ORIGINAL ARTICLE

Neonatal alloimmune thrombocytopenia in the Irish population: a discrepancy between observed and expected cases

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Aims: To estimate the rate of detection of neonatal alloimmune thrombocytopenia (NAITP) in the Irish population, to investigate clinical presentation and outcome in affected infants, and to determine the extent, if any, to which this condition is underdiagnosed at present.
Methods: Cases were collected in a retrospective fashion from a review of platelet serology laboratory records from January 1992 to December 2000. Clinical data were obtained from hospital records. Testing for maternal antiplatelet antibody was by one or more of the following: the platelet suspension immunofluorescence test, a commercial antigen capture enzyme linked immunosorbent assay (GTI-PakPlus®), and the monoclonal antibody immobilisation of platelet antigens assay. Platelet antigen typing was by the polymerase chain reaction technique with sequence specific primers.
Results: Twenty seven serologically verified cases of NAITP were identified in 18 families. Maternal antibody to human platelet antigen 1a accounted for 25 of the 27 confirmed cases. Twenty one of 26 infants were born with severe thrombocytopenia. Nineteen of 27 infants had bleeding manifestations at birth. Petechiae and bruising were most commonly observed (n = 17). There were no documented cases of intracranial haemorrhage in this group but systematic cranial ultrasound was not performed.
Conclusions: Screening studies in predominantly white populations have estimated the incidence of NAITP to be between 1 in 1000 and 1 in 2000 live births. With 50 000 births each year in Ireland, these results give a clinical detection rate for NAITP of just 1 case in 16 500 live births, strongly suggesting that NAITP is currently underdiagnosed. Antenatal screening to detect women at risk of having babies with NAITP is now scientifically feasible and should be considered.

Neonatal alloimmune thrombocytopenia (NAITP) is the platelet equivalent of haemolytic disease of the newborn (HDN), and is the most common cause of severe neonatal thrombocytopenia in otherwise well term infants.[1] NAITP is the result of maternal alloimmunisation to antigens on fetal platelets. The resultant transplacental passage of maternal IgG antibodies causes accelerated destruction of fetal/neonatal platelets, with resultant thrombocytopenia and bleeding manifestations. Maternal alloimmunisation to human platelet antigen 1a (HPA-1a) in a mother homozygous for the alternative allele, HLA-1b, accounts for most (85–90%) cases of NAITP in white individuals, followed at a much lower frequency by anti-HPA 5b.[2]

HPAs are polymorphic platelet surface glycoproteins. There are five well characterised biallelic platelet alloantigen systems, in addition to several low frequency or private antigens. HPA systems are named alphabetically, with the high incidence allele first (a) and the lower incidence allele second (b). The molecular basis of platelet glycoprotein polymorphisms is a single nucleotide substitution in the DNA coding for the relevant glycoprotein.[3]

> "Human platelet antigens are polymorphic platelet surface glycoproteins"

Platelet antigen typing or screening for platelet specific alloantibodies is not part of routine antenatal care. Therefore, NAITP is usually diagnosed only after the birth of a first clinically affected infant. Symptoms range from asymptomatic thrombocytopenia to intracranial haemorrhage (ICH). The latter can result in death of the fetus/neonate or residual brain damage.[2 4 5] Unlike HDN, NAITP affects first born and later born children equally.[2 6]

Screening studies in predominantly white populations estimate the overall incidence of NAITP to be between 1 in 1000 and 1 in 2000 live births.[1 2 7–9] The aim of our study was to estimate the current rate of clinical detection of NAITP in Ireland, to investigate clinical presentation and outcome in affected infants, and to determine the extent of possible underdiagnosis of the condition in routine clinical practice.

