ORIGINAL ARTICLE

Placental C4d deposition is a feature of defective placentation: observations in cases of preeclampsia and miscarriage

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Abstract Placental C4d deposition is frequent in preeclampsia, and shallow placentation is a characteristic of both preeclampsia and miscarriage. This study was conducted to determine the relationship among placental C4d, maternal human leukocyte antigen (HLA) antibodies, and placental pathology in preeclampsia and miscarriage cases. The patient population ($N=104$) included those with (1) preterm preeclampsia with fetal growth restriction (PE-FGR; $n=21$), (2) preterm preeclampsia (PE; $n=20$), (3) spontaneous preterm delivery (sPTD; $n=39$), and (4) miscarriage ($n=24$). C4d immunohistochemistry was performed, and the presence of maternal plasma HLA antibodies was examined. C4d staining of the syncytiotrophoblast was more frequent in PE-FGR patients (76.2 %) than in PE (10.0 %; $p<0.001$) and sPTD (2.6 %; $p<0.001$) patients. Maternal HLA antibody-positive rate was not different among the study groups. There was a significant correlation between C4d immunoreactivity and placental pathology consistent with maternal vascular underperfusion ($p<0.001$) but not with maternal HLA antibody status. In miscarriages, the positive rates of C4d, HLA class I, and HLA class II antibodies were 58.3, 25.0, and 12.5 %, respectively. There was no correlation between the presence of maternal HLA class I or II antibodies and placental C4d immunoreactivity. This study confirms frequent placental C4d deposition in preeclampsia with fetal growth restriction and miscarriage. The association between placental C4d deposition and pathological findings of maternal vascular underperfusion suggests that C4d staining of the syncytiotrophoblast is a consequence of defective placentation rather than of a specific maternal immune response against fetal HLA. The study also demonstrates the usefulness of C4d as a biomarker of placentas at risk.

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Introduction

Complement component 4d (C4d) is a split product of C4, and its deposition in an allograft is indicative of antibody-mediated rejection although there are some recent issues with its sensitivity [1, 2]. For example, both focal and diffuse immunoreactive C4d in peritubular capillary endothelial cells of renal allografts strongly associates with the presence of circulating donor-specific human leukocyte antigen (HLA) antibodies and poor allograft outcome [3, 4]. Studies have demonstrated C4d deposition in the syncytiotrophoblast in patients with certain placental lesions and pregnancy-related disorders,

including miscarriage [5], antiphospholipid syndrome [6], systemic lupus erythematosus [7], villitis of unknown etiology (VUE; anti-fetal cellular rejection of the placenta) [8–10], placental infarct [10], and preeclampsia. A systematic analysis of different complement components by Buurma et al. has provided evidence for placental complement dysregulation in patients with preeclampsia [9]. C4d deposition in the syncytiotrophoblast was evident in half of all preeclampsia cases, while it is present in only 3 % of healthy pregnant women. C4d placental deposition was likely a consequence of classical complement activation because mannose-binding lectin staining was not observed. Although the selective localization of C4d to VUE foci indicates that C4d deposition correlates with antibody-mediated rejection [8], it is not known if placental C4d deposition in patients with preeclampsia associates with the presence of circulating maternal HLA antibodies or if it is a non-HLA-related response induced by different types of placental injuries.

Preeclampsia is an enigmatic disorder defined by the onset of maternal hypertension and proteinuria during pregnancy [11]. Preeclampsia affects approximately 5 % of all pregnancies [12] and accounts for a large proportion of both maternal and neonatal morbidities [13, 14]. Patients with preeclampsia have a systemic vascular inflammatory response [15] and imbalance between pro- and anti-angiogenic factors, such as placental growth factor, sFlt-1, and soluble endoglin [16–18]. The primary characteristics of preeclampsia are shallow placentation and defective physiological remodeling of spiral arteries at the implantation site [19, 20]. Failure of physiological remodeling of spiral arteries results in increase of placental villous damage by turbulent intervillous blood flow jet and ischemia-reperfusion injury associated with spontaneous vasoconstriction [21]. These features are also common to fetal growth restriction, and preeclampsia associates with fetal growth restriction in more than one third of all cases [22, 23]. Furthermore, severe and early forms of defective placentation and placental oxidative stress may cause miscarriage, and it has been proposed that miscarriage and preeclampsia constitute a disease spectrum rather than being isolated disorders [24, 25]. Therefore, the placenta of patients with preeclampsia and miscarriage is a good model to study defective placentation, placental ischemia-reperfusion injury, and oxidative stress.

Major placental pathology clusters are composed of intra-amniotic infection and inflammation, maternal vascular underperfusion, fetal vascular thrombo-occlusive disease, and chronic placental inflammation [26–29]. Studies in chronic placental inflammation (chronic chorioamnionitis, chronic deciduitis, and VUE) clearly indicate that the presence of maternal HLA antibodies is a surrogate of maternal anti-fetal antibody-mediated rejection in patients with spontaneous preterm deliveries [30]. Therefore, the aim of this study was to determine if a correlation exists among the deposition of placental C4d, the presence of maternal HLA antibodies, and pathological changes of the placenta in patients with preeclampsia and miscarriage.

