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CONSISE REVIEW

Congenital Amegakaryocytic Thrombocytopenia: A Brief Review of the Literature

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Abstract: Congenital amegakaryocytic thrombocytopenia (CAMT) is a rare inherited autosomal recessive disorder that presents with thrombocytopenia and absence of megakaryocytes. It presents with bleeding recognized on day 1 of life or at least within the first month. The cause for this disorder appears to be a mutation in the gene for the thrombopoeitin (TPO) receptor, c-Mpl, despite high levels of serum TPO. Patients with severe Type I-CAMT carry nonsense Mpl mutations which causes a complete loss of the TPO receptor whereas those with Type II CAMT carry missense mutations in the Mpl gene affecting the extracellular domain of the TPO receptor. Differential diagnosis for severe CAMT includes thrombocytopenia with absent radii (TAR) and Wiskott-Aldrich syndrome (WAS). The primary treatment for CAMT is bone marrow transplantation. Bone Marrow/Stem Cell Transplant (HSCT) is the only thing that ultimately cures this genetic disease. Newer modalities are on the way, such as TPO-mimetics for binding towards partially functioning c-Mpl receptors and gene therapy. Prognosis of CAMT patients is poor, because all develop in childhood a tri-linear marrow aplasia that is always fatal when untreated. Thirty percent of patients with CAMT die due to bleeding complications and 20% -due to HSCT if it has been done.

Keywords: inherited, congenital, thrombocytopenia, amegakaryocytic

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Background

Congenital amegakaryocytic thrombocytopenia (CAMT) is a rare autosomal recessive bone marrow failure syndrome that presents with severe thrombocytopenia which can evolve into aplastic anemia and leukemia.[1–3] The disorder is expressed in infancy with or without physical anomalies.[4,5] It is often recognized on day 1 of life or at least within the first month. It is often initially confused with fetal and neonatal alloimmune thrombocytopenia, but the neonate fails to improve and responds only to platelet transfusion. Eventually, a diagnostic bone marrow is performed which can be technically difficult in a neonate.

A recent classification was proposed in 2005 supported by several other reports based on the course on outcome of the disease as follows;[1,5]

a. Type I—early onset of severe pancytopenia, decreased bone marrow activity and very low platelet counts. In this group, there is complete loss of functional c-Mpl. Median platelet count is usually 21 × 10^9/L or below.[5]
b. Type II—milder form with transient increases of platelet counts up to nearly normal values during the first year of life and an onset of bone marrow failure at age 3 to 6 years or later. In this group, there are partially functional receptors for the c-Mpl gene. Median platelet count is usually 35 × 10^9/L to 132 × 10^9/L.
c. Type III—there is ineffective megakaryopoeisis with no defects in the c-Mpl gene.

Presentation

The primary manifestations are thrombocytopenia and megakaryocytopenia or low numbers of platelets and megakaryocytes. There is an absence of megakaryocytes in the bone marrow with no associated physical abnormalities.[6] Platelet size is usually normal.[7] Bleeding is of primary concern: cutaneous, gastrointestinal, pulmonary, and intracranial hemorrhage.[5,7] However, the presence of a normal number of megakaryocytes on an initial bone marrow aspirate should not exclude CAMT from the differential diagnosis of thrombocytopenia within the first year of life.[3]

Platelet counts among neonates are usually in the level of 150 × 10^9/L. In patients with neonatal thrombocytopenia, infants may show a pattern of low/low-normal platelet counts (100–200 × 10^9/L), with levels falling to as much as 50–100 × 10^9/L at 4–5 days and returns to normal at 7–10 days. Late-onset neonatal thrombocytopenia presents usually after the first 3 days of life wherein platelet counts often drops rapidly to levels of 50 × 10^9/L or even more.[8]

The reasons why we have to suspect hereditary thrombocytopenia which includes;[7,9]

1. Familial history of thrombocytopenia, especially parent-child or maternal uncle-nephew.
2. Lack of platelet response to autoimmune thrombocytopenia therapies.
3. Diagnostic features on smear such as abnormal size platelets, absence of platelet alpha granules, Dohle-like bodies or microcytosis.
4. Bleeding out of proportion to the platelet count.
5. Onset at birth.
6. Associated features such as absent radii, mental retardation, renal failure, high tone hearing loss, cataracts or the development of leukemia.
7. Persistence of a stable level of thrombocytopenia for years. Some patients may present with petechial purpura, cranial hematoma or recurrent rectorrhagia.

