Diplomarbeit

The effects of smoking in neonates born to mothers with pre-eclamptic risk- A case series study

eingereicht von

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Graz, am 31.01.2022 *Karolina Marta Abramowicz eh*

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Abbreviations

AC	Abdominal circumference
AGA	Appropriate for gestational age
APGAR-Score	Appearance, -Pulse, -Grimacing, -Activity, -Respiration Score
ASS	Acetylsalicylic acid
BMI	Body mass index
BPDE-I	Benzo(a)pyrene diol epoxide-I
Case C-S	Case Current-Smoker
Case F-S	Case Former-Smoker
Case N-S	Case Non-Smoker
CO	Carbon-monoxide
CPR	Cerebroplacental ratio
CTG	Cardiotocography
EDF	End-diastolic flow
EPO	Erythropoietin
HbCO	Carbon monoxide haemoglobin
HC	Head circumference
HO	Haemoxygenase
IGF-1	Insulin like growth factor-1
IGFBP-3	Insulin like growth factor binding protein-3
ISSHP	International Society for the Study of Hypertension in Pregnancy
IUGR	Intrauterine growth restriction
MCA	Middle cerebral artery
MCV	Mean corpuscular volume
nACHR	Nicotine acetylcholine receptor
NK	Natural killer cell
oGTT	Oral glucose tolerance test
PCOS	Polycystic ovary syndrome
PE	Pulmonary embolism
PI	Pulsatility index
PlGF	Placental growth factor
RBC	Red blood cell
RI	Resistance index
sENG	Soluble endoglin

sFlt-1	Soluble fms-like tyrosine kinase-1
Th1/Th2	T helper cell 1,2
UCB	Umbilical cord blood
VEGF	Vascular endothelial growth factor
WBC	White blood cell
WHO	World Health Organisation

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Zusammenfassung

Einleitung: Mütterliches Rauchen während der Schwangerschaft und mütterlicher Prä-eklampsie haben beide negative Auswirkungen auf die neonatale Gesundheit. Zudem sind beide Faktoren mit negativen Folgen assoziiert, welche unter Anderem neonatale Wachstumsretardierung, Frühgeburtlichkeit, Fehlgeburten und neonatales Sterben beinhalten.

Zielsetzung: Es wurde untersucht, inwiefern mütterliches Rauchen während der Schwangerschaft postnatale Parameter von Neugeborenen beeinflusst, bei zusätzlichem Vorliegen eines positiven mütterlichen Prä-eklampsie Risikos.

Methoden: Diese Arbeit wurde im Zuge der größeren *"Does nicotine reduce the risk of pre-eclampsia? A prospective study"*-Studie, der Medizinischen Universität Graz durchgeführt. Drei Neugeborene wurden hinsichtlich der folgenden mütterlichen Klassifizierung inkludiert: *Case C-S,* dessen Mutter während der Schwangerschaft geraucht hat; *Case F-S*, dessen Mutter in der Schwangerschaft aufgehört hat zu rauchen; *Case N-S,* dessen Mutter Nicht-Raucherin war. Pränataler fetaler Ultraschall, postnatale neonatale Anthropometrie, APGAR-Score, venöse und arterielle Nabelschnurblutanalysen sowie postnatale neonatale Veränderungen und Adaptationen in der ersten Lebenswoche wurden verglichen.

Ergebnisse: Während der Zweit-Trimester Ultraschall Untersuchung zeigte die aktive Raucherin einen erhöhten utero-plazentaren Widerstand. Alle prä- und postnatal anthropometrischen Parameter des korrespondierenden Neugeborenen, sowie der übrigen Neugeborenen, wurden als normal eingestuft. Die Nabelschnurblutanalysen aller Neugeborenen wurden als physiologisch eingestuft. Auffallenderweise zeigte das Neugeborene der aktiv rauchenden Mutter die größten Differenzen zwischen venösen und arteriellem Nabelschnurblut. Daraus folgend wird eine Erhöhung des Metabolismus vermutet. Während des Wochenbetts wurden keine gesundheitlichen Komplikationen vermerkt. Alle Neugeborenen wurden in gutem klinischem Zustand entlassen.

Schlussfolgerung: Die Ergebnisse dieser Studie zeigten potenzielle Auswirkungen eines positiven prä-eklamptischen mütterlichen Risikos und mütterlichen Rauchen

während der Schwangerschaft auf den utero-plazentaren Widerstand sowie auf neonatale Nabelschnurblutwerte. In diesem Falle führten ein positives präeklamptisches mütterliches Risiko und mütterliches Rauchen zu keinen negativen Auswirkungen auf die neonatale Gesundheit. Für ein besseres Verständnis dieser Thematik wird empfohlen die beschriebenen neonatalen Parameter in einer größeren Studienpopulation zu vergleichen.

Abstract

Introduction: Maternal smoking during pregnancy and maternal pre-eclampsia have negative effects on neonatal health and are associated with adverse outcomes. These include foetal growth-restriction, pre-term delivery, miscarriage, and neonatal death.

Objective: To investigate how smoking influences neonatal parameters of infants born to mothers with pre-eclamptic risk.

Methods: The study was conducted as part of the larger *"Does nicotine reduce the risk of pre-eclampsia? A prospective study"* trial at the Obstetrics Ward at the Medical University of Graz. Three neonates were selected according to the initial study: Neonate of a mother who actively smoked during pregnancy (*Case C-S*), and neonate of a mother who was a former smoker prior to pregnancy (*Case F-S*), and neonate of mother who was a non-smoker (*Case N-S*). Prenatal foetal ultrasound examinations, postnatal neonatal biometry, appearance, -pulse, -grimacing, -activity, -respiration Score (APGAR), venous and arterial umbilical cord blood parameters (UCB), and neonatal changes and adaptation until discharge were compared amongst the three cases.

Results: During the 2nd trimester ultrasound examination, increased utero-placental resistance was noted in the actively smoking mother. Nonetheless, prenatal, and postnatal anthropometric parameters were classified as normal in the corresponding neonate, as well as in the other cases. Umbilical cord blood parameters of all cases were within the physiological range. However, the neonate born to the smoking mother displayed the highest differences between arterial and venous cord blood parameters, suggesting increased metabolism. During puerperium, none of the cases developed adverse health conditions. All neonates were discharged in good clinical health.

Conclusion: The results of this case series study have shown the potential effect of pre-eclamptic risk and maternal smoking on utero-placental resistance and neonatal cord blood parameters. Regarding the included cases, neonatal health was not affected more severely, when exposed to maternal smoking combined with pre-eclamptic risk.

For a better understanding, it is recommended to compare the investigated neonatal parameters in a larger study size.

1. Introduction

Pre-eclampsia and tobacco consumption in the form of smoking are well known to have negative effects on the mother and the neonate. Both factors can lead to changes in placental function and biology. In general, pre-eclampsia and smoking during pregnancy are associated with higher foetal mortality and morbidity rates. Underlying causes for increased morbidity and mortality include foetal intrauterine growth restriction, pre-term delivery, miscarriage, and neonatal death.

Furthermore, perinatal maternal smoking not only induces indirect placental changes, but also directly impacts the placental barrier via tobacco components. Although the harm of smoking during pregnancy is well known, smoking is believed to be associated with a reduction in pre-eclamptic risk.

Based on this study´s goal, the following chapters will elaborate the effect of pre-eclampsia and smoking on the placenta, the foetus, and the neonate separately. Also, the importance of this topic for neonatal life period, as well as for later adulthood, will be explained.

1.1. Pregnancy and pre-eclampsia

During pregnancy, the female body must undergo changes and adaptations to provide the needed environment for the child´s development. These include alterations to the organ systems as seen in figure 1. In pre-eclampsia, a pregnancy induced condition, cardiovascular changes play a major role. Peripheral vascular vasodilation accompanied by decreased vascular resistance (also in the uterine circulation) result in a reduction of blood pressure. Subsequently, cardiac output increases through higher stroke volume, heart rate and blood volume (1).

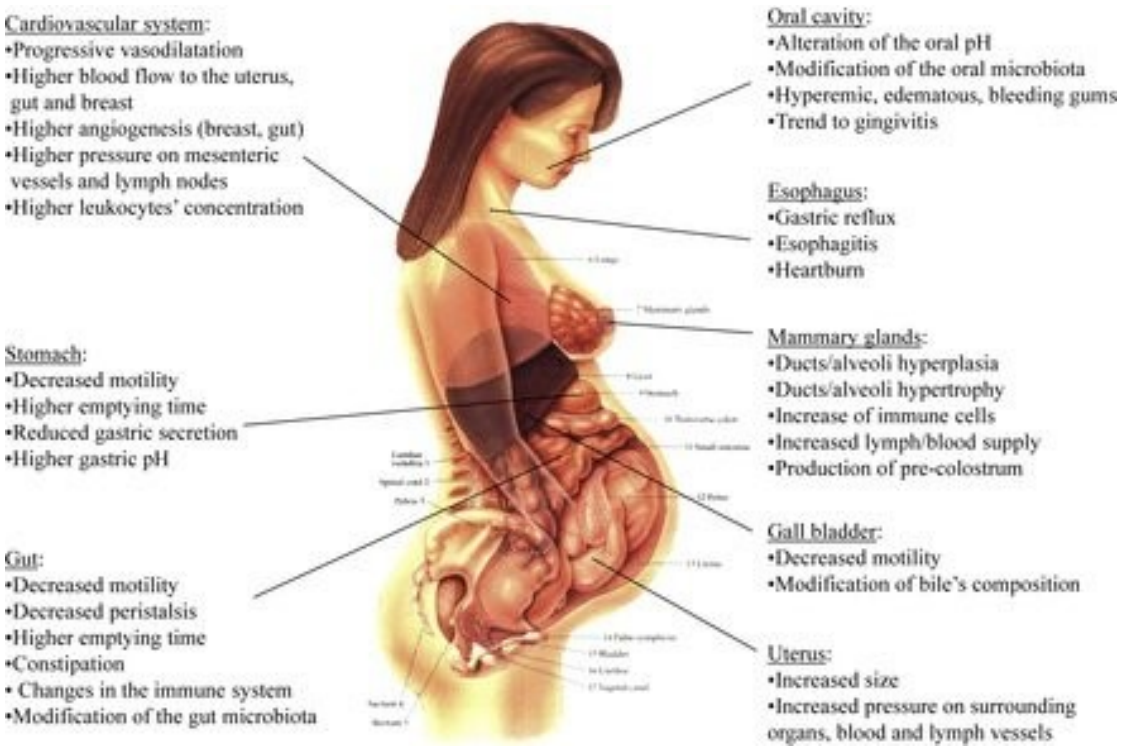

Figure 1: "Physiological adaptations of the female body during pregnancy" (2)

Even though blood pressure should decrease over the course of pregnancy, there are certain hypertensive disorders which specifically occur during pregnancy (figure 2).

Hypertensive disorders during pregnancy are divided into two subgroups: Pre-existing arterial hypertension (before pregnancy) or its manifestation before the 20th gestational week, and the development of hypertension at or after the 20th gestational week (3). As such, pre-eclampsia is among these hypertensive disorders (4).

Previously, pre-eclampsia was classified as a new onset of hypertension with a systolic blood pressure of >140mmHg and a diastolic blood pressure of >90 mmHg, accompanied by proteinuria at or after the 20th gestational week. Currently, this definition was extended to include other symptoms such as maternal organ or uteroplacental dysfunction. As seen in the figure below, proteinuria is not an essential criterion anymore (4).

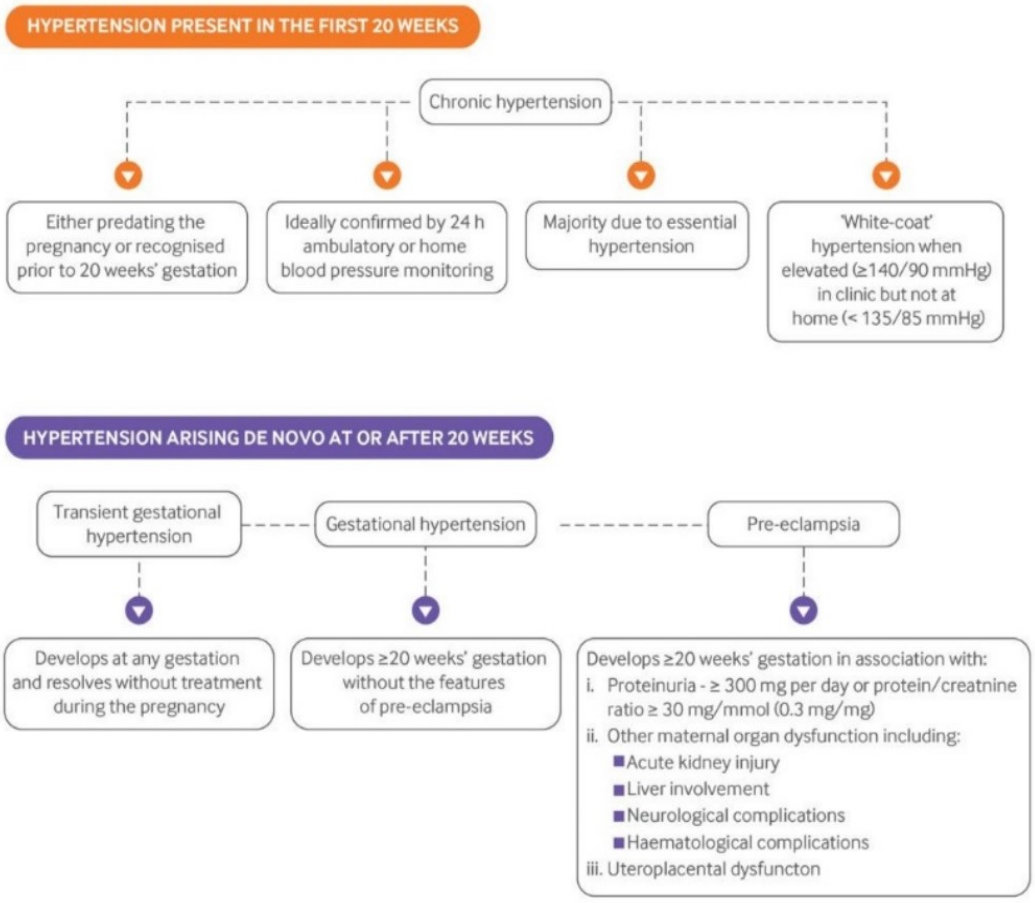

Figure 2: "Categories of hypertension in pregnancy recognised by the ISSHP" (4). Divided into pre-existing arterial hypertension (before pregnancy) or its manifestation before the 20th gestational week, and the onset of hypertension at or after the 20th gestational week (3).

The incidence rate of pre-eclampsia is hard to determine. Firstly, because of the complex diagnostic criteria and secondly, because of the differences in detection between low- and middle-income countries (5). A systematic review including data from nearly 39 million pregnancies throughout 40 different nations suggested an incidence rate of 4.6% (6).

