INHERITED BONE MARROW FAILURE SYNDROMES WITH PANCYTOPAENIA

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ABSTRACT

Bone marrow failure (BMF) is characterised by a reduction in the effective production of mature erythrocytes, granulocytes, and platelets by the bone marrow that leads to peripheral blood pancytopaenia. In some conditions, only one or two cell lines may be affected. The BMF syndromes include a group of disorders than can be either inherited or acquired. The inherited BMF syndromes include: Fanconi anaemia, dyskeratosis congenita, Diamond-Blackfan anaemia, and other genetic disorders. The most common cause of acquired BMF is aplastic anaemia.

Keywords: Fanconi anaemia, dyskeratosis congenita, Shwachman-Diamond syndrome, congenital amegakaryocytic thrombocytopaenia, genes, anaemia, pancytopaenia.

INTRODUCTION

The bone marrow (BM) has an enormous production capacity; it is estimated that 1,010 erythrocytes and 108-109 leukocytes are produced per hour in the steady state. Myeloid cells arise from a common stem cell whose development is regulated by stimulatory and inhibitory growth factors. Pluripotent1 hematopoietic stem cells are most influenced by interleukin-3 (IL-3), granulocyte-macrophage colony-stimulating factor (GM-CSF), and stem cell factor, while committed progenitor cells are regulated by variable concentrations of GM-CSF, granulocyte colony-stimulating factor (G-CSF), macrophage colony-stimulating factor (M-CSF), IL-5, erythropoietin (EPO), and thrombopoietin (TPO). Decreased transfer of mature cells from BM to blood may be due to a reduction in the number and the function of their progenitors; this results in a paucity of differentiated precursors causing such disorders as aplastic anaemia2 (pluripotent erythroid progenitors) or Diamond-Blackfan anaemia (committed erythroid progenitors). Sometimes the number of differentiated precursors may increase in the BM even if the number of their mature products has been diminished in the blood - referred to as ineffective hematopoiesis. Pancytopaenia is defined by the reduction of all the three formed elements of blood below the normal reference range. The presenting symptoms are often attributable to anaemia or thrombocytopaenia. Leukopaenia is often seen in the subsequent course of the disorder.

Pancytopaenia with a severe decrease of BM cellularity in children may be caused by a broad variety of underlying disorders; of these, acquired severe aplastic anaemia, refractory cytopena, and inherited BM failure (IBMF) disorders are three of the most common hematopoietic diagnoses. In this review we discuss the IBMF disorders - Fanconi anaemia (FA), dyskeratosis congenita (DC), Shwachman-Diamond syndrome (SDS), and amegakaryocytic thrombocytopaenia - and the importance of accurate diagnosis in the prerequisite for medical as well as genetic counselling, surveillance, and therapy options for these patients (Table 1).
In 1927 Fanconi described three brothers who had pancytopenia and congenital physical anomalies. FA is the most frequent inherited cause of BMF. The FA genes (genes that have been found to be mutated in FA patients) are called FANC, the most frequent being FANCA, FANCC, FANCG, and FANCD2. Except for the very rare FANCB, which is located on the X chromosome, all other FANC genes are autosomic and the disease is recessive. There are typically several clinical stages in FA that are related to age. Most children with FA are diagnosed between 6 and 9 years of age; the median age for boys is 6.5 years and for girls 8 years. Approximately 4% of cases are recognised between birth and 1 year of age, and 9% are diagnosed after 16 years of age. The diagnosis of FA may be delayed until adulthood, particularly for those patients for whom malignancy is the presenting clinical manifestation.

Moreover, they exhibit the classic anomalies of FA including short stature, microcephaly, abnormality of the thumbs, café au lait spots, and characteristic facial appearance: a broad nasal base, epicanthal folds, and micrognathia. Skin involvement is the most frequent sign followed by anomalies of the upper arms and structural renal anomalies. Patients often have mild or moderate thrombocytopenia or leukopenia before pancytopenia, but severe aplasia eventually develops in most cases. Examination of the blood smear shows macrocytosis, poikilocytosis, anisocytosis, and decreased numbers of platelets and leukocytes. The red cells may be larger, and foetal hemoglobin (HbF) levels may be higher. When aplastic, the BM is hypocellular and fatty, with increased number of lymphocytes, reticulum cells plasma, and mast cells.

