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Is the expression of placental epithelial and lymphoid markers associated with the perinatal outcomes in preeclampsia?

Short title: Expression of placental epithelial and lymphoid markers in preeclampsia

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ABSTRACT

Objectives: This study aimed to investigate the association of the epithelial and lymphoid immune markers with the adverse perinatal conditions such as early-onset preeclampsia (EOPE), fetal growth restriction (FGR) and intrauterine fetal death (IUFD) in preeclampsia in the placentae of preeclamptic patients.

Material and methods: A total of 60 pregnant patients were included in this study. The immunohistochemistry method was used to determine the expression levels of CD4, CD8, CD4 / CD8, CD68, P53, MDM2, CK18, CK19, E-cadherin, and β-catenin.

Results: In our study, the increase in E-cadherin expression in the preeclamptic fetal-maternal placental region was associated with EOPE and FGR development preeclampsia and the decrease in the expression of CD4 and CD8, which are involved in the local immunomodulation, was associated with IUFD.

Conclusions: Our data reveal that the increase in the expression of CK18, CK19, E-cadherin, and β-catenin and the decrease in CD4 and CD8 play a role in the pathogenesis of preeclampsia.

Key words: E-cadherin; CD4; CD8; β-catenin; early onset preeclampsia; adverse perinatal outcomes

INTRODUCTION

Preeclampsia is a pregnancy-specific disease that is a leading cause of FGR, IUFD, severe perinatal mortality, and morbidity [1]. Preeclampsia is a heterogeneous disease; EOPE occurs before the gestational age of 33 weeks and six days and the late-onset preeclampsia (LOPE) occurs at the gestational age of 34 weeks and later [2]. EOPE has severe symptoms such as platelet count < 100,000/mL, progressive disorder of renal function (serum creatinine > 1.1 mg/dL, or doubling of serum creatinine), liver dysfunction (doubling of liver transaminases), pulmonary edema, and cerebral or visual symptoms. Preeclampsia findings and adverse perinatal outcomes are more common [3, 4]. Whether preeclampsia is severe or not, the clinical symptoms, impaired laboratory findings, and adverse perinatal outcomes vary. This case shows that the onset of the disease has different etiopathogenesis depending on the early or late gestational age or severe or mild course, or in some cases, resulting in IUFD even with mild disease, or the absence of FGR in every severe or early-onset preeclamptic pregnancy.

The hypotheses are thought to be most related to the etiology of preeclampsia. Incomplete remodeling of spiral arterioles in the decidual phase of placenta during the first trimester, angiogenetic factors causing endothelial damage, placental apoptosis, and intravascular inflammation are considered to have roles in the pathogenesis of the disease [5, 6]. Cell adhesion and modulation of cell polarity occur through changes in cell-cell junction molecules such as cadherins. Cadherins, particularly Epithelial- I -cadherin, are important for maintaining the cell attachment and the villous cytotrophoblast [7]. Epithelial markers such as E-cadherin and β-catenin are associated with the development of both normal and pathological placenta and increase local tissue invasion [7]. FGR and PE involve trophoblast cells with apparently reduced invasion into the maternal environment [8]. E-cadherin and β-catenin are reduced or lost from cell-cell junctions [9, 10]. It has been shown that CK-18 is released from apoptotic epithelial cells and increases in patients with epithelial organ dysfunction such as preeclampsia [11].

It is also known that placental oxygenation is impaired and that a hypoxic environment occurs in preeclampsia due to impaired remodeling in spiral arteries. It has been shown that the activation of hypoxic signaling is triggered by the epithelial-mesenchymal transition (EMT) modulators accompanied by specific changes in gene expression such as down-regulation of E-cadherin, CK18 and CK19 [12]. Apoptosis, fibrinoid necrosis and perivascular immune cell infiltration are present in the remodeling of spiral arterioles in preeclampsia [13], especially these immune cells are maternal CD4 + and CD8 + T cells and fetal and maternal CD68 + macrophages [14, 15].

This study aimed to investigate the association of the epithelial and lymphoid immune markers with the adverse perinatal conditions such as early-onset preeclampsia (EOPE), fetal growth restriction (FGR) and intrauterine fetal death (IUFD) in preeclampsia in the placentae of the patients with preeclampsia. Therefore, we evaluated the immunohistochemical expressions of E-cadherin, β-catenin,

CK18, and CK19, which are involved in the adhesion of extravillous trophoblasts, p53, and MDM2, which is involved in apoptosis and epithelial-mesenchymal migration, as well as CD4, CD8 and CD 68, which are responsible for the immunomodulation in endothelial damage during angiogenesis.

MATERIAL AND METHODS

Design and study population

This is a prospective case-control study including 32 patients with preeclamptic placenta and 28 controls with healthy placenta. The groups were homogenized in terms of their ages and body mass indices (BMI). The diagnosis of preeclampsia depended on the following criteria; 1) Systolic blood pressure (SBP) ≥ 140 mmHg and/ or diastolic blood pressure (DBP) ≥ 90 mmHg in a previously normotensive patient after the 20^{th} gestational week with the persistency of signs in at least two measurements which were obtained at more than 4-hour intervals, 2) Presence of SBP ≥ 160 mmHg and/ or DBP ≥ 110 mmHg, and 3) New onset proteinuria [proteinuria ≥ 300 mg/24 hour or protein (mg/dL)/creatinine (mg/dL) ratio ≥ 0.3 in spot urine sample]. The preeclampsia group in our study included patients with any of the criteria above before the 34^{th} gestational week (EOPE) [16]. According to the sonographic assessment, the fetuses with biometric measurements of an estimated weight lower than 10% of their gestational ages had FGR [17].

Patients were excluded if any of the following disorders were present: multiple pregnancies, viral infections affecting the liver function tests, type I/II diabetes mellitus, hyper/hypothyroidism, chronic liver or renal disease, cholelithiasis, alcohol consumption history, chronic hypertension, smoking, history of thromboembolic disease or the other conditions or medications causing the elevation of the liver enzymes, history of maternal cancer or fetal chromosomal aneuploidy, and maternal infection diseases (urinary tract infection, upper respiratory tract infection, toxoplasmosis, rubella, cytomegalovirus, herpes, syphilis, varicella-zoster, parvovirus B19, *etc*.)

Clinical examination was performed for the patients and their anthropometric measurements and obstetric and medical history were recorded. Gestational age was calculated according to the last menstrual date and confirmed for all the patients by routine examination with ultrasonography performed during the 1^{st} trimester of gestation. The study protocol was performed and approved by the Local Ethical Committee of our hospital (approval date/number: 11.09.2020/313) in accordance with the Declaration of Helsinki principles.

Placental tissue preparation

The placentae were collected within two hours after delivery. For immunohistochemistry, chorionic and decidual surfaces were removed and placental tissue was sampled from two placental lobules at the center from each placenta. Placental tissues were then fixed by 10% Neutral Buffered

formaldehyde. Tissue sections with a thickness of 4 μm were obtained from the appropriate paraffin blocks. Immunohistochemistry was performed using monoclonal CD4, CD8, CD4/CD8, CD68, P53, MDM2, CK18, CK19, E-cadherin, and β-catenin. The antibodies used in this study were given in Tab. 1. The staining procedure was performed on the DAKO Omnisautostainer (Agilent, Santa Clara, CA 95051, USA). All the tissue sections were examined using an Olympus CX41 light microscope (Olympus, Tokyo, Japan).

Immunohistochemical data analysis

Semi-quantitative analysis of E-cadherin, β-catenin, CK18, and CK 19 expression was performed using a previously published method based on the combination of staining intensity of immunohistochemical images and the percentage of positive cells [5, 18]. In brief, no staining is scored as 0, 1–10% of the cells stained are scored as 1, 11–50% as 2, 51–80% as 3, and 81–100% as 4. Staining intensity is rated on a scale of 0 to 3, with 0 = negative; 1= weak; 2 = moderate, and 3 = strong. When there is multifocal immunoreactivity and there are significant differences in staining intensities between foci the average of the least intense and most intense staining was recorded. The scores could range from 0 to 12. An immunohistochemical score of 9–12 was considered as strong immunoreactivity, 5–8 as moderate, 1–4 as weak, and 0 as negative. In the immunohistochemical examination, p53 and MDM2 were stained with control tissues and evaluated negatively and positively. Images were taken using a digital camera mounted on an Olympus CX41 microscope to evaluate immunohistochemical results of CD4, CD8 and CD68. The Olympus software was used to measure the area at 40x. The number of stained cells was counted. Ten areas were used to analyze a minimum of 1 mm^2 of tissue for each tissue. The results were recorded as the numbers of cells/mm^2.

