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Maternal Floor Infarction/Massive Perivillous Fibrin Deposition: A Manifestation of Maternal Antifetal Rejection

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Abstract

Objective—Massive perivillous fibrin deposition (MPFD) and maternal floor infarction (MFI) are related placentallesions often associated with fetal death and fetal growth restriction. A tendency to recur in subsequent pregnancies has been reported. This study was conducted to determine whether this complication of pregnancy could reflect maternal anti-fetal rejection.

Methods—Pregnancies with MPFD were identified (n=10). Controls consisted of women with uncomplicated pregnancies who delivered at term without MPFD (n=175). Second-trimester maternal plasma was analyzed for panel-reactive anti-HLA class I and class II antibodies. The prevalence of chronic chorioamnionitis, villitis of unknown etiology, and plasma cell deciduitis was compared between cases and controls. Immunohistochemistry was performed on available umbilical vein segments from MPFD cases (n=4) to determine whether there was evidence of complement activation (C4d deposition). Specific maternal HLA-antibody and fetal HLA-antigen status were also determined in paired specimens (n=6). Plasma CXCL-10/IP-10 concentrations were measured in longitudinal samples of cases (n=28 specimens) and controls (n=749 specimens) by ELISA. Linear mixed models were used to test for differences in plasma CXCL-10 concentration.

Results—1) The prevalence of plasma cell deciduitis in the placenta was significantly higher in cases with MPFD than in those with uncomplicated term deliveries (40% vs. 8.6%, p=0.01); 2) patients with MPFD had a significantly higher frequency of maternal anti-HLA class I seropositivity during the second trimester than those in uncomplicated term deliveries (80% vs. 36%, p=0.01); 3) strongly positive C4d deposition was observed on umbilical vein endothelium in cases of MPFD; 4) specific maternal antibody against fetal HLA antigen class I or II was identified in all cases of MPFD; and 5) the mean maternal plasma concentration of CXCL-10 was higher in patients with evidence of MPFD than in those without evidence of MFPD (p <0.001).

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Conflicts of Interest
The authors have no financial conflicts of interest.

Conclusions—Collectively, the data presented herein suggest that a subset of patients with MPFD has a signature of maternal anti-fetal rejection as a mechanism of disease.

Keywords

PRA; MPFD; fibrinoid deposition; stillbirth; HLA; plasma cell deciduitis; villitis

Introduction

Massive perivillous fibrin deposition (MPFD) and maternal floor infarction (MFI) are related placental lesions characterized by extensive deposition of fibrinoid material in the intervillous space, and associated with hypoplastic and sclerosis of the engulfed villi.[1–3] Fibrin and/or fibrinoid material deposition interferes with perfusion and gas/nutrient exchange in the intervillous space, resulting in “chronic placental insufficiency”.[4–8] Pregnancies with MPFD are associated with serious obstetrical complications, such as spontaneous abortion,[3, 9, 10] fetal growth restriction,[3, 4, 6, 7, 11–15] and fetal death.[3, 4, 6, 7, 10, 12, 14–22] The mechanisms responsible for MPFD are unknown.[3, 23]

The fetus is the most successful semi-allograft. Therefore, maternal immune tolerance of the fetus is essential for successful pregnancy.[24–47] Failure of maternal tolerance to the fetus has been proposed to be a mechanism of disease in recurrent pregnancy loss,[48–52] preterm delivery,[18, 44, 53, 54] fetal growth restriction (FGR),[4, 12, 55] fetal death[4, 12, 56] and preeclampsia (PE).[44, 50, 52, 55, 57–60] Allograft rejection involves both the innate and adaptive limb of the immune response.[61, 62] The most important alloantigens are within the major histocompatibility complex (MHC) class I and class II, and are part of the human leukocyte antigen (HLA) system.[61, 63]

An important feature of humoral antibody-mediated rejection after allograft transplantation is the generation of donor-specific HLA. To screen for the presence of these antibodies, HLA panel reactive antibodies (PRA) can be used.[64–66] HLA sensitization is a risk factor for graft rejection.[67, 68] HLA panel-reactive antibodies (PRA) are used to determine the HLA sensitization status of recipients[69, 70] and to assess the likelihood of graft rejection in patients who undergo transplantation.[71–74] The presence of HLA-antibodies in early pregnancy is associated with a reduced chance of live birth.[75] Moreover, the presence of C4d deposition (a degradation product of complement factor C4) is considered to be an evidence of antibody-mediated rejection of the allografts.[76, 77] For example, in a renal transplant, immunostaining for C4d in glomerular endothelial cells and peritubular capillaries in renal allograft biopsies has been shown to be an important indication of graft pathology.[78, 79]

Recently, we and other investigators proposed that chronic chorioamnionitis (infiltration of maternal T cells in the chorioamniotic membranes)[54, 56, 80, 81], villitis of unknown etiology (VUE)[19, 20, 82, 83] and chronic deciduitis with plasma cell[74] are the placental lesions associated with maternal anti-fetal rejection. Prior reports showed that maternal HLA PRA positivity before 16 weeks[74] and at the time of diagnosis[54] are associated with the presence of chronic chorioamnionitis. Furthermore, maternal, fetal plasma and amniotic fluid concentration of CXCL-10, an anti-angiogenic T-cell chemokine, is higher in patients with

evidence of placental lesions associated with maternal anti-fetal rejection than in those without these lesions.[19, 20]

We postulated that if maternal anti-fetal rejection is involved in the mechanism of disease of MPFD, evidence in support of graft rejection may be present. Consequently, the objective of this study was to examine whether in MPFD there is: 1) a difference in the frequency of placenta lesions associated with maternal anti-fetal rejection; 2) an increased frequency of maternal anti-HLA seropositivity; 3) evidence of complement activation in the fetus (C4d deposition on umbilical cord vessels); and 4) a change in the maternal plasma concentration of CXCL-10.

