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Placental histological lesions in fetal and neonatal alloimmune thrombocytopenia: A retrospective cohort study of 21 cases

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ABSTRACT

Background: Alloimmunization against human platelet antigens (HPAs) can occur prenatally and induce fetal/neonatal alloimmune thrombocytopenia (FNAIT). The aim of this study was to identify placental histological features associated with platelet alloimmunization and their clinical significance.
Methods: This study examined 21 placentas from FNAIT-affected pregnancies and 42 age-matched control cases, all collected from pathology departments in the Rhône-Alpes region. Clinical and laboratory findings were collected for each FNAIT case. Two pathologists reviewed the placental slides of each FNAIT and control case. Histological features, with special emphasis on chronic inflammatory lesions, were evaluated. Differences between the two groups were calculated with odds ratios (ORs) and assessed with Wald's chi-square.
Results: FNAIT was associated with a significantly higher frequency of chronic chorioamnionitis (CC) (OR 14, 95%CI 1.7–113.8), basal chronic villitis (BCV) (OR 17, 95%CI 2–145.6) and chronic intervillositis (CIV). Chronic villitis (CV) (OR 3.7, 95%CI 0.9–15.2) and chronic deciduitis (CD) (OR 4.7, 95%CI 0.79–28.2) were also more frequent in the FNAIT than the control group, but these differences were not statistically significant.
Conclusions: FNAIT is significantly associated with CC, BCV, and CIV. This chronic inflammatory reaction is preferentially localized on the maternofetal interface. Anti-HPA alloimmunization may trigger an immunological conflict similar to graft-versus-host disease.

1. Introduction

Platelet alloimmunization induces fetal/neonatal alloimmune thrombocytopenia (FNAIT), which is the most common cause of severe thrombocytopenia in fetuses and newborns [1]. Its clinical presentation varies from asymptomatic thrombocytopenia, to mild petechiae and ecchymoses, to severe intracranial hemorrhage (ICH) that results in death or poor neurological outcome. Intrauterine growth restriction (IUGR) [2,3] and intrauterine fetal death (IUFD) are also recognized as complications of this disorder [4,5]. FNAIT is caused by maternal alloantibodies against paternally derived fetal platelet antigens and leads to fetal thrombocytopenia, defined by a platelet count below 150,000 platelets/μl. FNAIT, unlike erythrocyte alloimmunization, may appear during first pregnancies, with a high recurrence rate and often with progressively more severe manifestations in subsequent pregnancies [6]. Antigens capable of triggering FNAIT are carried on platelet membrane glycoproteins (GPs), which are remarkably polymorphic. To date, 37 human platelet specific alloantigens (HPA) have been described [7]. Antibodies against these antigens are called anti-HPA. Recent studies estimated that in white populations, anti-HPA-1a is the most common alloantibody (75–80%), followed by anti-HPA-5b (10–15%) [8]. The HPA-1 antigen is localized on platelet membrane glycoprotein (GP) GPIIIa and HPA-5 on GPIa. HPA-2, -3, and -15, respectively found on GPIb, GPIIb, and CD109, have also been associated with FNAIT.

The incidence of FNAIT is estimated around 1/1000 in live newborns [9]. Potential reasons to search for antiplatelet antibodies include IUFD, IUGR, and unexplained hydrocephaly, besides fetal/

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neonatal hemorrhage or thrombocytopenia. Although FNAIT is the object of many reports in the literature, only a few of them describe pathological examination of the placenta. Chronic inflammatory lesions such as chronic villitis and chronic intervillositis have occasionally been reported [2,10,11]. Recent studies have hypothesized that these chronic inflammatory lesions are linked to alloimmune reactions and might be similar to fetal graft-versus-host disease [12].

The aim of this study was to assess placental histological findings in pregnancies with FNAIT, regardless of the severity of its laboratory findings or clinical manifestations.

2. Methods

2.1. Case selection

After approval by its inhouse Institutional Review Board, the database of the blood transfusion center of the Rhône-Alpes region identified 147 cases of pregnancies between 1998 and 2014 in which the mother carried anti-HPA alloantibodies. FNAIT cases were selected when fetal/neonatal hemorrhage and/or thrombocytopenia and documented maternal anti-HPA alloantibodies were present. Fetomaternal incompatibility was confirmed by fetal and parental platelet antigen genotyping. In the absence of proven fetomaternal incompatibility, other causes of fetal/neonatal thrombocytopenia, including infection, autoimmunity, drug-related destruction, intravascular coagulation, necrotizing enterocolitis, hypersplenism, Kasabach-Merritt syndrome, and congenital hematologic disease were ruled out. Among these FNAIT cases we selected those for which placental histological examinations were available by requesting them from the initial pathology department. In all, 21 cases met these criteria and constituted the final study cohort. We selected 42 control cases matched for gestational age.