Statistical analysis

Data analysis was performed using SPSS for Windows, version 22.0 (SPSS Inc., Chicago, IL, USA). Whether the distribution of continuous variables was normal or not was determined with the Kolmogorov-Smirnov test and homogeneity of the variances was evaluated with the Levene test. Continuous variables were shown as mean ± standard deviation (SD) or median (min-max) where applicable. While the mean differences between preeclampsia and control groups were compared with the independent sample t-test Mann-Whitney U-test was used to compare the median values. Pearson's chi-square test was used to analyze the nominal data. Continuous variables were shown as percentages (%). Linear regression analysis was performed to determine the relationship of placental CD4, CD8, CD4 / CD8, CD68, P53, MDM2, CK18, CK19, E-cadherin, and β-catenin with EOPE, FGR and IUFD. Adjusted odds ratios and 95% confidence intervals were calculated for each variable and each specific adverse perinatal outcome (EOPE, FGR, IUFD). A p-value of less than 0.05 was considered as a statistically significant result.

RESULTS

Clinical and laboratory data of the study population

A total of 60 women were included in the study (32 patients with preeclampsia and 28 age- and BMI-matched healthy controls). The baseline characteristics of the patients with preeclampsia and healthy controls were given in Tab. 2. There were no statistically significant differences between the preeclampsia and control groups in terms of gravity, platelets, hemoglobin, and LDH, AST and ALT levels. Statistically significant differences were found in terms of SBP, DBP, birth week, and the levels of serum urea, creatinine and EFW. As expected, proteinuria (mg/24 hours) and Spot urine protein (mg/dL) / creatinine (mg/dL) were significantly higher in preeclamptic women compared with the normotensive pregnant women.

The levels of CD4, CD8, CD4/CD8, CD68, P53, MDM2, CK18, CK19, E-cadherin, and β-catenin in preeclamptic and healthy control placentae

CD4, CD8, CD4 / CD8, and CD68 were calculated as the total number of cells stained in 1 mm^2. CD4 was 4.14 ± 1.11, CD8 was 24.46 ± 6.66 and CD4/CD8 ratio was 0.17 ± 0.02 in preeclamptic placentae while CD4 was 20.38 ± 1.75, CD8 was 56.63 ± 5.29 and CD4 to CD8 ratio was 0.36 ± 0.05 in the control placentae. CD4, CD8 and CD4/CD8 ratio were significantly lower in the preeclampsia group than in the control group ($p < 0.001$, $p < 0.001$ and $p < 0.001$ respectively). The expression of CD68 significantly increased in the preeclampsia group compared with the control group (19.54 ± 1.85 vs 7.97 ± 2.58; $p < 0.001$) (Tab. 2, Fig. 1).

Twenty-two (68.75%) preeclamptic placentas and 7 (25%) control placentas were positively stained for p53. P53 expression in the preeclamptic placenta was significantly overexpressed compared with the control placenta ($p = 0.001$). MDM2 was significantly less expressed in preeclamptic placenta compared with the control placenta (Tab. 3, Fig. 2).

The expressions of CK18, CK19, E-Cadherin, and B-catenin in placental tissues were given in Table 3. The immune staining of CK18, CK19, E-cadherin, and β-catenin was significantly stronger in the preeclamptic placenta compared with the control placenta ($p < 0.001$, $p < 0.001$, $p < 0.001$, and $p < 0.001$) (Tab. 3, Fig. 3).

Placental CD4, CD8, CD4/CD8, CD68, P53, MDM2, CK18, CK19, E-cadherin, and β-catenin expressions associated with EOPE

Linear regression analysis of the placental CD4, CD8, CD4/CD8, CD68, P53, MDM2, CK18, CK19, E-cadherin, and β-catenin expression determined the association with EOPE. Only a strong expression of placental e-cadherin was independently associated with EOPE (Beta: 0.376, 95% CI:

0.001–0.185; p = 0.048). None of the placental CD4, CD8, CD4 / CD8, CD68, P53, MDM2, CK18, CK19, and β-catenin expressions was associated with EOPE (Tab. 4).

Each specific adverse perinatal outcome (FGR, IUFD) was calculated with the linear regression analysis. Only a strong expression of placental e-cadherin was independently associated with FGR in preeclampsia (Beta: 0.426, 95% CI:0.013–0.173; p = 0.024). None of the placental CD4, CD8, CD4 / CD8, CD68, P53, MDM2, CK18, CK19, and β-catenin expressions was associated with FRG in preeclamptic pregnant women (Tab. 5). Decreases in the placental CD4 and CD8 expressions were independently associated with IUFD in preeclampsia (Beta: –0.453, 95% CI: –0.322 to –0.037; p = 0.016 and Beta: –0.507, 95% CI: –0.056 to –0.011; p = 0.006) (Tab. 6).

DISCUSSION

In our study, the expression of E-cadherin, β-catenin, CK18, and CK19 increased in the preeclampsia group compared to the control group and the increased placental E-cadherin expression was also associated with both EOPE and FGR. Preeclampsia is a specific disease of the gestational period and can cause severe morbidity and mortality on the mother and the fetus. Commonly, impaired extravillous trophoblast invasion and maternal spiral artery remodeling constitute an essential part of the pathogenesis of preeclampsia in the first trimester [19]. The molecular mechanism driving trophoblast cell proliferation and invasion remains unclear. E-cadherin and β-catenin are cell-cell adhesion molecules, which are thought to play an important role in trophoblastic differentiation and remodeling during gestation [20]. E-cadherin is considered to be an inhibitor of trophoblast and cancer cell migration and invasion [21]. Li et al. [18] reported that E-cadherin's placental expression, CK18 and CK19 significantly increased in preeclampsia compared with the normotensive pregnancies. Al Nasiry et al. reported that E-cadherin was highly positive in trophoblasts in gestational hypertension (GH) and preeclamptic pregnancies compared with the normotensive pregnancies and that there was no difference in E-cadherin expression in HELLP syndrome [22]. Blechschmidt et al. [23] observed a 1.9-fold significant decrease in E-cadherin immunoreactivity in preeclamptic extravillous trophoblasts and a 1.3-fold decrease in HELLP placentas. However, they stated that the decline was statistically significant in the preeclamptic placenta, but the decrease was not statistically significant in the HELLP placenta. Brown et al. [24] showed that E-cadherin expression increased in preeclamptic placentas. In our study, we determined that E-cadherin expression increased in preeclampsia and that this increase was one of the risk factors for the development of EOPE. Although FGR is out of the diagnostic criteria of preeclampsia it is one of the frequent adverse perinatal outcomes in the EOPE clinic.

Similar uteroplacental histopathological changes are observed in FGR, EOPE and IUFD [25]. Yue et al. [26] showed that E-cadherin expression was lower in the placenta of FGR than in the control placentas. Huang et al. [27] reported that β-catenin decreased in the FGR placenta. Bahr et al. [28] stated that β-catenin expression increased in preeclamptic placentas compared with the placentas of

FGR and control groups. Our study showed that the expression of β-catenin levels in preeclamptic placentas significantly increased compared with the control group. We also found that it was not associated with EOPE and FGR.

