Original paper

Grading placental fetal vascular malperfusion and short-term perinatal outcome*

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This retrospective analysis was performed to seek possible associations of the global fetal vascular malperfusion (GFVM) and grade of segmental fetal vascular malperfusion (SFVM) with early perinatal outcome. Clinical associations of 538 consecutive cases of placental fetal vascular malperfusion (FVM), including 374 cases of SFVM (group 1: 308 low-grade, group 2: 66 high-grade), and 65 cases of GFVM without segmental villous changes (group 3), were statistically compared. Histological SFVM was graded on HE (Subgroups 1A and 2A) and on CD34 or histochemistry stains (segmental endothelial fragmentation, segmental hypovascularity and/or mineralization) (subgroups 1B and 2B). The short term neonatal outcome as evaluated by NICU stay and neurological complications was statistically significantly more frequently complicated in association with high-grade SFVM than in low-grade SFVM and GFVM in general, and equally frequently in high-grade SFVM diagnosed on HE slides only and on CD34 immunostain and/or mineralization histochemistry only (about 61% of cases each). High-grade SFVM portends a more complicated short-term perinatal outcome than low-grade SFVM or GFVM. CD34 immunohistochemistry and/or mineralization histochemistry diagnosed/upgraded high-grade SFVM has the same short-term prognostic significance as high-grade SFVM diagnosed on HE only, thus increasing the sensitivity of placental examination for FVM by these methods.

Key words: fetal vascular malperfusion, grade, placenta, umbilical cord compromise, histology, CD34 immunostain.

Introduction

Placental examination is the most valuable tool in determining the cause of fetal death (96%), followed by autopsy (73%) and cytogenetic analysis (29%) [1, 2, 3]. Fetal vascular malperfusion (FVM), caused by a variety of etiologies [4, 5], is one of the major placental correlates of complicated perinatal outcome [4, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16], particularly umbilical cord compromise [7, 17, 18], but confirmation of the clinical umbilical cord compromise should not be expected on placental examination if no morphological UC abnormality or abnormal UC insertion has been found [19]. Fetal vascular malperfusion is one of the basic patterns of hypoxic placental injury [20]. 5% of placentas from live births and 1% of all third trimester placentas show features of FVM [6].

Segmental FVM (SFVM) indicates thrombotic occlusion of chorionic or stem vessels or stem vessel obliteration. The diagnosis of low-grade SFVM requires finding at least 3 foci of 2-4 totally avascular

