Original paper

Placental recent/on-going foetal vascular malperfusion with endothelial fragmentation is diagnostically equivalent to established distal villous lesions of foetal vascular malperfusion

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CD34 immunostaining increases the sensitivity of placental diagnosis of foetal vascular malperfusion (FVM). This comparative retrospective study was performed to find out whether recent distal FVM lesions diagnosed with CD34 are diagnostically equivalent to remote FVM lesions diagnosed with haematoxylin-eosin (H&E).
Clinical and placental phenotypes of 562 placentas from ≥ 20-week, high-risk pregnancies were analysed: Group 1–158 placentas with remote distal villous FVM (by H&E only), Group 2–142 placentas showing clustered endothelial fragmentation by CD34 immunostaining, 98 of them also with H&E distal FVM lesions (on-going, temporal heterogeneity), and Group 3–262 placentas without distal villous FVM.
In Group 1, gestational age was the shortest, postnatal mortality most frequent, placental weight the smallest, and intra villous haemorrhage, erythroblasts in foetal blood, hypertrophic decidual arteriopathy, and foetal vascular thrombi most common. In Group 2, placental infarction, post-uterine pattern of chronic placental injury, and excessive extra villous trophoblasts of chorionic disc were most common ($p < 0.05$).
In this cohort of foetuses/neonates dominated by congenital malformations, distal villous FVM was the most common pattern of placental injury, and those diagnosed by CD34 and by H&E are diagnostically/prognostically equivalent. CD34 immunostaining is therefore a powerful tool in the diagnosis of distal villous FVM.

Key words: placenta, foetal vascular malperfusion, CD34 immunohistochemistry, congenital malformations.

Introduction

Placental examination is the most useful tool in determining complicated perinatal outcomes. Foetal vascular malperfusion (FVM) is one of 4 major patterns of placental injury, but in an unselected population of placentas its frequency follows that of inflammatory and hypoxic lesions and patterns [1]. Foetal vascular malperfusion may involve large proximal muscularized foetal vessels of the placenta or distal chorionic villi, the latter being more prognostically important. Distal FVM was seen in 1–5% of all pregnancies [2]. Antenatal findings in FVM include decreased foetal movements, foetal heart rate decelerations, and prolonged meconium exposure. Neonatal complications may manifest with end-organ thrombi, decreased platelets, foetal death, neonatal encephalopathy, foetal stroke, cerebral palsy, and mild fetal growth restriction (FGR) [1]. Those neonatal complications were more often seen in segmental and high-

grade than in global and low-grade FVM [3]. Mild FVM can also occur in pregnancies with a normal/uncomplicated outcome (19.7%), thus representing a subclinical pathological process, but high-burden lesions were reported in only 0.7% of placentas [4]. The overall recurrence risk of FVM is low, as is global FVM associated with umbilical cord (UC) compromise, but segmental FVM has a moderately increased risk of recurrence if predisposing maternal conditions are present [5].

Foetal vascular malperfusion is caused by foetal circulatory stasis or thrombophilia. Features of symptomatic thrombophilia with prenatal placental foetal thrombotic vasculopathy can be seen postnatally in neonatal organs [4]. Clinical cord entanglement and pathological cord abnormalities should be sought in placentas with FVM [6]. Umbilical cord complications as a group are associated with a significant increase in stasis-induced vasculopathy and foetal hypoxia [7], but confirmation of clinical UC compromise should not be expected on placental examination if no morphological UC abnormality or abnormal UC insertion has been found. In a group of cases with clinical UC compromise and UC pathology, multiple other abnormal clinico-placental phenotypes were also revealed [8]. Macerated third trimester stillbirths are more likely to have multifactorial aetiology than the second trimester stillbirths, and stasis-induced FMM secondary to occult UC compromise should also be considered in the placental examination in such cases [9].

To diagnose FVM that is histologically unapparent on haematoxylin-eosin (H&E), CD34 immunostaining and iron histochemistry can be used [10, 11]. For several years, the author has used such double immunostaining for the diagnosis of placental hypomaturity/dysmaturity (highlighting widening of vasculosyncytial syncytial membranes), chronic hypoxic patterns of placental injury (increased density of villous cytotrophoblasts by E cadherin, and villous vascularity by CD34), and, most recently, for the diagnosis of recent distal villous FVM when clusters of avascular villi are not seen on H&E staining, but endothelial fragmentation is highlighted by CD34 [10, 11]. In the latter application, the immunostaining increased the sensitivity of placental FVM diagnosis in stillbirth, was capable of upgrading low-grade FVM to high grade by increasing the number of involved distal chorionic villi (not seen on H&E staining) [11], and of disclosing temporal heterogeneity of FVM lesions by adding more recent lesions with clustered endothelial fragmentation (2 days) to other established lesions of FVM: clustered stromal vascular karyorrhexis (3 days), clusters of hypovascular distal villi (several days), clusters of sclerotic chorionic villi (2 weeks), or mineralized chorionic villi (> 2 weeks) [12, 13]. We also showed that distal villous lesions of FVM are clinically more relevant than proximal large-vessel FVM lesions [14] and that high-grade FVM is associated with adverse short-term perinatal outcome whether diagnosed by H&E, immunohistochemistry, or histochemistry staining [3].