Pre-eclampsia is one of the leading causes for maternal mortality and morbidity. An analysis by the World Health Organization observed hypertensive disorders during pregnancy to be the second most common cause of death after haemorrhagic complications. Every year, pre-eclampsia is responsible for 70.000 maternal and 500.000 foetal deaths worldwide (3-5).

Notably, there are far more foetal than maternal deaths. Another study, conducted by the World Health Organisation, showed that eclampsia/pre-eclampsia were the primary obstetrical cause for 1 out 4 perinatal deaths (perinatal death: foetal death at and after the 28th gestational week, neonatal death during labour, neonatal death during the first week of life). In 2014, another study including more than 300.000 pregnancies showed a similar mortality rate (7).

Due to the progressive tendency of pre-eclampsia, delivery is needed to halt progression and often results in iatrogenic pre-term delivery. In such cases, obstetric doctors must balance the negative effects of foetal prematurity and the ensuing consequences against the benefits of delivery and cessation of pre-eclampsia (8).

1.1.1. Pathophysiology of pre-eclampsia

Currently, there are two known subtypes of pre-eclampsia: early and late onset pre-eclampsia. Early onset pre-eclampsia emerges prior to the 34th gestational week and is believed to originate from a dysfunctional placentation. Late onset pre-eclampsia appears at or after the 34th gestational week and is thought to arise from an ageing placenta (9). In the end, both forms induce uteroplacental ischaemia and placental stress, although at different points in pregnancy. The inducement of placental stress, without the manifestation of maternal symptoms, is considered as the first stage of both forms of pre-eclampsia (10).

Placental stress leads to a placental release of nanoparticles into the maternal circulation, promoting the second stage of pre-eclampsia (3, 4). These secretions include harmful substances such as sFlt-1 and sENG, which have the potential to generate a dysfunctional maternal endothelium (5). Consequently, maternal systemic vascular dysfunction can emerge, manifesting as hypertension, proteinuria, visual disturbances, headaches, and cerebral oedema (3, 4). Pre-eclamptic conditions have the potential to evolve into other pregnancy associated disorders. These could include eclampsia, HELLP-Syndrome, or other coagulative abnormalities (5). Until now, only hypotheses exist about the origin of pre-eclampsia. Among others, the occurrence of certain pathologies such as prior pre-eclampsia, chronic hypertension (11), thrombophilia (12) and vascular conditions (e.g. Henoch-Schoenlein purpura) (13) were observed to correlate positively with the development of pre-eclampsia.

Early onset pre-eclampsia:

As mentioned, early onset pre-eclampsia is thought to be based on abnormal placental development, possibly due to a failure in interactions between foetal trophoblast cells and uterine structures. There are many hypotheses of what the cause of abnormal placentation might be, including maternal genetic, environmental, and immunological factors. However, the mechanisms behind the malfunctions are still unknown. One hypothesis describes the unsuccessful interaction between the shell of foetal trophoblast cells with the uterine decidua and uterine endometrial glands. These abnormal interactions can be compatible with pregnancy but can potentially lead to pre-eclampsia. In severe cases, this failed interplay might not be compatible with pregnancy and result in spontaneous miscarriage (4).

Another hypothesis suggests that foetal extra-villous trophoblast cells either fail to achieve the full range of invasion to remodel spiral arteries of the uterus, or that they do reach the uterine spiral arteries, yet fail to destroy their walls (4). This process would usually take place between the 8^{th} and 18^{th} gestational week (7). As seen in figure 3, remodelling is essential to reduce velocity of incoming blood to prevent damage to the placental villi (4).

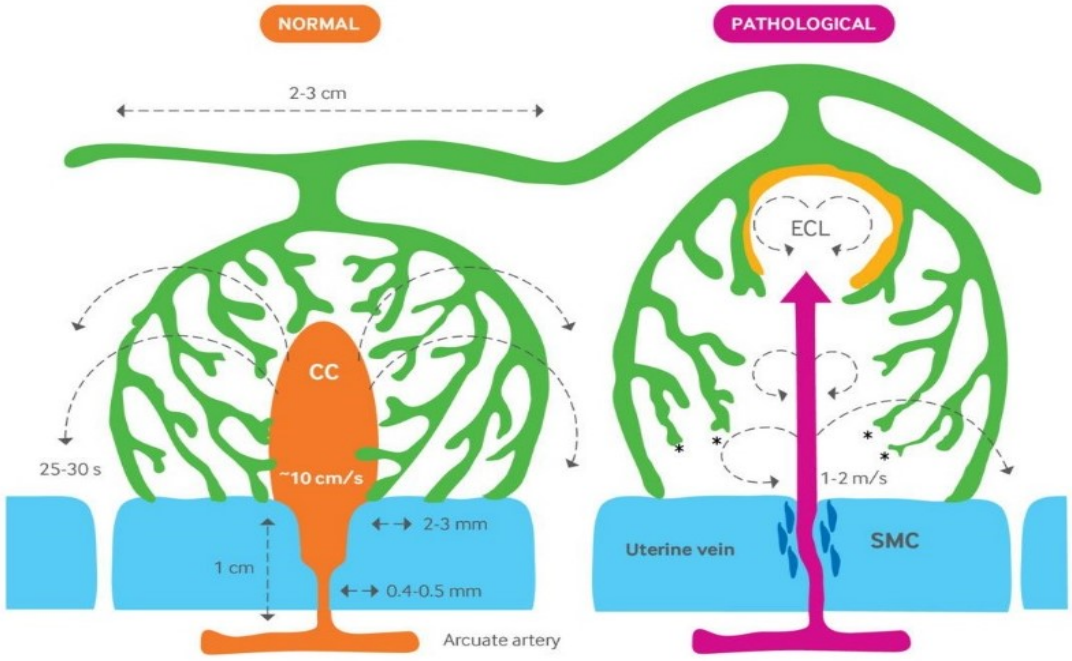

Figure 3: "Diagrammatic representation of the effects of spiral artery remodelling on the inflow of maternal blood into the intervillous space in normal and pathological pregnancies.". <u>Normal remodelling (left):</u> dilatated distal part of the spiral artery (from approximately 0,4 to 2-3mm); incoming speed 10cm/s into to the central cavity (CC) of a placental lobe; transit time to uterine vein approximately 25-30s. <u>Abnormal remodelling (right):</u> missing remodelling of the spiral arteries; increased incoming speed of 1-2m/s with reduced transit time and turbulent flow; consequent damage to villous tissue forms echogenic cystic lesions (ECL) lined by thrombus (stippled); retention of smooth muscle cells (SMC) increasing likelihood of spontaneous vasoconstriction (4).

Missing transformation of the spiral arteries´ hypercontractile segment (consisting of smooth muscle cells) was observed in pre-eclamptic placentas. In non-pregnant women, the hypercontractile segment restricts blood loss during menstruation. However, in pregnancy the retention of smooth muscle cells can lead to spontaneous vasoconstriction and, subsequently, reduced blood flow to the placental lobes (4).

<u>Late onset pre-eclampsia:</u>

In contrast to early onset pre-eclampsia, there is no evidence of poor spiral artery remodelling in late onset pre-eclampsia. Moreover, an imbalance between maternal supply and foetal demand is believed to induce placental stress. This imbalance increases "as the growing placenta reaches its size limit" (10). Maternal factors,

including genetic predispositions for cardiovascular diseases, predispositions to inflammation and metabolic diseases, play an important role in inducing late onset pre-eclampsia (4).

1.1.2. Pre-eclampsia: Intrauterine growth restriction

The definition of intrauterine growth restriction (IUGR) varies internationally. The definition by the "German Society of Gynaecology and Obstetrics" was used for this study and includes the following criteria:

1. estimated foetal birth weight lower than the 10th percentile AND/OR growth not according to the percentiles AND
2. a pathological doppler sonography of the umbilical artery OR
3. a pathological doppler sonography of the uterine arteries OR
4. oligohydramnios (14)

It is important to differentiate between an intrauterine growth restricted foetus and a small for gestational age foetus. Foetuses experiencing IUGR do not grow according to their genetic potential. The underlying pathology can be attributed to maternal, placental, or foetal causes. In the case of pre-eclampsia, placental and maternal factors can promote IUGR (14, 15).

Three types of IUGR are currently known:

- symmetrical IUGR,
- asymmetrical IUGR,
- symmetrical combined with asymmetrical IUGR (16).

Symmetrical IUGR occurs in early pregnancy due to genetic disorders or infections intrinsic to the foetus. These conditions result in a reduction in all foetal biometric parameters because of a decrease in the total cell count. Asymmetrical IUGR develops due to utero-placental insufficiency (as in pre-eclampsia), leading to a normal total cell count in the foetus but a reduction in its cell size. Indices evaluating foetal asymmetrical growth tendencies, such as ponderal or cephalization index, are abnormal in these cases. Mixed IUGR (symmetrical combined with asymmetrical

foetal growth restriction) can occur when early onset IUGR is affected by placental conditions in later pregnancy (16).

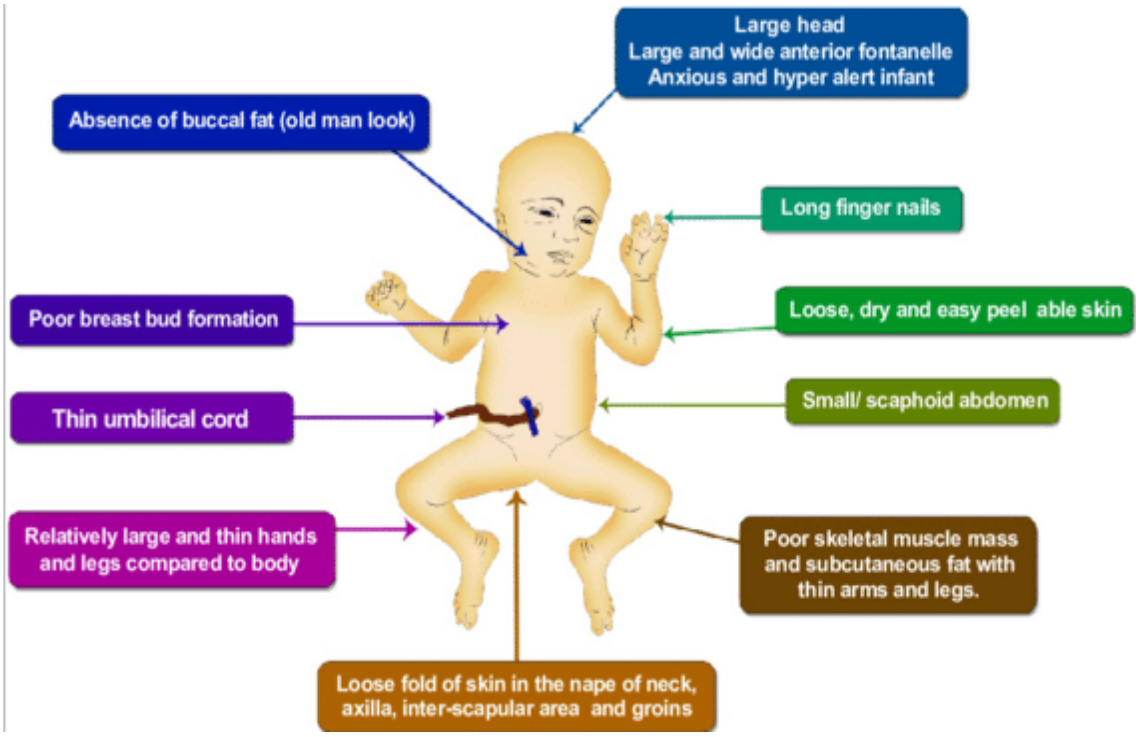

Figure 4: Clinical features of infants at birth that are having intrauterine growth restriction (16).

1.1.2.1. Pre-eclampsia: Changes promoting IUGR

Foetal growth depends on the availability of nutrients. As such, maternal diet, uteroplacental blood flow, placental villous development, the capacity of villous trophoblasts, and fetoplacental circulation form a fundamental base in foetal growth (15). Limited blood flow to the placenta due to alterations in uteroplacental blood vessels, can cause infarctions in the placental villous tissue. Villous free placental lakes and focal necrosis with a loss of microvilli were more likely to be found in pre-eclamptic placentas (15).

Another concern is the downregulation of important transporters, which impair the placental transfer of amino acids, fatty acids, and glucose to the foetus. The responsibility for the expression and activity of these transporters lies within the AKT/mTOR pathway, which is a central regulator in the translation of mRNA to proteins. A deficient remodelling of the spiral arteries has not been identified as the

cause of transporter-downregulation so far. However, evidence strongly suggests that it plays a central role in the emergence of IUGR (15).

The definition of IUGR also takes ultrasound parameters of the umbilical artery and the uterine arteries into consideration. Hereby, resistance indices of the blood vessels and Doppler waveforms of blood flow are assessed. In pre-eclamptic women, deficient remodelling of uterine spiral arteries is associated with increased resistance indices and abnormal Doppler waveforms of the umbilical and uterine arteries (4, 17). Regarding the umbilical artery´s flow pattern, heightened resistance patterns can present themselves as absent or reversed end-diastolic flows. This condition is further associated with foetal hypoxia (15).

1.1.2.2. Pre-eclampsia: Consequences of IUGR

Decreased placental perfusion can lead to low oxygen saturation in foetal blood and consequently to foetal hypoxaemia. The foetus reacts by centralising its systemic blood flow and providing more blood to oxygen sensitive organs such as the brain, heart, and adrenal glands. This mechanism is also known as brain-sparing growth. Vasoconstriction of foetal pulmonary, intestinal, renal, skeletal, and skin vessels, as well as vasodilatation in cerebral, cardiac, and adrenal vessels contribute towards this effect. Consequently, the afterload of the foetal right ventricle increases due to high systemic resistances while the afterload of the left ventricle decreases due to cerebral vasodilatation. Additionally, the blood flow through the ductus venosus surges, intensifying the bypass of the liver and therefore resulting in reduced liver fat (18).

This distributional process promotes specific neonatal anthropometric characteristics, making it possible to presume the occurrence of foetal IUGR postnatally (figure 4). Among others, these biometric features include a reduction in birthweight, large head circumference in relation to abdominal circumference, decreased overall and abdominal fat (16, 18).

1.1.3. Pre-eclampsia: Changes in foetal haematological parameters

Foetal haematological parameters can be affected through an insufficient exchange of oxygen and nutrients between the placenta and the foetus. These haematological

changes include a decrease in platelet and white blood cell counts, and an increase in red blood cell, reticulocyte and normoblast counts (8, 19).

Foetal hypoxia, induced by pre-eclampsia due to placental changes, is believed to have an oppressive effect on megakaryocyte proliferation. Due to suppressed megakaryocyte proliferation, the platelet count drops. Foetal hypoxia is further suggested to have an inhibitory effect on myeloid lineage, explaining the reduction in leukocyte count (8).

