International Undergraduate Journal of Health Sciences

Volume 4 | Issue 1 Article 2

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Recommended Citation

Horan, Mairead () "An Investigation into Aetiology, Detection and Treatment of Neonatal Alloimmune Thrombocytopenia," *International Undergraduate Journal of Health Sciences*: Vol. 4: Iss. 1, Article 2.
DOI: https://doi.org/10.61862/2811-5937.1080
Available at: https://sword.cit.ie/iujhs/vol4/iss1/2

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Cover Page Footnote

The author would like to thank the members of the blood transfusion team in UHK as well as those within the IBTS Cork and Dublin for their assistance and guidance throughout the process, and Laura Clifford of the pathology team in Bons Secours Tralee for their feedback and advice.

An Investigation into Aetiology, Detection and Treatment of Neonatal Alloimmune Thrombocytopenia

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ABSTRACT

Neonatal Alloimmune ThrombocytopeniaHDN (NAIT) is a rare disorder with a similar aetiology to Haemolytic Disease of the Newborn (HDN), however unlike its erythrocyte counterpart, thrombocyte immunisation can occur within the first pregnancy (Giouleka et al., 2023). The most common antibodies implicated are Human Platelet Antigen (HPA)-1a (Winkelhorst et al., 2017). Of the Caucasian population 2.5 % are HPA-1a negative, of this population 33% are Human Leucocyte Antigen (HLA) -DR- B3*0101 positive increasing the risk of producing an alloantibody upon encountering the HPA-1a antigen. The maternal system becomes alloimmunised to the foreign paternal antigens of the foetus/neonate, which cross the placenta causing low platelets of the foetus (Giouleka et al., 2023). One third of antigen-positive neonates born to antigen-negative mothers develop thrombocytopenia with a platelet count lower than $50x10^9$/L, thus there is a significant risk of ICH due to thrombocytopenia (Giouleka et al., 2023). The disorder is identified through bruising or other symptoms of thrombocytopenia, the neonate may also be asymptomatic, and the low platelet implicated incidentally in routine testing (Constantinesu et al. 2012). Using the success of prenatal screening programmes for HDN, non-invasive prenatal testing for NAIT would allow for early intervention and prevent harm to the foetus.

Currently, there is no consensus on the management of NAIT. A prophylactic treatment has seen success in clinical trials, if brought to market this drug could become the gold standard in preventing maternal immunisation (Giouleka et al., 2023) (Winkelhorst etal., 2017) (Zhi et al2022).

INTRODUCTION

Neonatal alloimmune thrombocytopenia (NAIT) is a rare disorder with a similar aetiology to haemolytic disease of the foetus/newborn (HDN). In this review, the current testing and treatment methods for this disorder will be discussed as well as future screening and prophylactic strategies that could be implemented.

NAIT occurs when the maternal system becomes immunised to foreign antigens of the foetal platelets, which are IgG in nature and could cross the placenta and destroy the platelets of the foetus (Giouleka et al., 2023). The most common antibodies implicated are HLA-1a, only 2.5% of the Caucasian population are HPA-1a negative increasing the risk of producing this alloantibody upon encountering the HPA-1a antigen (Winkelhorst et al., 2017). In the Asian population, HPA-4a is the most implicated antibody (Tao et al., 2019). Unlike HDN, NAIT

can occur within the first pregnancy and can have devastating effects on this pregnancy with a recurrence in subsequent pregnancies (Tiller et al., 2013).

A recent case of NAIT in UHK brought to light the lack of awareness of the condition in this hospital setting. An investigation into the thrombocytopenia of a newborn led to the discovery of maternal antibodies and a mismatch in the platelet antigen type of the newborn and mother regarding to the HPA-1a antigen. The antigen type of the family was involved as follows: Mother: HPA- 1a1b; 2a2a; 3a3a; 4a4a; 5a5a; 15a15b, Father: HPA- 1a1a; 2a2b; 3a3b; 4a4a; 5a5a; 15a15a and Child: HPA- 1a1a; 2a2b; 3a3a; 4a4a; 5a5a; 15a15a.

In this case, the newborn had a 50% chance of being the same antigenic type as the mother. The father being HPA-1a1a could only pass on the HPA-1a gene while the mother could pass on the HPA-1a or HPA-1b gene. The maternal system became sensitised to the antigen type presented by the father and anti-HPA-1a developed in the maternal system. This antibody is IgG in nature and can cross the placenta and enter the foetal system, opsonising the foetal platelets and causing the foetal system to destroy the platelets, leading to thrombocytopenia. A third of antigen-positive neonates born to antigen-negative mothers develop thrombocytopenia with a platelet count lower than 50 x 10^9/L. There is also a significantrisk (10%) of NAIT neonates developing intracranial haemorrhage (ICH). (Giouleka et al., 2023). Most cases are diagnosed at birth because of purpura or bruising that leads to the discovery of thrombocytopenia (Tiller et al., 2013).

Current testing involves foetal sampling through amniocentesis and paternal antigen typing, there are some issues with this line of testing including the risk associated with this method of foetal sampling as well as ethical considerations in paternal testing. Using HDN screening programmes as a road map there is the potential for a screening programme that would allow for the detection and possible prevention of NAIT and maternal immunisation. There is no current nationwide consensus on treatment, some centres administer intravenous immunoglobulin (IVIG) or corticosteroids to prevent maternal immunisation. Current clinical trials show promise in the development of a prophylactic treatment that would function similar to the Routine Antenatal Anti-D Prophylaxis (RAAPD) system of HDN. There is a clear motive for understanding and development of testing and treatment options regarding this condition.

