Enhancing the Retinopathy Of Prematurity Risk Profile Through Placental Evaluation of Maternal and Fetal Vascular Malperfusion

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Purpose. To determine the independent effect of uteroplacental malperfusion on the development of retinopathy of prematurity (ROP).

Methods. This cohort study included 591 neonates with a gestational age (GA) ≤ 32 weeks or birthweight (BW) ≤ 1500 g. Clinical data was retrospectively collected and placentas were prospectively examined for maternal vascular malperfusion (e.g., abruption, infarct, distal villous hypoplasia, ischemia, and decidual necrosis) and fetal vascular malperfusion (e.g., thrombosis, fetal hypoxia, and hydrops parenchyma). The primary outcome was ROP. Secondary outcomes were GA, BW, small for gestational age (SGA), mechanical ventilation duration, postnatal corticosteroids, sepsis, and necrotizing enterocolitis.

Results. Maternal vascular malperfusion was associated with higher GA, lower BW, and increased SGA rates, except placental abruption, which was associated with lower SGA rates. Fetal vascular malperfusion was associated with lower BW, increased SGA rates and lower duration of mechanical ventilation. Subgroup analysis of placentas without inflammation showed increased rates of distal villous hypoplasia (44% vs. 31%) and hydrops parenchyma (7% vs. 0%) in neonates with ROP. Multivariate regression analyses revealed three placenta factors to be independently associated with ROP: distal villous hypoplasia (OR = 1.7; 95% CI, 1.0–3.0), severe acute histological chorioamnionitis (OR = 2.1; 95% CI, 1.1–3.9) and funisitis (OR = 1.8; 95% CI, 1.0–3.1).

Conclusions. Placental evaluation of distal villous hypoplasia, severe acute chorioamnionitis and funisitis is a novel and valuable addition to the ROP risk profile. Evaluation of these placental risk factors shortly after birth can aid in identifying high-risk infants in an earlier stage than currently possible.

Keywords: retinopathy of prematurity, placenta, distal villous malperfusion, histological chorioamnionitis, funisitis

The placenta plays an essential role in providing nutrients and oxygen from mother to fetus. A healthy uterine environment and adequate placenta development is crucial for favorable short- and long-term perinatal outcomes.[1,2] The exact mechanism behind placental abnormalities remains complex but can occur due to maternal rejection of the fetus, infection by pathogens, or placental injury caused by altered uterine blood flow.[3,4] Impaired placenta development is strongly associated with fetal growth restriction and fetal death.[1,2]

Retinopathy of prematurity (ROP) is a sight-threatening disease of the retina caused by abnormal vessel development and occurs in 10% to 25% of all premature neonates born < 32 weeks of gestation.[5] The main risk factors used in ROP screening criteria are low gestational age (GA) at birth and low birthweight (BW). Several countries use extended screening criteria that include additional neonatal risk factors of ROP such as oxygen therapy, (cardio)respiratory support, and sepsis.[6,7] Recently, we confirmed that placental inflammation is directly associated with ROP through acute histological chorioamnionitis (HCA) and funisitis (FUN).[8] However, little is known regarding the association between uteroplacental malperfusion and its correlation with ROP.

Uteroplacental malperfusion can be divided into two groups: maternal vascular malperfusion (MVM) and fetal vascular malperfusion (FVM). MVM is an umbrella term for

histological changes in the maternal decidual vasculature or villous parenchyma, which can affect the maternal circulation to the fetus usually in the form of decreased uterine and intervillous blood flow.[4] FVM is a collective term for pathological lesions mainly caused by umbilical cord obstruction signifying absent or reduced perfusion of the villous parenchyma leading to hypoxia in the fetus.[9]

Insight into the effect of uteroplacental malperfusion may aid in refining the ROP risk factor profile directly after birth and identify high-risk infants in an earlier stage than currently possible. This study aims to explore the independent effect of maternal and fetal vascular malperfusion on the development of ROP.

Methods

Study Design

This cohort study was retrospectively performed according to STROBE guidelines and approved by the Biobank Research Ethics Committee of UMC Utrecht (TCBio 21-662).[10] The cohort consisted of neonates born in the Wilhelmina Children's Hospital in Utrecht in the years 1991 through 1995 and 2001 through 2005. The inclusion criteria were neonates born $\leq$ 32 weeks of GA and/or neonates with a BW $\leq$ 1500 grams. Neonates with the following characteristics were excluded: missing maternal/neonatal data, unarchived or missing placenta, and unattainable differentiation between placentas of twins/triplets.

