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Segmental villous mineralization: A placental feature of fetal vascular malperfusion

Jerzy Stanek

Division of Pathology, Cincinnati Children's Hospital Medical Center, 3333 Burnet Avenue, Cincinnati, OH, 45229, USA

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ABSTRACT

Introduction: This retrospective analysis was performed to find out if clusters of mineralized chorionic villi can be regarded as an independent feature of fetal vascular malperfusion (FVM).
Methods: Of all 1698 placentas reviewed by the author during the last 10 years, 39 (2.3%) showed clusters of mineralized chorionic villi (Group 1), 100 cases (5.9%) showed randomly scattered mineralized chorionic villi with without clustering (Group 2), and the remaining 1559 placentas showed no villous mineralization (comparative Group 3). In doubtful cases, histochemistry stains were performed to determine the pattern of villous mineralization. Twenty three independent clinical and 43 placental variables were statistically compared among the groups: descriptive statistics (Chi-square, Fisher test or signed rank test), and logistics regression model.
Results: Clinically, Group 1 featured shorter gestational age than Group 2, and in addition to shorter gestational age, more common oligohydramnios, polyhydramnios, induction of labor, macerated stillbirth and fetal growth restriction than Group 3. Of placental variables, fetal vascular ectasia, and clusters of avascular chorionic villi were more common in Group 1 than in Group 2, and in addition, segmental villous stromal vascular karyorrhexis was more common than in Group 3. By the logistics regression mode, segmental villous mineralization was independently associated with other histological features of FVM as a group and particularly with clusters of sclerotic chorionic villi.
Discussion: FVM is characterized by temporal heterogeneity, i.e. coexistence of lesions of various duration, and strongly and independently correlates with clusters of mineralized chorionic villi. Therefore, segmental villous mineralization should be included into the category of segmental FVM. It can be seen even in totally fibrotic placentas of prolonged stillbirth when other histological features of segmental vascular malperfusion can be obscured by global villous sclerosis.

1. Introduction

A spectrum of placental lesions is diagnostic of segmental fetal vascular malperfusion (FVM) on hematoxylin-eosin (H&E) stain. They gradually evolve to reach the avascular villi stage after approximately 2 weeks after the inciting event [1,2] (Fig. 1A–C). E cadherin/CD34 immunostain can help in diagnosis if the lesion is of shorter duration and total sclerosis of chorionic villi has not developed yet [3], but even with total villous avascularity on H&E, the segmental nature of FVM may be sometimes recognizable by the stain (Fig. 1D). However, when prolonged retained stillbirth follows the segmental FVM, the ensuing diffuse villous sclerosis can totally obscure the underlying segmental nature of FVM [4].

Placental mineralization is common, mostly presenting as dystrophic calcification of perivillous fibrin, sometimes massive, particularly at the maternal floor, which has no diagnostic or prognostic significance [5,6]. Placental necrotic or fibrotic foci may also secondarily calcify, e.g. laminar necrosis of membranes and maternal floor, necrotizing funisitis, villous infarction, old thrombi in placental vessels (umbilical cord, chorionic, stem and terminal villous capillaries) [2,7]. In these situations, they indicate long duration of the underlying pathology rather than the grade thereof as even non-mineralized multiple thrombi in large placental vessels alone permit the diagnosis of high grade FVM [1]. Calcification of such thrombi, however, permits timing the thrombosis to at least weeks prior to delivery [2].

A distinct type of villous microscopic mineralization is the blue purple discoloration of the trophoblastic basement membrane, trophoblast cytoplasm and villous stroma, formed by sedimentation of minerals which may be positive for iron and/or calcium/or phosphate by histochemistry [6,8] (Fig. 1C). It was reported in fetal hydrops, abortion specimens [6], fetal aneuploidies [9], polyhydramnios [10], thalassemia, anencephaly, Bartter syndrome [11], congenital nephrotic syndrome

E-mail addresses: jerzy.stanek@cchmc.org, jerzy.stanek@cchmc.org.

