



Fetal and Neonatal Alloimmune Thrombocytopenia: Advances in Laboratory Diagnosis and Management

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Abstract

Fetal and neonatal alloimmune thrombocytopenia (F/NAIT), caused by fetomaternal mismatch for human platelet (PLT) alloantigens (HPAs), is the commonest cause of severe thrombocytopenia in term neonates and is analogous to the fetal/neonatal anaemia caused by haemolytic disease of the newborn (HDN). The most feared complication of this syndrome is the occurrence of intracranial hemorrhage leading to death or neurological sequels. With advances in laboratory diagnosis and management of the index case and of pregnancies at risk, the prognosis of affected infants has improved. The study of F/NAIT have drawn attention to the fact that the first affected fetus/neonate in a family is only recognized after bleeding has occurred and this has raised the question of whether routine screening for F/NAIT would be advantageous. Further research must focus on the mechanisms of maternal sensitization and development of specific therapy.

Keywords

Platelet alloimmunization, Thrombocytopenia, Intracranial hemorrhage, HPA, MAIPA, PCR-SSP, Management

Introduction

Fetal and neonatal alloimmune thrombocytopenia (F/NAIT) is the most common cause of severe thrombocytopenia in the fetus and in otherwise healthy newborn [1]. The mother produces antibodies (IgG) against fetal HPA antigens inherited from the father. These alloantibodies (IgG) can cross the placenta, destroy fetal thrombocytes and may induce severe thrombocytopenia. It is most commonly caused by the HPA-1a antigen (80%). In contrast to haemolytic disease of the newborn (HDN), F/NAIT can be present in a first pregnancy [2]. Bleeding risk may be important throughout the severe thrombocytopenia phase, but the major risk is cerebral hemorrhage before 20 weeks of gestation [3,4]. Considerable progress has been made in the laboratory investigation of F/NAIT. There have also been improvements in its management, particularly in the antenatal management of women with a previous history of pregnancy(s) affected by F/NAIT, resulting from a better understanding of the risk of intracranial hemorrhage (ICH) and advances in fetal and transfusion medicine [5]. Predictive parameters for fetal and severe thrombocytopenia are important for the development of noninvasive strategy and appropriate intervention [1]. The incidence of F/NAIT

is estimated at 1 in 1000-2000 live births in most populations studied [6,8]. They represent 3% of all neonatal thrombocytopenia (NT) and 27% of severe NT [9] with occurrence of ICH in 7-26% of cases (50-80% *in utero*) [10-12]. These ICH are associated with high mortality in 10% of cases [13] and neurological sequels in 20% of cases. In Caucasians anti HPA-1a antibody is found in approximately 80% of severe cases of F/NAIT with fetal ICH in 10% of cases [4-14,15]. The incidence of NAIT with anti HPA-1a antibody is estimated at 1 per 1163 live births (86 per 100,000) [5-16]; whereas in Japanese populations anti HPA-4b appears most frequently involved [17]. Anti HPA-5b and -15a antibodies are involved in 20% of NAIT [18] (15% for the anti HPA-5b [19]). All other specificities, including the anti HPA-3a, -2a and -2b antibodies are reported in most of the studies in 2% of patients [20].

Pathogenesis

F/NAIT occurs in the first pregnancy in 50 to 60% of cases with a risk of recurrence in subsequent pregnancies in 88 to 95% [21]. Immunization involves mainly five biallelic platelet antigen systems: HPA-1, HPA-2, HPA-3, HPA-4 and HPA-5. Their frequencies vary among ethnic groups. The newly described antigens are essentially rare or private [22]. These antigens are located on platelet glycoproteins (GPIa, GPIIb, GPIIIa...) that are involved in platelet functional disorders causing hemorrhagic syndrome (Figure 1).