Material and Methods

Placental pathologic specimens from the Bank of Biological Materials of the Wayne State University/Detroit Medical Center/Perinatology Research Branch, *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, National Institutes of Health, U.S. Department of Health and Human Services, from 2006 to 2011 were reviewed. Cases meeting the criteria of MPFD were identified. The criterion for the diagnosis was the identification of perivillous fibrinoid material encasing at least 50% of the villi on a minimum of one slide[3]. Either cases with fibrinoid material only on the maternal floor side of the placenta or cases with transplacental fibrinoid deposition were eligible (n=10). Controls (n=175) were women without MPFD in the placenta who had uncomplicated pregnancies, delivered a neonate whose birth weight was appropriate for gestational age (10^{th} – 90^{th} percentiles)[84] and had plasma samples available for at least five of the following gestational age intervals: 6–15, 16–20, 20–24, 25–28, 28–32, 32–36 and ≥37 weeks. These patients had been enrolled in a longitudinal protocol to identify biological markers for the prediction of PE, SGA, and stillbirth. Venous samples were collected every 4 weeks until 24 weeks and every 2 weeks thereafter until delivery. Exclusion criteria were 1) multiple gestations; and 2) congenital fetal anomaly.

All women provided written informed consent before participating in the study, and the use of clinical data and the collection and utilization of biological samples for research purposes were approved by the Institutional Review Boards of Wayne State University and the *Eunice Kennedy Shriver* Nation al Institute of Child Health and Human Development, National Institutes of Health.

Placental Pathology

Histopathologic changes of the placenta were identified using the diagnostic criteria of the Perinatal Section of the Society for Pediatric Pathology.[85] Plasma cell deciduitis was defined as a lymphoplasmacytic infiltration of the decidua of the basal plate.[86] The diagnosis of VUE and chronic chorioamnionitis were based on previously published descriptions.[18, 20]

Briefly, the diagnosis of VUE is made when lymphohistiocytic infiltration is present in more than five villi on multiple slides. Chronic chorioamnionitis is confirmed when lymphocytic

infiltration into the chorionic trophoblast layer or chorioamniotic connective tissue is observed.

Flow Cytometry of Panel-Reactive Maternal Anti-HLA Antibodies

HLA PRA seropositivity was determined in plasma of both cases and controls. Flow cytometry was performed using the FlowPRA-I and FlowPRA-II Screening Tests (One Lambda Inc., Canoga Park, CA). The test represents a pool of 30 HLA antigens of each class I and class II and is designed to provide a non-specific assessment of the presence or absence of anti-HLA-I or anti-HLA-II antibodies. We followed the manufacturer recommendations for the assay; 20 μl of plasma were incubated with FlowPRA beads for 30 min at room temperature. The manufacturer's wash buffer was added and vortexed, and the supernatant was discarded a total of three times. The beads were then stained with 100 μl of 1X FITC labeled anti-human IgG antibody for 30 min. After washing the beads twice with wash buffer and adding fixing solution (PBS with 0.5% formaldehyde), the FL1 fluorescence of 5,000 events was analyzed using BD LSRII flow cytometry (BD Biosciences, San Jose, CA). Plasma that was anti-HLA IgG positive showed a fluorescent channel shift as compared with the negative serum; the percentage of PRA was represented by the percentage of beads that reacted positively with the serum, and a figure of >10% was considered a positive result.

The frequency of HLA PRA seropositivity in MPFD was compared to the control group. For this comparison, only the specimens collected closest to the time of delivery of MPFD cases were included. Samples from the control group were matched for gestational age at delivery (n=143) of the majority of cases with MPFD.

Flow Cytometry of Specific Maternal and Fetal Anti-HLA Antibodies

Specific antibody reactivity in maternal blood was then determined in the same cases using maternal blood collected just prior to delivery and fetal umbilical cord DNA from specimens obtained at the time of delivery (sample available in 6/10 cases). First, to assess the maternal HLA antibody, the LABScreen Single Antigen test (One Lambda Inc., Canoga Park, CA) was used. Five μL of each bead group were incubated in 20 μL of test plasma with gentle shaking. After washing, 1 μL of diluted FITC-conjugated anti-human IgG was added and incubated for another 30 min at room temperature. The sample was washed twice and then analyzed by flow cytometry. A negative control was used to generate flow cytometer settings prior to running prepared serum, according to the manufacturer's specifications. Data analysis was conducted with HLA Fusion 2.0 software (One Lambda Inc., Canoga Park, CA).

To assess fetal specificity of maternal HLA, the PRA LABType SSO typing kit (One Lambda Inc., Canoga Park, CA) was used to obtain the fetal HLA genotype. Genomic DNA was obtained from cord blood. To collect cord blood, a small piece of snap-frozen umbilical cord was ground with liquid nitrogen in a mortar. Genomic DNA was isolated from the ground tissue using the DNeasy Blood and Tissue Kit (Qiagen Inc., Valencia, CA). For fetal HLA genotyping, locus-specific polymerase chain reaction (PCR) amplification was performed with 2 μl of genomic DNA from the umbilical cord (20 to 50 ng/μl), D-mix (One

Lambda Inc., Canoga Park, CA), locus-specific amplification primers (One Lambda Inc., Canoga Park, CA) and Taq DNA Polymerase (Applied Biosystems, Carlsbad, CA). PCR was conducted during the following cycles: 1 cycle at 96 °C for 3 min; 5 cycles at 96 °C for 20 s, 60 °C for 20 s, and 72 °C for 20 s; 30 cycles at 96 °C for 10 s, 60 °C for 15 s, and 72 °C for 20 s; 1 cycle at 72 °C for 10 min. The amplified PCR products were then denatured and neutralized, followed by hybridization with LABType SSO beads at 60°C for 15 min. After three washes with wash buffer, the beads were labeled with R-PE Conjugated Streptavidin and washed twice. Data acquisition was performed with Luminex 100 (Luminex Corporation, Austin, TX), and data analysis was conducted with HLA Fusion 2.0 software (One Lambda Inc., Canoga Park, CA)

C4d Deposition Assessment

Immunohistochemistry was used to assess for C4d deposition on umbilical vein segments as specimens became available (n=4). Cases with a fetal death were excluded due to autolysis of tissue. Five-μm segments of formalin-fixed and paraffin-embedded umbilical cord were stained using a Ventana Discovery automatic staining system (Ventana Medical Systems, Tucson, AZ). A mouse monoclonal anti-human C4d antibody (1:100, ALPCO Diagnostics, Salem, NH) was used for immunostaining, and the Discovery DAB Map Kit (Ventana Medical Systems, Tucson, AZ) was used to detect the chromogen reaction of horseradish peroxidase. Positivity for C4d staining was defined by widespread circumferential venous epithelial staining.