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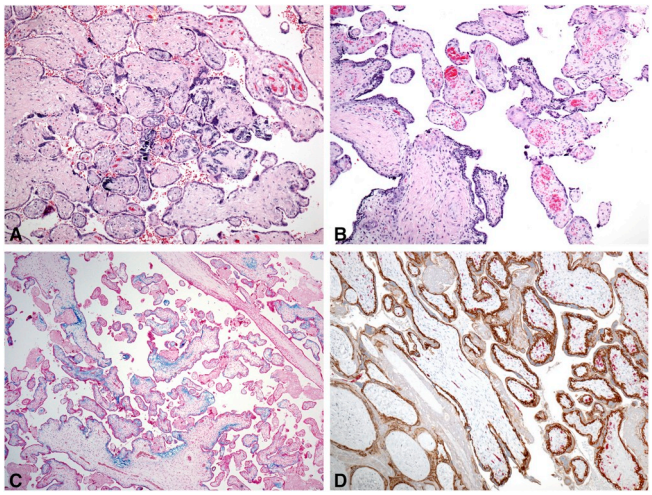

Fig. 1. Lesions of segmental fetal vascular malperfusion diagnosable without taking villous mineralization into consideration (all objective magnifications x10). A. Vascular and totally avascular/sclerotic villi with a superimposed cluster of focally mineralized chorionic villi in a retained 35 weeks stillbirth. B. A cluster of almost totally avascular villi neighboring villi with stromal vascular karyorrhexis at 28 weeks gestation, Treacher-Collins phenotype, died 24 min after birth. C. Hypovascular chorionic villi with focal mineralization, adjacent to totally avascular chorionic villi at 31 weeks gestation, stillbirth of several days duration, iron stain. D. Totally sclerotic villi on H&E examination at 27 weeks gestation, prolonged stillbirth, trisomy 18 syndrome, E cadherin/CD34 immunostain highlights villi with attenuated vessels adjacent to totally avascular villi. Although the E cadherin is not necessary for highlighting the villous hypovascularity, it delineated the clusters of hypovascular villi by highlighting the outlines thereof.

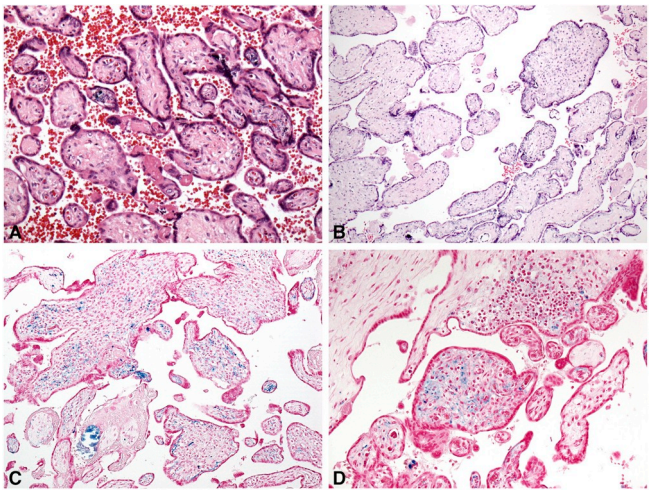

Fig. 2. Segmental mineralization of chorionic villi (A-C objective magnifications x10). A. Diffusely hypovascular chorionic villi with a cluster of mineralized chorionic villi, prolonged stillbirth at 31 weeks gestation, tight nuchal cord. B. Globally sclerotic chorionic villi with a cluster of chorionic villi with basement membrane mineralization, prolonged stillbirth, 30 weeks gestation, multiple pterygium syndrome. C. Globally avascular villi with features of placental hydrops (split between the villous core and trophoblastic shell), prolonged stillbirth, fetal growth restriction, 17 weeks gestation (iron stain). D. Stillbirth of several days' retention, 23 weeks, focal segmental cytomegalovirus villitis with stromal mineralization (iron stain) (x20).

[6,12], maternal smoking [13], stillbirth [14], and FVM, global [15] and segmental [3]; however, it has not been so far proved as a histological evidence of FVM and/or used for diagnosis thereof. The author feels that, in general, such placental villous mineralization is not uncommon, although underrepresented in placental textbooks [5,16,17].

The author observed clusters of mineralized chorionic villi with mineralization involving either only the trophoblast basement membrane or also the villous stroma or both in placentas with FVM [3,4] but statistical evaluation of such finding has never been published or this histological lesion has not been listed as a feature of FVM [1]. This retrospective analysis intends to prove that segmental villous mineralization is a histological sign of FVMM.

2. Materials and methods

The study was approved by the institutional review board (IRB #2016–7942). The placentas were submitted for examination at the discretion of the obstetricians because of high risk-pregnancy or its complications such as fetal distress, operative delivery, poor condition of the neonate, or grossly abnormal placenta, or were a part of autopsy. At least 2 sections of membrane roll and the umbilical cord and 2 paracentral sections of grossly unremarkable placenta were examined, but all gross abnormalities were sampled too. After sectioning, formalin fixation and paraffin embedding, slides were stained with H&E and were reviewed by the author using the same diagnostic criteria as in previous publications [7,18–21]. The nomenclature adopted by the 2016 consensus of the Amsterdam Conference was used [1].