Materials and methods

Patient materials

Placental tissues were obtained from the Department of Pathology, Seoul National University Hospital and the Infertility Registry of Hamchoon Women's Clinic. Corresponding patient plasma samples were obtained at the time of delivery or miscarriage. The patient population ($N=104$) included those with (1) preterm preeclampsia with fetal growth restriction (PE-FGR; $n=21$), (2) preterm preeclampsia (PE; $n=20$), (3) spontaneous preterm delivery (sPTD; $n=39$), and (4) miscarriage ($n=24$). Placental lesions were diagnosed by a pathologist (C.J.K.), who was masked to clinical findings, according to previously described criteria [27–29].

Preterm labor was diagnosed by the presence of regular uterine contractions and cervical dilation, followed by delivery prior to 37 weeks of gestation. Preterm prelabor rupture of membranes was defined by pooling of amniotic fluid in the vagina or a positive nitrazine test during the sterile speculum examination, which was conducted prior to 37 weeks of gestation in the absence of labor. Preeclampsia was defined as newly diagnosed high blood pressure (systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg) with onset after 20 weeks of gestation and proteinuria (of 24 hours urine protein ≥300 mg or a dipstick ≥1+) on at least two occasions taken at least 6 hours apart [31]. We classified the preeclampsia patients into two groups: those with preeclampsia with fetal growth restriction and those without fetal growth restriction, according to the birth weight of the newborn. Neonates weighing <10th percentile of the birth weight for the gestational age were diagnosed with fetal growth restriction. The diagnosis was made based on a new fetal-infant growth chart for preterm infants [32]. Gestational age at delivery and maternal age were adjusted by matching process between PE-FGR/PE and sPTD groups. The exclusion criteria were the followings: pregnancy with multifetal pregnancy, significant fetal anomaly, severe maternal medical disease, gestational diabetes on insulin, cerclage operation, placenta previa, placental abruption, fetal death in utero, intrauterine growth restriction by unknown cause, oligohydramnios only by unknown cause, cord prolapse, spontaneous fetal distress by unknown cause, and preterm labor combined with preeclampsia. Miscarriage cases

Table 1 Clinical characteristics of preterm delivery cases (*n*=80)

	PE-FGR (*n*=21)	PE (*n*=20)	sPTD (*n*=39)	*p* value
Maternal age (years)[a]	34.3 (32.0–37.5)	34.2 (31.0–37.6)	33.7 (31.0–36.0)	0.773
Multiparity (%)	9 (42.9 %)	7 (35.0 %)	17 (43.6 %)	0.836
Gestational age at delivery (weeks)[b]	32.0 (29.7–34.7)	34.2 (32.3–36.4)	33.3 (30.4–35.4)	0.092
Birth weight (g)[a]	1216 (830–1675)	2027 (1585–2567)	1971 (1640–2490)	<0.001
Cesarean delivery (%)	18 (85.7 %)	17 (85.0 %)	8 (20.5 %)	<0.001
Baby gender (male; %)	7 (33.3 %)	10 (50.0 %)	25 (64.1 %)	0.072

[a] Mean, interquartile range (25–75 %)

[b] Median, interquartile range (25–75 %)

were consecutively enrolled regardless of chromosomal status, because our previous study demonstrated frequent placental C4d immunoreactivity regardless of chromosomal status.

Tissue microarray

Tissue microarrays were prepared using formalin-fixed, paraffin-embedded blocks of preterm delivery cases. Two 2-mm-thick tissue cores were obtained from donor blocks and transferred onto recipient blocks after reviewing hematoxylin- and eosin-stained sections. Three tissue microarrays were constructed from preterm delivery cases and used for immunohistochemistry. For miscarriage cases, whole paraffin-embedded blocks were used for sectioning.

Immunohistochemistry

Four micron-thick paraffin sections were used for immunostaining. A rabbit polyclonal anti-C4d antibody was the primary antibody (diluted 1:200; Cell Marque Corporation, Rocklin, CA, USA), and immunostaining was performed with the BenchMark XT automated slide preparation system (Ventana Medical Systems, Inc., Tucson, AZ, USA). The positive control tissue was a renal biopsy specimen from a patient with documented acute allograft rejection. For the isotype control, rabbit IgG (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA, USA) was used at the same dilution. The degree of syncytiotrophoblast C4d immunoreactivity was scored as 0: none (<10 %), 1: focal (10–50 %), or 2: diffuse (>50 %) [9]. C4d scoring of placentas was performed by a pathologist (C.J.K.).

Detection of HLA antibodies

Maternal plasma samples were obtained at the time of delivery or miscarriage and stored at −80 °C until use.