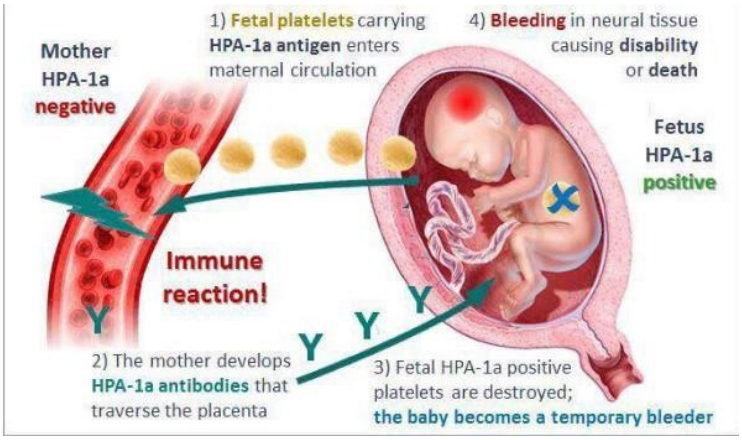

Figure 1: **Aetiology of NAIT HPA-1a positive foetal cells when entering the maternal system**
The mother's immune system recognises the cells as foreign and produces an antibody which pass into the foetal system and begins destruction of the foetal platelets leading to thrombocytopenia. (NAITbabies.org).

SIGNS AND SYMPTOMS

Intracranial Haemorrhage (ICH)

The more dangerous outcome of NAIT is ICH which occurs in approximately 1 in 10,000 live births, however, most cases occur antenatally and may not be discovered until later. According to one study which examined 592 cases of NAIT, 54% of ICH instances were documented before 28 weeks gestation (Tiller et al., 2013). The clinical outcome for neonates with ICH attributed to immune thrombocytopenia is poor, 35% of affected newborns pass away within four days of delivery, and the majority who survive face neurodevelopmental challenges which correlate with the neonate's maturity at the time of birth and the extent of the haemorrhage. Most cases of NAIT ICH are caused by bleeding diathesis, the low platelet count increases the chances of bleeding leading to intraventricular or intraparenchymal haemorrhage (Tan et al., 2018, Tiller et al., 2013). The preferred method of ICH detection is cranial ultrasound as it does notrequire transport, sedation, or exposure of the neonate to ionising radiation. (Tan et al., 2018).

Bruising

Other common symptoms include petechia, purpura and cephalohematoma (Kaplan, 2006). Petechiae is described as pinpoint non-blanching dots of discolouration that do not disappear when brief pressure is applied (McGrath and Barrett, 2020). Purpura is similar, an area of discolouration that occurs on the skin or mucous membranes. (Kaplan, 2006). Cephalohematoma is described as the accumulation of blood under the scalp which appears as a discoloured bruise (Raines and Sameer Jain, 2019). These types of bruising are caused by the thrombocytopenic bleeding diathesis which allows for bleeding in the microvasculature (Tan et al, 2018). These symptoms are all indications of thrombocytopenia and will resolve themselves over time. Some individuals may be asymptomatic, the danger in these cases is that the maternal system is now immunised to the paternal platelet antigens which could cause complications in subsequent pregnancies.

DETECTION

Based on clinical presentation and thrombocytopenia a NAIT investigation is carried out which identifies the platelet antigens of the individuals and the presence of an antibody in the maternal system. Blood samples can easily be taken from the mother for testing, but it is more difficult to obtain samples from a foetus *in utero* or a newborn.

Foetal Sample Retrieval: Amniocentesis

One method of obtaining foetal DNA for prenatal testing of NAIT is amniocentesis, the fluid containing foetal cells would be sent for testing to determine the HPA identity of the foetus. While the procedure is considered minimally invasive there are still associated risks. The earlier in pregnancy the procedure is carried out the higher the foetal risk. The procedure is not recommended for pregnancies under 16 weeks but the earlier certain disorders can be detected by the procedure the better the prognosis for foetal life (Cruz-Lemini et al., 2014: Jindal et al., 2023). In the case of NAIT, early determination of the condition could allow for early intervention such as intrauterine transfusion (IUT) to prevent severe thrombocytopenia

and hopefully prevent ICH. The procedure also carries a low risk of infection along with haemorrhage of the placenta or foetal lesions which can all be dangerous for both mother and foetus (Nizard, 2010; Cruz-Lemini et al., 2014)

The procedure is carried out under continuous ultrasound guidance, key locations are determined and used as reference points throughout the procedure, the pool of amniotic fluid, the placenta, the foetal position, and foetal movements are all closely observed (Figure 2). Transplacental needle insertion is avoided, if possible, to prevent damage to the placenta and risk of infection of the foetus if the maternal system carries viral infections like that of Human Immunodeficiency Virus (HIV) or hepatitis. A 20ml sample of the fluid is taken using a vacuum tube which avoids additional manipulation of the area, the needle is removed, and the procedure is complete, the fluid cannow be used to determine the foetal HPA type. (Cruz-Lemini et al., 2014; Jindal et al., 2023)