CXCL-10/IP-10 Expression Determination

Plasma CXCL-10 concentrations were determined in samples collected longitudinally from cases (n=10 patients; n=28 samples) and the entire control population (n=175 patients; n= 749 samples) by ELISA. Immunoassays for CXCL-10 (R&D Systems, Minneapolis, MN) were used and maternal plasma samples were added to assay diluent RD1-56 (R&D Systems, Minneapolis, MN) in duplicate to plates pre-coated with monoclonal antibody specific for human CXCL-10. After two hours of incubation, each well was aspirated and washed a total of four times with wash buffer. Two-hundred μL of CXCL-10 conjugate was added to each well and incubated for two hours. Aspiration and wash were repeated four times. Two-hundred μL of substrate solution (R&D Systems, Minneapolis, MN) were then added to each well and incubated for 30 min. After the addition of 50 μL of stop solution, the optical density of each well was determined using a microplate reader, according to the manufacturer's specifications. The inter-assay coefficient of variation (CV) was 3.6% while the intra-assay CV was 3.7%. The sensitivity of the assay for CXCL-10/IP-10 was <4.95 pg/mL.

Statistical Analysis

Medians and interquartile ranges were calculated for continuous variables and frequencies and percentages were calculated for categorical variables. The Fisher's exact test, Chi-square test, and Mann-Whitney U-test were used to compare groups as appropriate. A p-value <0.05 was considered significant. Statistical analyses were performed using SPSS Version 15.0 software (SPSS, Inc., Chicago, IL).

Linear mixed models were used to test for differences in the log-transformed average plasma CXCL-10 concentration overall and across four gestational length-defined periods (<14 weeks, 14–16 weeks, 17–19 weeks, and 20–30 weeks), adjusting for factors significantly associated with MPFD [maternal age, body mass index (BMI), African-American ethnicity] and using a robust covariance matrix estimator. Analysis was performed using SAS version 9.2 software (SAS Institute Inc., Cary, NC).

Results

Eighty percent of MPFD cases resulted in pregnancy loss (40% had a fetal death and 40% had a second-trimester spontaneous abortion). Patients with MPFD were significantly older, had a lower median gestational age at delivery, and lower median birthweight than those in the control group ($p < 0.001$ all) (Table I).

Figure 1 and 2 demonstrated gross and microscopic section of placenta from MPFD case, respectively. Pathologic findings in MPFD cases are summarized in Table II. There was a significantly higher rate of plasma cell deciduitis [40% (4/10) vs. 8.6% (15/175), p=0.011] and fetal vascular thrombo-occlusive disease [30% (3/10) vs. 6% (11/175), p=0.03] in MPFD than in the control group. There was also a trend towards increased frequency of villitis of unknown etiology in pregnancies complicated by MPFD, although the difference did not reach statistical significance [10% (1/10), vs. 0.6% (1/175), p=0.1]. The frequency of chronic chorioamnionitis in MPFD, however, was not significantly different from that in the control group [20% (2/10) vs. 17% (30/175); p=0.7].

Figure 3 demonstrates the frequency of HLA Class I and HLA Class II seropositivity in patients with MPFD compared to women in the control group. Patients with MPFD had a significantly higher frequency of HLA Class I seropositivity than women with uncomplicated pregnancy [(80% (8/10) vs. 36.4% (52/143); p=0.01)]. There was no significant difference in the frequency of HLA class II seropositivity among 2 groups [40% (4/10) vs. 18.2% (26/143); p=0.1]. Furthermore, fetal specific HLA genotyping and identification of specific maternal HLA antibodies demonstrated the presence of maternal antibodies against fetal specific HLA antigen in all cases for which fetal tissues (umbilical cord and umbilical cord blood) were available (n=6). Table III and Table IV show the results for each MPFD case for fetal HLA specific HLA antigens and identification of maternal specific HLA antibodies.

In four cases, the fetus was liveborn and umbilical cord specimens were available for immunostaining–all four cases demonstrated strongly positive C4d deposition in the umbilical vein specimen (Figure 4). Finally, the mean maternal plasma concentration of CXCL-10 was significantly elevated in MPFD cases relative to controls both overall ($p<0.001$) and further as a function of gestational age (<14 weeks, 14–16 weeks, 17–19 weeks, and 20–30 weeks) (p=0.01); both relationships remain significant after adjustment for maternal age, African-American ethnicity, and pre-pregnancy BMI (p=0.04, p=0.03 respectively).

The mean maternal plasma concentration of CXCL-10 remained relatively stable over time and negligibly declined following the 16th week of gestation among controls, while, the mean plasma concentration among cases increased over time starting from 17–19 weeks (p=0.006) and became significantly higher than that in control group at 20–30 weeks of gestation (p=0.004; Figure 5). Thus, evidence of increasing maternal plasma concentration of CXCL-10 was present in patients with MPFD affected pregnancies before the diagnosis of MPFD. Table V summarizes the clinical course, placental pathology, and laboratory assays for cases with MPFD.

Discussion

Principal findings of the study

1) The frequency of plasma cell deciduitis and fetal vascular thrombo-occlusive disease was significantly higher in the placenta of patients with MPFD than in the control group; 2) patients with MPFD had a significantly higher frequency of maternal anti-HLA class I seropositivity during the second trimester than in those in the control group; 3) maternal plasma antibodies against fetal HLA antigens class I or II were identified in all cases of MPFD; 4) C4d deposition in the umbilical vein was documented in all cases of MPFD; and 5) the mean maternal plasma concentration of CXCL-10 in MPFD cases was significantly higher than in the control group. Taken together, these findings suggest that antibody-mediated maternal anti-fetal rejection operates in cases of maternal floor infarction.

Maternal floor infarction and massive perivillous fibrin deposition

Maternal floor infarction (MFI) was originally described by Benirschke in 1961 as a passing comment accompanied by a figure in an article focused on the examination of the placenta.[1] He described a lesion on the maternal surface of the placenta, in which the thin layer of the decidua basalis is covered by small amounts of calcium and fibrin.[1] Benirschke suggested that lack of fissuring of the decidua basalis in a term placenta was abnormal, and he coined the term “maternal floor infarction” to emphasize that the deposition of material occurred mainly in the villous tree next to the placental floor.[1] Subsequently, Benirschke and Driscoll in 1967 described the lesion in greater detail.[2] Fox proposed in 1978 that MFI was a postmortem change of the placenta of stillborns,[16] a concept which has been abandoned.