It is still unclear, however, whether the lesions diagnosed with CD34 are diagnostically/prognostically equivalent to those diagnosed with H&E staining only, which is the purpose of this study.

Material and methods

This comparative retrospective study has been approved by the institutional review board, ID 2011–3136. The material consisted of 562 consecutive placentas from ≥ 20-week, high-risk pregnancies in which at least one slide of grossly normal placental parenchyma was stained with E-cadherin/CD34 immunostaining to disclose the potential recent distal villous FVM (clustered endothelial fragmentation by CD34 immunostaining). The placentas were submitted for pathology examination at the discretion of the clinicians because of a high-risk pregnancy or complicated perinatal outcome. Placental examination was performed according to standard criteria: at least 2 sections of umbilical cord, 2 sections of placental membranes, and at least 2 sections of grossly normal placental parenchyma were submitted, in addition to sections of all grossly identifiable lesions. The samples were fixed in buffered formalin, followed by paraffin-embedding and staining with haematoxylin-eosin. Definitions of placental lesions and patterns were according to the standard criteria provided by the Amsterdam consensus conference [15], but other lesions not included there and defined in other authors' publications were also analysed [16–24].

The material was divided into 3 groups. Group 1 comprised 158 placentas with FVM diagnosed on H&E staining of at least 3 days' duration (stromal-vascular karyorrhexis) or longer, such as clustered hypovascularity of distal villi (several days), clustered sclerotic chorionic villi (2 weeks), or clustered segmental villous mineralization (more than 2 weeks) [5]. The criteria of at least low-grade FVM were fulfilled, i.e. at least 3 clusters of 2–4 involved distal villi [15] (Fig. 1). Group 2 included 142 placentas with recent (2 days duration) or ongoing (recent lesions present together with established/remote changes) distal villous FVM diagnosed based on endothelial fragmentation highlighted by the CD34 component of the dual E cadherin/CD34 immunostaining (clustered villous endothelial fragmentation or hypovascularity chorionic villi by CD34 immunostaining in comparison to adjacent normovascular chorionic villi) [11], also fulfilling the above-mentioned quantitative criteria of at least low-grade FVM as in Group 1 (Fig. 2). Grading of FVM or the presence of large vessel lesions (global

