8 Inherited Bone Marrow Failure Syndromes

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8.1 Introduction

The inherited bone marrow failure syndromes are a heterogeneous group of disorders characterized by bone marrow failure which may or may not be associated with one or more somatic abnormality. The bone marrow failure can involve all or a single cell lineage resulting in pancytopenia or single cytopenias, respectively. Their true incidence is not clear since most of them are misdiagnosed as cases of acquired aplastic anemia. These syndromes often present in childhood but may not do so until adulthood in some cases. Inherited marrow failure syndromes need to be considered in the differential diagnosis of patients with characteristic physical abnormalities when present, along with idiopathic aplastic anemia, myelodysplastic syndrome, acute myeloid leukemia, or other characteristic solid cancers at an unusually early age. Diagnosis is confirmed by the identification of pathogenic mutations associated with each syndrome.

8.2 Classification

1. Pancytopenia
   (a) Fanconi anemia
   (b) Dyskeratosis congenita
2. Single cytopenias
   (a) Anemia
      - Diamond–Blackfan anemia
   (b) Neutropenia
      - Schwachman–Diamond syndrome*
      - Severe congenital neutropenia
   (c) Thrombocytopenia
      - Congenital amegakaryocytic thrombocytopenia*
      - Thrombocytopenia absent radii

*Although they present with neutropenia/thrombocytopenia to begin with, they eventually progress to pancytopenia (hence discussed along with pancytopenia syndromes).

8.3 Fanconi Anemia

Fanconi anemia (FA) is the most common among the inherited bone marrow failure syndromes, in which cells cannot properly repair a particularly deleterious type of DNA damage known as interstrand crosslinks (ICLs). ICLs can be caused by chemotherapeutic agents and endogenous metabolites such as acetaldehyde, malon-di-aldehyde, and nitrous acid. ICLs pose a major challenge to DNA replication and transcription. ICL lesions cause stalling of replication forks and activation of the DNA damage response and DNA repair processes, all of which are coordinated to repair ICLs and resume DNA replication. Unrepaired ICLs are par-

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ticularly deleterious, as they prevent strand separation. This defect in DNA repair results in genomic instability and inability to repair these errors. This in turn leads to increased sensitivity to cytotoxic therapies and a predisposition to certain malignancies. This defect also causes loss of hematopoietic stem cells, resulting in bone marrow failure. The incidence of FA is approximately 1 in 100,000 to 250,000 births [1]. This condition is more common among the people of Ashkenazi Jews, with a carrier frequency of 1 in 89 [2], and black South Africans where the carrier frequency is 1 in 83 [3].

FA was first described in 1927 by the Swiss pediatrician Guido Fanconi when he described a family with three boys with birth defects and anemia [4]. The median age at diagnosis of FA in literature is around 6.6 years, with cases being reported up to the age of 49 years, the delay being due to the variable clinical presentation. Determining whether FA is the cause of bone marrow failure has important implications for management because individuals with FA require increased surveillance for hematologic and nonhematologic malignancies and other organ dysfunction. They also need to be managed with dramatically reduced doses of chemotherapy for treating malignancies and in the preparative regimen for hematopoietic cell transplantation (HCT). Additionally, the presence of FA must be confirmed or excluded when evaluating siblings as HCT donors, so that the patient does not receive hematopoietic stem cells from a sibling with FA.

8.3.1 Pathophysiology

FA is caused by mutations in one of at least 17 different FA genes (*FANCA to FANCQ*), although pathogenicity of mutations in *FANCM* has been called into question [5–9]. Mutations in these genes account for more than 95% of all known patients with Fanconi anemia. Additional genetic subtypes may be added, including those affecting RAD51 (*FANCR*), BRCA1 (*FANCS*), UBE2T (*FANCT)*, and XRCC2 (*FANCU*) [10–17]. The latter group of genes are called Fanconi anemia-like genes since they do not present with the classical phenotype of FA.

In most cases, FA is inherited in an autosomal recessive manner through homozygous or compound heterozygous mutations affecting an individual FA gene. Two exceptions are the rare FA subtypes associated with mutation in *FANCB*, which is X-linked recessive, and *FANCR* (*RAD51)*, which is autosomal dominant [18, 19]. Heterozygotes for mutations in FA genes other than *FANCB* and *FANCR* are considered to be unaffected carriers, although some of these individuals may have an increased susceptibility to cancer.