The term “MFI” recognizes that the major feature is deposition of fibrinoid material around the villous tree.[1] This material prevents normal gas and nutritive exchange between the maternal and fetal circulations, and consequently can lead to fetal growth restriction[3, 4, 6, 7, 11–15] and death.[3, 4, 6, 7, 10, 12, 14–18, 20–22] Naeye R et al. reported that some cases develop rapidly in the third trimester because fetal death was not accompanied by fetal growth restriction.[6]

MPFD and MFI are considered to represent the same process with different severity. Although the term “infarction” has been used repeatedly, several pathologists have emphasized that the lesion does not represent an infarction; yet the term continues to be used.[6]

Since the original descriptions, the diagnosis of these lesions has been subjective. In 2002, Katzman and Genest proposed semi quantitative histologic criteria based on the review of 80 placentas.[3] The frequency of these lesions (MPFD/MFI) has been estimated to be 0.03–0.5% of all deliveries.[3]

Several mechanisms of disease leading to MPFD have been proposed, including: 1) infection;[87, 88] 2) cytoxicity due to extravillous trophoblast (formerly referred to as "X cells");[8] 3) autoimmune disorders;[7] 4) coagulation or fibrinolytic disorders;[89] and 5) imbalance of angiogenic/antiangiogenic factors.[90] The hypothesis that polymorphisms in fibrinolysis or fibrinolysis inhibitor genes might be more frequent in MPFD placentas has not been supported in a study focusing on DNA variants in genes involved in fibrinolysis (PAI-1, thrombin activated fibrinolysis inhibitor, plasminogen activator urokinase, and tissue plasminogen activator).[89]

Plasma cell deciduitis in MPFD/MFI

We report herein an association between MPFD/MFI and plasma cell deciduitis, a placental lesion associated with maternal anti-fetal rejection.[80] Previous reports also demonstrated the association of this placental lesion and preterm birth.[53, 80]

Antibody-mediated maternal anti-fetal rejection

Antibody-mediated maternal anti-fetal rejection is thought to result from maternal anti-fetal IgG crossing the placenta and inducing a fetal systemic inflammatory response.[54] A requirement for antibody-mediated rejection is the presence of antibodies against fetal antigens. We screened for such antibodies using the HLA PRA assay against HLA Class I and HLA Class II antigens. This test has been used to test the recipient for the presence of circulating antibodies before organ transplantation. These antibodies identify patients who are already sensitized against antigens of potential donors and their presence identify patients at risk for rejection and graft failure.[91, 92]

Sensitization against HLA Class I and Class II antigens may occur through prior transfusions.[93, 94] However, pregnancy is a unique state in which there is evidence of bidirectional cell traffic between the mother and fetus.[95–98] Indeed, this sensitization against fetal antigens of paternal origin may explain why patients with a prior pregnancy have a higher rate of rejection or graft failure than women who have not been pregnant. We have previously shown that the likelihood of HLA PRA positivity is greater in multiparous than in nulliparous women, and males.[99, 100] In the study herein, we demonstrated a higher HLA-class I seropositivity in women with MPFD than in the control group. While HLA-class II seropositivity was higher in patients with MPFD, compared to controls, this finding did not reach statistical significance.

A positive HLA PRA test indicates the presence of circulating antibodies, but does not provide specificity as to the nature of the antibody and the presence of the antigen in the fetus, both of which would be a requirement for maternal anti-fetal rejection to occur. To address this, we determined the fetal HLA status at the A, B, and C loci from umbilical cord DNA and then compared the results to specific HLA antibodies detected in maternal serum using multiplex technology. Among the six patients in whom umbilical cord specimens were

available to assess the fetal HLA genotype, all exhibited antibodies specific to the fetal HLA class I or HLA class II antigens.

Even if specific antibodies against fetal antigens are present, activation of complement is required for tissue damage. Indeed, complement activation is an integral part of antibody mediated rejection. We report here C4d immunostaining of the umbilical vein endothelium. C4d deposition is indicative of activation of the classical pathway of complement, and is unique in that it covalently binds to the endothelial basement membranes, leaving immunologic evidence that antibody-mediated complement activation has occurred.[101] C4d deposition in peritubular capillaries has been shown to be key in identifying evidence of graft rejection in renal transplants.[78, 102] In the current study, all cases with MPFD examined (n=4) showed strong immunoreactivity for C4d in the umbilical vein endothelium. We have previously reported that C4d deposition in umbilical vein endothelium was significantly more frequent in preterm labor (77.1%) than in term labor (11.4%).[54]

Chemokine associated with rejection is elevated in the maternal circulation in MPFD

CXCL-10 is a chemokine of the CXC family which has both pro-inflammatory and anti-angiogenic properties.[103] Overexpression of this chemokine has been demonstrated in serum/plasma,[104, 105] urine,[106] peripheral white blood cells of patients who have received a transplant.[107–112] In addition, this chemokine has also been found within transplanted organs during the course of rejection.[103, 107, 108, 110, 113, 114] These findings, coupled with gene deletion experiments have shown that CXCL-10 is an important mediator in allograft rejection.[115] During normal pregnancy, the plasma concentration of this chemokine is higher than in non-pregnant women. In the current study, we found that the mean maternal plasma CXCL-10 concentration was higher in MPFD than in the control group.[116]

Conclusion

Massive perivillous fibrin deposition is associated with: 1) plasma cell deciduitis; 2) the presence of specific anti-HLA antibodies in maternal blood to fetal antigens; 3) evidence of antibody-mediated complement activation on umbilical vein endothelium; and 4) elevation of a maternal plasma concentration of CXCL-10. Collectively, our results support the concept that MPFD is a unique state in which there is maternal anti-fetal rejection. This work has implication for understanding the mechanism of disease, the discovery of biomarkers for patient at risk and potential therapeutic intervention in this serious placental disorder.