Screening for the presence of HLA class I, HLA class II, and MHC class I polypeptide-related sequence A (MICA) antibodies was performed with the LABScreen® Mixed Class I and II assay (One Lambda Inc., Canoga Park, CA, USA) and LABScan™ 200 flow analyzer (Luminex Corporation, Austin, TX, USA) according to the manufacturers' instructions. For each test, 20 μl of plasma was mixed with 5 μl of beads. Data analysis was performed using HLA Fusion software (One Lambda Inc.). A normalized background ratio greater than 3 was defined as positive.

Table 2 Clinical characteristics of miscarriage cases

	n=24
Maternal age (years)[a]	35.0 (31.7–37.4)
Multiparity	8 (33.3 %)
Gestational age at abortion (weeks)[b]	7 (6–9)
Diagnosis	
Blighted ovum	2 (8.3 %)
Missed abortion	22 (91.7 %)
Chromosomal study	
Normal chromosome	4 (16.7 %)
Abnormal chromosome	20 (83.3 %)
Presence of Y chromosome	12 (50.0 %)
History of infertility (>12 months)	6 (25.0 %)
Antithyroid antibody	2 (8.3 %)
Anticardiolipin antibody	1 (4.2 %)
Congenital uterine anomaly	1 (4.2 %)
Pregnancy after ART	2 (8.3 %)
Previous spontaneous abortion	17 (70.8 %)
Previous preterm delivery	1 (4.2 %)
Previous spontaneous abortion ≥2	13 (54.2 %)
Previous spontaneous abortion ≥3	7 (29.2 %)
Previous elective abortion	3 (12.5 %)

ART assisted reproductive technology (i.e., in vitro fertilization and intracytoplasmic sperm injection)

[a] Mean, interquartile range (25–75 %)

[b] Median, range (minimum-maximum)

Statistical analysis

The Kolmogorov–Smirnov test was used to analyze the distributions for normality of continuous variables. If data were not normally distributed, Kruskal–Wallis analysis and the Mann–Whitney U test were used for multiple comparisons among groups. If data were normally distributed, one-way analysis of variance and an unpaired t test were used. For post-hoc analysis, the Tukey test was applied.

For categorical variables, proportions were analyzed by the Fisher's exact test or Chi-square test. Means and medians with interquartile ranges were calculated for continuous variables, whereas frequencies and percentages were reported for categorical variables. SPSS (version 18.0, SPSS Inc., Chicago, USA) was used for all statistical analyses. All p values were two-sided, and p values <0.05 were considered statistically significant.

Results

Placental C4d immunoreactivity

Tables 1 and 2 summarize the clinical characteristics of patients with preterm deliveries and miscarriages, respectively. The gestational ages of preeclampsia cases ranged from 25.1 and 36.7 weeks (Online resource 1). Initial clinical assessments of deliveries in the spontaneous preterm group consist of preterm premature rupture of the membrane (n=21, 53.8 %) and preterm labor (n=18, 46.2 %). *Ureaplasma urealyticum* or *Mycoplasma hominis* was found (by culture or PCR of amniotic fluid or umbilical cord blood) in seven out of thirty-eight patients tested (18.4 %). Placental C4d immunoreactivity was linear along the syncytiotrophoblast and cytoplasmic in villous cytotrophoblasts in patients with preterm deliveries (Fig. 1). In addition, there were significant differences in C4d immunoreactivity in patients with
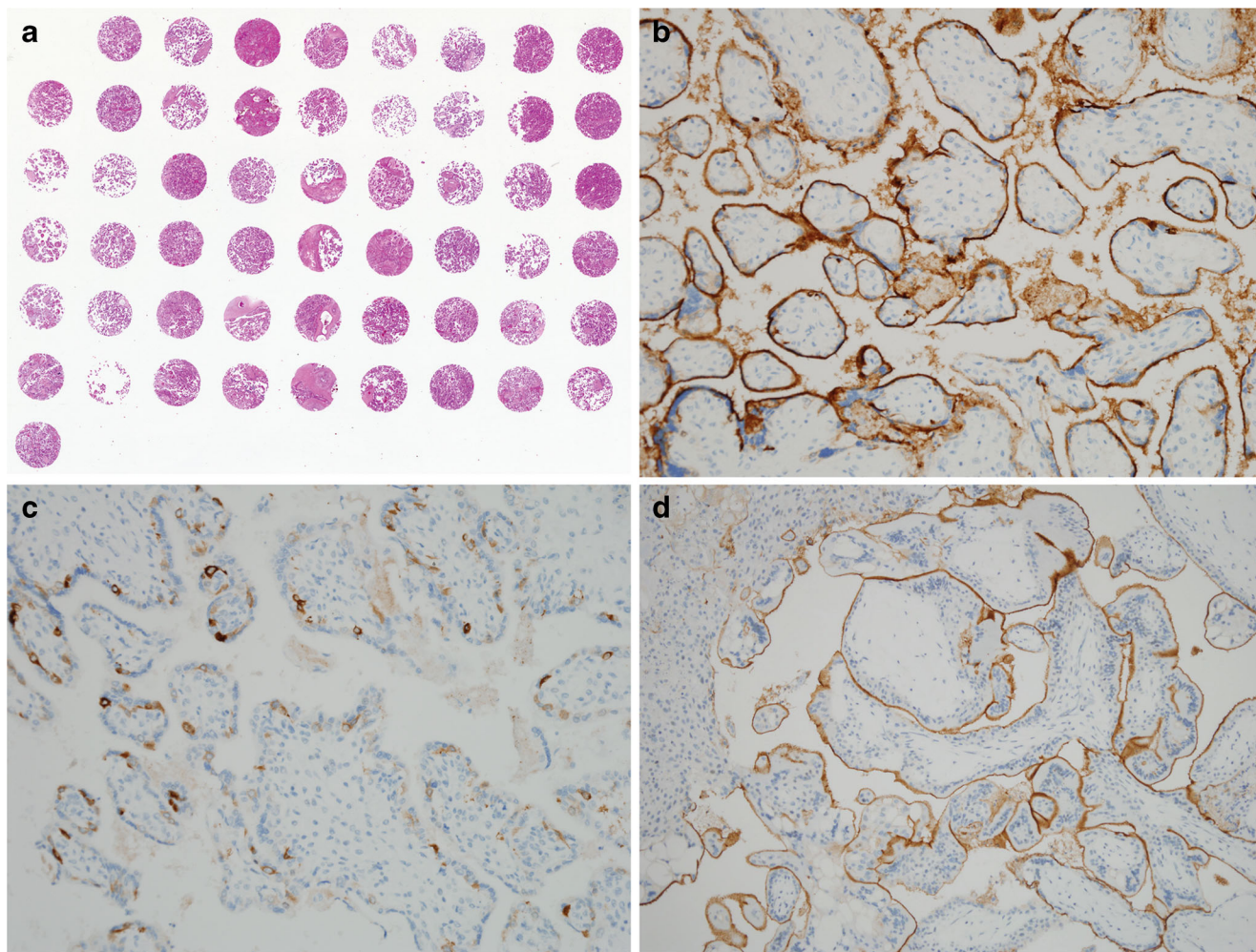