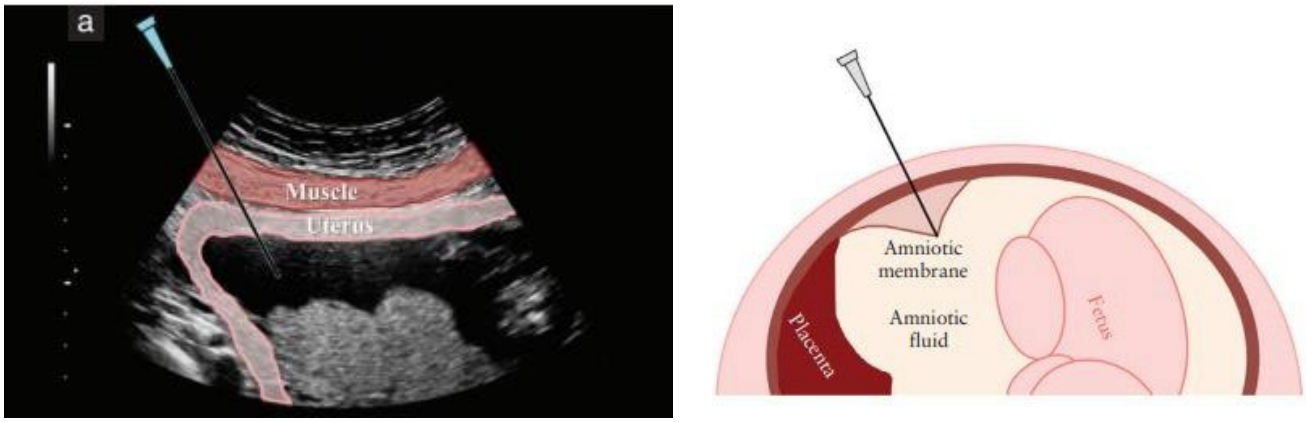

Figure 2: Amniocentesis procedure.
a) Shows correct needle entry guided by ultrasound as to prevent damage of surrounding maternal tissue.
b) The needle is inserted at a right angle to the ultrasound probe and proceeds through the four stages of entry: abdominal skin puncture, uterine puncture, amniotic cavity entry and advancement of the needle, avoiding both the placenta and foetus. Once within the amniotic pool the needle is advanced to the anterior wall to avoid interaction with the foetus, foetal movements are continuously monitoredto avoid harm. (Cruz-Lemini et al., 2014).

Antibody Identification: MAIPA

Monoclonal antibody-specific immobilization of platelet antigens (MAIPA) is the gold standard for detecting the NAIT causing HPA antigens in maternal circulation (Kaplan et al., 2007). Other techniques like western blotting were previously used in antibody identification but lack the specificity of MAIPA as the technique can destroy some of the epitopes of interest (Kiefel et al., 1987). MAIPA uses monoclonal HPA-specific antibodies in an Enzyme Linked Immunoassay (ELISA) like technique to allow for the optical density to be measured and therefore detection of the antibody of interest (Figure 3) (Kaplan et al., 2007; Kiefel et al., 1987). The indirect MAIPA method was used by the reference centre in the case observed in UHK.

The indirect method uses commercial typed platelets that are incubated with the test serum (Brighton et al., 1996). The commercial platelets have specific antigens which correlate to

different epitopes of interest in platelet immune disorders. In the case of NAIT, the epitope of interest in the GPIIb/IIIa which holds the HPA-1a antigen as well as other HPAantigens associated with NAIT (Tiller et al., 2013).

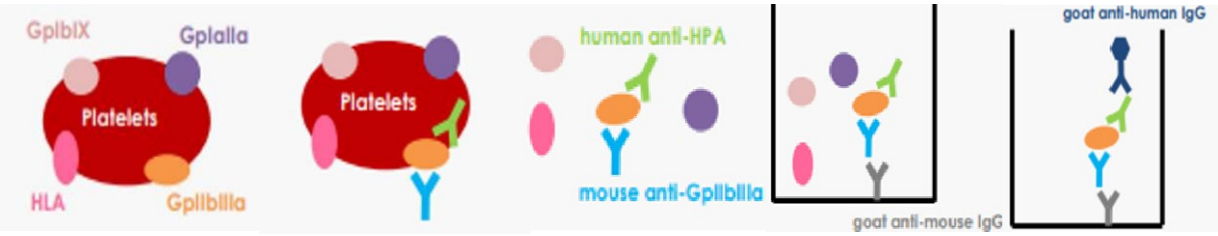

Figure 3: MAIPA Test Procedure.
The HPA antibody in the patient's serum (green) binds to the platelet, commercial mouse antibody (blue) binds to the GPIIb/IIIa antigen backbone. The platelet is lysed, cell debris removed, and goat anti-mouse antibody (grey) immobilises the complex before the addition of labelled goat anti-human antibody (navy) which allows for detection. Signal production can only occur in the presence of thepatient platelet antibody. (MAIPA Procedure, 2019).

While this method is considered the gold standard for detecting anti-platelet antibodies the sensitivity varies between test centres (Brighton et al., 1996) (Kaplan et al., 2007). There are few commercially available reagents for use in clinical diagnosis which leaves laboratories using their own in-house monoclonal antibodies whose effectiveness can vary (Kaplan et al., 2007). Cross contamination is also another potential issue (MAIPA Procedure, 2019). Other limitations of this testing method include its use of monoclonal IgG, the use of poly-specific antibodies as opposed to IgG alone could increase the specificity of the test. (Brighton et al., 1996). False-negative results could be because of the competition between the antibodies for similarly structured binding sites which could result in improper treatment (Kaplan et al., 2007). Despite the limitations, this method is highly specific and remains the gold standard in detecting and diagnosing NAIT.

