REVIEW ARTICLES

IMMUNE NEONATAL THROMBOCYTOPENIA – REVIEW
TROMBOCITOPENIA NEONATAL IMUNE – REVISÃO

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ABSTRACT

Introduction: Thrombocytopenia is the most frequent hematological change in the neonatal period, with immune thrombocytopenia as the main cause of moderate-to-severe thrombocytopenia in apparently healthy newborns. Immune thrombocytopenia in the fetus or newborn may result from platelet alloantibodies against paternal antigens inherited by the fetus (alloimmune thrombocytopenia) or platelet autoantibodies due to maternal autoimmune disease (autoimmune thrombocytopenia).

Objectives: To review published literature about immune thrombocytopenia in newborns, including the latest advances in pathogenesis, diagnosis, treatment, and prevention.

Results: Neonatal alloimmune thrombocytopenia is the most common cause of severe thrombocytopenia and intracranial hemorrhage in term neonates. Clinical presentation varies from mild thrombocytopenia to life-threatening bleeding and death. As maternal screening is not routinely performed, most cases are diagnosed in the first child. Despite intensive research, a consensus strategy for prevention and treatment of the condition is lacking. Diagnosis of neonatal autoimmune thrombocytopenia is usually apparent from maternal medical history and thrombocytopenia. Although maternal immune thrombocytopenic purpura does not carry a high risk of perinatal hemorrhage, it may lead to thrombocytopenia in the newborn, mostly mild-to-moderate. Clinical presentation varies from no symptoms to mucocutaneous signs of thrombocytopenia and may persist for weeks to months requiring long-term monitoring.

Conclusions: Fetal and Neonatal alloimmune thrombocytopenia can cause severe disease in the affected fetus or newborn. Facing the lack of routine antenatal screening, the strategies currently proposed for pregnancies at risk. We also discussed the latest research and therapies in development, aiming at potential improvements in diagnosis, treatment, and prevention of this disease. Neonatal autoimmune thrombocytopenia may cause long-lasting low platelet count, that need regular checking.

Keywords: autoimmune thrombocytopenia; alloimmune thrombocytopenia; newborn

RESUMO

Introdução: Trombocitopenia é a alteração hematológica mais frequente no período neonatal, sendo trombocitopenia imune a principal causa de trombocitopenia moderada a grave em recém-nascidos aparentemente saudáveis. O desenvolvimento de trombocitopenia imune no feto ou no recém-nascido pode dever-se à passagem transplacentária de anticorpos plaquetários maternos, nomeadamente aloanticorpos dirigidos a antígenos parentais herdados pelo feto (aloimune) ou autoanticorpos sintetizados por patologia autoimune materna (autoimune).

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Objetivos: Rever a literatura publicada até à data sobre o tema, incluindo os últimos avanços sobre patogénese, diagnóstico, tratamento e prevenção de trombocitopenia imune em recém-nascidos.

Resultados: Trombocitopenia neonatal aloimune é a causa mais comum de trombocitopenia grave e hemorragia intracraniana em recém-nascidos de termo. A apresentação clínica varia entre trombocitopenia ligeira isolada e hemorragia intracraniana letal. Dado que o rastreio pré-natal não é efetuado por rotina, a maioria dos casos são diagnosticados no primeiro filho. Apesar de intensa investigação, não existe atualmente uma estratégia consensual de prevenção e tratamento da condição.

Deve existir suspeita de trombocitopenia neonatal autoimune em presença de história materna sugestiva ou trombocitopenia materna. Púrpura trombocitopénica imune materna não comporta um risco elevado de hemorragia perinatal, embora possa originar trombocitopenia –na maioria dos casos, ligeira a moderada – no recém-nascido. A apresentação clínica varia entre ausência de sintomas a presença de sinais mucocutâneos de trombocitopenia e pode persistir durante semanas a meses, requerendo monitorização a longo prazo.

Conclusões: A trombocitopenia aloimune fetal e neonatal é causa de doença grave no feto ou no recém-nascido afetado. Dada a inexistência de rastreio pré-natal de rotina, é efectuada a revisão das estratégias propostas actualmente para as gestações de risco, assim como a discussão da investigação mais recente e das terapias em desenvolvimento, cujo objectivo é melhorar o diagnóstico, tratamento e prevenção dessa doença. A trombocitopenia neonatal autoimune pode levar a baixa contagem de plaquetas persistente, sendo necessário um controlo regular.