Immunization appears from 14 weeks of pregnancy. After placental transfer of these antibodies, they bind to the surface of the fetal platelet and the destruction of antigen antibody complex by the macrophage system, mainly in the spleen, results in thrombocytopenia of variable intensity according to the antibodies concentration and precocity of installation. Fetal and maternal factors defining the immunogenicity and immunoreactivity contribute with these processes in maternal fetal bidirectional relationship. It has been demonstrated that the HLA class I related neonatal Fc receptor (FcRn) plays an important role in maternal-fetal passageway of IgG antibodies. FcRn prevents degradation of antibodies during the transfer by binding to regions IgG CH2 and CH3. A genetic background is involved for HPA-1a and the human leukocyte antigen (HLA)-DRB3*0101 and/or -DQB1*201 allele and showing that HPA-1a-specific-T-cells can be isolated from HPA-1a-immunized women [23-25]. In studies examining the mediation of T cells to the regulation of the humoral

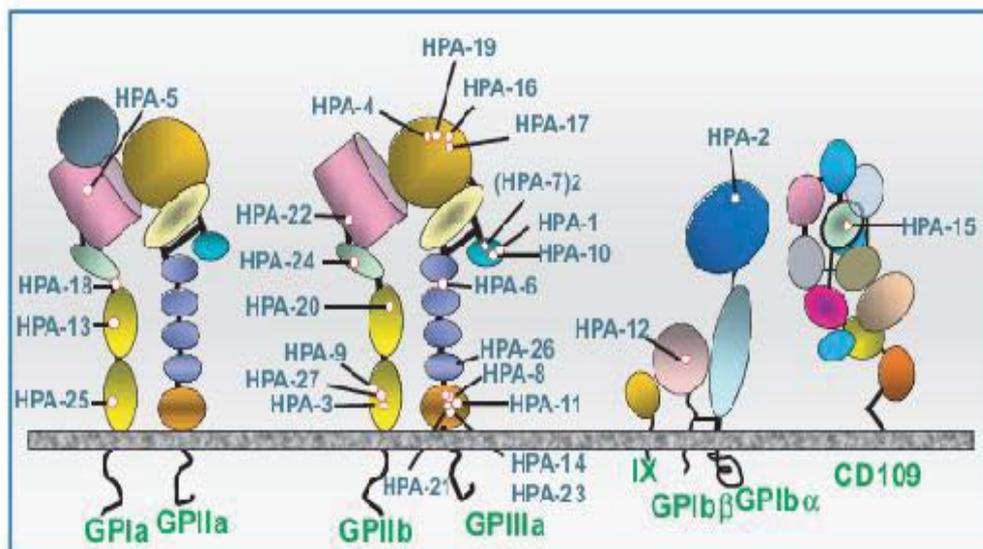


Figure 1: Antigens known to trigger maternal sensitization leading to neonatal alloimmune thrombocytopenia are carried on four different platelet membrane glycoproteins (GP) and glycoprotein complexes. Structural domains identified by crystallographic studies are shown schematically [29].

response in the alloimmune response, it has been found that the polymorphic residue 33 of the GPIIIa integrin $\beta 3$, where the allele HPA-1 is located, is responsible of dual response of B and T cells. This suggests that the Leu / Pro33 substitution on GPIIIa glycoprotein contributes in antigen presentation. The anti HPA-5b immunization is associated with a group of HLA-DR molecules that share an amino acid sequence in position 69-70 [23-26]. Recently a novel model to study antigen-specific CD4+T cells during alloimmunization to PLT transfusion has supported a critical role for CD4+Tcell help in the humoral response to PLT transfusion and established the spleen as a required microenvironment for effective CD4+T-cell priming against donor PLT- derived HLA I [27,28]. As regards the other platelet antigens, immunized women series are not large enough to enable such analysis.

Clinical Data

There are two types of clinical appearance 1) Fetal thrombocytopenia 2) Neonatal Thrombocytopenia