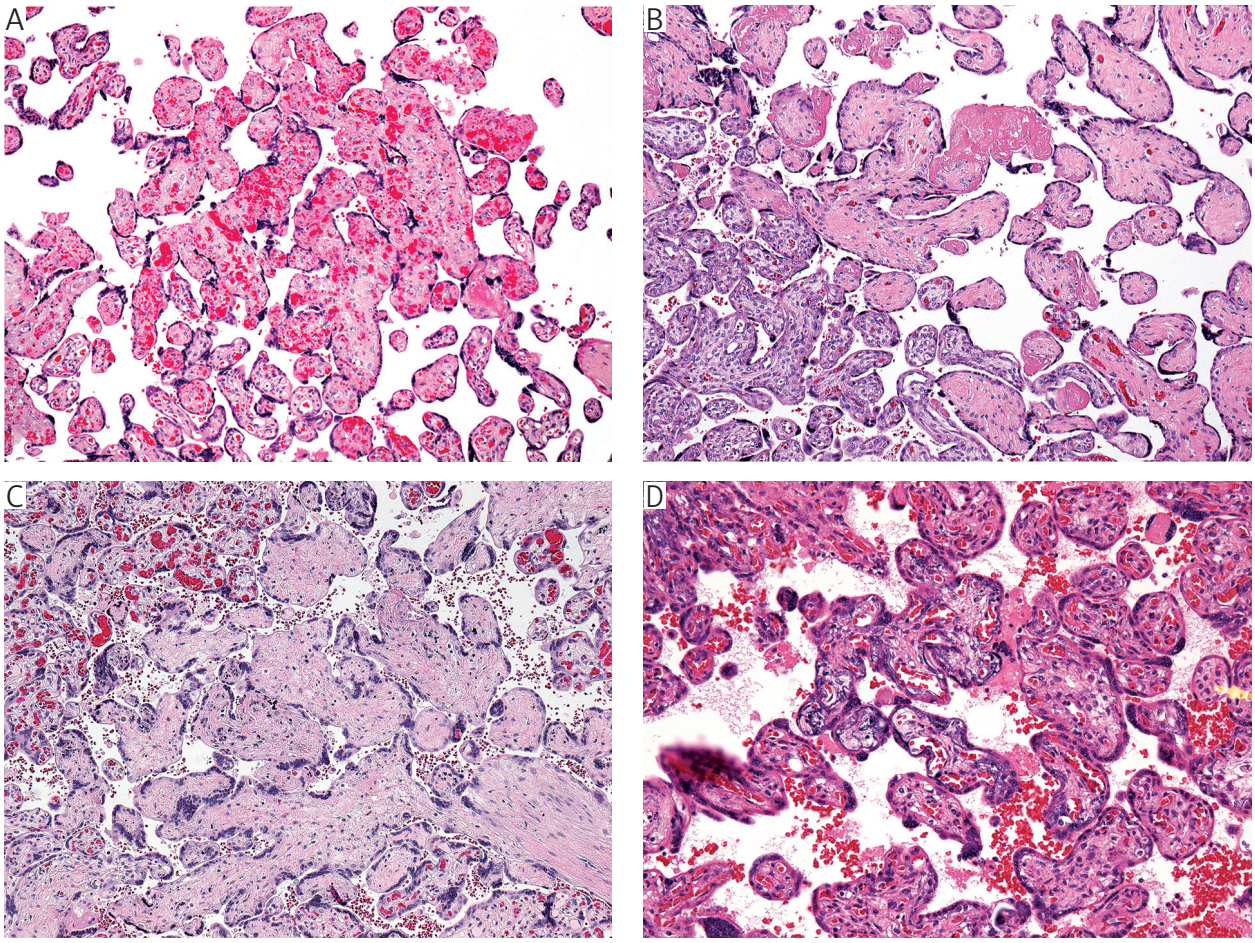

Fig. 1. Segmental fetal vascular malperfusion recognizable on routine HE stains (Subgroups 1A and 2A), the actual grade depending on quantitative assessment of villous involvement. A) Stromal-vascular karyorrhexis, 10×, 39 weeks, double outlet right ventricle, nuchal cord 2×. B) Villous hypovascularity, 10×, 21 weeks, nonmacerated stillbirth, amniotic sac infection syndrome, cystic dysplastic kidney. C) Villous segmental avascularity, 10×, 39 weeks, nonmacerated stillbirth, acute intrapartum hypoxia, hypercoiled umbilical cord. D) Segmental basement membrane mineralization in non-sclerotic placenta, 20×, 35 weeks, fetal genital anomalies

terminal villi, while high-grade segmental FVM is determined by more than 1 focus of avascular villi (> 45 avascular villi over 3 sections or an average of > 15 such villi per section) with or without thrombus, or 2 or more occlusive or nonocclusive thrombi in chorionic plate or major stem villi, or multiple nonocclusive thrombi [7] (Fig. 1). Recently we reported that recognizing segmental villous mineralization [21] and/or endothelial fragmentation by CD34 immunohistochemistry [22] can also detect SFVM in cases not diagnosable on HE stained placental sections (Fig. 2).

For the diagnosis of global FVM intramural fibrin deposition in large vessels, and/or fetal vascular ectasia (Fig. 3), and/or small foci (< 5 villi per focus) of avascular or karyorrhectic villi was recommended. This type of malperfusion is due to partial or intermittent occlusion of umbilical blood [7]. While global FVM can also be low grade or high grade, the criteria are not very clear as distal villous changes are allowed in both segmental and global FVM. Histological finding of FVM is particularly vital to explain perinatal mortality, morbidity and future child development [14, 23]; however, its impact on more immediate perinatal complications is less documented. It was suggested that non-severe lesions of FVM may not produce a significant difference from control in regard to morbidity and neurological injury [20]. Contrary to some reports [15], some of our cases with placental FVM, even low grade, showed brain thrombi on autopsy [21].

As the associations of SFVM (particularly low grade) and global FVM without segmental changes, and the impact of FVM grade on short-term perinatal complications, are not well documented, the aim of this retrospective analysis is to compare the relation of the grade of segmental and global FVM to early clinical outcome and other abnormal placental findings. In addition, it is not clear whether the recently reported techniques (segmental endothelial

