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Correlations of placental histopathology, clinical signs and perinatal outcomes in preeclampsia and fetal growth restriction

PhD Thesis

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LIST OF ABBREVIATIONS

AC	abdominal circumference
ACOG	American College of Obstetricians and Gynecologists
AEDF	absent end diastolic flow
AFI	amniotic fluid index
AUM	umbilical artery
AVM	accelerated villous maturation
BMI	body mass index
BPM	beats per minute
CTG	cardiotocography
CVR	capillary-villus ratio
DVH	distal villous hypoplasia
DVM	delayed villous maturation
EO	early-onset
FGR	fetal growth restriction
FIGO	International Federation of Obstetricians and Gynecologists
FL	femoral length
FVM	fetal vascular malperfusion
HE	hematoxylin and eosin
HELLP	Haemolysis, Elevated Liver enzymes, Low Platelet count
LO	late-onset
MCA	middle cerebral artery
MVM	maternal vascular malperfusion
PE	preeclampsia
PITV	percentage of intact terminal villi
REDF	reverse end diastolic flow
RI	resistance index
VUE	villitis of unknown etiology

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1. INTRODUCTION

Placental histopathology has a great importance in the field of perinatology, as placental changes give us significant information about the intrauterine milieu in which the fetus was in the antepartum period. The information provided by the histopathological evaluation of placental tissue is crucial in the assessment of possible intrauterine insults and pregnancy-associated pathological entities [1]. Placental histopathological examination after birth should be performed in all cases where the pregnancy was associated with significant pathology or in the presence of any kind of adverse pregnancy outcome(s), indicated by explicit criteria [2]. Moreover, placental histopathological examination could provide additional information about intrauterine fetal wellbeing in cases when litigation is initiated, and in this way, it could also have medico-legal significance [3].

The placenta is the metabolic interface between the mother and the fetus; it develops from the cells of the basal decidua and chorion frondosum during the process of hemochorial placentation [4]. Human placentation and placental vasculogenesis is delicately regulated by the immune system; the role of uterine natural killer cells and macrophages in the process is highly important, as the invasion of trophoblasts and the remodelling of the spiral arteries are regulated by these cells [5]. Placental vasculogenesis is modulated by the balance of pro-angiogenic and anti-angiogenic factors, and the imbalance of these regulating factors leads to defective placental vessel formation, with clinically relevant consequences [6].

Preeclampsia (PE) is a common complication of pregnancy with potential transition to eclampsia and significant morbidity and mortality affecting both the mother and the fetus. Although the exact pathogenesis of PE is not fully understood, several details have been described on molecular and histopathological levels. Numerous histopathological changes have been reported in placentae from PE and it is also known that oxydative stress, pathologic placentation and pathologic angiogenesis have an important role in the development of the disease [7].

Early-onset (EO) and late-onset (LO) PE are two distinct entities with different maternal and neonatal prognosis and a distinct pathology; the 34th week of gestation at the time of diagnosis is the boundary between the two subtypes [8]. Association of fetal growth restriction (FGR) with PE is the main cause of unfavourable perinatal outcome, especially if FGR is evident at the onset of PE [9].

The placenta is always affected in PE, and has a key role in its development: delivery of the fetus and the placenta is the efficient cure of PE. On the other hand, the placenta is not the only responsible element in the pathogenesis: the maternal cardiovascular system also has an important role in developing the systemic symptoms of the disease [10]. The maternal immune system and

genetic factors also contribute to the development of the syndrome [11]; as the systemic symptoms of PE develop, placental involvement becomes obvious.

Correlating placental histology with clinical signs and symptoms of PE could be a useful approach to characterize the disease.

PE has a broad clinical spectrum in severity ranging from mild disease to severe conditions [12].

The prevalence of PE is between 2% and 10% of pregnancies according to the statistics provided by the World Health Organisation [13], and PE is still a leading cause of maternal perinatal morbidity and mortality in developed and developing countries worldwide [14]. Therefore, early detection and diagnosis of PE has a crucial role in its clinical management [15].

FGR is a common complication of pregnancy, with a significantly increased risk of perinatal death [16] and is associated with several consequences of neonatal and perinatal morbidity [17]. FGR is defined as fetal weight below the 10th percentile for the given week of gestation; it is a severe condition, in which the fetus does not grow according to its genetically expected percentile (which is characteristic to the given population) [18, 19]. Adequate monitoring of fetal wellbeing has a crucial role in the assessment of intrauterine fetal condition; in case of deteriorizing intrauterine fetal wellbeing, preterm delivery is inevitable, consequently an increased rate of preterm delivery is associated with FGR [20].

Cardiotocography (CTG) is a non-invasive and widely accessible tool for the assessment of fetal conditions, although its interpretation has a poor inter-observer agreement [21]. CTG monitoring of preterm fetuses does not have reference standards and well-defined guidelines in contrast with the CTG interpretation of term fetuses [22]. CTG interpretation in the early preterm (between the 24th and 28th weeks of pregnancy) population (especially, if pregnancy is also complicated by FGR) is especially characterized by inadequate monitoring [23] and lack of evidence.

The placenta is affected by numerous histopathological alterations in FGR [24]. These changes lead to chronic intrauterine hypoxia and malnutrition. The result of this altered placental metabolic and transport function is FGR with all of its consequences. At the point when fetal hypoxia as final outcome of these placental changes manifests, alterations of CTG patterns could signal the necessity of obstetrical intervention.

Similarly to PE, early-onset and late-onset FGR have been distinguished, and their pathogenesis, histopathological background and prognosis seem different, suggesting that these two entities are different [25]. Several studies have examined the boundary between EO-FGR and LO-FGR [26, 27, 28].

FGR has a notable tendency to recur, and recurring FGR in a consecutive pregnancy is also a clinically important subtype worth to be distinguished from non-recurrent cases [29].

Ultrasound is a helpful tool in the follow-up and management of FGR; the amniotic fluid index (AFI) [30], biophysical profile of the fetus [31], fetal biometry, femoral length/abdominal circumference (FL/AC) ratio [32], umbilical (AUM) and middle cerebral artery (MCA) Doppler indices [33] are highly useful methods helping the clinician in the assessment of intrauterine fetal wellbeing, recognizing the deterioration of fetal conditions *in utero*, and making possible delivery before manifestation of irreversible fetal damage or death.

Postpartum histopathological examination of placenta has an emphasized role in the clinical interpretation of pathologic pregnancies with adverse perinatal outcomes, and the Amsterdam Placental Workshop Group Consensus Statement in 2016 made it possible to standardize and unify the terminology and classification of placental histopathological entities [34]; it creates a uniform scientific nomenclature in placental pathology [35]. This approach allows to maintain a complex yet traceable classification in this field, which yields important and clinically relevant information about perinatal pathological entities both to the obstetricians and the pathologists [36, 37].

2. AIMS

1. To analyze the placental histopathological background of early- versus late-onset PE, early- versus late-onset FGR, and to investigate the placental histological background of PE complicated with FGR, recurring FGR and recurring PE

2. To assess the clinicopathological implications of placental histopathology in cases with PE and/or FGR on neonatal outcomes

3. To find possible connections between the rate of villous capillarization, the percentage of intact terminal villi and pregnancy outcomes

4. To analyze the possible connections between placental histopathology and CTG baseline variability and acceleration patterns in the growth restricted preterm population

5. To investigate the connections of placental histopathological changes and different Doppler indices measured by prenatal ultrasound, and to assess the possible correlations of placental microscopic changes and ultrasound biometry parameters

3. MATERIALS AND METHODS

3.1 Correlations between placental histopathology, clinical signs of preeclampsia and neonatal outcome

All placentae from preeclamptic pregnancies submitted for histological evaluation between 2007 and 2022 in our tertiary medical centre were retrieved from the archives of the Pathology Department, and were evaluated by the same observer according to a uniform scheme. The retrospective review of placental signs was blinded to clinical details and neonatal outcomes, the only information that was given for the examiner was the gestational age at the time of delivery.

The clinical diagnosis of PE was made according to the ACOG (American College of Obstetricians and Gynecologists) guideline: elevated blood pressure in a previously normotensive pregnant woman on two occasions four hours apart and proteinuria >300 mg 24-hour-urine collection [38]; we studied only cases in which gestational age was beyond the 24th week. Exclusion criteria included twin pregnancies, placental abruption, fetal genetic or structural disorders, pregnancies complicated with intrauterine infection, and presence of gestational diabetes.

All placentae were fixed in neutral buffered formalin after delivery, and tissue blocks were taken from three different places. All slides represented basal plate, villous tree and chorionic plate in each instance. Formalin fixed and paraffin embedded material was used and 3-4 micrometer thick sections were stained with hematoxylin and eosin (HE).

Histological signs of maternal vascular malperfusion (MVM; the studied MVM-associated placental changes were: chorionic villous infarction, accelerated villous maturation (AVM), distal villous hypoplasia (DVH)), decidual arteriopathies, fetal vascular malperfusion (FVM; the studied FVM-associated placental chages were chorionic plate and/or stem vessel thrombosis, fibrinoid necrosis of large fetal vessels, avascular villi), delayed villous maturation (DVM), villitis of unknown etiology (VUE) and chorangiosis were studied. All of the above mentioned lesions were diagnosed according to the histological definitions of the Amsterdam Placental Workshop Group Consensus Statement [39]; chorangiosis was studied by using the criteria of Altshuler [40].

As far as decidual arteriopathies are concerned, acute atherosis, fibrinoid necrosis of vessel wall, medial hypertrophy of decidual vessels, perivasculitis and arterial thrombosis were separately recorded as components of this entity [41, 42, 43]; in all cases, maternal decidual vessels were extensively studied.

Avascular villi were categorized into three groups (small foci: three or more foci of 2 to 4 terminal villi showing loss of villous capillaries and bland hyaline fibrosis of the villous stroma; intermediate foci: 5 to 10 villi; large foci: more than 10 villi) [39, 44]. Diagnosis and grading of VUE were also made following explicit criteria (low grade: presence of inflammation affecting

fewer than 10 contiguous villi in any focus, with more than one focus required for the diagnosis; high grade: presence of multiple foci, on more than one section, at least one of which shows inflammation affecting more than 10 contiguous villi) [39, 45, 46]. In case of the other histopathological changes, focal and diffuse appearance of the lesions was distinguished: a lesion was considered focal if it was present in one full-thickness placental slide, and it was considered diffuse if it appeared on two or more slides.

Avascular and hypovascular villi were carefully distinguished, as well as infarcted areas differentiated from avascular villi.

DVM was only studied after the 36th week of pregnancy. Diagnosis of AVM was only made before the 36th week of gestation.

The percentage of intact terminal villi (PITV) was determined according to our own methodology: ten non-overlapping areas of placenta were evaluated under high power magnification, and 100 adjacent villi were counted in each area to determine the percentage of intact villi. PITV was analyzed in randomly selected areas from the central part of placental parenchyma, away from the fetal and maternal surfaces. In each instance, a central terminal villous was randomly selected, which was surrounded by a population of other terminal villi; with the use of a clockwise approach, 100 neighbouring terminal villi were evaluated (the counting always involved the closest adjacent villi step by step, until 100 villi were reached in the given randomly selected area). Villous infarction, DVH, AVM, avascular villi, DVM and VUE have well-defined histopathological characteristics, and if any of these placental changes were identified, the affected villus was considered non-intact. Villi with the signs of chorangiosis in itself, without any evidence of the other mentioned placental changes were considered intact. If one capillary of a single villus had a vascular thrombus but was otherwise intact, it was considered intact; if multiple capillaries in a single villus were thrombotised, the villus was considered obliterated and non-intact (Figure 1).

The number of intact capillaries in 100 adjacent villi in five different, randomly selected areas of the placental slides were also determined: the capillary-villus ratio (CVR; also our own method for the purpose of quantitative studies) was calculated using the number of capillaries (identified on HE stain) in these villi (CVR = capillary number in 500 villi/500). In this retrospective study, we did not have the opportunity to use vascular specific immunostains to help counting the capillaries in the terminal villi.

Histological signs of ascending intrauterine infection in the placenta were not described because of the lack of infected cases.

The corresponding clinical data, obstetrical and neonatal history were retrieved from the digital charts. EO-PE and LO-PE were compared for histological features, then we studied the distinct character of PE complicated with FGR. FGR was defined as birth weight under the 10th

percentile for gestational age [47]. PE was considered recurring when previous pregnancy (or pregnancies) were also complicated with PE. Different ranges of proteinuria, antihypertensive treatment of PE (number of required antihypertensive medicines), grade of maternal hypoalbuminemia were studied and correlations were examined with histological features. We used clinical and laboratory data from the last 72 hours of pregnancy; criteria of combined antihypertensive treatment were met when two or three different drugs were used for at least 72 hours in a PE patient.

Ultrasound scans and Doppler measurements were done with a transabdominal transducer (GE Voluson 730 Pro and GE Voluson E8 machines, 2-D mode and conventional colour Doppler studies of fetal vessels) to assign the resistance index (RI) of umbilical arteries (done in 30 cases) and to diagnose the persistence of diastolic notch of uterine artery (done in 27 cases); definition of increased umbilical artery RI was RI beyond the 95th percentile for gestational age.