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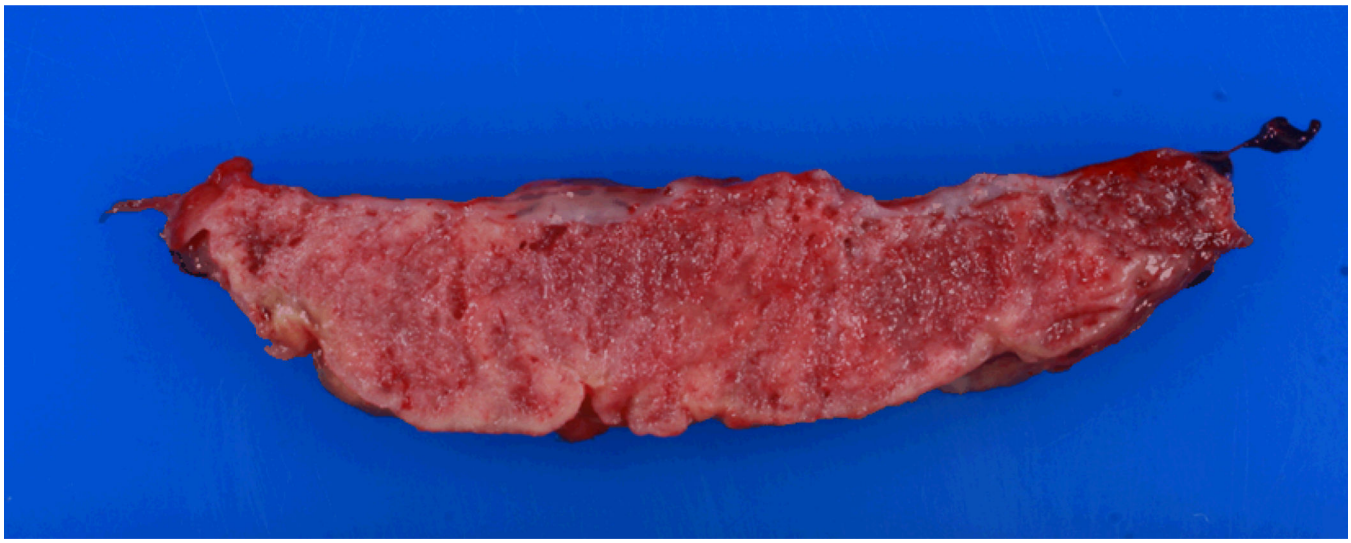

Figure 1.
Gross placental specimen from MPFD case #1. Note thickening and rubber-like texture. Fibrin deposition visualized grossly as yellow/avascular appearing tissue.

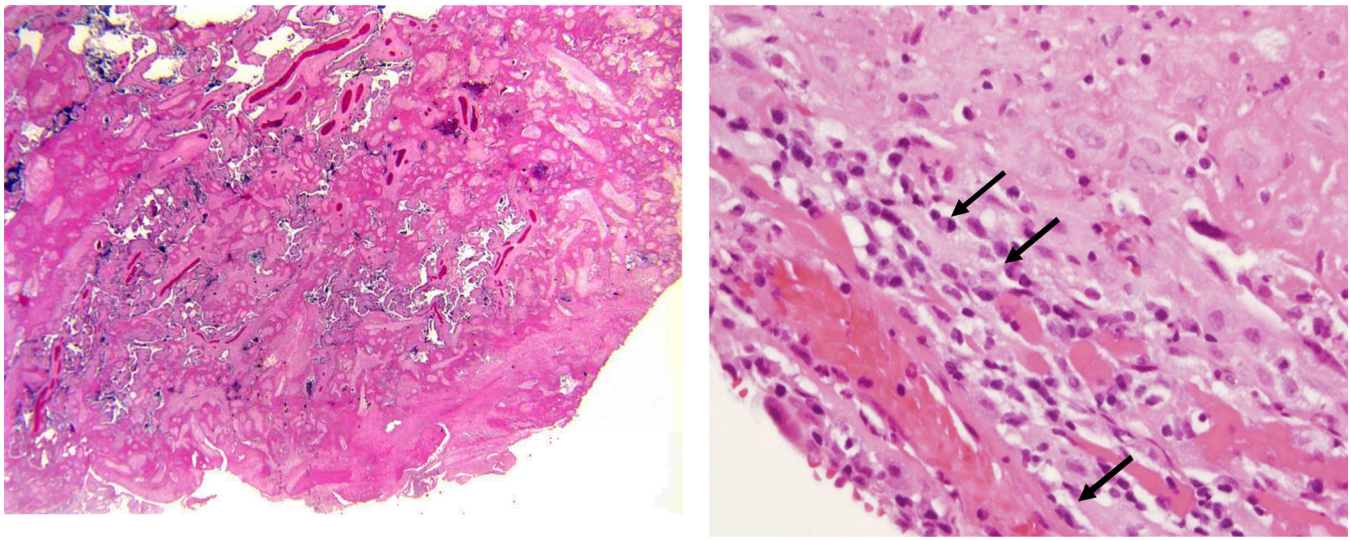

Figure 2.

Microscopic section of placenta from MPFD case #1. Note loss of normal villous architecture and encasement of remaining villi in pale-pink fibrin material (left). Area of chronic deciduitis with plasma cells (denoted by arrows) within large amount of fibrin deposition.This lesion is suggestive of maternal anti-fetal rejection (right).