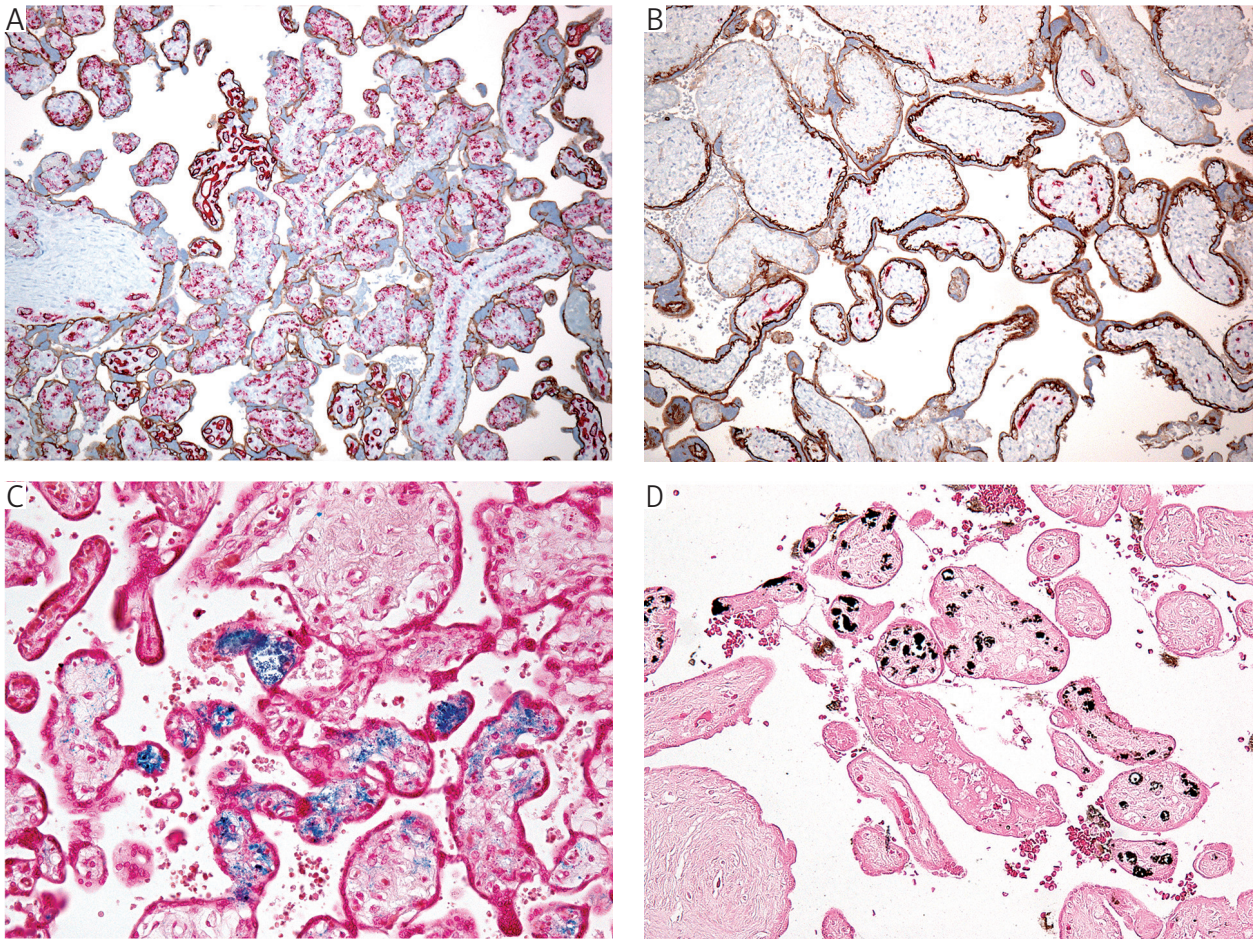

Fig. 2. Segmental fetal vascular malperfusion recognizable on immunohistochemistry/histochemistry stains (Subgroups 1B and 2B), the actual grade depending on quantitative assessment of villous involvement. A) Endothelial fragmentation (E-cadherin brown, CD34 red), 10×, 40 weeks, multiple umbilical cord entanglements. B) Villous hypovascularity visualized by CD34 in a totally sclerotic placenta by HE, 10×, (E-cadherin brown, CD34 red), 26 weeks, macerated fetus with oligohydramnios sequence. C) Villous ferrugination, 20×, iron stain, 38 weeks, neonate with ischemic brain damage. D) Endothelial mineralization, 20×, 20 weeks, von Kossa stain, stillborn twin, totally sclerotic placenta on HE

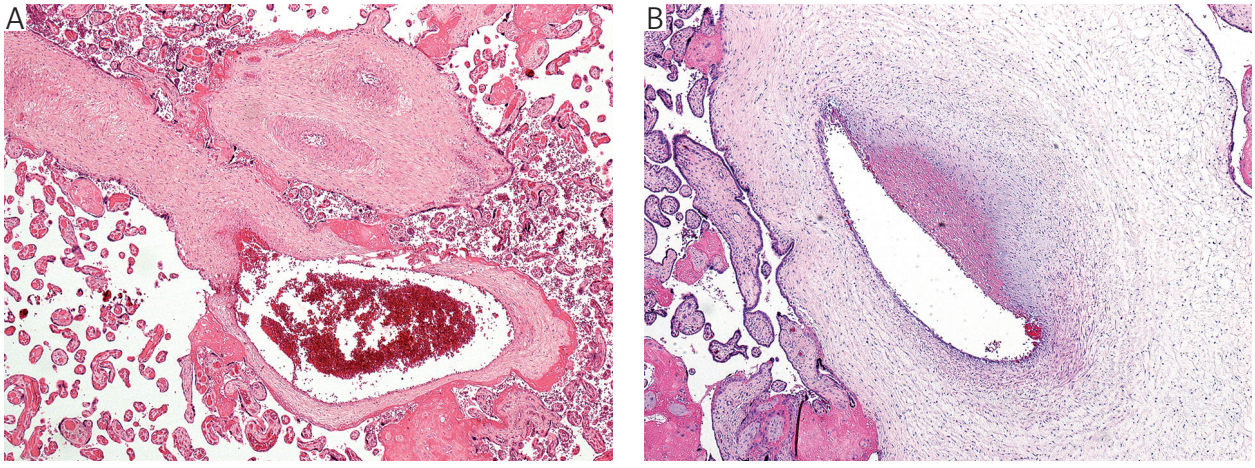

Fig. 3. Global fetal vascular malperfusion (Group 3). A) Stem fetal vascular ectasia, 4×, 33 weeks, low Apgar scores, single umbilical artery. B) Intramural fibrin deposition, 4×, stillbirth

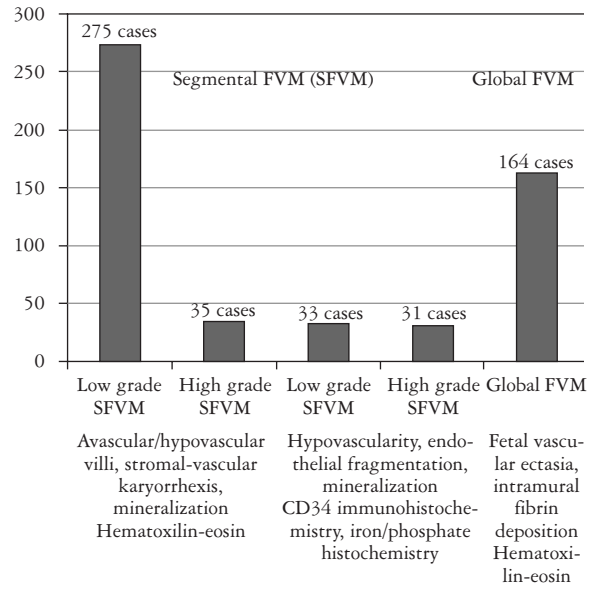

Fig. 4. Classification of the material: 538 cases with fetal vascular malperfusion (FVM)

fragmentation, hypovascularity, and villous mineralization) [21, 22, 23, 24, 25, 26] can identify cases with the same potential of perinatal complications as cases with totally sclerotic chorionic villi seen on HE sections only. For the purpose of this study, the FVM Amsterdam criteria were modified and expanded by including those stains.