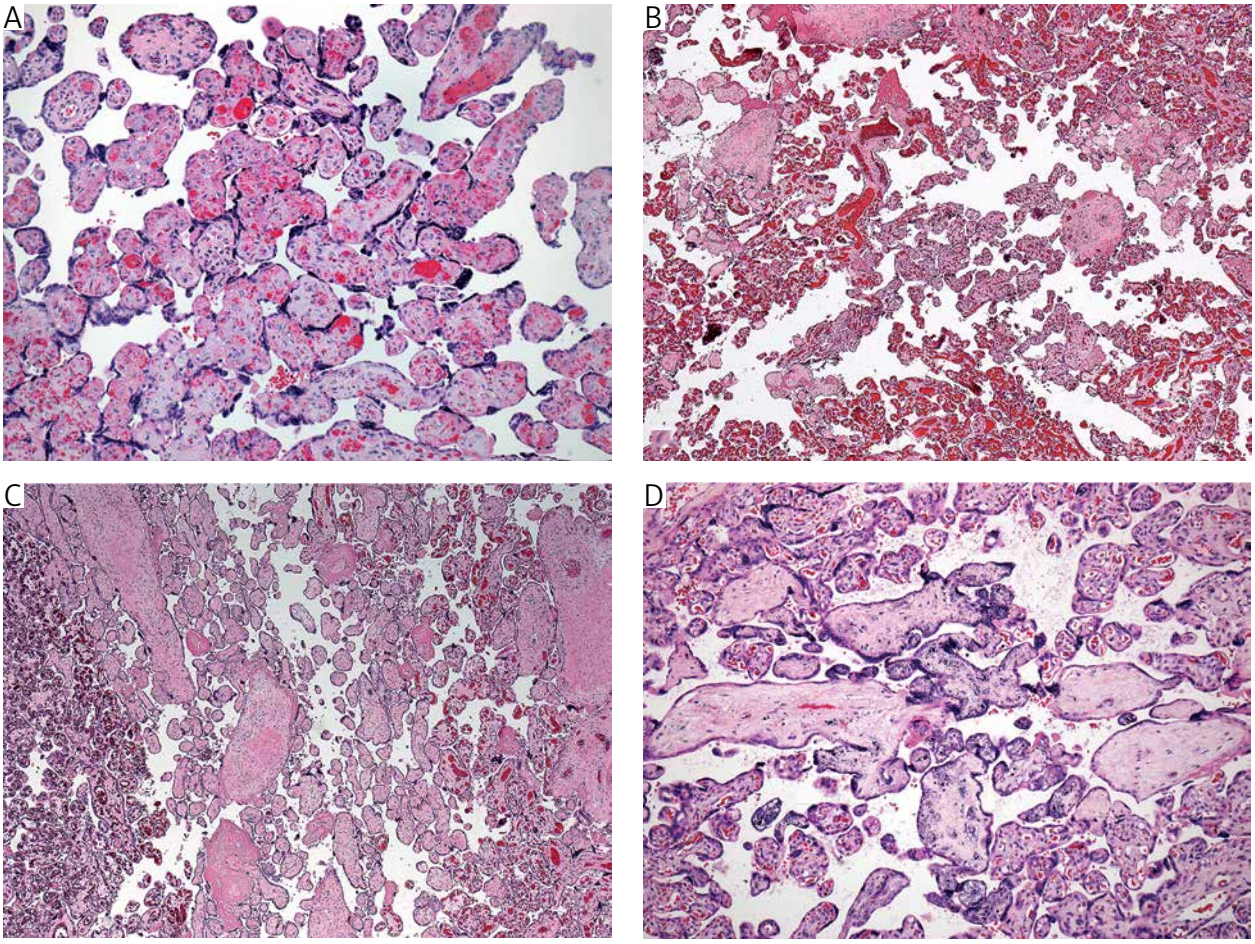

Fig. 1. Distal villous foetal vascular malperfusion diagnosable on haematoxylin-eosin staining (Group 1), objective magnifications given. A) Stromal vascular karyorrhexis, 10×, 39 weeks, bladder outlet obstruction, double nuchal cord; B) low grade, 4×, 38 weeks, intrapartum foetal demise, diaphragmatic hernia, Goltz syndrome; C) high grade, 4×, 39 weeks, MTFHR (methylenetetrahydrofolate reductase) mutation, gestational diabetes mellitus; D) almost avascular and focally mineralized villi, 10×, same case as (A), indicative of temporal heterogeneity

FVM) (Fig. 3) was not used for group allocation, but Group 2 thus included either solely a recent FVM (low or high grade) or low-grade FVM by H&E but upgraded to high grade by CD34 immunostaining (temporal heterogeneity, ongoing) [13]. Group 3 included 262 placentas without distal villous FVM.

Differences in clinical variables (25 independent phenotypes) and placental variables (48 independent phenotypes) among the groups were evaluated with the χ^2 test or analysis of variance where appropriate.

Results

Gestational age was the shortest in Group 1 and the longest in Group 3, all averages being in the preterm range, and neonatal deaths were most common in Groups 1 and 2. Congenital malformations dominated all 3 groups, being present in 58.5% of all material, and there were no significant differences in clinical signs or symptoms of UC compromise among the groups (Table I).

Distal villous FVM was the most common pattern of placental injury in this material (53.4%). More differences were seen in placental phenotypes than in clinical phenotypes (Table I vs. Table II) ($p < 0.05$). In Groups 1–3, the placental weight was proportional to the average gestational age at delivery, being the lowest in Group 1 and the highest in Group 3. An acute placental hypoxic change (intra-villous haemorrhage), stress-related hypoxic change (erythroblasts in foetal blood), hypertrophic decidual arteriopathy, and foetal vascular thrombi were more common in Group 1 than in Groups 2 and 3, while the post-uterine pattern of chronic hypoxic (developmental) placental injury [21] and excessive amount of extravillous trophoblasts of the chorionic disc (a lesion of shallow placental implantation) [24] were more common in Group 2 than in Groups 1 and 3. There were no differences in frequencies of lesions of large-vessel FVM (other than foetal vascular thrombi), UC abnormalities, or features of shallow placental implantation (other than