Fetal thrombocytopenia

This is the most severe of all fetal thrombocytopenia. It can occur early and severe thrombocytopenia exposed to the risk of ICH. It has been shown in a survey of 5194 samples of fetal blood that alloimmune thrombocytopenia was reported in the majority of severe fetal thrombocytopenia and no spontaneous correction of fetal thrombocytopenia was observed during the gestation period [23]. During pregnancy, F/NAIT should be suspected in various situations. Unfortunately it is usually discovered after deleterious complications of ICH. A change in fetal movement or fetal heart rate should alert the clinician to seek ICH. These ICH are diagnosed by fetal ultrasound and magnetic resonance revealing a ventriculoperitoneal megalia, fetal hydrocephalus....Retrospective studies have shown that 80% of ICH occur *in utero* with 13,8% to 20% diagnosed before 20 weeks of gestation and 27,6% to 28% before 30 weeks of gestation. Fetal ICH are mainly observed when the anti HPA-1a antibody is involved and the anti HPA-3a antibody is also linked to severe F/NAIT cases but ICH are less frequently reported. The ICH can give serious consequences; deaths were documented in 10% of affected children and neurological sequels in 20%. Other F/NAIT complications such as hydrops *in utero* or unexplained fetal anemia have been reported [23-30].

Neonatal thrombocytopenia

In the absence of an F/NAIT screening program, this pathology is usually discovered when a child is born after a first uncomplicated pregnancy with petechiae or purpura at birth or occurring in the first

hours of life. The diagnosis of F/NAIT is suspected when other causes of thrombocytopenia such as fever, infections, splenomegaly, DIC ... are excluded. However some cases of NAIT have been reported associated with HDN, infections, chromosomal abnormalities, DIC (related to anoxia and shock) and alloimmune thrombocytopenia (AIT) may be suspected if thrombocytopenia is severe and prolonged [4-23]. Hemorrhagic disease is more severe when it comes to anti-HPA-1a immunization (ICH: 25.5% of cases) or anti HPA-3a (ICH: 24% of cases) than when antigen HPA-5b is involved (ICH: 15% of cases). The child is at risk of bleeding throughout the duration of severe thrombocytopenia and it is important to quickly make the diagnosis of NAIT to implement appropriate treatment [31]. The NAIT with anti-HPA-9bw antibody gives a severe clinical syndrome and must have an early diagnosis [32]. Observational cohort study of all recorded cases of ICH caused by F/NAIT from the international No Intra Cranial Haemorrhage (NOICH) registry during the period 2001-2010 shows clinical characteristics of ICH pregnancies in figure 2.

Laboratory Diagnosis of F/NAIT

Proper laboratory diagnosis of F/NAIT requires sophisticated testing, a thorough understanding of platelet serology and, often, personal communication between the testing laboratory and the attending physician and should be undertaken only by laboratories that possess these capabilities. For the most informative evaluation, it is important to study blood samples from both mother and father [34]. Detailed laboratory investigations are required for confirmation of a provisional clinical diagnosis, and should be performed by an experienced reference laboratory.

Diagnosis is based on clinical and serological findings; it includes a low platelet count, bleeding and detection of alloantibodies. In addition, determination of the HPA genotype of mother, father and, if needed, the child (or fetus) is useful for confirming the diagnosis, especially in cases without detectable antibodies [5].

Serological methods

Research and identification of maternal alloantibodies are realized with ELISA antigen capture assays. Monoclonal Antibody Immobilization of Platelet Antigen (MAIPA) is currently considered the reference test; it is based on the immobilization of platelet glycoprotein (GP) on a plate, the GP are sensitized with murine monoclonal antibodies before solubilization. Only antibodies having specificity for glycoproteins are detected. Maternal sera will be tested first against paternal platelets (crossmatch) and against a panel of typed platelets from normal group O donors to remove any reaction