Material and methods

The study was approved by the institutional review board (IRB #2016-7942). The clinical data were extracted from the available information in the hospital computer database until the day of discharge or neonatal death. The placentas were obtained for examination at the discretion of obstetricians because of high risk-pregnancy, its complications, or were a part of autopsy. All gross abnormalities were sampled and in addition at least 2 sections of membrane roll and the umbilical cord and 2 paracentral sections of grossly unremarkable placenta were taken. Formalin-fixed and paraffin-embedded sections were stained with HE and were reviewed by the author using the same diagnostic criteria as in previous publications [1, 22, 23, 24, 25, 26, 27, 28, 29, 30].

The 538 most recent consecutive cases of placental FVM diagnosed by the author, including 374 cases with SFVM – 308 low-grade (group 1), 66 high-grade (group 2), and 164 cases of global FVM without segmental distal villous changes (group 3) – were compared. SFVM was diagnosed on HE slides based on the finding of more than one focus of avascular or hypovascular distal villi or segmental stromal vascular karyorrhexis (Fig. 1), or segmental endothelial fragmentation or segmental mineralization (linear basement membrane or speckled villous core staining) [31, 32] based on CD 34 immunostain, or histochemistry (iron, von Kossa) [21, 22] (Fig. 2). The Amsterdam criteria for FVM were thus expanded. Diffuse villous mineralization [33] was not taken into consideration. Avascular or hypovascular villi of chronic villitis were not considered for the purpose of diagnosis or grading FVM.

The indications for performing the CD34 immunostain were: 1) high risk conditions for FVM, such as clinical evidence of cord compromise, pathological cord abnormalities, EXIT procedures, unexplained stillbirth, or mass forming fetal anomalies, even when HE sections were negative for clusters of sclerotic or karyorrhectic distal villi, or if there was a global component without segmental changes on HE, 2) low-grade SFVM on HE (a potential for upgrading). We do not perform the stains on all placentas or on all blocks of a given placenta [25], particularly when there is no clinicopathologic suspicion of FVM. We perform CD34 on one least abnormal on HE block of grossly normal placenta as the stain is intended to reveal the earliest segmental changes not yet diagnosable on HE staining.

High-grade SFVM was defined based on cumulative assessment of 45 ≥ affected villi (i.e. villi affected by the above processes) over 3 sections, or an average of >15 villi per section (with or without thrombus). Low-grade SFVM was defined when segmental villous changes were seen in at least 3 clusters of 2-4 affected terminal villi but did not reach the threshold of high-grade SFVM [7]. For purpose of this analysis, the presence of occlusive or non-occlusive thrombi was not used for grading. In the two segmental FVM groups, the A subgroups showed distal segmental changes on HE slides (Fig. 1), and B subgroups showed villous hypovascularity, endothelial fragmentation or segmental mineralization by immunohistochemistry/histochemistry (CD34 immunostain and/or iron of von Kossa histochemistry stains) (Fig. 2) with the same grading numerical criteria of involvement as for totally sclerotic chorionic villi [7]. The definition of global FVM was modified from that proposed by the Amsterdam criteria, by inclusion only of cases with fetal vascular ectasia and/or intramural fibrin deposition (Fig. 3), but no segmental villous changes. No attempt was made to subclassify global FVM into low-grade and high-grade. The criteria for the group and subgroup classification are illustrated in Fig. 4. Frequencies of 33 independent clinical and 40 placental phenotypes were statistically compared by the chi-square test or ANOVA among the groups and subgroups.

Table I. Clinical phenotypes in low-grade and high-grade segmental fetal vascular malperfusion and global fetal vascular malperfusion without distal villous changes (on HE or immunohistochemistry)