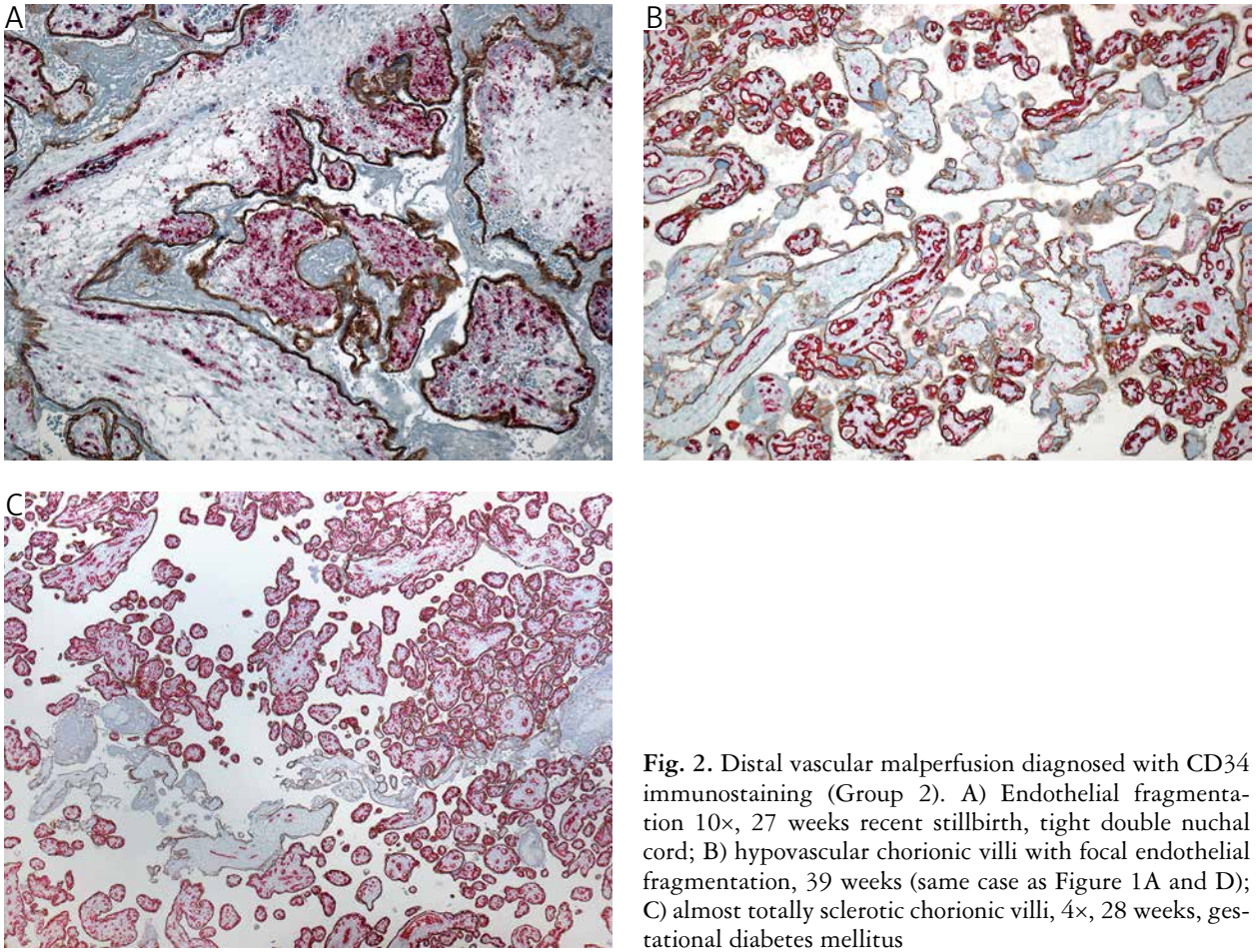

Fig. 2. Distal vascular malperfusion diagnosed with CD34 immunostaining (Group 2). A) Endothelial fragmentation 10×, 27 weeks recent stillbirth, tight double nuchal cord; B) hypovascular chorionic villi with focal endothelial fragmentation, 39 weeks (same case as Figure 1A and D); C) almost totally sclerotic chorionic villi, 4×, 28 weeks, gestational diabetes mellitus

the excessive amount of extravillous trophoblasts in the chorionic disc) (Table II).

Discussion

We previously reported clusters of avascular villi in 7% of our otherwise unselected material of high-risk pregnancies [25]. Other authors reported 8.7%, with a similar frequency of global and segmental FVM, with high-grade FVM more common than low-grade FVM [26]. We reported FVM in 10–24% of placentas from pregnancies complicated by congenital malformations, depending on the type of malformation, but in that material distal FVM was diagnosed traditionally based on clustered avascularity of distal villi on H&E staining (27). In the currently analysed population of high-risk pregnancies dominated by foetal congenital malformations, the prevalence of FVM was substantially higher because of expanding the inclusion criteria (endothelial fragmentation and hypovascularity by CD34, and segmental villous mineralization in addition to clusters of sclerotic distal villi and clusters of villi with stromal vascular karyorrhexis). Although the UC compromise is regarded as the most common cause of FVM, in the currently analysed material, the clinical signs of UC compromise were seen in 10–14% of cases in the 3 groups, 3 times or less frequently than congenital anomalies (Table I), and various anatomical abnormalities of the UC and its insertion were similarly less common (Table II).

The absence of statistically significant differences in many clinical or placental phenotypes between Groups 1 and Group 2, but also among all 3 groups, indicates that FVM is only one of the patterns of placental injury having an impact on the foetal condition in this specific type of pathology dominated by frequently severe foetal congenital anomalies. Previously we reported that foetal anomalies in the second half of pregnancy show abnormal clinical phenotypes much more frequently than abnormal placental phenotypes, the mass-forming anomalies featuring diffuse chronic hypoxic patterns of placental injury and lesions of FVM, which are probably stasis-induced [27]. Our current material obtained in the Children's Hospital contained few hypertensive conditions of pregnancy, diabetes mellitus, infections, chronic FHR abnormalities, genetic thrombophilia, FGR, and oligohydramnios, i.e. the conditions known to be associated with FVM [28–30], but less frequently in our material than in UC compression [31]. Foetal growth restriction was reported to be associated with maternal background morbidities during pregnancy [32],

