

# ARCHIVES OF DISEASE IN CHILDHOOD

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## Leading article

### Management of severe alloimmune thrombocytopenia in the newborn

Petechiae or echymoses and severe thrombocytopenia ( $< 20 \times 10^9$  platelets/litre) is a worrying and serious condition in newborn infants. Rapid correction of the platelet count is essential to prevent cerebral bleeding and associated life long disability, and this should be combined with laboratory investigations to confirm the clinical diagnosis. Prospective studies have revealed that the most likely cause of severe thrombocytopenia in a term and otherwise healthy neonate is immune mediated destruction of fetal/neonatal platelets by maternal alloantibodies.<sup>1,2</sup> Antibodies can be formed against human platelet alloantigens (HPAs)<sup>3,4</sup> present on fetal but not maternal platelets (table 1). Leakage of fetal platelets and possibly other HPA alloantigen expressing fetal cells<sup>5</sup> into the maternal circulation during pregnancy can stimulate the mother's immune system to produce IgG alloantibodies against "non-self" HPAs inherited from the father. Maternal HPA alloantibodies of the IgG class cross the placenta and bind to fetal platelets, shortening their survival.

#### Is neonatal alloimmune thrombocytopenia a rare disease?

Neonatal alloimmune thrombocytopenia is the platelet homologue of haemolytic disease of the newborn and was initially thought to be a rare disease. However, a recent prospective screening study in 25 000 non-selected pregnant women showed an incidence of severe thrombocytopenia ( $< 50 \times 10^9$  platelets/litre) caused by anti-HPA-1a

antibodies (see below) of one in 1100 (95% confidence interval, 684 to 2910).<sup>6</sup> No antenatal screening procedure is in place to identify women at risk of HPA alloimmunisation because it has not been proved that this would significantly reduce morbidity and mortality.<sup>6</sup> Thus, neonatal alloimmune thrombocytopenia is diagnosed in utero if a pregnancy is complicated (for example, cerebral bleeds, hydrocephalus,<sup>7,8</sup> or hydrops fetalis) or in the newborn by obvious signs of bleeding or abnormal neurological features pointing towards a possible cerebral bleed.<sup>3,4</sup> Infrequently, a diagnosis is made when a blood count is performed for an alternative reason.

#### Laboratory diagnosis

That maternal platelet alloantibodies could cause neonatal thrombocytopenia was first reported in 1959 by van Loghem *et al*,<sup>9</sup> but laboratory investigations to confirm a clinical diagnosis were cumbersome. Over the past two decades immunological, biochemical, and molecular biological studies have resulted in major improvements in anti-HPA antibody detection and in the determination of parental HPA genotypes.

The molecular basis has been determined for 14 of the 19 "platelet specific" alloantigen systems described so far, and in all but one, the difference between the two alleles is based on a single nucleotide difference that results in a single amino acid substitution.<sup>4</sup> Table 1 shows the clinically most relevant HPA systems. Some of the HPA alloantigens are relatively non-immunogenic and alloantibodies are only formed sporadically.<sup>3,4</sup> HPA-1a and HPA-5b are the most immunogenic alloantigens and are implicated in more than 85% and 10% of clinically diagnosed cases of neonatal alloimmune thrombocytopenia, respectively. Interestingly, the ability of an HPA-1a negative mother to form anti-HPA-1a is controlled by the HLA DRB3\*0101 allele.<sup>6</sup> The chance of antibody formation in an HLA DRB3\*0101 negative individual is small when compared with a DRB3\*0101 positive individual, with an odds ratio of 140.<sup>6</sup> The risk of having a severely affected child ( $< 50 \times 10^9$  platelets/litre) in the latter group is one in 15.