The methods of inactive gene function include point mutations, large deletions, and duplications. Genotype–phenotype correlations have occasionally been identified. However, it has been found that the presence of congenital malformations in one sibling does not imply that all affected siblings will have similar congenital malformations indicating the phenotypic heterogeneity of these mutations.

8.3.2 The Fanconi Anemia Pathway

The Fanconi anemia genes encode proteins involved in a common DNA repair pathway known as the Fanconi anemia pathway. These proteins are also involved in homologous recombination. This pathway functions during the S phase of the cell cycle and involves the various steps of ICL repair, namely lesion recognition, DNA incision, lesion bypass, and lesion repair [20].

The appearance of ICLs is identified by FANCM along with Fanconi anemia-associated protein 24 (FAAP24) and the histone fold proteins MHF1 (also known as FAAP16 or CENPS) and MHF2 (also known as FAAP10 or CENPX). Once bound to chromatin, FANCM forms the platform for the assembly of the Fanconi anemia core complex consisting of 14 proteins (FANCA, FANCB, FANCC, FANCE, FANCF, FANCG, FANCL, FANCM, FANCT, FAAP100, MHF1, MHF2, FAAP20, and FAAP24). The Fanconi anemia core complex serves as an ubiquitin ligase for two other Fanconi anemia proteins, FANCD2 and FANCI, which together form a heterodimer (referred to as FANCD2–I).

At the site of attachment, ubiquitylated FANCD2–Ire moves nucleotides at the replication forks to release the ICL from one of the two parental strands. This process is known as "unhooking." BRCA1 is also required for FANCD2 recruitment. FANCD2Ub through SLX4 (also known as FANCP) recruits and activates several endonucleases. After unhooking, polymerases carry out lesion bypass by inserting nucleotides opposite the ICL and extending the nascent strand (known as "extension"). This process inherently has low infidelity and hence can cause potentially introducing mutations in the genome.

Double strand breaks caused by the nucleolytic incision step of the Fanconi anemia pathway must be repaired by homologous recombination for the completion of ICL repair. The BRCA2–FANCN complex promotes RAD51mediated formation of singlestranded DNA (ssDNA) nucleofilaments. FANCJ along with BRCA1 mediates homologous recombination, thereby completing the process of ICL repair.

In patients with FA, loss of FA gene function leads to disruption of this normal repair process, genomic instability, aberrant cell cycle regulation, and cell death. These cellular effects occur both during development, leading to congenital anomalies, as well as during childhood and into adulthood leading to increased risk of bone marrow failure, organ susceptibility to toxic exposures, and cancer.

Bone marrow failure in FA is thought to occur due to premature, selective attrition of $CD34^+$ HSCs possibly due to defective DNA repair leading to increased DNA damage and cell cycle arrest; increased levels of reactive oxygen species and circulating inflammatory cytokines affecting bone marrow microenvironment; and excessive damage caused by reactive aldehydes in the absence of intact FA repair pathways.

8.3.3 Clinical Features

A wide range of congenital anomalies have been reported in patients with FA. However, a study of 370 patients by the International Fanconi Anemia Registry (IFAR) found that nearly 40% of them had no physical findings [21]. Patients with biallelic mutations in *FANCD1/BRCA2* have a very severe phenotype, including features of the vertebral, anal, cardiac, tracheal, esophageal, and limb (VACTERL) association.

1. *Skeletal*
   - Short stature is seen in almost half of all FA patients.
   - Upper limb abnormalities (40%)—most common are thumb abnormalities.
     - Thumbs can be absent or hypoplastic, supernumerary, bifid, rudimentary, short, triphalangeal or tubular.
     - Radii—Absent or hypoplastic (In FA, hypoplastic radii are associated with abnormal thumbs unlike in TAR).
     - Hands—Clinodactyly, hypoplastic thenar eminence, polydactyly, absent first metacarpal, enlarged or short fingers.
     - Ulnae can also be occasionally dysplastic.
   - Spine—Spina bifida, scoliosis, abnormal ribs, sacrococcygeal sinus, Klippel–Feil syndrome, extra vertebrae.
   - Lower limbs—Toe syndactyly, polydactyly, abnormal toes, flat feet, clubfoot, hip dislocation, Perthes disease, coxa vara, abnormal femur, thigh osteoma.
2. *Skin*
   - Hyperpigmentation or hypopigmentation on the trunk, neck, back, and intertriginous areas (café au lait spots) (>50%)
3. *Genitourinary*
   - Renal defects (20%)—such as horseshoe kidney
   - **Gonadal** abnormalities
     - Males (30%)—Undescended testes, hypospadias, abnormal or absent testis, azoospermia, phimosis, abnormal urethra, small penis, delayed development
     - Females (<5%)—Structural anomalies of the uterus; aplasia of vagina; absence of uterus, vagina, or ovary/ovaries