PATIENTS AND METHODS

Cases were collected in a retrospective fashion from a review of records of the Irish Blood Transfusion Service Platelet Serology Laboratory for the time period 1 January 1992 to 31 December 2000. This is the only facility that performs the investigations for a diagnosis of NAITP in the Republic of Ireland. The method used for maternal antiplatelet antibody investigation before 1995 was the platelet suspension immunofluorescence test (PSIFT).[10] In 1995, a commercially available solid phase enzyme linked immunosorbent assay kit (GTI-PakPlus® ELISA)[11] replaced PSIFT as a platelet antibody test. In cases where no antibody was detected by the GTI-PakPlus kit, maternal serum was further investigated by

Abbreviations: ELISA, enzyme linked immunosorbent assay; HDN, haemolytic disease of the newborn; ICH, intracranial haemorrhage; IVIG, intravenous immunoglobulin; MAIPA, monoclonal antibody specific immobilisation of platelet antigen assay; NAITP, neonatal alloimmune thrombocytopenia; PCR-SSP, polymerase chain reaction technique with sequence specific primers; PSIFT, platelet suspension immunofluorescence test;

Table 1 Characteristics of pregnancies associated with neonatal alloimmune thrombocytopenia

Case	Parity	Antigen involved	Sibling platelet count (×10⁹/l)	Platelet count at birth (×10⁹/l)	Mode of delivery
1	P3+0	HPA-1a	NK	13	SVD
2	P2+0	HPA-1a	NK	14	Vacuum assisted delivery
3a	P1+0	HPA-1a		NK	SVD
3b	P2+0		NK	45	SVD
3c	P3+0		45	14	Elective CS
4	P3+0	HPA-1a	NK	21	SVD
5a	P1+2	HPA-1a		10	SVD
5b	P2+2		16	27	SVD
5c	P3+2		27	3	Elective CS
6a	P6+2	HPA-1a	NK	9	Emergency CS
6b	P7+2		9	20	SVD
7	P2+0	HPA-1a	NK	70	SVD
8a	P2+0	HPA-1a	NK	<10	SVD
8b	P3+0		<10	9*	Elective CS
9	P2+1	HPA-1a	NK	23	SVD
10a	P1+0	HPA-1a		18	SVD
10b	P2+0		18	10	SVD
11	P1+0	HPA-1a		8	SVD
12a	P3+3	HPA-1a	NK	77	SVD
12b	P4+3		77	100	SVD
12c	P5+3		100	57	SVD
13	P2+0	HPA-1a	NK	10	SVD
14	P3+0	HPA-1a	NK	13	SVD
15	P3+0	HPA-1a		39	Elective CS
16	P1+0	HPA-1a		48	SVD
17	P4+0	HPA-3a	NK	29	Elective CS
18	P4+0	HPA-5b	NK	65	SVD

*Intravenous immunoglobulin (1g/kg/week) given from 30 weeks gestation.
CS, caesarean section; HPA, human platelet antigen; NK, not known; SVD, spontaneous vaginal delivery;

the more sensitive monoclonal antibody specific immobilisation of platelet antigens (MAIPA) assay (which was performed by the International Blood Group Reference Laboratory, Bristol).[12] HPA genotyping was performed using the polymerase chain reaction technique with sequence specific primers (PCR-SSP).[13] Clinical data were obtained from hospital records, as well as directly from the parents of affected infants or from their family doctors in some cases.

Eleven of the 18 women included in our study and six partners were fully genotyped for HPA-1–5. Three mothers and three partners were genotyped for HPA-1 only. In the four remaining cases, the diagnosis of NAITP was based on a positive test for anti-HPA-1a in the maternal serum and otherwise unexplained neonatal thrombocytopenia.

RESULTS

Between 1 January 1992 and 31 December 2000, 27 cases of NAITP were identified in 18 families.

Serological evaluation

Twenty five of 27 were caused by maternal anti-HPA-1a alloimmunisation. The remaining two cases were caused by anti-HPA-5b and anti-HPA-3a antibodies, respectively. Paternal genotyping for relevant HPAs was determined in only nine of 18 cases. Eight partners were found to be homozygous and one heterozygous for the relevant HPA antigen.