It has been shown that inflammation and immunity are effective in the pathogenesis of preeclampsia and this situation is mostly caused by CD4 and CD8 imbalance [29] Wilczyński et al. [29] showed that the percentage of CD8 increased and that the rate of CD4 significantly decreased in preeclamptic decidua. Nakabayashi et al. [30] said that CD8(+) T cells, CD4(+) T cells, CD56(+) NK cells, and CD68(+) macrophages in preeclampsia were significantly smaller than those in normal pregnancy. Proportions of $CD4^+$ / $CD8^+$ cells did not differ between preeclamptic and healthy pregnant women [31, 32]. In our study, we found that CD4, CD8 and CD4 / CD8 expressions were significantly lower and that CD68 was significantly higher in preeclamptic placentas compared with the control placentas. Still, we found that it was not associated with EOPE and FGR, but the decreased expression of CD4 and CD8 was significantly associated with IUFD. Leukocytes, macrophages and CD4 (+) and CD8 (+) T cells increased throughout the placenta in IUFD [33]. However, the number of natural killer cells (uNK), macrophages and T cells of the total CD immune cells on the localized maternal-fetal surface in the uterus decreases in preeclampsia, resulting in the disruption of placental extravillous trophoblasts invasion and remodeling of spiral arteries [33]. In our study, CD4, CD8 and CD4 / CD8 placental expression decreased in preeclampsia. This decrease was associated with IUFD in preeclamptic pregnant women, but it was not associated with EOPE and FGR.

CONCLUSIONS

According to our study, the increase in E-cadherin expression in the preeclamptic fetal-maternal placental region was associated with EOPE and FGR development preeclampsia and the reduced CD4 and CD8 expression involved in local immunomodulation was associated with IUFD. This shows that clinical worsening of preeclampsia, its onset in the early or late weeks and adverse perinatal outcomes that may occur may have different mechanisms in the etiopathogenesis. An approach considering the different close interactions between maternal, fetal and placental factors and cells should be chosen today, because no single factor can explain preeclampsia and the resulting adverse perinatal outcomes.

Conflict of interest

The authors report no conflicts of interest.

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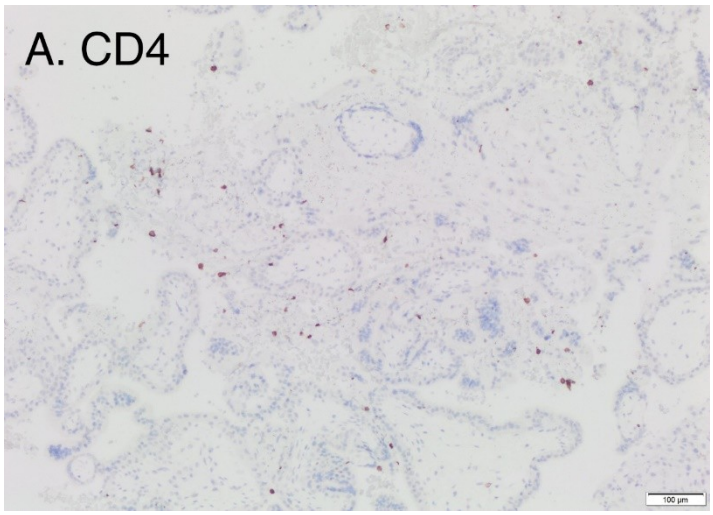
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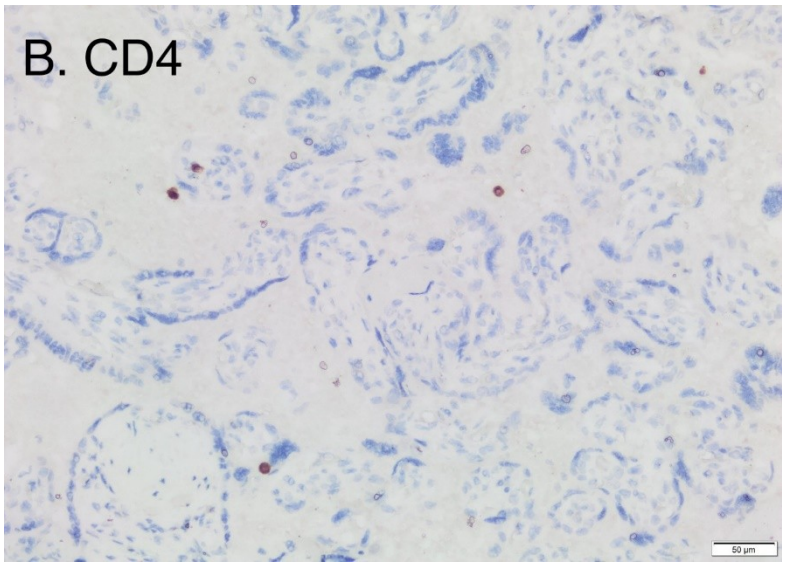
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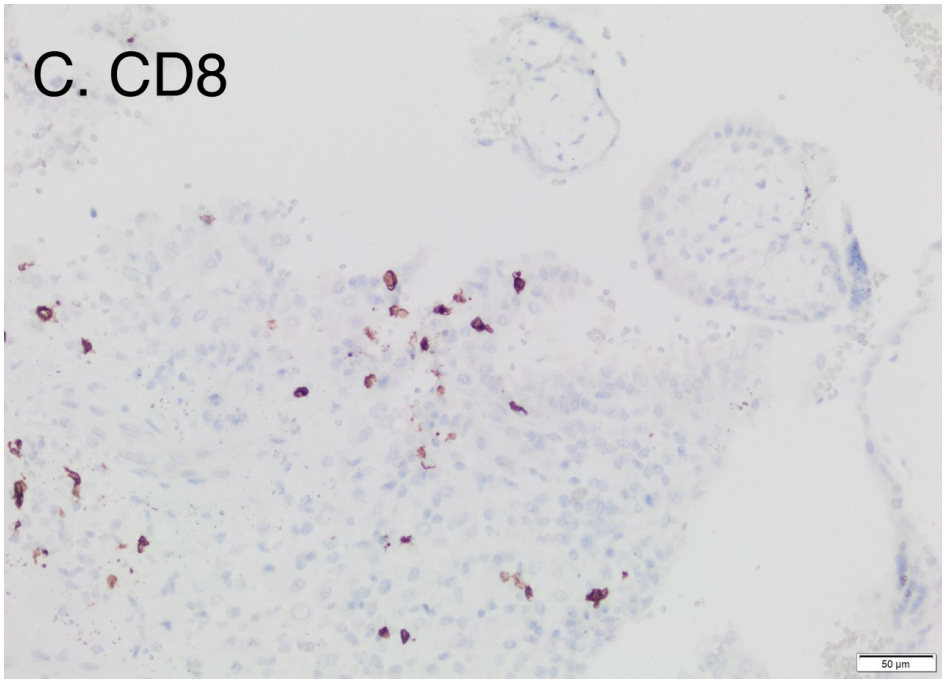
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50 µm