Foetal red blood cell production is regulated by erythropoietin (EPO). It is responsible for maintaining a steady-state erythropoiesis and normal haemoglobin levels. In the event of hypoxia, EPO accelerates erythropoiesis. A correlation was seen between high EPO levels in umbilical cord blood plasma and foetal hypoxia in growth restricted foetuses. High EPO levels were also linked to low base excess, pH, and pO2 values, as well as high pCO2 and lactate values in arterial cord blood (20).

In general, studies observing changes in foetal haematological parameters of neonates born to pre-eclamptic mothers have detected a rise in red blood cell counts, reticulocyte counts, and haemoglobin. On the other hand, foetal white blood cell counts, and platelet counts were lowered (19, 21-23).

Table 1: Publication by El-Sayed et al: Comparison between parameters of neonates born to pre-eclamptic mothers and neonates born to healthy controls. Legend: Hb, haemoglobin; **MCV**, mean corpuscular volume; **MCH**, mean corpuscular haemoglobin; **MCHC**, mean corpuscular haemoglobin concentration; **RBC**s, red blood cells; **WBC**s, white blood cells; **PT**, prothrombin time; **PC**, prothrombin concentration; **PTT**, partial thromboplastin time; *, significant ($p<0.05$) (21).

Variables	Full term			Preterm		
	Patient N=30	Control N=15	p-value	Patient N=30	Control N=15	p-value
Birth weight (kg)	2.2±0.2	3.1±0.1	0.001*	1.8±0.2	2.2±0.1	0.001*
APGAR (1 minute)	7.7±0.8	8.8±0.7	0.001*	6.5±0.7	7.4±0.3	0.001*
APGAR (5 minute)	8.3±0.6	9.5±0.5	0.001*	7.5±0.9	8.5±0.8	0.001*
Hematocrit value						
Hb (gm/ml)	16.2±0.9	15.4±0.8	0.004*	16.6±1.1	15.1±0.5	0.001*
MCV	89.1±2.2	91.6±3.1	0.67	82.1±3.2	89.6±1.1	0.77
MCH	24.1±1.8	23.9±1.9	0.78	25.1±2.7	24.9±1.9	0.68
MCHC	32.7±3.3	33.8±4.1	0.67	32.7±1.3	34.8±1.1	0.67
RBCs (106/ml)	4.6±0.4	3.6±0.2	0.001*	4.7±0.4	3.8±0.4	0.001*
WBCs (103/ml)	10.7±2.1	13.1±1.9	0.001*	10.8±2.2	13.8±1.7	0.001*
Plateletcount (109/L)	186.1±91.5	223.1±43.4	0.001*	133.7±58.9	216.4±51.8	0.001*
PT (second)	28.03±6.9	14.1±1.8	0.001	21.7±3.1	13.4±1.5	0.001*
PC (%)	43.2±7.8	70±3.5	0.001	61±7.7	74.2±19	0.001*
PTT (second)	52.9± 6.7	50.3±2.6	0.003	51.2±9.7	31.8±6.5	0.001*

As seen in the table above, neonates born to pre-eclamptic mothers (pre-term and term) had significantly increased red blood cell levels (RBC) and haemoglobin as well as significantly decreased white blood cell levels (WBC) and platelet counts when compared to neonates born to healthy control groups. Besides platelet counts, the haematological values did not differ between the term and pre-term pre-eclamptic group (21).

1.1.4. Pre-eclampsia: Changes in umbilical cord blood gases

Najib et al´s study compared umbilical cord blood gas parameters of neonates born to pre-eclamptic mothers with those neonates born to healthy controls. In this study,

correlations between low O_2 partial pressure and low pH-values were detected in neonates born to pre-eclamptic mothers. Furthermore, they observed associations between neonates born to pre-eclamptic mothers and heightened umbilical cord blood CO_2 partial pressure levels. Umbilical cord blood bicarbonate concentrations showed no significant differences between the pre-eclamptic group and the controls (24).

Table 2 presents umbilical cord blood gas profiles of appropriate for gestational age neonates (AGA) born to pre-eclamptic mothers compared to neonates born to healthy controls (25). Hereby a novel placental oxygenation parameter was introduced, calculated by deducting the absolute arterial O_2 partial pressure from the absolute venous O_2 partial pressure (A-V pO_2). By the same principle, placental clearance parameters were introduced by deducting atrial pCO_2 from venous pCO_2 (A-V pCO_2) (25).

Table 2: Publication by Matsuo et al: Umbilical cord gas analyses in appropriate for gestational age (AGA) neonates born to pre-eclamptic mothers compared to AGA neonates born to healthy controls. Legend: APGAR-Score, appearance-pulse-grimace-activity-respiratory Score; **pO_2**, oxygen partial pressure; **pCO_2**, carbon dioxide partial pressure; **$HCO3^-$**, bicarbonate; **A-V**, arterio-venous difference; significant ($p<0,05$). **(25)**

Subjects	Pre-eclampsia n=33	Non-pre-eclampsia n=66	P-value
Gestational age (weeks)	30.4±3.6	30.4±3.6	1.0
Birth weight (g)	1518±648	1685±811	0.4
Apgar 1 min	6 (1–9)	6 (1–9)	0.68
Apgar 5 min	8 (2–9)	8 (2–9)	0.77
Arterial pH	7.20±0.12	7.22±0.1	0.49
Arterial pO_2	13.9±6.7	15.3±6.5	0.29
Arterial pCO_2	60.3±16.7	59.8±13.6	0.68
Arterial HCO_3	22.4±3.8	23.5±3.9	0.08
Arterial base excess	−6.0±4.8	−4.8±4.4	0.11
Venous pH	7.24±0.12	7.29±0.09	0.03
Venous pO_2	21.0±5.9	26.6±8.1	0.001
Venous pCO_2	52.5±15.1	49.1±12.6	0.29
Venous HCO_3	22.1±4.1	23.0±2.5	0.26
Venous base excess	−5.0±5.2	−3.4±3.2	0.06
A-V pO_2 difference	7.1±3.8	11.3±5.9	0.001
A-V pCO_2 difference	7.8±5.7	10.7±5.9	0.01

Mean±SD or median (range) is shown. Mann-Whitney *U*-test for all statistic comparisons.

In agreement with Najib et al´s study, Matsuo et al´s study showed a significant reduction in venous pO_2. Also, A-V pO_2 and A-V pCO_2 were significantly lowered in

neonates born to mothers with pre-eclampsia. Matsuo et al further compared these blood gas parameters between IUGR neonates born to pre-eclamptic mothers and IUGR neonates born to healthy controls.

Table 3: Publication by Matsuo et al: Umbilical cord gas analyses in intrauterine growth restricted neonates born to pre-eclamptic mothers compared to intrauterine growth restricted neonates born to healthy controls. Legend: APGAR-Score, appearance-pulse-grimace-activity-respiration Score; **pO_2**, oxygen partial pressure; **pCO_2**, carbon dioxide partial pressure; **$HCO3^-$**, bicarbonate; **A-V**, arterio-venous difference; significant (p<0,05). **(25)**

Subjects	Pre-eclampsia n=67	Non-pre-eclampsia n=67	P-value
Gestational age (weeks)	30.2±3.0	30.2±3.0	1.0
Birth weight (g)	989±360	964±406	0.44
Apgar 1 min	5 (1–9)	6 (1–9)	0.73
Apgar 5 min	8 (2–9)	8 (2–9)	0.82
Arterial pH	7.23±0.09	7.20±0.10	0.12
Arterial pO_2	13.7±5.4	14.5±7.9	0.97
Arterial pCO_2	57.8±10.6	60.1±11.7	0.25
Arterial HCO_3	23.3±3.5	22.1±3.8	0.044
Arterial base excess	−6.3±4.8	−4.6±4.6	0.039
Venous pH	7.27±0.09	7.26±0.09	0.49
Venous pO_2	20.3±6.3	25.4±11.9	0.003
Venous pCO_2	51.2±9.8	49.2±11.9	0.36
Venous HCO_3	22.9±3.6	22.3±3.0	0.23
Venous base excess	−4.7±4.1	−3.8±4.2	0.27
A-V pO_2 difference	6.6±3.1	11.4±8.0	<0.001
A-V pCO_2 difference	6.7±4.5	10.9±10.3	0.044

Mean±SD or median (range) is shown. Mann-Whitney *U*-test for all statistic comparisons.

As with the results in table 2, venous pO_2, A-V pO_2, and A-V PCO_2 were significantly decreased in the umbilical cord blood of growth restricted neonates born to pre-eclamptic mothers when compared to the umbilical cord blood of growth restricted neonates born to non-pre-eclamptic women. Overall, pre-eclampsia appeared to induce changes in blood gas parameters regardless of foetal growth restriction (25).

The mentioned studies also investigated the effects of maternal pre-eclampsia on neonatal acid-base balances, which seemed to be not affected by this condition. The missing impact on acid-base statuses could have been due to preventive deliveries. In case of maternal clinical deterioration as a consequence of pre-eclamptic progression, dysbalanced foetal acid-base statuses would have been expected (25).

1.1.5. Pre-eclampsia: APGAR-Score

The APGAR- (Appearance-, Pulse-, Grimace-, Activity, and Respiration) Score was introduced to rapidly assess the neonates´ clinical status after delivery. Skin colour, heart rate, reflexes, muscle tone and respiration are evaluated, each given a score of 0, 1 or 2. The score is usually taken after the 1st and 5th minute and repeated in 5 minutes intervals until 20 minutes if the infant scores 7 or less in the first two evaluations. The APGAR-Score also provides an estimation of the infant´s response in the unlikely event of resuscitation (26).

Table 4: APGAR-Score: Skin colour, heart rate, reflexes, muscle tone and respiration evaluation at 1st and 5th minute, continued in 5 minute intervals (up to 20 minutes, if the infant scores 7 or less in the first two evaluations); also to estimate the infant´s response if being resuscitated (26).

Sign	0	1	2
Appearance/ Colour	Blue or pale	Acrocyanotic	Completely pink
Pulse/ Heart rate	Absent	<100/min	>100/min
Grimace/ Reflex irritability	No response	Grimace	Cry or active withdrawal
Activity/ Muscle tone	Limp	Some flexions	Active motion
Respiration	Absent	Weak cry/Hypoventilation	Good crying

A definition by *The Neonatal Encephalopathy and Neurologic Outcome* suggests that a 5th minute Apgar-Score of 7 to 10 is reassuring, between 4 and 6 moderately abnormal and from 0 to 3 low, thus indicating a sign of nonspecific illness (26).

Various circumstances such as maternal sedation, congenital malformations, and gestational trauma must be considered when analysing the APGAR-Score. Among others, the mentioned factors could result in a lowered APGAR-Score. When examining neonates born to pre-eclamptic mothers, it is important to consider the foetus´ physiological maturity, which could be decreased because of iatrogenic pre-term delivery. Even healthy pre-term neonates might score lower compared to healthy term neonates due to immaturity (26).

Studies have shown varying results regarding influences on the APGAR-Score of neonates born to pre-eclamptic mothers. As seen in table 2 and table 3, there were no significant differences in the APGAR-Score between the pre-eclamptic and the control groups (25). On the other hand, table 1 demonstrated a statistically significant lowered 1st and 5th minute APGAR-Score in pre-eclamptic term and pre-term groups compared to term and pre-term controls (21). In Saadat et al´s study, 94 neonates born to pre-eclamptic mothers were compared to 94 neonates born to healthy mothers. The 1st and 5th minute APGAR-Scores were significantly reduced in the pre-eclamptic group (27).

1.1.6. Pre-eclampsia: Neonatal adaptations and adverse outcomes

During the progression of pre-eclampsia, uteroplacental insufficiency might emerge, creating a deprived environment for the foetus. Furthermore, the foetus might endure restrictions in development and growth. After delivery, growth restricted neonates have a higher risk of developing immediate acute complications, as seen in figure 5. The outcome highly depends on the aggravating circumstances. Short term complications can lead to long term damage, such as poor neurodevelopmental progression (28).

Also, prematurity itself represents a high risk of developing adverse health events. Conversely, neonates are often delivered prematurely to halt the progression of pre-eclampsia (28).

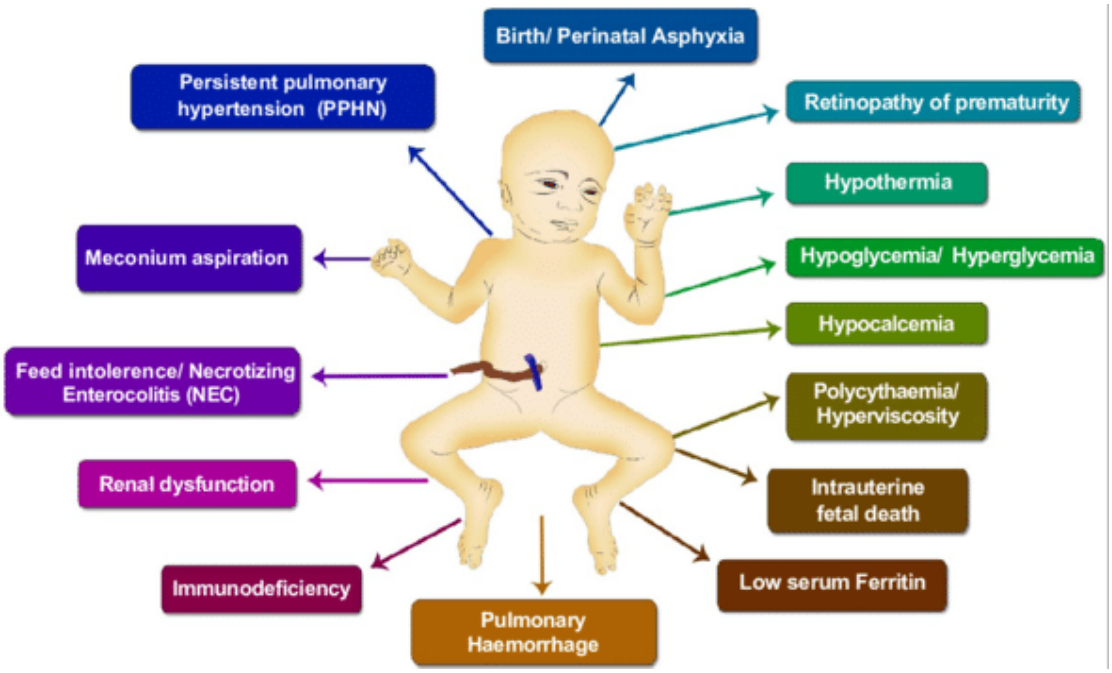

Figure 5: Overview of immediate neonatal complications seen in intrauterine growth restricted neonates (16).

The table below (table 5) lists an excerpt of possible short-term consequences of growth restricted neonates, with a focus on the potential underlying causes.

Table 5: Excerpt of complications in intrauterine growth restricted neonates (16).