Fig. 1 C4d immunoreactivity in the villous trophoblast. **a** A section of tissue microarray stained with hematoxylin and eosin. **b** A microscopic image of C4d immunostaining in the syncytiotrophoblast. Placental C4d immunoreactivity is linear along the syncytiotrophoblast in a case of preeclampsia with fetal growth restriction at 27 weeks of gestation. **c** Cytoplasmic C4d immunoreactivity in the villous cytotrophoblasts in a case of spontaneous preterm delivery at 28 weeks of gestation. **d** C4d immunoreactivity is linear along the syncytiotrophoblast in a case of miscarriage at 7 weeks of gestation. There is no C4d immunoreactivity in the villous cytotrophoblasts (**b**, **c** ×200; **d** ×100)

PE-FGR ($n=21$), PE ($n=20$), and sPTD ($n=39$; $p<0.001$; Fig. 2). C4d staining of the syncytiotrophoblast was more frequent in PE-FGR patients (76.2 %) than in PE (10 %, $p<0.001$) and sPTD (2.6 %, $p<0.001$) patients. The severity of preeclampsia in PE-FGR and PE cases is shown in Table 3, and C4d immunoreactivity was not associated with the severity of preeclampsia. C4d staining in the cytotrophoblast was also frequently detected; however, there were no differences in C4d staining in the cytotrophoblast among patients with PE-FGR (66.7 %), PE (90.0 %), and sPTD (89.7 %). C4d staining of the syncytiotrophoblast was also detected in 14 out of 24 patients with miscarriages (58.3 %; Fig. 2). Furthermore, C4d

Fig. 2 Syncytiotrophoblast C4d immunoreactivity in different groups. **a** There is a significant difference in C4d immunoreactivity in cases of preeclampsia with fetal growth restriction (*PE-FGR*; $n=21$), preeclampsia (*PE*; $n=20$), and spontaneous preterm delivery (*sPTD*; $n=39$, $p<0.001$). C4d staining of the syncytiotrophoblast was detected in 58.3 % of patients with miscarriages. The rate of C4d staining in PE-FGR patients was higher than that in PE and sPTD patients ($p<0.001$ for each). **b** The distribution patterns of C4d immunostaining score in each group

staining of the cytotrophoblast was not observed in all miscarriage cases in contrast to preterm deliveries. No difference was noted between preterm deliveries and miscarriages in terms of the C4d immunoreactive pattern on the syncytiotrophoblast.

Based on the C4d immunopositive patterns on the syncytiotrophoblast, we additionally immunostained the full thickness placental sections of PE-FGR cases ($n=3$) to see if the intermediate trophoblasts at the implantation site and those at the basal plate were C4d negative in all cases (Online resource 2).

