54 Fetal alloimmune thrombocytopenia

Hung N. Winn

PATHOGENESIS

Alloimmune thrombocytopenia (AIT) is a syndrome that develops as a result of maternal sensitization to fetal platelet antigens. These women who themselves lack the specific antigen produce immunoglobulin G (IgG) antibodies directed against the platelet antigen that the fetuses inherit from their father. The antibodies are then transported across the placenta to the fetal circulation where they result in the destruction of the fetal platelets. Thus, the pathogenesis of AIT is analogous to that of hemolytic (Rh) disease of the newborn. However, unlike Rh disease, prior sensitization to the platelet antigen is not necessary since approximately half of the clinically evident cases of AIT occur in the first pregnancies. This suggests that either the fetal platelets gain access to the maternal circulation more easily than red blood cells, and/or the platelet antigens are highly provocative and therefore stimulate production of IgG antibodies during the first pregnancy (1). In a family with a previously affected infant, the rate of recurrence with an antigen-positive fetus is estimated to be at least 90% (2,3). The recurrence risk of neonatal intracranial hemorrhage (ICH) in a subsequent pregnancy after having a child with ICH due to AIT is about 77% (4). Additionally, it has been a uniform finding that the severity of thrombocytopenia in subsequent infants is either the same or worse than that of the first affected child.

PLATELET SEROLOGY

Platelets possess specific alloantigens that are expressed on the surface membrane glycoproteins. There are five major platelet alloantigen systems, all with biallelic polymorphism and inherited as codominant traits (5). The difference in alleles results from a single base substitution in the glycoprotein deoxyribonucleic acid (DNA) sequence, which ultimately results in a protein recognized as different from its allele (6). The most common antigen associated with AIT is HPA-1A, accounting for approximately 80% of reported cases (7). Other platelet antigen systems involved in AIT are shown in Table 1. The frequency of phenotypes and genotypes for each antigen differs in different ethnic groups.

INCIDENCE/GENETIC ASPECTS OF MATERNAL IMMUNE RESPONSE

Based on prospective analysis, the incidence of AIT is estimated to be between 1 per 1000 and 1 per 2000 births (7–9). The expected incidence in Caucasians based on the 98% frequency of the HPA-A1 allele is 1 to 2 per 100 births. This discrepancy between expected and observed incidence suggests that additional factors contribute to the maternal immune response. The risk of sensitization in HPA-A1 negative women is correlated with the presence of specific maternal human leukocyte antigen (HLA)-associated immune response genes, namely HLA-DR3, and more specifically

HLA-DRW52 (10). The immune response markers are contributory, but not sufficient for the development of alloimmunization, as not all HPA-A1 negative women with an HLA-DRW52 haplotype will deliver thrombocytopenic infants (11). The role of immune responsiveness for other platelet antigens has not yet been established.

CLINICAL PRESENTATION

In typical cases of AIT, the mother is healthy (without thrombocytopenia) and her pregnancy, labor, and delivery are uncomplicated. The neonates who are otherwise normal present with symptomatic thrombocytopenia within several hours of birth. Affected infants often develop a generalized distribution of petechiae, as well as ecchymoses over the presenting fetal part. Visceral bleeding and bleeding during circumcision or venipuncture may be present. Of greatest consequence is the risk of ICH, occurring in up to 30% of cases (3) and responsible for the 6.5% mortality rate (12) and the significant long-term neurologic sequelae, including hydrocephaly and porencephaly. Laboratory data are remarkable for an isolated and often severe thrombocytopenia, with normal or increased numbers of megakaryocytes found on bone marrow aspirate. The hematocrit may be decreased if significant bleeding has occurred. A definitive diagnosis can be made by phenotyping parental platelets and demonstrating antiplatelet antibodies in the maternal serum, which are specific for the antigen incompatibility identified.