Perinatal asphyxia	chronic foetal hypoxia due to placental abnormalities; possibly worsened by acute events; thus, resulting in acidosis
Meconium aspiration	reduced perfusion of the intestines with reactive hyperperistalsis and relaxation of the anal- sphincter; aspiration through amniotic fluids
Hypoglycaemia	poor glycogen stores of the liver/muscles and poor alternative energy sources; decreased adipose tissue; deficient gluconeogenesis
Hyperglycaemia	low insulin levels subsequently lead to an immature pancreas
Feeding intolerance and necrotizing enterocolitis	poor motility and reduced intestinal perfusion resulting from blood redistribution to vital organs

1.1.6.1. Pre-eclampsia: Long-term complications

The origin of long-term complications was established by the Barker hypothesis. The hypothesis states that, early life environments have effects on health in later life. Also, it represents the most accepted hypothesis of *"Developmental Origin of Health and Disease"* (16).

While growing and developing in a deprived intrauterine environment, the foetus must undergo adaptations to survive. This process results in permanent epigenetic changes in the genome. Postnatal lifestyles involving normal/excessive nutrition and decreased physical activity, aggravates these intrauterine adaptations, leading to abnormal growth and increased propensity to develop diseases in later life (16).

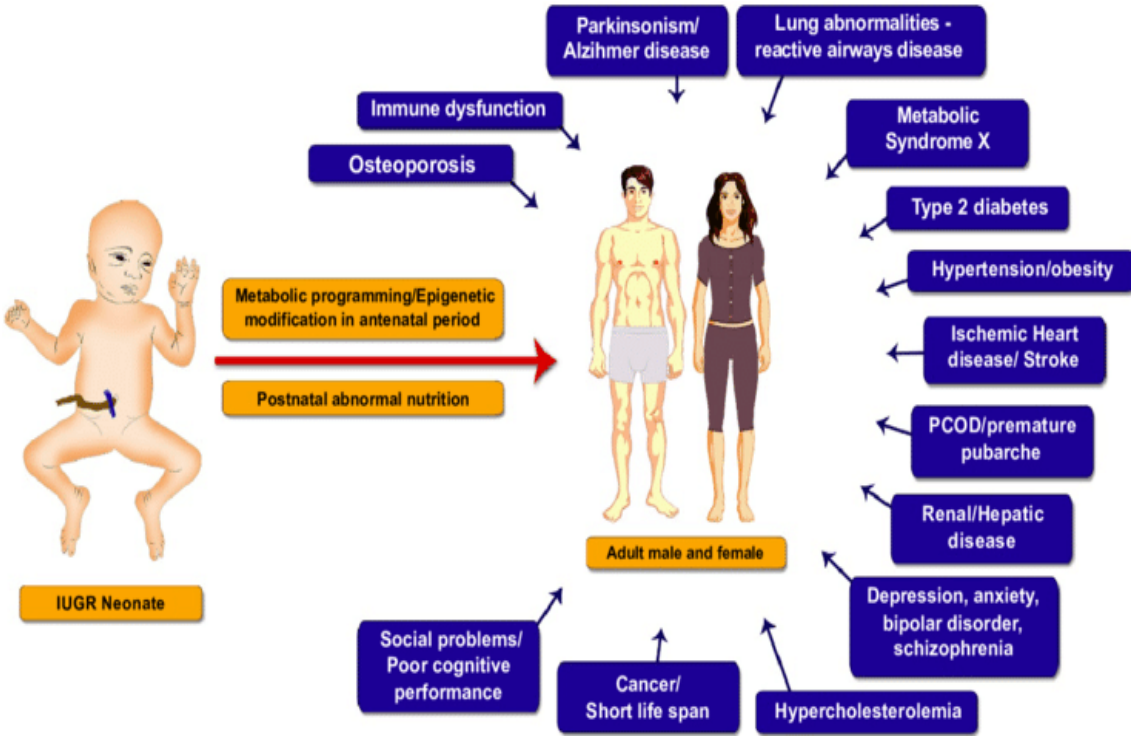

Figure 6: "Showing various adult diseases the IUGR infant is prone to develop in his adulthood as per *Developmental origin of health and diseases (DoHaD).* IUGR infants undergo epigenetic modification in-utero and postnatally have abnormal nutrition and growth leading to various disease of adulthood in these infants" (16).

Studies have shown that maternal pre-eclampsia, irrespective of foetal growth restriction, increases the children´s risk of developing hypertension and cardiovascular diseases. AGA neonates of pre-eclamptic mothers are associated with increased systolic blood pressure in childhood and adolescence. Also, a heightened prevalence

for strokes was detected in individuals who were born to pre-eclamptic mothers, even if they were not growth restricted (29).

As seen in figure 6, these offspring are prone to developing subtle or major cognitive and neurodevelopmental abnormalities. As a consequence, difficulties in school, behavioural problems, lower strength, and work capacity are more common in such children(16).

1.2. Tobacco smoking

Tobacco is usually smoked in the form of commercial cigarettes and contains approximately 5,000 different chemicals (30). Less common forms of smoking include cigarillos, cigars, pipes, or water pipes. Also, tobacco can be consumed in smokeless forms such as chewing of tobacco, orally as a wad between the cheek and the gums, and by nasal absorption (31). Even though the harmful consequences of smoking are well known, the WHO estimates that there are 1,27 billion smokers worldwide. Approximately 24,8% of the male and 14,6% of the female population worldwide smoke regularly (32).

Smoking is the leading preventable cause of mortality. It is responsible for approximately 6 million deaths per year. Mortality is mainly caused by respiratory pathologies, such as lung and upper airway cancer, chronic obstructive pulmonary diseases, and cardiovascular diseases including heart conditions and strokes (figure 8) (33).

Even though cigarettes contain many different harmful substances, comparatively few of these components were linked to specific pathologies. Airway-disorders are mainly attributed to substances released during the burning process of the cigarettes (e.g., acrolein, acetaldehyde, formaldehyde). On the other hand, cardiovascular disorders are mainly caused by carbon monoxide, nicotine, oxidants (such as nitrogen oxides and free radicals), hydrogen cyanide, arsenic, and acrolein. Of importance are substances that act as carcinogens. Tobacco-specific N-nitrosamines, polycyclic aromatic hydrocarbons (e.g. benzo[a]pyrene) and metals or metalloids are known to vastly increase the risk of cancer development (34).

While other constituents are responsible for the toxicity of cigarettes, nicotine is the main component causing addiction. A commercial cigarette contains 10mg-14mg of nicotine, of which 1mg-1,5mg are absorbed systemically while smoking. Entering the brain, nicotine binds to nicotine cholinergic receptors, activating cation channels and consequently calcium channels. Subsequently, the release of neurotransmitters such as dopamine, glutamate, and gamma aminobutyric acid are induced. These transmitters play a major role in the development of dependency and addiction (35, 36). Exclusive nicotine consumption in the form of chewing-gum, patches, e-cigarettes and inhalable agents only make up for 1% of the worldwide nicotine intake (32).

Although it is widely known that smoking can also harm foetuses, studies have shown that 14% to 17% of the women in western countries smoke during pregnancy. The prevalence of pregnant smokers is influenced by circumstances such as maternal age and educational status. Young mothers under 20 or 25, as well as routine and manual workers, have a higher prevalence of smoking during pregnancy compared to mothers older than 35 or who have tertiary education (37).

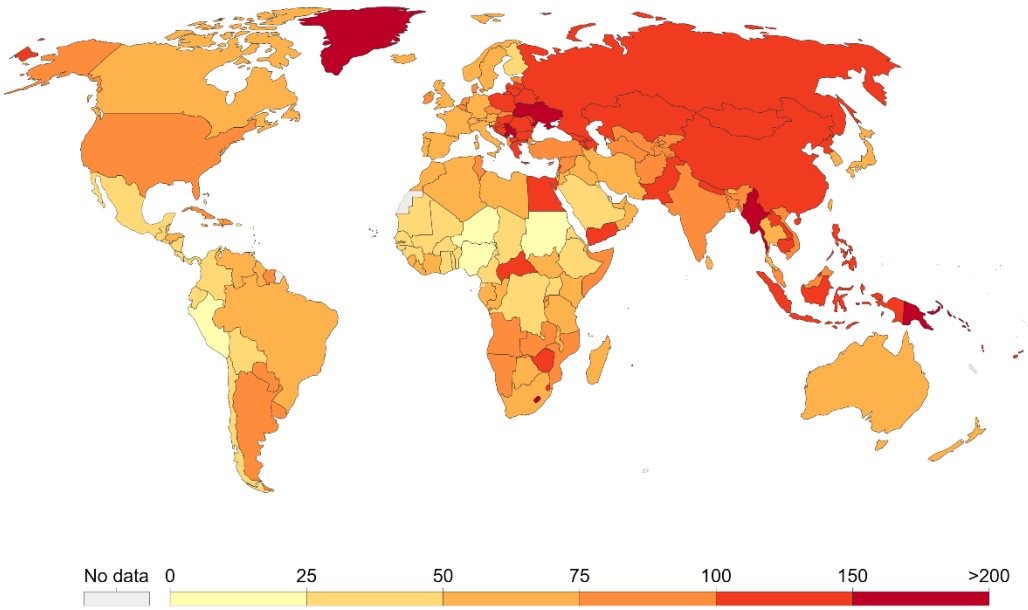

Figure 7: "Worldwide death rate from smoking (2017). The annual number of deaths attributed to smoking per 100,000 people" (38)

1.2.1. Smoking: Pathophysiological consequences

Direct as well as indirect smoking afflicts the human body with various chemicals. Some of them act as oxidants, pro-inflammatory agents, carcinogens, or a combination of such. These chemical substances may lead to oxidative damage in macromolecules, therefore compromising or altering their function (39).

Besides the apparent induced pathologies seen in figure 8, smoking also promotes contemporary alterations in maternal body systems during pregnancy. These changes affect the cardiovascular system, immune system, endocrine system, and metabolic processes. Subsequently, these smoking induced maternal changes can affect the foetus´ health, growth and development (40).

Possible foetal complications of maternal smoking during pregnancy include the risk of miscarriage, foetal growth restriction, placental abruption, and neonatal death (33).

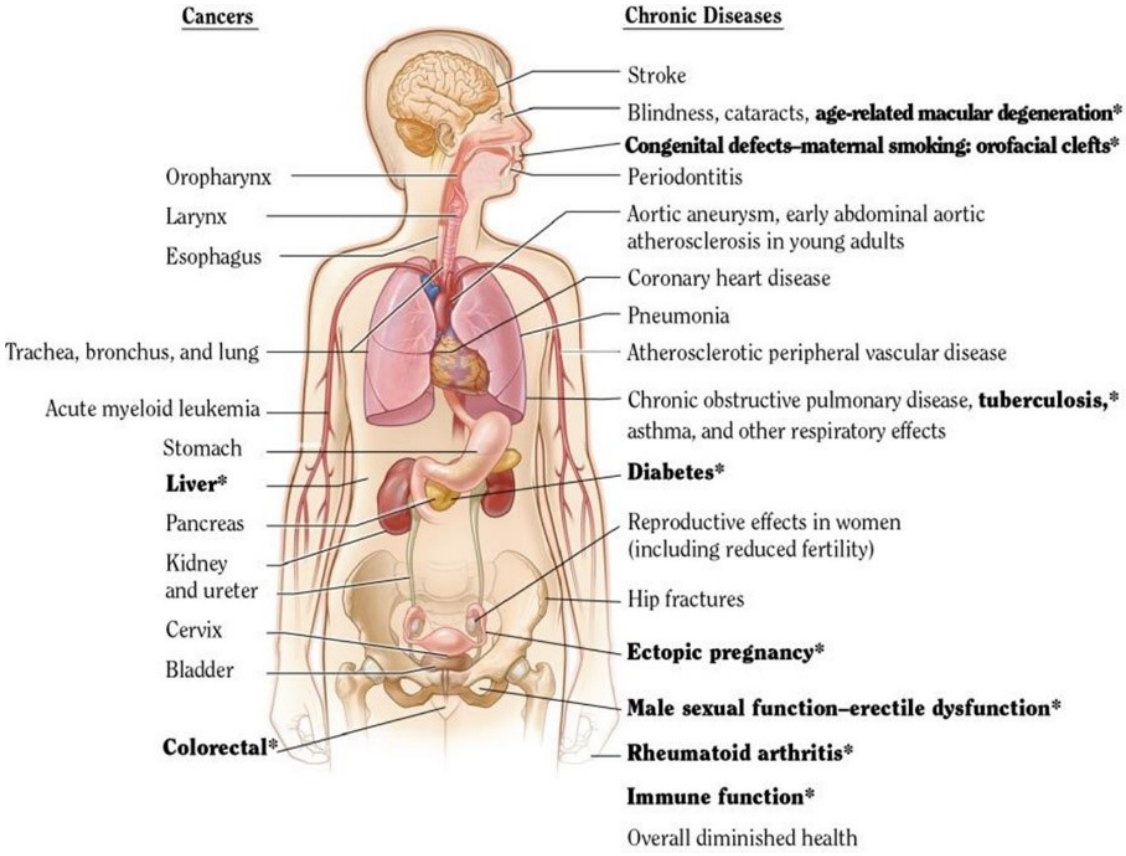

Figure 8: Cancers and chronic diseases promoted by smoking. (41).

1.2.1.1. Smoking: Changes in placental morphology and biology

In general, placentas of smoking mothers were observed to have a reduced number of cytotrophoblast cells, increased villous membrane thickness, and an overall decline in foetal capillary volume. These alterations consequently lead to a decreased exchange capacity of nutrients and oxygen between the placenta and the foetus. Additionally, smoking induces vasoconstriction in placental blood vessels and, thus, lead to constrained blood flow (40, 42).

In table 6 an excerpt of smoking induced morphological and biological changes of placentas are listed (43).

Table 6: Morphological and biological changes in placentas associated with maternal smoking (excerpt) (36). Additional explanations: VEGF, vascular endothelial growth factor, stimulates vascular endothelial cell growth; **Metallothionein,** protects trophoblast cells from metal- or oxidative stress induced apoptosis; **92kDa type IV collagenase,** essential mediator of invasion of cytotrophoblast cells; **1-selectin and TRA-1-81 reactive carbohydrate ligands,** essential in 1-selectin adhesion system; **Enzymatic activity complex III and mitochondrial DNA,** essential for mitochondrial function and energy supply.

Increased	Decreased
Trophoblast and villous membrane thickness	Villous capillary volume fraction
Syncytiotrophoblast necrosis	Total surface of syncytial knots
Thickness of the trophoblast basal membrane	Synthesis and activation of 92kDa type IV collagenase
Apoptosis in the syncytiotrophoblast	Cytotrophoblast expression of l-selectin and TRA-1-81 reactive carbohydrate ligands
Expression of VEGFs	Progesterone synthesis
Stimulation of nicotinic acetylcholine receptors	Enzymatic activity of complex III and mitochondrial DNA
Level of metallothionein	-
Vasoconstrictive response to endothelin-1	-

VEGF stimulates vascular endothelial cell growth and is therefore an essential mediator in placental angiogenesis. Metallothionein, a metal-binding protein, protects trophoblast cells from metal- or oxidative stress induced apoptosis. Nicotine induces a decrease in 92kDa type IV collagenase which acts as an essential mediator of cytotrophoblast cell invasion. Furthermore, it is believed that nicotine also reduces the expression of 1-selectin and TRA-1-81 reactive carbohydrate ligands in cytotrophoblasts, which are essential for the 1-selectin adhesion system. Downregulation is associated with impacted development of cell columns which connect the foetal portion of the placenta to the uterus, possibly resulting in unsustainable pregnancies. Enzymatic activity complex III and mitochondrial DNA are inversely related to cigarette consumption, suggesting impaired mitochondrial function in placentas which possibly provide less energy (43).