Histopathological Examination of the Placenta

Histological examination of tissue samples of the placenta parenchyma and membranes was prospectively performed by an experienced perinatal pathologist (L.E.M.). Tissue samples were sliced into blocks of four µm and were formalin-fixed and paraffin-embedded and subsequently stained with hematoxylin and eosin. The placenta was retrospectively examined for the following macroscopic data: placental weight percentile,[11,12] umbilical cord insertion, coiling index (coiling/umbilical cord length), and single umbilical artery.

The placenta was structurally evaluated for abnormalities according to the Amsterdam Placental Consensus statement.[13] Microscopic examination of the placenta included accelerated maturation of placental parenchyma, delayed villous maturation, MVM, FVM, and hydrops parenchyma. MVM was defined as one of the following: abruption, infarct, distal villous hypoplasia (DVH), ischemia, decidual arteriopathy, and decidual necrosis. FVM was characterized by fetal thrombosis, avascular villi, thrombi, intervillous fibrin ($\geq$10%) or fetal hypoxia (i.e., $\geq$1 nucleated red blood cells within villous capillaries per high-power field). Severity of fetal hypoxia was categorized in mild (1–2 cells), moderate (3–5 cells), and severe (>5 cells) fetal hypoxia.[14,15]

Because inflammatory placental lesions have been reported to increase the risk of ROP and thus can influence associations found between uteroplacental malperfusion and ROP, placentas were also evaluated for acute and chronic placental inflammation. A subgroup analysis was performed in which all placentas with inflammatory placental lesions were excluded. Structural microscopic examination of inflammatory placental lesions included: severe acute HCA (i.e., necrotizing chorioamnionitis),[16] FUN (in umbilical vein/artery or chorionic plate),[16] chronic chorioamnionitis,[17] villitis of unknown etiology (i.e., inflammation in contiguous villi in any one focus),[15] resolved villitis (i.e., stromal changes and chronic inflammation in villi with lymphocytes adjacent to villi), focal perivillous fibrin deposition, massive perivillous fibrinoid deposition (>50% of slides), and chronic deciduitis (i.e., presence of lymphocytes/plasma cells in decidual tissue). Severe acute HCA was defined as the presence of necrotizing chorioamnionitis.[16]

Data Collection

Data on perinatal and neonatal outcomes were retrospectively collected from medical records and included: prolonged preterm rupture of membranes $\geq$ 24 hours, age mother at birth, GA at birth, BW, sex, small for gestational age (SGA) (BW < 10th centile),[18] multiple pregnancy, mechanical ventilation duration, antenatal maternal corticosteroids, postnatal corticosteroids, necrotizing enterocolitis $\geq$ stage 2 (NEC),[19] sepsis (positive blood culture and clinical signs), and ROP (most severe stage included).[20] The primary outcome was ROP, and secondary outcomes were GA, BW, SGA, mechanical ventilation duration, postnatal corticosteroids, sepsis, and NEC.

ROP Staging

The International Classification of ROP was used to diagnose ROP and classify severity in five stages.[20] Data on location of retinal involvement in the three retinal zones and presence of plus disease were not available because of the earlier time period of our cohort. The first ROP screening was performed five weeks after birth but not before 31 weeks post menstrual age.

Statistical Analysis

Data was statistically analyzed in IBM SPSS Statistics for Windows, version 29.0 (IBM Corp., Armonk, N.Y., USA). Data is reported as n/N (%) or median with interquartile ranges (IQR). Numerical continuous data was analyzed by the Mann-Whitney U test, whereas the binary categorical data were analyzed by the χ^2 test. Univariate regression analysis was performed for risk factor analysis of ROP. Variables explored in the risk factor analysis of MVM and FVM were based on literature.[8,21] A correlation analysis was performed to detect multicollinearity, and, when present, only one predictor was included. Multivariate regression analysis was performed with significant variables found in univariate analysis. Multivariate regression analysis adjusted for the following: GA at birth, BW, severe acute HCA, distal villous hypoplasia, hydrops parenchyma, mechanical ventilation days, postnatal steroids and sepsis. P values < 0.05 were deemed statistically significant.

Results

A total of 1083 neonates were born with a GA $\leq$ 32 and/or BW $\leq$ 1500 grams and were eligible for inclusion. The application of exclusion criteria (n = 492) resulted in 591 neonates who were included in the final analysis (Fig. 1). Of the included neonates, 145 (25%) had placental inflammation and uteroplacental malperfusion, 160 (27%) had placental inflammation, 211 (36%) had uteroplacental malperfusion, and 75 (13%) had no pathology.