Clinical evaluation

Table 1 outlines the clinical characteristics of affected pregnancies. Five cases were diagnosed after first live births. Twenty one of 27 babies were delivered vaginally (20 spontaneous vaginal deliveries and one vacuum assisted). One baby was delivered by emergency caesarean section because of maternal pre-eclampsia. Three infants with a sibling history of NAITP were delivered by elective caesarean section and two caesarean sections were performed for obstetrical indications.

Table 2 shows the platelet count distribution at birth of the affected infants. Twenty one of 26 infants for whom information was available had platelet counts of $< 50 \times 10^9$/litre at birth, with five infants having platelet counts between 50 and 100×10^9/litre.

At birth, 19 of 27 infants had bleeding manifestations (table 3). Petechiae and bruising were most frequently observed (17

Table 2 Distribution of infants with platelet counts in categories <30, 30–50, >50 (×10⁹/litre) born to mothers with antiplatelet antibodies

Category	No. of infants
<30	18
30–50	3
>50	5

Table 3 Haemorrhagic symptoms in infants with neonatal alloimmune thrombocytopenia as a result of anti-HPA-1a antibodies

Haemorrhagic symptom	No. of infants*
None	10
Petechiae/purpura	17
Visceral bleed	2
Subaponeurotic haemorrhage	1
Palatal haematoma	1
Subconjunctival haemorrhage	1
Retinal haemorrhage	1

*Some infants had more than one bleeding manifestation.
HPA, human platelet antigen.

Table 4 Treatment

Treatment		No. of infants
None		12
Single	Platelet transfusion alone	
	Random donor platelets	3
	1a antigen negative platelets	5
Combined		
	Platelet transfusion/IVIG	3
	Platelet transfusion/IVIG/steroids	3
	IVIG/steroids	1

IVIG, intravenous immunoglobulins.

infants). One infant (case 2) was born with a massive subaponeurotic haemorrhage, associated with severe hypotensive shock, and required intensive resuscitation with platelets, red blood cells, and steroids. There were two cases of visceral haemorrhage and one infant with a large palatal haematoma.

A total of 14 infants received platelet transfusions at birth. Eight of these infants required no additional treatment (table 4). Six infants received intravenous immunoglobulin (IVIG) in addition to platelets, three of whom also received steroids. Five platelet transfusions were antigen matched (HPA-1a negative). Two of the latter were washed maternal platelets and three were from HPA-1b homozygous voluntary donors. A documented poor response to random donor platelets necessitating a second transfusion was recorded in the case of two infants. One infant was treated with IVIG and steroids only

DISCUSSION

Although NAITP is usually a transient passive disease of the newborn, serious bleeding problems do sometimes occur. ICH has been reported to occur in up to 10% of cases, and up to half of these incidents occur in utero.[2 14] The consequences of severe ICH are devastating and include death, seizures, learning disability, and cerebral palsy.[4 5]

Inadequacies in both the diagnosis and management of NAITP have been described previously,[15] and our data support this view.

It can be estimated that about 25–50 cases of NAITP occur in Ireland each year (based on 50 000 live births annually and a reported incidence of 1 in 1000 to 1 in 2000 live births).[1 7–9]

Twenty seven laboratory confirmed cases of NAITP in nine years (giving a detection rate of 1 in 16 500 live births) indicates that the condition is under-recognised in Ireland. Because of the fairly discrete health care system in Ireland, the lack of additional capabilities for diagnosing the condition in the country, and the lack of other references to known cases by Irish haematologists or obstetricians to the authors, we are confident that all diagnosed cases have been captured.