Palavras-chave: trombocitopenia aloimune; trombocitopenia autoimune; recém-nascido

INTRODUCTION

Neonatal thrombocytopenia is defined by platelet count below 150,000/µL and can be subdivided into mild (100,000 to 150,000/µL), moderate (50,000 to 99,000/µL), and severe (<50,000/µL), the latter being associated with an increased risk of bleeding resulting in morbidity or death.1 However, the largest study conducted using neonatal platelet count has recently shown that values between 100,000 and 150,000/µL are more common among newborns than children. Platelet count increases with advanced postnatal age, thus prematurity is an important risk factor for neonatal thrombocytopenia.2,3 Other reported predisposing factors for neonatal thrombocytopenia include preterm birth, sepsis, asphyxia, intrapartum growth restriction, and necrotising enterocolitis.2

Although thrombocytopenia is a rare event in the general newborn population, it occurs in 18–35% of neonates admitted to neonatal intensive care units.2 The reported incidence of neonatal thrombocytopenia is 1.8 per 1,000 live births.1

Prevalence of cutaneous bleeding in thrombocytopenic neonates has been reported to be as high as 81%.2 Mortality rate in these newborns is significantly related to the underlying pathological condition and thrombocytopenia severity.

The most common pathophysiological mechanism is increased platelet destruction, either by immunological cause or peripheral consumption.1

Immune neonatal thrombocytopenia is due to placental crossing of maternal antibodies, which destroy fetal and/or neonatal platelets. It is a common cause of early thrombocytopenia in healthy newborns (before 72 hours of life) and can be subdivided into alloimmune or autoimmune.1,3

OBJECTIVES

The aim of this study was to review recent evidence on neonatal immune thrombocytopenia, namely alloimmune and autoimmune thrombocytopenia. Pathogenesis, diagnosis, and treatment are discussed, as well as prevention and current medical research. Literature search included articles published over the last ten years in English and Portuguese retrieved from several databases, including PubMed.

DEVELOPMENT

Alloimmune thrombocytopenia

Neonatal alloimmune thrombocytopenia (NAIT), also referred to as fetal and neonatal alloimmune thrombocytopenia (FNAIT), is the result of maternal antibodies directed towards fetal and neonatal platelets. NAIT is the main cause of early and severe thrombocytopenia, as well as intracranial hemorrhage (ICH) in term neonates.2,6

Epidemiology

NAIT incidence is 0.5–1.5 per 1,000 births. However, as mild cases may go undetected and most severe cases can lead to intrauterine death, the real incidence may be higher.3 Recent studies have shown a severe NAIT incidence of 63 per 100,000, with six per 100,000 ICH cases, all reported in utero. A recent study by Tiller et al. indicated that most ICH cases occur before the 28th week of gestation.2 A 2014 systematic review of prospective studies reported a 0.15% incidence of severe thrombocytopenia in the general newborn population, with NAIT diagnosed in one-quarter of these patients.1

Pathophysiology
NAIT is triggered when fetal platelets express a paternally-inherited antigen that is lacking in the mother (human platelet antigen, HPA). As a result, the mother develops immunoglobulin G (IgG) antiplatelet antibodies against the “foreign” antigen, which cross the placenta and destroy fetal platelets containing the paternal antigen.1,3

Two mechanisms have been proposed to explain maternal alloimmunization: maternal exposure to HPA on fetal platelets due to fetomaternial bleeding (most frequent during delivery) and/or maternal exposure to β-3 integrin on placental syncytiotrophoblast cells during pregnancy.4,7

HPAs are the result of single nucleotide polymorphisms in the gene encoding any of the main glycoproteins located on platelet surface, particularly glycoprotein (GP) IIb/IIla. Antigen epidemiology varies according to ethnic group.5 In the Caucasian population, 85% of immunizations are caused by alloantibodies against HPA 1a.6 Only approximately 2% of Caucasians are HPA-1a-negative, with anti-HPA-1a antibodies detected in about 10% of HPA-1a-negative pregnant women. HPA-5b antigen is the second most common platelet antigen in the Caucasian population, while HPA-4 antigen system is most frequent in the Asian population.6

The mother of an affected newborn is asymptomatic and has normal platelet count, although personal or family history of previously affected pregnancies may be present.1

NAIT severity may be affected by several factors:

- **Pregnancy order** – Evidence from retrospective studies suggest that in most cases the second pregnancy is more severely affected than the first. Repeated immune stimulation results in strong maternal immunization against fetal HPA-1a platelet antigens.8

- **Maternal HPA-1a antibody values** – Low and stable maternal HPA-1a antibody levels appear to be associated with a low NAIT risk, while high and rising levels are predictive of neonatal thrombocytopenia.4