Neonatal AIT is usually a self-limiting disorder, often resolving over the course of several weeks, as maternal antiplatelet antibodies disappear from the neonatal circulation. The most serious and feared complication is ICH, and until recently it had been believed that the greatest risk of ICH was at the time of labor and delivery. Hence, obstetric management had focused on the peripartum period and elective cesarean section at term was advocated (13,14). In the neonatal period, the goal was to replenish the neonate's circulating platelets in order to prevent complications secondary to bleeding. These infants should receive compatible antigen-negative platelets from either the mother or compatible donors (15). If the mother's platelets are used, they must be carefully washed in order to avoid introducing additional antiplatelet antibodies into the neonatal circulation. High-dose intravenous immunoglobulin therapy (1–2 g/kg over 1–5 days) may provide an additional or alternative therapy (16,17) especially when matched platelets are unavailable.

EVIDENCE OF ANTENATAL ICH

These approaches, however, have no impact on fetal bleeding in utero prior to the onset of labor. In 1979, Zalneratis et al. reported two cases of AIT in which there was good evidence that ICH had occurred in utero (18). Both infants had enlarged

Table 1 Platelet-Specific Alloantigen Systems Involved in Alloimmune Thrombocytopenia

Nomenclature		Platelet glycoprotein (GP)	Alleles
New	Old		
HPA-1	PL, Zw	GP IIIa	HPA-1a, 1b
HPA-2	Ko	GP Ib	HPA-2a, 2b
HPA-3	Bak	GP IIb	HPA-3a, 3b
HPA-4	Pen, Yuk	GP IIIa	HPA-4a, 4b
HPA-5	Br	GP Ia	HPA-5a, 5b

heads (>97%) at birth. The first had bifrontal and left posterior fossa cysts with obstructive hydrocephalus, and the second had a large right parieto-occipital hematoma followed by the development of hydrocephaly and a porencephalic cyst in the same location. Examination of the cyst contents obtained at the time of shunt placement revealed fluid filled with hemosiderin-laden macrophages in the first case and liquefied, “rusty” fluid in the second. The pathologic findings placed the time of bleeding at several months, and 1 to 2 weeks prior to delivery in these two cases, respectively.

Stronger confirmation that AIT could be responsible for cerebral hemorrhage in utero was presented in a series of cases where the diagnosis of porencephaly was made with ultrasound examination in the third trimester in fetuses suspected, and later confirmed, to have this disorder (19).

It is now known that up to 80.5% of ICHs secondary to AIT occur in utero (20). Fetal platelet antigens may be expressed as early as 13 weeks of gestation and ICH may be present by 16 weeks of gestation (21). Hence, in order to be maximally effective, the treatment of AIT must begin in utero.

ANTENATAL MANAGEMENT

Since routine screening for fetomaternal platelet incompatibility is not performed, identifying those fetuses at risk for AIT almost always consists of obtaining the history of a previously affected sibling or other positive family history. The risk to future pregnancies depends on the zygosity of the fetus's father. When the father is a heterozygote for the alloantigen, each fetus has a 50% chance of inheriting the antigen. In these cases, DNA-based testing of fetal amniocytes or chorionic villi has enabled typing of fetal platelet antigens (6,10). Those fetuses who are antigen negative can be managed routinely, while those possessing the antigen are managed aggressively.

The optimal antenatal management of AIT is still debated in the literature. One approach consists of serial fetal platelet transfusions with antigen-negative platelets (22,23). Evaluation of the fetal platelet counts before and after these procedures reveals that platelet transfusions are effective. However, the platelet count remains normal for less than 10 days, and hence platelet transfusions must be administered weekly. This approach subjects the pregnancy to the risk of fetal loss due to repetitive invasive procedures, which is especially significant since fetuses with AIT are at increased risk for fetal exsanguination associated with cordocentesis (24). The estimated risk for fetal loss from fetal transfusion of platelets through cordocentesis is about 6% and depends on the operator's experiences (25). A less invasive alternative involves the use of maternally administered intravenous gamma

globulin (IVIG). In the pilot study of 18 patients (26), IVIG with and without steroid therapy was found to significantly increase the fetal platelet count and decrease the risk of ICH. No cases of ICH occurred in that series, in contrast to 10/21 (48%) in untreated siblings.