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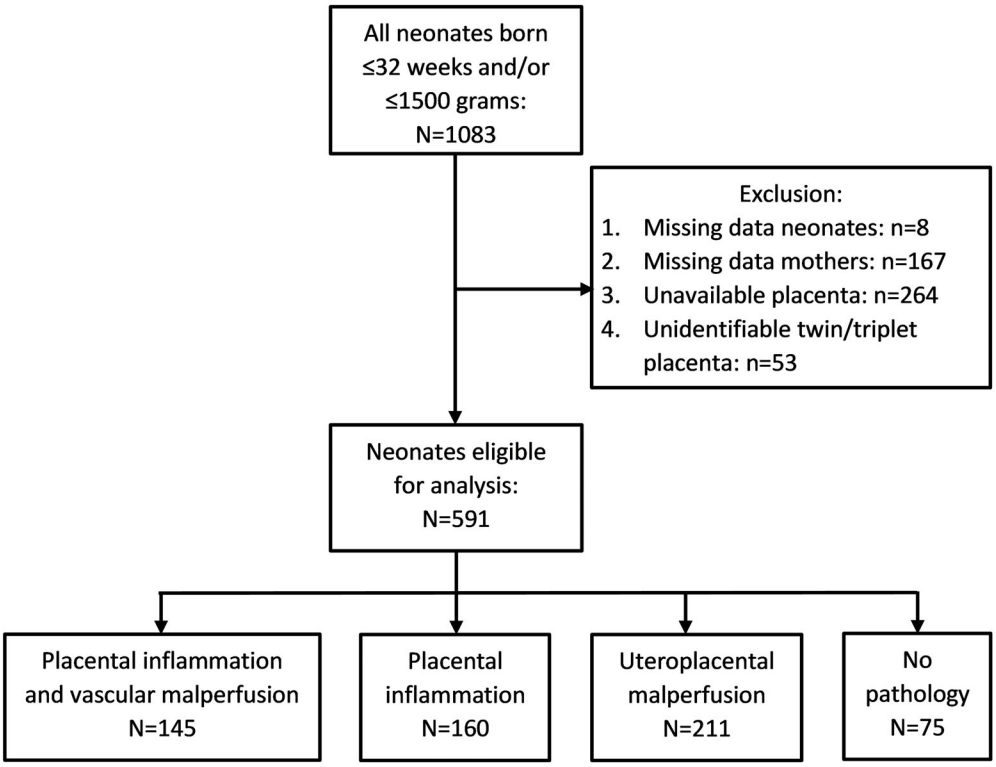

Figure 1. Flowchart of study inclusion.

Table 1. Baseline Characteristics of Total Cohort

Characteristics	Total Cohort ($N = 591$)	Uteroplacental Malperfusion ($N = 356$)	No Uteroplacental Malperfusion ($N = 235$)
Macroscopy			
Placental weight < p10	201/571 (35)	178/348 (51)	23/223 (10)
Umbilical cord insertion			
Central	168/576 (29%)	99/352 (28%)	69/224 (31%)
Paracentral	294/576 (51%)	178/352 (51%)	116/224 (52%)
Marginal	81/576 (14%)	52/352 (15%)	29/224 (13%)
Velamentous	33/576 (6)	23/352 (7)	10/224 (4%)
Coiling index	0.15 (0.1–0.2)	0.17 (0.1–0.2)	0.14 (0.1–0.2)
Single umbilical artery	11/581 (2%)	9/349 (3%)	2/232 (1%)
Microscopy			
Accelerated maturation parenchyma	354/591 (60%)	287/356 (81%)	67/235 (29%)
Delayed villous maturation	42/591 (7%)	25/356 (7%)	17/235 (7%)
Clinical			
Age mother at birth (yrs)	30 (27–34)	30 (27–34)	30 (27–34)
Antenatal steroids	371/583 (64%)	235/353 (67%)	136/230 (59%)
Prolonged rupture of membranes	97/591 (16%)	33/356 (9%)	64/235 (27%)
Multiple pregnancy	162/591 (27%)	64/356 (18%)	98/235 (42%)
Male	307/591 (52%)	181/356 (51%)	126/235 (54%)

Baseline characteristics of the total cohort, categorized by placentas with and without uteroplacental malperfusion (MVM and FVM) are illustrated in Table 1. ROP occurred in 28% (166/591) of the total cohort, of whom 74% (123/166) was classified as stadium 1, 22% (36/166) as stadium 2, and 4% (7/166) as stadium 3. Placentas with uteroplacental malperfusion had a placental weight <p10 in 51% (178/348) of cases compared to in only 10% (23/223) of placentas without uteroplacental malperfusion. Accelerated maturation of the parenchyma is a characteristic feature of maternal vascular malperfusion. This was more prevalent in cases with uteroplacental malperfusion, namely 81% (287/356) compared to 29% (67/235) in placentas without uteroplacental malperfusion.