1.2.2. Smoking: foetal IUGR

Studies analysing whether smoking is an independent factor for IUGR (intrauterine growth restriction) compared various maternal characteristics such as body mass index (BMI), age, and pre-existing diseases to biometric parameters of foetal growth restriction (44). Results have shown that smoking is an independent and significant contributor to growth restriction. Its impact on foetal development is strongest when mothers smoke during the third trimester. Neonates born to mothers who ceased smoking in the first trimester are just as likely of being growth restricted as neonates born to non-smokers (44).

Further, a dose dependent association between cigarette smoking and birthweight was detected. Mothers who self-reportedly smoked more than 20 cigarettes daily, gave birth to infants weighing 277g less on average. If mothers smoked 11-20 cigarettes per day, neonatal birthweight was reduced by 190g, and 1-10 cigarettes per day reduced the weight by 86g. However, whether the association is linear is unclear. Not only active smoking, but also maternal second-hand smoke exposure was observed to correlate with lowered neonatal birthweights (44).

In addition to birthweight, various neonatal anthropometric parameters were decreased in neonates born to smoke exposed mothers:

- femur-, limb, and total body length,
- occipitofrontal and biparietal diameters,
- head-, chest, and abdominal circumferences.

1.2.2.1. Smoking: Direct acting agents and foetal growth

Maternal smoking mainly influences foetal metabolic and enzymatic systems. Ascorbic acid, thyroxine, IGF-I, IGFBP-3 were lowered in neonates born to smoking mothers while leptin, lipid peroxides were increased. Also, lower levels of amino acids during the 12^{th} and 17^{th} gestational week, as well as at term, were detected in infants of smoking mothers. Correspondingly, in-vitro studies have shown that tobacco smoke lowers the activity of sodium dependent transporters of neutral amino acids. (43).

Erythropoietin (EPO) increases counter-regulatorily in hypoxic conditions to increase erythropoiesis. Higher EPO levels were observed in umbilical cord blood of neonates born to smoking mothers compared to neonates born to non-smoking mothers. Following this observation, it is assumed that maternal smoking during pregnancy induces hypoxic conditions in foetuses (45).

The passage of tobacco-toxins mainly depends on placental age and placental detoxification capacity. During pregnancy, placental permeability increases due to its natural development, making the transport of harmful substances easier. Also, if placental detoxification capacity is overwhelmed, toxins can be transferred to the foetus (43).

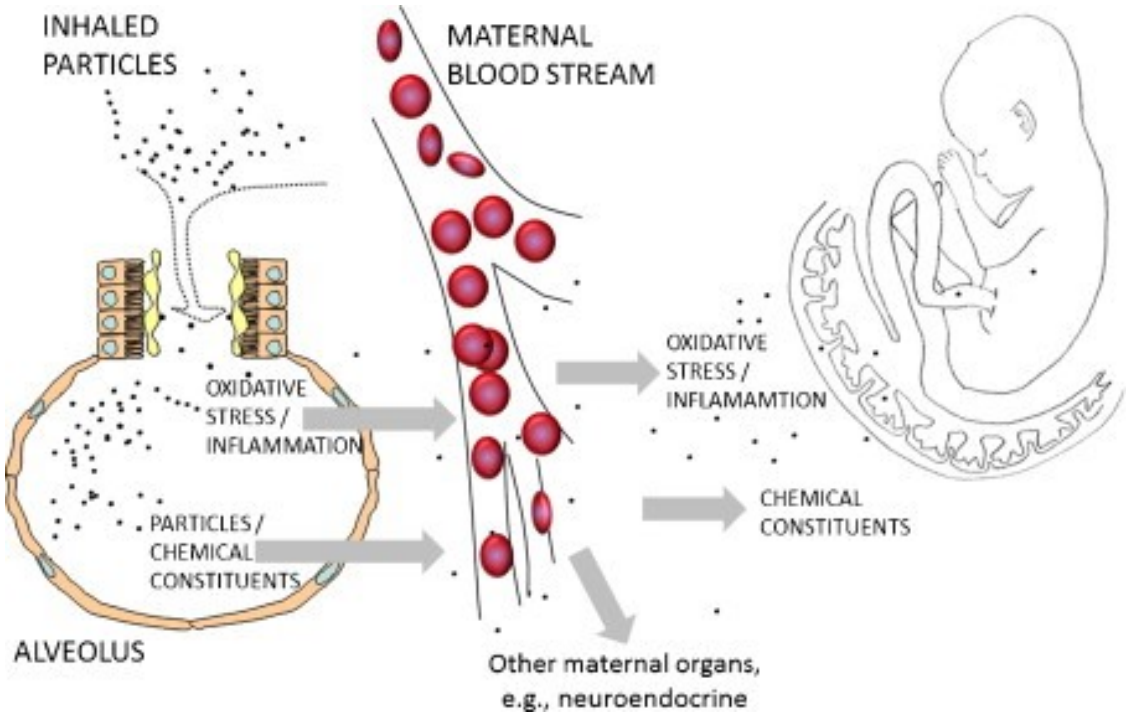

Figure 9: Pathways of tobacco inhaled particles and development of foetal toxicity (46).

1.2.3. Smoking: Changes in ultrasound parameters

Studies evaluating the ultrasound parameters in pregnant smokers detected increased placental and uterine vascular resistances compared to pregnant non-smokers. Hereby, the pulsatility index, resistance index, and systolic to diastolic ratios were evaluated. Also, pregnant smokers were more likely to have diastolic "Notch Signs" in uterine artery flow, indicating elevated vascular resistance. Increased resistance was further observed in umbilical-placental and foetal cerebrovascular flow parameters in neonates of smokers (44).

The foetal medial cerebral artery (MCA) physiologically displays high resistance flow patterns. However, this flow pattern drops to low resistance flow if blood is distributed according to brain sparing growth mechanisms (vasodilatation in oxygen sensitive organs) (47). Foetal brain sparing growth can occur as a response to smoking induced intrauterine growth restriction (15). Nevertheless, in cases of severe foetal growth restriction, the flow pattern of the foetal MCA can revert to high resistance flow patterns (16, 47).

Foetuses were also observed to have an immediate reaction after maternal smoking. Foetal heart rate, as well as systolic to diastolic ratio of the umbilical artery, increases and remains temporarily elevated (48).

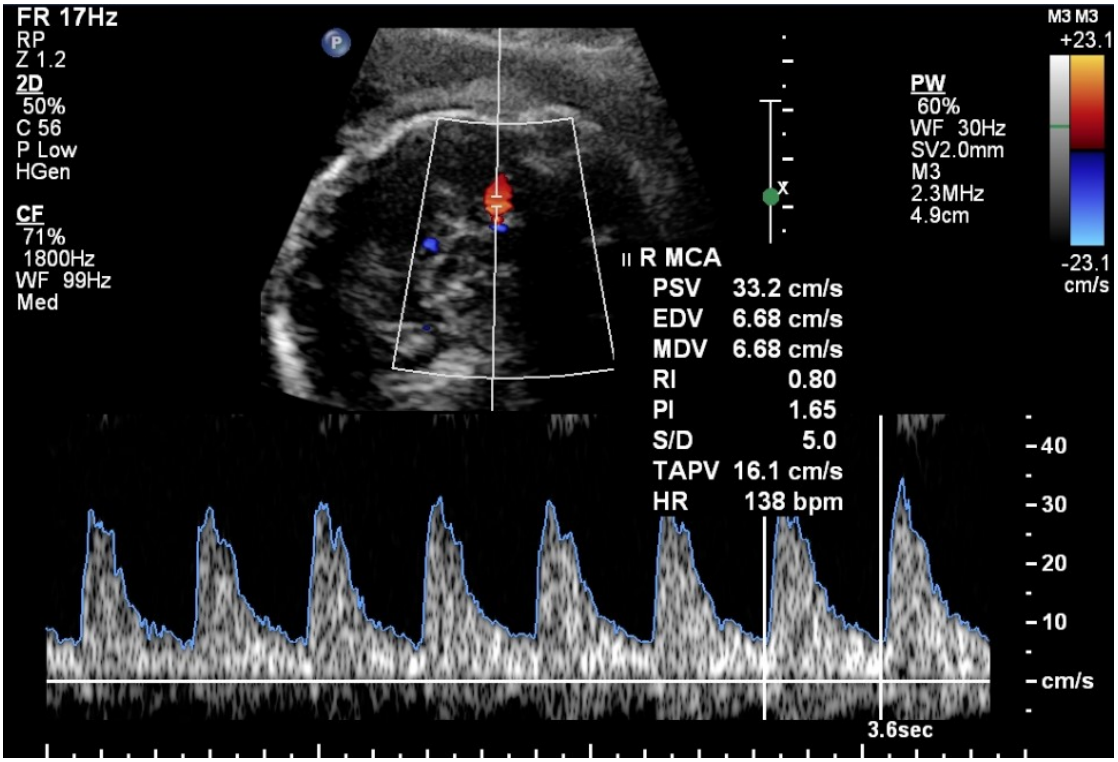

Figure 10: Doppler ultrasound of right medial cerebral artery in a severe case of foetal growth restriction. Abnormal pulsatility index was detected. Anthropometric parameters (head and abdominal circumference; biparietal diameter; expected foetal weight) under 2nd percentile (49).

1.2.4. Smoking: Effects on foetal blood parameters

Smoking induced placental changes can hinder the exchange of nutrients and oxygen to the foetus. This lack of nutrients and oxygen has an impact on the infants´ haematological system. Cadmium, an embryotoxic and teratogenic component found in tobacco (40), accumulates in the placentas of smoking mothers (50). Placental-foetal transfer of zinc, an essential factor for erythropoiesis (51), can be hindered by the accumulating cadmium (50). Also, negative correlations between maternal cigarette smoking and foetal ferritin and total body iron were observed (52).

Iron, also an essential element of erythropoiesis, can promote microcytic hypochromic forms of anaemia when lowered (51). Carbon monoxide (CO), an agent released during cigarette ignition, has the potential to bind to haemoglobin more effectively

than oxygen, forming carboxyhaemoglobin (Hb-CO). Subsequently, oxygen attachment to haemoglobin decreases along with systemic oxygen availability (53).

Neonates born to smoking mothers are observed to have higher levels of carboxyhaemoglobin than neonates born to non-smoking mothers. Furthermore, the percentage of neonatal Hb-CO can be up to twice as high as the percentage of Hb-CO in the corresponding mother (54). Depending on the amount of foetal haemoglobin that is transformed into carboxyhaemoglobin, hypoxic conditions can emerge which ultimately, harm the foetus (40).

Table 7 represents an overview of the differences in haematological parameters in neonates born to smoking mothers compared to those of neonates born to non-smoking mothers (55, 56). A study performed by Dollberg et al also detected a rise in nucleated red blood cells in neonates born to mothers who did not smoke during pregnancy but were exposed to second-hand smoke. Also, small-for-gestational-age neonates or cases of severe hypoxia were excluded in Dollberg et al´s study. According to their results, absence of foetal growth restriction should not be used as a marker for normal placental-foetal transmission of nutrients and oxygen (57).

Table 7: Differences in haematological parameters of neonates born to smoking mothers compared to neonates born to non-smoking mothers. (55, 56)

Decrease in	Increase in	No differences in
reticulocyte count	nucleated red blood cells	red blood cell count
lymphocyte count	erythroblast levels	haematocrit
neutrophile count	eosinophile fraction	haemoglobin
CD4 cells	CD8 cells	reticulocyte subsets

Surprisingly, neither the red blood cell count, nor the haematocrit or haemoglobin differed in neonates born to smokers when compared to neonates born to non-smokers. It is suspected that only heavy maternal smoking (more than 20 cigarettes per day) induces severe enough hypoxia in the foetus, subsequently stimulating its erythropoiesis (50).

A study conducted by Habek et al, could also detect changes in the acid-base status in neonates born to smoking mothers. The pH-value of arterial umbilical cord blood in neonates born to mothers who smoked more than 20 cigarettes per day, was significantly lower than the corresponding pH-values in neonates born to mothers who smoked less than 20 cigarettes per day or who were non-smokers (50).

1.2.5. Smoking: Effects on APGAR-Score

Studies observed that the APGAR-Score (Appearance-, Pulse-, Grimace-, Activity, and Respiration) of neonates born to smoking mothers is lower than those of neonates born to non-smokers. Yet, results vary regarding the point of assessment (50, 56, 58). As seen in table 8, Karadeniz et al detected a lowered 1st minute APGAR-Score in infants born to smokers compared to infants born to non-smokers. However, 5th minute APGAR-Scores did not differ (58). On the other hand, Marin et al´s study observed a lowered 5th minute APGAR-Score in neonates born to smokers while the remaining evaluations did not vary between neonates born to smokers and neonates born to non-smokers (56).

Table 8: "Placental and foetal parameters of smokers and non-smokers". Lowered 1st minute APGAR-Score in infants born to smokers compared to infants born to non-smokers; 5th minute APGAR-Score did not differ between the infants born to smokers and non-smokers. (58)

Parameters	Smokers (n=46)	Nonsmokers (n=212)	p
	Mean+ Standard deviation	Mean+ Standard deviation	
Placental surface area	1925 ± 974 mm^2	1202 ±302 mm^2	0.04*
Coiling index of the umbilical cord	0.36 ± 0.15 coils/cm	0.32 ± 0.15 coils/cm	0.40
Number of cotyledon	15.28 ± 1.96	15.24± 2.23	0.49
Placental weight	494± 38 g	503 ± 46 g	0.55
Fetal length	50.78±3.13 cm	50.50 ± 3.01 cm	0.92
Fetal weight	3295± 520 g	3189 ± 510 g	0.90
Apgar 1 minute	7.05± 0.55	7.99 ± 0.73	0.03*
Apgar 5 minute	9.91±0.28	9.89 ± 0.32	0.38

** Statistically significant difference.*

1.2.6. Smoking: Adverse outcome

Maternal smoking during pregnancy can lead to short and long-term consequences in neonates. Short-term complications include pre-term birth, intrauterine growth restriction, small-for-gestational-age infants, and reduced pulmonary alveolarization in the foetus. Generally, each complication on its own has the potential to induce a variety of different outcomes (59). For instance, complications related to intrauterine growth restriction are displayed in figure 5, 6 and table 5. Comparatively, pre-term delivery can be complicated (among others) by foetal intraventricular haemorrhage, necrotising enterocolitis, retinopathy of prematurity, and bronchopulmonary dysplasia (60).