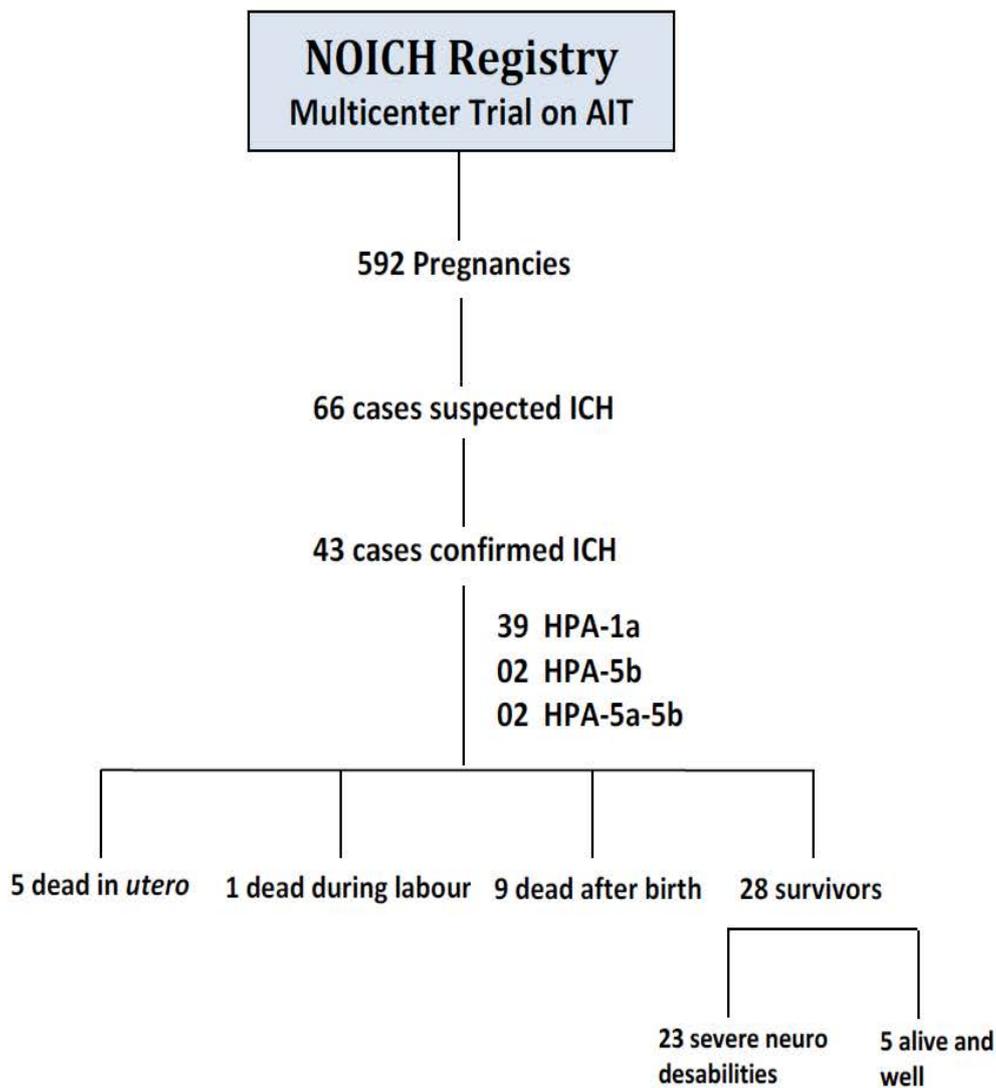


Figure 2: Clinical characteristics of ICH pregnancies [33].

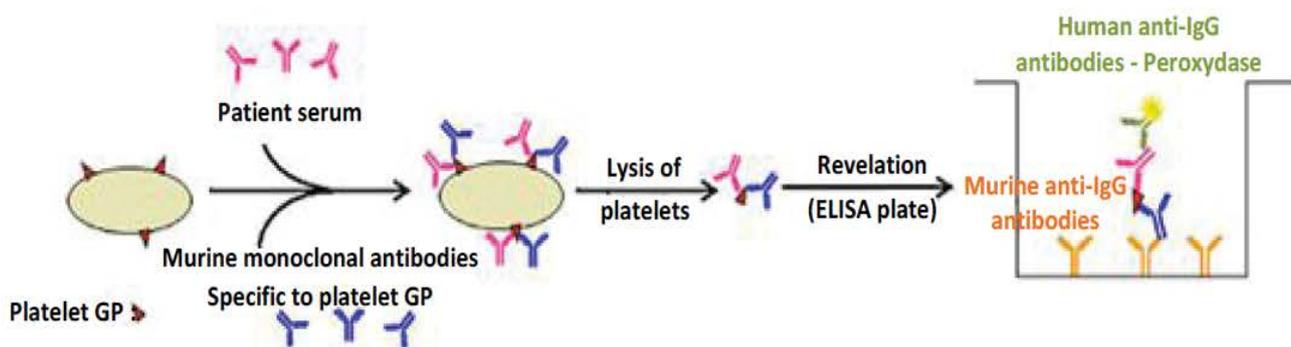


Figure 3: Detection of anti-HPA antibodies by MAIPA test

due to the presence of anti-A or anti-B antibodies in maternal sera. We must not overlook the fact that alloantibodies are heterogeneous and that the reactivities observed vary according to the monoclonal antibodies used [35]. MAIPA test allows the identification both of alloantibodies directed specifically against platelet antigens (anti-HPA) and anti- HLA antibodies that may appear from the first pregnancy [36] (Figure 3).