Maternal HLA class I, HLA class II, and MICA antibodies

The rates of PE-FGR, PE, and sPTD patients positive for maternal HLA class I antibodies were 57.1 % (12/21), 35.0 % (7/20), and 53.8 % (21/39), respectively, with no difference in rates among groups ($p=0.349$; Fig. 3a). The rates of PE-FGR, PE, and sPTD patients positive for maternal HLA class II antibodies were 19.0 % (4/21), 20.0 % (4/20), and 30.8 % (12/39), respectively. Similarly, there was no difference in positive rates among groups ($p=0.564$; Fig. 3b). MICA antibodies were detected in only one patient in each group. There was no correlation between the presence of maternal HLA class I or class II antibodies and C4d immunoreactivity in the placenta (Fig. 3c, d).

The rates of patients with miscarriages positive for maternal HLA classes I and II antibodies were 25 % (6/24) and 12.5 % (3/24), respectively. There was no association between the status of maternal HLA antibodies and C4d immunoreactivity in the placenta of patients with miscarriages (Fig. 3e, f).

Placental pathology, C4d, and maternal HLA antibodies

Pathological changes of the placenta in patients with preterm deliveries (PE-FGR, PE, and sPTD) were classified into four categories: (1) amniotic fluid infection (acute chorioamnionitis), (2) maternal vascular underperfusion, (3) fetal vascular thrombo-occlusive disease, and (4) chronic placental inflammation (chronic chorioamnionitis, VUE, and chronic deciduitis). When C4d immunoreactivity in the syncytiotrophoblast was analyzed with pathological changes of the placenta, there was a significant correlation between C4d immunoreactivity and maternal vascular underperfusion ($p<0.001$; Fig. 4), while there was no association between C4d immunoreactivity and acute chorioamnionitis, fetal vascular thrombo-occlusive disease, or chronic placental inflammation. Furthermore, there was a significant correlation between the presence of maternal HLA class I antibodies and chronic placental inflammation when the maternal HLA antibody status was compared with pathological changes of the placenta
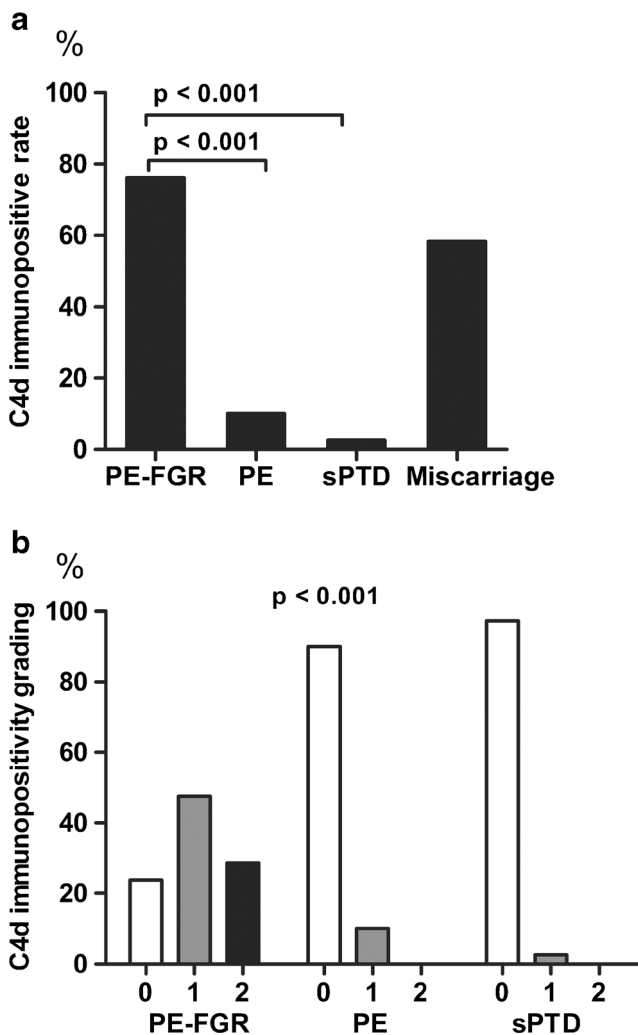
Table 3 The clinical parameters of preeclampsia cases

	PE-FGR (n=21)	PE (n=20)	Total (n=41)
Diastolic BP ≥110 mmHg	13 (61.9 %)	12 (60.0 %)	25 (61.0 %)
Systolic BP ≥160 mmHg	12 (57.1 %)	10 (50.0 %)	22 (53.7 %)
Eclampsia	0 (0 %)	0 (0 %)	0 (0 %)
Persistent severe headache or visual changes	9 (42.9 %)	4 (20.0 %)	13 (31.7 %)
AST/ALT>twice than normal	1 (4.8 %)	2 (10.0 %)	3 (7.3 %)
Proteinuria ≥3+	11 (52.4 %)	10 (50.0 %)	21 (51.2 %)
24 h urine protein (mg)	2623.0 (735.0–6089.0)[a]	810.0 (380.0–8744.0)[a]	2196.0 (400.0–6278.0)[a]
Oliguria <500 mL/24 hours	1 (4.8 %)	0 (0.0 %)	1 (2.4 %)
Serum creatinine ≥1.5 mg/dL	0 (0.0 %)	1 (5.0 %)	1 (2.4 %)
Persistent platelet counts <100,000/μL	1 (4.8 %)	3 (15.0 %)	4 (9.8 %)
Shortness of breath and/or SaO$_2$ <94 % breathing room air; pulmonary edema	6 (28.6 %)	1 (5.0 %)	7 (17.1 %)