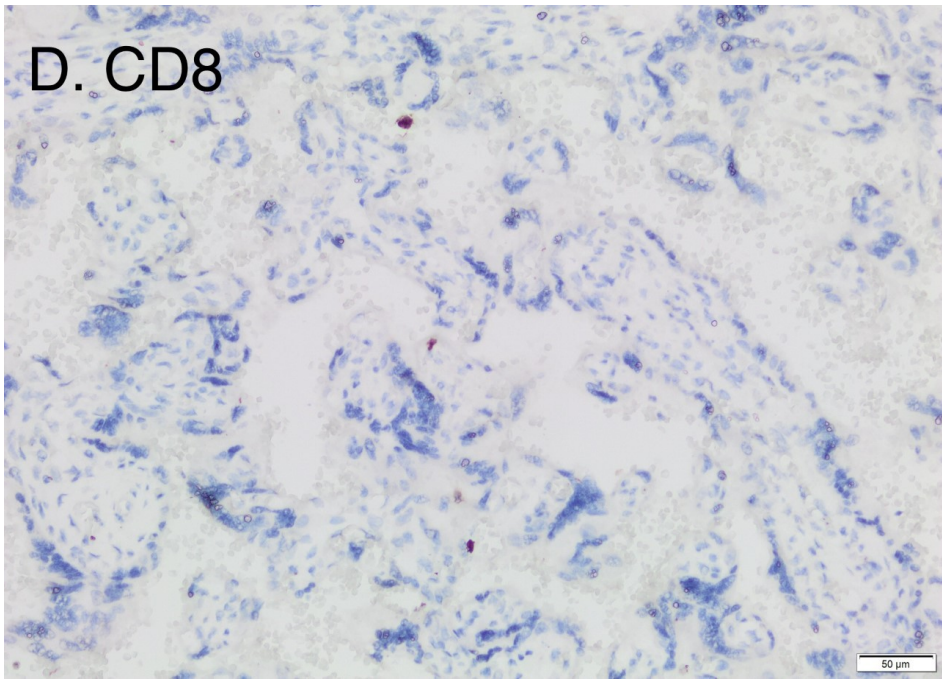
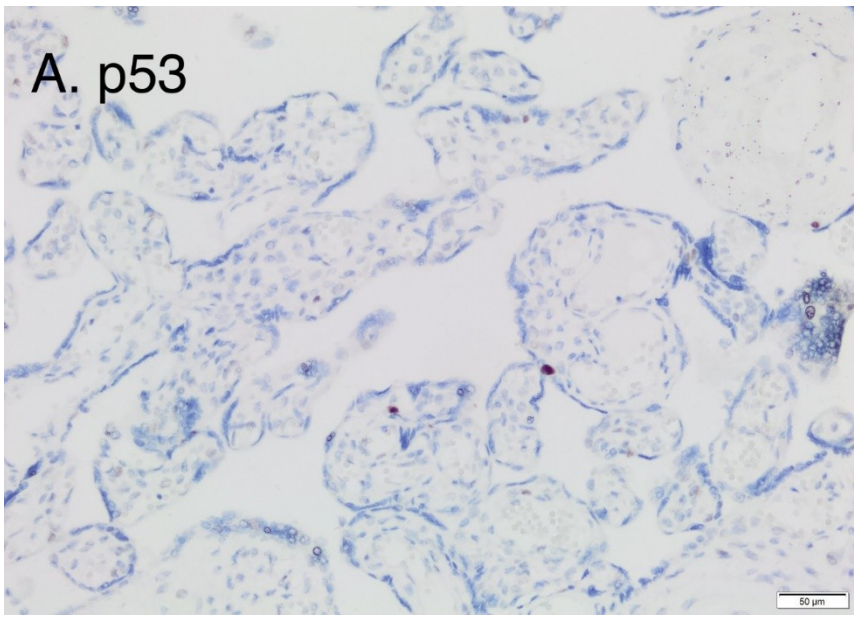
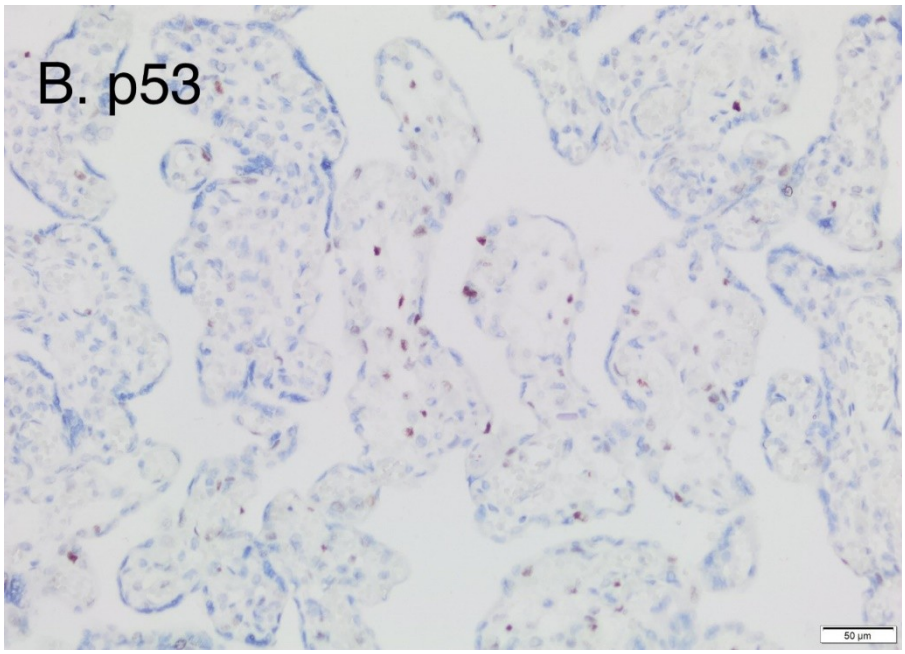
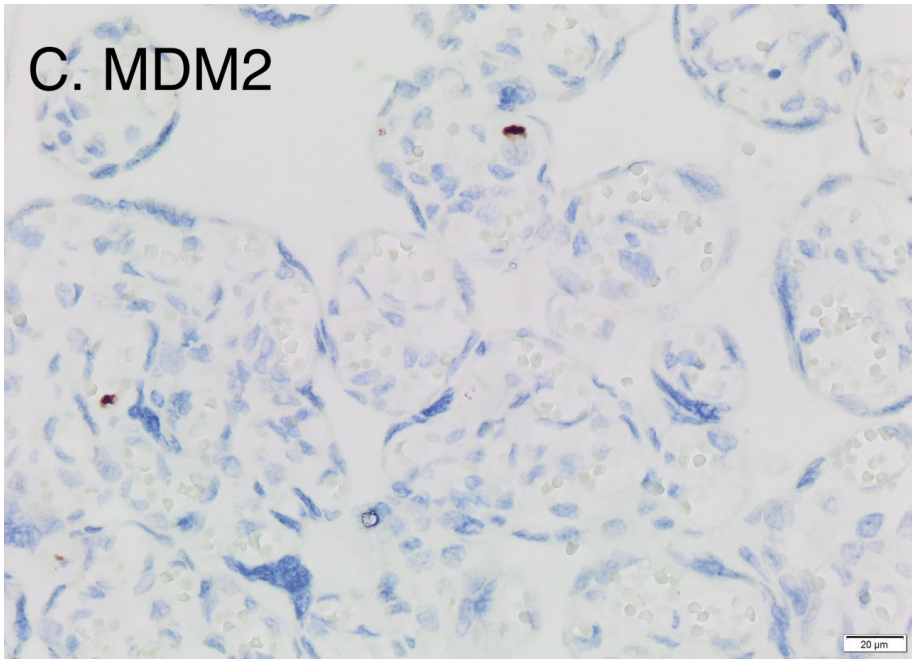
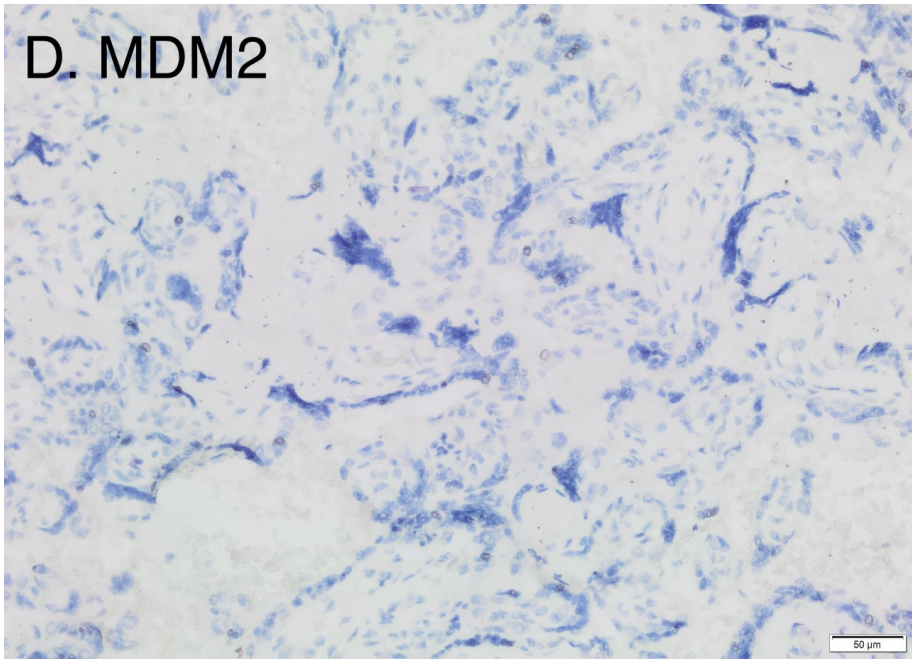