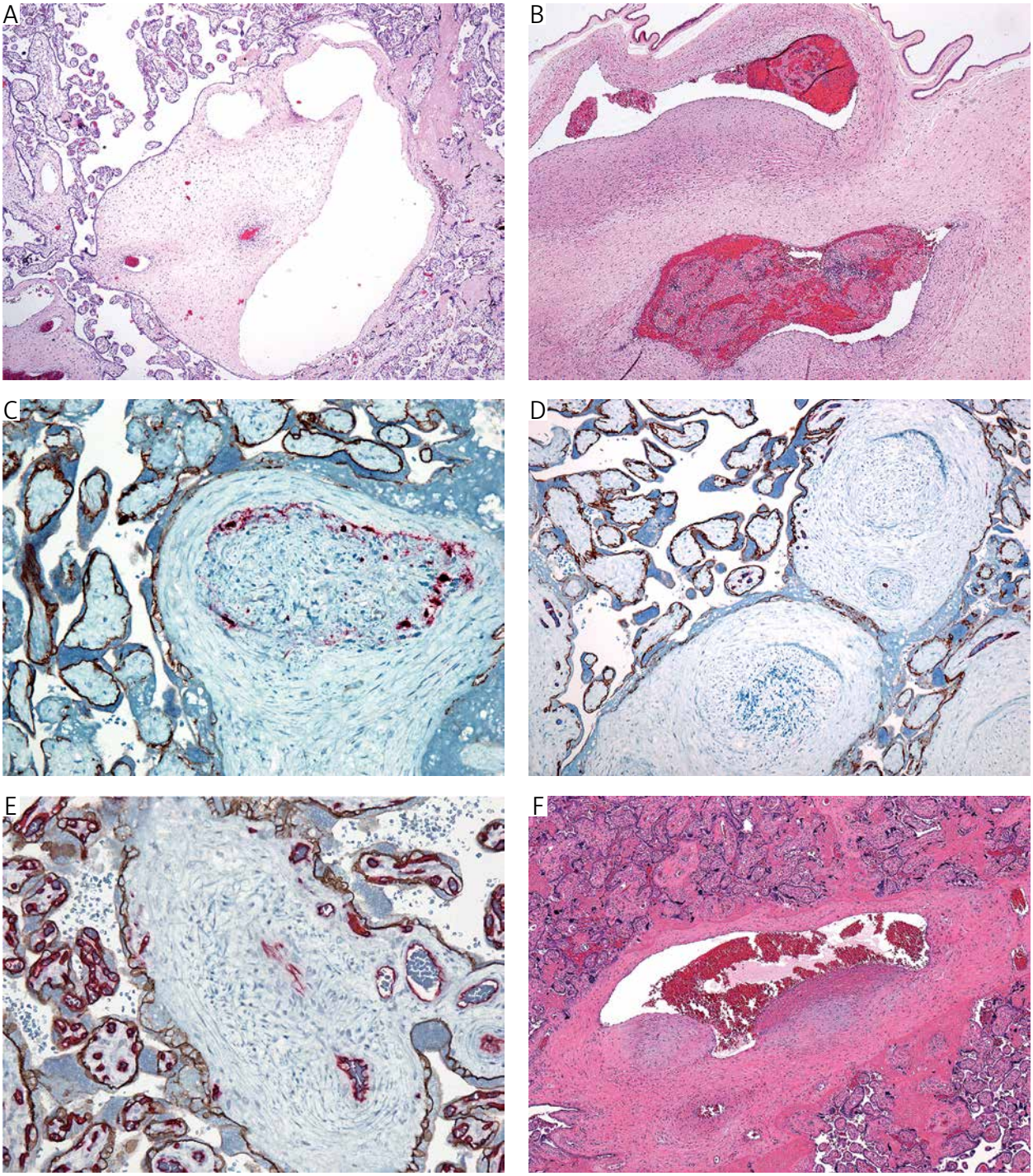

Fig. 3. Large vessel, proximal foetal vascular malperfusion. A) Stem vascular ectasia, 4×, 23 weeks, hyaline membrane disease, neonatal death; B) non-occluding thrombi of chorionic plate, 4×, 36 weeks, foetal hydrops; C) occluding stem thrombus, 20×, 36 weeks, open myelomeningocele, E cadherin (brown) CD34 (red) immunostaining; D) stem vessel obliteration, 10×, 36 weeks – same case as (C); E) stem vessel obliteration 20×, E cadherin (brown) CD34 (red), 35 weeks, repeat variable decelerations, aqueductal stenosis; F) intramural fibrin deposition 4×, 37 weeks, succenturiate lobe, giant omphalocele, pulmonary hypoplasia

representing a chronic repeated insult while "new" FGR cases (those following an appropriate for gestational age pregnancy) were characterized by a higher rate of FVM lesions and lower birth rate, probably representing "an accident" in placentation [33]. Our material comprised twice as many FGR cases in Groups 1 and 2 than in the general population and the comparative Group 3, but we did not distinguish between sporadic and recurrent FGR (Table I).

Of other complications of pregnancy, stillbirth has a special position as far as FVM is concerned. Placental histology can help in determining timing of foetal