Multiple genes appear to be responsible for FA. To date, 15 FANCs have been identified: FANCA, FANCB, FANCC, FANCD1, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCL, FANCM, FANCN, FANCO, and FANCP. The majority of FA genes are located on autosomes, with the exception of...
FANCB, which is on the X chromosome. FA patients with mutations in any of these FA genes share a characteristic clinical and cellular phenotype, and these 15 gene products appear to function in a common cellular pathway, termed the FA pathway. Indeed, mutations in eight FA subtypes (FANCA, FANCB, FANCC, FANCE, FANCF, FANCG, FANCL, and FANCM) result in loss of FANCD2 and FANCI monoubiquitylation, the central regulatory event in the FA pathway. Mutations in these eight upstream genes account for approximately 90% of patients. Identification of the breast cancer susceptibility gene, BRCA2, as an FA gene (FANCD1) reaffirm the close cooperative relationship between the FA pathway and the breast and ovarian tumour-suppressive BRCA proteins BRCA1 and BRCA2 in DNA repair mechanisms. The gene responsible for FA-A, which occurs in 60-65% of patients with FA, is located on chromosome 16q24.3. FA-C and FA-G are the next most common subtypes, each occurring in 10% of patients with FA. The normal FA gene product may contribute to the maintenance of genomic stability, control of apoptosis, oxidant sensitivity, and response to cytokines. Restoration of the expression of the normal FA protein in cells from patients with FA normalises cellular growth and corrects their sensitivity to chromosomal breakage in the presence of mitomycin C.

The FA gene FANCD1 is identical to BRCA2, one of the breast cancer susceptibility genes involved with DNA repair. Disruption of this repair process may result in the chromosomal abnormalities and propensity to malignancy (e.g. medulloblastoma, head and neck tumours, acute leukaemia) that are common in FA. It may also contribute to the susceptibility to breast, ovary, and pancreas cancers etc. seen in some patients with FA. The presence of FA should be considered in any patient presenting with or without the congenital malformations associated with FA, together with otherwise unexplained cytopenias or aplastic anaemia, unexplained macrocytosis, or an affected sibling. The diagnosis is made by the presence of increased chromosomal breakage in lymphocytes cultured in the presence of DNA cross-linking agents such as mitomycin C or diepoxybutane.

Management can be divided into therapy directed towards reversal of BMF and supportive therapy. Supportive therapy includes replacement therapy for each of the cell lines based upon the clinical status of the patient. Transfusion should be given when the patient is symptomatic. Only red cells that have been leukodepleted, to minimise the risk of allo sensitisation, and irradiated should be used. Platelet transfusion is indicated in patients with severe bruising, bleeding, or undergoing invasive procedures. The use of single donor apheresis platelets minimises exposure to multiple donors. Androgen therapy is effective for treating BMF in some FA patients. Synthetic androgens, such as oxymetholone and danazol, have been effective in treating hematopoietic defects in FA patients, although long-term androgen use has side-effects that include hirsutism and increased liver tumour incidence. G-CSF may raise the neutrophil count in most neutropaenic patients, but the platelet count and Hb concentration generally are unaffected.

Hematopoietic stem cell transplantation is the only curative approach for the hematological complications of FA. Because of the underlying DNA repair defect in FA, extensive chemoradiation used in the transplantation procedure can be highly toxic. In 1984, Gluckman developed the first successful preparative regimen for FA patients consisting of low-dose cyclophosphamide and single-fraction irradiation. Histocompatible matched sibling donor transplant remains the best treatment for FA and gives the best outcome if performed early prior to the development of myelodysplastic syndrome (MDS) or leukaemia. Hematopoietic cell transplantation (HCT) from alternate (unrelated or human leukocyte antigen mismatched) donors is associated with a higher risk of complications and a lower survival rate.