A large randomized trial was designed to determine whether the addition of daily low-dose steroid therapy (1.5-mg dexamethasone) to IVIG (1gm/kg/week) was more efficacious than IVIG alone in preventing severe thrombocytopenia and ICH in the antenatal management of AIT (3). Of the 54 women treated, the majority responded to therapy with success rates between 62% and 85%, depending on the definition of response. The addition of low-dose dexamethasone was not found to increase the effectiveness of IVIG alone. In this series, there were no cases of ICH compared with 10 previously affected siblings, and there were no differences in response when steroids were used concomitantly. Ten initial non-responders were entered into the “salvage arm,” which consisted of 60mg of prednisone daily in addition to IVIG. Half of these patients responded with an increased platelet count.

The largest prospective study of antenatal treatment of 33 pregnant patients (37 fetuses) who had AIT and previous siblings with ICH demonstrated good neonatal outcomes with stratifying patients according to the timing of the previous child's ICH (4). The patients were classified as extremely high risk (EHR), very high risk (VHR), and high risk (HR) if ICH in the previous child occurred in the second trimester (less than 28 weeks of gestation), the third trimester (28–36 weeks of gestation), or during the perinatal period, respectively. The patients received primary treatment with intravenous immunoglobulin (IVIG) and salvage therapy with prednisone and/or more intravenous immunoglobulin. Only 5 of 37 fetuses developed ICH: (i) two had platelet counts greater than 100,000/ μ L, (ii) one had grade I ICH, and (iii) two had grade III–IV ICH and were demised. The investigators recommended the following guidelines in treating patients with AIT and ICH in a previous child. First, the treatment should be started no later than 12 weeks of gestation. Second, fetal blood sampling should be delayed until 32 weeks of gestation to minimize the complications from the procedure. Third, the fetal platelet counts obtained from the fetal blood sampling would dictate the need for additional salvage therapy (prednisone or additional IVIG) or delivery. The therapeutic regimen includes (i) intravenous IgG (IVIG) 2g/kg/wk for the EHR group or 1g/kg/wk for the VHR and HR groups starting at 12 weeks of gestation plus (ii) prednisone 1mg/kg/day starting at 24 to 26 weeks of gestation for the EHR group or prednisone 1mg/kg/day or additional IVIG 1g/kg/wk starting at 20 weeks of gestation for the VHR and HR groups (Table 2).

While these findings are quite promising, there have been reports of unsuccessful response (i.e., ICH in utero) to antenatal IVIG therapy (4,27,28). This could be due to an inconsistent increase in fetal platelets to IVIG infusion despite of a significant increase in maternal and fetal IgG levels (29). The knowledge that (i) not all fetuses will respond to IVIG therapy and (ii) salvage therapy (high-dose prednisone, additional IVIG, or platelet transfusions) is available underscores the need to assess response to therapy with fetal blood sampling to obtain fetal platelet counts. Since fetuses with AIT are at increased risk for

Table 2 Treatment Based on Risk Stratification

Variable	Extremely high risk	Very high risk	High risk
Definition	Previous sibling: in utero ICH in second trimester	Previous sibling: in utero ICH in third trimester	Previous sibling: perinatal ICH
Start	12 wk GA Initially IVIG 2g/kg/wk Add prednisone 1mg/kg/day at 24–26 wk GA First FBS at 32 wk GA Additional salvage treatment and/or early delivery based on FBS results	12 wk GA Initially IVIG 1g/kg/wk Add prednisone 1mg/kg/day or second IVIG 1g/kg/wk at 20 wk GA First FBS at 32 wk GA Additional salvage treatment and/or early delivery based on FBS results	
Regimen		If FBS unable to be performed or not performed for other reasons including patient and/or physician choice, salvage therapy could be empirically instituted	
Abbreviations: FBS, fetal blood sampling; GA, gestational age; ICH, intracranial hemorrhage; IVIG, intravenous immunoglobulin. Source: From Ref. 4.			

fatal bleeding at the time of cordocentesis (24,25), it seems prudent to have compatible platelets available and to transfuse if the platelet count is found to be $<50,000/\mu\text{L}$.