2.2. Antibody analysis

Detection of specific antiplatelet antibodies was determined by a monoclonal antibody immobilization of platelet antigens (MAIPA) assay. Paternal and fetal/neonatal platelet genotypes were determined from genomic DNA, by polymerase chain reaction testing with sequence-specific primers (ssp) for the *a* and *b* alleles of HPA systems 1, 3, 5, and 15. Testing was performed by the Rhônes-Alpes Blood Center.

2.3. Data collection

For each FNAIT case and each control, we retrieved and recorded the following specific information from the electronic medical database: type of pregnancy (singleton or twin), maternal age, maternal race, gravidity, parity, gestational age at delivery, preeclampsia-toxemia (PET), preterm labor (PTL), IUGR, premature prelabor rupture of membranes (PPROM), placental weight, and neonatal/fetal weight.

For FNAIT cases, we also collected the maternal antiplatelet antibody identification, and if available, the fetal and parental platelet antigen genotypes, fetal/neonatal platelet counts, hemorrhagic events, and outcomes.

2.4. Pathologic evaluation

Placentas were formalin-fixed. Standard sampling included at least one section of the umbilical cord and membrane roll and a minimum of 2 sections of the placental parenchyma. Hematoxylin-eosin-saffron (HES) stained slides were prepared for histological examination. When diagnosis was difficult, immunohistochemical staining for CD3, CD20, CD68, and CD138 was performed on paraffin-embedded tissue sections. For each case and control, the slides were reviewed by two referent pathologists blinded to the clinical and laboratory information, except for gestational age.

Chronic inflammatory lesions included chronic chorioamnionitis (CC), chronic villitis (CV), basal chronic villitis (BCV), chronic deciduitis (CD), and chronic intervillositis (CIV) [13].

CC was defined by the presence of mononuclear infiltrate in the fetal membranes, which can be seen either in the free membranes or the chorionic plate [13]. The severity of CC was graded as follows: grade 1, when there were more than two foci or patchy inflammation, and grade 2 when diffuse inflammation was present. The stage of inflammation was scored as stage 1 if lymphocytic infiltration was only seen in the chorionic trophoblast layer, sparing the chorioamniotic connective tissue, and stage 2 if the chorioamniotic connective tissue was involved [14].

CV was defined by the presence of a lymphohistiocytic infiltrate in the chorionic villous tree [15]. We distinguished it from BCV, in which the infiltrate was present in the basal villi along the basal plate, subjacent to the decidua [13]. We classified these lesions as follows: low grade when fewer than 10 villi were involved, either focal (seen in only one slide) or multifocal (if visible on more than one slide), and high-grade when the process was seen in more than 10 villi per focus. It was separated into patchy (less than 5% of all distal villi involved) or diffuse (when more than 5% of all distal villi were involved) [15,16].

CD was defined by the presence of plasma cell infiltration in the decidua basalis, seen either in the free membrane roll or along the maternal surface [13].

CIV was defined by the presence of mononuclear inflammatory cell infiltrate with variable amounts of fibrinoid deposition in the intervillous space [13,17]. The semi-quantitative grading proposed by Rota et al. was used, based on the extent of cell infiltration and fibrinoid deposition: grade 1, when less than 10% of the intervillous space contained infiltrate and fibrin deposits, grade 2 for 10%–50% of the intervillous space, and grade 3 for more than 50% [18].

These findings were tabulated with all the clinical data collected.

2.5. Statistical analysis

Quantitative characteristics were described by their median, minimum, and maximum values. Qualitative characteristics were described by their frequency and percentages. The two groups were compared with the Mann Whitney test for the quantitative characteristics and Fisher's exact test for qualitative characteristics. To take matching into account, the comparisons of the placenta abnormalities between the FNAIT group and the control group were compared with the Mantel Haenszel chi-2 test, stratified for the matched triplets. To quantify the effects of platelet alloimmunization on the placenta abnormalities, stratified odds ratios were estimated with their 95% confidence intervals. Bilateral p values < 0.05 were considered significant. The analysis was conducted with SPSS software (IBM Corp. Released 2010. IBM SPSS Statistics for Windows, Version 19.0. Armonk, NY: IBM Corp).

3. Results

The FNAIT group included 18 singleton and 3 dichorionic-diamniotic twin pregnancies. The control group included 36 singleton and 6 dichorionic-diamniotic twin pregnancies. The clinical characteristics of the 21 cases and 42 controls are shown in Table 1. The mean maternal age was 32 years (range 26–42 years) in the FNAIT group and 31.4 (range 18–40) in the control group. The