4. *Craniofacial*
   - Head and face—Microcephaly (25%), hydrocephalus, micrognathia, bird facies, flat head, frontal bossing, scaphocephaly, sloped forehead, choanal atresia
   - Neck—short neck, low hairline, webbed neck
   - Ophthalmic abnormalities (20%)—Microphthalmia, epicanthal folds, strabismus, hypertelorism, ptosis, slanted eyes, cataracts, epiphora, nystagmus, proptosis, small iris
   - Otic anomalies (10%)—Conductive deafness, atresia, low-set, large, small, abnormal middle ear, absent drum, canal stenosis
5. *Gastrointestinal malformations*
   - High-arched palate, atresia, imperforate anus, tracheoesophageal fistula, Meckel diverticulum, umbilical hernia, abnormal biliary radicles, megacolon, Budd–Chiari syndrome
6. *Congenital heart disease (5%)*
   - Patent ductus arteriosus, ventricular septal defect, coarctation of aorta, truncus arteriosus
7. *Low birth weight (10%)*
8. *Developmental delay (10%)*
9. *Bone marrow failure*
   Bone marrow failure in FA usually begins with single-lineage cytopenias which then progresses to pancytopenia. This progression may take several years or may occur rapidly or may not progress at all. Thrombocytopenia is usually the first cytopenia to develop. Anemia is usually the last to develop. The anemia is usually macrocytic. Sometimes there can be macrocytosis without anemia. The cumulative incidence of bone marrow failure is about 90% by the age of 40 years. However, all are not affected, for example, patients with biallelic *FANCD2/BRCA2* mutations appear less likely to develop bone marrow failure. Also, in 25% of patients, factors like additional mutations and somatic mosaicism ameliorate bone marrow failure symptoms though the risk of malignancy remains.
10. *MDS/Leukemia*
    Patients with FA are at an increased risk of developing MDS and acute myeloid leukemia, sometimes maybe the presenting finding. By the age of 50, the cumulative incidence of MDS and AML is around 40 and 15%, respectively [22]. Rarely, these patients may also develop lymphoid malignancies like ALL, Burkitt's lymphoma, etc. The risk is much higher in patients with biallelic mutations of FANCD1/BRCA2 (about 80% by the age of 10).
11. *Solid tumors*
    Patients with FA have a higher risk of developing solid malignancies and usually present at an earlier age. The median age of developing malignancy in FA patients is 16 years [23], the risk increasing with age. The most common were squamous cell cancers (SCCs) of the head, neck, esophagus, anus, and urogenital region, liver tumors as well as renal, brain, breast cancers, and other tumor types including germ cell tumors and sarcomas [21]. Yet again, patients with biallelic mutations of FANCD1/BRCA2 have an increased risk, with about 97% of patients developing at least one solid malignancy by the age of 5 years.
12. *Endocrine manifestations*
    These may be due to anatomical disruption of the hypothalamic-pituitary axis or due to conditioning regimens.
    - Short stature
    - Primary hypothyroidism
    - Adrenal dysfunction due to low ACTH secretion
    - Altered glucose metabolism, including diabetes mellitus and impaired glucose tolerance
    - Dyslipidemia
    - Infertility and delayed or abnormal progression of puberty

8.3.4 Diagnosis

Testing for FA is absolutely and urgently indicated in any child or young adult meeting any of the following criteria:

- Two or more moderate to severe cytopenias (absolute neutrophil count [ANC] <1000/μL, platelet count <50,000/μL, hemoglobin<10 g/dL with absolute reticulocyte count <40,000/μL), persistent for more than 2 weeks, and a hypocellular bone marrow (<25% of normal cellularity) in the absence of malignancy, cytotoxic therapy, or other known cause.
- Findings that satisfy criteria for the VACTERL-H association or multiple other malformations such as short stature, café au lait spots, or hypospadias, which are strongly associated with FA.
- Relative of a known patient with FA who is being evaluated as a potential donor for HCT.