Neonatal alloimmune thrombocytopenia is, a priori, a clinical diagnosis, to be considered in an otherwise healthy term neonate with a low platelet count and a normal

Table 1 Clinically most relevant human platelet alloantigen (HPA) systems associated with neonatal alloimmune thrombocytopenia

System	Antigen	Alternative names	Glycoprotein	Nucleotide change	Amino acid change
HPA-1	HPA-1a	Zw <sup>a</sup> , p <sup>1A1</sup>	GPIIIa	T <sup>196</sup>	Leucine <sup>33</sup>
	HPA-1b	Zw <sup>b</sup> , p <sup>1A2</sup>		C <sup>196</sup>	Proline <sup>33</sup>
HPA-2	HPA-2a	Ko <sup>b</sup>	GPIIb	C <sup>524</sup>	Threonine <sup>145</sup>
	HPA-2b	Ko <sup>a</sup> , Sib <sup>a</sup>		T <sup>524</sup>	Methionine <sup>145</sup>
HPA-3	HPA-3a	Bak <sup>a</sup> , Lek <sup>a</sup>	GPIIb	T <sup>2622</sup>	Isoleucine <sup>843</sup>
	HPA-3b	Bak <sup>b</sup>		G <sup>2622</sup>	Serine <sup>843</sup>
HPA-4*	HPA-4a	Yuk <sup>a</sup> , Pen <sup>a</sup>	GPIIIa	G <sup>526</sup>	Arginine <sup>143</sup>
	HPA-4b	Yuk <sup>b</sup> , Pen <sup>b</sup>		A <sup>526</sup>	Glutamine <sup>143</sup>
HPA-5	HPA-5a	Br <sup>a</sup> , Zav <sup>a</sup>	GPIa	G <sup>1648</sup>	Glutamic acid <sup>505</sup>
	HPA-5b	Br <sup>b</sup> , Zav <sup>b</sup> , Hc <sup>a</sup>		A <sup>1648</sup>	Lysine <sup>505</sup>

\*Not important in the white population because the mutation has only been detected in populations from the Far East.

coagulation screen. The hospital laboratory diagnosis is made by an isolated thrombocytopenia on a whole blood count and is confirmed by investigations on parental blood samples in a platelet immunology reference laboratory experienced in the appropriate techniques. The mother's serum will be investigated for anti-HPA antibodies; in general, two techniques are used: an indirect immunofluorescence test<sup>3</sup> and a sandwich enzyme linked immunosorbent assay (monoclonal antibody specific immobilisation of platelet antigens, MAIPA).<sup>10</sup> The current second generation immunoassays are sensitive but "antibody negative" cases have been reported. An initial antibody negative case might become positive when a repeat sample taken 2–4 weeks after delivery is tested. In addition, a clinical case might remain serologically unconfirmed because of variations in the proficiency of diagnostic laboratories, although this unsatisfactory situation has improved more recently.<sup>11</sup> Genotyping of the parents for the HPA 1, 2, 3, and 5 systems by the polymerase chain reaction using sequence specific primers<sup>12</sup> aids the interpretation of the serological investigations, determines whether parental HPA incompatibility exists, and is crucial for counselling. In women with HPA alloantibodies and an already affected child, the knowledge of the zygosity of the father for the relevant HPA allele is important. In cases of paternal heterozygosity, antenatal HPA genotyping of the fetus from amniocytic DNA<sup>13</sup> can be planned for the first trimester, with the option of termination or the provision of well managed antenatal care (see below).

#### Treatment of the neonate with thrombocytopenia

The optimal treatment of a neonate with severe thrombocytopenia ( $< 20 \times 10^9$  platelets/litre), and a probable diagnosis of neonatal alloimmune thrombocytopenia, is an urgent correction of the platelet count. Transfusion of a neonatal dose of ABO, RhD compatible, and HPA-1a (and HPA-5b) negative platelets is treatment of choice, because these will be compatible in approximately 95% of cases with an alloimmune cause. In the UK, HPA-1a (and HPA-5b) negative platelets can be obtained from the National Blood Service and treatment should be started as soon as practically feasible. Many authorities now accept that the cytomegalovirus status of the donor is not important because, in the UK, leucocytes are now removed routinely from platelet concentrates. Irradiation is indicated if platelets are of maternal origin or where intrauterine transfusions preceded the transfusion.

The outcome of serological investigations should not be awaited because these are time consuming and the risk of a cerebral bleed is highest in the first days after delivery. HPA compatible donor platelets for neonatal use should be used where available, but when these are unavailable, maternal platelets (from which the plasma has been removed and replaced with donor plasma) can be considered. Correction of the platelet count might be lasting, although it can again dip below  $20 \times 10^9$ /litre, warranting close monitoring of the platelet count and possible subsequent platelet transfusions.<sup>6</sup> High dose intravenous IgG (1 g/kg body weight/day for two consecutive days) seems to be effective in approximately 65% of cases with severe thrombocytopenia, but the delay in achieving a "safe" platelet count is significantly longer when compared with platelet transfusion. Transfusion of HPA incompatible platelets should only be contemplated when compatible ones are not available, but their survival will be poor in most cases. Corticosteroids for the neonate are not advised. The platelet count should be monitored if it is between 20 and  $50 \times 10^9$ /litre, but platelet transfusion is recommended only if there is evidence of bleeding.