Table I. Clinical phenotypes

	Group 1 Remote distal villous FVM (by H&E staining)	Group 2 Recent or on-going FVM (segmental endothelial fragmentation by CD34)	Group 3 No distal villous FVM
Number of cases	158	142	262
Gestational hypertension, *n* (%)	9 (5.7)	11 (7.7)	17 (6.5)
Preeclampsia, *n* (%)	18 (11.4)	13 (9.1)	13 (5.0)
Chronic hypertension, *n* (%)	5 (3.2)	6 (4.2)	13 (5.0)
Gestational age (weeks, average ± standard deviation)**, *n* (%)	33.6 ±5.5	34.7 ±4.8	35.2 ±4.7
Poor or absent prenatal care, *n* (%)	3 (1.9)	3 (2.1)	7 (2.7)
Substance abuse, *n* (%)	19 (12.0)	9 (6.3)	22 (8.4)
Maternal diabetes mellitus, *n* (%)	21 (13.3)	16 (11.3)	23 (8.8)
Oligohydramnios, *n* (%)	16 (10.1)	8 (5.6)	26 (9.9)
Polyhydramnios, *n* (%)	12 (7.6)	10 (7.0)	20 (7.6)
Premature rupture of membranes, *n* (%)	17 (10.8)	11 (7.7)	25 ((.5)
Antepartum haemorrhage, *n* (%)	9 (5.7)	3 (2.1)	16 (6.1)
Meconium-stained amniotic fluid, *n* (%)	21 (13.3)	17 (12.0)	27 (10.3)
Abnormal foetal heart rate tracing[a], *n* (%)	26 (16.5)	26 (18.3)	33 (12.6)
Abnormal umbilical artery Dopplers, *n* (%)	13 (8.2)	10 (7.0)	5 (1.9)
Induction of labour, *n* (%)	37 (23.4)	30 (21.8)	57 (21.8)
Caesarean section, *n* (%)	88 (55.7)	76 (53.2)	140 (53.4)
EXIT procedure, *n* (%)	10 (6.3)	16 (11.3)	26 (9.9)
Multiple pregnancy, *n* (%)	6 (3.8)	8 (5.6)	5 (1.9)
Neonatal deaths*,**, *n* (%)	24 (15.2)	8 (5.6)	31 (11.8)
Non-macerated stillbirth	8 (5.1)	5 (3.5)	9 (3.4)
Macerated stillbirth	24 (15.2)	18 (12.7)	27 (10.3)
Foetal growth restriction[b], *n* (%)	36 (22.7)	33 (23.2)	30 (11.4)
Umbilical cord compromisec, *n* (%)	18 (11.4)	21 (14.8)	25 (9.5)
Congenital malformations, *n* (%)	86 (54.4)	75 (52.8)	168 (64.1)
Stay in neonatal intensive care unit (survivors), *n* (%)	75 (73.5)	77 (69.4)	145 (70.7)
Abnormal 3rd stage of labour (prolonged, haemorrhage), *n* (%)	10 (6.3)	16 (11.3)	16 (6.1)

FVM – foetal vascular malperfusion, H&E – haematoxylin-eosin
[a]abnormal non-stress test and/or abnormal contraction stress test and/or abnormal intrapartum cardiotocography (prolonged bradycardia and/or prolonged tachycardia and or decrease of foetal heart rate variability and/or late decelerations), [b]birth weight < 10th centile, [c]variable decelerations, encirclement, true knot, or prolapse
*Statistical significance: $p < 0.05$: * Group 1 vs. 2, ** Group 1 vs. 2 vs. 3.*

death: Villous intravascular karyorrhexis (6 or more hours), vascular luminal abnormalities of stem villi (2 or more days), and extensive fibrosis of terminal villi (2 or more weeks) [34]. The diffuse vs. focal nature of lesions may help with the distinction between FVM and regressive foetal post-mortem changes [10, 34, 35], which can be used to date the onset of FVM before delivery or stillbirth. In macerated stillbirth, evidence of occult cord compromise should be sought, particularly in the third trimester [9]. Clustered linear basement membrane hemosiderosis, or speckled villous core staining, is a feature of FVM as opposed to diffuse deposition secondary to stillbirth because iron uptake by the placenta is an autonomous process, independent of the foetus, which continues after foetal death [36]. In stillbirth, FVM is the most common pattern of placental injury when CD34 immunostaining is applied [37]. When compared with H&E staining, the CD34 increased the sensitivity and/or upgraded the FVM in stillbirths but not in livebirths, significantly improving the detection of focal or subtle findings of FVM in placental specimens, partic-