Of all 1698 placentas reviewed by the Author during last 10 years, 39 showed clusters of chorionic villi with mineralization, defined as one or more cluster of chorionic villi with villous basement membrane mineralization (linear or stippled) and/or villous stromal mineralization and/or presence of mineral deposits in villous macrophages (Hofbauer cells), and absence of diffuse villous mineralization (global villous mineralization), i.e. mineralization of chorionic villi in the adjacent placental parenchyma (Fig. 2). In doubtful cases, Prussian blue or von Kossa histochemistry stain was performed (Group 1). 100 cases showed randomly scattered chorionic villi with villous mineralization without clustering (Group 2) (Fig. 3), and the remaining 1559 placentas showed no villous mineralization (Group 3, comparative group). Other types of placental mineralization (dystrophic mineralization of cell islands, perivillous fibrin, and maternal floor, mineralization of intramural fibrin deposition or large placental vessels mural thrombi, decidua, placental membranes, umbilical cord, or syncytiotrophoblast

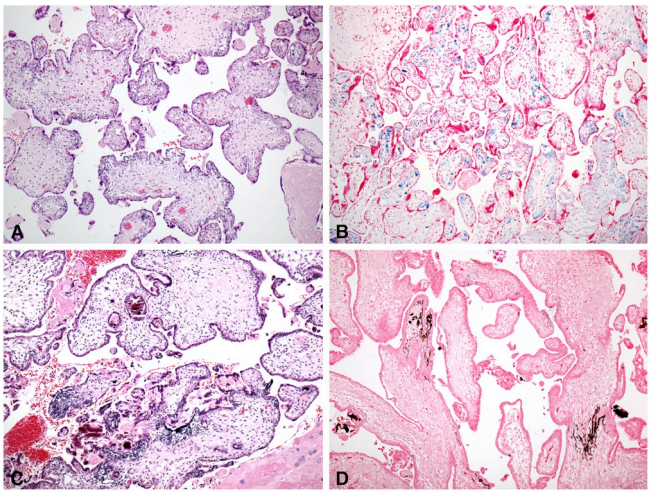

Fig. 3. Diffuse villous mineralization (all objective magnifications x10). A. Prolonged stillbirth, 25 weeks, villi are still vascular, oligohydramnios sequence, mineralization of liver and kidneys (H&E stain). B. Prolonged stillbirth, villi still vascular, 30 weeks, tight nuchal cord ×2 (iron stain). C. Stillbirth of several days duration, villi still focally vascular, villous edema and fibrosis, scattered diffuse mineralization of basement membranes, (H&E stain, double trisomy X and 18, 17 weeks gestation). D. Prolonged stillbirth, Turner syndrome, 21 week gestation, scattered diffuse villous mineralization (von Kossa stain).

pseudoinclusions) were not considered the basis for the classification.

Twenty-three independent clinical and 43 placental variables were statistically compared between Groups 1 and 2, and then between Groups 1 and 3 (for categorical variables, using the likelihood Chi-square test, or Fisher exact test; and for continuous variables the Wilcoxon signed-rank test. To account for multiple comparisons, significance was set a priori at alpha (α) = 0.002.)

In order to assess whether the odds of being diagnosed with FVM differed between the groups independent of possible confounders, covariates or other predictors – a logistic regression model was run, modeling the probability (log-odds) of FVM by groups and variables that are possibly medically/biologically associated. Two different outcome variables were used in separate models: (1) FVM defined as being positive for one or more of the following: fetal vascular ectasia, fetal vascular thrombi, intramural fibrin deposition in chorionic or stem veins, clusters of at least 3 avascular chorionic villi, segmental villous stromal vascular karyorrhexis; (2) positive for clusters of at least 3 avascular chorionic villi.

The initial predictors/covariates for inclusion in the model were: Group, gestational age, preeclampsia, chronic hypertension, diabetes mellitus, maternal smoking/substance abuse, fetal growth restriction, oligohydramnios, polyhydramnios, abnormal Dopplers, stillbirth, clinical umbilical cord compromise, congenital anomalies, abnormal coiling of umbilical cord, other umbilical cord pathology, uterine chronic hypoxic injury, chronic villitis of unknown etiology, acute chorioamnionitis (fetal inflammatory reaction).

Univariate associations of each predictor with FVM were first tested using a likelihood-ratio chi-square test (2 × 2) table. If the result was $p < 0.10$, then that covariate was initially included in the logistic regression model (along with all other covariates meeting that threshold). Using backward, step-wise elimination, a final model was produced for both outcomes (FVM and clusters of at least 3 avascular chorionic villi), with only those covariates included with a $p < 0.10$.