Flow cytometry provides a rapid and sensitive means of detecting platelet-reactive antibodies and is used to test maternal sera against washed paternal and maternal platelets and a small panel of typed

platelets from normal group O donors [34-37]. Because of the clinical importance of HPA-1a system in the platelet alloimmunization; platelet HPA-1a typing assay using flow cytometry or sandwich ELISA has been developed. Indeed, Norwegian investigators typed women pregnant for the first time for HPA-1a and screened those found to be negative for HPA-1a antibody at various times during gestation in an attempt to identify infants at risk for F/NAIT [38]. Some have argued that it may be cost-effective to perform such screening routinely and offer special case management to the 10% of HPA-1a-negative women who produce antibody [39] but at the

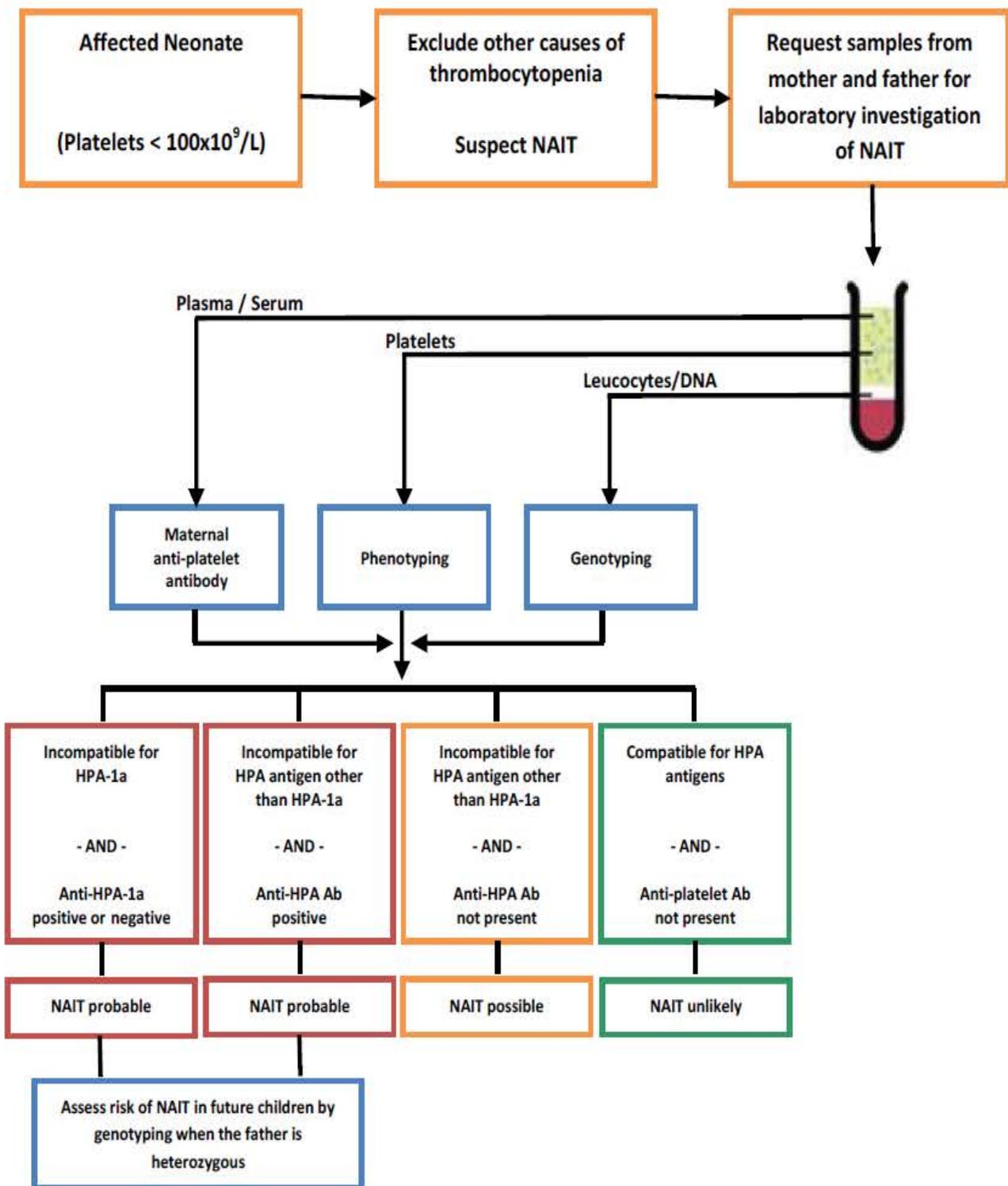


Figure 4: Algorithm of a F/NAIT diagnosis protocol [20]. "Testing for Human Platelet Antigens (HPA) and Antibodies"

present time this is not practiced in the absence of a family history of F/NAIT, e.g., in a sister [34].

Platelet typing

Development of DNA-based methods has made serological typing for HPA antigens obsolete [34]. However, mutations may interfere with the genotyping techniques. That is why it is recommended to not neglect the achievement of phenotyping for the most common antigens or to perform two different genotyping techniques to avoid misdiagnosis [40]. Today, polymerase chain reaction-sequence specific primers (PCR-SSP) is the most used molecular biology method,

although other older methods such as PCR-restriction fragment length polymorphism (PCR-RFLP) or more recently the real time-PCR is used sometimes [41]. For miniaturization and automation of analysis, the high-throughput genotyping by microarrays, fluorescent or colored microbeads technology is currently developed (Figure 4).

Treatment

The treatment of thrombocytopenia varies considerably depending on the installation period (ante- or post-natal), responsible pathology, the eventual combination of coagulation abnormalities, and mainly on the risk of occurrence of intracranial hemorrhage. This

