the topic of this review. RUPP changes the expression of bioactive factors like sFlt-1, VEGF, PlGF and CAMs, causing an imbalance in the pro- and anti-angiogenic metabolism. Additionally, proinflammatory cytokines, like CRP and interleukins, hypoxia-inducible factor, reactive oxygen species, and angiotensin $AT_1$ receptor agonistic autoantibodies are released, damaging the endothelium, smooth muscle cells, the glycocalyx and the extracellular matrix. As a result, generalized endotheliosis develops, leading to an imbalance of vasoconstrictive and vasodilative factors. Furthermore, studies presented a decreased NO bioavailability, causing impaired vasodilatation. Enhanced intracellular $Ca^{2+}$ stimulates contraction in smooth muscle cells, increasing vasoconstriction further. Dyslipidaemia and changes in the complement system also seem to play a part in the pathophysiology. New indicators for diagnosis and/or possible therapeutical targets include lipocalin-2, sAxl, galectin-3 and HtrA4. Compromised functions of matrix metalloproteinases cause arterial stiffness and inadequate vascular remodelling, leading to hypertension. (30,32,38,51-53,58,60,64-66)

Future scientific research should take the following aspects into consideration: First of all, there is a need for further understanding of the interaction between the bioactive factors, molecular mechanism and vascular mediators, which may differ between early- and late-onset PE. (70) For example, sFlt-1 serum levels differ between mild and severe PE, as well as early- and late-onset courses. (40) Secondly, a deeper understanding of the pathophysiology of PE hopefully leads to better therapeutic options and prolonged gestation, since the only curative treatment still is the delivery of the child. Further research is necessary on treatment strategies, like restoring the angiogenic imbalance or reducing PlGF levels, as well as targeted drug delivery and regenerative therapy, which may even prevent PE. (8,69) A potential therapeutical strategy could include L-arginine supplementation for example. (32) Thirdly, new diagnostic criteria need to be established, as the definition of hypertension and proteinuria is obsolete. New onset hypertension and altered angiogenic status are much more accurate diagnostical parameters, providing the possibility of earlier diagnosis and better prevention of fatal outcomes. (71) Lastly, as women with pre-eclampsia not only have a high-risk level for complications during pregnancy, it seems that their future, post-partum cardiovascular risk is elevated as well. This makes prevention, diagnosis and therapy, during and after birth, even more important. (30)

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