[a] Median, interquartile range (25–75 %)

(p=0.041; Fig. 4); however, there was no association between the maternal HLA antibody status and other pathological changes.

Discussion

We studied placental C4d immunoreactivity and maternal HLA antibodies in preeclampsia and miscarriage cases. The primary findings of our study are as follows: (1) placental C4d staining is linear along the syncytiotrophoblast or diffusely cytoplasmic in the cytotrophoblast; (2) C4d staining of the syncytiotrophoblast is more frequent in patients with preeclampsia and fetal grow restriction (PE-FGR) than in patients with isolated preeclampsia or spontaneous preterm delivery; (3) there is no correlation between placental C4d immunoreactivity and circulating maternal HLA class I or class II antibodies; 4) there is no difference in C4d staining in the cytotrophoblast in the different preterm groups; 5) there are significant correlations between C4d staining of the syncytiotrophoblast and maternal vascular underperfusion, as well as between the presence of maternal HLA class I antibodies and chronic placental inflammation; and 6) C4d staining of the syncytiotrophoblast is frequent in patients with miscarriages, and there is no association with maternal HLA status.

This study confirms frequent C4d deposition in the syncytiotrophoblast of preeclampsia cases. Importantly, syncytiotrophoblast C4d immunoreactivity is more frequent in cases of preeclampsia with fetal growth restriction than in isolated preeclampsia cases. Although Buurma et al. showed no correlation between isolated fetal growth restriction and placental C4d deposition [9], our

observations are consistent with the patterns of C4d immunoreactivity seen in cases of pregnancy-induced hypertension (PIH) and systemic lupus erythematosus (SLE) [7]. Furthermore, Minamiguchi et al. found an inverse correlation between neonatal birth weight and placental C4d immunoreactivity in patients with PIH or SLE [7]. Girardi et al. demonstrated that complement activation leads to fetal rejection and growth restriction in a mouse model, and complement inhibition can rescue these cellular processes [33]. Therefore, there seems to be a clear relationship among C4d deposition, immunological placental injury, and growth restriction in patients with SLE, PIH, and preeclampsia. We studied preterm preeclampsia cases because spiral arteries are not remodeled adequately due to shallow trophoblast invasion in this condition. This is a primary feature of early onset preeclampsia, which occurs before 34 weeks of gestation [34, 35]. Minamiguchi et al. have also shown an association between placental C4d deposition and lower gestational age at the time of delivery [7]. Of note, previous studies have shown that the frequency of syncytiotrophoblast C4d immunoreactivity in first trimester elective abortions and term deliveries is relatively uncommon (5 % for each) [5, 8].

We also investigated if there is an association between maternal HLA antibody status and placental C4d deposition. Maternal HLA classes I and II antibodies were present in all groups. Although there is a relationship between C4d deposition and HLA antibody-mediated rejection in solid organ allografts [36, 37], there was no correlation between the presence of HLA classes I and II antibodies and C4d staining of the syncytiotrophoblast. Therefore, placental C4d deposition is different from the situations seen in conventional allografts. These results further support a view of Buurma et al. that placental C4d deposition is likely a