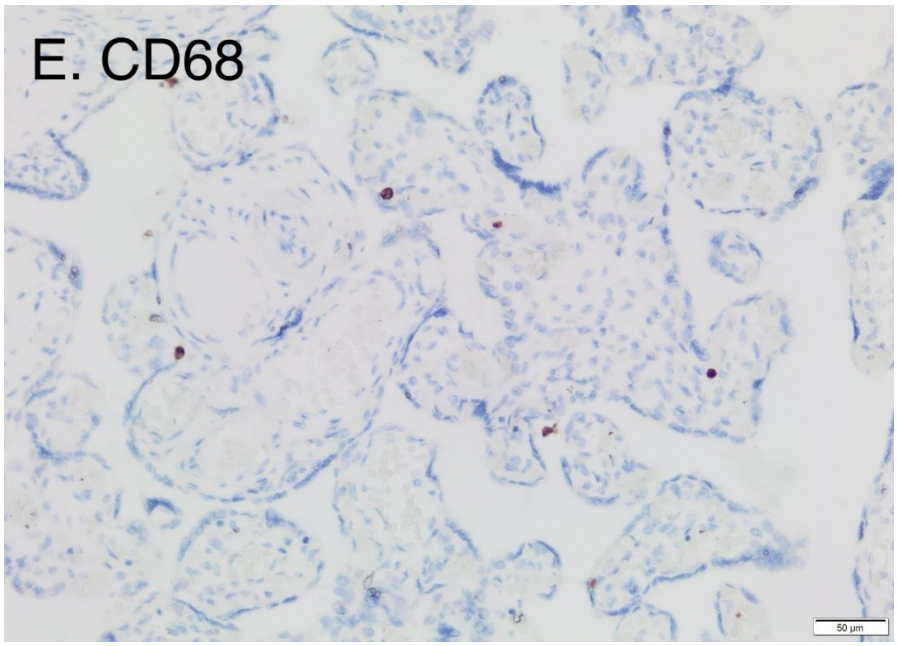
Figure 1. A, B. Immunohistochemical staining for CD4+ cells. **A.** CD4+ cells in normotensive placenta, 4x power field, scale bar 100 µm. **B.** CD4+ cells in preeclamptic placenta, 20x power field, scale bar 50 µm. **1. C, D**. Immunohistochemical staining for CD8+ cells. **C**. CD8+ cells in normotensive placenta, 20x power field, scale bar 50 µm. **D**. CD8+ cells in preeclamptic placenta, 20x power field, scale bar 50 µm. **1. E, F**. Immunohistochemical staining for CD68+ cells. **E.** CD68+ cells in normotensive placenta, 20x power field, scale bar 50 µm. **F.** CD68+ cells in preeclamptic placenta, 20x power field, scale bar 50 µm




B. p53
50 µm


C. MDM2
20 µm


D. MDM2
50 µm


E. CD68
50 µm

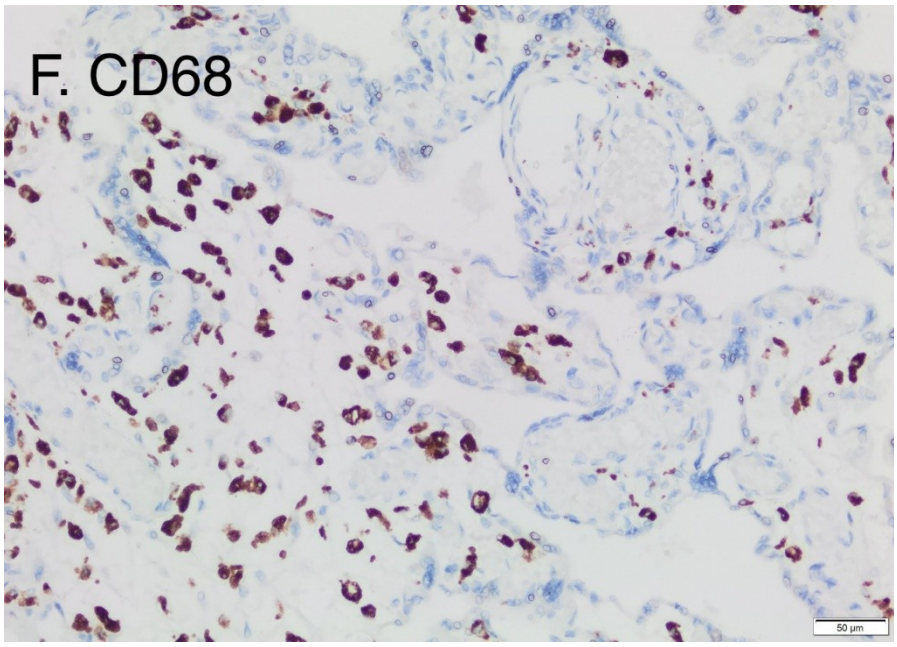
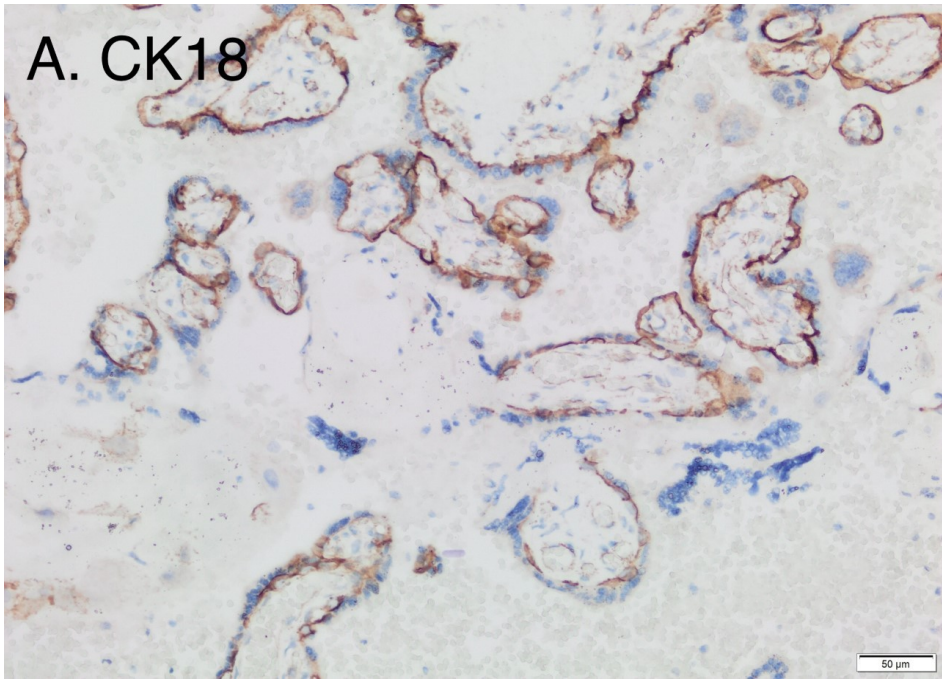
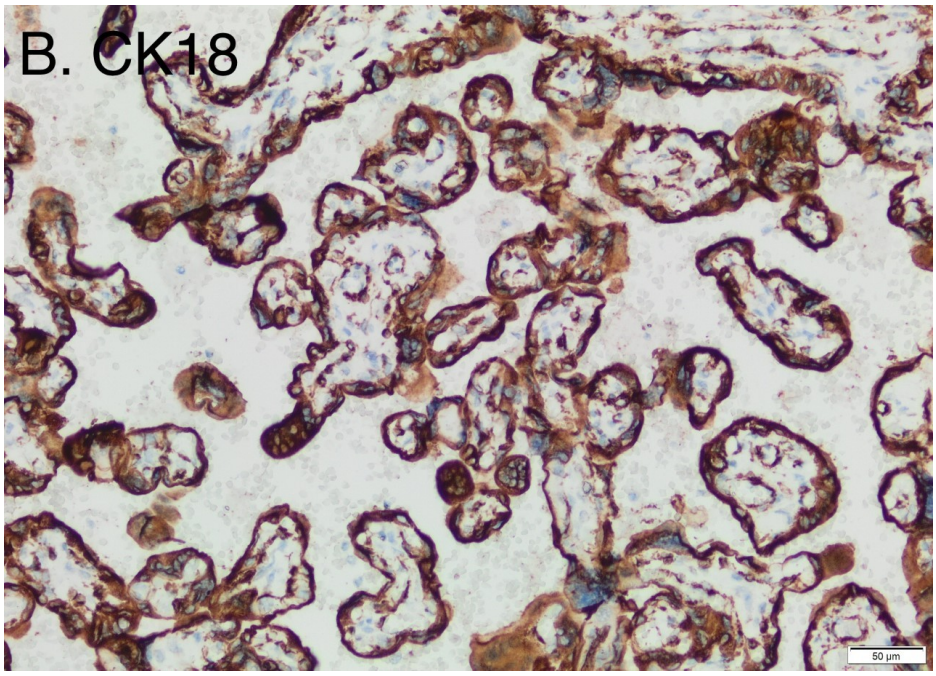
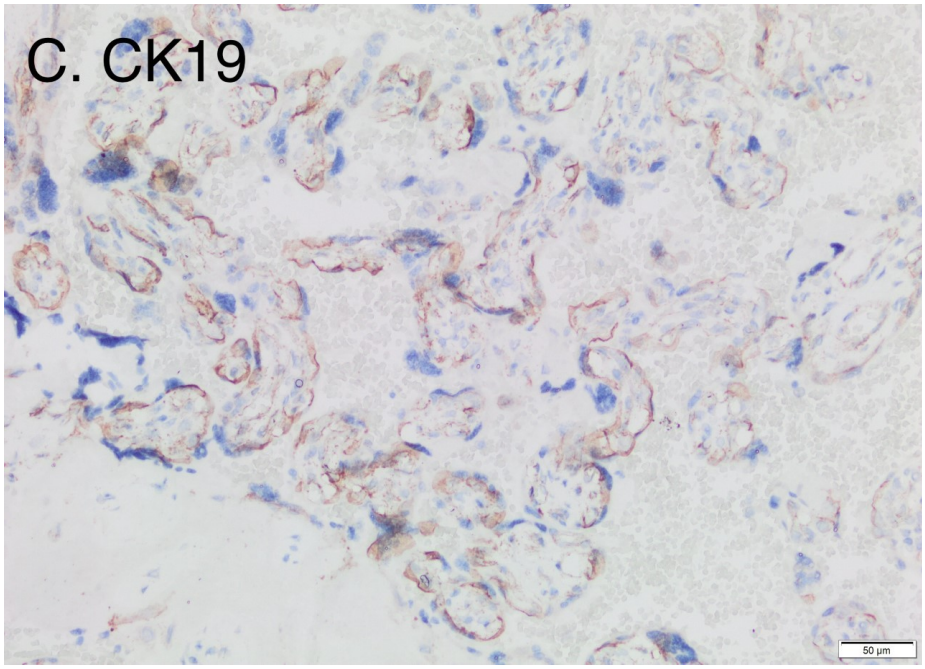
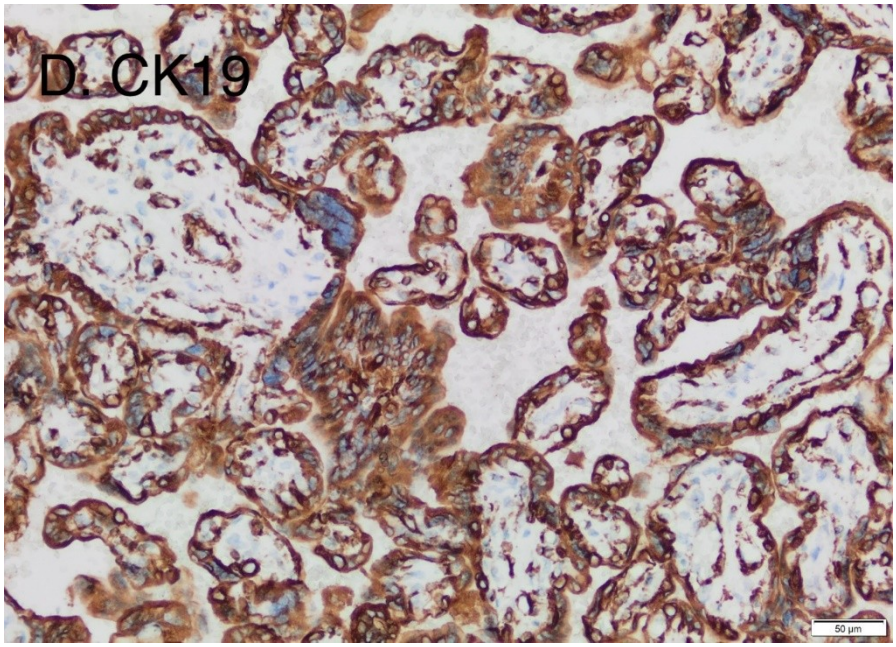