- **Intracranial hemorrhage in previous pregnancies** – Fetal ICH due to alloimmune thrombocytopenia increases the probability of fetal ICH in subsequent pregnancies. About half of cases occur before 28 weeks of gestation and the risk of recurrent hemorrhage in a subsequent pregnancy is as higher as earlier in index gestation ICH occurred.4

- **Type of platelet antigen** – HPA-1a alloimmunization causes severe disease, while HPA-5b alloimmunization is associated with less severe condition.4

- **Human leukocyte antigen (HLA) type** – HPA-1 antigenic system is defined as an amino acid change at residue 33 of the mature glycoprotein IIa (integrin β-3). HPA-1a peptide presentation and subsequent anti-HPA-1a alloantibody production are associated with a particular HLA: DRB3*01:01. In 90% of HPA-1a fetomaternal alloimmunization cases, the mother carries this HLA-class II molecule. Some authors suggest that HLA DRB4*01:01 also plays a role in HPA-1a peptide presentation and the combination of both DRB3*01:01 and DRB4*01:01 is associated with NAIT, affecting response to prenatal treatment with intravenous (IV) immunoglobulin.6,9

- **Presence of anti-HPA-1a antibodies of α-v β-3 subtype specificity in maternal serum** – α-v β-3-specific anti-HPA-1a antibodies induce endothelial cell apoptosis, which affects fetal vessel wall integrity, a critical factor in fetal ICH development, even in absence of thrombocytopenia. This occurs by inhibition of angiogenic signaling, inducing endothelial cell apoptosis and decreasing blood vessel density in the brain and retina, potentially explaining why ICH does not occur in all severe fetal thrombocytopenia cases. ICH can appear in occasional cases with normal fetal platelet count.6

**Clinical findings and management**

Clinical findings in affected newborns depend on thrombocytopenia severity. Findings may include petechiae, bruising, and bleeding in neonates with moderate-to-severe thrombocytopenia. Bleeding risk is highest within the first 96 hours of life.1,10

NAIT diagnosis should be considered in neonates presenting with severe thrombocytopenia at birth or in the first 24–48 hours of life, particularly in absence of other risk factors, clinical signs, or abnormalities in physical examination.1,3 Additionally, diagnosis should also be considered in ill-appearing infants, especially if severe thrombocytopenia seems out of proportion to the clinical illness or persists despite clinical improvement.1

ICH is the most severe complication of NAIT. It occurs in approximately 10–20% of affected newborns – 25–50% of these in utero (mostly before 28 weeks of gestation) – and is fatal in 59% of cases.1,3,4,6,8,11 Association of severe neonatal thrombocytopenia with parenchymal, rather than intraventricular, ICH is highly suggestive of NAIT.3 Extracranial fetal hemorrhage is extremely rare.4 Knight and colleagues estimated that one in every 25 infants with FNAIT die antenatally through miscarriage, stillbirth, or pregnancy interruption, and an estimated one in every 12 infants dies or becomes severely disabled before the age of one year. The authors also estimated that this occurs in approximately one in every 100,000 total births.12

Neonatal platelet count is often less than 100,000/µL and typically decreases within the first few days after birth then increasing until four weeks, as antibody levels decline.1

Current recommendations state that all neonates with platelet count bellow 50,000/µL in the first days of life should be investigated for NAIT.3

When NAIT is suspected, blood should be collected from the mother and father and submitted to antigen screening (HPA). Positive cases show maternal plasma antibodies directed against specific paternal platelet antigens. If blood cannot be collected from parents, neonatal serum may be screened for presence of antiplatelet antibodies. However, if antibody concentration in the newborn is low, it may lead to false negative results. It remains unclear whether a correlation exists between antibody affinity and severity of disease. These evaluations should be performed in an experienced reference.
laboratory, with required technology and resources. As antibodies can be difficult to detect in samples collected during delivery, follow-up serology tests should be performed when clinical diagnosis of NAIT is suspected. NASCER E CRESCER

— Alternatively, donor platelets typed and matched to maternal antiplatelet antibodies.

— If transfusion of ABO-incompatible platelets is required, plasma component should be reduced to avoid potential hemolysis.

— Administration of HPA-1a-negative/HPA-5b-negative platelet concentrates is an option, although costly and not generally available.

When a neonate is born to a mother with a previously NAIT-affected pregnancy, genotypically matched platelets should be available in the blood bank at time of delivery and should represent the first-line treatment if infant is thrombocytopenic.

However, it is important to remember that some NAIT infants fail to respond to random donor platelets and other therapies. For this reason, the blood bank should be immediately warned about a suspected NAIT infant, and measures should be taken to secure an antigen-negative platelet source as soon as possible. Platelets from the mother and HPA-1b1b or HPA-5a5a donors are compatible in more than 90% of cases.