risk is a major in postpartum, especially when delivery is difficult or traumatic. The optimal postnatal management of AIT depends on its rapid recognition, and prompt correction by transfusion of platelet concentrates to neonates who are severely thrombocytopenic (platelet count lower than 30 Giga/l) or bleeding during the first 24 hours of life [42]. Transfused platelets should not be destroyed by maternal antibodies, and the mother is the best donor [41]. If maternal platelets are used, it is absolutely essential that they be washed to remove antibody containing maternal plasma [34]. However, it is not appropriate to wait for the laboratory confirmation of the diagnosis in suspected cases to select the HPA compatible platelets. Platelet HPA-1a and -5b negative donors should be used initially, if they are available, on the basis of the certainty of their effectiveness in the more than 90% of cases of AIT that are due to anti-HPA-1a or anti-HPA-5b [43]. Random donor platelets appropriate for neonates (ABO compatible, volume reduced, if indicated cytomegalovirus negative, and irradiated) will usually elevate the platelet count at least transiently and reduce the likelihood of bleeding even when they are incompatible with the maternal antibody. In addition, intravenous immunoglobulin (IVIG) at 0.4-1.0g/kg/d for 2-5d can be given to potentially prolong the survival of the incompatible platelets and lessen the overall period of thrombocytopenia [34]. Note that IVIG are by no means an alternative to platelet transfusions; Indeed, the action of immunoglobulins is observed that after a period of 18 hours during which the child severely thrombocytopenic remains at risk of bleeding [41]. Moderately severe thrombocytopenia (e.g. PLT between 50 and 30Giga/l) without obvious hemorrhage can be managed with IVIG treatment alone. Typically, total doses of 2g/kg are given over 2-5d [11-44]. When a second transfusion is needed, the laboratory results may allow the production of platelets compatible with maternal antibodies. Monitoring will be carried out until a platelet count over 50 Giga/L. It will of course be necessary to ensure the absence of even minimal intracranial hemorrhage.