Pathophysiology

The majority of patients have a mutation in the gene for the thrombopoeitin (TPO) receptor, c-Mpl, resulting in high levels of serum TPO.[1,10] TPO binding stimulates both early and late phases of megakaryocytopoiesis, increasing the number, size, and ploidy of megakaryocytes, and promoting the expression of platelet-specific markers. TPO also maintains the numbers of hematopoietic stem cells thus, any abnormalities contribute to the occurrence of both thrombocytopenia and pancytopenia in CAMT patients. In addition, there may be abnormalities with the central nervous system including the cerebrum and cerebellum which could cause symptoms.[10] Animals deficient for TPO or c-Mpl have a 90% reduction of megakaryocytic precursor cells and a 60%–80% decrease of both erythroid and myeloid progenitors.[11]

TPO, also known as c-Mpl ligand is a cytokine that plays a central role in megakaryopeoiesis by

influencing the development and maturation of megakaryocytes and platelet production from hematopoetic stem cells. TPO exerts its biological effects through the TPO receptor, c-Mpl. C-Mpl is a member of the cytokine receptor superfamily and its function is restricted to hematopoietic tissues and cells, such as bone marrow, spleen, fetal liver and CD34+ cells. Stimulation of c-mpl with TPO results in the activation of tyrosine kinase (Tyk2) and Janus kinase (JAK2) family members, which in turn phosphorylate Stat5 and Stat3, causing their nuclear translocation and the transcription of Stat responsive genes.[12–18]

Bone marrow failure in CAMT is congenital. The pathophysiology of bone marrow failure in CAMT is not completely understood. It has been hypothesized that TPO and c-Mpl are necessary for the growth and maintenance of the hematopoietic stem cell population.[1,19,] This has been shown both *in vitro* and *in vivo* with murine models. C-mpl –/– mice have demonstrated a deficiency in megakaryocytes with severe thrombocytopenia and high serum TPO levels similar to humans with CAMT.[20] While widely thought to be a platelet disorder, amegakaryocytic thrombocytopenia frequently is associated with the development of aplastic anemia. Of the congenital bone marrow failure syndromes causing aplastic anemia, CAMT is the most likely to present in the neonatal period. Although the mechanisms leading to aplasia are unknown, the age of onset has been reported to depend on the severity of the c-MPL functional defect. The percentage of bone marrow cells expressing tumor necrosis factor-a and interferon-g increases during pancytopenia. As in other bone marrow failure diseases, these inhibitory cytokines contributed to the pancytopenia.[19] A global reduction in hematopoietic progenitor cells was also seen in these c-mpl knockout mice. Marrow repopulation studies in c-mpl –/– mice have also shown decreased engraftment compared to c-mpl + mice. These studies indicate that c-mpl is necessary for hematopoietic progenitor cell development.[21,22]

Genetics

The myeloproliferative (Mpl) genes in patients with CAMT were analyzed and reported in some literatures. The defects concern the incapacity of the TPO to fulfill its normal thrombopoeitic role as a result of the abnormalities in the c-Mpl gene that encodes the TPO receptor.[23–25] Two heterozygous mutations of the c-Mpl gene: a gln 186-to-ter substitution in exon 4 and a single nucleotide deletion in exon 10 were found in some patients with CAMT.[10] Patients with severe Type I CAMT carries nonsense MPL mutations which causes a complete loss of the thrombopoeitin (TPO) receptor whereas those with Type II CAMT carries missense mutations in the Mpl gene affecting the extracellular domain of the TPO receptor.[5] Some patients have inherited two different mutations of the c- Mpl gene resulting in heterozygous states.[12,26,27] However, a G304C mutation has been suggested as seen more frequently in patients with CAMT. They described a homozygous nonsense mutation (C268T) upstream of the mutation in the rare G304C polymorphism.[1] There is a high rate of consanguinity between of parents of children with CAMT, leading to the inheritance of homozygous c-mpl mutations.[28–30]

A genotype to phenotype correlation has been made with the type of mutation corresponding to the severity of disease. Patients with frameshift or nonsense mutations had a rapid progression of bone marrow failure, whereas patients with heterozygous or homozygous missense mutations had milder disease.[1] Perhaps, this is related to residual c-Mpl protein function. The discovery of the c-mpl gene mutation as the cause for CAMT has lead to the development of diagnostic genetic testing and prenatal diagnosis. The overall hematopoietic progenitor cells suggest that in the future, gene therapy may be a possible treatment option for this disorder. It is currently unknown what effect c-mpl transduction would have on c-mpl-deficient animal models. Retroviral-mediated gene transfer of c-mpl in normal mouse bone marrow cells has shown a reduction in megakaryopoiesis with enhanced erythropoiesis.[25]

Diagnosis and Testing

Diagnostic features include:

1. Defective signal and response to thrombopoeitin in megakaryocyte-colony formation—in the absence of a signal from thrombopoeitin, megakaryocytes do not proliferate.[4]
2. Decreased numbers of erythroid and myelocytic progenitors in clonal cultures.[4]

3. Lack of Mpl mRNA in bone marrow monocellular cells.[4]
4. Elevated serum level of TPO.[1,4]
5. White blood cells and red blood cells gradually decrease with age.[10]
6. No reactivity to TPO by the hematopoietic progenitor cells.[1,10]
7. Absent surface expression of the TPO receptor Mpl.[10]
8. Hypoplastic bone marrow without dysplasia.[31]

Mpl gene analysis is performed by bi-directional sequencing of the coding regions and splice sites of exons 1–12 of the Mpl gene. Mutations found in the first person of a family to be tested are confirmed by repeat analysis using sequencing, restriction fragment analysis, or other appropriate method. Using the GeneDx (GeneDx, DNA diagnostic experts, MD, USA) has 95%–97% sensitivity because it can detect almost all expected types of the Mpl gene, except for some promoter mutations, large deletions or duplications that are outside the regions sequenced that can not be detected. Specimens usually are taken as a single sample with 1.5 ml whole blood in ethylenediamineteraacetic acid (EDTA), buccal brushes as alternative samples or 10 mL of amniotic fluid.[32] Cytogenic investigations and analysis can be done also by fluorescent in-situ hybridization (FISH) on interphase nuclei. Chromosome analyses are performed on bone marrow and peripheral blood- simulated cultures. Skin fibroblasts can be cultured and quinacrine fluorescence (QFQ) banding can be used for chromosome analysis.[33]

Differential Diagnoses

Differential diagnosis for severe CAMT includes thrombocytopenia with absent radii (TAR) and Wiskott-Aldrich syndrome (WAS).[24,34–36] These can be distinguished from CAMT on the basis of associated skeletal hypoplasia of the arms (TAR) and microthrombocytes (WAS).[34,35] In addition, antiplatelet antibodies maybe transferred from the mother to the child in-utero or at the time of delivery, causing severe thrombocytopenia. Women who lack common platelet antigens may produce antibodies against paternal antigens that are expressed in the developing fetus.

Treatment

Treatment options for CAMT are limited. Supportive care includes judicious use of unrelated donor platelet transfusions (leucocyte-induced, and irradiated), avoidance of non-steroidal anti-inflammatory medications and aspirin, adjunctive therapies such as fibrinolytic agents for minor bleeding, and monitoring for the development of additional cytopenias. Platelets transfusions may be efficient in increasing the platelet count up to 10 days. Frequent platelet transfusions are required to ensure that platelet levels do not fall to dangerous levels, although this is not always the case.[5] It is known for patients to continue to create very small numbers of platelets over time. Desmopressin acetate (DDAVP) should be avoided in small infants due to the risk of hyponatremia but may be useful in older children and adults. Cytokine therapy with interleukin-3 and granulocyte-macrophage colony-stimulating factor has been attempted but only resulted in transient responses.[1,36] Steroids and immunoglobulins does not have an effect on thrombocytopenia, thus is not advised.[5]

The primary treatment for CAMT is bone marrow transplantation.[5,37] Bone Marrow/Stem Cell Transplant is the only thing that ultimately cures this genetic disease. Children and their family members should be human leukocyte antigen (HLA)-typed to identify possible matched related donors. Siblings that are heterozygous for a c-mpl mutation may have abnormal megakaryocytes despite normal peripheral platelet counts,[12] and usually a sibling can be used as a donor.[4] If a matched sibling is not available, transplantation may still be necessary, especially when marrow failure ensues, but reported outcomes using matched unrelated donors have been poor and rates of graft failure are high.[5,7] Newer modalities are on the way, such as TPO-mimetics for binding towards partially functioning c-Mpl receptors[38] and gene therapy.[39]

Prognosis and Mortality

Although HSCT has been implicated to treat ultimately CAMT, preliminary studies showed that the prognosis of CAMT patients becomes poor when tri-linear marrow aplasia develops in childhood.[5]

Furthermore, a single study showed that 30% of CAMT patients die to bleeding complications and 20% is actually due to HSCT if it has been done.[5] Moreover, the same study showed that in patients who underwent HSCT, conditioning regimen with busulfan (BU) and cyclophosphamide plus antithymocyte globulin and graft-versus-host prophylaxis with cyclosporine and methotrexate improves survival up to 80% of patients.[40]

Disclosure

This manuscript has been read and approved by the author. This paper is unique and is not under consideration by any other publication and has not been published elsewhere. The author and peer reviewers of this paper report no conflicts of interest. The author confirms that they have permission to reproduce any copyrighted material.

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