	All FVM cases	Group 1 Low-grade segmental FVM	Group 2 High-grade segmental FVM	Group 3 Global FVM without segmental component	F or chi square	p-value
	538	308	66	164		
Gestational hypertension	33 (6.1%)	21 (6.8%)	3 (4.5%)	9 (5.5%)		
Preeclampsia	51 (9.5%)	26 (8.4%)	5 (7.6%)	20 (12.2%)		
Chronic hypertension	25 (4.65%)	11 (3.6%)	3 (4.5%)	11 (6.7%)		
Gestational age (weeks, average ± standard deviation)	33.0 ±6.1	32.3 ±7.0	35.4 ±5.9	33.4 ±5.8		
Poor or absent prenatal care	12 (2.2%)	8 (2.65%)	0 (0%)	4 (2.4%)		
Substance abuse	56 (10.4%)	35 (11.4%)	4 (6.1%)	17 (10.4%)		
Maternal diabetes mellitus	56 (10.4%)	28 (9.1%)	11 (16.7%)	17 (10.4%)		
Oligohydramnios	62 (11.5%)	43 (14.0%)	5 (7.6%)	14 (8.5%)		
Polyhydramnios	59 (11.0%)	32 (10.4%)	6 (9.1%)	21 (12.8%)		
Premature rupture of membranes	78 (14.5%)	44 (14.3%)	6 (9.1%)	28 (17.1%)		
Antepartum hemorrhage	46 (8.5%)	26 (8.4%)	4 (6.1%)	16 (9.8%)		
Meconium-stained amniotic fluid	64 (11.9%)	39 (12.7%)	8 (12.1%)	17 (10.4%)		
Abnormal fetal heart rate tracing[a]	106 (19.7%)	59 (19.1%)	14 (21.2%)	33 (20.1%)		
Abnormal umbilical artery Dopplers	37 (6.9%)	21 (6.8%)	7 (10.6%)	9 (5.5%)		
Induction of labor	113 (21.0%)	78 (25.3%)	13 (19.7%)	22 (13.4%)		
Cesarean section	289 (53.7%)	159 (51.6%)	37 (56.1%)	93 (56.7%)		
EXIT procedure[b]	80 (14.9%)	49 (15.9%)	2 (3.0%)	29 (17.7%)	$^{1\text{-}2}$6.6	0.0057
Multiple pregnancy	39 (7.2%)	24 (7.8%)	1 (1.5%)	14 (8.5%)		
Umbilical cord compromise[c]	64 (11.9%)	33 (10.7%)	14 (21.2%)	17 (10.4%)	$^{1\text{-}2}$5.4	0.019
					$^{2\text{-}3}$4.7	0.029
Congenital malformation	148 (27.5%)	77 (25.0%)	24 (36.4%)	47 (28.7%)		
Perinatal mortality	220 (40.9%)	134 (43.5%)	24 (36.4%)	62 (37.8%)		
Neonatal mortality[d]	81 (15.1%)	48 (15.6%)	5 (7.6%)	28 (17.1%)		
Nonmacerated stillbirth	24 (4.5%)	14 (4.5%)	3 (4.5%)	7 (4.3%)		
Macerated stillbirth	115 (21.4%)	72 (23.4%)	16 (24.2%)	27 (16.4%)		
Fetal growth restriction[e]	115 (21.4%)	79 (25.6%)	19 (28.8%)	15 (9.1%)	$^{1\text{-}3}$18.3	0.00002
					$^{2\text{-}3}$14.4	0.0001
Apgar score after 1 minute	5.2 ±3.1	5.2±3.1	6.8 ±2.7	4.3 ±3.4	$^{2\text{-}3}$8.3	0.005
Apgar score after 5 minutes	7.2 ±3.1	7.1±3.1	7.5 ±2.9	5.2 ±3.6	$^{1\text{-}3}$12.4	0.0006
					$^{2\text{-}3}$6.8	0.011
Venous pH	7.3 ±0.1	7.3±0.1	7.2 ±0.2	7.2 ±0.2		
Venous BE	–6.0 ±8.2	–5.9±8.4	–6.6 ±7.7	–6.1 ±6.6		
Arterial pH	7.2 ±0.2	7.2 ±0.2	7.2 ±0.1	7.0 ±0.8		
Arterial BE	–4.3 ±3.8	–4.2 ±3.9	–4.6 ±3.1	–3.8 ±2.4		
Stay in NICU	158 (318) (49.7%)	79 (174) (45.4%)	26 (42) (61.9%)	53 (102) (52.0%)	$^{1\text{-}2}$3.7	0.0548
Hypoxic-ischemic encephalopathy or other neurological sequelae	21 (318) (6.6%)	8 (174) (4.6%)	7 (42) (16.7%)	6 (102) (5.9%)	$^{1\text{-}2}$7.6	0.006
					$^{2\text{-}3}$4.2	0.040

[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 fetal heart rate variability and/or late decelerations), [b] EXIT procedures are commonly performed in our children's hospital (for ECMO, to secure airway or (rarely) for primary resection), [c]variable decelerations, encirclement, true knot, or prolapse, [d]death of a liveborn infant in 1st month of age, [e]birth weight <10 centile

Results

Clinical variables

Three infants who were not included in the perinatal mortality died after 1 month of age: 2 from group 1A (one with sepsis and one with diffuse alveolar damage in the setting of congenital heart disease) and 1 from group 2B (sepsis). Group 3 distinguished itself by the lowest rate of fetal growth restriction (statistically significant), low Apgar scores and a lower rate of neurological sequelae and umbilical cord compromise than group 2. Group 1 had a higher frequency of EXIT procedures but a lower rate of umbilical cord compromise than group 2. The high-grade FVM group (group 2) showed more common clinical umbilical cord compromise, NICU stay and neurological sequelae than the low-grade SFVM group. In general, global FVM was less commonly associated with abnormal short-term outcome than SFVM (Table I).

When groups 1 and 2 were subclassified according to FVM grade based on HE staining versus CD34 and/or histochemistry staining, most differences between group 1A and group 1B were not statistically significant, except for the interrelated EXIT procedures and congenital malformations, which were statistically significantly more common in the latter. Meconium-stained amniotic fluid was more commonly observed in group 2A than in group 2B, and oligohydramnios was less common in group 2B than in group 1A and 1B. Umbilical cord compromise was seen more commonly in groups 2A than 2B. Fetal growth restriction was statistically significantly less common in group 3 than in groups 1A, 2A and 2B. Congenital malformations were most common in group 2A. 5 min Apgar score was the lowest in group 3. High-grade SFVM diagnosed only on HE showed the same high percentage of NICU stay as that diagnosed on CD34, both statistically significantly higher than in group 3. Neurological sequelae were statistically significantly more common in group 2A than in 1A and group 3 (Table II).