If NAIT is confirmed or strongly suspected, intravenous immunoglobulin (IVIg) (1 g/kg per day for two consecutive days or 400 mg/kg per day for three to four days) may be administered immediately following platelet transfusion to increase patient’s own platelets and potentially protect transfused platelets. Because in NAIT platelet counts usually fall after birth, IVIg may be administered when platelet count is between 30−50,000/µL in a stable neonate. Although intravenous methylprednisolone (1 mg every eight hours for one to three days) has been used as additional therapy, its effectiveness has not been proven, with some authors considering it in cases of persisting refractory and life-threatening thrombocytopenia.

Fetal and neonatal alloimmune thrombocytopenia is analogous to fetal and neonatal anaemia caused by anti-red cell antibodies in hemolytic disease of the fetus and newborn. In contrast, in FNAIT first pregnancies are often severely affected and diagnosis is usually made at birth of the first affected infant. Therefore, the debate about the usefulness of antenatal screening for the condition persists. Tiller et al. estimated that testing for FNAIT only in presence of clinical symptoms misses 86% of cases. Another study showed that timely FNAIT diagnosis was not performed in 15% of neonates born with severe thrombocytopenia, with severe consequences for subsequent pregnancies, including ICH. Women giving birth to NAIT infants should be followed for all future pregnancies in a perinatal referral center, as recurrence rate is high and they can benefit from prenatal treatment at those centers. Maternal treatment with weekly IVIg infusions, with or without steroids, may be indicated, as well as fetal therapy with platelet transfusion in utero (weekly or immediately before delivery); timed nearterm delivery (by labor induction or cesarean delivery) and birth in a perinatal center with immediate availability of matched platelets are other measures that should be taken. In families with an affected fetus or infant, recurrence rate is as high as 75−90%. Thrombocytopenia in the second affected
newborn is equally or more severe than in the first.1

Other therapeutic alternatives are being studied to reduce newborn exposure to blood products, including hematopoietic growth factors and thrombopoietin.26 Current medical research is focused on FNAIT prevention, namely in development of biological drugs.

One of investigated drugs is purified hyperimmune anti-HPA-1a Ig, from plasma collected from HPA-1a-immunized women with a previous FNAIT-complicated pregnancy. The hyperimmune anti-HPA-1a IgG is used for disease prevention through antibody-mediated immune suppression. The underlying concept was to apply the same prophylactic principle by which hyperimmune anti-D IgG has been used for hemolytic disease prevention. The drug is administered within the first six hours after delivery to negative HPA-1a women with antigen-positive newborns. This resulted from a European project started in 2011 (www.profnait.eu) and approved by the Food and Drug Administration (FDA) in December 2017. Drug’s licensing for clinical use is anticipated for 2020.19-22

It has been shown that neonatal Fc receptor (FcRn) regulates immunoglobulin G homeostasis and plays an important role in transplacental IgG transport. This receptor is expressed in placental syncytiotrophoblast. Recombinant monoclonal antibodies directed at FcRn are currently being investigated in animal models. They act by blocking receptor and preventing transplacental transport of alloantigens and represent a potential prenatal drug. However, further studies are required to confirm this hypothesis.21-25

Another potential prenatal therapy under development is human recombinant high-affinity HPA-1a antibody, which competes with maternal alloantibodies for fetal platelet binding. This therapy has already been tested in volunteers, showing a platelet destruction reduction and survival increase. However, further studies are warranted regarding its benefits for the fetus.21,25

**Autoimmune thrombocytopenia**

Autoimmune thrombocytopenia is due to maternal autoantibodies reacting with antigens expressed on both maternal and neonatal platelets. This occurs in maternal autoimmune disorders, including immune thrombocytopenia purpura (ITP) and systemic lupus erythematosus.1,3,26

Autoimmune thrombocytopenia is estimated to occur in one to two of every 1,000 pregnancies.26

Diagnosis is usually apparent from maternal medical history and thrombocytopenia. However, platelet count of affected mothers may be normal after a splenectomy or in cases of sufficient compensatory thrombopoiesis.1

ITP mothers have an only 10% risk of having thrombocytopenic infants.13 Mothers of infants with unexplained neonatal thrombocytopenia should be investigated for presence of an autoimmune disorder, since neonatal thrombocytopenia can be the presenting sign. However, healthy women without history of autoimmune disorders may also develop gestational thrombocytopenia. This is considered a benign mild and transient form of ITP, with neonatal thrombocytopenia being a rare event.1