Testing for FA is also recommended in the following scenarios, with the rationale that the diagnosis of FA should be established (or eliminated) prior to administration of cytotoxic chemotherapy for cancer or HCT; and if FA is present, related family members should be tested before being considered as HCT donors:

- Any patient with single-lineage or multilineage cytopenias without known cause with one or more congenital malformations strongly associated with FA.
- Any patient less than 40 years of age diagnosed with myelodysplastic syndrome (MDS) not attributable to other known genetic cause or to prior cytotoxic radiation or chemotherapy.
- Any patient less than 40 years of age with de novo acute myeloid leukemia (AML) not caused by another known germline predisposition and associated with the following cytogenetics: monosomy 7, deletion 7q, complex cytogenetics, gain of 1q, 3q, or 13q. The rationale is that doses of chemotherapy and/or cytotoxic agents given as part of the HCT conditioning regimen need to be dramatically reduced in a patient with FA.
- Any patient with unexplained severe toxicity to cytotoxic agents indicative of increased sensitivity without other known cause.
- Any child or young adult who develops head/neck or anorectal squamous cell carcinoma with no known attributable exposures.
- Family members of known FA patients who request genetic testing.

8.3.4.1 Stress Cytogenetics

The hallmark of FA is defective DNA repair that results in extreme sensitivity to DNA interstrand crosslinking agents. The screening laboratory test for this defect involves assessment of chromosomal breakage upon exposure of cells to diepoxybutane (DEB) or mitomycin C (MMC) [1]. This testing is performed on T lymphocytes; thus, peripheral blood is preferred as a test source over bone marrow. In settings of severe leukopenia, the testing can still be performed on cells expanded in culture. It can be performed on skin fibroblasts too.

Heparinized venous blood is taken and expanded in culture. The cells are then treated with 150 nM and 300 nM of mitomycin C. The cells are arrested at metaphase using colcemid and harvested. Increased chromosomal sensitivity results in chromatid gaps, breaks, triradial and quadriradial chromosomes. These aberrations are scored as "break events." In a typical FA patient, at 300 nM MMC, no undamaged cells should be left and most cells should have "≥10 breaks/cell." This should be run along with a healthy control in whom at 300 nM, around 30% of the cells may show 1 to ≤5 break events/cell [24].

FA gene sequencing is recommended for all patients with a positive result from chromosomal breakage testing. The differential diagnosis of FA includes acquired aplastic anemia, paroxysmal nocturnal hemoglobinuria (PNH), other inherited bone marrow failure syndromes, other causes of pancytopenia, other chromosomal breakage syndromes, and de novo MDS. Sequencing not only helps to confirm the diagnosis but also serves to screen family members and allows for genotype–phenotype correlations.

8.3.5 Treatment

Allogeneic hematopoietic cell transplantation (HCT) is the only established curative therapy for FA-associated bone marrow failure, myelodysplastic syndromes (MDS), and leukemia. Androgen therapy is not curative, but it may be appropriate for patients with bone marrow failure awaiting HCT or those who cannot undergo HCT. Transfusion and growth factor support may be necessary in patients. These patients need to be screened routinely for MDS, leukemias, and other solid malignancies from time to time. If a patient with FA develops a malignancy that requires chemotherapy and/or radiation therapy, dose reductions or alternative regimens are likely to be necessary. These patients also require a multispeciality approach for other associated anomalies such as endocrine dysfunction and congenital abnormalities.

8.4 Dyskeratosis Congenita

Dyskeratosis congenita (DKC), also known as Zinsser-Engman-Cole syndrome, was first described in 1906. It is estimated to occur in 1 in one million people with a male to female ratio of 3:1. Approximately 2–5% of patients with bone marrow failure are identified to have DKC. It is an inherited disorder characterized by bone marrow failure, cancer predisposition, and additional somatic abnormalities. DKC and related short telomere syndromes are caused by mutations that interfere with normal maintenance of telomeres, the regions at the ends of the chromosomes that protect nucleated cells from the loss or gain of genetic material.