Maternal smoking during pregnancy increases the likelihood of the following neonatal long-term consequences (43, 59, 61):

- Malformations,
- Cardiovascular related diseases,
- Neurological changes,
- Diseases related to the respiratory system,
- Genetic instability.

Neonatal malformations induced by maternal smoking can include various somatic systems. Among others, malformations of the heart (e.g., septal defects), face (e.g., lip/palate cleft), central nervous system, vascular system, musculoskeletal system (e.g. clubfoot), eyes, limbs, and gastrointestinal tract were confirmed in a meta-analysis as positively associated with maternal smoking during pregnancy (61).

Studies have shown an association between maternal smoking during pregnancy and a heightened risk for developing cardiovascular diseases in the corresponding offspring. These children were observed to have a higher risk for obesity, along with hypertension in childhood. "Endothelial dysfunction, changes in renal structure, and alterations in perivascular adipose tissue" are believed to be the causing factors of hypertension in children born to smoking mothers. Notably, if mothers stopped smoking during pregnancy, the risk for cardiovascular diseases diminished in the corresponding offspring (59). Also, low birth weight neonates (as seen in smoking mothers) were observed to have an increased risk for cardiovascular diseases. Undergoing catch-up growth, these neonates gain fat mass rather than lean body mass. Furthermore, an accumulation of intrabdominal fat instead of subcutaneous fat was noted (62).

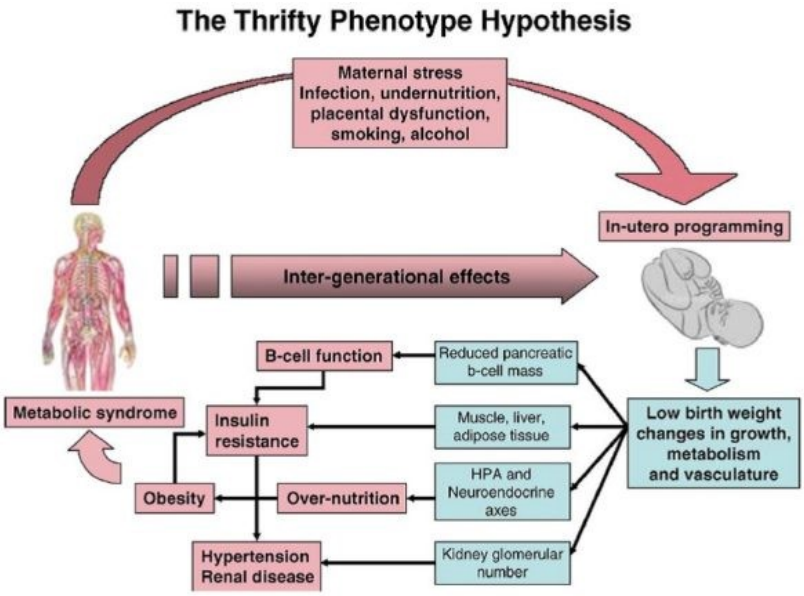

Figure 11: "Foetal programming and the risk of cardiometabolic disorders". Maternal smoking induces intrauterine changes in foetuses as in lowered birth weight, changes in growth, metabolism, and vasculature. These alterations increase the risk for cardiovascular diseases in later life (63).

Benzopyrene, a component of tobacco, acts as a carcinogen when metabolised to BPDE-I (= Benzo(a)pyrene diol epoxide-I). BPDE-I on the other hand has the potential to bind to DNA. It was further found to accumulate in the placentas of smoking mothers and umbilical cord blood of the corresponding foetuses. More mutations were found in T-lymphocytes and amniocytes of infants born to smokers, suggesting increased genetic instability and, thus, a higher risk for developing malignancies (43).

Nicotine passes through the placental barrier, resulting in relatively higher concentrations in foetal blood than in maternal serum levels (35). In the foetal system, nicotine can bind to nicotinic acetylcholine receptors in the foetal brain. By connecting to these receptors, nicotine induces alterations in cell proliferation and differentiation, potentially resulting in a decreased neuronal cell count (43). These neurological effects can also lead to a 0,5cm reduction in foetal head circumference compared to infants born to non-smokers (59).

Neonates born to smokers are prone to developing asthma and wheezing. This is likely caused by a higher activity and reactivity of Th2 cells to allergens. Also, more IgE antibodies were observed to be produced in infants born to smokers. In general, IgE antibodies play an essential role in allergic reactions (59).

1.2.7. Smoking and the risk of pre-eclampsia

Tobacco smoking is negatively associated with the risk of developing pre-eclampsia. It is believed that the clinical phenotype of pre-eclampsia emerges from an imbalance between pro-angiogenic factors and anti-angiogenic factors (64).

Table 9: Imbalance between pro-angiogenic factors and anti-angiogenic factors due placental to overproduction of sFlt-1, VEGFR-1 as a reaction to placental ischemia or placental stress (3, 4). An Excerpt of pro- and anti-angiogenic factors:

Imbalance between

Pro-angiogenic factors		**Anti-angiogenic factors**
vascular endothelial growth factor (VEGF)	**AND**	fms-like tyrosine kinase-1 (sFlt-1)

placental growth factor (PlGF)		vascular endothelial growth factor receptor 1 (VEGFR-1).

SFlt-1 inhibits proangiogenic growth factors such as VEGF or PlGF, by binding to them and consequently interfering with blood vessel development. Studies showed an association between smoking and reduced levels of sFlt-1 and subsequent higher VEGF/sFLT-1 ratios (64).

A study including more than 600,000 Nordic women from the Swedish medical birth register published positive associations between tobacco smoking and a reduced risk of pre-eclampsia. They also observed that snuff, a smokeless tobacco, did not cause a reduction in pre-eclamptic risk, consequently leading to the hypothesis that combustion products, such as carbon-monoxide, might provide the protective effect. Additionally, they examined the period in which tobacco smoking decreased the risk of pre-eclampsia. They were able to reveal that women who had ceased smoking until the 30-32nd gestational week had, compared to women who continued, a higher risk of developing pre-eclampsia (64).

Furthermore, no reduction of pre-eclamptic risk (due to smoking) was seen in severe cases of pre-eclampsia, small-for-gestational-age foetuses, or cases of stillbirths. However, women who already had developed pre-eclampsia, had worse pregnancy outcomes if smoking was continued compared to pre-eclamptic women who stopped smoking (64). Correspondingly, the risk of intrauterine growth restriction doubled when moderate to severe forms of pre-eclampsia were combined with maternal smoking (44).

1.3. Aims and objectives

The aim of this case series study was to analyse the effects of maternal smoking on neonatal parameters of infants born to mothers, who were tested positive for pre-eclamptic risk. For this purpose, the following cases were included:

- Case "Current-Smoker": Case C-S represents a neonate born to a woman who was tested positive for pre-eclamptic risk, smoked before pregnancy, and continued smoking during pregnancy.

- Case "Former-Smoker": Case F-S represents a neonate born to a woman who was tested positive for pre-eclamptic risk, did smoke before pregnancy, and discontinued at the beginning of pregnancy.

- Case "Non-Smoker": Case N-S represents a neonate born to a woman who was tested positive for pre-eclamptic risk and did not smoke before or during pregnancy.

In this thesis it was hypothesised that a maternal pre-eclamptic risk combined with maternal smoking during pregnancy, can have a more severe effect on neonatal parameters than without smoking.

The study was performed as part of the bigger "*Does nicotine reduce the risk of Pre-eclampsia? A prospective study*"-trial at the Department of Obstetrics, Medical University of Graz. The *"The effects of smoking in neonates born to mothers with pre-eclamptic risk. A case-series study"*- trial focused on factors affecting the infants´ in utero development, neonatal postnatal parameters, and neonatal postnatal adaptations and changes until hospital discharge.

Therefore, the following foetal/neonatal parameters were compared between the cases:

- Intrauterine ultrasound measurements,
- Postnatal biometry,
- Umbilical cord blood analysis,
- APGAR-Score,
- Postnatal changes and adaptation after birth.

2. Materials and Methods

All women included in the "*Does nicotine reduce the risk of Pre-eclampsia? A prospective study"*-trial had given their informed consent before participation. This study was performed in accordance with the Declaration of Helsinki (1989) of the WMA. An ethics permission was obtained before commencing this study.

Inclusion criteria:

Infants born to mothers who were classified as high-risk patients for developing pre-eclampsia at the first trimester screening (i.e., mothers between the 11^{th} and 16^{th} gestational week), which was conducted in February 2020. The score calculating pre-eclamptic risk included maternal demographic characteristics, medical and obstetric histories, uterine artery pulsatility index (=PI), mean arterial blood pressure, placental growth factor (=PlGF) and pregnancy-associated plasma protein A (=PAPP-A) levels. Acetylsalicylic acid was prescribed to all mothers and taken over the course of pregnancy as a preventive against pre-eclampsia.

Exclusion criteria:

Maternal discontinuation of acetylsalicylic acid.

2.1. Protocol of maternal measurements

To examine how smoking influences the risk of pre-eclampsia, maternal parameters were measured between the 11^{th}-16^{th}, 20^{th}-28^{th}, after the 28^{th} and before the 40^{th} gestational week, as well as three days and six months after delivery. Hereby, ultrasound examinations were conducted including Doppler-Ultrasound of the maternal uterine arteries. Vascular resistance in the uterine arteries was assessed measuring the pulsatility index(=PI), the resistance index (=RI) and post-systolic indentations of the flow curve (=Notch-sign).

The PI was calculated by dividing the difference between systolic and diastolic flow velocity by the time-average flow velocity. On the other hand, the RI was calculated by dividing the difference between peak systolic and end-diastolic flow by peak

systolic flow. The Notch sign was determined as a post-systolic indentation of flow curve in the uterine artery. (65, 66)

History and quantity of smoking was categorised and assessed during the initial clinical interview.

Further maternal measurements included retinal imaging, heart rate and heart rate variability, pulse wave velocity, index of arterial stiffness and vascular age (Vicorder), hair nicotine concentration and hair cortisol levels, serum parameters such as sFlt-1, PlGF, sFlt-1/PlGF-ratio and coagulation parameters, relative telomere length, ADMA, SDMA and mircoRNA.

2.2. Protocol of neonatal measurements

During the above-mentioned maternal ultrasound examinations (up until delivery), Doppler-ultrasound of the foetal umbilical arteries and the foetal medial cerebral arteries were conducted. Also, intrauterine foetal biometric parameters were measured during those examinations.

Doppler-ultrasound of the foetal umbilical arteries and foetal cerebral arteries included the calculation of the pulsatility indices (=PI), the resistance indices (=RI) (see protocol of maternal measurements) and the measurement of the end-diastolic flows (=EDF). The EDF was either categorised as existent (=positive, physiological), non-existent (=negative, pathological) or reversed (=pathological). The PI of the foetal middle cerebral artery enabled the calculation of the cerebroplacental-ratio (=CPR), which was established by dividing the PI of the foetal middle cerebral artery by the PI of the foetal umbilical artery. CPR was considered as an early marker of placental dysfunction, when lowered (67). In one case, Doppler ultrasound of the ductus venosus was conducted for evaluation of the a-wave. A-waves were classified as normal if they were positive during atrial contraction, abnormal if absent or negative.

During the ultrasound-measurements of the intrauterine foetal biometric parameters, head-circumferences (=HC), abdominal- circumferences (=AC) and HC/AC-ratios were obtained.

For the umbilical cord blood analysis, the method of delayed clamping was used. Here, a period of 30 to 60 seconds had to be waited before the umbilical cord was clamped. The umbilical cord was clamped approximately at 30cm. Pre-heparinised syringes were used to aspirate blood from the umbilical vein and umbilical artery separately. The cord blood analysis was carried out by Radiometer ABL90Serie.

Umbilical cord blood analysis included arterial and venous O_2 partial pressures, CO_2 partial pressures, pH-values, haematocrit, haemoglobin, and oxygen saturations. Also, FO_2Hb, the fraction of oxygenated haemoglobin in relation to total haemoglobin (= oxy- and deoxygenated haemoglobin, carboxyhaemoglobin, and other forms of haemoglobin), was measured. Furthermore, sBEc (= standard base excess) was evaluated. The sBEc measurement was adjusted for 37°C, a CO_2 partial pressure of 40mmHg and a haemoglobin value of 5g/dl by the Radiometer ABL90Serie. $cHCO_3$-(P)c (standard bicarbonate) was measured according to the same adjustments as in sBEc. Additionally, lactate and glucose parameters were evaluated. These values were compared to those published in other studies (68, 69).

Directly after birth, an APGAR-Score was evaluated for rapid assessment of the neonates´ clinical status. The values were taken in the 1^{st}, 5^{th}, and 10^{th} minute after delivery. The assessment was conducted by the present midwife.

Postnatal neonatal biometric parameters including birthweight, body length and head circumference were noted in absolute values and percentiles before the neonates´ admission to puerperal. These parameters were also used to calculate the ponderal index and cephalization index. The ponderal index was calculated by dividing the weight (grams) x100 by the body length $(cm)^3$. If the ponderal index was lowered, it indicated intrauterine malnutrition. On the other hand, the cephalisation index was calculated by dividing the head circumference (cm) by the body weight (grams). A high cephalisation index was used as an indicator for intrauterine growth restriction.

Postnatal changes and adaptational progresses were observed by monitoring weight progression, eating behaviour, transcutaneous bilirubin values, and adverse outcomes in the neonates.

3. Results

3.1. Maternal/Prenatal results

In table 10, the maternal characteristics are presented. The mother who smoked during pregnancy was the youngest, followed by the mother who discontinued smoking during pregnancy and finally the non-smoking mother. Also, the BMI of the smoking mother was higher than the former smoker´s but lower than the non-smoker´s BMI. The smoking mother also gained the most weight compared to the others. All three mothers were workers and were in a stable relationship with their partners.

Table 10: Maternal characteristics. Case C-S, "Case Current-Smoker" refers to the neonate born to the current smoker; **Case F-S**, "Case Former-Smoker" refers to the neonate born to the former smoker; **Case N-S,** "Case Non-Smoker" refers to the neonate born to the non-smoker; **BMI**, body mass index.

Mother of	**Case C-S**	**Case F-S**	**Case N-S**
Age	19a	30a	35a
Height	165cm	165cm	170cm
Pre-eclamptic risk factors	previous Henoch-Schoenlein Nephritis	previous early onset pre-eclampsia and HELLP-Syndrome	medically suspected thrombophilia due to previous leg vein thrombosis and pulmonary embolism
Weight (beginning of pregnancy)	62kg	51kg	76kg
BMI (beginning of pregnancy)	22,8	18,7	26,3
Weight before delivery	81kg	66kg	83kg
Gravida	2	2	1

Parity	1	2	1
Social-economic status	worker/in a stable relationship	worker/ in a stable relationship	worker/ in a stable relationship

Pre-eclamptic risk evaluation showed an increased pre-eclamptic risk due to previous Henoch-Schoenlein nephritis in the smoking mother, because of previous early onset pre-eclampsia and HELLP-Syndrome in the prior smoking mother, and because of medically suspected thrombophilia due to previous deep vein thrombosis and pulmonary embolism in the non-smoking mother. All women were under strict observation by the corresponding Departments. However, no reactivation or reoccurrence of the mentioned conditions were detected. Thrombophilia screening of the non-smoking mother remained negative.