Maternal splenectomy, maternal platelet count below 50,000/µL at some time during pregnancy, and history of an older sibling with neonatal thrombocytopenia are risk factors associated with autoimmune neonatal thrombocytopenia.1,26,27

Most affected infants seem healthy, as more than half of infants born to ITP mothers have either mild thrombocytopenia or normal platelet counts. However, a relevant minority of infants develops severe thrombocytopenia.27 Clinical signs are consistent with moderate-to-severe thrombocytopenia and include petechiae, bruising, and bleeding. Clinical manifestations are less severe than in alloimmune thrombocytopenia and ICH risk is lower than 1% and decreases between the third and fourth days of life.1,28

Platelet counts of infants born to ITP mothers often decrease sharply during the first days after birth, with nadir typically occurring between the age of two and five days. All infants with maternal history of autoimmune disorders should perform early postnatal platelet count, which should be monitored if below the normal range.1,3,13 If the neonate has mild thrombocytopenia, platelet count should be repeated within two to three days; if it is lower than 30,000/µL, first-line treatment with IVIg should be provided.3 Asymptomatic infants with stable platelet counts above 30,000/µL may be discharged, with appropriate outpatient follow-up.1

Cranial ultrasound should be performed to all infants with platelet count below 30,000/µL for ICH assessment.3 Importantly, neonatal thrombocytopenia secondary to maternal ITP may last for weeks to months, requiring long-term monitoring.2 It has recently been reported that antiplatelet antibody transfer from ITP mothers through breastfeeding (immunoglobulin-A type) can be associated with persistent neonatal thrombocytopenia (beyond four months) and resolve when breastfeeding is discontinued.1,2,9

Platelet transfusion is provided to infants with severe thrombocytopenia or clinical bleeding. However, transfusions may be ineffective since autoantibodies usually react with donor platelets, including from the mother. For this reason, some authors recommend administering IVIg after transfusion.1,2,7

IVIg should be offered to infants with severe thrombocytopenia at a 1 g/kg dose, which may be repeated. Since it may last weeks to months, a second IVIg dose is sometimes required at four to six weeks of life.1,3 This treatment typically produces a rapid response. If thrombocytopenia is severe and persists after IVIg therapy, some clinicians consider administering a short course of prednisone (2 mg/kg per day) or methylprednisolone (1 mg/kg twice a day for five days), although the efficacy of this approach has not been proven.1

History of neonatal thrombocytopenia in the previous infant represents the only reliable predictor of neonatal thrombocytopenia in ITP mothers. Type of delivery in ITP patients should be selected according to obstetric indication, avoiding interventions that may increase fetal bleeding risk.3
CONCLUSIONS

FNAIT is the most frequent cause of severe isolated thrombocytopenia in neonates, and ICH its most devastating outcome. In absence of routine antenatal screening, most diagnoses are performed when the fetus or newborn are already affected. For this reason, implementation of a routine antenatal screening program has been widely discussed over the last decade, aiming at HPA typing and detection of pregnant women at risk, similarly to Rh immunization screening instituted in the 1960s. However, despite several published studies supporting screening cost-effectiveness, no European or American country has adopted this strategy to date, one of the main arguments being the lack of consensus regarding the optimal treatment for pregnant women at risk. Proposed intervention strategies for screening-positive pregnancies include maternal IVIg infusions (with or without steroids), timed nearterm delivery, and birth in a perinatal center with immediate availability of matched platelets.

Among other aspects, current research aims to better understand the mechanisms involved in ICH development in the context of alloimmune thrombocytopenia, such as angiogenesis interference via cross-reaction of antiplatelet antibodies with α-V β-3 integrin present in endothelial cells. Detection of maternal anti-α-V β-3 antibodies may be crucial for ICH prevention and diagnosis. New therapies are also under investigation, namely human Ig-specific anti-HPA-1a immunoglobulins, recombinant monoclonal antibodies directed at FcRn, and recombinant anti-HPA-1a monoclonal antibodies. Neonatal autoimmune thrombocytopenia does not carry a serious risk of perinatal bleeding, but may cause moderate neonate thrombocytopenia persisting for weeks to months and requiring long-term monitoring.

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ABBREVIATION | DEFINITION
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FcRn | Fc receptor
FNAIT | Fetal and neonatal alloimmune thrombocytopenia
HPA | Human platelet antigen
ICH | Intracranial hemorrhage
IgG | Immunoglobulin G
IVIg | Intravenous immunoglobulin
ITP | Immune thrombocytopenia purpura
NAIT | Neonatal alloimmune thrombocytopenia

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Trombocitopenia.


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