24-hour-proteinuria in PE is a useful prognostic marker of severity [48]; we classified proteinuria values of patients in three groups (300-1000 mg/L/24h, 1001-2000 mg/L/24h, >2000 mg/L/24h), using the last known laboratory results from the last one-week-period of gestation before childbirth. The severity of hypertension in PE was characterized by the number of medicines needed to control hypertension (alpha-methyldopa only, alpha-methyldopa+nifedipine combination therapy and alpha-methyldopa+nifedipine+beta-blocker combination therapy).

Comparative statistical analysis was made by using the Pearson’s Chi-square test and when the numbers were low (<10 in any subgroup) the Fisher exact test was used instead. Statistical significance was defined as $p<0.05$. The Mann-Whitney U test was used for the comparison of PITV and CVR in different subgroups.

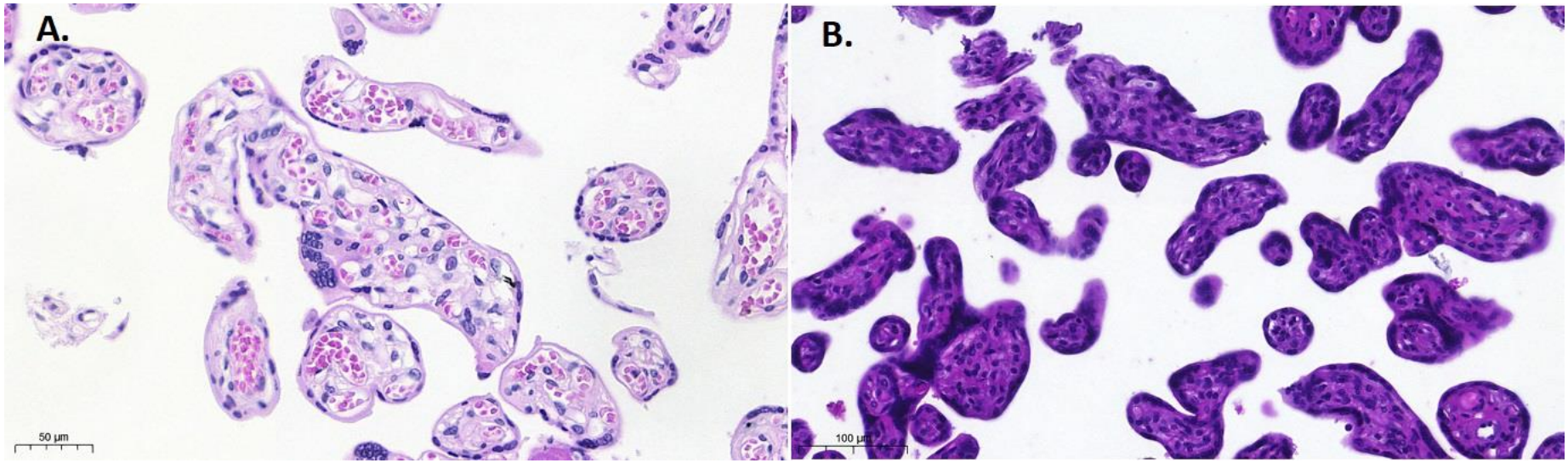

Figure 1.

Placental slides: intact versus non-intact villi

Description:

1A: Placental histology of a late-onset growth restricted fetus delivered at the 38th week of gestation (x28.5, HE staining). Full-thickness tissue sample was taken from the placenta, which was macroscopically normal. The photomicrograph represents villi from the central part of placental parenchyma; these villi do not have any pathologic histological alterations. However, other tissue sections of this placenta had histopathological changes.

1B: Placental histology of an early-onset fetal growth restriction case (x20, HE staining). Gestational age at the time of delivery was 33 weeks. Full-thickness tissue sample was taken from the central part of the placenta adjacent to the umbilical cord insertion; the placenta was macroscopically normal. In this focus the villi are slender, hypoplastic, some of them were elongated, distal villous tree was poorly developed – distal villous hypoplasia was diagnosed.

3.2 Correlations of placental histopathology, neonatal outcome, and cardiotocogram baseline variability and acceleration patterns in the growth restricted preterm population

In this retrospective study, we examined FGR cases delivered before the 37th week of gestation. In each case, placental histopathological examination was performed after delivery, and continuous, uninterrupted CTG monitoring of the baby was done before vaginal delivery or before transportation of the mother to the operative suite for Cesarean section. The average duration of CTG monitoring was 354.6±124.2 minutes.

EO-FGR and LO-FGR cases were studied separately; in case of EO-FGR, growth restriction of the fetus was diagnosed prenatally before the 32th week of gestation, while in LO-FGR it happened after the 32th week [49]. Corresponding clinical data were coupled with each case (Apgar scores at 5 and 10 minutes; umbilical artery pH after birth (AUM pH), umbilical artery blood lactate level after birth (AUM lactate), birth weight percentile); AUM pH and AUM lactate results were studied from the first 15 minutes after birth of the newborn. The practice of our perinatal intensive care unit applies the cutoff value of 3.75 mmol/L for AUM lactate as a proper marker for intrapartum asphyxia, and this cutoff value was found to have a good specificity and sensitivity for asphyxia by a recent study [50]. Higher AUM lactate levels were split into the ranges of 3.75-4.99 mmol/L, 5.0-9.99 mmol/L and >10 mmol/L, in order to distinguish between severe and more severe lactic acidosis of the newborn. Similarly, in our everyday routine, we consider AUM pH ≤7.2 as acidotic, and AUM pH <7.01 as critically acidotic; these pH values are also considered proper cutoff values by international studies [51].

To exclude inter-observer variability, each cardiotocogram was assessed by the same person according to a uniform scheme using the definitions of the FIGO (International Federation of Gynecology and Obsterics) CTG guidelines [52, 53]. Evaluation of the cardiotocogram reads was performed retrospectively, the study was done by a licensed obstetrician; in each case, the examiner was blinded to clinical details, gestational age, neonatal outcomes and placental findings, respectively.

Baseline fetal heart rate was between 120 and 160 beats per minute (bpm) in each case, and none of the studied cardiotocograms had any form of decelerations or bradycardia. (To note, decelerations on CTG in preterm fetuses could be physiological [54, 55]). The presence of accelerations was evaluated in each cardiotocogram – if acceleration was not registered in the last 60 minutes (but at least one acceleration was present in every 30 minutes in the previous segments of cardiotocograms), ‘lack of accelerations’ was diagnosed.

In all of the studied cases, independent from the duration of CTG monitoring, the diagnostic criteria of reduced or increased baseline variability were met only in the last 60 minutes of

cardiotocography, therefore, baseline variability was normal in all cases before the last hour of monitoring.

Histopathological study of placental slides was accomplished the same way as in the previously described study of preeclampsia; qualitative (MVM- and FVM-associated histopathological entities, VUE, chorangiosis) and quantitative (PITV and CVR) placental histological studies were recorded.

Exclusion criteria included stillbirth, twin pregnancies, indeterminate gestational age, genetic or structural abnormalities of the fetus, intrauterine infection diagnosed before or after delivery, gestational diabetes or disorders of placental implantation, cases where any medications or general anaesthetics (except for intravenous antibiotics, intramuscularly administered dexamethasone as a prophylaxis against infant respiratory distress syndrome, saline infusion, and anaesthetics used for intradural analgesia) were administered in the last 48 hours of pregnancy (to avoid potential overlapping effects of medications on CTG patterns). The use of intravenous oxytocin was also excluded. We did not study cases with umbilical cord complications (e.g. nuchal cord, cord knot) in order to eliminate the possible effect of cord compression on CTG patterns. There were no cases with acute placental inflammation in this cohort.

All patients had regular uterine contractions in the studied interval. There were no vacuum-assisted or forceps deliveries in the series.

We looked for any associations between neonatological parameters with CTG in the EO-FGR and LO-FGR groups separately and together. Possible connections of the studied placental changes and CTG baseline variability and acceleration patterns were evaluated. The percentage of intact terminal villi was evaluated in different subgroups. The CVR of different groups was also statistically analyzed.

For the comparison of continuous variables in different subgroups, the t-test for independent samples was applied. For the associations of categorical variables, as case numbers were low, we applied the Fisher exact test, with a level of significance at $p<0.05$. The Mann-Whitney U test was used to determine statistically significant differences between the PITV and CVR in different groups.

As we applied multiple statistical tests in the study, we performed a multivariate logistic regression analysis (with backward selection of variables) as a correction at the end of the study (all variables studied in univariate analyses were entered in the logistic regression analysis).

3.3 Placental pathology and its associations with clinical signs in different subtypes of fetal growth restriction

In this retrospective study, we examined placentae from FGR pregnancies from the interval between 2007 and 2022. The clarification of gestational age was done in all cases using the crown-rump length (CRL) of the embryo from the ultrasound scan conducted in early pregnancy: age of gestation (week/days) was determined with the help of a percentile table containing the corresponding gestational age to the measured CRL [56].

We studied only singleton pregnancies with a gestational age over the 24th week at the time of birth. Exclusion criteria included: twin pregnancies, genetic or structural disorder(s) of the fetus, stillbirth, pregnancies complicated with gestational diabetes; maternal thrombophilia; uncertain age of gestation; placental abruption; pregnancies with signs of intrauterine infection.

The methodology of qualitative and quantitative histopathological study of placental slides was the same as in our previously described studies.

We analyzed the differences between histopathological alterations in EO-FGR versus LO-FGR, FGR with PE versus FGR without PE, and recurring versus non-recurring FGR.

We also studied the connections between placental histological changes and clinical features or neonatal outcome, notably AFI, AUM and MCA Doppler indices; fetal birth weight; FL/AC ratio diagnosed with ultrasound at the time of diagnosis of FGR; and maternal body mass index (BMI) at the time of birth. In our study, poor perinatal outcome was defined as umbilical artery pH <7.2 and/or lactate >3.75 mmol/L, respectively. AFI and Doppler indices were used only from the period of the last 48 hours of pregnancy. The AUM Doppler indices were considered pathologic in case of absent end diastolic flow (AEDF), reverse end diastolic flow (REDF) or when the resistance index (RI) was beyond the 95th percentile of the reference values for gestational age. MCA Doppler indices were considered pathologic when RIs were below the 5th percentile for gestational age. Ultrasound scans and Doppler measurements were done with a transabdominal transducer (GE Voluson 730 Pro or GE Voluson E8 machines, 2-D mode and conventional color Doppler studies of fetal vessels), using a free umbilical cord loop and the proper area of MCA.

The presented studies were approved by the Scientific and Research Ethics Committee of the Medical Research Council, Hungary (ETT-TUKEB, IV/2992-3/2022/EKU).

4. RESULTS

4.1 Correlations between placental histopathology, clinical signs of preeclampsia and neonatal outcome

The study population characteristics are summarized in Table 1. Altogether, 49 cases were analyzed.

Histological characteristics of EO-PE and LO-PE were compared (Table 2.); numerous features of PE were detected in both groups. Histopathological changes were more often diffuse than focal. Comparing the two groups, diffuse DVH ($p=0.01$), diffuse AVM ($p<0.01$), diffusely described medial hypertrophy of maternal decidual arteries ($p<0.01$), diffusely described perivasculitis ($p<0.01$), and large foci of avascular villi ($p<0.01$) were significantly more common in EO-PE, whereas LO-PE had significantly more frequent villous infarction ($p=0.04$) and chorionic plate or stem vessel thrombosis ($p=0.02$).

When comparing neonatal outcomes of EO-PE versus LO-PE, the former had worse prognosis: babies delivered from EO-PE pregnancies had lower Apgar scores, lower umbilical artery pH values, more commonly occurring umbilical artery lactate level of >3.75 mmol/L at birth, and all the stillbirth cases were from this group.

Analyzing PE with FGR versus PE without FGR, their histology showed several differences. We found a significant increase of diffuse DVH ($p<0.01$), diffuse AVM ($p<0.01$), diffuse perivasculitis ($p=0.02$), diffuse arterial thrombosis of maternal decidual vessels ($p<0.01$), diffuse chorionic plate or stem vessel thrombosis ($p<0.01$), large foci of avascular villi ($p<0.01$) and multifocal high grade VUE ($p=0.01$) in PE with FGR (in other words, a combination of diffuse MVM and FVM lesions are characteristic to PE with FGR). Surprisingly, from the aspects of neonatal outcomes, significant differences were only noted in the proportion of birth weight under the 2nd percentile for gestational age in the group of PE with FGR (7/22 vs. 1/27, $p=0.01$).