All women were prescribed ASS, with a daily dosage of 150mg. Due to the leg vein thrombosis and subsequent pulmonary embolism, the non-smoking mother additionally took Enoxaparin (1x60mg) during pregnancy. Before pregnancy, the non-smoking mothers was on metformin therapy (1x500mg) because of a light form of PCO-syndrome. OGTTs were conducted twice with normal results.

According to the anamnesis, only the mother of Case C-S smoked during pregnancy. Her smoking habits decreased from 10 cigarettes daily to “occasionally” from the third gestational month onwards.

Frequency and the examined structures of ultrasound investigations varied between the women. The smoking mother had positive Notch-signs in the right and left uterine artery, which were detectable until the 20th gestational week. Also, during the 27th week ultrasound examination, an increased resistance in the utero-placental unit of the smoking mother was noted. The non-smoking mother displayed a non-recurring Notch-sign in the 16th gestational week examination. Ultrasound examination of the venous duct was conducted once in the foetus of the non-smoking mother. During this examination, a positive a-wave was detected and was thus classified as physiological. The former smoking mother and the corresponding foetus did not show any abnormalities during the ultrasound investigations.

All pulsatility and resistance indices of uterine arteries, umbilical arteries, and foetal medial cerebral arteries were within the norm in all mothers and foetuses during all examination points. End-diastolic flows (EDF) of umbilical arteries and foetal cerebral medial arteries were positive in all foetuses during all ultrasound measurements. Foetal head circumferences (HC), abdominal circumferences (AC) and HC to AC ratios were according to gestational age in all foetuses during all ultrasound examinations.

Table 11: Ultrasound parameters of mother of Case C-S. Case C-S, "Case Current-Smoker"; Positive **Notch-sign** at the 15+4 examination in the right and left uterine artery; indicated **Notch-sign** at the 20+1 examination; all pulsatility indices **(PI)** and resistance indices **(RI)** of uterine arteries, umbilical artery, and foetal cerebral medial artery are within the norm at all examinations; detection of increased resistance in the utero-placental unit at the 27+6 examination; end-diastolic flows **(EDF)** of umbilical artery and foetal cerebral medial artery positive in all examinations; normal cerebroplacental-ratio **(CPR)** at 27+6 ultrasound; foetal head- **(HC)**, abdominal circumference **(AC)**, and **HC to AC ratio** according to gestational age at all examination points.

Gestational week	**Left uterine artery (mother)**	**Right uterine artery (mother)**	**Umbilical artery (foetus)**	**Left medial cerebral artery (foetus)**	**Intrauterine biometry (mm) (foetus)**
15+4	PI:1,17 pos. Notch	PI:1,20 pos. Notch	-	-	-
20+0/+1	PI:0,81 RI:0,51 indicated Notch	PI:0,73 RI: 0,49	PI:1,63 RI:0,80 EDF: pos.	-	HC:179,7 AC:153 HC/AC Ratio: 1,175
23+6	PI: 0,81	PI: 0,63	-	-	HC:225,3 AC:201 HC/AC Ratio: 1,121
27+6	PI: 0,96	P:0,65	PI:1,11 EDF: pos.	PI:2,15 EDF: pos. CPR: 1,937	-
31+6	PI: 0,88	PI: 0,56	PI:1,14 EDF: pos.	-	HC:306 AC:276 HC/AC Ratio: 1,109

35+4	-	-	PI:0,95 EDF: pos.	-	HC:333 AC:326 HC/AC Ratio: 1,021
38+3	-	-	PI:0,67 RI:0,50	-	-

Table 12: Ultrasound parameters of Case F-S. Case F-S, "Case Former-Smoker". All pulsatility indices **(PI)** and resistance indices **(RI)** of uterine arteries, umbilical artery, and foetal cerebral medial artery are within the norm at all examination points; end-diastolic flows **(EDF)** of umbilical artery and foetal cerebral medial artery positive during all ultrasound examinations; normal cerebroplacental-ratio **(CPR)** at 26+3 and 30+3 ultrasound; foetal head circumference **(HC)**, abdominal circumference **(AC)**, and **HC to AC ratio** according to gestational age at all examination points.

Gestational age	**Left uterine artery (mother)**	**Right uterine artery (mother)**	**Umbilical artery (foetus)**	**Left medial cerebral artery (foetus)**	**Intrauterine biometry (mm) (foetus)**
13+6	PI: 2,04	PI: 1,74	-	-	-
16+3	PI: 0,84	PI: 1,02	-	-	-
22+3	PI: 0,84	PI: 0,57	-	-	-
26+3	PI: 0,84	PI: 0,49	PI: 0,99 EDF: pos.	PI: 1,56 EDF: pos. CPR:1,576	HC: 255 AC: 277 HC/AC Ratio: 1,123
30+3	PI: 0,84	PI: 0,46	PI: 0,99 EDF: pos.	PI: 2,41 EDF: pos. CPR: 2,133	HC: 296 AC: 255 HC/AC Ratio: 1,161
35+3	-	-	PI: 0,81 EDF: pos.	PI: 2,14 EDF: pos.	HC: 323 AC: 311 HC/AC Ratio: 1,039
38+3	-	-	-	-	HC: 356

					AC: 350 HC/AC Ratio: 1,017
39+3	-	-	PI: 0,63 RI: 0,46 EDF: pos.	-	-

Table 13: Ultrasound parameters of Case N-S. Case N-S, "Case Non-Smoker". All pulsatility indices **(PI)** and resistance indices **(RI)** of uterine arteries are within the norm at all examination points; indicated Notch sign at the 16+0 ultrasound in both uterine arteries; positive a-wave detected in the venous duct at the 12+1 examination (physiological); normal PI and positive end-diastolic flow **(EDF)** of umbilical artery at the 38+5 examination; foetal head circumference **(HC)**, abdominal circumference **(AC)**, and **HC to AC ratio** according to gestational age at all examination points.

Gestational age	**Left uterine artery (mother)**	**Right uterine artery (mother)**	**Umbilical artery (foetus)**	**Left middle cerebral artery (foetus)**	**Intrauterine biometry (mm) (foetus)**
12+1	PI: 2,05 RI: 0,79	PI: 1,96 RI: 0,78	-	-	HC: 76,1 AC: 54,5 HC/AC Ratio: 1,369
14+5	PI: 1,80	PI: 0,87	-	-	HC: 116 AC: 103 HC/AC Ratio: 1,126
16+0	PI: 1,6 indicated Notch	PI: 0,75 indicated Notch	-	-	-
36+5	-	-	-	-	HC: 342 AC: 332 HC/AC Ratio: 1,030
38+5	-	-	PI:0,70 EDF: pos.	-	-

Pre-eclampsia related markers such as sFlt-1 and PlGF were drawn at different points during pregnancy. The latest measurements are shown in table 14. The smoking

mother displayed the lowest sFlt-1/PlGF ratio, due to the lowest sFlt-1 and highest PlGF values. In contrast, the mother who discontinued smoking demonstrated the highest sFlt-1/PlGF ratio, because of highest sFlt-1 and lowest PlGF values. sFlt-1 and PlGF levels of the non-smoking mother were between the smoker´s and the former smoker´s.

Table 14: Maternal pre-eclampsia markers. Case C-S, "Case Current-Smoker"; **Case F-S**, "Case Former-Smoker"; **Case N-S**, "Case Non-Smoker"; **sFlt-1**, soluble Fms-like thyrosinkinase-1; **PlGF**, placental growth factor. **Results:** Lowest sFlt-1/PlGF ratio due to the lowest sFlt-1 and highest PlGF values in the smoking mother at 32+1; highest sFlt-1/PlGF ratio due to highest sFlt-1 and lowest PlGF values in the former smoking mother at 39+3; sFlt-1 and PlGF levels of the non-smoking mother located between those of the smoker´s and the former smoker´s.

	Gestational age	**sFlt-1 (pg/ml)**	**PlGF (pg/ml)**	**sFlt-1/PlGF-ratio**
Mother of Case C-S	32+1	1494,0	1310,0	1,1
Mother of Case F-S	39+3	24887,0	205,0	121,4
Mother of Case N-S	36+1	2107,0	823,3	2,5

3.2. Birth/Neonatal results

All mothers experienced a premature rupture of membranes without the need of prophylactic treatment. All neonates were born vaginally in cephalic presentation. None of the neonates had congenital pathologies or abnormalities. There were no signs of intrauterine meconium passage, nor infection of the amniotic fluids. Treatment during delivery (in all cases) consisted of intravenous administration of nalbuphine, buscopan, oxytocin, and inhalation of livopan.

The neonate born to the smoking mother (Case C-S) was delivered earliest (i.e., 38+6th gestational week) compared to the neonate of the former smoking mother (Case F-S) and the neonate of the non-smoking mother (Case N-S). Also, the duration of birth was shortest in the smoking mother, followed by the former smoking mother, then the

non-smoking mother. No abnormalities were noted in Case C-S´s and Case N-S´s delivery. Case F-S had to be extracted via vacuum, due to a pathological CTG.

Neonatal parameters such as APGAR-Score, birthweight, birth length and head circumference were physiological in all neonates. However, the birthweight of the non-smoker´s neonate was borderline on the 11th percentile. It was, therefore, considered as lowered when compared to the birthweight of the smoker´s and the former smoker´s neonates. All neonatal calculated ponderal indices and cephalization indices showed no signs of intrauterine growth restriction nor asymmetrical growth tendencies.

Table 15: Neonatal parameters and birth characteristics. APGAR-Score, birthweight, birth length and head circumference were physiological in all neonates; birthweight of Case N-S borderlines on the 11th percentile; calculated ponderal indices and cephalization indices (of all neonates) showed no signs of asymmetrical growth. **Legend: Case C-S**, "Case Current-Smoker"; **Case F-S**, "Case Former-Smoker"; **Case N-S**, "Case Non-Smoker"; **APGAR-Score**, Appearance-, Pulse-, Grimace-, Activity-, Respiration-Score.

	Case C-S	**Case F-S**	**Case N-S**
Gestational age	38+6	39+3	39+2
Duration of birth	2h	5h	11h
APGAR-Score (1st /5th /10th)	9/10/10	9/10/10	9/10/10
Gender	male	male	male
Birthweight (grams/percentile)	3140g/ 23rd p.	3290g/28th	2990g/ 11th
Birth length (cm/percentile)	52cm/ 52nd p.	52cm/ 46th p.	51cm/ 31st
Head circumference (cm/percentile)	36cm/ 74th p.	36,5cm/ 82nd	36cm/ 71st
Ponderal index/ cephalization index	2,23/ 0,011	2,34/ 0,011	2,25/0,12

Analysis of venous umbilical cord blood gas showed similar pO_2, pCO_2, sO_2, and FO_2Hb levels in the neonate born to the smoking mother and the neonate born to the former smoking mother. The neonate born to the non-smoker (Case N-S) had a lowered pO_2 and decreased percentage of sO_2 and FO_2Hb. Also, pCO_2 of Case N-S was higher compared to the other neonates. Evaluation of the haematological parameters showed heightened levels of haemoglobin and haematocrit in the neonate born to the non-smoker, compared to the neonates born to the smoking and the former smoking mother. Besides the mentioned differences, venous cord blood gas and haematological parameters were physiological in all neonates.

Parameters of acid-base status such as pH, HCO_3^-, and BE were similar in the neonate born to the smoker and the neonate born to the non-smoker. Their acid-base values showed no abnormalities. In contrast, pH, HCO_3^-, and BE were lowered in the neonate born to the former smoker (Case F-S), therefore showing acidic tendencies. Also, Case F-S´s glucose levels were highest, followed by the neonate born to the smoker and the neonate born to the non-smoker.

Table 16: Venous umbilical cord blood analysis. "*", out of reference; Abnormal values: marginally increased *haematocrit and *haemoglobin in Case N-S; acidic tendency in Case F-S seen as lowered *bicarbonate, *base excess and pH-value. **Legend: Case C-S**, "Case Current-Smoker"; **Case F-S**, "Case Former-Smoker"; **Case N-S**, "Case Non-Smoker"; **UCB-V**, Umbilical Cord blood-Venous; **pO_2**, oxygen partial pressure; **pH**, pH-value; **pCO_2**, carbon dioxide partial pressure; **ctHb**, haemoglobin concentration; **Hct**, haematocrit; **sO_2**, oxygen saturation; **FO_2Hb**, fraction of oxyhaemoglobin; **sBEc**, standard base excess concentration; **$cHCO_3^-$(P,st)c**, standard bicarbonate concentration.

	Case C-S	**Case F-S**	**Case N-S**
UCB-V pO_2 (mmHg)	22,9	26,3	18,6
UCB-V pH	7,335	7,253	7,304
UCB-V pCO_2 (mmHg)	41	41,6	45,6
UCB-V ctHb (g/dl)	16,8	16,7	*18,8
UCB-V Hct (%)	51,6	51,2	*57,5
UCB-V sO_2 (%)	53,9	52,9	33,7

UCB-V FO_2Hb (%)	52,5	51,7	32,9
UCB-V sBEc (mmol/L)	-4	*-8,9	-3,7
UCB-V $cHCO_3^-$(P,st)c (mmol/L)	20,2	*16,8	19,4
UCB-V Lactate (mmol/L)	3,2	5,3	4,4
UCB-V Glucose (mg/dl)	102	123	80

Case C-S, who was born to the smoking mother, showed the highest CO_2 partial pressure in the arterial umbilical cord blood analysis. However, Case C-S´s pO_2, sO_2, and FO_2Hb were lowered compared to the neonate born to the former smoking mother, yet higher when compared to the neonate born to the non-smoking mother. As expected, arterial haematological parameters were analogous to venous haematological parameters in all neonates. Again, acid-base values (pH, HCO_3^-, BE) were similar in the neonate born to the smoking mother and the neonate born to the non-smoking mother. According to the venous parameters, the neonate born to the former smoking mother (Case F-S) presented lowered pH, HCO_3^-, and BE values, thus presenting acidic tendencies. Again, Case F-S displayed the highest glucose values.

Table 17: Arterial umbilical cord blood analysis. "*", out of reference; Abnormal values: marginally increased *haematocrit and *haemoglobin in Case N-S; acidic tendency in Case F-S seen in lowered *bicarbonate, *base excess and pH-value. **Legend: Case C-S**, "Case Current-Smoker"; **Case F-S**, "Case Former-Smoker"; **Case N-S**, "Case Non-Smoker"; **UCB-A**, Umbilical Cord blood-Arterial; **pO_2**, oxygen partial pressure; **pH**, pH-value; **pCO_2**, carbon dioxide partial pressure; **ctHb**, haemoglobin concentration; **Hct**, haematocrit; **sO_2**, oxygen saturation; **FO_2Hb**, fraction of oxyhaemoglobin; **sBEc**, standard base excess concentration; **$cHCO_3^-$(P,st)c**, standard bicarbonate concentration.