Table II. Placental variables

	Group 1 Remote FVM (by H&E)	Group 2 Recent or on-going FVM (including CD34)	Group 3 No distal FVM
Number of cases	158	142	262
Placental weight (grams, average ± standard deviation)**	363.5 ±175.5	373.9 ±150.0	404.5 ±166.3
Inflammatory patterns			
Acute chorioamnionitis, *n* (%)	35 (22.1)	36 (25.3)	62 (23.7)
Maternal inflammatory response	26 (16.5)	27 (19.0)	41 (15.6)
Foetal inflammatory response	9 (5.7)	9 (6.3)	21 (8.0)
Chronic villitis of unknown aetiology	28 (17.7)	19 (13.4)	29 (11.1)
Plasma cell deciduitis, *n* (%)	8 (5.1)	11 (7.7)	20 (7.6)
Hypoxic patterns and related lesions			
Acute hypoxic lesions			
Meconium (histological), *n* (%)	67 (42.4)	64 (45.1)	117 (44.7)
Deep (decidual)	5 (3.2)	11 (7.7)	13 (5.0)
Shallow (amnionic or chorionic)	62 (39.2)	53 (37.3)	104 (39.7)
Intravillous haemorrhage*,**, *n* (%)	10 (6.3)	2 (1.4)	3 (1.1)
Villous infarction (> 5% of placental parenchyma)**, *n (%)*	21 (13.3)	24 (16.9)	18 (6.9)
Laminar necrosis of membranes[a], *n* (%)	51 (33.5)	51 (35.9)	68 (25.9)
Chronic hypoxic lesions/patterns			
Erythroblastosis of foetal blood**, *n* (%)	37 (23.4)	22 (15.5)	35 (13.4)
Hypertrophic decidual arteriopathy**, *n* (%)	53 (33.5)	35 (24.6)	49 (18.7)
Atherosis of spiral arterioles, *n* (%)	15 (9.5)	11 (7.7)	16 (6.1)
Patterns of chronic hypoxic injury, *n* (%)	29 (18.3)	31 (21.8)	47 (17.9)
Pre-uterine	8 (5.1)	2 (1.4)	13 (5.0)
Uterine	12 (7.6)	17 (12.0)	28 (10.7)
Post-uterine**	9 (5.7)	12 (8.4)	6 (2.3)
Retroplacental haematoma, *n* (%)	5 (3.2)	5 (3.5)	13 (5.0)
Intervillous thrombus, *n* (%)	59 (37.3)	51 (35.9)	95 (36.3)
Patterns/lesions of shallow placental implantation			
Membrane chorionic microcysts[b] (%)	24 (15.2)	20 (14.1)	44 (16.8)
Chorionic disc chorionic microcysts[c] (%)	23 (14.6)	17 (12.0)	34 (13.0)
Maternal floor multinucleate trophoblastic giant cells (%)	46 (29.1)	35 (24.6)	53 (20.2)
Excessive amount of extravillous trophoblasts in chorionic disc[d],**, *n* (%)	41 (25.9)	40 (28.2)	40 (15.3)
Placenta creta (including basal plate myometrial fibres), *n* (%)	28 (17.7)	20 (14.1)	47 (17.9%)
Lesions related to foetal vascular malperfusion			
Low-grade distal segmental FVM, *n* (%)	94 (59.5)	70 (49.3%)	0
High grade distal segmental FVM, *n* (%)	63 (39.9)	72 (50.7)	0
Clusters of sclerotic/stromal vascular karyorrhectic/hemosiderotic distal villi (H&E)	158 (100)	98 (69.0)	0
Clusters of villi with endothelial fragmentation (CD34)	0 (0.0)	142 (100)	0

Table II. Cont.

	Group 1 Remote FVM (by H&E)	Group 2 Recent or on-going FVM (including CD34)	Group 3 No distal FVM
Large vessel FVM lesions, *n* (%)	120 (75.9)	101 (71.1)	189 (72.1)
Foetal vascular ectasia	95 (60.1)	71 (50.0)	148 (56.5)
Foetal vascular thrombi*,**	70 (44.3)	42 (29.6)	81 (30.9)
Stem vessel obliteration	53 (33.5)	45 (31.7)	76 (29)
Intramural fibrin deposition	26 (!6.5)	24 (16.9)	34 (13)
Luminal vascular abnormalities of chorionic villi, *n* (%)	11 (7.0)	14 (9.9)	15 (5.7)
Diffusely increased extracellular matrix of chorionic villi, *n* (%)	14 (8.9)	22 (15.5)	28 (10.7)
Other patterns/lesions			
Massive perivillous fibrin deposition (> 30% of placental parenchyma), *n* (%)	7 (4.4)	5 (3.5)	7 (2.7)
Chorangiosis, *n* (%)	20 (12.7)	16 (11.3)	37 (14.1%)
Chorangioma/chorangiomatosis , *n* (%)	4 (2.5)	4 (2.8)	4 (1.5)
Choriodecidual hemosiderosis, *n* (%)	6 (3.8)	6 (4.2)	10 (3.8)
Villous oedema, *n* (%)	9 (5.7)	11 (7.7)	19 (7.2)
Two-vessel umbilical cord, *n* (%)	19 (12.0)	13 (9.1)	15 (5.7)
Hypercoiled umbilical cord, *n* (%)	50 (31.6)	45 (31.7)	82 (31.3)
Hypocoiled umbilical cord, *n* (%)	17 (10.8)	9 (6.3)	15 (5.7)
Perivascular stem oedema, *n* (%)	13 (8.2)	11 (7.7)	13 (5.0)
Marginal insertion of umbilical cord, *n* (%)	4 (2.5)	10 (7.0)	10 (3.8)
Velamentous insertion of umbilical cord, *n* (%)	5 (3.2)	2 (1.4)	6 (2.3)
Other umbilical cord abnormalities[e], *n* (%)	28 (17.7)	30 (21.2)	45 (17.2)
Amnion nodosum/chorion nodosum, *n* (%)	12 (7.6)	4 (2.8)	15 (5.7)
Marginate or vallate placenta, *n* (%)	20 (12.7)	12 (8.4)	12 (4.6)
Gross chorionic cyst(s), *n* (%)	3 (1.9)	4 (2.8)	3 (1.1)
Succenturiate lobe, *n* (%)	3 (1.9)	4 (2.8)	7 (2.8)

FVM – foetal vascular malperfusion, H&E – haematoxylin-eosin, N/A – not applicable
[a]at least 10% of membrane rolls, [b]at least 3 pseudocysts per membrane roll, [c]at least 3 pseudocysts per a section of grossly unremarkable chorionic disc, [d] > 5 cell islands/placental septa per chorionic disc section, [e]too long, too short, too thin, stricture, aneurysm, varix, haematoma, vessel unprotected by Wharton jelly, chorda, ulcer, harber pole funisitis, amniotic band, meconium toxicity, furcate insertion, oedema
*Statistical significance ($p < 0.05$): *Group 1 vs. 2, ** Group 1 vs. 2 vs. 3.*

ularly in cases of high-risk pregnancy, placentas with hypoxic lesions, and poor foetal/infant outcome [11]. In the current material, the frequency of stillbirth was decreasing from Group 1 to Group 2 to Group 3, which indicates that remote distal villous FVM is more likely to produce an unexpected stillbirth.