Meticulous examination of maternal decidual vessels (vascular lumen, vascular wall and perivascular space, see Table 2.) disclosed several histological changes in the studied placentae. Large number of histological alterations were found (37 cases of medial hypertrophy, 26 cases of fibrinoid necrosis of vessel wall, 21 cases of acute atherosis, 13 cases of perivasculitis and 10 cases of arterial thrombosis, respectively). Comparing the occurrence of these features in EO-PE versus LO-PE (Table 2.), medial hypertrophy and perivasculitis were significantly more prevalent in EO-PE. Perivasculitis was remarkably more common among recurring PE cases (7/8 in recurring cases of PE, 6/41 in non-recurring cases; $p<0.01$), but there were no differences in other vascular lesions.

Table 1. Maternal, neonatal and pregnancy-associated characteristics of the preeclamptic study population

Maternal characteristics		Neonatal characteristics		Pregnancy-associated characteristics	
Proportion of gravida aged >37 years (%)	16/49 (33)	Stillbirth (%)	4/49 (8)	Preeclampsia complicated with fetal growth restriction (%)	22/49 (45)
Primipara (%)	35/49 (71)	Delivery before 37th week of gestation (%)	27/49 (55)	Early-onset-preeclampsia (%)	20/49 (41)
Delivery via Cesarean section (%)	39/49 (80)	Neonatal death (%)	1/49 (2)	Maternal proteinuria 300-1000 mg/L/24h (%)	20/49 (41)
Maternal tobacco smoking (%)	20/49 (41)	Apgar 1 min median (mean ± SD)	8.54 ± 1.11	Maternal proteinuria 1001-2000 mg/L/24h (%)	19/49 (39)
Group B Streptococcus colonisation (%)	12/49 (24)	Apgar 5 min median (mean ± SD)	9.02 ± 0.64	Maternal proteinuria >2000 mg/L/24h (%)	10/49 (20)
Hepatitis B surface antigene positive (%)	1/49 (2)	Umbilical artery pH 7.01-7.2 at birth (%)	19/49 (39)	Preeclampsia treated with alpha-methyldopa only (%)	23/49 (47)
Venereal Disease Research Laboratory test for syphylis positive (%)	2/49 (4)	Umbilical artery pH <7.0 at birth (%)	5/49 (10)	Preeclampsia treated with alpha-methyldopa+nifedipine combination (%)	12/49 (24)
Hospitalization for at least one week before delivery (%)	31/49 (63)	Umbilical artery lactate level >3.75 mmol/L at birth (%)	22/49 (45)	Preeclampsia treated with alpha-methyldopa+nifedipine+beta-blocker combination	14/49 (29)
		Neonatal Intensive Centre admission (%)	36/49 (73)	Maternal serum albumin <33 g/L (%)	12/49 (24)
		Hypoxic-Ischemic Encephalopathy (%)	3/49 (6)	Placental weight <5th percentile (%)	9/49 (18)
		Fetal weight <2nd percentile at birth for gestational age (%)	8/49 (16)	Placental weight between 5th and 95th percentile (%)	32/49 (65)
				Placental weight >95th percentile (%)	8/49 (16)

Table 2. Histological features of early-onset-preeclampsia versus late-onset-preeclampsia

	Early-onset preeclampsia (n=20)	Late-onset preeclampsia (n=29)	p value
SIGNS OF MATERNAL VASCULAR MALPERFUSION OF THE PLACENTAL BED			
Infarction			
Focal	2/20	3/29	0.67
Diffuse	**6/20**	**17/29**	**0.04**
Distal villous hypoplasia			
Focal	2/20	2/29	0.54
Diffuse	**8/20**	**3/29**	**0.01**
Accelerated villous maturation (studied only before 36th week of gestation)			
Focal	4/20	3/29	0.29
Diffuse	**14/20**	**9/29**	**<0.01**
Decidual arteriopathy of maternal decidual vessels			
Acute atherosis			
Focal	1/20	0/29	0.41
Diffuse	9/20	11/29	0.42
Fibrinoid necrosis of vessel wall			
Focal	2/20	3/29	0.66
Diffuse	11/20	10/29	0.13
Medial hypertrophy			
Focal	2/20	1/29	0.36
Diffuse	**18/20**	**16/29**	**<0.01**
Perivasculitis			
Focal	1/20	2/29	0.64
Diffuse	**8/20**	**2/29**	**<0.01**
Arterial thrombosis			
Focal	1/20	0/29	0.41
Diffuse	4/20	5/29	0.55
SIGNS OF FETAL VASCULAR MALPERFUSION			
Chorionic plate or stem vessel thrombosis			
Focal	1/20	2/29	0.64
Diffuse	**3/20**	**14/29**	**0.02**
Avascular villi			
Small foci	1/20	1/29	0.65
Intermediate foci	2/20	1/29	0.36
Large foci	**13/20**	**4/29**	**<0.01**
Intramural fibrin deposition			
Focal	1/20	2/29	0.64
Diffuse	4/20	5/29	0.55
DELAYED VILLOUS MATURATION (STUDIED ONLY AFTER 36TH WEEK OF GESTATION)			
Focal	2/20	2/29	0.53
Diffuse	1/20	4/29	0.31
VILLITIS OF UNKNOWN ETIOLOGY (VUE)			
Focal/Patchy			
Low grade	1/20	0/29	0.40
High grade	1/20	0/29	0.40
Diffuse/Multifocal			
Low grade	2/20	1/29	0.36
High grade	5/20	3/29	0.16

Significant differences are highlighted in bold.

Comparing the rate of the studied vascular lesions in mothers with an increased RI of umbilical artery and those with physiologic RI of umbilical artery, we found a significant association between both medial hypertrophy of maternal decidual vessels (12/12 vs. 11/18, p=0.02) and acute atherosis (9/12 vs. 5/18, p=0.02) and an increased RI. We could also demonstrate that the presence of medial hypertrophy of maternal decidual vessels is significantly associated with the persistence of diastolic notch of the uterine artery compared to cases without it (9/9 vs. 9/18, p=0.01).

Of the 13 cases with VUE, 10 were concomitantly diagnosed with large foci of avascular villi; all cases had decidual arterial thrombosis simultaneously. Of these VUE-associated avascular villi cases, all were described in EO-PE, 8/10 were PE cases with FGR, and 7/10 were recurring PE cases, respectively. Therefore, the presence of VUE-associated avascular villi has a strong connection with EO-PE (p<0.01), PE with FGR (p=0.01) and recurring PE (p<0.01).

Of the histopathological phenomena investigated, only the presence of large foci of avascular villi was correlated with the degree of proteinuria (p<0.01). Stillbirth (p=0.01), HELLP-syndrome (Haemolysis, Elevated Liver enzymes, Low Platelet count syndrome, p<0.01) and eclampsia (p=0.03) were significantly more frequent in the group of patients with the highest proteinuria range.

There were no qualitative pathological features in placentae to show an association with antihypertensive medication of PE.

Maternal serum albumin below 33 g/L diagnosed in the last 168 hours of PE pregnancy showed no correlation with any of the studied placental histopathological features.

Splitting placental weight into three categories using percentiles for gestational age (<5th percentile, between 5th and 95th percentile, >95th percentile), we found that a remarkable portion of placentae (47%) were below the 5th percentile or beyond the 95th percentile calculated for the actual gestational age at the time of birth. In case of placental weight <5th percentile, diffuse villous infarction (p=0.01), diffuse DVH (p=0.01) and diffuse AVM (p=0.04) were significantly more common, whereas we found no histological changes associated with placental weight beyond the 95th percentile. However, we found no significant differences in the proportion of placental histological changes in the three groups of placental weight to birth weight ratios of <1:5, between 1:5 and 1:7, and >1:7.

There were significant differences in the PITV in case of hypertension requiring a three-drug combination of antihypertensive medications versus hypertension treated with one or two drugs (p=0.03), and stillbirth versus live birth (p=0.02). Surprisingly, we found no significant associations between PITV and birth weight below versus beyond the 2nd percentile, PE concurrence with FGR, placental weight or onset of PE.

The mean±SD CVR in the study population was 3.98±1.02 (range: 2.02-5.12). CVR was significantly lower in case of hypertension requiring a three-drug combination of antihypertensive medications (p=0.01), PE with FGR (p=0.03), and stillbirth versus live birth (p=0.02) (Table 3.). Surprisingly, we found no significant association of villous capillarization reflected by CVR and fetal weight below versus beyond the 2nd percentile, placental weight or onset of PE. There was a notable difference in CVR in cases of stillbirth versus live births. We found no significant differences of CVR in different subgroups of placental weight percentiles, or in the three groups of placental weight to birth weight ratios of <1:5, between 1:5 and 1:7, and >1:7, respectively.

Table 3. Capillary-villus ratio (CVR) in different types and subgroups of preeclampsia

Capillary-villus ratio (CVR)		p value
Early-onset-preeclampsia average 3.88	Late-onset-preeclampsia average 4.08	0.71
Preeclampsia with fetal growth restriction average 3.14	**Preeclampsia without fetal growth restriction average 4.84**	**0.03**
Proteinuria >2000 mg/L/24h average 3.86	Proteinuria ≤2000 mg/L/24h average 4.1	0.39
Preeclampsia treated with 3 antihypertensive medications average 3.08	**Preeclampsia treated with <3 antihypertensive medications average 4.9**	**0.01**
Placental weight <5th percentile average 3.71	Placental weight ≥5th percentile average 4.27	0.48
Placental weight ≤25th percentile average 3.92	Placental weight >25th percentile average 4.06	0.85
Maternal serum albumin <33 g/L average 3.86	Maternal serum albumin ≥33 g/L average 4.12	0.48
Maternal weight gain ≥20 kg average 3.66	Maternal weight gain <20 kg average 4.32	0.23
Maternal body mass index ≥28 average 3.59	Maternal nody mass index <28 average 4.39	0.68
Umbilical artery pathologic resistance index average 3.56	Umbilical artery normal resistance index average 4.42	0.55
Uterine artery persistence of early diastolic notch average 3.62	Uterine artery no early diastolic notch average 4.36	0.49
Fetal weight below 2nd percentile for gestational age average 3.50	Fetal weight beyond 2nd percentile for gestational age average 4.48	0.87
Stillbirth average 3.09	**Live birth average 4.89**	**0.02**

Significant differences are highlighted in bold.
CVR: capillary number of 500 villi/500

4.2 Correlations of placental histopathology, neonatal outcome, and cardiotocogram baseline variability and acceleration patterns in the growth restricted preterm population

Patient characteristics are summarized in Table 4. Birth weight below the 2nd percentile for gestational age and delivery via Cesarean section were common. Average time of cessation of CTG monitoring to delivery of the baby (time of transportation to operating suite and preparation for surgery) was 18.8 minutes in case of Cesarean sections.

A total of 50 cases were studied from the period between 2010 and 2020; FGR pregnancies are common in our centre, however, the described inclusion and restrictive exclusion criteria made possible the analysis of only 50 cases. 24 cases were EO-FGR, and 26 cases were LO-FGR.

Comparing the EO-FGR and LO-FGR subgroups in Table 4, Apgar scores at 1 and 5 minutes after birth, AUM pH, AUM lactate and gestational age at the time of delivery mean±SD values, we found no statistically significant differences between the two subgroups. There were also no statistically significant differences in birth weight below the 2nd percentile, hypoxic-ischemic encephalopathy, intraventricular hemorrhage and percentage of delivery by Cesarean section between the EO-FGR and LO-FGR subgroups.

As far as the CTG baseline variability patterns and neonatal parameters are concerned, we found that reduced baseline variability on CTG in the whole study population was significantly associated with 10-minute Apgar score <7 ($p=0.03$), AUM pH 7.01-7.2 ($p=0.02$), AUM pH <7.01 ($p<0.01$), AUM lactate ≥10 mmol/L ($p<0.01$), and birth weight below the 2nd percentile ($p<0.01$).

According to our findings, absence of accelerations was associated with 5-minute Apgar <7 (<0.01), AUM pH <7.01 ($p=0.04$) and AUM lactate level 3.75-4.99 mmol/L ($p=0.04$) in the EO-FGR subgroup; it was associated with 5-minute Apgar <7 ($p<0.01$) and birth weight below 2nd percentile ($p=0.02$) in the LO-FGR subgroup; in the whole study population it was significantly associated with 5-minute Apgar score below 7 ($p<0.01$), 10-minute Apgar score below 7 ($p=0.04$), AUM pH <7.01 ($p<0.01$), AUM lactate level between 3.75 and 4.99 mmol/L ($p<0.01$), and birth weight below the 2nd percentile ($p<0.01$), respectively.

We found no associations of increased baseline variability with any of the studied neonatological parameters.

Birth weight below 2nd percentile had an association with woeful perinatal outcome: average±SD 5-minute Apgar score was 6.3±2.1, AUM pH was 7.11±0.14, and AUM lactate level was 5.9±3.35 mmol/L in our study.