Luminex has presented a more sensitive method of MAIPA using similar concepts but with bead technology as opposed to microtitre plates. The Luminex beads are coated with specific antibodies that bind the commercial platelets, human IgG of the patient sera binds theplatelet that is then lysed, and a labelled enzyme which binds the human IgG allows for detection by flow cytometry (Tao et al., 2019) While based on similar concepts the Luminex technology has a higher sensitivity, and the bead technology allows for the potential detectionof more HPA and HLA antigens using coupled beads. This method, however, is currently unsuccessful in detecting HPA-15 which has a low surface expression but does have the potential to cause NAIT (Masalanka et al., 2011). The commercially available beads could mitigate the variation in sensitivity seen between laboratories using the traditional MAIPA method.

Antibody Identification: PIFT

Platelet immunofluorescence test (PIFT) allows for the detection of platelet antibodies in serum. Indirect PIFT was used as a screening test to determine if antibodies were present in the maternal serum while MAIPA was used to identify further. PIFT has been described as

the thrombocyte equivalent of the Coombs test which allows for the determination of antibodieswhich pertain to erythrocyte immune conditions (Gabe et al., 2023; Nebe, 2015). This technique can also be used to crossmatch units of platelets prior to transfusion which in the case of alloimmune reactions such as NAIT would offer the best platelet increment (Gabe et al., 2023).

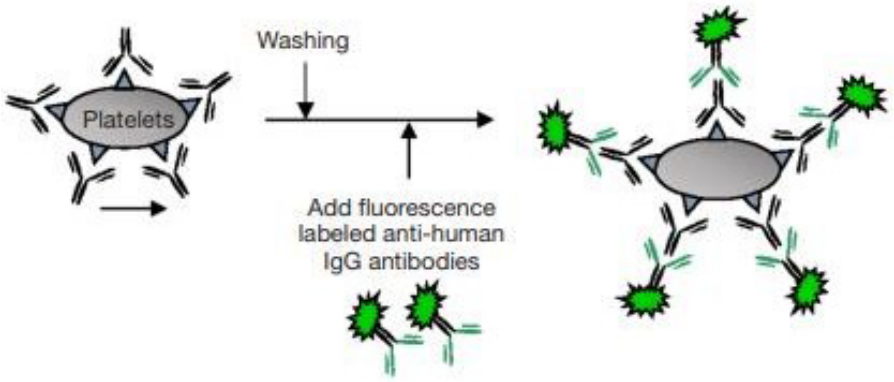

Figure 3: Platelet immunofluorescence test PIFT.
Antigen-specific platelets were added to the patient serum and incubated to allow for the antibody antigen reactions to occur. Antihuman immunoglobulin with a radiolabel was then added which binds to the human antibody present in the serum and the fluorescent signal is detected through flow cytometry. The fluorescence allows for detection of the antibody and the use of platelets determines if the antibody in question effects these blood cells. (Matsuhashi and Tsuno, 2018).

Antigen Identification: PCR-SBT

Polymerase chain reaction sequence-based typing (PCR-SBT) is the gold standard in platelet antigen identification. The technique utilises the specificity of PCR primers to amplify the sequence before NGS techniques are used to elucidate the DNA sequence of the sample. Locus-specific primers are used in the PCR processto amplify the sequence, through electrophoretic methods the size of the fragments is identified which indicates the position of the DNA base within the sequence. The larger the DNA fragment, the longer the sequence of the fragment and dyes are used to identify the different bases. When all fragments are lined up according to size the entire sequence can be identified (Jaramillo, Fernande and Marino, 2008). In this way, single base pair differences and subtle amino acid substitutions are accurately identified which allows for the full HPA type of the individual to be known, combined with antibody testing the cause of NAIT can be determined. This method while highly accurate in determining the DNA sequence is very labour-intensive (Gersuk and Nepom, 2006)

Future Testing: Cell Free Foetal DNA (cffDNA)

Currently NAIT is diagnosed at birth based on the newborns symptoms but there is potential for a prenatal screening programme similar to that seen in HDN to allow for the risk of NAIT to be determined early in pregnancy and possibly prevent maternal immunisation (Tiller et

al., 2013). Amniocentesis as mentioned is one method of retrieving foetal cells for testing, however, could less invasive testing similar to HDN based on cffDNA testing would allow for a lower risk approach to detection and the possibility of a screening programme? Testing is currently based on the mothers HPA type, if the mother is HPA-1a negative then the father is tested to determine the need for amniocentesis to take place (Kjeldsen-Kragh and Hellberg, 2022). There are ethical challenges associated with this line of testing, the presumed father could potentially not be the biological father of the foetus and so an incorrect genetic picture could leave the foetus still in danger and lead to maternal immunization. Additionally, the strides made in recent years in reproductive technologies means that the mother carrying the foetus or the father involved may have no biological relation to the foetus and so testing would yield inaccurate results (Kjeldsen-Kragh and Bengtsson, 2020; Kjeldsen-Kragh and Hellberg, 2022). If the foetal HPA type could be determined from cffDNA in a maternal blood sample, this would eliminate both the risks associated with amniocentesis and the ethical difficulties in paternal testing. Several methods have been proposed but the low concentration of foetal DNA in the maternal system in conjunction with the similarity in structure of the HPA-1a and HPA-1b antigens serves as complications in testing. PCR-SBT testing may not be able to overcome this barrier. (Ouzegdouh Mammasse et al., 2020; Kjeldsen-Kragh and Hellberg, 2022).