**DYSKERATOSIS CONGENITA**

DC is an IBMF syndrome. The disease was first described by Zinsser in 1906 and recognised as a clinical entity by Engman in 1926 and Cole in 1930. In the past DC was clinically characterised by the classic diagnostic triad of reticular skin pigmentation, nail dystrophy, and mucosal leukoplakia. BMF develops in 80-90% of patients with DC by the age of 30 and is the leading cause of death. In addition to BMF, individuals with DC have increased risk of malignancy, with a 40-50% cumulative incidence by the age of 50; the risks of squamous cell carcinoma of the tongue, acute myeloid leukaemia, and MDS, which may evolve into acute myeloid leukaemia, are markedly increased. Additional ‘minor’ features of DC include intraterine growth retardation, developmental delay, microcephaly, abnormalities involving the eyes, teeth, and hair, including premature greying.
excessive sweating, short stature, hypogonadism, enteropathy, liver disease, oesophageal and urethral stenosis, osteoporosis, and avascular necrosis of the hips or shoulders.

The disease is heterogeneous at the genetic level. As well as the most common X-linked form, autosomal recessive and dominant forms occur. In recent decades great progress has been made in the genetics and molecular biology of DC with about half of DC patients now assigned to mutations in known genes. Mutations in TERC or TERT were subsequently identified in the autosomal dominant form of DC. These patients may have classic DC with many complications, whereas others may be less severely affected. Functional analysis of several disease-associated TERC and TERT mutations revealed that they result in short telomeres due to haploinsufficiency of telomerase rather than a dominant negative effect, indicating that telomerase is limiting in normal cells. A small number of severely affected patients have also been shown to have DC due to autosomal recessive inheritance of TERT mutations which results in significantly decreased levels of telomerase. Patients with biallelic TERT mutations appear to be more severely affected (i.e. Hoyeraal-Hreidarsson variant) than those with the autosomal dominant TERT mutations.

Mutations in DKC1, which encodes the protein dyskerin, are associated with the X-linked form of DC. Most DKC1 mutations are missense mutations. Dyskerin is an evolutionarily conserved protein that is expressed throughout all tissues. Dyskerin associates with the box H/ACA class of RNAs, which includes the telomerase RNA, and is important for their nuclear accumulation and stability. Dyskerin also shares structural similarities to pseudouridine synthases. Telomeres stabilise the chromosome ends to prevent their shortening during replication, to distinguish chromosome ends from DNA damage-induced breaks, and to inhibit end-to-end fusions. Telomerase is highly expressed in tissues with a high replicative state, such as hematopoietic cells, germ cells, and tissue stem cells. The clinical phenotype of DC mirrors those tissues where there is a high rate of cell turnover and telomerase is highly expressed. Telomerase is also present in many cancer cells. When telomerase levels are low or absent, as is the case in most somatic tissues, telomeres progressively shorten with each cell division. When a critically short telomere length is reached, a checkpoint is triggered causing the cells to stop dividing and senesce to prevent chromosomal rearrangements.

In most patients the first hematologic findings are thrombocytopenia or anaemia, followed by pancytopenia. BM aspiration reveals increased cellularity at the outset, suggesting an element of hypersplenism. Then hypocellularity ensues, coinciding with the diagnosis of aplastic anaemia. Macrocytosis and elevated levels of HbF are common. Decreased immunoglobulin levels or cellular immunity is found inconsistently. Treatment options in DC are limited. Androgens (e.g. oxymetholone, danazol) may improve the blood counts but HCT remains the only curative option for BMF.

SHWACHMAN-DIAMOND SYNDROME

SDS is characterised by exocrine pancreatic dysfunction with malabsorption, malnutrition, and growth failure; hematologic abnormalities with single or multi-lineage cytopenia, and susceptibility to MDS and acute myelogenous leukaemia (AML), and bone abnormalities. In almost all affected children, persistent, or intermittent neutropenia is a common presentation, often before the diagnosis of SDS is made. Short stature and recurrent infections are common. On physical examination the most common findings are malnourishment and short stature. The combination of pancreatic dysfunction and BMF was noted by Ozsoyglu and Argun, who found decreased duodenal levels of trypsin, amylase, and lipase in patients with aplastic anaemia.

Neutropenia may be chronic, intermittent, or cyclic and it presents early in childhood. Anaemia occurs in more than one-third of patients and transfusions are required. Thrombocytopenia is seen in fewer than one-third of cases. BM cellularity is decreased with a myeloid maturation arrest and hyperplastic erythroid series. Sometimes the marrow aspiration is normal. HbF levels are often increased even without anaemia, suggesting the presence of BM stress.