Fig. 3 Maternal HLA antibody status in different study groups and its relationship with C4d staining of the syncytiotrophoblast. **a**, **b** There is no difference in rates in PE-FGR, PE, and sPTD cases with regard to the presence of maternal HLA classes I and II antibodies (p=0.349 and p=0.564 for each). **c**, **d** C4d staining of the syncytiotrophoblast in relation to the presence or absence of maternal HLA classes I and II antibodies in preterm delivery cases (n=80; PE-FGR, PE, sPTD). There is no significant difference in the presence of HLA classes I and II antibodies between placental C4d-positive and -negative cases (p=0.189 and p=0.768, respectively). **e**, **f** There is also no significant correlation between HLA classes I and II antibody status and C4d staining of the syncytiotrophoblast in patients with miscarriages
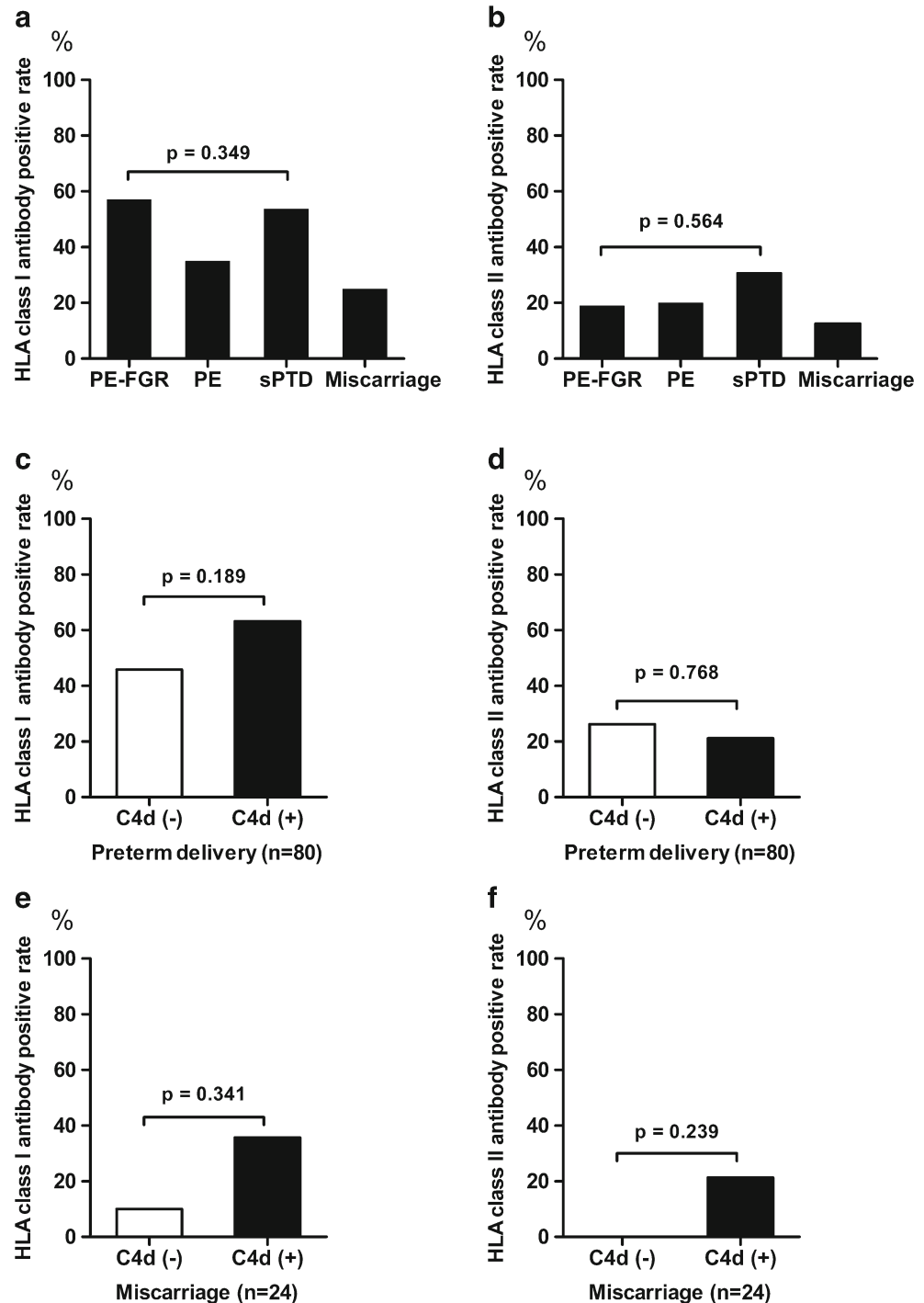
consequence of ischemia-reperfusion injury [9]. They proposed that activation of the classical complement pathway in patients with preeclampsia is a nonspecific reaction to placental damage where IgM antibodies, instead of fetal antigen-specific IgG HLA antibodies, play a role. In fact, ischemia-reperfusion can induce complement activation [38], and Weiser et al. have proposed that preexisting natural antibodies are responsible for classical complement

activation in a mouse hindlimb ischemia model. The hindlimbs of mice deficient in C3, C4, and serum immunoglobulin were all protected from ischemia-reperfusion injury [39]. Therefore, it is plausible that C4d immunoreactivity is closely associated with biological challenges such as oxidative stress and endoplasmic reticulum stress in placental cells as a consequence of shallow placentation and ischemia-reperfusion injury. This postulate is further

supported by a significant correlation between the placental
findings of maternal vascular underperfusion and C4d de-
position found in this study.