Droplet digital PCR (ddPCR) holds the most potential for non-invasive prenatal testing, the process involves dividing the sample into partitions containing varying concentrations of DNA. PCR coupled with fluorescence allows for quantification and software is used to determine the HPA type ofthe sample (Ouzegdouh Mammasse et al., 2020) RASSF1a, a marker of foetal DNA, along with single nucleotide polymorphisms act as internal controls lending credibility to the results obtained. Other proposed test methods like Cold PCR which takes advantage of the differing DNA sequences having different melting points lack internal control methods which make these methods less desirable for use in a screening programme (Kjeldsen-Kragh and Hellberg, 2022; Ouzegdouh Mammasse et al., 2020).

The risk of NAIT is also linked to the HLA type of the mother, HLA-DRB3*01:01 negative mothers have a significantly lower risk of birthing a thrombocytopenic baby even ifthe HPA type of the mother and child differ, HLA-DRB3*01:01 positive mothers have a 25% increased chance of NAIT (Kjeldsen-Kragh and Hellberg, 2022). This could also be considered in a screening programme to determine the risk of developing NAIT.

TREATMENT

Platelet Transfusion

Current treatment of NAIT often involves platelet transfusion upon discovery of thrombocytopenia, the symptoms are immediately addressed and confirmational diagnosis is performed later. Platelet transfusion of specific HPA-type platelets is preferred but any platelet unit can be used to increase drastically low counts, a higher increment is achieved from platelets that lack the antigen causing the response (Giouleka et al., 2023; Moise, 1993). Maternal platelets that are washed to remove the antibody will not be effected by the antibody in the babies system (JPAC, 2014a). The unit must be CMV (cytomegaly virus) negative and come from one single donor to prevent transfusion transmitted infection and irradiated to prevent a graft versus host effect. Additionally the concentration of white cells in the platelet unit should be low but irradiation destroys the lingering leucocytes removing this

risk (JPAC,2014a; JPAC, 2014b). The platelets are hyper concentrated to a concentration of $2000x10^9$/Land a small volume is given so as to not overwhelm the circulatory system of the baby (JPAC, 2014a; Moise, 1993). In the UHK case, antigen specific platelets were transfused postnatally, and the infant made a full recovery, genetic counselling will be offered to the parents in the case of future pregnancies.

Neonatal transfusion can correct the low platelet count at birth, but intrauterine transfusion is required in cases where birth would be detrimental to the baby's health. Intrauterine transfusion (IUT) involves a similar process to amniocentesis where a needle guided by ultrasound is inserted through the mother's abdomen, the needle is inserted into the vessels of the umbilical cord and the platelets passed into foetal circulation (Moise, 1993). As the maternal antibody leaves the newborn system after birth the platelet count rises and reaches normal levels within six weeks (JPAC, 2014a).

Prophylaxis

Intravenous immunoglobulins are administered in some treatment centres, but the amount and timing of administration vary widely (Kamphuis and Oepkes, 2011; Tiller et al., 2013). The concept behind administering immunoglobulin is like that of HDN prophylaxis, destroying the foetal platelets that cross the placenta before the maternal system can be immunised prevents the production of IgG. Corticosteroids like prednisone are also used by some centres as they dampen the immune response, if the maternal system is unable to produce the antibody or the concentration is lessened, less immunoglobulin crosses the placenta and reduces platelet destruction resultingin higher platelet counts at birth (Kamphuis and Oepkes, 2011). While some success is seen with these treatment methods there is variation between treatmentcentres and the course of drugs given. A universal clinically trailed course of treatment is needed to prevent maternal immunisation.

RLYB211

Currently, there is no approved prophylactic treatment for FNAIT (Semple and Kapur, 2022). Recently procured by the company RallyBio, a polyclonal human-derived HPA-1a antibody isin clinical trials and showing great promise in removing antigen-positive platelets from circulation in a timely manner which would prevent the recipient system from developing an alloimmune antibody, thus preventing NAIT (Geisen et al., 2023; NAIT Babies, 2023; Zhi et al., 2022). The antibody designated RLYB211 has recently completed phase 1/2 proof of concept clinical trials after success in murine models. This antibody is retrieved from the plasma of immunised women who were introduced to the antibody during pregnancy (Geisen et al., 2023)

Proof of concept studies in the murine model showed the potential for administration of HPA-1a antibodies for use in the prevention of NAIT (Zhi et al., 2022). Developments in recombinant DNA technologies allow the production of transgenic organisms with the desired antigen expression, CRISPR/CAS technology created mouse-to-human amino acid substitution in the GPIIIa genes produced by APLDQ GPIIIa mice. (T30→A, S32→P, Q33→L, N39→D, and M470→Q) (Zhi et al., 2021). The human HPa-1a antibody binds to this form of the antigen and does not bind to wild-type (WT) platelets. WT female mice were

exposed to APLDQ homozygous platelets and subsequently administered RLYB211 (Geisen et al., 2023). The administered antibody was effective in removing antigen-positive platelets within five hours. A second dose of RLYB211 was given 21 days after initial exposure to the antigen and the female WT mice in this cohort were subsequently bredwith APLDQ-positive male mice with offspring showed platelet counts that were elevated in proportion to the dose given. (Zhi et al., 2022). The developments in the field of recombinant DNA allowed for the APLDQ murine model to establish that prophylactic antibodies can be used in a comparable way to HDN to prevent FNAIT (Zhi et al., 2021; Zhi et al., 2022; NAIT Babies, 2023).