Table 4. Patient characteristics of the cardiotocogram study population

	Early-onset fetal growth restriction (n=24)	**Late-onset fetal growth restriction (n=26)**
5-minute Apgar score mean ± SD	7.5 ± 2.1	8.1 ± 1.6
10-minute Apgar score mean ± SD	8.1 ± 1.4	8.3 ± 0.8
Umbilical artery pH mean ± SD	7.19 ± 0.15	7.23 ± 0.14
Umbilical artery lactate mean ± SD (mmol/L)	5.1 ± 3.24	3.83 ± 2.62
Mean gestational age at the time of delivery (days ± SD)	217 ± 21	238 ± 15
Birth weight below the 2nd percentile	10/24 (0.42)	7/26 (0.27)
Hypoxic-ischemic encephalopathy	1/24 (0.04)	0/26 (0.00)
Intraventricular hemorrhage	2/24 (0.08)	1/26 (0.04)
Perinatal death	0/24 (0.00)	0/26 (0.00)
Delivery via Cesarean section	18/24 (0.75)	17/26 (0.65)

Numbers in parentheses represent proportions (1.00=100%).

As far as the associations of the studied placental histopathological changes and CTG baseline variability is concerned (Table 5), villous infarction, DVH, AVM, decidual arterial thrombosis, chorionic plate thrombosis, avascular villi, and VUE were significantly more common in the whole study population when baseline variability was reduced; however, there were differences in the distribution of significant associations between EO-FGR and LO-FGR.

Absence of accelerations was significantly associated with arterial thrombosis, avascular villi and chorangiosis in the EO-FGR subgroup; it was associated with DVH, avascular villi and chorangiosis in the LO-FGR subgroup; in the whole study population absence of accelerations was significantly associated with DVH, VUE and chorangiosis (Table 6).

The average PITV was 65.8% in the study population; the average CVR was 3.65. We found no significant differences in PITV and CVR between EO-FGR and LO-FGR. Quantitative analysis of the studied placentae revealed that in case of lower AUM pH (AUM pH ≤7.2 vs. >7.2, p=0.02; AUM pH <7.01 vs. ≥7.01, p=0.01), higher AUM lactate levels (AUM lactate <3.75 mmol/L vs. ≥3.75 mmol/L, p=0.02; AUM lactate <10 vs. ≥10 mmol/L, p<0.01), birth weight below vs. beyond 2nd percentile (p=0.01), and reduced vs. normal (p=0.02) baseline variability on CTG, the PITV was significantly lower (Table 7). When AUM pH was <7.01, and AUM lactate ≥10 mmol/L, PITV was characteristically lower than when the threshold of pH was ≤7.2 and the threshold of lactate was ≥3.75 mmol/L.

Capillarization of villi reflected by CVR also showed significant differences, being reduced with AUM pH <7.01 (p=0.02), AUM lactate ≥10 mmol/L (p=0.01), birth weight below 2nd percentile (p=0.01), absence of accelerations (p=0.03) and decreased baseline variability (p=0.02). Absence of accelerations was associated with a significantly lower CVR (but not with a lower PITV). We found no differences in this quantitative analysis between normal vs. increased baseline variability on CTG.

The multivariate logistic regression analysis revealed birth weight below the 2nd percentile, reduced baseline variability and absence of accelerations on CTG, the presence of at least two types of MVM and/or FVM placental changes, VUE, chorangiosis, PITV and CVR below the average values of the cohort as independent factors for poor perinatal outcome (defined as umbilical artery pH ≤7.2 and umbilical artery lactate ≥3.75 mmol/L after birth) in the whole study population (with a p value <0.05).

Table 5. Baseline variability of cardiotocogram (CTG) and placental histopathological changes

	Early-onset fetal growth restriction (n=24)				Late-onset fetal growth restriction (n=26)				All cases (n=50)			
CTG baseline variability	Normal (5-25 bpm)	Reduced or absent (<5 bpm)	Increased or saltatory (>25 bpm)	p value	Normal (5-25 bpm)	Reduced or absent (<5 bpm)	Increased or saltatory (>25 bpm)	p value	Normal (5-25 bpm)	Reduced or absent (<5 bpm)	Increased or saltatory (>25 bpm)	p value
Villous infarction	**1/10**	**7/8**	**2/6**	**<0.01**	1/9	6/12	3/5	0.11	**2/19**	**13/20**	**5/11**	**<0.01**
Distal villous hypoplasia	**0/10**	**3/8**	**0/6**	**0.04**	0/9	5/12	2/5	0.05	**0/19**	**8/20**	**2/11**	**<0.01**
Accelerated villous maturation	**3/10**	**7/8**	**4/6**	**0.04**	4/9	9/12	3/5	0.34	**7/19**	**16/20**	**7/11**	**0.02**
Medial hypertrophy of decidual arteries	3/10	4/8	4/6	0.39	5/9	5/12	4/5	0.40	8/19	9/20	8/11	0.28
Fibrinoid necrosis of vessel walls of decidual arteries	3/10	4/8	3/6	0.66	2/9	2/12	3/5	0.19	5/19	6/20	6/11	0.28
Perivasculitis of decidual arteries	3/10	1/8	3/6	0.38	3/9	3/12	2/5	0.87	6/19	4/20	5/11	0.32
Acute atherosis of decidual arteries	1/10	2/8	1/6	0.80	1/9	2/12	0/5	1.0	2/19	4/20	1/11	0.67
Arterial thrombosis of decidual arteries	**0/10**	**3/8**	**0/6**	**0.04**	0/9	3/12	0/5	0.27	**0/19**	**6/20**	**0/11**	**<0.01**
Chorionic plate thrombosis	**0/10**	**5/8**	**1/6**	**<0.01**	**0/9**	**8/12**	**0/5**	**<0.01**	**0/19**	**13/20**	**1/11**	**<0.01**
Avascular villi	**4/10**	**7/8**	**6/6**	**0.02**	**2/9**	**10/12**	**5/5**	**<0.01**	**6/19**	**17/20**	**11/11**	**<0.01**
Fibrin deposition	1/10	3/8	3/6	0.20	2/9	5/12	2/5	0.66	3/19	8/20	5/11	0.14
VUE	**1/10**	**7/8**	**2/6**	**<0.01**	**0/9**	**7/12**	**1/5**	**<0.01**	**1/19**	**14/20**	**3/11**	**<0.01**
Chorangiosis	**1/10**	**7/8**	**2/6**	**<0.01**	**0/9**	**8/12**	**2/5**	**<0.01**	**1/19**	**15/20**	**4/11**	**<0.01**

Significant differences are highlighted in bold.

Table 6. Absence of accelerations on cardiotocogram (CTG) and placental histopathological changes

	Early-onset fetal growth restriction (n=24)			Late-onset fetal growth restriction (n=26)			All cases (n=50)		
Accelerations on cardiotocogram	Absent	Present	p value	Absent	Present	p value	Absent	Present	p value
Villous infarction	6/12	4/12	0.34	6/12	4/14	0.24	12/24	8/26	0.14
Distal villous hypoplasia	2/12	1/12	0.5	**6/12**	**1/14**	**0.02**	**8/24**	**2/26**	**0.02**
Accelerated villous maturation	8/12	6/12	0.34	8/12	8/14	0.46	16/24	14/26	0.26
Medial hypertrophy of decidual arteries	6/12	5/12	0.5	8/12	6/14	0.21	14/24	11/26	0.2
Fibrinoid necrosis of vessel walls of decidual arteries	5/12	5/12	0.66	4/12	3/14	0.4	9/24	8/26	0.42
Perivasculitis of decidual arteries	4/12	3/12	0.5	5/12	3/14	0.25	9/24	6/26	0.21
Acute atherosis of decidual arteries	3/12	1/12	0.29	2/12	1/14	0.44	5/24	2/26	0.18
Arterial thrombosis of decidual arteries	**3/12**	**0/12**	**0.02**	2/12	1/14	0.44	5/24	1/26	0.08
Chorionic plate thrombosis	4/12	2/12	0.32	5/12	3/14	0.25	9/24	5/26	0.13
Avascular villi	**12/12**	**5/12**	**<0.01**	**12/12**	**5/14**	**<0.01**	**24/24**	**10/26**	**<0.01**
Fibrin deposition	4/12	3/12	0.5	5/12	4/14	0.39	9/24	7/26	0.31
VUE	7/12	3/12	0.11	6/12	2/14	0.06	**13/24**	**5/26**	**0.01**
Chorangiosis	**8/12**	**2/12**	**0.01**	**9/12**	**1/14**	**<0.01**	**17/24**	**3/26**	**<0.01**

Significant differences are highlighted in bold.

Table 7. Percentage of intact terminal villi (PITV) and capillary-villus ratio (CVR) in different subgroups of the studied cases

		p value
Percentage of intact terminal villi (PITV, average %)		
Early-onset fetal growth restriction: 66.4%	Late-onset fetal growth restriction: 65.2%	0.56
5-minute-Apgar score <7: 64.2%	5-minute-Apgar score ≥7: 67.4%	0.45
10-minute-Apgar score <7: 62.5%	10-minute-Apgar score ≥7: 69.1%	0.33
Umbilical artery pH ≤7.2: 56.4%	Umbilical artery pH >7.2: 75.2%	**0.02**
Umbilical artery pH <7.01 : 52.5%	Umbilical artery pH ≥7.01: 79.1%	**0.01**
Umbilical artery lactate <3.75 mmol/L: 58.7%	Umbilical artery lactate ≥3.75 mmol/L: 72.9%	**0.02**
Umbilical artery lactate <10 mmol/L: 48.2%	Umbilical artery lactate ≥10 mmol/L: 83.4%	**<0.01**
Birth weight below 2nd percentile: 53.7%	Birth weight beyond 2nd percentile: 77.9%	**0.01**
Absence of accelerations on CTG: 62.5%	Presence of accelerations on CTG: 69.1%	0.48
Reduced/absent baseline variability on CTG: 54.1%	Normal baseline variability on CTG: 77.5%	**0.02**
Increased/saltatory baseline variability on CTG: 64.9%	Normal baseline variability on CTG: 77.5%	0.65
Reduced/absent baseline variability on CTG: 54.1%	Increased/saltatory baseline variability on CTG: 64.9%	0.55
Capillary-villus ratio (CVR):		
Early-onset fetal growth restriction: 3.83	Late-onset fetal growth restriction: 3.49	0.32
5-minute-Apgar score <7: 3.56	5-minute-Apgar score ≥7: 3.74	0.67
10-minute-Apgar score <7: 3.22	10-minute-Apgar score ≥7: 4.08	0.08
Umbilical artery pH ≤7.2: 3.15	Umbilical artery pH >7.2: 4.15	0.06
Umbilical artery pH <7.01 : 2.89	Umbilical artery pH ≥7.01: 4.41	**0.02**
Umbilical artery lactate <3.75 mmol/L: 3.34	Umbilical artery lactate ≥3.75 mmol/L: 3.96	0.18
Umbilical artery lactate <10 mmol/L: 2.85	Umbilical artery lactate ≥10 mmol/L: 4.45	**0.01**
Birth weight below 2nd percentile: 2.84	Birth weight beyond 2nd percentile: 4.46	**0.01**
Absence of accelerations on CTG: 3.03	Presence of accelerations on CTG: 4.27	**0.03**
Reduced/absent baseline variability on CTG: 2.99	Normal baseline variability on CTG: 4.31	**0.02**
Increased/saltatory baseline variability on CTG: 3.49	Normal baseline variability on CTG: 4.31	0.46
Reduced/absent baseline variability on CTG: 2.99	Increased/saltatory baseline variability on CTG: 3.49	0.68

Significant differences are highlighted in bold.

4.3 Placental pathology and its associations with clinical signs in different subtypes of fetal growth restriction

A total of 61 placentae from pregnancies complicated with FGR were studied. Table 8 describes patient characteristics. There was no perinatal death in the series.

Numerous histological alterations were identified in all compared groups, and they were predominantly diffuse, implying extensive involvement of the placenta.

Comparative histopathological analysis of EO-FGR versus LO-FGR (Table 9) revealed that diffuse DVH, AVM, fibrinoid necrosis and medial hypertrophy of decidual vessel walls, large foci of avascular villi and multifocal high grade VUE were significantly more frequent in EO-FGR. None of the pathological alterations studied occurred more frequently in the LO-FGR group.

Classification of the cases was based on gestational age at the time of diagnosis of FGR; 28.6% of EO-FGR (n=8) cases and 51.6% of the LO-FGR (n=17) cases were delivered after the 37th week of pregnancy.

Since half of LO-FGR cases were delivered after the 37th week of pregnancy, we subdivided the LO-FGR cohort into a term delivery and preterm delivery subset, and we found that there were markedly fewer placental histopathological changes in the term delivery subgroup (we have found any histopathological changes in 15/16 of the LO-FGR cases delivered preterm, and 7/17 of the LO-FGR cases delivered in term, p=0.003) (Table 10).

With regard to neonatal outcome, the 5-minute-Apgar score <7 (10/28 vs. 5/33, p=0.01), umbilical artery pH below 7.2 at birth (15/28 vs. 8/33, p=0.02), umbilical artery lactate >3.75 mmol/L at birth (12/28 vs. 5/33, p=0.02) and neonatal birth weight under the 2nd percentile (21/28 vs. 7/33, p=0.01) were significantly more common in the EO-FGR group.