3. Results

Results of descriptive statistics are presented in Tables 1 and 2. Villous mineralization was observed in 8.2% placental cases in this material (2.3% clustered/segmental- Group 1, and 5.9% diffuse/global-Group 2) (Table 1). The frequency of abnormal clinical phenotypes in these groups were similar between Group 1 and Group 2, except for gestational age which was shorter in Group 2, likely due to higher number of early 2nd trimester deliveries in Group 2. There were, however, several statistically significant differences between Groups 1 and Group 3: gestational age was longer in Group 3, while oligohydramnios, polyhydramnios, induction of labor, perinatal mortality, and macerated stillbirth were more common in Group 1. Overall, most cases of Groups 1 and 2 ended in perinatal mortality.

Of placental phenotypes (Table 2), at least one component of FVM was seen 87.2% in Group 1, 47.0% in Group 2, and 37.7% in Group 3. The only two statistically significant differences between Group 1 and Group 2, were increased frequencies of fetal vascular ectasia and clusters of avascular chorionic villi. Frequencies of the following abnormal placental phenotypes were more common in Group 1 than in Group 3: increased amount of extravillous trophoblasts in the chorionic disc (a lesion of shallow placental implantation) and five features of FVM: fetal vascular ectasia, clusters of sclerotic chorionic villi, segmental stromal vascular karyorrhexis, luminal vascular abnormalities of stem chorionic villi and diffusely increased extracellular matrix of chorionic villi, the latter two the features of global FVM, associated with prolonged stillbirth, however. Of note, there were no statistically significant differences in inflammatory or hypoxic lesions/patterns among the three groups. Erythroblastosis of fetal blood, diffuse patterns of chronic hypoxic placental injury and various umbilical cord abnormalities were more common in Group 1 than in Group 3, albeit not statistically significantly.

Table 3 shows that group is a significant and independent predictor of FVM ($p < 0.0001$). Group 1 has greater odds than Group 2 (OR 5.5, 95% confidence interval 1.9, 15.8) and greater odds than Group 3 (OR 8.2, 95% confidence interval 3.1, 21.9) of having FVM, even accounting for other significant predictors. Those covariates are: gestational age ($p < 0.0001$), polyhydramnios ($p = 0.0003$), stillbirth ($p = 0.0042$), clinical umbilical cord compression ($p = 0.0041$), congenital anomalies ($p = 0039$), hypercoiling of umbilical cord ($p = 0.0313$), other umbilical cord abnormalities ($p = 0.001$), chronic villitis of unknown etiology ($p = 0.0007$), and uterine pattern of chronic hypoxic placental injury ($p = 0.0016$).

By the same token, Group is a significant and independent predictor of clusters of at least 3 avascular chorionic villi ($p < 0.0001$). Group 1 has greater odds than Group 2 (OR 5.1, 95% confidence interval 2.2, 11.8) and greater odds than Group 3 (OR 2.0, 95% confidence interval 1.3, 3.2) (Table 4). Several covariates have also have significant associations with clusters of at least 3 sclerotic chorionic villi: fetal growth restriction ($p = 0.0016$), polyhydramnios ($p = 0.0026$), clinical umbilical cord compression ($p = 0.0220$), other umbilical cord abnormalities ($p = 0.0066$), chronic villitis of unknown etiology ($p < 0.0001$), and uterine type of chronic hypoxic placental injury ($p = 0.009$).

4. Discussion

The most valuable test for determination of cause of fetal death is placental examination [22,23]. FVM is commonly regarded as one of the most important causes of perinatal morbidity/mortality, but only extensive FVM involving 40–60% of the placental mass is regarded as causative of fetal death [24]. Multiple other factors may be operative in perinatal morbidity/mortality and unexpected umbilical cord compromise at term is regarded as major cause of perinatal mortality at term [25]; therefore even smaller involvement of the placenta by FVM may be important if other etiologies co-exist [26]. This is reflected by observations that mixed placental pathologies are causative of third-trimester stillbirth, with three quarters of the placentas demonstrating combinations of maternal vascular pathology, fetal vascular pathologies, umbilical cord abnormalities, or inflammatory lesions [27], such as e.g. cytomegalovirus focal segmental plasmacytic villitis [28].