A difference in the epidemiology of NAITP is unlikely: in a recent HPA-1a prospective screening study of 4090 pregnant women, the prevalence of the HPA-1a negative platelet phenotype was 2%, comparable to the published prevalence of this phenotype in other predominantly white populations.[16]

It is noteworthy that although 21 of 26 infants in our study were severely thrombocytopenic at birth (platelet count $< 50 \times 10^9$/litre), in only 11 of the 21 infants can we confirm that a cranial ultrasound was performed. It has been recommended by some experts that all infants with NAITP and a platelet count of $< 50 \times 10^9$/litre should have a cranial ultrasound to rule out clinically silent ICH.[17]

Optimal postnatal management of NAITP requires the provision of antigen matched platelets because the response to random donor platelets is usually poor and the effect of IVIG is delayed for 24 to 48 hours.[15 18] Maternal platelets are suitable but take time to prepare and must be washed to remove the pathogenic antibody. Because platelet antigen typing of blood donors is not performed routinely, voluntary donor HPA-1b platelets are rarely available for immediate use. However, some blood centres, including our own, have now established panels of HPA-1b/1b donors, and this should lead to the improved postnatal management of infants with the most common form of NAITP.[18]

Take home messages

- Human platelet antigen 1a (HPA-1a) polymorphism mismatch between a mother and her fetus is the most common cause of neonatal alloimmune thrombocytopenia (NAITP)
- NAITP complicates 1 in 1000 to 1 in 2000 pregnancies
- The observed incidence of NAITP (approximately 1 in 16 500 live births) in Ireland strongly suggests that the condition is underdiagnosed in routine clinical practice
- Reliable HPA-1a phenotyping kits are now available commercially and routine antenatal screening to detect pregnancies at risk should be reconsidered in an attempt to avert some of the more devastating sequelae of this disorder
- Methods to predict the subset of pregnancies at greatest risk for severe NAITP and most likely to benefit from invasive procedures are required

> "The consequences of severe intracranial haemorrhage are devastating and include death, seizures, learning disability, and cerebral palsy"

Paternal HPA genotyping was only performed in 50% of cases in this series. Determination of paternal zygosity is important in counselling mothers regarding the risk of recurrence of NAITP in a future pregnancy with the same partner. If the father is heterozygous, HPA-1a/1b, the risk of recurrence will be 50%, and the fetal HPA genotype can be determined by PCR-SSP on DNA isolated from amniocytes early in the second trimester.[19] Sensitive, reliable, and relatively inexpensive HPA-1a (most clinically relevant fetomaternal incompatibility) phenotyping assays are now available.[20 21] However, antenatal screening to detect women at risk has not yet become a standard of care. Hindering the introduction of a routine antenatal screening programme have been the lack of a consistently effective antenatal treatment, continuing controversy among different groups regarding optimal management of alloimmunised pregnancies, particularly where there is no history of a previously affected infant, and inability to predict non-invasively which fetuses of alloimmunised mothers are most at risk of a severe outcome.[14 22 23] Fetal blood sampling to determine the initial fetal platelet count carries a procedure related mortality of 1–1.5%, even in experienced centres.[24 25] A recent study by one group of European experts urged a more restrictive approach in the selection of patients for invasive treatment, especially where there is no history of a previous child with ICH.[26] Our findings would tend to support the view that a conservative approach in such cases may be reasonable. In this small series of cases, no fetal blood sampling was performed and only one of five mothers (case 8b) with a history of a previously affected infant received antenatal treatment. Although about three quarters of the infants in this series had normal births no serious neurological sequelae appear to have occurred. Follow up information on 19 infants now ranging in age from 3 to 9 years, from contact with parents or through family doctors, confirm they are all now doing well, with no evidence of developmental problems. It can be estimated that there will be two to four severe cases of NAITP leading to death or disability in Ireland each year. The care needs of children with severe disabilities are much greater than those of non-disabled children and the emotional burden on families and the children themselves

immeasurable in monetary terms.[27 28] Routine antenatal screening for NAITP is now feasible and the cost to the Health Service may well be offset if even one severe case each year could be averted.

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