CONCLUSIONS

At present, the prospective diagnosis of a fetus affected with AIT relies almost exclusively on the history of a previously affected sibling or other positive family history. Since this disorder can potentially result in death or permanent neurologic sequelae as a result of ICH, the concept of routine screening of pregnant women to detect fetomaternal platelet incompatibility has been proposed. There are limitations to routine antenatal screening. All affected pregnancies may not demonstrate detectable anti-HPA-A1 antibodies and conversely the presence of anti-HPA-A1 antibodies does not invariably predict fetal thrombocytopenia. In addition, not all fetuses with AIT will develop ICH. Fetuses at greatest risk include those with severe thrombocytopenia and those with a previously affected sibling with ICH. Antenatal therapy needs to be directed at this small group of fetuses at risk for ICH in utero. A recent systemic review of the literature and analysis suggested that screening pregnant women for AIT would be cost-effective with a saving of at least 9 million euros per year in Netherlands (30). The analysis was based on i) the incidence of severe fetal AIT being about 40/100,000 pregnancies; ii) the incidence of severe ICH being about 4 children/100,000 pregnancies; and iii) including the costs of initial HPA typing, antibody detection, maternal treatment, and care for the neurologically impaired children.

The optimal antenatal management for AIT remains to be determined. Serial platelet transfusions are efficacious, but necessitate weekly invasive procedures with the additive risks of pregnancy loss. Medical management with maternally administered intravenous gamma globulin appears to be effective in the majority of, but not all, cases. The mechanism(s) of action of IVIG in preventing severe thrombocytopenia is unknown but may reflect i) decreased maternal antibody production, ii) antibody Fc receptor blockade in the placenta, and/or iii) antibody Fc receptor blockade in the fetal reticuloendothelial system. The mechanism by which IVIG prevents ICH in cases where it does not prevent severe thrombocytopenia has also not been elucidated. It is interesting to note that the HPA-A1 antigen is expressed on human endothelial cells as well

as platelets. Hence, it has been proposed that HPA-A1 antibodies may damage these cells resulting in vascular injury (24) and predisposition to intracranial bleeding. IVIG may interfere with this endothelial cell–antiplatelet antibody interaction. Lastly, therapy should be initiated as early as 12 weeks of gestation given the fact that severe thrombocytopenia has been documented as early as 13 weeks of gestation.

REFERENCES

- Shulman NR, Jordon VJ. Platelet immunology. In: Coleman RW, Hirsch J, Marder VJ, et al., eds. Hemo-stasis and Thrombosis. Philadelphia: JB Lippincott, 1982: 274–342.
- Kaplan C, Daffos F, Forestier F, et al. Management of alloimmune thrombocytopenia: antenatal diagnosis and in utero transfusion of maternal platelets. *Blood* 1988; 72: 340–3.
- Bussell JB, Berkowitz RL, Lynch L, et al. Antenatal management of alloimmune thrombocytopenia with intravenous gamma globulin: a randomized trial of the addition of low dose steroid to gamma globulin. *Am J Obstet Gynecol* 1996; 174: 1414–23.
- Bussell JB, Berkowitz RL, Hung C, et al. Intracranial hemorrhage in alloimmune thrombocytopenia: stratified management to prevent recurrence in the subsequent affected fetus. *Am J Obstet Gynecol* 2010; 203: 135.e1–14.
- Menell JS, Bussell JB. Antenatal management of the thrombocytopenias. *Clin Perinatol* 1994; 21: 591–614.
- Khouzami A, Kickler TS, Bray PF, et al. Molecular genotyping of fetal platelet antigens with uncultured amniocytes. *Am J Obstet Gynecol* 1995; 173: 1202–6.
- Mueller-Eckhardt C, Kiefel V, Grubert A, et al. 348 cases of suspected neonatal alloimmune thrombocytopenia. *Lancet* 1989; 1: 363–6.
- Blanchette V, Chen L, Defreidberg Z, et al. Alloimmunization to the PLA1 platelet antigen: results of a prospective study. *Br J Haematol* 1990; 74: 209–15.
- Williamson LM, Hackett G, Rennie J, et al. The natural history of fetomaternal alloimmunization to the platelet-specific antigen HPA-1a (PLA1, Zwa) as determined by antenatal screening. *Blood* 1998; 92: 2280–7.
- Valentin N, Vergracht A, Bignon J, et al. HLA-DRW52 is involved in alloimmunization against PL-A1 antigen. *Hum Immunol* 1990; 27: 73–9.
- Panzer S, Auerbach L, Cechova E, et al. Maternal alloimmunization against fetal platelet antigens: a prospective study. *Br J Haematol* 1995; 90: 655–60.
- Murphy MF, Metcalfe P, Waters AH. Antenatal management of severe fetomaternal alloimmune thrombocytopenia: HLA incompatibility may affect responses to fetal platelet transfusions. *Blood* 1993; 81: 2174–9.
- Mennuti M, Schwarz RH, Gill F. Obstetric management of isoimmune thrombocytopenia. *Am J Obstet Gynecol* 1974; 118: 565–6.
- Sitarz AL, Driscoll JM, Wolff JA. Management of isoimmune neonatal thrombocytopenia. *Am J Obstet Gynecol* 1976; 124: 39–42.