Although correlation does not mean causation, strong association of clustered villous mineralization and clusters of sclerotic/hypovascular chorionic villi (Table 2) indicates that that this lesion is likely of same etiopathogenesis and has likely similar diagnostic value in diagnosing FVM, therefore present even without associated clusters of sclerotic but not mineralized chorionic villi, is equivalent to segmental FVM. The mechanism of mineral deposits in sclerotic chorionic villi appears to be due to the fact that transport of minerals into chorionic villi continues for a time, but without fetal circulatory removal and uptake, particularly in the 2nd trimester [29]. Right ventricular failure is the mechanism of placental hydrops and villous mineralization in fetal aneuploidies [30] but other mechanisms were also suggested, such as FVM secondary to fetal artery thrombosis [9], particularly in stillbirths [14], thalassemic patients [8], and mutations in calcium iron channels, such as in Bartter syndrome [31]. In addition, the mechanism may be in some cases different for basement membrane mineralization, which is commonly associated with villous edema (like in Groups 1 and 2, imbalance in the iron transport from maternal blood through the vasculosyncytial membrane, particularly in the 2nd trimester due to poor development thereof, and of villous core iron deposits usually due to microhemorrhage, like in cytomegalovirus infection. Both types, however occur in FVM [28]. Likewise, both types of mineralization occur after experimental interplacental bridging vessel ligation in bilobed placenta [32]. I think that the avascular villi seen in the succenturiate lobe may mineralize due to similar mechanisms as those in our Group 1, but the reason for stromal villous calcification in the main lobe is unclear to me.

In fact, clusters of sclerotic chorionic villi were 4.4 times more frequent in Group 1 than in Group 3, but they were also 2.4 times more frequent in Group 2 than in Group 3, the latter explainable by more

Table 1
Clinical phenotypes.

	Group 1 Villous clustered mineralization	Group 2 Diffuse villous mineralization	Group 3 No villous mineralization	χ^2 test or Fisher:1 vs. 2 (Signed-rank for G. age)	P-value for χ^2 test:1 vs. 2. (Values in bold indicate $p < 0.002$)	χ^2 test or Fisher:1 vs. 3 (Signed-rank for G. age)	P-value for χ^2 test:1 vs. 3. (Values in bold indicate $p < 0.002$)
Number of cases	39	100	1559				
Gestational hypertension	2 5.1%	5 5%	61 3.9%		NS		NS
Preeclampsia	4 10.3%	4 4%	136 8.7%		NS		NS
Chronic hypertension	4 10.3%	1 1%	41 2.6%	6.1	0.02	5.0	0.02
Gestational age (weeks, average ± standard deviation)	29.8 ± 6.3	24.0 ± 8.0	32.9 ± 7.0	15.4	< **0.0001**	10.9	**0.001**
Early second trimester (< 20 weeks)	3 7.7%	40 40%	98 6.3%	16.2	**0.0002**		NS
Poor or absent prenatal care	2 5.1%	7 7%	83 5.3%		NS		NS
Substance abuse	6 15.4%	16 16%	143 9.2%		NS		NS
Maternal diabetes mellitus	4 10.3%	7 7%	97 6.2%		NS		NS
Oligohydramnios	10 25.6%	15 15%	133 8.5%		NS	9.6	**0.0019**
Polyhydramnios	8 20.5%	11 11%	80 5.1%		NS	10.8	**0.001**
Premature rupture of membranes	8 20.5%	5 5%	237 15.2%	7.1	0.008		NS
Antepartum hemorrhage	5 12.8%	4 4%	208 13.3%		NS		NS
Meconium-stained amniotic fluid	6 15.4%	4 4%	186 11.9%	4.8	0.03		NS
Abnormal fetal heart rate tracing[a]	5 12.8%	10 10%	329 21.1%		NS		NS
Abnormal umbilical artery Dopplers	4 10.3%	7 7%	92 5.9%		NS		NS
Induction of labor	15 38.5%	36 36%	255 16.4%		NS	10.6	**0.001**
Cesarean section	15 38.5%	19 19%	730 46.8%	5.4	0.02		NS
Multiple pregnancy	2 5.1%	10 10%	127 8.1%		NS		NS
Perinatal mortality	27 69.2%	76 76%	427 27.4%		NS	28.6	< **0.0001**
Neonatal mortality	7 17.9%	14 14%	148 9.5%		NS		NS
Nonmacerated stillbirth	3 7.7%	8 8%	70 4.5%		NS		NS
Macerated stillbirth	17 43.6%	54 54%	209 13.4%		NS	20.5	< **0.0001**
Fetal growth restriction[b]	13 33.3%	31 31%	266 17.1%		NS	5.9	0.015
Umbilical cord compromise[c]	6 15.4%	10 10%	113 7.2%		NS		NS
Congenital malformations	14 35.9%	28 28%	250 16.0%		NS	17.4	0.003
Abnormal 3rd stage of labor (prolonged, hemorrhage)	6 15.4%	2 2%	120 7.7%	8.1	0.004		NS

All p-value results < 0.05 are shown.