Of the 591 included neonates, 205 neonates were born in 1991 through 1995 and 386 in 2001 through 2005. The 2001–2005 cohort had increased placental inflammation rates (58% vs. 48%, $P = 0.035$), lower GA (28.9 vs. 29.6, $P = 0.042$), lower duration of mechanical ventilation (4 vs. 9 days, $P < 0.001$), increased postnatal steroids rates (44% vs. 23%, $P < 0.001$), and increased ROP rates (40% vs. 22%, $P < 0.001$) compared to the 1991–1995 cohort.

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Table 2. Maternafl Vascular Malperfusion Predictors of Clinical Risk Factors of ROP

Characteristics	GA	BW	SGA (N = 279)	Mechanical Ventilation	Postnatal Steroids (N = 179)	Sepsis (N = 243)	NEC (N = 42)	ROP (N = 166)
MVM	29.7 (28.2–31.1)‡	1035 (867–1270)‡	222/348 (64)‡	5 (0–14)	110/347 (32)	149/347 (43)	24/348 (7)	96/348 (28)
No MVM	28.7 (27.4–30.4)	1200 (990–1440)	57/243 (23)	6 (0–17)	69/242 (29)	94/243 (39)	18/242 (7)	70/243 (29)
Abruption	29.1 (27.7–30.3)	1125 (915–1354)	26/74 (35)*	7 (0–15)	25/74 (34)	26/74 (35)	6/74 (8)	18/74 (24)
No abruption	29.4 (27.7–31.0)	1100 (905–1358)	253/517 (49)	5 (0–15)	154/515 (30)	217/516 (42)	36/516 (7)	148/517 (29)
Infarction	29.8 (28.6–31.1)‡	963 (793–1160)‡	162/196 (83)‡	5 (0–15)	63/195 (32)	99/195 (51)‡	16/196 (8)	56/196 (29)
No infarction	29.1 (27.4–30.7)	1180 (970–1430)	117/395 (30)	6 (0–15)	116/394 (29)	144/395 (36)	26/394 (7)	110/395 (28)
DVH	30.0 (28.6–31.1)‡	960 (795–1207)‡	114/149 (77)‡	5 (0–15)	47/149 (32)	67/149 (45)	11/149 (7)	45/149 (30)
No DVH	29.1 (27.6–30.8)	1150 (950–1400)	165/442 (37)	6 (0–16)	132/440 (30)	176/441 (40)	31/441 (7)	121/442 (27)
Ischemia	29.9 (28.4–31.1)‡	1028 (850–1246)‡	215/310 (69)‡	5 (0–13)*	99/309 (32)	126/309 (41)	21/310 (7)	85/310 (27)
No ischemia	28.7 (27.3–30.4)	1200 (985–1430)	64/281 (23)	6 (0–17)	80/280 (29)	117/281 (42)	21/280 (8)	81/281 (29)
DA	30.1 (29.0–32.3)	1070 (780–1120)	18/19 (95)‡	0 (0–8)	2/19 (11)	10/19 (53)	4/19 (21)*	2/19 (11)
No DA	29.3 (27.7–30.9)	1110 (910–1365)	261/572 (46)	6 (0–15)	177/570 (31)	233/571 (41)	38/571 (7)	164/572 (29)
Decidual necrosis	29.6 (28.3–30.4)	925 (750–1080)‡	55/64 (86)‡	6 (0–18)	27/64 (42)*	27/64 (42)	5/64 (8)	20/64 (31)
No decidual necrosis	29.3 (27.7–31.0)	1130 (925–1380)	224/527 (43)	5 (0–15)	152/525 (29)	216/526 (41)	37/526 (7)	146/527 (28)

P values are based on the difference between presence and absence of a placental risk factor.
* $P < 0.05$.
† $P < 0.01$.
‡ $P < 0.001$.

MVM and its association with clinical risk factors of ROP was analyzed in Table 2. Neonates with placental infarction, DVH and ischemia were delivered at a later GA, had a lower BW and increased SGA rates ($P < 0.001$). Sepsis occurred more often in neonates with placental infarction (51% vs. 36%, $P < 0.001$). Decidual arteriopathy was the only variable associated with increased NEC rates (21% vs. 7%, $P = 0.039$) and had the highest SGA incidence (95% vs. 46%, $P < 0.001$). Neonates with decidual necrosis had a lower BW and increased SGA rates ($P < 0.001$). Decidual necrosis was the only variable associated with increased postnatal steroids rates (42% vs. 29%, $P = 0.030$).

In Table 3, FVM and its association with clinical risk factors of ROP are presented. Neonates with thrombosis and avascular villi were born with a lower BW ($P < 0.05$), increased SGA rates ($P < 0.001$) and lower duration of mechanical ventilation ($P < 0.05$). Fetal hypoxia was associated with a higher GA ($P = 0.017$), lower BW ($P < 0.001$) and increased SGA rates ($P < 0.001$). Severe fetal hypoxia was associated with a lower birthweight (785 vs. 1115 grams, $P < 0.001$) and increased SGA rates (100% vs. 46%, $P < 0.001$). Neonates with parenchymal hydrops had lower SGA rates (19% vs. 48%, $P = 0.021$) and increased mechanical ventilation duration (13 vs. 5 days, $P = 0.037$).