Genetic testing is helpful in confirming the clinical diagnosis of SDS, but as previously mentioned, 10% of diagnosed patients with SDS will not have an SBDS gene mutation. Whether these patients without the SBDS gene mutations have mutations of another gene is unknown. Thus, a positive test confirms the diagnosis of SDS, but a negative genetic test does not exclude the diagnosis. Genetic testing is available in Europe.
and North America. In addition, national registries and an international collaborative database have been established to better understand the genetic basis of SDS and, if possible, correlate genetic abnormalities with clinical disease and outcome.

Treatment options are also limited. The malabsorption in these patients responds to treatment with oral pancreatic enzymes. G-CSF has been used in patients with recurrent infections or in cases of life-threatening infections. Although there is a theoretical concern of stimulating a malignant preleukaemic clone, resulting in an increased risk of myelodysplasia and acute myeloid leukaemia, no clear association has been established. There is no clear consensus on when to perform HCT in a patient with SDS, and clinical decisions also depend on the availability of an appropriate donor.

**TREATMENT AND PREVENTION**

Thrombocytopenia is a relatively common clinical problem in hospitalised neonates, and it is critical to distinguish infants with rare congenital thrombocytopenias from those with acquired disorders. Two well-described inherited thrombocytopenia syndromes that present in the new-born period include congenital amegakaryocytic thrombocytopenia (CAMT) and thrombocytopenia with absent radius (TAR).

CAMT is an IBMF syndrome caused by mutations in the receptor for TPO, c-Mpl. Affected children typically present with thrombocytopenia at birth, with BM analysis revealing severely reduced or absent megakaryocytes. Within the first decade of life, isolated thrombocytopenia progresses to pancytopenia due to trilineage BMF, and most children require treatment by stem cell transplantation. Mutations have been described throughout the c-Mpl receptor, although mutations in exons 2 and 3 are the most frequent. Mutations of c-Mpl have been classified as either Type 1, in which the receptor has lost all activity, or Type 2, in which the receptor retains some degree of function. Type 1 mutations completely eliminate receptor signalling through disruption of all or most of the intracellular domain, often through creation of a stop codon or frameshift. Type 2 mutations typically create amino acid substitutions or altered splice sites that result in a small degree of residual receptor signalling. Clinically, Type 2 patients have a slightly delayed onset of BMF compared to Type 1 patients. Nevertheless, with rare exceptions, most patients with CAMT go on to develop BMF. Importantly, patients with CAMT are also at an increased risk for the development of myelodysplasia and acute myeloid leukaemia. Supportive care in patients with CAMT consists primarily of platelet transfusion as well as adjunctive therapies such as fibrinolytic inhibitors to manage bleeding symptoms, as well as red cell transfusions, and antibiotics if needed.

TAR is a clinically-defined syndrome characterised by thrombocytopenia and bilateral radial aplasia with thumbs present. BM examination typically demonstrates a reduction in the size and number of BM megakaryocytes, and if obtained, plasma TPO levels are usually elevated. Although bilateral radial aplasia is the defining skeletal feature in TAR, additional skeletal abnormalities are frequently observed, including more extensive upper limb malformations, phocomelia, and lower limb malformations in as many as 50% of the patients. Non-skeletal abnormalities are also common, including gastroenteritis and cow’s milk intolerance, renal malformations, cardiac defects, facial dysmorphism, short stature, macrocephaly, and capillary hemangioma. Thus, when a child is born with absent radius, a multi-system evaluation with genetics and orthopaedics consultations should be initiated to detect and manage associated congenital malformations.

The inheritance pattern of TAR is complex, and autosomal recessive as well as autosomal dominant with variable penetrance inheritance patterns have been reported. As with CAMT, plasma TPO levels in patients with TAR are elevated, and signalling in response to TPO is abnormal; nevertheless, no mutations in c-Mpl or its associated kinase JAK2 have been identified, and the underlying reason for this signalling defect is not understood. Because thrombocytopenia tends to remit, the treatment of TAR is largely supportive with platelet transfusions in the first year of life as needed to control bleeding symptoms and facilitate orthopaedic or other procedures. SCT is a very rare requirement.

**CONCLUSIONS**

Much remains to be understood about the genetics, pathophysiology, and treatment of IBMF syndromes. This requires correct diagnoses, proper treatment and careful follow-up. The prognosis and the quality of life for most of these diseases have improved with recent therapeutic advances.
REFERENCES