[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] birth weight < 10 centile.

[c] variable decelerations, encirclement, true knot, or prolapse, NS statistically not significant.

common macerated stillbirth and villous edema of right ventricular failure in the former (the mechanism of fetal death in chromosomal abnormalities and congenital malformations) [30] (Fig. 3). However, in totally fibrotic placentas of prolonged stillbirth, clusters of sclerotic chorionic villi may not be easily visible on hematoxylin-eosin stained slides. CD34 immunostain is occasionally helpful in visualizing segmental FVM in stillbirths [3] (Fig. 1D), but the test may be more applicable to stillbirth of shorter duration and not to totally and diffusely sclerotic placenta, therefore it may not always have utility in very prolonged stillbirth and long lasting diffuse/global villous avascularity.

Clusters of mineralized chorionic villi are less common than non-mineralized clusters of sclerotic chorionic villi diagnosed by E-cadherin/CD34 immunostain [3] because not all stillbirths are retained long enough for the mineralization to occur. The clusters can occasionally be seen on H&E slides (Fig. 2A and B), but may be better visible only on histochemistry slides (Fig. 2C and D). This becomes even more striking when compared with our overall population of placentas from high-risk pregnancies [21]: clusters of avascular villi were seen 7% of placentas in general, but in the group 1 of the current analysis 10 times more frequently (Table 2). This is due most likely to the fact that that the clusters of avascular villi would eventually mineralize if delivery were delayed by a few more weeks after formation of clusters of sclerotic chorionic villi. Temporal heterogeneity of villous lesions in FVM, i.e. still vascular chorionic villi adjacent to sclerotic chorionic villi and mineralized chorionic villi (Fig. 1A) illustrates not only the reason for our approach, but also the ongoing process of FVM (Fig. 4). Eventually, mineralized thrombi in brain vessels may develop [33]. However, the CD34 immunostain may reveal residual capillaries even in chorionic villi that appear to be totally avascular on hematoxylin-eosin stained placental sections (Fig. 1D). Global villous mineralization seems to be more associated with villous edema and/or global (diffuse) postuterine hypoxic pattern [18] of stillbirth [15] (Fig. 3). In fact, higher percentages of macerated stillbirth in Group 2 than in Group 3 (Table 1) correlated with higher percentages of global placental regressive changes in Group 2 including diffuse villous mineralization rather than with segmental villous mineralization (Table 2).

The results of the current analysis indicate, that the stasis-induced FVM of umbilical cord compromise may be more frequent than reported 10.4% [33–35], also in the second trimester and preterm third trimester pregnancies, as various umbilical cord abnormalities were observed in equal frequency in Groups 1 and 2 (almost a third of placentas of those groups, twice as frequently as in Group 3) (Table 2), as clusters of avascular chorionic villi show a strong correlation with gestational age [7]. In fact, although higher rate of fetal vascular supply abnormalities were found throughout pregnancy, the involvement of placental fetal vascular supply lesions is more extensive in early fetal death than in late fetal death [36].

It is unclear why increased amount of extravillous trophoblast is associated with both segmental and global villous mineralization. It is a

Table 2
Placental phenotypes.