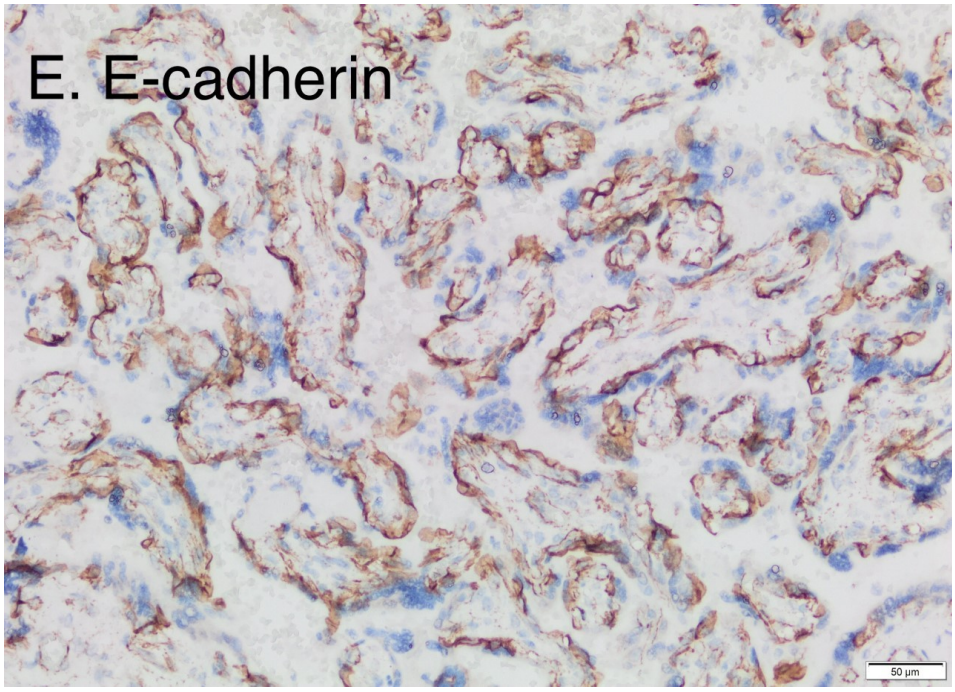
Figure 2. A, B. Immunohistochemical staining for P53+ cells. **A.** P53+ cells in the normotensive placenta, 20x power field, scale bar 50 µm. **B.** P53+ cells in preeclamptic placenta, 20x power field, scale bar 50 µm. **2. C, D**. Immunohistochemical staining for MDM2 **C.** Positive reaction with MDM2 in very few cells in the normotensive placenta, 20x power field, scale bar 50 µm. **D**. Absence of MDM2 staining in the preeclamptic placenta, 20x power field, scale bar 50 µm