In the absence of ICH, the evolution is favorable; a normal platelet count is obtained in a few days. The severity of maternal alloimmunization and recurrence of fetal damage during subsequent pregnancy with platelet maternofetal incompatibility led to the establishment of a specific management of risk pregnancies [41].

Management of High-Risk Pregnancies

As noted, NAIT tends to be more severe in infants born subsequently to a mother who previously gave birth to an infant with this condition [34]. The care of women at risk of F/NAIT and their children requires a multidisciplinary collaboration between hematologists, obstetricians and neonatologists, seconded and supported by transfusion medicine services and platelet immunology laboratories. The purpose of antenatal management is to prevent the occurrence of severe thrombocytopenia and the concomitant risk of ICH with its sequels including deaths and severe disabilities [6-20]. The subsequent pregnancies of HPA-1a alloimmunized women with a history of a previously affected infant with AIT are well recognized to be associated with a high risk of recurrence of AIT and poor outcome [5]. Several steps should be considered in such cases. One is to determine whether the infant being carried incompatible with the maternal alloantibody is previously demonstrated, whether or not it is still detectable. Second (if the infant is incompatible) is to estimate the degree of fetal thrombocytopenia so as to gauge the risk of antenatal intracranial hemorrhage. Third is to offer risk-stratified antenatal therapy to the mother to ameliorate fetal thrombocytopenia and reduce the likelihood of prenatal and postnatal bleeding [34]. If the father is homozygous all subsequent fetuses will be obligate heterozygous and will be incompatible with the maternal antibody. If the father is heterozygous the fetus will have a 50% chance of inheriting the marker. In the latter case, fetal genotyping can be performed on amniotic cells or recently from on fetal DNA in maternal blood to determine whether the fetus is at risk [45]. When a fetus has been determined to be at risk for F/NAIT, an estimate should be made of its likely severity. Non-invasive methods for estimating the severity of F/NAIT during pregnancy include testing of the mother's serum

for the strength of the anti-HPA antibody and considering the severity of disease in previously affected siblings [34]. If conflicting results have been reported regarding the meanings of the titration of maternal antibodies during pregnancy, a study has shown that the titration of antibodies before 28 weeks, without any treatment, carried out in well-standardized conditions was correlated with severe fetal thrombocytopenia [3]. Different treatments have been proposed: transfusion *in utero*, early cesarean section and maternal treatment. Currently, there is a consensus in favor of maternal treatment in first line. The proposed treatment during pregnancy is infusion of IVIG at a dose of 1 g/kg per week. A retrospective study comparing several maternal treatments, corticosteroids alone, only IVIG and IVIG with corticosteroids, showed that the most effective treatment was the latter [1]. At birth, a platelet count of the child will be immediately realized and in cases of severe thrombocytopenia, transfusion of genotyped platelets is immediately performed. If the currently proposed protocols relate to women who had a history, the problem is posed for a first pregnancy in a woman that may be at risk of alloimmunization (e.g., sister of a woman who has given birth to a child with alloimmune thrombocytopenia).

The prenatal treatment may be proposed if there was detection of alloantibodies. No invasive procedure will be made for these cases [41].

Antenatal Screening for F/NAIT

Advances in the laboratory diagnosis and antenatal management of AIT have drawn attention to the fact that the first affected fetus/neonate is usually only recognized after bleeding has occurred or severe thrombocytopenia detected by chance. This raises the question of whether routine screening for AIT should be considered [5]. Antenatal screening for F/NAIT is possible through the identification of maternal alloantibodies during the first pregnancy and the study of the fetal platelet genotype. This approach allows an antenatal intervention to prevent the occurrence of ICH [46]. Prospective studies have been conducted showing that routine screening could be a public health option. However, the implementation of such a program raises several issues such as sufficient sensitivity in diagnosis tests of maternal immunization, existence of maternal predictive parameters of fetal damage, and optimizing management of high-risk pregnancies [41].

Conclusion

Major progresses have been made to better diagnose and therapeutic management of F/NAIT. However, many questions are still unanswered about prevention and mechanisms of maternal immunization.

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