	Group 1 Villous segmental mineralization	Group 2 Diffuse villous mineralization	Group 3 No villous mineralization	χ^2 test or Fisher:1 vs. 2 (Signed-rank for G. age)	P-value for χ^2 test: 1 vs. 2. (Values in bold indicate $p < 0.002$)	χ^2 test or Fisher:1 vs. 3 (Signed-rank for G. age)	P-value for χ^2 test: 1 vs. 3. (Values in bold indicate $p < 0.002$)
Number of cases	39	100	1559				
Placental weight (grams, average ± standard deviation)	351.3 ± 345.3	249.6 ± 290.8	369.4 ± 185	7.4	0.007	5.25	0.02
Inflammatory lesions							
Acute chorioamnionitis	16 41.0%	44 44%	556 35.7%		NS		NS
Chronic villitis of unknown etiology	7 17.9%	4 4%	201 12.9%	6.6	0.01		NS
Plasma cell deciduitis	1 2.6%	8 8%	79 5.1%		NS		NS
Hypoxic lesions/patterns							
Acute							
Meconium (histological)	12 30.8%	21 21%	574 36.8%		NS		NS
Deep (decidual)	0 0%	9 9%	129 8.3%		NS		NS
Shallow (amnionic or chorionic)	12 30.8%	12 12%	445 28.5%	6.4	0.012		NS
Intravillous hemorrhage	3 7.7%	7 7%	81 5.2%		NS		NS
Villous infarction (> 5% of placental parenchyma)	6 15.4%	12 12%	134 8.6%		NS		NS
Laminar necrosis of membranes[a]	9 23.1%	33 33%	431 27.6%		NS		NS
Chronic							
Erythroblastosis of fetal blood	12 30.8%	17 17%	236 15.1%		NS	5.85	0.02
Hypertrophic decidual arteriopathy	12 30.8%	17 17%	286 18.3%		NS		NS
Atherosis of spiral arterioles	4 10.3%	6 6%	73 4.7%		NS		NS
Patterns of chronic hypoxic injury	16 41.0%	25 25%	319 20.5%		NS	8.3	0.0041
Preuterine	5 12.8%	4 4%	82 5.3%		NS		NS
Uterine	8 20.5%	13 13%	153 9.8%		NS	3.8	0.0499
Postuterine	3 7.7%	8 8%	84 5.4%		NS		NS
Retroplacental hematoma	2 5.1%	10 10%	98 6.3%		NS		NS
Intervillous thrombus	9 23.1	22 22%	254 16.3%		NS		NS
Lesions of shallow placental implantation							
Membrane chorionic microcysts[b]	3 8.0%	6 6%	204 13.1%		NS		NS
Chorionic disc extravillous trophoblast microcysts[c]	6 15.4%	18 18%	258 16.5%		NS		NS
Maternal floor multinucleate trophoblastic giant cells	11 28.2%	25 25%	339 21.7%		NS		NS
Excessive amount of extravillous trophoblasts in chorionic disc	16 41.0%	30 30%	249 16.0%			13.5	**0.0002**
Placenta creta (including basal plate myometrial fibers)	3 7.7%	9 9%	141 9.0%		NS		NS
Fetal vascular malperfusion							
Fetal vascular ectasia	18 46.2%	17 17%	204 13.1%	11.9	**0.0006**	24.4	< **0.0001**
Fetal vascular thrombi	12 30.8%	25 25%	288 18.5%		NS		NS
Intramural fibrin deposition in chorionic or stem vein	7 17.9%	16 16%	90 5.8%		NS	6.8	0.009
Clusters of at least 3 avascular chorionic villi	28 71.8%	30 30%	252 16.2%	20.3	< **0.0001**	57.4	< **0.0001**
Segmental villous stromal vascular karyorrhexis	10 25.6%	16 16%	77 4.9%		NS	17.9	< **0.0001**
Luminal vascular abnormalities of chorionic villi	15 38.5%	47 47%	147 9.4%		NS	22.7	< **0.0001**

(*continued on next page*)

Table 2 (*continued*)

	Group 1 Villous segmental mineralization	Group 2 Diffuse villous mineralization	Group 3 No villous mineralization	χ^2 test or Fisher:1 vs. 2 (Signed-rank for G. age)	P-value for χ^2 test: 1 vs. 2. (Values in bold indicate $p < 0.002$)	χ^2 test or Fisher:1 vs. 3 (Signed-rank for G. age)	P-value for χ^2 test: 1 vs. 3. (Values in bold indicate $p < 0.002$)
Diffusely increased extracellular matrix of chorionic villi	19 48.7%	60 60%	205 13.1%		NS	27.7	< **0.0001**
Other							
Massive perivillous fibrin deposition (> 30% of placental parenchyma)	1 2.6%	3 3%	92 5.9%		NS		NS
Chorangiosis	3 7.7%	4 4%	167 10.7%		NS		NS
Obliterative endarteritis	5 12.8%	2 2%	97 6.2%	6.0	0.014		NS
Choriodecidual hemosiderosis	5 12.8%	9 9%	107 6.9%		NS		NS
Villous edema	4 10.3%	25 25%	96 6.2%	4.1	0.043		NS
Two-vessel umbilical cord	4 10.3%	3 3%	64 4.1%		NS		NS
Hypercoiled umbilical cord	13 33.3%	22 22%	341 21.9%		NS		NS
Hypocoiled umbilical cord	0 0%	17 17%	156 10.0%	12.1	0.003	8.1	0.004
Stem perivascular stem edema	5 12.8%	4 4%	122 7.8%		NS		NS
Marginal insertion of umbilical cord	1 2.6%	7 7%	116 7.4%		NS		NS
Velamentous insertion of umbilical cord	0 0%	4 4%	50 3.2%		NS		NS
Other umbilical cord abnormalities[d]	12 30.8%	30 30%	260 16.7%		NS	4.6	0.033
Amnion nodosum/chorion nodosum	5 12.8%	19 19%	74 4.7%		NS		NS
Marginate or vallate placenta	1 2.6%	2 2%	71 4.6%		NS		NS
Gross chorionic cyst(s)	1 2.6%	0 0%	15 1.0%		NS		NS
Succenturiate lobe	2 5.1%	2 2%	32 2.1%		NS		NS

All p-value results < 0.05 are shown.