	Case C-S	**Case F-S**	**Case N-S**
UCB-A pO2 (mmHg)	17,4	31,2	16
UCB-A pH	7,213	7,177	7,243

UCB-A pCO2 (mmHg)	60,3	49,8	55,8
UCB-A ctHb (g/dl)	16,9	16,8	*18,8
UCB-A Hct (%)	51,9	51,3	*57,5
UCB-A sO2 (%)	28,7	55,9	22,7
UCB-A FO2Hb (%)	27,8	54,4	22,1
UCB-A sBEc (mmol/L)	-3,5	*-9,9	-3,3
UCB-A cHCO3- (P,st)c (mmol/L)	18,8	*15,7	18,7
UCB-A Lactate (mmol/L)	3,8	6,7	5,1
UCB-A Glucose (mg/dl)	77	110	75

The neonate born to the smoking mother (Case C-S), showed the highest differences in pO_2 and pCO_2 compared to the others. Also, differences in Case C-S´s saturation parameters, such as sO_2 and FO_2Hb, were increased compared to the neonate born to the former smoker (Case F-S) and the neonate born to the non-smoker (Case N-S). Furthermore, pH-value and glucose differences were highest in the neonate born to the smoking mother. However, differences in base excess and lactate levels were higher in the neonate born to the former smoker compared to the values of Case C-S and Case N-S.

Table 18: Differences between venous and arterial cord blood parameters. Legend: Case C-S, "Case Current-Smoker"; **Case F-S**, "Case Former-Smoker"; **Case N-S,** "Case Non-Smoker"; **V-A**, Venous-Arterial (difference); **pO_2**, oxygen partial pressure; **pCO_2**, carbon dioxide partial pressure; **pH**, pH-value; **sO_2**, oxygen saturation; **FO_2Hb**, fraction of oxyhaemoglobin; **sBEc**, standard base excess concentration; **$cHCO_3^-$(P,st)c**, standard bicarbonate concentration.

	Case C-S	**Case F-S**	**Case N-S**
V-A pO_2 (mmHg)	5,5	-4,9	2,6

V-A pCO_2 (mmHg)	-19,3	-8,2	-10,2
V-A pH	0,122	0,076	0,061
V-A sO_2 (%)	25,2	-3	11
V-A FO_2Hb (%)	24,7	-2,7	10,8
V-A sBEc (mmol/L)	-0,5	1	-0,4
V-A $cHCO_3^-$ (P,st)c (mmol/L)	1,4	1,1	0,7
V-A Lactate (mmol/L)	-0,6	-1,4	-0,7
V-A Glucose (mg/dl)	25	13	5

During puerperium, all neonates developed sub-icteric skin alterations. In addition, the neonate born to the smoking mother developed petechial skin lesions. Analysis of weight progression showed the highest weight loss in the neonate born to the former smoker, followed by the neonate born to the non-smoker and the neonate born to the smoker. The percentage of weight loss did not exceed 10%, therefore no treatment was induced. All neonates were breast fed ad-libidum, adding hypoallergenic formula if needed. All conducted transcutaneous bilirubin measurements were within physiological range. No adverse health events were noted during puerperium. All neonates and mothers were discharged on the 3rd day post-partum.

Table 19: Neonatal changes and adaptations in puerperium. Legend: Case C-S, "Case Current-Smoker"; **Case F-S**, "Case Former-Smoker"; **Case N-S,** "Case Non-Smoker"; **pp.,** postpartum; **PreHa**, hypoallergenic formula.

	Case C-S	**Case F-S**	**Case N-S**
Weight on day of discharge	2960g	3065g	2774g

Weight loss	180g	225g	216g
Transcutaneous bilirubin measurement (2nd day pp./3rd day pp.)	8,8mg/dl; 11,4 mg /dl	-; 7,3 mg/dl	6,6 mg/dl; 8,9 mg/dl
Nutrition	Breast milk + PreHa	Breast milk +PreHa	Breast milk + PreHa
Abnormalities during puerperal	Sub-icteric skin; petechial lesions	Sub-icteric skin	Sub-icteric skin, scratch marks

4. Discussion

Pre-eclampsia and maternal smoking during pregnancy are both known to adversely affect foetal/neonatal parameters such as intrauterine growth (15, 44), placental-foetal transfer of nutrients and oxygen (7, 20, 45), and postnatal clinical status (APGAR-Score) (27, 56, 58). Furthermore, each condition is strongly associated with increased neonatal morbidity and mortality (7, 33). Despite the negative impacts on the foetus, maternal smoking during pregnancy might reduce the risk of developing pre-eclampsia (44). Previous studies evaluating neonatal parameters of infants born to pre-eclamptic mothers who smoked during pregnancy primarily focused on the effects on neonatal birthweight, generally showing conflicting results (70-72). Those studies did not emphasise the impact on neonatal parameters such as APGAR-Score, anthropometric sizes, umbilical cord blood analyses and postnatal neonatal changes and adaptations. Therefore, this case series was conducted comparing the above-mentioned parameters of three neonates born to mothers with positive pre-eclamptic risk. More specifically, one of the mothers represented an active smoker, one a former smoker and one a non-smoker.

In this case studies, increased maternal pre-eclamptic risks were observed due to the following risk factors: Previous Henoch-Schoenlein nephritis (in the smoking mother) (13), previous early onset pre-eclampsia and HELLP-Syndrome (in the former smoking mother) (11), and suspected thrombophilia (in the non-smoking mother) (12). However, only the former smoking mother developed pre-eclamptic tendencies, as seen in her increased sFlt-1/PlGF ratio (table 14) (3, 4). Studies evaluating the protective effect of smoking against pre-eclampsia, only observed a decrease in pre-

eclamptic risk when smoking was continued during the third trimester (64). The discontinuation of smoking, in the former smoking mother, potentially could have influenced the mother´s pre-eclamptic markers (sFlt-1). Nonetheless, neither a rise in resistance indices, nor abnormal Doppler flow waveforms of utero-placental vessels were noted in ultrasound examinations of the former smoking mother, which could have been expected due to her pre-eclamptic tendencies (4). On the other hand, during the 27+6 ultrasound examination, an increased utero-placental resistance was noted in the active smoking mother. The rise of utero-placental vascular resistance in pregnant smokers was already described in many studies, as summarised in Reeves et al´s publication. (44). Yet, against expectations, no signs of increased utero-placental resistance were detected after the 27+6 ultrasound examination in the smoking mother. This could have potentially been due to her self-reported reduction in smoking. Even though all women had a positive pre-eclamptic risk, no further abnormalities (outside of the physiological range) were noticed during ultrasound examinations. Evaluations of intrauterine foetal biometry showed appropriate prenatal biometric parameters in all neonates.

The comparison of neonatal APGAR-Scores showed no differences between the neonate born to the active smoking mother and the neonates born to the mothers who did not smoke during pregnancy. This data contradicts observations published in Karadeniz et al´s (58) and Marin et al´s (56) studies, which detected a decrease in the 1st and 5th minute APGAR-Scores of neonates born to active smoking mothers when compared to neonates born to non-smoking mothers. In contrast to Saadat et al´s study, in which maternal pre-eclampsia was associated with a lowered neonatal APGAR-Score (27), a positive pre-eclamptic risk did not seem to affect neonatal APGAR-Scores. All neonates included in this study were born with an APGAR-Score of 9/10/10 and, hence, presented themselves in good clinical condition.

The analysis of neonatal anthropometric parameters showed no differences between the neonate born to the active smoking mother (Case C-S) and the neonate born to the former smoking mother (Case F-S). Signs of growth restriction or asymmetrical growth could have been expected in the neonate born to the active smoking mother (44) and in the neonate born to the former smoking mother (due to high pre-eclamptic risk (16)). Yet, their anthropometric parameters were classified as normal.

Unexpectedly, the birthweight of the neonate born to the non-smoker (Case N-S) was borderline on the 11th percentile and, therefore, the lowest compared to the other cases´ birthweights. However, this data could also indicate a physiologically small for gestational age neonate.

During the analyses of umbilical cord blood parameters, hypoxic tendencies induced by maternal pre-eclamptic risk could have been expected in all neonates. Hypoxic conditions would have presented themselves as decreased oxygen partial pressures and pH-values, and increased carbon-dioxide partial pressures (short term parameters), as well as increased haemoglobin and haematocrit levels (long term parameters). In the case of the neonate born to the smoking mother, more severe hypoxic tendencies could have been anticipated. However, umbilical cord blood gas analysis neither revealed signs of hypoxic conditions due to positive pre-eclamptic risk regarding all neonates nor due to maternal smoking during pregnancy in the neonate born to the smoker. This data is in contrast to Najib et al s´ (24) and Matsuo et al s´ (25) studies, who detected associations between pre-eclampsia and signs of neonatal hypoxia in umbilical cord blood (UCB) gas analysis, as well as in contrast to Habek et al´s (50) data, who noted hypoxic changes in UCB gas analyses induced by maternal smoking during pregnancy.

Nonetheless, acidic tendencies were detected in the umbilical cord blood analysis of the neonate born to the former smoker (Case F-S). These presented themselves as a decreased pH-value, base excess, and bicarbonate. Presumably, these changes were induced through complications during birth, as displayed in the pathological CTG, which ultimately resulted in a vacuum extraction delivery of the neonate. Interestingly, the neonate born to the non-smoking mother (Case N-S) showed the lowest oxygen partial pressure and the highest haemoglobin and haematocrit levels (in the UCB analysis). Considering the already observed association between lowered oxygen partial pressure and heightened haemoglobin and haematocrit levels (published by Teramo et al (20)), a mild form of oxygen undersupply in the neonate born to the non-smoker (Case N-S) could be suspected. However, since Case N-S´s haematological parameters were only minimally out of range, it was considered as physiological.

Differences between arterial and venous umbilical cord blood parameters were analysed to calculate placental oxygenation capacity (difference in arterial and venous

oxygen partial pressure) and placental clearance (difference in arterial and venous carbon-dioxide partial pressure), as conducted in Matsuo et al´s study (25). In this case series the mentioned parameters do not represent placental oxygenation capacity or clearance due to umbilical cord blood being drawn after delayed cord clamping, therefore enabling the neonate to breath while still being connected to the placenta. Thus, gas exchange could have already been induced by pulmonary (neonatal) breathing and, therefore, altered the umbilical blood gas parameters. Even though placental oxygenating capacity or placental clearance capacity could not be calculated, disparities in arterio-venous differences were detected between the cases. The neonate born to the smoking mother showed the highest differences (except in lactate and base excess) between umbilical venous and umbilical arterial blood parameters. Thus, an increased systemic metabolism was suspected. In contrast, the neonate born to the non-smoker had the lowest differences between arterial and venous umbilical blood parameters, suggesting a potentially lowered systemic metabolism. However, no literature was found on how interpretate the differences between arterial and venous umbilical cord blood parameters.

During the puerperium, the postnatal changes and adaptational progresses of the neonates were observed (table 18). No adverse health events such as feeding intolerance, necrotizing enterocolitis or hypo-/hyperglycaemia were noted. This data contradicts the ones described in Sharma et al´s publication regarding immediate complications in growth restricted neonates (16). However, none of the neonates included in this study were classified as growth restricted.

The neonate born to the smoking mother did not show immediate complications associated with maternal smoking during pregnancy. Among others, these could have had included malformations of the heart, central nervous system, vascular system, and musculoskeletal system as described in Raghuveer et al´s publication (61). It is important to note that maternal smoking during pregnancy might have induced silent clinical changes such as genetic changes due to carcinogens (43) or alterations in foetal neurons due to nicotine binding to nicotinic acetylcholine receptors (located in the brain) (43). Also, the neonate born to the smoking mother was the only case that developed petechial skin lesions during puerperium.

In the end, neonatal check-up conducted by paediatricians showed no abnormalities besides the observations shown in table 18. All neonates were discharged in good clinical condition.

4.1. Limitations

Originally, this case series study should have been carried out as a pilot study including 15 participants. However, due to the SARS-CoV-2 pandemic and difficulties in recruiting pregnant smokers with pre-eclamptic risk, the sample size was reduced to three subjects. Also, the original title of this thesis, "*The effects of nicotine consumption in new-born infants born to pre-eclamptic mothers"*, was changed to "*The effects of smoking in neonates born to mothers with pre-eclamptic risk"* since the participating mothers consumed commercial cigarettes rather than nicotine alone. Additionally, all mothers who participated in this study were prescribed acetylsalicylic acid. As such, the risk of thrombosis and embolisms was reduced in all mothers. Furthermore, examination dates (ultrasound examinations, sampling of pre-eclampsia markers) varied during the lockdown. As a result, certain conditions could have been missed. Also, the investigated data represents specific time points and no prospective observations. Neonatal intrauterine changes and adaptations which did not manifest/were not visible during the time of observation could have not been detected.

4.2. Conclusion and further directions

This case series study was conducted as part of the bigger *"Does nicotine reduce the risk of pre-eclampsia? A prospective study"* trial which analysed pre-eclamptic risk in smoking mothers. However, the focus of this case series was to analyse the possible effects of a positive (maternal) pre-eclamptic risk combined with maternal smoking during pregnancy on the neonates. Therefore, neonatal parameters of three infants born to mothers with a positive pre-eclamptic risk were compared. More specifically, one of the mothers smoked during pregnancy, one mother represented a former smoker, and the last mother was a non-smoker. The analysed neonatal parameters included the APGAR-Score, postnatal neonatal biometry, umbilical cord blood analyses and neonatal changes and adaptations during the first week of life. These neonatal parameters were investigated for changes induced by maternal pre-eclamptic risk or, in one case, by maternal pre-eclamptic risk combined with smoking during pregnancy. The neonate born to the smoking mother with a positive pre-eclamptic risk represented the focal point of this study. Regarding the results of this case series, a positive pre-eclamptic risk along with maternal smoking during pregnancy could have potentially increased utero-placental resistance, as this effect was not seen in the non-smoking cases. However, intra-uterine growth of the corresponding foetus did not seem to be affected, as the foetus developed according to gestational age. Also, there was no impact on the neonate´s APGAR-Score or umbilical cord blood values, which ended up being physiological. Surprisingly, maternal smoking combined with a positive maternal pre-eclamptic risk seemed to increase the differences between the corresponding neonate´s arterial and venous cord blood parameters. This could potentially be an indicator for a heightened metabolism as a response by the neonate. However, the corresponding neonate´s health was not affected more adversely when exposed to a positive pre-eclamptic risk and maternal smoking during pregnancy. In the end, all cases were discharged in good clinical condition.

Diverse data was collected during this case series study, yet no statistically significant conclusions could be drawn due to the nature of the study design. For statistically significant results, it is recommended to compare the investigated neonatal parameters in a larger study size.

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