An intriguing feature of placental C4d immunoreactiv-
ity is the frequent staining of the villous cytotrophoblasts
in preterm delivery cases. We have also previously
shown cytoplasmic C4d immunoreactivity in preterm pla-
centas affected by cytomegaloviral villitis [10]. Lack of
cytoplasmic C4d staining in the first trimester and full-
term placentas strongly suggested that C4d immunoreac-
tivity in the cytotrophoblast is a gestational age-
dependent change. The trophoblast is a known source
of different complement proteins, and recent studies
showed C4 synthesis by human microvascular and glo-
merular capillary endothelial cells [40]. Therefore, C4d
immunoreactivity is possibly associated with the produc-
tion of C4 by cytotrophoblasts. Bulla et al. have demon-
strated an increase in the expression and secretion of C4
by HTR8/SVneo trophoblast cells and primary
cytotrophoblasts when treated with interferon-γ [41]. A
significant association between maternal HLA class I an-
tibody status and chronic placental inflammation is con-
sistent with previous observations that chronic placental
inflammation is a placental manifestation of maternal
anti-fetal cellular rejection [29, 30].

In summary, we have investigated placental C4d depo-
sition in major pregnancy disorders with special reference
to maternal HLA status and pathological changes. C4d
deposition in the syncytiotrophoblast was frequently de-
tected in cases of preeclampsia with fetal growth restric-
tion. It was also common in patients with miscarriages,
which further confirmed our previous observation [5]. Ap-
proximately two thirds of first trimester miscarriages are
the result of shallow placentation and incomplete plug-
ging of spiral arteries, which leads to premature
intervillous circulation and placental oxidative stress
[42]. The results of the present study are consistent with
the view that preeclampsia and miscarriage share a com-
mon pathogenesis of shallow placentation. A major limi-
tation of the study is that it is retrospective and explorato-
ry. A prospective study based on a larger sample size
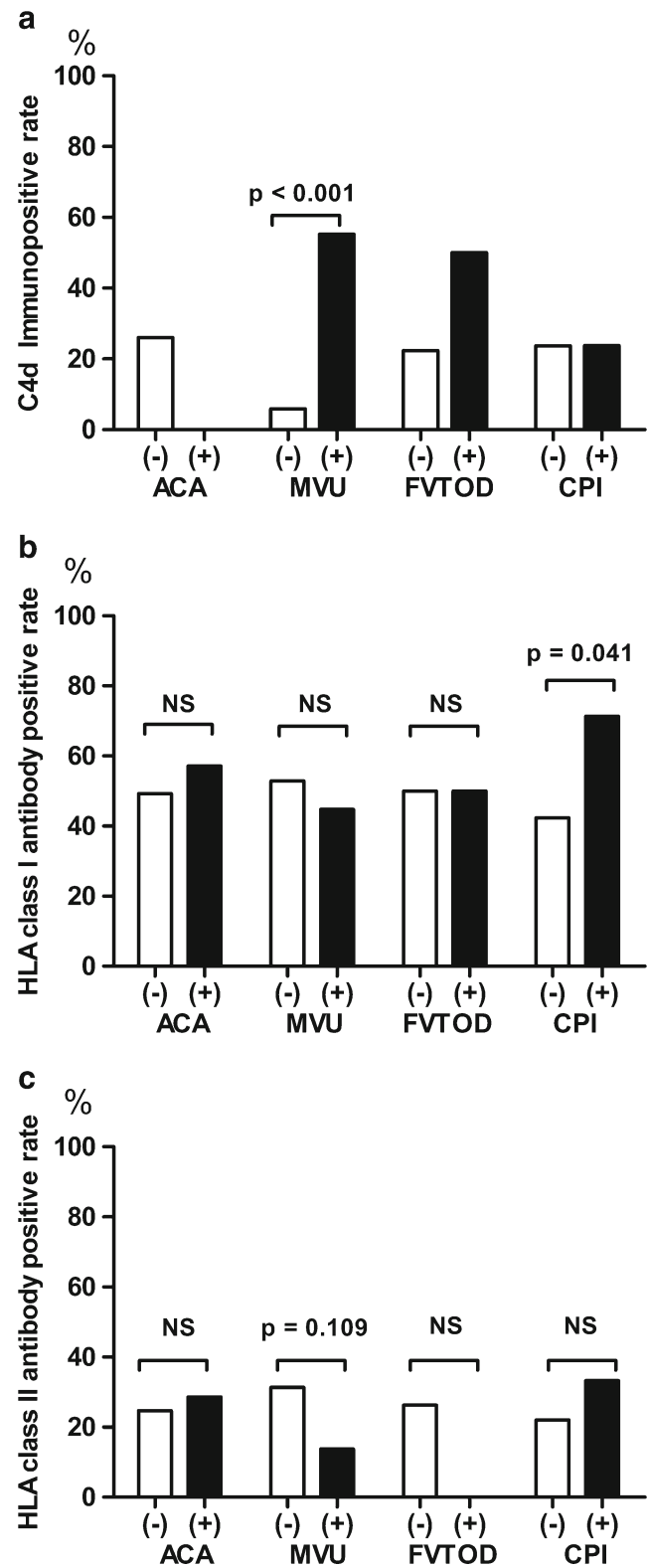
would be required, along with mechanistic investigations.
Collectively, C4d seems to be a biomarker for at-risk pla-
centas due to defective placentation during pregnancy.

Conflict of interest The authors declare that they have no conflict of interest.

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