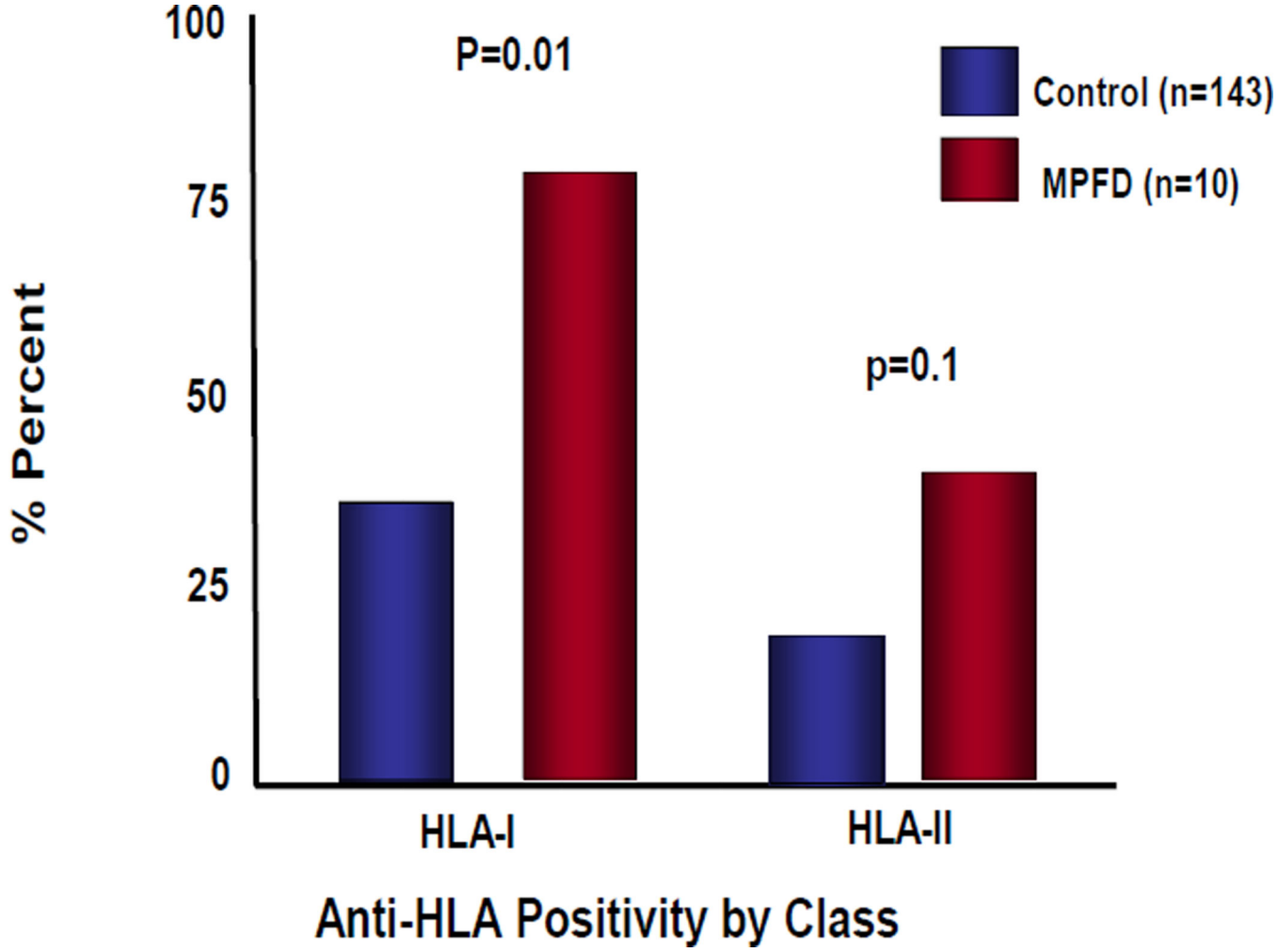

Figure 3.
Rates of anti-HLA Class I and Class II positivity in maternal blood from MPFD cases and control, uncomplicated term-delivery patients.

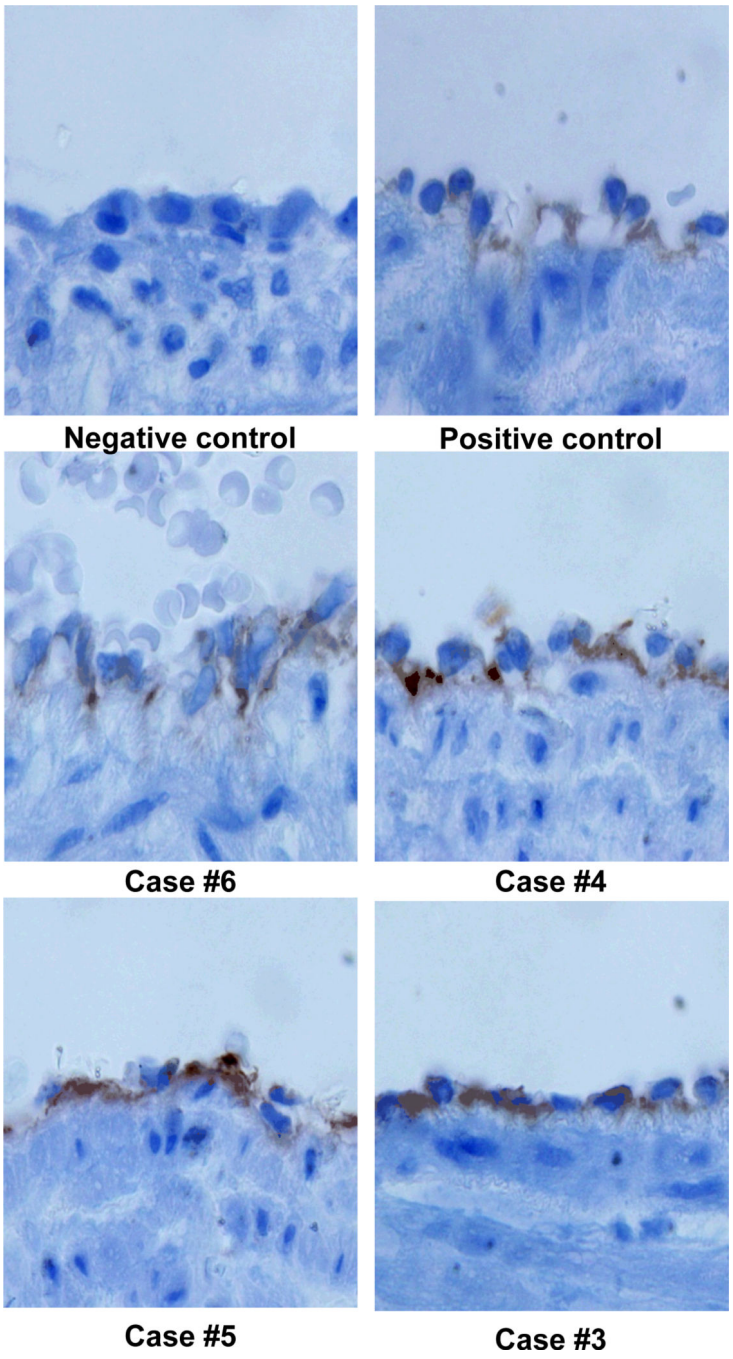

Figure 4.
C4d deposition on umbilical vein endothelium. All 4 cases demonstrate strong reactivity by immunohistochemistry (brown).