Recurring FGR (12/28 vs. 6/33, p=0.03) and complication of FGR with PE (15/28 vs. 8/33, p=0.02) were more common in case of EO-FGR than in LO-FGR. There was no association between maternal smoking and onset of FGR in this population.

There were 18 recurring FGR cases. Diffuse high grade VUE was significantly more common among recurring FGR cases than non-recurring ones (9/18 vs. 8/43, p=0.01). There were no differences in neonatal outcomes between recurring and non-recurring FGR. Twelve of the 18 recurring cases of FGR were of EO. Among these recurring FGR cases, one patient had a pregnancy loss in the second trimester of her previous pregnancy (that was her first pregnancy terminating as a missed abortion in the 19th week of gestation; unfortunately, histopathological evaluation of placenta was not available because it happened in another institute). There was no previous third trimester pregnancy loss in the cohort.

Table 8. Maternal, pregnancy-associated and neonatal characteristics of the fetal growth restriction study population

Maternal and pregnancy-associated characteristics	
Mean maternal age	26.1 years (15 – 43)
Gravida age under 18 years	7/61 (0.11)
Gravida age beyond 37 years	13/61 (0.21)
Primiparity	33/61 (0.54)
Delivery before 37th week of gestation (%)	36/61 (0.59)
Delivery via Caesarean section (%)	52/61 (0.85)
Maternal tobacco smoking (%)	20/61 (0.33)
HBsAg positive (%)	0/61 (0.00)
VDRL positive (%)	1/61 (0.01)
Intrauterine growth restriction complicated with preeclampsia	22/61 (0.36)
Recurring fetal growth restriction	18/61 (0.29)
Hospitalization of gravida for at least one week before childbirth (%)	49/61 (0.80)
Neonatal characteristics	
Apgar 1 min mean ± SD	7.43 ± 1.15
Apgar 5 min mean ± SD	8.36 ± 0.75
Umbilical artery pH ≤7.0 at birth (%)	3/61 (0.05)
Umbilical artery pH 7.01-7.2 at birth (%)	20/61 (0.32)
Umbilical artery blood lactate >3.75 mmol/L at birth	17/61 (0.11)
Perinatal death (%)	0/61 (0.00)

HBsAg: Hepatitis-B surface antigen serology; VDRL: Veneral Disease Research Laboratory test for Treponema pallidum (syphilis); numbers in parentheses represent proportions (1.00=100%).

Table 9. Early-onset versus late-onset fetal growth restriction histological findings

	Early-onset fetal growth restriction (n=28)	Late-onset fetal growth restriction (n=33)	p value
SIGNS OF MATERNAL VASCULAR MALPERFUSION OF THE PLACENTAL BED			
Infarction			
Focal	3/28	4/33	0.59
Diffuse	14/28	16/33	0.56
Distal villous hypoplasia			
Focal	1/28	1/33	0.71
Diffuse	**11/28**	**5/33**	**0.03**
Accelerated villous maturation (studied only before 36th week of gestation)			
Focal	1/28	3/33	0.37
Diffuse	**19/28**	**8/33**	**<0.01**
Decidual arteriopathy			
Acute atherosis			
Focal	0/28	0/33	1.0
Diffuse	3/28	3/33	0.58
Fibrinoid necrosis of vessel wall			
Focal	1/28	3/33	0.37
Diffuse	**11/28**	**5/33**	**0.03**
Medial hypertrophy			
Focal	1/28	1/33	0.71
Diffuse	**20/28**	**8/33**	**<0.01**
Perivasculitis			
Focal	1/28	1/33	0.71
Diffuse	5/28	6/33	0.62
Arterial thrombosis			
Focal	1/28	0/33	0.46
Diffuse	10/28	9/33	0.33
SIGNS OF FETAL VASCULAR MALPERFUSION			
Chorionic plate or stem vessel thrombosis			
Focal	1/28	1/33	0.71
Diffuse	4/28	10/33	0.12
Avascular villi			
Small foci	0/28	1/33	0.54
Intermediate foci	1/28	0/33	0.46
Large foci	**24/28**	**13/33**	**<0.01**
Intramural fibrin deposition			
Focal	2/28	2/33	0.63
Diffuse	10/28	10/33	0.43
DELAYED VILLOUS MATURATION (STUDIED ONLY AFTER 36TH WEEK OF GESTATION)			
Focal	1/28	1/33	0.71
Diffuse	2/28	1/33	0.44
VILLITIS OF UNKNOWN ETIOLOGY (VUE)			
Focal/Patchy			
Low grade	1/28	1/33	0.71
High grade	1/28	1/33	0.71
Diffuse/Multifocal			
Low grade	1/28	1/33	0.71
High grade	**13/28**	**4/33**	**<0.01**

Significant differences are highlighted in bold.

Table 10. Placental histopathological changes in the late-onset fetal growth restriction cohort by gestational age at the time of delivery (preterm versus term delivery)

	Late-onset fetal growth restriction cases preterm delivery (<37 weeks) (n=16)	Late-onset fetal growth restriction cases term delivery (≥37 weeks) (n=17)
SIGNS OF MATERNAL VASCULAR MALPERFUSION OF THE PLACENTAL BED		
Infarction		
Focal	3/16	1/17
Diffuse	11/16	5/17
Distal villous hypoplasia		
Focal	1/16	0/17
Diffuse	3/16	2/17
Accelerated villous maturation (studied only before 36th week of gestation)		
Focal	3/16	0/17
Diffuse	8/16	0/17
Decidual arteriopathy		
Acute atherosis		
Focal	0/16	0/17
Diffuse	2/16	1/17
Fibrinoid necrosis of vessel wall		
Focal	2/16	1/17
Diffuse	3/16	2/17
Medial hypertrophy		
Focal	1/16	0/17
Diffuse	5/16	3/17
Perivasculitis		
Focal	1/16	0/17
Diffuse	4/16	2/17
Arterial thrombosis		
Focal	0/16	0/17
Diffuse	6/16	3/17
SIGNS OF FETAL VASCULAR MALPERFUSION		
Chorionic plate or stem vessel thrombosis		
Focal	1/16	0/17
Diffuse	6/16	4/17
Avascular villi		
Small foci	1/16	0/17
Intermediate foci	0/16	0/17
Large foci	9/16	4/17
Intramural fibrin deposition		
Focal	1/16	1/17
Diffuse	8/16	2/17
DELAYED VILLOUS MATURATION (STUDIED ONLY AFTER 36TH WEEK OF GESTATION)		
Focal	0/16	1/17
Diffuse	0/16	1/17
VILLITIS OF UNKNOWN ETIOLOGY (VUE)		
Focal/Patchy		
Low grade	1/16	0/17
High grade	1/16	0/17
Diffuse/Multifocal		
Low grade	1/16	0/17
High grade	3/16	1/17

Cases with AFI below 2 cm were more commonly associated with decidual arterial thrombosis (9/17 vs. 11/44, p=0.03), chorionic villous infarction (14/17 vs. 23/44, p=0.03), AVM (15/17 vs. 16/44, p<0.01), DVH (10/17 vs. 8/44, p=0.01) and avascular villi (15/17 vs. 24/44, p=0.01) than cases with AFI beyond 2 cm. When birth weight was below the 2nd percentile for gestational age, AVM (16/19 vs. 15/42, p<0.01), chorionic villous infarction (18/19 vs. 19/42, p<0.01), chorangiosis (15/19 vs. 16/42, p<0.01) and avascular villi (16/19 vs. 23/42, p=0.02) were significantly more common.

There were no significant differences in histological placental alterations according to maternal BMI.

Doppler flowmetry on AUM 0-48 hours before delivery was performed in 52 cases (normal AUM RI in 34, increased AUM RI in 11, AUM AEDF/REDF in 7cases). Medial hypertrophy of decidual vessels (10/11 vs. 18/34, p=0.02), AVM (11/11 vs. 20/34, p<0.01) and DVH (11/11 vs. 3/34, p<0.01) were more common in the background of increased AUM RI compared to the normal AUM RI group. The same histological alterations plus avascular villi (7/7 vs. 18/34, p=0.03) were found more commonly in cases of AEDF or REDF on AUM, of which the prognosis is much worse. Pathological MCA Doppler indices were associated with the same alterations.

In this cohort there were regular uterine contractions in 39 cases (all of the other cases were Cesarean sections without uterine contractions - pathologic Doppler indices and/or pathologic CTG signs were the indications of Cesarean delivery). Of these, 30/39 (76.9%) had a Cesarean section; distress signs (any form of decelerations during labor, fetal bradycardia and/or decreased/absent baseline variability) on the cardiotocogram of cases with regular contractions were common (29/39 cases, 74.4%).

Clinocopathological study of the possible connections of FL/AC ratio and perinatal outcomes was also performed; we divided the study population by FL/AC <0.25 and ≥0.25 (we considered FL/AC ratio high above 0.25, and very high above 0.26). Fetal weight estimated by ultrasound at the time of diagnosis was below the 2nd percentile in all cases of EO-FGR with FL/AC ratio >0.26, and all of these cases had pathologic AUM Doppler indices, suggesting poor prognosis. All cases had distress signs on CTG in the LO-FGR subgroup with FL/AC ratio >0.26. In general, all of the fetuses in our FGR cohort with FL/AC ratio >0.26 were born within 72 hours after the diagnosis, because pathological CTG and/or pathologic Doppler indices made the delivery necessary. In the background of the highest FL/AC ratio group among EO-FGR cases, infarction, DVH, and decidual arterial thrombosis were significantly more common than for lower FL/AC values, while in LO-FGR the highest FL/AC cases were associated with chorionic villous infarction and avascular villi (Table 11).

On average, 62% of terminal villi were intact. As stillbirths were excluded, no comparisons of PITV could be made with live births, but fetuses were born alive even when the PITV was as low as 20-30%. We found significantly fewer intact villi in placentae from pregnancies with pathologic AUM Doppler flowmetry versus normal flowmetry (45% versus 69%, p=0.01). In contrast, there were no significant differences in the PITV in case of placental weight below 2nd percentile (versus above 2nd percentile), maternal smoking (versus its lack), fetal weight below 2nd percentile (versus greater), FGR complicated with PE (versus those without PE) or FGR subtypes by onset.

The mean±SD CVR in the study population was 3.48±0.88 (1.02-5.42). CVR was significantly lower in case of EO-FGR (p=0.01), PE with FGR (p=0.02), birth weight below the 2nd percentile (p=0.01), and pregnancies with pathologic AUM Doppler flowmetry versus normal flowmetry (p=0.04). Surprisingly, we found no significant differences of CVR in different subgroups of placental weight percentiles, or in the three groups of placental weight to birth weight ratios of <1:5, between 1:5 and 1:7, and >1:7, respectively.

Table 11. Femoral length/abdominal circumference (FL/AC) ratio and histology of placenta

	Early-onset FGR			**Late-onset FGR**		
	FL/AC ratio <0.25 at the time of diagnosis (n=14)	FL/AC ratio ≥0.25 at the time of diagnosis (n=14)	p value	FL/AC ratio <0.25 at the time of diagnosis (n=18)	FL/AC ratio ≥0.25 at the time of diagnosis (n=15)	p value
SIGNS OF MATERNAL VASCULAR MALPERFUSION OF THE PLACENTAL BED						
Infarction	**4/14**	**13/14**	**<0.001**	**5/18**	**15/15**	**<0.001**
Distal villous hypoplasia	**2/14**	**10/14**	**0.003**	2/18	4/15	0.24
Accelerated villous maturation	11/14	9/14	0.34	4/18	7/15	0.13
Decidual arteriopathies						
Fibrinoid necrosis of vessel walls	6/14	6/14	0.65	5/18	3/15	0.46
Acute atherosis	1/14	2/14	0.5	1/18	2/15	0.43
Medial hypertrophy	11/14	10/14	0.5	5/18	4/15	0.63
Perivasculitis	3/14	3/14	0.68	4/18	3/15	0.61
Arterial thrombosis	**2/14**	**9/14**	**0.01**	3/18	6/15	0.13
SIGNS OF FETAL VASCULAR MALPERFUSION						
Chorionic plate/stem vessel thrombosis	3/14	2/14	0.5	6/18	5/15	0.65
Avascular villi	11/14	14/14	0.11	**2/18**	**12/15**	**<0.001**
Intramural fibrin deposition	6/14	6/14	0.65	7/18	5/15	0.51
DELAYED VILLOUS MATURATION (STUDIED ONLY AFTER 36TH WEEK OF GESTATION)						
	2/14	1/14	0.5	2/18	0/15	0.29
Villitis of unknown etiology (VUE)						
Focal/Patchy	1/14	1/14	0.76	2/18	0/15	0.29
Diffuse/Multifocal	6/14	8/14	0.35	3/18	2/15	0.59

FGR: fetal growth restriction, FL/AC ratio: femoral length/abdominal circumference ratio; **Significant differences are highlighted in bold.**

5. DISCUSSION

5.1 Correlations between placental histopathology, clinical signs of preeclampsia and neonatal outcome

Our goal was to correlate histology with subtypes of PE and clinical findings. We were looking for connections between placental histopathology and objective clinical signs of PE. For this, placental sections from pregnancies complicated with PE were examined by the same person to exclude interobserver variability, and histological alterations were collected in a uniform manner for each placenta.