8.4.1 Pathophysiology

Telomeres are specialized structures at the end of chromosomes that protect the natural ends of chromosomes from loss of DNA, abnormal fusion to other chromosomes, and from activation of DNA damage pathway responses that normally would occur in response at free ends of DNA created by strand breaks. Telomeric DNA consists of tandem repeats of the TTAGGG sequence. Most of the telomeric DNA exists as duplex DNA, with a terminal single-stranded overhang of typically approximately 150–200 nucleotides of the G-rich strand. The shortening of the duplex DNA portion of telomeres is most characteristic of telomere biology disorders.

At birth, the telomere length is around 8–14 kilobase pairs. With each cell division, this length shortens. When the telomere length reaches a critical minimum, the cells can no longer divide. For this reason, the telomeres are referred to as biological clocks that keep track of the number of divisions a cell undergoes. In most somatic cells, this shortening is a normal part of ageing. However, some cells such as germ cells, epithelial cells, and malignant cells require unlimited replicative potential. This increased replicative capacity is made possible by the action of the telomerase complex, a ribonuclear protein complex (RNA and proteins) that counteracts telomere shortening by adding back DNA to the ends of chromosomes. The ability of telomerase to lengthen telomeres is regulated by several other mechanisms. The nucleoprotein factors contributing to these mechanisms, and consequently the genes encoding these factors, mutations of which lead to telomere biology disorders, have been subdivided into five categories [25]:

- *Telomerase activity*—Telomerase is a reverse transcriptase enzyme consisting of TERT, a catalytic protein; and TR, an RNA template. Its assembly requires small nucleolar ribonucleoproteins including dyskerin, NHP2, NAF1, and NOP10.
- *Telomerase trafficking and recruiting to telomeres*—Once the telomerase has been assembled, its recruitment to the telomeric site is mediated by TCAB1 and the shelterin complex which consists of the proteins TRF1, TRF2, RAP1, TIN2, TPP1, and POT1.
- *Telomere replication*—The CST complex causes extension of the telomere end in conjunction with the telomerase enzyme.
- *Telomere stability*—The RTEL1 is a DNA helicase that maintains the integrity of the

newly formed DNA duplex end of the telomere.
- *Unknown or multifactorial*—This group includes other proteins whose functional and pathologic significance have not yet been elucidated.

In telomere biology disorders (also called short telomere syndromes or telomeropathies), mutations in the genes encoding any of the above factors implicated in telomere function lead to abnormally short telomeres. This group of disorders exhibit the phenomenon of "disease anticipation" in which successive generations of affected individuals may be born with progressively shorter telomeres. Premature telomere shortening leads to premature cell death, senescence, or genomic instability, which in turn leads to impaired organ and tissue function, altered homeostasis, or inappropriate growth.

DKC is the prototypic short telomere syndrome defined by the bone marrow failure and the classic clinical triad of abnormal skin pigmentation, nail dystrophy, and oral leukoplakia. The inheritance pattern varies depending on the gene involved. It can be autosomal dominant (mutations in *ACD*, *RTEL1*, *TERC*, *TERT*, *TINF2*, and *NAF1*), autosomal recessive (mutations in *CTC1*, *NHP2*, *NOP10*, *PARN*, *RTEL1*, *STN1*, *TERT*, and *WRAP53*), or X-linked (also called Hoyeraal–Hreidarsson syndrome caused by mutations in DKC1 gene and characterized by severe phenotype with symptoms beginning in early childhood). The prevalence of DKC has been estimated to be approximately 1 in one million people in the general population [26] with a median age at diagnosis of 15 years. Approximately 2–5% of patients with bone marrow failure are identified to have DKC.

8.4.2 Clinical Features

The classical clinical findings are:
- Abnormal lacy reticular skin hyperpigmentation of the upper chest and neck (89%)
- Nail dystrophy with longitudinal ridges (88%)
- Oral leukoplakia (78%)

The other findings include:
- Bone marrow failure (86%)
- Premature graying/hair loss (16%)
- Hyperhidrosis (15%)
- Epiphora
- Developmental delay
- Pulmonary fibrosis (31%)—typically presenting in adulthood
- Endocrine manifestations such as short stature and hypogonadism
- Gastroenterological manifestations such as esophageal strictures and liver cirrhosis
- Increased predisposition to cancers including squamous cell carcinoma of the head and neck; stomach, rectal, and other gastrointestinal cancers; leukemia and myelodysplastic syndrome; and cancers of the skin, lung, and liver [27]

8.4.3 Diagnosis

The diagnosis of DKC has evolved from a purely clinical one based on classic mucocutaneous findings with or without bone marrow failure to the demonstration of telomeric dysfunction in these diseases by assays for telomere length and genetic testing for specific abnormalities that affect telomeric function. This approach enables diagnosis of probands with subtle clinical presentations.