The association between uteroplacental malperfusion and ROP was analyzed in placentas without placental inflammation ($n = 286$) in Table 4. Placentas with placental inflammation ($n = 160$) and placentas with both placental inflammation and uteroplacental malperfusion ($n = 145$) were excluded from this analysis. ROP occurred more often in neonates with DVH (44% vs. 31%, $P = 0.039$) and parenchymal hydrops (7% vs. 0%, $P = 0.005$).

In Table 5, risk factor analysis for ROP is performed. Correlation analysis revealed significant correlations between GA and BW (coefficient: 0.484, $P < 0.001$) and between severe acute HCA and FUN (coefficient: 0.707, $P < 0.001$). When adjusting for GA and risk factors of ROP, multivariate analysis showed four factors to be independently associated with ROP: DVH (OR 1.7; 95% CI, 1.0–3.0), lower GA (OR = 1.3; 95% CI, 1.1–1.5), mechanical ventilation days (OR = 1.1; 95% CI, 1.0–1.1) and postnatal steroids (OR = 3.4; 95% CI, 2.0–5.6). When adjusting for BW and risk factors of ROP, multivariate analysis showed that severe acute HCA (OR = 2.1; 95% CI, 1.1–3.9), lower BW (OR = 1.0; 95% CI, 1.0–1.0), mechanical ventilation days (OR = 1.1; 95% CI, 1.0–1.1) and postnatal steroids (OR = 3.2; 95% CI, 2.0–5.4) were independently associated with ROP. Separate multivariate analysis for FUN showed an independent association with ROP (OR = 1.8; 95% CI, 1.0–3.1). Histological imaging of DVH, severe acute HCA and FUN are illustrated in Figure 2.

Discussion

This retrospective cohort study illustrated that MVM is associated with higher GA at birth, lower BW and increased SGA rates, and that FVM is associated with lower BW, increased SGA rates and lower duration of mechanical ventilation. To our knowledge, this is the first study that has structurally investigated the role of all individual uteroplacental malperfusion pathologies, while also adjusting for acute placental inflammation and known perinatal and neonatal risk factors of ROP in a large cohort. Multivariate risk factor analysis revealed three independent placental risk factors of ROP: DVH, severe acute HCA and FUN.

Table 3. Fetal Vascular Malperfusion Predictors of Clinical Risk Factors of ROP

Characteristics	GA	BW	SGA ($N = 279$)	Mechanical Ventilation
FVM	29.6 (28.9–31.0)	968 (788–1153)†	34/46 (74)‡	2 (0–8)*
No FVM	29.3 (27.7–30.9)	1120 (915–1370)	245/545 (45)	6 (0–15)
Thrombosis	29.6 (28.9–31.0)	968 (788–1153)†	34/46 (74)‡	2 (0–8)*
No thrombosis	29.3 (27.7–30.9)	1120 (915–1370)	245/545 (45)	6 (0–15)
Avascular villi	29.6 (28.7–31.0)	970 (790–1160)*	31/43 (72)‡	2 (0–8)*
No avascular villi	29.3 (27.7–30.9)	1120 (911–1369)	248/548 (45)	6 (0–15)
Thrombi	29.5 (27.4–30.8)	950 (780–1071)	4/6 (67)	2 (0–19)
No thrombi	29.3 (27.7–30.9)	1110 (910–1360)	275/585 (47)	6 (0–15)
Intervillous fibrin	29.7 (27.9–31.6)	1123 (881–1400)	46/88 (52)	5 (0–11)
No intervillous fibrin	29.3 (27.7–30.9)	1110 (910–1355)	233/503 (46)	6 (0–15)
Fetal hypoxia	29.7 (28.0–31.1)*	1000 (795–1205)‡	118/165 (72)‡	6 (0–14)
No fetal hypoxia	29.1 (27.7–30.7)	1150 (948–1400)	161/426 (38)	5 (0–16)
Severe fetal hypoxia	29.4 (27.7–30.9)	785 (671–944)‡	14/14 (100)‡	2 (0–16)
No/mild fetal hypoxia	29.3 (27.7–30.9)	1115 (915–1365)	265/577 (46)	6 (0–15)

P values are based on the difference between presence and absence of a placental risk factor.
* $P < 0.05$.
† $P < 0.01$.
‡ $P < 0.001$.