Involvement of the placental vasculature, terminal villi, intervillous and perivillous space in PE is obvious; many previous studies have described that reduced placental perfusion precedes clinical signs of PE [57] – this is the two-stage-model of PE. In the first stage, placental malperfusion and chronic hypoxia occur, and maternal systemic signs of PE manifest in the second stage of the disease, depending on maternal responsiveness, maternal and even paternal genetic and environmental factors, making this second stage widely different in severity [58]. The clinical diagnosis of PE is made exclusively in the second stage, in presence of the clinical signs and symptoms of the disease. Consequently, PE cannot be diagnosed in its first stage when placental involvement is already present without systemic maternal signs. Thus, placental involvement is inevitable in PE; but the association between severity of PE and placental histology are less characterized.

The two onset-dependent subtypes of PE have many similarities at the microscopic level, but distinguishing histological features seem to point out to the fact that EO-PE and LO-PE are two distinct entities (Table 2.). Our findings regarding histological differences of EO-PE versus LO-PE are similar to several earlier observations [59].

The exact pathogenesis of PE is not completely understood, but several mechanisms leading to PE have been identified. In EO-PE, incomplete trophoblast invasion and inadequate remodelling of maternal spiral arteries are the main pathogenetic elements [60], whereas LO-PE is considered to be induced by placental maturation: i.e., placental hypoxia is caused by altered blood flow in the placenta, and specific maternal factors contribute to systemic signs and symptoms of PE [61]. In other words, in EO-PE, placentation and placental vasculogenesis is *ab ovo* altered, while in LO-PE placentation in itself is physiologic.

In our wide-spectrum (gestational ages from 24th to 40th week) study population, numerous histopathologic alterations were identified in PE placentae, but the two entities of EO-PE and LO-PE were different in some respects: EO-PE cases were characterised by diffuse DVH, AVM, certain decidual arteriopathies (i.e. signs of MVM), and large foci of avascular villi, while LO-PE cases

had significantly increased infarcted areas and chorionic plate/stem vessel thrombosis compared to EO-PE. DVH and avascular villi – accurately differentiated from infarcted areas – could be an evidence of pathological placentation in EO-PE, while stem vessel thrombosis in the background of LO-PE could be considered as consequence of different forms of umbilical cord pathology, and as a placental change associated with fetal cardiac failure, anaemia, or fetal hypercoaguability [62]: in our study population, the presence of these entities could be found in the background of all of the LO-PE cases (22 cases had thrombosis in the umbilical cord vessels, five cases had fetal cardiac failure, and two cases had fetal anemia; none of these backgrounds were found in the EO-PE group).

Our observations and conclusions coincide with results of the latest review about PE [63]: inadequate placentation and poor villous development are characteristic to EO-PE, while in LO-PE microscopic placental changes altering placental blood flow in term or near-term are the representative entities; this placental milieu leads to reduced uteroplacental blood supply and uteroplacental mismatch, and consecutive syncytiotrophoblast stress-derived factors and angiogenic imbalance lead to maternal systemic endothelial dysregulation and inflammation.

The above described distinctive histopathological features of EO- versus LO-PE, however, were not exclusively found in only one subgroup of PE; a few cases demonstrated the features of both EO and LO forms of the disease, at least at the microscopic level of placentae. Specifically, PE manifesting between the 33th and 35th weeks of gestation could be characterized by overlapping attributes of EO-PE and LO-PE, pointing to a possibly mixed etiology or the arbitrary separation of EO-PE and LO-PE at the 34th week gestational age. Furthermore, the concurrence of VUE and avascular villi is another remarkable issue in the background of EO-PE with FGR and a characteristic predisposition for recurrence; average CVR in placentae with VUE-associated avascular villi was as low as 2.55 (mean±SD 2.55±0.42, 2.02 – 2.87), suggesting a negative prognostic significance to the concurrence of VUE and avascular villi.

As far as CVR (our indicator of capillarization of villi) is concerned, we consider it a proper index of quantitative placental histological examination at the time of delivery; the absence of significant differences of CVR in different subgroups of placental weight percentiles, or in the three groups of placental weight to birth weight ratios makes it an ideal, weight-independent marker of capillarization. Although, CVR gives us information only after the time of delivery, and it does not reflect timing and dynamics of placental damage.

Examining decidual arteriopathies, medial hypertrophy and perivasculitis of maternal decidual vessels seemed to be more characteristic to EO-PE (Table 2.). Medial hypertrophy of maternal decidual vessels also had connection with increased RI of umbilical artery and persistence of uterine artery diastolic notch, reflecting its impact on placental hemodynamics. These

associations of MVM lesions with abnormal fetoplacental Doppler findings (such as increased RI of umbilical artery and persistence of diastolic notch on uterine artery) are congruent with the observations of Paules et al. [64], but in contrast to their results, we found these associations statistically significant in PE independently from the concurrence of FGR.

Acute atherosis of vessel wall was particularly prevalent in our study population compared to previous studies [65]. Acute atherosis and fibrinoid necrosis of vessel walls showed no connection with onset of PE: these phenomena could be secondary changes due to altered placental perfusion.

The presence of medial hypertrophy was significantly associated with EO-PE, but was also found in a significant portion of LO-PE cases. If medial hypertrophy of maternal decidual vessels were a characteristic sign of LO-PE, it could be speculated that at least a proportion of LO-PE cases might carry the histological characteristics of EO-PE, indicating that these could be primarily ’asymptomatic’ EO-PE cases which after additional phenomena affecting placental tissue (e.g. multiple villous infarctions), would manifest as LO-PE at a certain point of gestation.

Our results furthermore describe the possible connection between perivasculitis and recurrent character of PE, suggesting a presumable immune-mediated mechanism; fibrinoid necrosis also could be concerned as a change with immunological background [66].

The combination of PE with FGR was not rare. These combined PE and FGR cases had significant differences in the prevalence of histopathological alterations when compared to PE cases without FGR; these alterations were peculiar histological characteristics of EO-PE, especially the diffuse distribution of DVH and AVM (Figure 2.). Our finding of combined MVM and FVM lesions in PE with FGR is corroborated by the previously described results and findings of Kovo et al [52]; histopathological changes involving both fetal and maternal placental vasculature lead to restricted growth potential of fetus with all of its consequences. This is reflected in the significantly more common birth weight below the 2nd percentile in PE with FGR, although we did not find significant differences in Apgar scores, umbilical artery pH and lactate levels in PE with and without FGR, in contrast to the findings of Kovo et al [67, 68].

Multifocal high grade VUE – diagnosed after exclusion of infectious etiology – was also associated with the concurrence of PE with FGR: chronic destructive inflammation of the placenta could be the additional mechanism leading to fetal malnutrition in such cases. Perivasculitis of decidual vessels in the background of recurring PE cases found in the multiparous population of our study further anticipates the role of immune-mediated mechanism in PE; additional investigation is needed to elucidate the role of VUE and perivasculitis in the pathogenesis of PE, especially recurring PE. It is also important to note that 12 of the 13 cases with perivasculitis also had VUE.

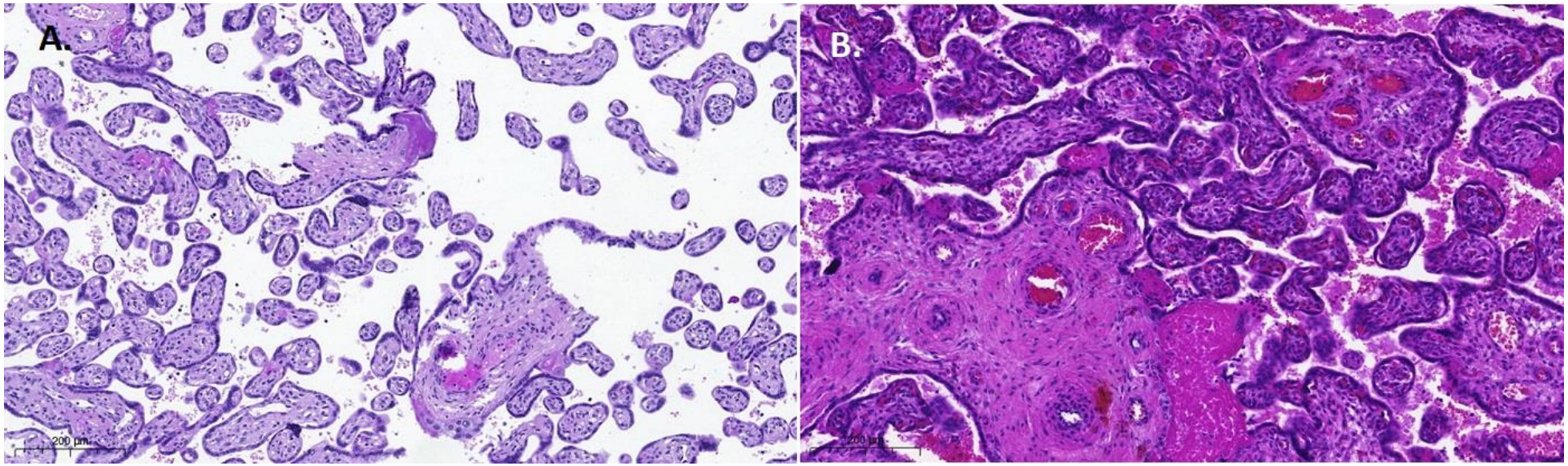

Figure 2.

Placental histopathology in cases of early-onset (EO-PE) and late-onset preeclampsia (LO-PE)

Description:

2A: Placental histopathology of an EO-PE case complicated with severe fetal growth restriction (FGR) (x10, HE staining): accelerated villous maturation (AVM), expanded intervillous space with villus paucity (gestational age was 31^{+4} weeks at the time of delivery).

2B: Placental histopathology of a LO-PE case (x20, HE staining): agglutinated villi with syncytial knots and intervillous fibrin deposition – this finding could be normal histology of term placenta, accentuating the fact that histopathological findings of LO-PE are often common entities of normal term placental histology. This case was not complicated with FGR. Gestational age was 38^{+1} weeks at the time of delivery.

The previously described higher rates of signs of severe placental MVM and FVM signs in recurring PE [69] and their association with or possible cause being VUE and perivasculitis require further investigations.

EO-PE had significantly worse neonatal outcome from many aspects; this is not surprising in conjunction with the high rate of pregnancies terminated preterm. On the other hand, the only neonatal difference between PE with and without FGR – irrespectively of onset of PE – was birth weight below the 2nd percentile. Presumably, histopathological changes in the background of PE with FGR lead to a placental milieu resulting in more severe fetal malnutrition. Nevertheless, in case of fetal weight below the 2nd percentile, the distinctness of placental lesions in the background are qualitative, but not quantitative: the calculated CVR revealed no significant difference between the two groups.

Classifying proteinuria results into three groups, we found that only large foci of avascular villi were significantly increased in the >2000 mg/L/24h proteinuria group. In the light of this, large foci of avascular villi could indicate an advanced stage of the disease. However, we found no connection between higher proteinuria and more severe MVM lesions in contrast to an earlier report suggestive of such an association [70]. The connection of avascular villi with proteinuria is an interesting aspect of PE: as avascular villi were found to be characteristic of EO-PE and could be an evidence of pathologic placentation or – at least in a proportion of cases – an accompanying entity of VUE; and besides, avascular villi could be associated with prolonged MVM as Genest et al. described [70].

We hypothesised that the number of medicines needed to treat hypertension in PE could be used as a marker of hypertension severity. In case of antihypertensive treatment with three medicines, CVR was significantly lower than in case of one or two medicines used for blood pressure control (Table 3.).

The results, therefore, suggest that in case of preeclamptic placentae, not only the quality of histolopathologic changes, but also the reduced capillarization of villi has a role in pregnancy outcome and clinical signs.

Trying to answer our own question, the placenta is not the one and only ’troublemaker’ in PE, but it is also not an ‘innocent bystander’: it is the two at the same time, because several histopathological changes of the placenta show significant association with adverse pregnancy outcomes.

The main limitation of our study was the small sample of studied placentae, however, the application of the terminology described by the Amsterdam Placental Workshop Group Consensus Statement, the study of clinicopathological associations in the field of placentology and perinatology, the high number of separately investigated histological changes and the distinction

between focal and diffuse lesions allowed a more detailed study of the difference between PE subtypes.

5.2 Correlations of placental histopathology, neonatal outcome, and cardiotocogram baseline variability and acceleration patterns in the growth restricted preterm population

Adequate CTG evaluation of a preterm fetus is a problematic issue in perinatology, as the lack of guidelines and evidence-based recommendations make it complicated [71]. Baseline fetal heart rate in preterm fetuses is often tachycardiac (>160 bpm) [22] and decelerations – especially in the early preterm (24-28 weeks) population – can be found more often and could be normal compared to term fetuses [72].