Following the success seen in the mouse model the antibody was entered into a randomised, single-blind, placebo-controlled, phase 1/2 proof of concept study. This phase marked the introduction of RLYB211 into human testing. Two groups of men meeting the pretesting requirements including antigen status, HLA status and Body mass index (BMI) requirements were randomly assigned either the placebo or the antibody. Cohort 1 consisted of eight men, six receiving the antibody and two placebo cases. This group were administered the drug or placebo following antigen-positive platelet transfusion to determine the effectiveness of the antibody in preventing immunisation following a sensitising event (Geisen et al., 2023).

Group 1B consisted of four men, three receiving the antibody and one placebo, these individuals received the drug and were subsequently transfused with platelets to determine the prophylactic potential of RLBY211 when administered before a sensitising event (Geisen et al., 2023). The HLA nature of the platelets transfused contrasted with the HLA status of the individuals which allowed for flow cytometry detection and the quantification. The HPA genotype of the platelets allowed for RLBY211 antibody-mediated destruction of the donor platelets. In cohort 1 all donor platelets were removed from circulation within two hours showing the potential of the drug to prevent alloimmunisation following a sensitising event. Group 1B saw total elimination of donor platelets within 3 hours showing the potential to prevent immunisation when administered before sensitising event (Geisen et al., 2023). While this study shows promise it is not without limitation, the small sample size limits the reliability of results which may be remedied as the clinical trials continue.

CONCLUSION

NAIT is a serious condition that can have harmful effects on individuals, unlike its erythrocyte equivalent NAIT can affect first pregnancies which furthers the need for prophylactic and screening programmes. There is an abundance of studies regarding the aetiology and dangers of NAIT, however, there is a distinct lack of study into treatment or preventative measures. While NAIT is a rare disorder occurring in 1/1000 live births there are other disorders screened for that have a lower occurrence. Every newborn is screened for cystic fibrosis, phenylketonuria, and a number of other disorders where early intervention reduces morbidity despite low incidence: cystic fibrosis and phenylketonuria affecting 1/2500 and 1/10.000 live births respectively, making the disorders less common than NAIT. With the success seen in the clinical trials, it seems a prophylactic treatment for this condition is on the horizon for NAIT caused by the HPA-1a antigen, but this is not the only antigen implicated simply the most common, more research is needed in testing and treatment for other HPA antigens that cause this condition before an all-inclusive screening program can be rolled out. Using the success of screening and prophylactic programs for HDN the stage is set to tackle NAIT.

REFERENCES

Antonov, N.K., Ruzal-Shapiro, C.B., Morel, K.D., Millar, W.S., Kashyap, S., Lauren, C.T. and Garzon, M.C. (2017). Feed and Wrap MRI Technique in Infants. *Clinical Pediatrics*, [online] 56(12), pp.1095–1103. doi:https://doi.org/10.1177/0009922816677806.

Brighton, T., Evans, S., Pa, C., Chesterman, C.N. and Chong, B. (1996). Prospective evaluation of the clinical usefulness of an antigen- specific assay (MAIPA) in idiopathic thrombocytopenic purpura and other immune thrombocytopenias. *Blood*, 88(1), pp.194–201. doi:https://doi.org/10.1182/blood.v88.1.194.194.

Campbell, S. (2013). A Short History of Sonography in Obstetrics and Gynaecology. *Facts, Views & Vision in ObGyn*, [online] 5(3), pp.213–29. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3987368/ [Accessed 17 Oct. 2023].

Constantinescu, S., Zamfirescu, V. and Vladareanu, P.R. (2012). Fetal and neonatal alloimmune thrombocytopenia. Maedica, [online] 7(4), pp.372–6. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3593293/.

Cruz-Lemini, M., Parra-Saavedra, M., Borobio, V., Bennasar, M., Goncé, A., Martínez, J.and Borrell, A. (2014). How to . . . Practical Advice on imaging-based Techniques and Investigations with Accompanying Slides Online How to Perform an Amniocentesis.

Ultrasound Obstetrics Gynecology, [online] 44. doi:https://doi.org/10.1002/uog.14680.

Gabe, C., Ziza, K.C., Durazzo, N., Pagani, F.M., Conrado, Marina-C.A.V., Dezan, M.R., Mendrone, A., Villaça, P.R., Dinardo, C.L. and Rocha, V. (2023). Detection of Alloimmunization in Glanzmann Thrombasthenia and Bernard-Soulier Syndrome: Data from a Brazilian Center. *Hematology, Transfusion and Cell Therapy*, 45(S2), pp.S101–S107. doi:https://doi.org/10.1016/j.htct.2022.06.005.

Geisen, C., Mette Kjær, Fleck, E., Skogen, B., Armstrong, R., Behrens, F., Zubin Bhagwagar, Braeuninger, S., Mörtberg, A., Olsen, K.J., Martin, S., Walter, C., Seifried, E., Wikman, A., Jens Kjeldsen-Kragh and Köhm, M. (2023). An HPA-1a–positive Platelet–depleting Agent for Prevention of Fetal and Neonatal Alloimmune thrombocytopenia: a randomized, single-blind, placebo–controlled, single-center, Phase 1/2 proof-of-concept Study. *Journal of Thrombosis and Haemostasis*, [online] 21(4), pp.838–849. doi:https://doi.org/10.1016/j.jtha.2022.11.041.

Gersuk, V.H. and Nepom, G.T. (2006). A Real-time PCR Approach for Rapid High Resolution Subtyping of HLA-DRB1*04. *Journal of Immunological Methods*, 317(1-2), pp.64–70. doi:https://doi.org/10.1016/j.jim.2006.09.003.