Table 4. Comparison of MVM and FVM Between ROP and No ROP

Characteristics	ROP ($N = 75$)	No ROP ($N = 211$)	*P* Value
MVM			
Infarction	37/75 (49%)	91/211 (43%)	0.353
Distal villous hypoplasia	33/75 (44%)	65/211 (31%)	**0.039**
Ischemia	53/75 (71%)	136/211 (64%)	0.329
Decidual necrosis	11/75 (15%)	23/211 (11%)	0.387
FVM			
Thrombosis	9/75 (12%)	16/211 (8%)	0.245
Fetal hypoxia	35/75 (47%)	76/211 (36%)	0.104
Other placenta morbidity			
Hydrops parenchyma	5/75 (7%)	1/211 (0%)	**0.005**

P values < 0.05 are depicted in bold.

The first phase of ROP is initiated directly after birth, in which the retina is exposed to a hyperoxic environment compared to intrauterine, resulting in attenuation and cessation of retinal vessel growth.[22] Proangiogenic factors play a crucial role in this phase of ROP, since insulin-like growth factor-1 (IGF-1) levels are decreased due to placental disruption and concentration of vascular endothelial growth factor (VEGF) is decreased because of hyperoxia and decreased IGF-1 levels.[23] Thus far, the incidence of ROP is still high, and its onset cannot be prevented because many of the known clinical risk factors of ROP cannot be avoided during the treatment of extremely preterm neonates.

Literature suggests that its pathophysiology may begin before birth due to an unhealthy uterine environment.

Table 5. Risk Factor Analysis for Any Stage ROP

	Univariate Analysis			Multivariate Analysis*			Multivariate Analysis†		
Characteristics	OR (95% CI)	SE	*P* Value	OR (95% CI)	SE	*P* Value	OR (95% CI)	SE	*P* Value
Severe acute HCA (%)	2.4 (1.5–3.9)	0.249	**<0.001**	1.5 (0.6–3.6)	0.446	0.378	2.1 (1.1–3.9)	0.320	**0.021**
Funisitis (%)‡	2.0 (1.3–3.0)	0.213	**0.001**	*1.4 (0.8–2.4)*	*0.288*	*0.297*	*1.8 (1.0–3.1)*	*0.278*	***0.040***
Distal villous hypoplasia (%)	1.1 (0.8–1.7)	0.208	0.507	1.7 (1.0–3.0)	0.267	**0.037**	1.2 (0.7–2.0)	0.269	0.548
Hydrops parenchyma (%)	2.6 (1.0–7.2)	0.509	0.056	1.7 (0.5–6.2)	0.655	0.411	2.0 (0.5–8.0)	0.705	0.321
Lower GA at birth (wks)	1.7 (1.5–2.0)	0.060	**<0.001**	1.3 (1.1–1.5)	0.078	**<0.001**	—	—	—
Lower BW (gm)	1.0 (1.0–1.0)	0.000	**<0.001**	—	—	—	1.0 (1.0–1.0)	0.000	**<0.001**
Mechanical ventilation (d)	1.1 (1.1–1.1)	0.011	**<0.001**	1.1 (1.0–1.1)	0.013	**<0.001**	1.1 (1.0–1.1)	0.012	**<0.001**
Postnatal steroids (%)	9.0 (6.0–13.6)	0.208	**<0.001**	3.4 (2.0–5.6)	0.258	**<0.001**	3.2 (2.0–5.4)	0.259	**<0.001**
Sepsis (%)	1.8 (1.2–2.5)	0.185	**0.002**	1.4 (0.9–2.1)	0.231	0.177	1.3 (0.8–2.0)	0.233	0.312

P values < 0.05 are depicted in bold.
* Variables used in this model are GA at birth, severe acute HCA, distal villous hypoplasia, hydrops parenchyma, mechanical ventilation days, postnatal steroids and sepsis.
† Variables used in this model are BW, severe acute HCA, distal villous hypoplasia, hydrops parenchyma, mechanical ventilation days, postnatal steroids and sepsis.
‡ Separate multivariate analysis was performed for funisitis to avoid multicollinearity with severe acute HCA.