Placental variables

Statistically significantly ($p < 0.05$), group 3 showed lower frequency of villous infarction than groups 1 and 2 (16.2%, 18.2%, and 67.7% in groups 1, 2, and 3 respectively), and Group 2 showed higher frequency of hypertrophic decidual arteriopathy than group 1 (22.7% and 34.8%, in groups 1 and 2 respectively). Group 3 featured higher frequencies of hypercoiled umbilical cord than groups 1 and 2 (39.6%, 23.7%, and 25.8%, respectively).

After the material was subdivided into subgroups, statistically significantly ($p < 0.05$), erythroblastosis of fetal blood was more frequent in group 1A than group 1B (26.2% and 6.1%, respectively), and hypertrophic decidual arteriolopathy was more common in group 2B than in each of the remaining subgroups (22.5%, 20.0%, 24.2%, 51.6%, and 23.2%, in groups 1A, 1B, 2A, 2B, and 3, respectively). Hypercoiled umbilical cord was more common in group 3 than in groups 1A and 2B (39.6%, 22.9%, and 19.3%, respectively), while other umbilical cord abnormalities were more common in group 2B than in group 3 (38.7%, and 24.4%, respectively).

Discussion

As compared with our total placental database [27], this material of FVM contained more high-risk pregnancies such as gestational hypertension, polyhydramnios, congenital malformations, maternal substance abuse, diabetes mellitus, oligohydramnios, abnormal umbilical artery Dopplers, umbilical cord compromise, perinatal mortality, particularly neonatal, cesarean sections, and fetal growth restriction. Placental phenotypes were also more frequently abnormal: fetal vascular ectasia, fetal vascular thrombosis, excessive extravillous trophoblasts, uterine pattern of chronic hypoxic injury, erythroblastosis of fetal blood, intervillous thrombi, stem vessel obliteration, diffusely increased extracellular matrix of chorionic villi, and vascular intramural fibrin deposition, chronic villitis of unknown etiology, intravillous hemorrhage, postuterine hypoxic pattern of chronic placental injury, extravillous trophoblast microcysts, maternal floor multinucleate trophoblasts clusters, stem luminal vascular abnormalities, villous edema, stem perivascular edema, hyper- and hypocoiled umbilical cord and other umbilical cord abnormalities, placenta creta, and amnion nodosum.

This analysis mutually compared the short-term perinatal outcome associated with global, low-grade and high-grade SFVM, and their subtypes derived from the Amsterdam criteria modified by the author mainly by adding the recently described SFVM lesions (villous hypovascularity/endothelial fragmentation by CD 34 immunohistochemistry, and segmental villous mineralization) [21, 22]. Two other modifications included defining high-grade SFVM solely based on number of involved distal chorionic villi, without taking into consideration occluding/non-occluding thrombi in large fetal vessels, and considering global fetal vascular malperfusion solely based on intramural fibrin deposition and fetal vascular ectasia, but without inclusion of scattered distal villous changes (Fig. 4), as the author agrees that once the segmental component is present, there is no need to make an additional diagnosis of global FVM [34]. The numerical Amsterdam criteria (based on number of avascular villi) were not changed, but

Table II. Clinical phenotypes comparing segmental fetal vascular malperfusion subclassified using CD34 immunohistochemistry and/or histochemistry, and global fetal vascular malperfusion without segmental changes (on HE or immunohistochemistry)

	Group 1A Low-grade segmental FVM by HE	Group 2A High-grade segmental FVM by HE	Group 1B Low-grade segmental FVM by CD34 or histochemistry	Group 2B High-grade segmental FVM by CD34 or histochemistry	Group 3 Global FVM without segmental changes	F or chi square	p
Number of cases	275	35	33	31	164		
Gestational hypertension	17 (6.2%)	3 (8.6%)	4 (12.1%)	0 (0%)	9 (5.5%)		
Preeclampsia	25 (9.1%)	2 (5.7%)	1 (3.0%)	3 (9.7%)	20 (12.2%)		
Chronic hypertension	10 (3.6%)	1 (2.9%)	1 (3.0%)	2 (6.4%)	11 (6.7%)		
Gestational age (weeks, average ± standard deviation)	32.2 ±7.1	33.1 ±5.7	33.3 ±6.1	33.2 ±6.4	33.4 ±5.8		
Poor or absent prenatal care	8 (2.9%)	0 (0%)	0 (0%)	0 (0%)	4 (2.4%)		
Substance abuse	30 (10.9%)	3 (8.6%)	5 (15.1%)	1 (3.2%)	17 (10.4%)		
Maternal diabetes mellitus	26 (9.4%)	5 (14.3%)	2 (6.1%)	6 (19.3%)	17 (10.4%)		
Oligohydramnios	39 (14.2%)	5 (14.3%)	4 (12.1%)	0 (0%)	14 (8.5%)	$^{1A-2B}$5.0	0.025
						$^{2A-2B}$4.8	0.041
Polyhydramnios	31 (11.3%)	4 (11.4%)	1 (3.0%)	2 (6.4%)	21 (12.8%)		
Premature rupture of membranes	39 (14.2%)	3 (8.6%)	5 (15.1%)	3 (9.7%)	28 (17.1%)		
Antepartum hemorrhage	25 (9.1%)	4 (11.4%)	1 (3.0%)	0 (0%)	16 (9.8%)		
Meconium-stained amniotic fluid	35 (12.7%)	7 (20.0%)	4 (12.1%)	1 (3.2%)	17 (10.4%)	$^{2A-2B}$4.3	0.037
Abnormal fetal heart rate tracing[a]	56 (20.4%)	8 (22.9%)	3 (9.1%)	6 (19.3%)	33 (20.1%)		
Abnormal umbilical artery Dopplers	18 (6.5%)	4 (11.4%)	3 (9.1%)	3 (9.7%)	9 (5.5%)		
Induction of labor	70 (25.4%)	7 (20.0%)	8 (24.2%)	6 (19.3%)	22 (13.4%)		
Cesarean section	139 (50.5%)	19 (54.3%)	20 (60.6%)	18 (58.1%)	93 (56.7%)		
EXIT procedure[b]	25 (9.1%)	1 (2.9%)	8 (24.2%)	1 (3.2%)	29 (17.7%)	$^{1A-1B}$7.1	0.0078
						$^{2B-3}$4.2	0.041
Multiple pregnancy	21 (7.6%)	1 (2.9%)	3 (9.1%)	0 (0%)	14 (8.5%)		
Umbilical cord compromise[c]	29 (10.5%)	9 (25.7%)	4 (12.1%)	5 (16.1%)	17 (10.4%)	$^{1A-2A}$7.7	0.0054
Congenital malformation	63 (22.9%)	12 (34.3%)	14 (42.4%)	10 (32.3%)	47 (28.7%)	$^{1A-1B}$5.0	0.014
Perinatal mortality	120 (43.6%)	14 (40.0%)	14 (42.4%)	10 (32.3%)	62 (37.8%)		
Neonatal mortality[d]	41 (14.9%)	3 (8.6%)	7 (21.2%)	2 (6.4%)	28 (17.1%)		
Nonmacerated stillbirth	12 (4.4%)	3 (8.6%)	2 (6.1%)	0 (0%)	7 (4.3%)		
Macerated stillbirth	67 (24.4%)	8 (22.9%)	5 (15.1%)	8 (25.8%)	27 (16.4%)		
Fetal growth restriction[e]	73 (26.5%)	9 (25.7%)	6 (18.2%)	10 (32.3%)	15 (9.1%)	$^{1A-3}$19.4	0.00001
						$^{2A-3}$7.5	0.006
						$^{2B-3}$12.5	0.0004