Giouleka, S., Ioannis Tsakiridis, Fotios Zachomitros, Apostolos Mamopoulos, Ioannis Kalogiannidis, Athanasiadis, A. and Themistoklis Dagklis (2023). Fetal and neonatal alloimmune thrombocytopenia: A rare case report of prenatal treatment. Clinical Case Reports, 11(8). doi:https://doi.org/10.1002/ccr3.7806.

Grover, V.P.B., Tognarelli, J.M., Crossey, M.M.E., Cox, I.J., Taylor-Robinson, S.D. and McPhail, M.J.W. (2015). Magnetic Resonance Imaging: Principles and Techniques: Lessons for Clinicians. *Journal of Clinical and Experimental Hepatology*, [online] 5(3), pp.246–255. doi:https://doi.org/10.1016/j.jceh.2015.08.001.

Jaramillo, A., Fernande, M. and Marino, S. (2008). *The Human Major Histocompatibility Complex and DNA-Based Typing of Human Leukocyte Antigens for Transplantation*.

Handbook of Human Immunology .

Jindal, A., Sharma, M., Karena, Z.V. and Chaudhary, C. (2023). *Amniocentesis*. [online] www.ncbi.nlm.nih.gov. Available at: https://www.ncbi.nlm.nih.gov/books/NBK559247/?report=reader#_NBK559247_pubdet_ [Accessed 19 Nov. 2023].

JPAC (2014a). *JPAC - Transfusion Guidelines Fetal Transfusion*. [online] transfusionguidelines.org.uk. Available at: http://www.transfusionguidelines.org/transfusion-handbook/10-effective-transfusion-in-paediatric-practice/10-1-fetal-transfusion [Accessed 20 Nov. 2023].

JPAC (2014b). *JPAC - Transfusion Guidelines Neonatal Transfusion*. [online] transfusionguidelines.org.uk. Available at: http://www.transfusionguidelines.org/transfusion-handbook/10-effective-transfusion-in-paediatric-practice/10-2-neonatal-transfusion [Accessed 20 Nov. 2023].

Kamphuis, M.M. and Oepkes, D. (2011). Fetal and Neonatal Alloimmune thrombocytopenia: Prenatal Interventions. *Prenatal Diagnosis*, 31(7), pp.712–719. doi:https://doi.org/10.1002/pd.2779.

Kaplan, C. (2006). Foetal and Neonatal Alloimmune Thrombocytopaenia. *Orphanet Journal of Rare Diseases*, 1(1). doi:https://doi.org/10.1186/1750-1172-1-39.

Kaplan, C., Freedman, J., Foxcroft, Z., Husebekk, A., Metcalfe, P., Muniz-Diaz, E., Ouwehand, W., Panzer, S., Rozman, P. and Skogen, B. (2007). Monoclonal platelet antigen capture assays (MAIPA) and reagents: a statement. *Vox Sanguinis*, 93(4), pp.298–299. doi:https://doi.org/10.1111/j.1423-0410.2007.00943.x.

Kiefel, V., Santoso, S., Weisheit, M. and Mueller-Eckhardt, C. (1987). Monoclonal antibody-specific Immobilization of Platelet Antigens (MAIPA): a New Tool for the Identification of platelet-reactive Antibodies. *Blood*, 70(6), pp.1722–1726. doi:https://doi.org/10.1182/blood.v70.6.1722.1722.

Kjeldsen-Kragh, J. and Bengtsson, J. (2020). Fetal and Neonatal Alloimmune Thrombocytopenia—New Prospects for Fetal Risk Assessment of HPA-1a–NegativePregnant Women. *Transfusion Medicine Reviews*, 34(4), pp.270–276. doi:https://doi.org/10.1016/j.tmrv.2020.09.004.

Kjeldsen-Kragh, J. and Hellberg, Å. (2022). Noninvasive Prenatal Testing in Immunohematology—Clinical, Technical and Ethical Considerations. *Journal of Clinical Medicine*, 11(10), p.2877. doi:https://doi.org/10.3390/jcm11102877.

MAIPA MAIPA Procedure. (2019). [online] *apDia*. Available at: https://apdiagroup.com/wp-content/uploads/2020/03/IFU-900006-MAIPA-procedure-V12-2019-RUO-1.pdf [Accessed 12 Nov. 2023].

MAŚLANKA, K., MICHUR, H., GUZ, K., WRÓBEL, A., UHRYNOWSKA, M., MISIAK, A., EJDUK, A., BROJER, E. and ŻUPAŃSKA, B. (2011). The relevance of HPA-15 antigen

expression for anti-HPA-15 antibody detection. International Journal of Laboratory Hematology, 34(1), pp.65–69. doi:https://doi.org/10.1111/j.1751-553x.2011.01358.x.

Matsuhashi, M. and Tsuno, N.H. (2018). Laboratory Testing for the Diagnosis of immune-mediated Thrombocytopenia. *Annals of Blood*, 3(41), pp.41–41. doi:https://doi.org/10.21037/aob.2018.09.02.

McGrath, A. and Barrett, M.J. (2020). *Petechiae*. [online] PubMed. Available at: https://www.ncbi.nlm.nih.gov/books/NBK482331/ [Accessed 20 Nov. 2023].

Moise, K.J. (1993). Intrauterine Transfusion with Red Cells and Platelets. *The Western Journal of Medicine*, [online] 159(3), pp.318–24. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1011345/ [Accessed 20 Nov. 2023].

NAIT Babies. (2023). *Rallybio | naitbabies.org*. [online] Available at: https://www.naitbabies.org/resources/rallybio/ [Accessed 11 Nov. 2023].