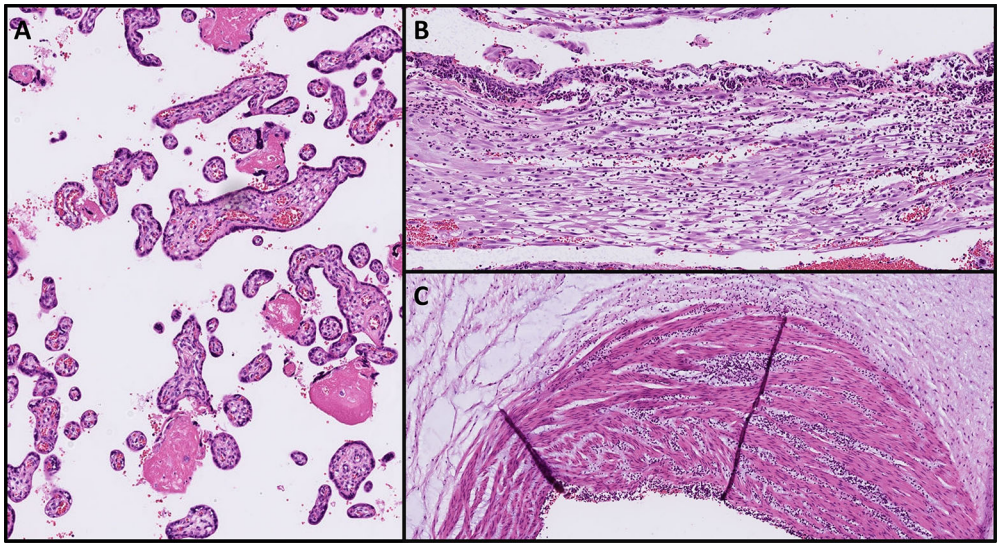

Figure 2. (**A**) Distal villous hypoplasia with elongated long villi, hematoxylin-eosin (HE) staining (5 mm). (**B**) Severe acute chorioamnionitis in membranes (amnion and chorion), HE staining (10 mm). (**C**) Funisitis, neutrophil infiltrate in the vascular wall of the umbilical vein, HE staining (5 mm).

An extensive meta-analysis by our team confirmed acute placental inflammation in the placental membranes (HCA) and umbilical cord (FUN) as risk factors of ROP (OR = 1.8).[8] To further explore this association, our current cohort study structurally investigated the independent role of placental inflammation and determined a 2.1-fold increased risk of ROP in neonates with severe HCA and 1.8-fold increased risk in neonates with FUN after adjusting for known risk factors of ROP. Some studies have reported contrasting results regarding the role of HCA/FUN, and this is presumably due to not using the golden standard for placenta histology and not adjusting for crucial neonatal risk factors of ROP such as mechanical ventilation and sepsis.[8,24] Furthermore, it is plausible to assume that FUN, an additional fetal inflammatory response, would increase the risk of ROP because this could result in early-onset neonatal sepsis, which is a crucial risk factor of ROP.[8,24]

In the current study, we explored whether uteroplacental malperfusion also played a role in ROP development while taking HCA/FUN into account and we have now introduced a new placental risk factor: uteroplacental malperfusion in the distal villi (DVH). Because uteroplacental malperfusion can lead to hypoxia-reperfusion injury in the fetus, the increased risk of ROP in neonates with DVH could perhaps be explained by the higher difference in retinal oxygen exposure before and after birth, resulting in the initiation of phase I. Preclinical studies have reported increased IGF-1 and retinal VEGF levels in rat pups/mice with maternal uteroplacental insufficiency and ROP compared to controls.[25,26] However, these results are very difficult to translate to preterm neonates with ROP because premature birth was excluded and severe uteroplacental malperfusion was induced by bilateral uterine artery ligation.

Remarkably, a study by Agrawal et al.[27] found that mothers who developed MVM experienced gradually declining maternal circulating placental growth factor (PlGF) levels as pregnancy progressed (81.4% had PlGF levels < 10th percentile in week 28–32 of gestation).[27] PlGF is synthesized in the placental villi and damage to the placental villi, which occurs in DVH, prevents the physiological rise in circulating PlGF levels. The role of PlGF in retinal angiogenesis is still unclear, but it is an important cofactor of FLT-1 (a VEGF receptor), which affects the angiogenic response by increasing VEGF activity and expression.[28] Hence, reduced levels of PlGF, which occur in pregnancies with DVH, could in theory decrease retinal VEGF levels and induce the first phase of ROP.

MVM is most commonly found in pregnancies with preeclampsia, fetal death, intrauterine growth restriction and spontaneous preterm birth.[4] Budal et al.[29] (n = 123) reported no association between MVM and severe ROP in neonates born < 28 weeks of GA. Besides MVM as a whole, our study also explored individual pathological lesions classified as MVM and found a lower BW and increased SGA rates in neonates with placental infarction (extensive necrosis of villous cells), DVH (inadequately developed distal villous tree), ischemia (restricted/reduced blood flow in placenta), and decidual necrosis (band of coagulative necrosis at the choriodecidual interphase). MVM can result in severe fetal hypoxia which was associated with the lowest BW and highest SGA rates in our cohort and, thus, emphasizes the importance of adequate placenta development in perinatal outcomes.