15. Adner MM, Fisch GR, Starobin SG, et al. Use of “compatible” platelet transfusions in treatment of congenital isoimmune thrombocytopenic purpura. *N Engl J Med* 1969; 280: 244–7.
16. Sidiropoulos D, Straume B. The treatment of neonatal isoimmune thrombocytopenia with intravenous immunoglobulin. *Blut* 1984; 48: 383–6.
17. Derycke M, Dreyfus M, Roper JC, et al. Intravenous immunoglobulin for neonatal isoimmune thrombocytopenia. *Arch Dis Child* 1985; 50: 667–9.
18. Zalneratis EL, Young RKS, Krishnamoorthy KS. Intracranial hemorrhage in utero as a complication of isoimmune thrombocytopenia. *J Pediatr* 1979; 95: 611–14.
19. Herman JH, Jumbelic MI, Ancona RJ, et al. In utero cerebral hemorrhage in alloimmune thrombocytopenia. *Am J Pediatr Hematol Oncol* 1986; 8: 312–17.
20. Spencer JA, Burrows RF. Feto-maternal alloimmune thrombocytopenia: a literature review and statistical analysis. *Aust NZ J Obstet Gynecol* 2001; 41: 45–55.
21. Johnson JM, MacFarland JG, Blanchette VS, et al. Prenatal diagnosis of neonatal alloimmune thrombocytopenia using an allele specific oligonucleotide probe. *Prenat Diagn* 1993; 13: 1037–42.
22. Nicolini U, Rodeck CH, Kichenour NK, et al. In utero platelet transfusions for alloimmune thrombocytopenia. *Lancet* 1988; 2: 506.
23. Murphy MF, Pullon HW, Metcalfe P, et al. Management of fetal alloimmune thrombocytopenia by weekly in utero platelet transfusions. *Vox Sang* 1990; 58: 45–9.
24. Paidas MJ, Berkowitz RL, Lynch L, et al. Alloimmune thrombocytopenia: fetal and neonatal losses related to cordocentesis. *Am J Obstet Gynecol* 1995; 172: 475–9.
25. Overton TG, Duncan KR, Jolly M, et al. Serial aggressive platelet transfusion for fetal alloimmune thrombocytopenia: platelet dynamics and perinatal outcome. *Am J Obstet Gynecol* 2002; 186: 826–31.
26. Lynch L, Bussel JB, McFarland JG, et al. Antenatal treatment of alloimmune thrombocytopenia. *Obstet Gynecol* 1992; 80: 67–71.
27. Kroll H, Kiefel V, Giers G, et al. Maternal intravenous immunoglobulin treatment does not prevent intracranial hemorrhage in fetal alloimmune thrombocytopenia. *Transfusion Med* 1994; 4: 293–6.
28. Bussel JB, Zabusky MR, Berkowitz RL, et al. Fetal alloimmune thrombocytopenia. *N Engl J Med* 1997; 337: 22–6.
29. Giers G, Wenzel F, Stockschröder M, et al. Fetal alloimmune thrombocytopenia and maternal intravenous immunoglobulin infusion. *Haematologica* 2010; 95: 1921–6.
30. Kamphuis M, Paridaans N, Porcelijn L, et al. Screening in pregnancy for fetal or neonatal alloimmune thrombocytopenia: systematic review. *BJOG* 2010; 117: 1335–43.