Nebe, C.T. (2015). Platelet Analysis in Laboratory Hematology. *LaboratoriumsMedizin*, 38(5). doi:https://doi.org/10.1515/labmed-2015-0044.

Nizard, J. (2010). Amniocentesis: Technique and Education. *Current Opinion in Obstetrics and Gynecology*, [online] 22(2), pp.152–154. doi:https://doi.org/10.1097/GCO.0b013e32833723a0.

Ouzegdouh Mammasse, Y., Chenet, C., Drubay, D., Martageix, C., Cartron, J.-P., Vainchenker, W. and Petermann, R. (2020). A new efficient tool for non-invasive diagnosisof fetomaternal platelet antigen incompatibility. *British Journal of Haematology*, [online] 190(5), pp.787–798. doi:https://doi.org/10.1111/bjh.16593.

Raines, D.A. and Sameer Jain (2019). *Cephalohematoma*. [online] Nih.gov. Available at: https://www.ncbi.nlm.nih.gov/books/NBK470192/ [Accessed 20 Nov. 2023].

Revive (n.d.). *Phase 1, 2, 3, 4 Clinical Trials*. [online] Revive GARDP. Available at: https://revive.gardp.org/resource/phase-1234-trials/?cf=encyclopaedia [Accessed 11 Nov. 2023].

Scotet, V., Duguépéroux, I., Saliou, P., Rault, G., Roussey, M., Audrézet, M.-P. and Férec, C. (2012). Evidence for Decline in the Incidence of Cystic fibrosis: a 35-year Observational Study in Brittany, France. *Orphanet Journal of Rare Diseases*, [online] 7(1), p.14. doi:https://doi.org/10.1186/1750-1172-7-14.

Semple, J.W. and Kapur, R. (2022). Protecting the Fetus from FNAIT. *Blood*, [online] 140(20), pp.2097–2099. doi:https://doi.org/10.1182/blood.2022017937.

Shao, Y.-H., Tsai, K., Kim, S., Wu, Y.-J. and Demissie, K. (2019). Exposure to Tomographic Scans and Cancer Risks. *JNCI Cancer Spectrum*, [online] 4(1). doi:https://doi.org/10.1093/jncics/pkz072.

Tan, A.P., Svrckova, P., Cowan, F., Chong, W.K. and Mankad, K. (2018). Intracranial Hemorrhage in neonates: a Review of etiologies, Patterns and Predicted Clinical Outcomes. *European Journal of Paediatric Neurology*, [online] 22(4), pp.690–717. doi:https://doi.org/10.1016/j.ejpn.2018.04.008.

Tao, S., Chen, S., Hong, X., He, J. and Zhu, F. (2019). Novel Method for Simultaneously Detecting HPA and HLA Antibodies Using Luminex Microbeads. *Journal of Translational Medicine*, 17(1). doi:https://doi.org/10.1186/s12967-019-2002-4.Tiller, H., Kamphuis, M.M., Flodmark, O., Papadogiannakis, N., David, A.L., Sainio, S., Koskinen, S., Javela, K., Wikman, A.T., Kekomaki, R., Kanhai, H.H.H., Oepkes, D., Husebekk, A. and Westgren, M. (2013a). Fetal Intracranial Haemorrhages Caused by Fetaland Neonatal Alloimmune thrombocytopenia: an Observational Cohort Study of 43 Cases from an International Multicentre Registry. *BMJ Open*, [online] 3(3). doi:https://doi.org/10.1136/bmjopen-2012-002490.

Tiller, H., Kamphuis, M.M., Flodmark, O., Papadogiannakis, N., David, A.L., Sainio, S., Koskinen, S., Javela, K., Wikman, A.T., Kekomaki, R., Kanhai, H.H.H., Oepkes, D., Husebekk, A. and Westgren, M. (2013b). Fetal Intracranial Haemorrhages Caused by Fetal and Neonatal Alloimmune thrombocytopenia: an Observational Cohort Study of 43 Cases from an International Multicentre Registry. *BMJ Open*, [online] 3(3). doi:https://doi.org/10.1136/bmjopen-2012-002490.

Winkelhorst, D., Murphy, M.F., Greinacher, A., Shehata, N., Bakchoul, T., Massey, E., Baker, J., Lieberman, L., Tanael, S., Hume, H., Arnold, D.M., Baidya, S., Bertrand, G., Bussel, J., Kjaer, M., Kaplan, C., Kjeldsen-Kragh, J., Oepkes, D. and Ryan, G. (2017). Antenatal management in fetal and neonatal alloimmune thrombocytopenia: a systematic review. Blood, 129(11), pp.1538–1547. doi:https://doi.org/10.1182/blood-2016-10-739656.

www.thermofisher.com. (n.d.). *Documents and Certificates - US*. [online] Available at: https://www.thermofisher.com/ie/en/home/support/documents-certificates.html [Accessed 16 Nov. 2023].

Zhi, H., Ahlen, M.T., Skogen, B., Newman, D.K. and Newman, P.J. (2021). Preclinical Evaluation of Immunotherapeutic Regimens for fetal/neonatal Alloimmune Thrombocytopenia. *Blood Advances*, [online] 5(18), pp.3552–3562. doi:https://doi.org/10.1182/bloodadvances.2021004371.

Zhi, H., Sheridan, D., Newman, D.K. and Newman, P.J. (2022). Prophylactic Administrationof HPA-1a-specific Antibodies Prevents fetal/neonatal Alloimmune Thrombocytopenia in Mice. *Blood*, [online] 140(20). doi:https://doi.org/10.1182/blood.202201