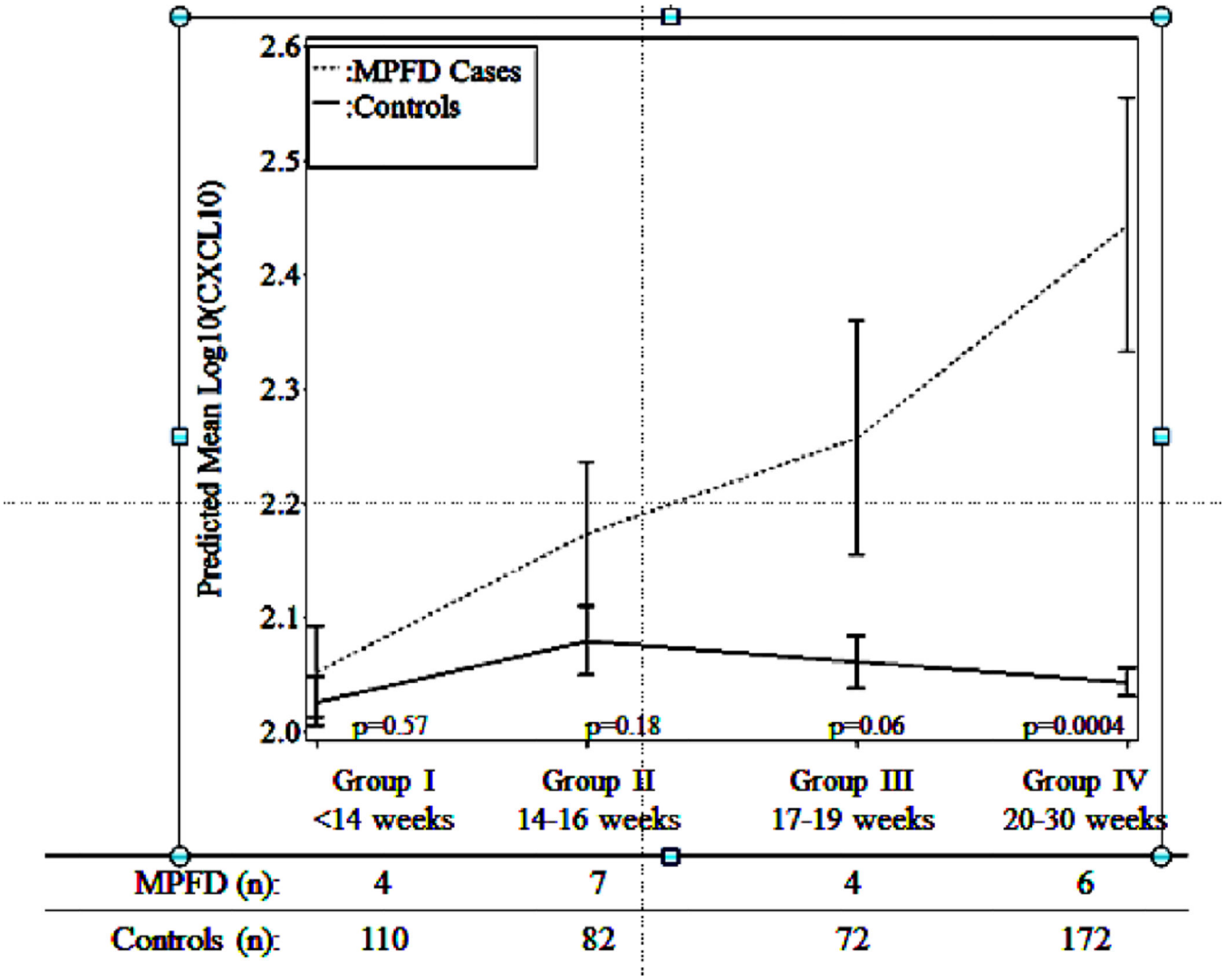

Figure 5.

Estimated mean +/− standard error of plasma concentrations (log 10) of CXCL-10 in MPFD and uncomplicated pregnancies by gestational age interval. Estimated mean CXCL-10 concentrations over time are adjusted by maternal age, body mass index and African-American ethnicity; P-values characterize the difference in estimated mean concentrations at each gestational age interval determined by the linear mixed effects model.

Table I

Demographics and clinical characteristics of patients with uncomplicated pregnancy and massive perivillous fibrin deposition

	Uncomplicated pregnancies (n=175)	MPFD (n=10)	p-value
Maternal Age (years)	23 (20–26)	31 (26–35)	<0.001
African American	151 (86%)	10 (100%)	0.4
Nulliparity	62 (35%)	0 (0%)	0.03
Pre-pregnancy BMI (kg/m^2)	27 (23–32)	29 (28–35)	0.04
Gestational Age at Delivery (weeks)	39 (39–40)	23 (17–29)	<0.001
Birth weight (grams)	3330 (3150–3555)	277 (175–605)	<0.001
Stillbirth (> 20 weeks)	0	4 (40%)	---
Miscarriage in the Second Trimester (<20 weeks)	0	4 (40%)	---
Fetal Growth Restriction	0	4 (40%)	---
Placental Abruption	0	2 (20%)	---

Data are expressed as median (interquartile range) or number (percent).
MPFD: Massive perivillous fibrin deposition
BMI: Body mass index

Table II

Frequency of pathologic diagnosis in massive perivillous fibrin deposition group and uncomplicated pregnancy group.

	Uncomplicated pregnancies (n=175)	MPFD (n=10)	p-value
Deciduitis with plasma cells	15 (8.6%)	4 (40%)	0.011
Villitis of Unknown Etiology	1 (0.6%)	1 (10%)	0.1
Chronic chorioamnionitis	30 (17%)	2 (20%)	0.7
Acute chorioamnionitis	42 (24%)	5 (50%)	0.1
Fetal vascular thrombo-occlusive disease	11 (6%)	3 (30%)	0.03
Chronic villitis	18 (10%)	3 (30%)	0.1
Evidence of fetal rejection	45 (26%)	4 (40%)	0.5

Data are expressed as number (percent).
Evidence for fetal rejection was defined as positive for either chronic chorioamnionitis, villitis, or deciduitis.

Table III

Fetal HLA-class I type at A, B, and C loci as determined from umbilical cord DNA compared to specific maternal anti-HLA class I antibodies. Maternal antibodies corresponding to fetal antigen are high-lighted/underlined. In all cases except one, maternal antibodies show specificity against fetal HLA antigens.