Table II. Cont.

	Group 1A Low-grade segmental FVM by HE	Group 2A High-grade segmental FVM by HE	Group 1B Low-grade segmental FVM by CD34 or histochemistry	Group 2B High-grade segmental FVM by CD34 or histochemistry	Group 3 Global FVM without segmental changes	F or chi square	p
Apgar score after 1 minute	5.3 ±3.1	6.7 ±3.1	4.3 ±2.8	6.9 ±2.3	4.5 ±3.4		
Apgar score after 5 minutes	7.2 ±3.1	7.3 ±3.2	7.1 ±2.7	7.8 ±2.5	5.5 ±3.5	$^{1A-3}$8.3	0.0045
Venous pH	7.3 ±0.1	7.2 ±0.4	7.2 ±0.2	7.3 ±0.1	7.2 ±0.2		
Arterial pH	7.2 ±0.2	7.3 ±0.1	7.3 ±0.2	7.2 ±0.1	7.0 ±0.8		
Venous BE	–5.7 ±8.4	–9.4 ±14.3	–7.2 ±8.8	–5.2 ±2.5	–6.1 ±6.6		
Arterial BE	–4.2 ±3.9	–4.3 ±4.0	–4.2 ±3.8	–5.4 ±3.1	–3.8 ±2.4		
Stay in NICU	71 (155) (45.8%)	13 (21) (61.9%)	8 (19) (42.10%)	13 (21) (61.9%)	53 (102) (52.0%)	$^{2A-3}$5.4	0.02
						$^{2B-3}$5.4	0.02
Hypoxic-ischemic encephalopathy or other neurological sequelae	5 (155) (3.2%)	5 (21) (23.8%)	3 (19) (15.8%)	2 (21) (9.5%)	6 (102) (5.9%)	$^{1A-2A}$14.6	0.0001
						$^{2A-3}$6.9	0.009

Footnotes same as for Table I

now the author expanded them qualitatively by including segmental hypovascularity, endothelial fragmentation, stromal vascular karyorrhexis and mineralization. Only cases with global FVM without segmental changes (on HE, immunohistochemistry or histochemistry) were included in group 3, while those with associated segmental lesions meeting at least the criteria of low-grade SFVM were distributed in groups 1 and 2.

The most interesting findings were in the frequencies of NICU admissions and neurologic complications, including hypoxic-ischemic encephalopathy, which were most common in high-grade SFVM than low-grade SFVM, the latter also more common than in global FVM (Table I). The differences persisted for the high-grade SFVM diagnosed both based on HE and immunohistochemistry and histochemistry (Table II), which supports the usefulness of the latter methods in diagnosing SFVM early [21, 22]. It seems, therefore, that the early and more advanced FVM have similar contribution to neonatal morbidity. However, the reason for stay in the NICU cannot be ascribed solely to FVM as this material of high-risk pregnancies included multifactorial reasons for admissions to NICU such as preterm deliveries, congenital anomalies, EXIT procedures, etc. Fetal growth restriction (FGR) was more frequent in segmental than global FVM, as was clinical umbilical cord compromise in high-grade SFVM (two times more frequent than in low-grade segmental and global FVM) (Table I). This is only in part inconsistent with partial obstruction of umbilical cord blood flow as an explanation of the global FVM [23], as hypercoiled umbilical cord was significantly more common in group 3. This stresses the significance of umbilical cord hypercoiling in producing fetal distress in the last moments of labor, which probably resulted in lower 5 min Apgar scores in group 3. However, confirmation of clinical UC compromise should not be expected on placental examination if no morphological UC abnormality or abnormal UC insertion has been found [19]. It is believed that the frequency of unrecognized umbilical cord compromise increases towards term [35], but gestational age did not differ in group 3 from that of groups 1 and 2. There were no significant differences in base excess between the groups and subgroups, although many cases fell into the area of metabolic acidosis.