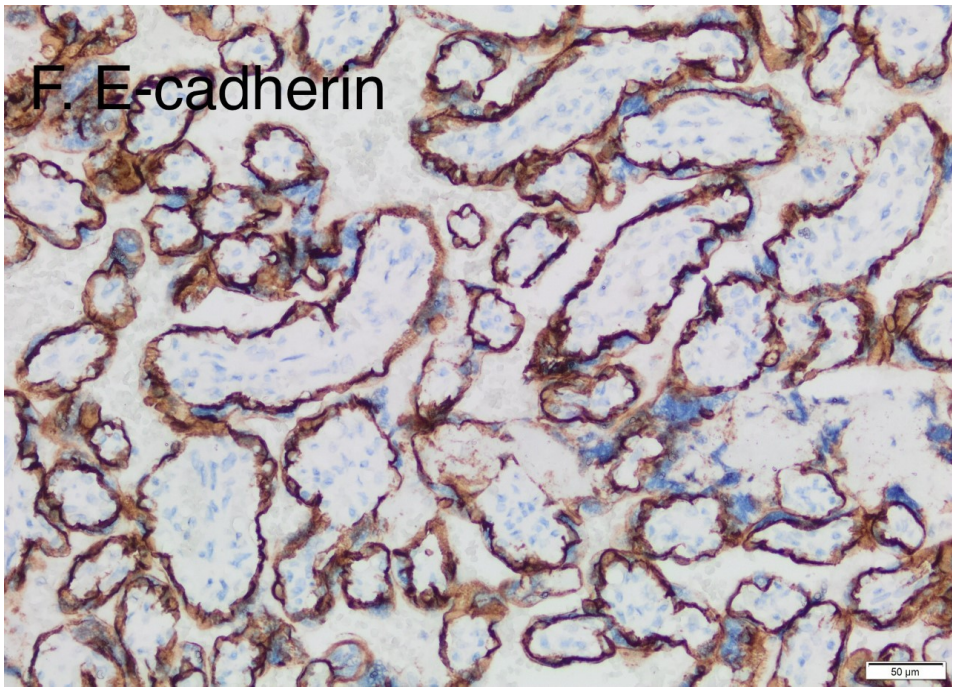


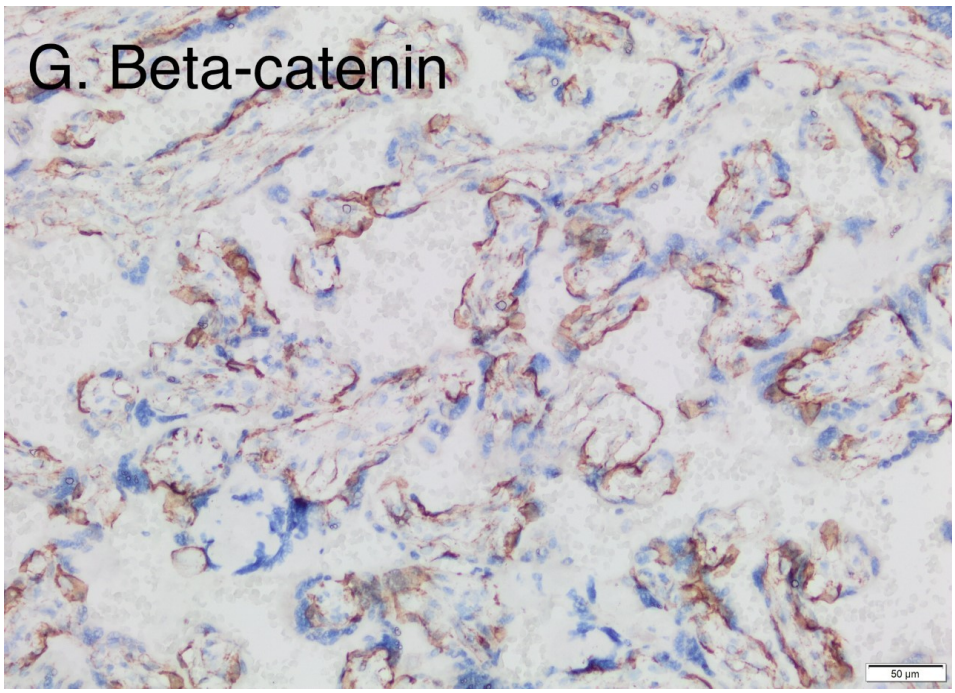

B. CK18
50 µm


C. CK19
50 µm


D. CK19
50 µm


E. E-cadherin
50 µm


F. E-cadherin
50 µm


G. Beta-catenin
50 µm

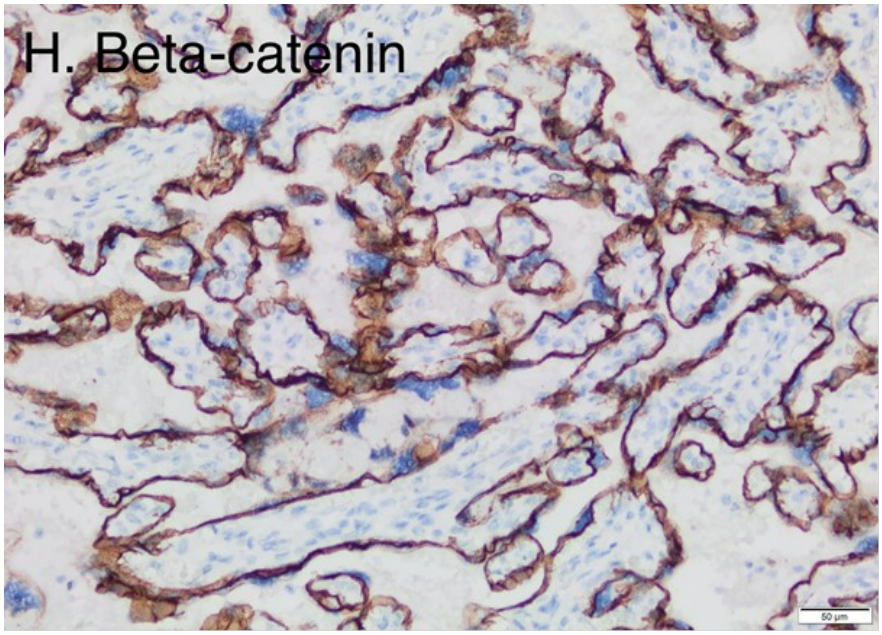


Figure 3. A, B. Immunohistochemical staining for CK18 cells. **A.** CK18 cells in normotensive placenta, 20x power field, scale bar 50 µm. **B.** CK18 cells in the preeclamptic placenta, 20x power field, scale bar 50 µm. **3. C, D**. Immunohistochemical staining for CK19 cells. **C.** CK19 cells in normotensive placenta, 20x power field, scale bar 50 µm. **D.** CK19 cells in the preeclamptic placenta, 20x power field, scale bar 50 µm. **3. E, F**. Immunohistochemical staining for E-cadherin cells. **E.** E-cadherin cells in normotensive placenta, 20x power field, scale bar 50 µm. **F.** E-cadherin cells in the preeclamptic placenta, 20x power field, scale bar 50 µm. **3. G, H**. Immunohistochemical staining for Beta-catenin cells**. G.** Beta-catenin cells in the normotensive placenta, 20x power field, scale bar 50 µm. **H.** Beta-catenin cells in preeclamptic placenta, 20x power field, scale bar 50 µm

Table 1. The primary antibody used in the study

Primary antibody	**Type**	**Host**	**Clone**	**Producer**
CD 4	Monoclonal	Mouse	4B12	DAKO
CD 8	Monoclonal	Mouse	C8/144B	DAKO
E-Cadherin	Monoclonal	Mouse	NCH-38	DAKO
β-catenin	Monoclonal	Mouse	β -Catenin-1	DAKO
CK 18	Monoclonal	Mouse	DC 10	DAKO
CK 19	Monoclonal	Mouse	RCK108	DAKO

P53	Monoclonal	Mouse	DO-7	DAKO
MDM-2	Monoclonal	Mouse	SMP14	Medaysis
CD68	Monoclonal	Mouse	KP1	DAKO

Table 2. Clinical and laboratory data of preeclampsia and control groups

		Preeclampsia n = 32	**Control n = 28**	**p value***
Age (year)*		30.68 ± 6.21	30.75 ± 6.01	0.964
SBP (mm-Hg)*		173.21 ± 12.48	117.50 ± 11.91	< **0.001**
DBP (mm-Hg)*		111.25 ± 6.02	65.63 ± 7.59	< **0.001**
Gravidy***	**Primigravidy**	5 (17.9%)	5 (15.6%)	0.544
	Multigravidy	23 (82.1%)	27 (84.4%)	
Birth week**		32 [25–37]	39 [37–40]	< **0.001**
Platelets (/mm^3)*		218.72 ± 55.03	222.32 ± 49.71	0.792
Hemoglobin (g/dL)*		12.66 ± 1.87	12.45 ± 1.66	0.640
Urea (mg/dL)*		24.46 ± 7.70	16.53 ± 4.55	< **0.001**
Creatinine (mg/dL)*		0.71 ± 0.13	0.59 ± 0.07	< **0.001**
AST (U/L)*		55.29 ± 9.88	22.09 ± 4.43	0.123
ALT (U/L)*		37.82 ± 5.78	12.47 ± 4.28	0.100
LDH (U/L)*		375.91 ± 81.17	291.38 ± 72.14	0.215
EFW**		1416 [400–3225]	3108 [2200–4200]	< **0.001**
Proteinuria (mg/24 hours)**		4127 [350–17444]	Not tested	< **0.001**
Spot urine protein (mg/dL) / creatinine (mg/dL)**		2.67 [0.29–8.61]	Not tested	< **0.001**