#### Counselling and treatment options in next pregnancies

Counselling of couples with an index case of neonatal alloimmune thrombocytopenia about the risks of severe fetal/neonatal thrombocytopenia in a next pregnancy needs to be based on the severity of disease in the index case and the outcome of immunological investigations. First, thrombocytopenia in subsequent infants is as severe or generally more severe. Second, if the father is heterozygous, there is a 50% chance that his offspring will not be affected. Third, the antibody specificity and titre have some correlation with severity.<sup>6</sup> For example anti-HPA-5b antibodies generally cause mild disease, which rarely results in cerebral bleeds, whereas most severe cases are associated with anti-HPA-1a antibodies.<sup>4</sup> A high titre HPA antibody is more likely to be associated with severe thrombocytopenia, but cerebral bleeds have also been seen with low titres. A decision needs to be taken on whether treatment of the fetus, the mother, or both is indicated, or whether conservative management is acceptable. In the latter case, the pregnancy should be closely monitored, and the mother advised to avoid any non-steroidal anti-inflammatory drugs, as well as aspirin. The delivery needs careful planning by obstetric and paediatric teams in close consultation with the haematologist to arrange availability of compatible platelets for the neonate and compatible blood for the mother. Treatment during pregnancy should be reserved for the cases in which the estimated risk of severe fetal/neonatal thrombocytopenia is considerable, and treatment should be carried out in collaboration with a fetal medicine unit with an interest in the disease. The available treatments during pregnancy are: (1) intrauterine intravascular transfusion of compatible platelets by peri-umbilical blood sampling at weekly intervals or just before delivery; and (2) the administration of intravenous IgG or corticosteroids, or a combination of both, to the mother.<sup>14–16</sup> Because no randomised trials have been performed using either treatment, firm evidence of efficacy is lacking. However, weekly platelet transfusions via peri-umbilical blood sampling, although invasive and technically demanding, has shown good outcome in families with previously severely affected children.<sup>16</sup> Repeated infusion of intravenous IgG to the mother remains highly controversial because the initial report of its possible effectiveness made use of a historical control group,<sup>17, 18</sup> the costs are high, and there is a small risk of transmission of infectious agents by a pooled plasma product. The precise mechanism of action, if any, of maternally administered corticosteroids on the severity of fetal disease is poorly understood. In one study, no significant benefit was seen from the addition of low dose steroids to high dose intravenous IgG treatment.<sup>19</sup>

#### Emerging novel treatments

Despite a thorough understanding of the molecular and immunological basis of neonatal alloimmune thrombocytopenia, its treatment relies on the use of donor derived platelets or immunoglobulins. However, it is expected that with the advent of molecular immunology it should be possible to develop a specific treatment for fetal and neonatal thrombocytopenia caused by anti-HPA-1a antibodies. A detailed understanding of the molecular interactions between maternal antibody and its antigen might lead to the design of competitor molecules that could out compete the binding of harmful maternal antibody.<sup>20</sup> More speculative is the possible use of peptides that would inactivate antigen specific T cells, or DNA based V gene vaccines, which might lead to an active immune response against B cells producing harmful antibodies.

In conclusion, maternal alloantibodies against the platelet specific alloantigen HPA-1a are the most frequent cause of severe thrombocytopenia in the neonate, with an estimated incidence of one in 1100 births. Over the past two decades, confirmation of a clinical diagnosis of neonatal alloimmune thrombocytopenia by serological and genetic tests has improved greatly, allowing optimal counselling of affected couples. Parents of an index case should receive advice on the risk of severe disease in subsequent pregnancies, and need to be given an explanation of the advantages and disadvantages of possible interventions. In serologically confirmed patients with a severe history, care should be provided by a team of fetal medicine experts, including neonatologists, haematologists, and immunohaematologists.

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*Arch Dis Child Fetal Neonatal Ed* 2000 82: F173-F175  
doi: 10.1136/fn.82.3.F173

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