FVM is most common in pregnancies with fetal growth restriction, fetal death and fetal cardiac insufficiency.[9] Çakir et al.[30] investigated the overall effect of maternal and fetal vasculopathy and reported no association with severe ROP and significant associations with lower BW and increased SGA rates. Our study found similar results in placentas with thrombosis (obstruction of umbilical arteries or vein) and avascular villi (loss of villous capillaries). Fetal vascular thrombosis is induced by the Virchow's triad, namely: stasis, hypercoagulability and damage of the endothelial/vessel wall.[30] The endothelial/vessel wall can be damaged by severe inflammation such as FUN, which is an independent risk factor of ROP.[8]

This study reported a significant association between MVM and higher GA, whereas previous studies have shown

a significant association between placental inflammation and lower GA.[8] In clinical practice, gynecologists prefer to leave the fetus in utero for as long as possible if the mother's condition and cardiotocography are stable in order to allow the lungs to further mature. Pregnancies will be induced earlier if placental inflammation is suspected, which could explain the opposite found results. Additionally, our study reported increased SGA rates in neonates with FVM, but lower mechanical ventilation duration. An identical twin study by Groene et al.[31] demonstrated that neonates with SGA have a reduced odds of respiratory distress syndrome, but a more than doubled odds of bronchopulmonary dysplasia. Neonates with SGA are exposed to prolonged prenatal stress, which could increase the production of corticosteroids and result in a decreased risk of acute lung injury and thus decreased mechanical ventilation duration.

Remarkably, when excluding neonates with placental inflammation, increased rates of DVH and hydrops parenchyma were found in infants with ROP. Acute severe HCA and FUN are independently associated with ROP, which could affect the results when present in placentas with uteroplacental malperfusion. This was confirmed in multivariate regression analysis, which showed an independent association between DVH and ROP after adjusting for placental inflammation. Thus, when investigating the effect of uteroplacental malperfusion on ROP, it is crucial to adjust for acute placental inflammation and vice versa. DVH is characterized by maldevelopment of the distal villous tree with sparse villi and widening of the intervillous space, which can lead to intrauterine growth restriction.[32] The villous tree plays a crucial role in maternal-fetal blood exchange, which can result in reduced blood flow to the fetus when maldeveloped. Hence, neonates with DVH are at risk of initiating phase I of ROP.

Even though GA and BW are both necessary components for the onset of ROP and modify the likelihood of occurrence, it is not the cause of ROP.[24] Oxygen exposure together with inflammation/infection are the causal initiators of ROP.[24] Neonatal outcomes were comparable when adjusting for the risk factors GA or BW because of their collinearity. With regard to placental abnormalities, GA and BW are outcomes and can therefore differ. We demonstrated that GA at birth plays a role in placental inflammation, whereas BW plays a role in uteroplacental malperfusion in multivariate regression analysis.

The main limitation of this study was its retrospective design, which may have introduced bias. Additionally, the small sample size of severe ROP ($n = 7$) hampered risk factor analysis. Furthermore, our cohort consists of placentas from 20 to 30 years ago and treatment of pregnant women may have improved since then. Nevertheless, this study provides the first complete evaluation of uteroplacental malperfusion and placental inflammation in a large cohort of neonates eligible for ROP screening, and novel and detailed information regarding the direct effect of placental abnormalities on ROP and the indirect effect through established and crucial clinical risk factors of ROP.

To expand our understanding of the intrinsic mechanism of ROP progression, future studies should further investigate this found effect of DVH, severe acute HCA and FUN on ROP development, specifically their influence on proangiogenic growth factors such as VEGF, IGF-1 and PlGF during pregnancy in a preclinical setting. Because circulating serum levels of proangiogenic growth factors are not specific to the retina, retinal growth factor levels after birth should be compared between rat pups/mice with and without placenta pathology. Additionally, it would be plausible to identify the molecular placenta profiles of the established placental risk factors by comparing the methylation profiles of placentas with and without ROP in a case-control study. Subsequently, the identified molecular placenta profiles can be used as biomarkers during pregnancy in the form of a maternal blood test.[33] Furthermore, it would be informative to test, in a preclinical study, if there is less ROP progression in successfully treated cases with placental pathology. In the future, these results from preclinical and clinical studies could be used to develop additional treatment options targeting DVH, severe acute HCA and FUN, which can potentially be implemented before birth in order to prevent ROP development and progression.

In conclusion, this study shows that placental evaluation of DVH can be valuable in predicting the development of ROP. Histological confirmation of DVH, severe acute HCA and FUN in the first postnatal days of life will be a novel and valuable tool to identify high-risk neonates in an earlier stage than currently possible, which can then be used in a personalized neonatal treatment approach for ROP prevention. In the future, these newly found placental risk factors may be used as placental therapy targets to prevent ROP in these vulnerable extremely premature neonates.

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