Although perinatal mortality, the most severe fetal pregnancy complication, is 3 times more frequent in this material of FVM than in our database of high-risk pregnancy [27], there were no statistically significant differences in this outcome rate among the 3 groups (Table I), which reflects various time intervals between the onset of FVM and delivery (temporal heterogeneity) more than etiology. We documented previously the characteristic association with mass-forming

fetal anomalies and the lesions of FVM [36]. Some authors observed a trend towards more segmental FVM and higher grade FVM in cases of neonatal encephalopathy [11], but we observed intracerebral vascular thrombi even in low-grade SFVM diagnosed by CD34 immunohistochemistry only [32]. Clinical meconium staining, which may be of hypoxic origin, was more frequent in association with SFVM on HE than on CD34, likely because of a more protracted disease process in the former. Clinical umbilical cord compromise was statistically significantly more common in high-grade than low-grade SFVM and global FVM, consistent with data previously published [7, 17]. EXIT procedures were statistically significantly more frequent in low-grade segmental FVM, also consistent with our previous observation [13]. Although fetal congenital anomalies are 3 times more common in this material than in our total database (Table I), there was only an increase of recent (on immunohistochemistry) low-grade FVM over that diagnosed on HE, likely because of higher frequency of EXIT procedures (elective cesarean section) in this population of cases (Table II).

The differences in abnormal placental phenotypes other than umbilical cord abnormalities were conspicuously scant among the 3 groups studied. This is likely because of low prevalence of hypertensive diseases and maternal diabetes mellitus in this study group in comparison with our database of high-risk pregnancies [27]. The lower rate of villous infarction in global FVM may be due to partial umbilical cord compromise in the etiology of this type of FVM that is known not to be associated with lesions of maternal vascular malperfusion. The same is true about the 2 features of shallow placental implantation which were previously reported in association with mass-forming fetal anomalies [36], but not with umbilical cord compromise [23]. It is difficult to explain why hypertrophic decidual arteriolopathy is more common in high-grade SFVM (Table II), particularly as there is no characteristic distribution in maternal hypertensive conditions among the 3 groups (Table I).

In the present author's database of high-risk pregnancies, the frequency of SFVM is 7%, based on clusters of avascular chorionic villi on HE [27]. Adding SFVM diagnosable by CD34 immunostain and/or histochemistry not only increased the overall prevalence of SFVM but also threw some light on observable new statistically significant differences in frequencies of clinical and placental phenotypes. SFVM lesions diagnosable on HE stained slides are older than those diagnosable only on CD34 immunostained slides (endothelial fragmentation or hypovascularity), a reflection of their temporal heterogeneity as various specific FVM lesions share etiologies but tend to differ in temporality [21]. Nucleated red blood cells were observed more commonly in Group 1A than 1B, probably due to prolonged low-grade in-utero hypoxia [16]. Congenital malformations were more common in low-grade SFVM by CD34 than in low-grade SFVM diagnosable on HE only, likely due to the effect of EXIT procedures in the latter group (without trial of labor). Other associated abnormal placental phenotypes were remarkably scant in this context, proving that FVM is mostly a lesion independent from other placental pathologies in this particularly high-risk subgroup of high-risk pregnancy. However, in many individual cases, a mixed pathology was observed, but the majority of placental lesions were relatively mild, as was observed by others [37, 38].

Co-existing multiple placental pathologies are usually associated with severely compromised fetal/neonatal condition [37]. As one third of fetal deaths remain unexplained [35], CD34 immunohistochemistry and iron/phosphate histochemistry-diagnosed/upgraded lesions increase the sensitivity of placental examination for FVM by adding more cases to those diagnosed on HE only, in particular in totally sclerotic placentas of retained stillbirth [24, 25, 32], in which the segmental distribution of avascular villi can be lost on HE slides [15]. Indeed, placentas from macerated stillbirths cluster with regressive postmortem changes [19] and with no other abnormal phenotypes when evaluated only by HE stain [12]. Now we know that CD34 immunostaining may visualize at least partial/diminished vascularity (Fig. 2B) and histochemistry the segmental mineralization in such cases (Fig. 2C, D), which can increase the sensitivity of placental examination in diagnosing SFVM and/or upgrading SFVM both in not-yet sclerotic or diffusely sclerotic chorionic villi [24, 26]. Therefore, the likely stasis induced FVM of occult umbilical cord compromise should be sought in macerated 3rd trimester stillbirth [32].

In summary, global FVM without a segmental component has similar short-term prognosis as low-grade SFVM. High-grade SFVM is associated with increased likelihood of admission to NICU and neurological complications, no matter whether diagnosed only by HE stain or by immunohistochemistry and/or histochemistry staining. The use of CD34 immunohistochemistry and iron/phosphate histochemistry results in increased sensitivity of placental examination for SFVM (more cases diagnosed), which confirms the usefulness of those stains in diagnosis of FVM. The prognostic significance of SFVM with and without occlusive and non-occlusive thrombi in large fetal vessels and global FVM with and without small clusters of distal villi still have to be determined.

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