In our study, baseline variability pattern and absence of accelerations were studied to determine the reassuring or non-reassuring nature of cardiotocograms in the light of neonatal parameters after birth, with the concurrent study of the corresponding placental histopathological changes. We found that reduced baseline variability is strongly associated with bad perinatal outcome.

Absence of accelerations and reduced baseline variability proved to be good markers of poor perinatal outcome. Reduced baseline variability in combination with lack of accelerations for 60 minutes was associated with AUM pH ≤7.2 and AUM blood lactate level ≥3.75 mmol/L in each case, strenghtening the linkage of these patterns with bad prognosis. Our findings suggest that absence of accelerations could have relevance in predicting fetal acidemia, in contrast to prior findings in the near-term/term pregnant population [73]. In short, as far as CTG evaluation is considered in preterm cases, baseline variability and lack of accelerations could be useful markers of altered intrauterine fetal wellbeing and these parameters could help the assessment of fetal state, especially in a scenario when other diagnostic possibilities (e.g. ultrasound with Doppler flowmetry, fetal blood sampling) are not available, as in this series. At the same time, these results also confirm that adequate CTG evaluation and timely diagnosis could prevent serious hypoxia-related neonatal conditions such as hypoxic-ischemic encephalopathy and perinatal death, also in the growth restricted preterm population.

According to our findings, reduced baseline variability and absence of accelerations diagnosed on cardiotocograms of growth restricted preterm fetuses signal potential fetal distress reflected by postnatal indicators such as lower Apgar scores, lower AUM pH and higher lactate levels. The presence of significantly more common placental histopathological alterations in the background of cases with pathologic CTG signs further supports this conclusion. The multivariate regression further confirmed the correlations of placental histopathological changes, villous undercapillarization and poor perinatal outcomes.

Although timing, onset and dynamics of placental changes have the key role in the pathomechanism of FGR, we assumed that the study of PITV and CVR in our cohort could be interesting, as the studied placental slides representated placental histology at the static time of delivery – at the point when FGR has already been established as a complication of pregnancy; as no placentae from normal pregnancies were studied for given gestational ages, no normal ranges were available for these parameters.

The lack of any forms of decelerations and fetal bradycardia or tachycardia in the study made it possible to focus on the connection of baseline variability and acceleration patterns with perinatal outcomes.

Our results suggest, that in case of growth restricted preterm fetuses, in the presence of regular uterine contractions, reduced baseline variability diagnosed using the FIGO CTG evaluation guidelines and/or absence of accelerations on CTG could be interpreted as distress signs reflecting potential placental insufficiency and altered placental function. We consider it important to note, that in this study scenario, the role of umbilical cord compression and/or cord anomalies were minimalized (at least on a theoretical basis), as it is indicated in the exclusion criteria; the absence of any forms of decelerations on cardiotocograms further supports this.

The common finding of infarction, DVH, AVM, chorionic plate thrombosis and avascular villi in placental histopathology accent an altered placental milieu in the background of pathologic CTG findings (and FGR itself) and poor neonatal outcome. This is reasonable, as the placenta is the metabolic interface between mother and fetus. The role of VUE in the background of pathological CTG signs and poor outcome could be in the immune-mediated destruction of placental tissue [74], while chorangiosis seems to be a placental change with a compensational character as a response to chronic, low-grade hypoxia [75]. Namely, cardiotocogram features are affected by placental function which is altered by these histological changes (Figure 3).

Chorangiosis was a common histological finding in our cohort of growth restricted preterm placentae. The presence of villi with the signs of chorangiosis, as a compensational phenomenon to hypoxic environment, could be evident in preterm cases, and villi with chorangiosis could be found next to non-intact villi.

PITV and villous capillarization indicated by CVR are parameters which are dependent on the gestational age, however, the mean±SD gestational age at the time of delivery was relatively close to each other in the EO-FGR and LO-FGR groups (Table 7), making these parameters comparable in the study population.

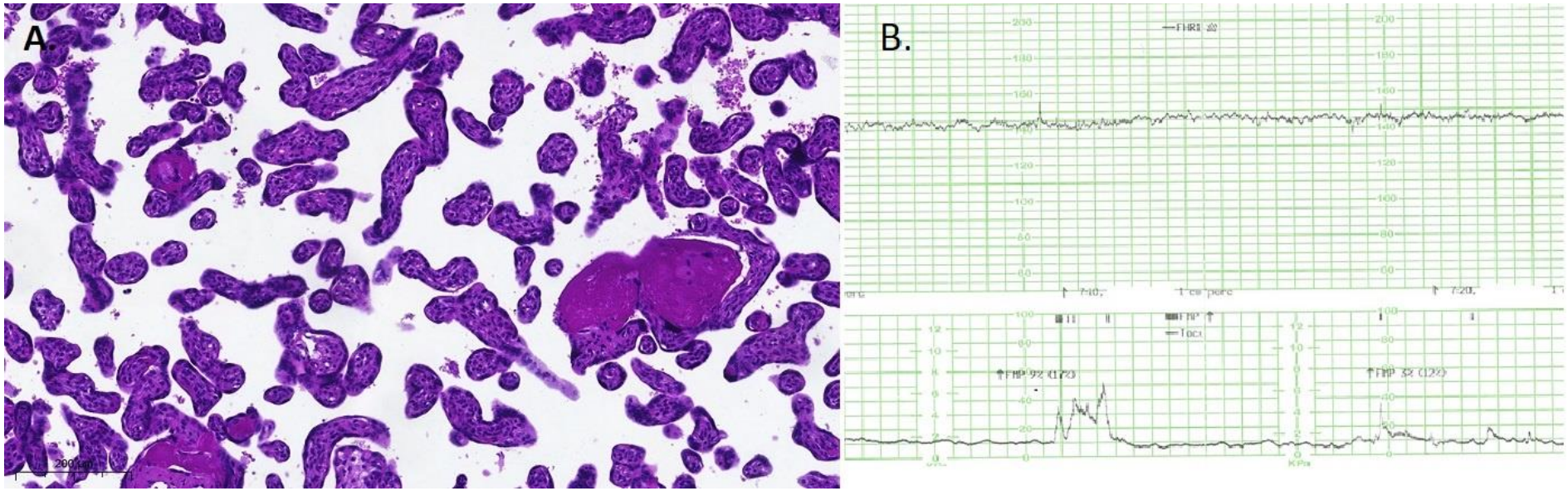

Figure 3.

Placental slide and cardiotocogram (CTG) of an early-onset growth restricted preterm baby

Description:

3A: Placental histology of a growth restricted fetus delivered at the 28th week of gestation (x10, HE staining). Full-thickness tissue sample was taken from the central part of the placenta, which was macroscopically normal. The photomicrograph represents the central part of placental parenchyma. Distal villous hypoplasia with expanded intervillous space, most of the villi are avascular, fibrin is present in the intervillous space.

3B: The CTG corresponding to placental slide 3A: last segment of the CTG trace recorded before Cesarean section; absence of accelerations and reduced baseline variability are obvious. Speed of the tracing was 1 cm/60 sec.

The significantly decreased PITV and significantly lower CVR in the background of cases with neonatal acidemia (reflected by pH ≤7.2 and AUM lactate level ≥3.75 mmol/L) strongly point to the possibility that the described histopathological alterations could influence neonatal outcome as a measure of undercapillarization of placental villi; whereas other histological changes affecting the perivillous- and intervillous space (e.g. AVM by fibrin deposition) and the decidual vasculature (e.g. decidual arterial thrombosis) further aggravate this placental damage cummulatively leading to FGR and placental impairment. Disorders of villous maturation affect capillarization of villi, and this phenomenon is not uncommon in preterm birth [76].

The limitations of this study include its low case numbers, and the fact that for Cesarean sections, CTG registration ended minutes before delivery, therefore we do not have information about fetal state from that interval. However, our method of combined evaluation and statistical analysis of baseline variability and acceleration patterns of CTG, placental histopathology and neonatal outcome points at interesting correlations in this area of perinatology: the findings of this study lead us to the conclusion that pathologic CTG signs in the growth restricted preterm population have their own characteristic pathomorphological background at the microscopic level of placenta.

5.3 Placental pathology and its associations with clinical signs in different subtypes of fetal growth restriction

Histopathological involvement of the placenta in FGR is well-known, and several histopathological alterations have been described [77, 78].

Prenatal hospitalisation of patients with FGR is a common practice in our institute, because timely recognition of deteriorating intrauterine fetal wellbeing has a key role in the management of FGR. As prevalence of umbilical artery pH ≤7.0 at birth was only 5% and there was no perinatal death in our cohort (Table 8), we consider our practice safe and effective in the prevention of neonatal hypoxic impairment.

In this series, a large proportion of the abnormal histopathological features identified were diffuse, but on average, 62% of terminal villi were intact. Focal distribution of the studied histological signs of MVM or FVM and VUE was not typical, and focal changes seemed to show no difference in occurrence in various subtypes of FGR. According to our knowledge, no previous work has studied the focal or diffuse distribution of placental changes in the study of FGR with a similar quantitative approach.

Although timing, onset and dynamics of placental changes have the key role in the pathomechanism of FGR, we assumed that the quantitative study of the PITV and CVR in our cohort could be interesting, as these placental slides representated placental histology at the time of delivery – at the point when FGR has already been a manifest complication of pregnancy; the study of the associations of these quantitative placental markers and clinical and perinatal parameters could point at further clinicopathological correlations.

The placenta is the interface between the mother and the fetus, it separates and connects at the same time; permanent placental malperfusion leads to chronic placental hypoxia, and fetal malnutrition is the core of FGR [79]. Oligohydramnios, pathological CTG signs and pathological Doppler indices develop at a certain point of pregnancy as manifestations of placental insufficiency.

Uterine contractions physiologically lead to impaired maternal oxygen delivery to the placenta, and the described histopathological changes contribute to decreased placental reserve - distress signs on CTG, elevated umbilical artery lactate level and low umbilical artery pH are features of this altered placental reserve. Subsequently, the high rate of Cesarean section among cases with uterine contractions is not surprising in our cohort.

Our results on EO-FGR versus LO-FGR concur with results of previous observations describing that histological alterations of LO-FGR as less characteristic than those of EO-FGR [80]. The LO-FGR cases delivered after the 37th week of gestation sparsely had placental histopathological changes - this further strengthens the less characteristic placental pathology of LO-FGR.

EO-FGR and LO-FGR seem to be two different entities from the aspect of histopathology (Figure 4). EO-FGR is more commonly complicated with PE and/or recurrence than LO-FGR. The relatively frequent finding of VUE in the background of EO-FGR suggests immunological mechanisms in its pathogenesis, and this could explain its recurring character. The concept of VUE is infiltration of the semiallogenic placenta by maternal immune cells leading to a chronic, non-infectious, destructive, immune-mediated inflammation [81]. Eventually, this destructive inflammation leads to pathological changes of the villous tree, causing placental damage. Furthermore, VUE is known to be associated with avascular villi, although the mechanism for obliteration of the fetal vasculature in chronic villitis is unclear [82]; therefore, in cases where VUE and avascular villi were present at the same time, on the theoretical level, avascular villi were presumably associated with VUE.

As far as the co-occurrence of EO-FGR and PE is concerned, Staff et al. described a biphasic PE pathogenesis model in which the first phase of PE is placental involvement

without systemic signs, and in the second phase, systemic symptoms develop depending on maternal responsiveness [83]. This biphasic PE model distinguishes between EO-PE and LO-PE; EO-PE is the result of pathologic placentation, while in case of LO-PE, placentation is normal. We identified several histological changes in FGR complicated with PE and we also described that concurrence of EO-FGR with (per definition) EO-PE is significantly more common than co-occurrence of LO-FGR and LO-PE. The morphological evidence of the pathological placentation in cases of EO-PE concurrent with EO-FGR could be the more common presence of DVH and avascular villi outside infarcted areas, assuming that these villi were *ab ovo* hypoplastic or avascular.

In the background of the most severe clinical symptoms of FGR (severe oligohydramnios, birth weight below the 2nd percentile, pathological Doppler indices), we found that chorionic villous infarction, DVH, AVM and avascular villi were significantly more common. According to these findings, it seems that these histopathological changes collectively reflect or are collectively responsible for generating the placental milieu leading to chronic malperfusion and malnutrition of the fetus, causing chronic hypoxia. Therefore, these histological characteristics determine the chronic placental insufficiency at the microscopic level.

There were 18/52 cases (34.6%), including 12 EO-FGRs with pathologic AUM flowmetry. This finding correlates with the results of a previous study, stating that the AUM flowmetry is pathologic only in a proportion of FGR cases [84]. Our results further support the finding that pathologic AUM Doppler indices in FGR could have a connection with diffuse MVM-associated placental changes [64, 85] - in our cohort the high prevalence of DVH, AVM and diffuse infarction among the EO-FGR cases unequivocally demonstrates this histopathological background. We found that DVH had a strong association with pathologic Doppler indices of AUM; this finding further strengthens the previously described associations of villous maldevelopment and non-branching placental angiogenesis with FGR [86].