In postnatal complications, FVM seems to be a less sensitive tool for the prediction of complications [38]. The NICU stay in the infants analysed here was around 70% in all groups (Table I), but high-grade distal FVM is associated with complicated short-term perinatal outcome, no matter if remote or recent [3]. CD34 immunohistochemistry and/or mineralization histochemistry diagnosed/upgraded to high-grade FVM has the same short-term prognostic significance as high-grade segmental FVM diagnosed by H&E only [3], but not global FVM, which was also confirmed by this analysis (Table II). Because of the longer time needed for its development, distal FVM portends poorer prognosis for the foetus than large-vessel FVM [14]. Foetal vascular malperfusion may be a cause of a neurological deficit in childhood [15] but some authors indicated that FVM lesions are associated with adverse perinatal outcome but not neurological complications [39]. We reported that perinatal outcome and neurological complications are particularly common in high-grade FVM (by H&E or CD34 only), and calcified brain microthrombi may be seen even in incipient FVM (by CD34) [3]. Subacute/chronic FVM can

prime the foetal death for acute peri-partum hypoxic-ischaemic injury [40, 41] under conditions that might not lead to hypoxic ischaemic encephalopathy in the absence of FVM, being implicated in sometimes severe sequelae: FGR, poor perinatal outcome, foetal demise, and neurodevelopmental sequelae. In 50% of cases with neonatal stroke FVM was diagnosed, as compared with 17% of controls (both arterial and venous stroke) [42, 43]. 24% of cases of neonatal hypoxic-ischaemic encephalopathy were associated with FVM. Another potentiating factor may be systemic infection/inflammation [40]. Foetal vascular malperfusion of subacute and chronic origin on the foetal side of the placenta was associated with increased risk of neonatal encephalopathy [44]. There was a trend toward more segmental FVM and high-grade FVM among such cases [5, 43].

Our current analysis adds to the understanding of the pathomechanism of FVM. Although numerically predisposing to FVM, as discussed above, various clinical conditions are not specifically associated with various types of FVM, which reflect the temporal heterogeneity thereof related to the time of onset before delivery or stillbirth [13]. The pathomechanism of FVM is therefore mostly similar to UC compromise (stasis-induced FVM), the difference being the absence of associated features of shallow placental implantation and chronic hypoxic patterns of placental injury [8, 27] but not in this material dominated by congenital anomalies. The discussed distal lesions are the manifestation of the same continuous process [1, 38] as stromal vascular karyorrhexis and clusters of sclerotic villi cluster together [25]. Sclerotic chorionic villi were introduced first into the diagnosis of FVM [29], followed by stromal vascular karyorrhexis, endothelial fragmentation (the most sensitive), before the stromal component of stromal vascular karyorrhexis and villous hypovascularity [10] and segmental mineralization, which may be seen even in totally sclerotic placentas [10]. Segmental mineralization of distal villi, seen even in totally sclerotic placentas, is an independent feature of FVM even when other features of FVM are not seen [12]. The coexistence of different FVM lesions indicates the on-going process or repeat occurrences of FVM that may be useful in dating the occurrence thereof [13]. Unless identified at gross examination, diagnosis of foetal vascular thrombosis is subject to considerable sampling error and may not be identified in the planes of section (thrombi or mural lesions) although foetal vascular thrombi are essential in the pathogenesis of FVM. Global FVM is frequently a precursor of segmental FVM, and features of both can be present in the same placenta. Less commonly, thrombi may be seen without stasis-related changes. Because of its longer duration, distal villous FVM portends poorer prognosis for the foetus than large-vessel FVM [14].

In this analysis, there were few statistically significant differences in clinical phenotypes not only between Groups 1 and Group 2, but also among all 3 groups, the most conspicuous difference being the shortest gestational age at delivery in Group 1 and the longest in Group 3, which resulted in a higher neonatal death rate in Group 1. In placental phenotypes, the recent FVM diagnosed by CD34 immunostaining was associated with other placental lesions and patterns, similarly to those associated with remote FVM diagnosed by H&E, in particular similar proportions of high-grade and low-grade foetal vascular malperfusion, large vessel FVM component, percentage of UC abnormalities, hypoxic, or inflammatory lesions. These findings indicate that the recent distal villous FVM by CD34 has same diagnostic/prognostic significance as the time-honoured distal FVM lesions diagnosed by H&E that develop much earlier and are of longer standing. We agree that once segmental FVM is recognized, it is not necessary to make an additional diagnosis of global FVM [5].

Conclusions

CD34 immunostaining is a powerful tool in diagnosing the distal villous FVM. By using the immunostaining, FVM became the most common type of placental injury, more common than the inflammatory and maternal vascular malperfusion patterns, not only in stillbirth but also in other types of high-risk pregnancy, like foetal anomalies. This indicates that the CD34 immunostaining is a powerful tool expanding the capabilities of placental examination for FVM. We apply this test in all cases of high-risk pregnancy now.

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