Patient Number	HLA A	HLA B	HLA C	Maternal Serum anti-HLA class I Antibodies
1	23,74	53,57	4,7	A1,A11,A2,A25,A26,A29,A3,A30,A31,A32,A33,A34,A36,A43,A66,A68,A69,A74,A80,B13,B41,B44,B45,B46,B47,B49,B50,B57,B58,B60,B61,B62,B63,B73,B75,B76,B77,Cw1,Cw10,Cw12,Cw14,Cw15,Cw16,Cw17,Cw18,Cw2,Cw5,Cw6,Cw7,Cw9
3	2,30	7,81	8,15	A1,A11,A2,A24,A25,A26,A29,A3,A30,A31,A32,A33,A34,A36,A43,A66,A68,A69,A74,A80,B13,B41,B42,B44,B45,B46,B47,B49,B50,B51,B52,B55,B57,B58,B60,B61,B62,B63,B64,B65,B7,B71,B72,B73,B75,B76,B77,B78,B8,Cw1,Cw10,Cw12,Cw14,Cw15,Cw16,Cw17,Cw18,Cw2,Cw5,Cw6,Cw7,Cw9
4	1,24	8,45	6,7	A25,A26,A29,A31,A33,A34,A66,A68,A69,A74,B18,B35,B37,B38,B39,B46,B48,B49,B50,B51,B52,B53,B54,B56,B57,B58,B59,B62,B63,B67,B71,B72,B75,B77,B78,B8,Cw10,Cw9
5	2,3	35,45	4,16	A1,A2,A24,A36,A68,A69,B13,B41,B44,B45,B46,B47,B49,B50,B57,B58,B60,B61,B62,B76,B82,Cw12,Cw15,Cw16,Cw17,Cw6
6	2,26	8,49	3,7	B13,B27,B47,B48,B57,B60,B61,B7,B81
7	30,34	14,49	7,8	A2,B13,B27,B41,B42,B44,B45,B47,B48,B49,B50,B55,B56,B60,B61,B62,B67,B7,B71,B72,B75,B76,B81

Table IV

Fetal HLA-class II type at DQ, DR B1, and DR B345 loci as determined from umbilical cord DNA compared to specific maternal anti-HLA class II antibodies. Maternal antibodies corresponding to fetal antigen are high-lighted/underlined. Maternal specificity against fetal class II antigen was shown in 4/6 cases.

Patient Number	DQ	DR B1	DR B345	Maternal Serum anti-HLA class II Antibodies
1	2	13,17	52	DP1,DP10,DP11,DP13,DP14,DP15,DP17,DP18,DP19,DP20,DP23,DP28,DP3,DP5,DP6,DP9,DQ4,DQ6,DQ7,DQ8,DQ9,DR1,DR103,DR11,DR12,**DR13**,DR14,DR15,DR16,DR4,DR51,DR7,DR8
3	2,6	9,15	51,53	DP1,DP10,DP11,DP13,DP14,DP17,DP19,DP20,DP3,DP5,DP6,DP9,DQ4,DQ7,DQ8,DQ9,DR103,DR11,DR12,DR13,**DR15**,DR16,DR4,**DR51**,DR7,DR8
4	2,6	15,17	51,52	DP2,DR11,DR12,DR13,DR14,**DR17**,DR18,**DR52**,DR7,DR8,DR9
5	5,6	13,15	51,52	Negative
6	3,5	1,13	52	DP1,DP10,DP28,DP3,DQ4,DQ6,DQ7,DQ8,DQ9,DR12,**DR52**
7	6	15	51	Negative

Table V

Clinical description of massive perivillous fibrin deposition cases.

Case Number	Age	Gravida, Parity	GA at Delivery (weeks)	Clinical Description	Birth Weight (grams, Percentile for GA)	Placental Pathology			+ HLA		Genotype Confirmation		+C4d Umbilical Vein
						VUE	PD	Other	I	II	I	II	
1	35	G 12 P 8-2-1-8	23+1	The fetus has decreased growth and progressive deterioration of Doppler parameters starting at 20 weeks gestation. Intrauterine fetal death diagnosed at 23 weeks.	274 (1%)	Yes	Yes		Yes	Yes	Yes	Yes	N/A
2	34	G 11 P 8-1-1-8	28+2	The fetus has decreased growth and progressive deterioration of Doppler parameters starting at 20 weeks gestation. Fetal death diagnosed and labor was induced at 28 weeks	454 (1%)	Yes	Yes	Acute Chorioamnionitis	Yes	Yes	N/A	N/A	N/A
3	33	G 10 P 7-1-1-7	38+1	Spontaneous labor at term.	3285 (51.5%)	Yes	Yes	Chronic Chorioamnionitis	Yes	Yes	Yes	Yes	Yes
4	24	G 4 P 2-0-1-2	15+6	Presented with ruptured membranes and was induced for inevitable abortion.	150	No	No	Acute Chorioamnionitis; Chronic Deciduitis without Plasma Cells	Yes	Yes	Yes	Yes	Yes
5	27	G 3 P 0-0-2-0	30+0	Presented with fetal growth restriction, heavy vaginal bleeding and clinical placental abruption. Emergency cesarean delivery was performed.	755 (1%)	No	No		Yes	No	Yes	No	Yes
6	22	G 2 P 0-0-1-0	22+3	Short cervix was noted at 20 weeks; membranes ruptured with spontaneous labor at 22 weeks. Intrapartum demise with delivery of a stillborn infant.	448 (34%)	No	No	Acute Chorioamnionitis	Yes	No	No	Yes	Yes
7	28	G 11 P 0-1-9-1	23+6	The fetus has thickening placenta, multiple placental lacunae, and oligohydramnios at 18 weeks. Fetal death diagnosed and labor was induced at 23+ weeks	277 (1%)	No	No	Acute Chorioamnionitis Chronic Deciduitis without Plasma Cells	Yes	No	Yes	No	N/A
8	43	G 13 P 3-3-6-4	16+4	Presented with rupture of fetal membranes and fetal demise.	Unknown	No	Yes		Yes	No	N/A	N/A	N/A
9	35	G 7 P 0-0-6-0	17+3	Cervical length of 0 mm; A rescue cerclage was placed but membranes ruptured shortly	160	No	No		No	No	N/A	N/A	N/A

Case Number	Age	Gravida, Parity	GA at Delivery (weeks)	Clinical Description	Birth Weight (grams, Percentile for GA)	Placental Pathology			+ HLA		Genotype Confirmation		+C4d Umbilical Vein
						VUE	PD	Other	I	II	I	II	
				afterwards. Induction for inevitable afterwards. Induction for inevitable	abortion. abortion.								
10	29	G 3 P 1-1-0-1	17+2	Presented with abdominal pain and vaginal bleeding. Fetal demise was diagnosed and patient was induced.	190	No	No	Acute Chorioamnionitis Chronic Deciduitis without Plasma Cells	No	No	N/A	N/A	N/A

** Cases #1–3 are pregnancies in the same patient, VUE: Villitis of Unknown Etiology, PD: Deciduitis with Plasma Cells