[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] too long, too short, too thin, stricture, aneurysm, varix, hematoma, vessel unprotected by Wharton jelly, chorda, ulcer, barber pole funisitis, amniotic band, meconium toxicity, furcate insertion, edema; NS statistically not significant.

Table 3
Logistic regression results: Fetal vascular malperfusion: Odds-ratios for group.

Odds Ratio Estimates and Wald Confidence Intervals			
Odds Ratio	Estimate	95% Confidence Limits	
Group 1 vs Group 2	5.462	1.893	15.758
Group 1 vs Group 3	**8.194**	**3.067**	**21.897**
Group 2 vs Group 3	1.500	0.943	2.387

Group is a significant and independent predictor of FVM ($p < 0.0001$), irrespective of other covariates, even accounting for other significant predictors.

Table 4
Clusters of at least 3 avascular chorionic villi: Logistic regression results: Odds-ratios for group.

Odds Ratio Estimates and Wald Confidence Intervals			
Odds Ratio	Estimate	95% Confidence Limits	
Group 1 vs Group 2	5.067	2.179	11.781
Group 1 vs Group 3	**10.249**	**4.919**	**21.353**
Group 2 vs Group 3	**2.023**	**1.266**	**3.232**

Group is a significant and independent predictor of clusters of avascular chorionic villi ($p < 0.0001$), irrespective of other covariates.

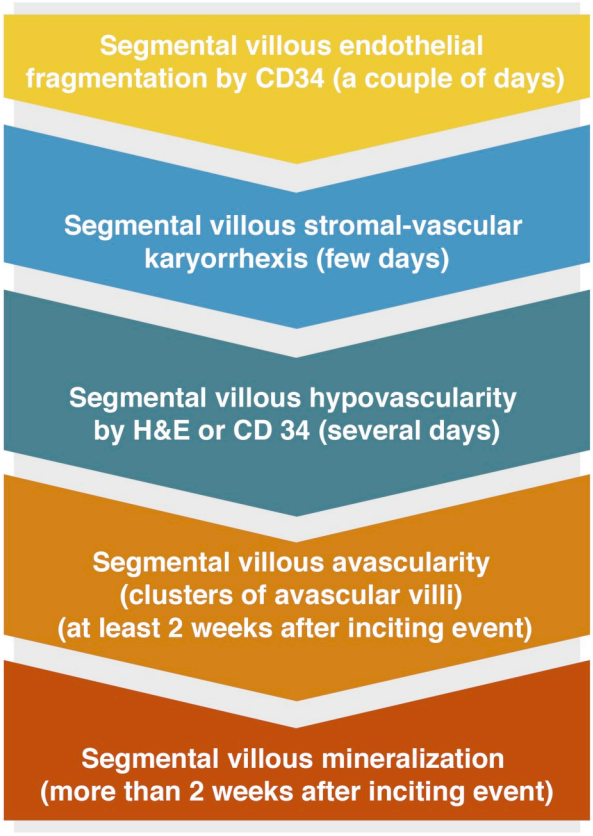

Fig. 4. Tentative time frame of temporal heterogeneity of lesions of segmental FVM. The type of lesion reflects the time of duration of FVM, but the grade depends on the extent of villous involvement. By the Amsterdam criteria, the grading of segmental FVM refers only to avascular villi, but it appears that total villous avascularity/sclerosis reflects mainly a time point rather than the extent of the process. All lesions presented on the diagram may also appear diffusely (global fetal malperfusion) after fetal death.

lesion of shallow placental implantation that was found to be more common not only in mass forming fetal congenital anomalies, and in heart malformations resulting in placental hydrops [37], but also in association with clinical and placental features of umbilical cord compromise [25], which is consistent with fetal vascular ectasia being common in Group 1 (Table 2). The two etiologies may have an additive impact resulting in an increase of extravillous trophoblast and diffuse and segmental villous mineralization (Table 2).

In summary, results of both descriptive statistics and logistic regression indicate strong correlation of segmental villous mineralization with other lesions of segmental FVM and non-segmental FVM and that segmental villous mineralization is an independent lesion of FVM which can be the only lesion identifiable in totally sclerotic placenta of retained stillbirth, particularly useful in the differential diagnosis of etiology of prolonged stillbirth. This histological placental lesion seems to be the last and least frequent of FVM lesions but nevertheless an important stage of FVM which should be added to the list of segmental lesions of FVM (Fig. 4).

Declaration of competing interests

None to report.

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