*Results were analyzed by independent Samplet-test. (mean ± standard deviation). **Results were analyzed by Kruskal- Wallis test. (median [min-max]). ***Results were analyzed by; Pearson chi-square test. (percent). P value; statistical significance, < 0.05 statistically significant. Statistically, significant p values are marked as bold text. SBP — systolic blood pressure; DBP — diastolic blood pressure; AST — aspartate aminotransferase; ALT — alanine aminotransferase; LDH — lactate dehydrogenase; EFW — estimated fetal weight

Table 3. Placental CD4, CD8, CD4/CD8, CD68, P53, MDM2, CK18, CK19, E-cadherin and Beta-catenin expression between preeclampsia and control groups

	Preeclampsia n = 32	**Control n = 28**	**p value**
CD4 (total number of cells stained in 1 mm^2)	4.14 ± 1.11	20.38 ± 1.75	< **0.001**
CD8 (total number of cells stained in 1 mm^2)	24.46 ± 6.66	56.63 ± 5.29	< **0.001**
CD4/CD8	0.17 ± 0.02	0.36 ± 0.05	< **0.001**
CD68 (total number of cells stained in 1 mm^2)	19.54 ± 1.85	7.97 ± 2.58	< **0.001**

p53	**Negative**	10 (31.35%)	21 (75%)	**0.001**
	Positive	22 (68.75%)	7 (25%)	
MDM2	**Negative**	18 (56.25%)	7 (25%)	**0.022**
	Positive	14 (43.75%)	21 (75%)	
CK18	**0 (negative)**	none	13 (46.4%)	**< 0.001**
	1–4 (weak positive)	28 (87.5%)	12 (42.9%)	**< 0.001**
	5–8 (moderate positive)	4 (12.5%)	none	**N/A**
	9–12 (strong positive)	none	3 (10.7%)	**< 0.001**
CK19	**0 (negative)**	none	10 (35.7%)	**< 0.001**
	1–4 (weak positive)	26 (81.3%)	14 (50%)	**< 0.001**
	5–8 (moderate positive)	6 (18.8%)	4 (14.3%)	**< 0.001**
	9–12 (strong positive)	none	none	**N/A**
E-Cadherin	**0 (negative)**	none	17 (60.7%)	**< 0.001**
	1–4 (weak positive)	32 (100%)	5 (17.9%)	**< 0.001**
	5–8 (moderate positive)	none	6 (21.4%)	**< 0.001**
	9–12 (strong positive)	none	none	**N/A**
Beta-Catenin	**0 (negative)**	none	7 (25%)	**< 0.001**
	1–4 (weak positive)	9 (28.1%)	16 (57.1%)	**< 0.001**
	5–8 (moderate positive)	20 (62.5%)	5 (17.9%)	**< 0.001**
	9–12 (strong positive)	3 (9.4%)	none	

Results were analyzed by Pearson chi-square test (percent). P value; statistical significance, < 0.05 statistically significant. Statistically, significant p values are marked as bold text. MDM2 — murine double minute 2; CK18 — citoceratin 18; CK19 — citoceratin 19; N/A — not applicable

Table 4. Placental CD4, CD8, CD4/CD8, CD68, P53, MDM2, CK18, CK19, E-cadherin and Beta-catenin expression related to EOPE in Preeclampsia

	Unstandardized coefficients		**Standardized coefficients**			**95.0% Confidence interval for B**	
Model	**B**	**Std. Error**	**Beta**	**t**	**Sig.**	**Lower bound**	**Upper bound**
CD4 (Total number of cells stained in 1 mm^2)	0.090	0.076	0.226	1.185	0.247	-0.066	0.245
CD8 (Total number of cells stained in 1 mm^2)	0.019	0.012	0.293	1.561	0.131	–0.006	0.045
CD4/CD8	–1.915	3.463	–0.108	–0.553	0.585	–9.033	5.202
CD68 (Total number of cells stained in 1 mm^2)	0.051	0.046	0.215	1.123	0.272	-0.042	0.145

p53 (positive)	–0.350	0.175	–0.365	–2.000	0.056	–0.710	0.010
MDM2 (positive)	0.292	0.162	0.333	1.803	0.083	–0.041	0.624
CK18	0.048	0.050	0.185	0.961	0.345	–0.055	0.151
CK19	0.011	0.047	0.046	0.236	0.816	–0.086	0.109
E-Cadherin	0.093	0.045	0.376	2.072	**0.048**	0.001	0.185
Beta-Catenin	–0.038	0.037	–0.200	–1.042	0.307	–0.114	0.037

Table 5. Placental CD4, CD8, CD4/CD8, CD68, P53, MDM2, CK18, CK19, E-cadherin and Beta-catenin expression related to FGR in Preeclampsia

	Unstandardized coefficients		**Standardized coefficients**			**95.0% Confidence interval for B**	
Model	**B**	**Std. Error**	**Beta**	**t**	**Sig.**	**Lower bound**	**Upper bound**
CD4 (total number of cells stained in 1 mm^2)	–0.009	0.069	–0.024	–0.124	0.902	–0.150	0.133
CD8 (total number of cells stained in 1 mm^2)	0.002	0.011	0.033	0.169	0.867	–0.022	0.025
CD4/CD8	–0.956	3.075	–0.061	–0.311	0.758	–7.276	5.365
CD68 (total number of cells stained in 1 mm^2)	–0.025	0.041	–0.119	–0.610	0.547	–0.109	0.059
p53 (positive)	–0.075	0.166	–0.088	–0.453	0.654	–0.415	0.265
MDM2 (positive)	0.167	0.148	0.215	1.124	0.271	–0.138	0.471
CK18	0.018	0.045	0.078	0.398	0.694	–0.074	0.110
CK19	0.008	0.042	0.037	0.190	0.851	–0.078	0.094
E-Cadherin	0.093	0.039	0.426	2.399	**0.024**	0.013	0.173
Beta-Catenin	0,009	0,033	0,056	0,285	0,778	–0,059	0,077

Table 6. Placental CD4, CD8, CD4/CD8, CD68, P53, MDM2, CK18, CK19, E-cadherin and Beta-catenin expression related to IUFD in Preeclampsia

Model	Unstandardized coefficients		Standardized coefficients	t	Sig.	95.0% Confidence interval for B	
	B	Std. Error	Beta			Lower bound	Upper bound
CD4 (total number of cells stained in 1 mm^2)	–0.179	0.069	–0.453	–2.590	**0.016**	–0.322	–0.037
CD8 (total number of cells stained in 1 mm^2)	–0.034	0.011	–0.507	–2.998	**0.006**	–0.056	–0.011
CD4/CD8	1.592	3.469	0.090	0.459	0.650	–5.539	8.722
CD68 (total number of cells stained in 1 mm^2)	0.013	0.047	0.057	0.289	0.775	–0.082	0.109
p53 (positive)	0.350	0.175	0.365	2.000	0.056	–0.010	0.710
MDM2 (positive)	–0.146	0.169	–0.167	–0.862	0.397	–0.494	0.202
CK18	0.042	0.050	0.160	0.829	0.415	–0.062	0.145
CK19	0.045	0.047	0.185	0.958	0.347	–0.051	0.141
E-Cadherin	–0.023	0.048	–0.094	–0.482	0.634	–0.122	0.076
Beta-Catenin	0.003	0.037	0.018	0.093	0.927	–0.074	0.080