The FL/AC ratio investigated by ultrasound is a diagnostic tool for FGR; the higher this ratio, the poorer the prognosis [87]. It seems that a FL/AC ratio ≥0.25 in FGR, besides the estimated fetal weight under the 2nd percentile, is a useful predictor of worse perinatal prognosis (Figure 5). Most babies with FL/AC ratio ≥0.25 at the time of diagnosis of FGR had an umbilical artery blood lactate level >3.75 mmol/L at birth, reflecting intrauterine hypoxia [50]. In the EO-FGR group, we identified infarction, DVH, and decidual arterial

thrombosis more commonly in the background of high FL/AC ratio. We are not aware of prior investigations of the connection of the FL/AC ratio with histopathological alterations.

Terminal villi are the functional unit of the placenta and their main role is in metabolism, transplacental transport and gas exchange. The quantitative analysis of the PITV pointed to the fact that the reduction of terminal villi has a direct connection with pathologic CTG signs and pathologic AUM flowmetry. We found no significant differences in the PITV between different FGR subtypes (by onset or recurrency) and in relation to the presence of other signs and symptoms of FGR. This lack of difference remains unexplained, as both FGR subtypes and the presence of clinical signs were associated with several diffuse histopathological changes described above. To our knowledge, this kind of quantitative analysis of terminal villi has never been used in placental histological studies.

Villous capillarization reflected by CVR, however, had a significant association with EO-FGR, FGR with PE, and birth weight below the 2nd percentile; this leads us to the conclusion, that CVR could be a better quantitative marker of placental pathology in FGR compared to PITV, especially in EO-FGR, because it has a statistically significant connection with perinatal markers of villous undercapillarization such as low birth weight or concurring PE in EO-FGR. Furthermore, CVR seems to have no connection with placental weight or placental weight to birth weight ratio, this way it could be an appropriate quantitative marker of placenta independently from placental weight. Our method of placental capillary quantification with the use of CVR seems to be a feasible though demanding practical way to study villous capillarization, however other methodologies such as stereology [88, 89] and placental CT micro-angiography [90, 91] have been previously described which more rigorously characterize villous growth and fetoplacental angiogenesis.

Chorangiosis was frequently identified (31/61 cases); villi with signs of chorangiosis were found simultaneously with DVH, AVM and avascular villi. The common occurrence of chorangiosis in cases of FGR suggests that certain villi compensate chronic hypoxia with this alteration, as previously described [92], and this phenomenon is prevalent both in EO-FGR and LO-FGR.

In this study we succeeded to prove connection between FL/AC ratio of the fetus at the time of diagnosis of FGR and placental histopathology, and the study of the percentage of intact terminal villi and CVR also lead to novel findings; furthermore, the comprehensive study of different connections of clinical data and placental histopathology also facilitated the understanding of FGR.

The limitations of our study include the relatively small number of cases and relatively broad spectrum of gestational ages; on the other hand, the distribution of EO- and LO-FGR cases is relatively balanced, making their comparison possible.

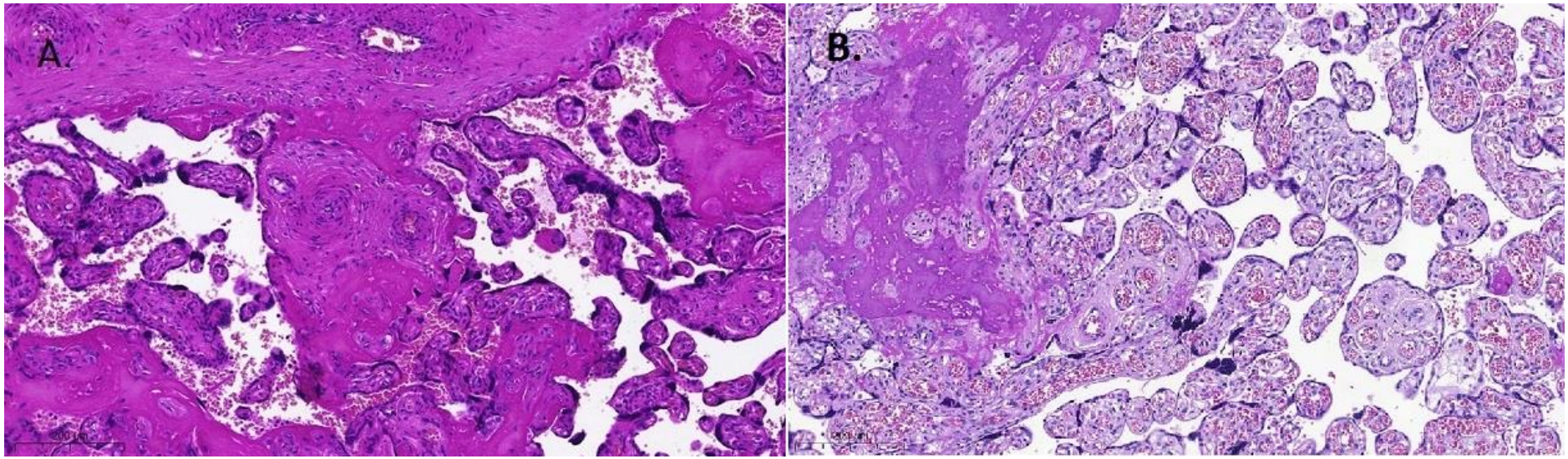

Figure 4.

Placental histopathology of early-onset (EO-) versus late-onset (LO-) fetal growth restriction (FGR)

Description:

4A: Placental histopathology of an EO-FGR case (x10, HE staining, full-thickness tissue sample was taken from the central part of the placenta): accelerated villous maturation (AVM) and perivillous fibrin deposition. This case was complicated with preeclampsia (PE). Gestational age at the time of diagnosis was 28 weeks, Caesarean section was performed at 33^{+4} weeks for absent end diastolic flow on umbilical artery.

4B: Placental histopathology of a LO-FGR case (x10, HE staining, full-thickness tissue sample was taken from the placenta adjacent to the umbilical cord insertion site, placental parenchyma was macroscopically normal-appearing): syncytial knot formation and perivillous fibrin – these findings recall histology of normal term placenta. This case was not complicated with PE. Diagnosis of FGR was made at the 35^{th} week of pregnancy, vaginal delivery happened at 40^{+3} weeks.

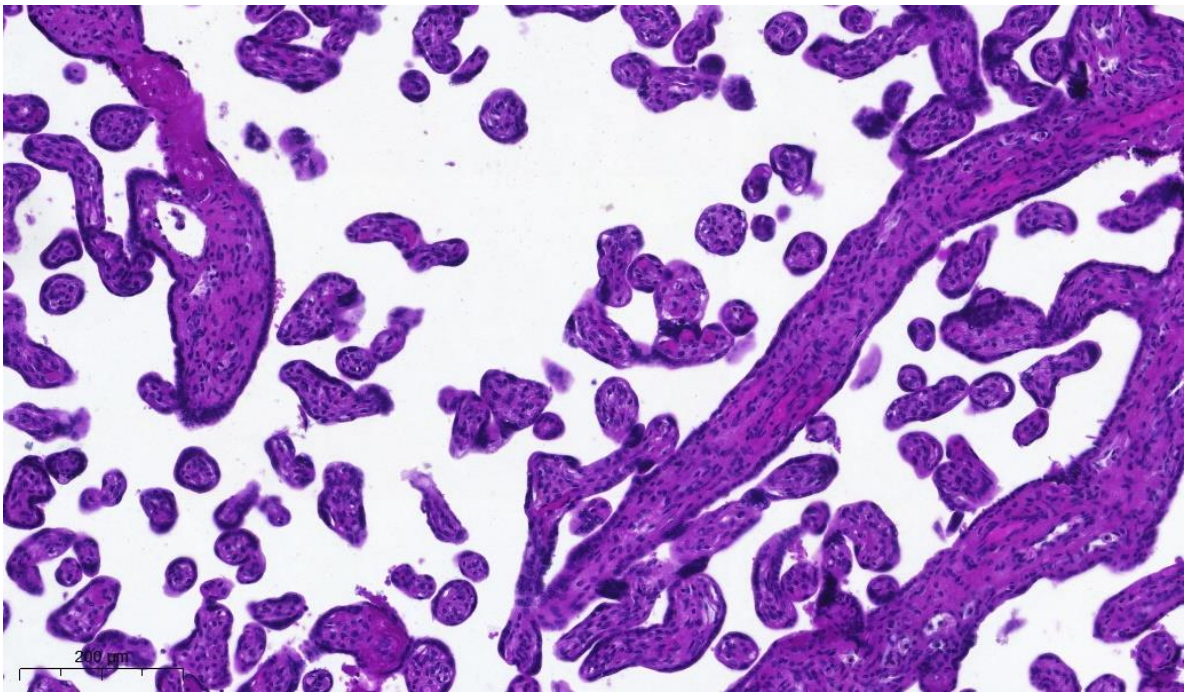

Figure 5.

Placental histology of an early-onset fetal growth restriction (EO-FGR) case with FL/AC ratio >0.26 at the time of diagnosis

Description:

Placental slide of an EO-FGR case (x10, HE staining, full-thickness tissue sample was taken from the central part of the placenta, which was macroscopically normal; the photomicrograph represents the central part of placental parenchyma): distal villous hypoplasia (DVH) with an expanded intervillous space. Gestational age at the time of diagnosis was 28^{+2} weeks, oligohydramnios was diagnosed, estimated fetal weight was under the 2nd percentile for the age of gestation. This case was complicated with preeclampsia (PE). Three days later, pregnancy was terminated via Caesarean section for absent end diastolic flow on umbilical artery and severe maternal hypertension.

6. CONCLUSIONS

6.1 EO-PE and LO-PE are two distinct entities from the viewpoint of placental histopathology: in EO-PE placentation and placental vasculogenesis is *ab ovo* altered, and poor villous development has a key role in its pathogenesis, while in LO-PE, placentation in itself is physiologic. DVH and avascular villi reflect pathological placentation in EO-PE, while in LO-PE placental changes altering placental blood flow in term or near-term are the most characteristic features.

6.2 Decidual arteriopathies, medial hypertrophy and perivasculitis of maternal decidual vessels are more characteristic to EO-PE. Medial hypertrophy of maternal decidual vessels also has a connection with increased RI of umbilical artery and persistence of uterine artery diastolic notch, reflecting its impact on placental hemodynamics.

6.3 In EO-PE with FGR, the concurrence of VUE and avascular villi is a remarkable issue, and a characteristic predisposition for recurrency.

6.4 In PE and FGR, CVR (our indicator of capillarization of villi) is a proper index of quantitative placental histological examination at the time of delivery; the absence of significant differences of CVR in different subgroups of placental weight percentiles, or in different groups of placental weight to birth weight ratios makes it an ideal, weight-independent marker of placental villous capillarization.

6.5 In the growth restricted preterm population, the significantly decreased PITV and significantly lower CVR in the background of cases with absence of accelerations, decreased baseline variability on CTG and neonatal acidemia strongly point to the fact that undercapillarization of placental villi has a key role in fetal distress; other histological changes affecting the perivillous- and intervillous space (e.g. AVM by fibrin deposition) and the decidual vasculature (e.g. decidual arterial thrombosis) further aggravate this placental damage cummulatively leading to FGR and placental impairment.

6.6 In the background of the most severe clinical symptoms of FGR (severe oligohydramnios, birth weight below the 2nd percentile, pathological CTG and pathological Doppler indices), chorionic villous infarction, DVH, AVM and avascular villi are significantly more common than other placental histopathological changes.

6.7 DVH has a strong association with pathologic Doppler indices of AUM; this finding further strengthens the previously described associations of villous maldevelopment and non-branching placental angiogenesis with FGR.

6.8 In FGR, FL/AC ratio ≥0.25 and estimated fetal weight under the 2nd percentile are useful predictors of bad perinatal prognosis. In the background of high FL/AC ratio in the EO-FGR group, villous infarction, DVH, and decidual arterial thrombosis were described more commonly; villous infarction and avascular villi were found more commonly in the LO-FGR group when FL/AC ratio was high.

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9. APPENDIX

Printed articles:

I. Dankó I, Tankó A, Kelemen E, Cserni G. Placental pathology of preeclampsia from a clinical point of view: Correlation between placental histopathology, clinical signs of preeclampsia and neonatal outcome. J Obstet Gynaecol Res. 2023;49(6): 1471–80.

II. Dankó I, Kelemen E, Tankó A, Cserni G. Correlations of Placental Histopathology, Neonatal Outcome, and Cardiotocogram Baseline Variability and Acceleration Patterns in the Growth Restricted Preterm Population. Pediatric and Developmental Pathology. 2023;26(5): 447-457.

III. Dankó I, Kelemen E, Tankó A, Cserni G. Placental Pathology and Its Associations With Clinical Signs in Different Subtypes of Fetal Growth Restriction. Pediatric and Developmental Pathology. 2023;26(5): 437-446.