- *Telomere length analysis*—This is done by flow-FISH (multi-color flow cytometry with fluorescence in situ hybridization) peripheral blood lymphocytes using peptide nucleic acid probes for telomeric DNA. Average telomere length below the first percentile for age is considered indicative of abnormally short telomeres and is consistent with DKC or a related telomere biology disorder.
- *Molecular sequencing*—This testing is critical for definitive establishment of a genetic diagnosis and enabling testing of first-degree relatives for potential carrier or disease status, and to determine family member eligibility to be an HCT donor. This could be done by sequential single gene testing, next generation sequencing panels or by whole exome sequencing.

8.4.4 Treatment

Historically, treatment options for patients with DKC have been limited by the rarity of the disease, few prospective studies, and predisposition to excess organ toxicities seen with conventional treatments used for other bone marrow failure conditions. However, the treatment outlook may be improving with active exploration of the roles for certain telomere-directed treatments, including androgen therapy, as well as hematopoietic cell transplantation (HCT) with reduced intensity conditioning regimens specifically designed for patients with telomere disorders.

8.5 Schwachman–Diamond Syndrome

Shwachman–Diamond syndrome (SDS, also known as Shwachman–Bodian–Diamond syndrome, Shwachman–Diamond–Oski syndrome, or Shwachman syndrome) is a rare inherited disorder associated with neutropenia which progresses to bone marrow failure along with exocrine pancreatic dysfunction and skeletal abnormalities. The disease usually presents in infancy. Among inherited bone marrow failure syndromes, SDS comes third after Fanconi anemia and DKC in frequency.

8.5.1 Genetics and Pathophysiology

SDS is an autosomal recessive disorder. About 90% of affected individuals harbor mutations in the SBDS gene situated on the long arm of chromosome 7 [28]. The protein encoded (SBDS protein) is involved in ribosomal biogenesis and mitotic spindle function. How these defects contribute to the clinical manifestations of SDS has not been established. Defects in maintaining cellular ploidy may contribute to the increased risk of leukemic transformation.

8.5.2 Clinical Features

- Bone marrow failure—usually presents as neutropenia (98%), but can also present as anemia (42%), thrombocytopenia (34%), or pancytopenia (19%).
- Steatorrhea—86%. SDS is the second most common cause of exocrine pancreatic insufficiency after cystic fibrosis. Interestingly, pancreatic function improves with age.
- Serum hepatic aminotransferase abnormalities—60%.
- Skeletal abnormalities such as osteopenia, metaphyseal dysplasia, thoracic/rib and pelvic dystrophies, short arms and legs, and duplicated distal thumb—49%.
- Short stature—56%.
- MDS/AML—The cumulative risk of developing MDS or AML in individuals with SDS was estimated to be 19% at 20 years and 36% at 30 years.
- The other less common findings include hepatic, cardiac, endocrine, neurocognitive abnormalities and increased HbF due to stress erythropoiesis.

8.5.3 Diagnosis

SDS should be suspected in an infant with growth failure, feeding difficulties, steatorrhea, neutropenia, and/or recurrent infections [29]. Diagnosis is established by demonstrating bone marrow failure with exocrine pancreatic dysfunction. Molecular diagnosis is established by identifying biallelic mutations in SBDS genes known to be deleterious, though their absence does not rule out the disease.

8.5.4 Treatment

Treatment of patients with SDS is directed at specific clinical manifestations. Management by a multidisciplinary team (e.g., gastroenterologist and hematologist, with other subspecialists as

clinically indicated) provides optimal care. Allogeneic hematopoietic cell transplantation (HCT) is the only curative therapy for SDS-associated bone marrow dysfunction and/or progression to MDS or AML. However, HCT will not improve pancreatic exocrine function or a predisposition to non-hematologic abnormalities.

8.6 Congenital Amegakaryocytic Thrombocytopenia

Congenital amegakaryocytic thrombocytopenia (CAMT) is a rare bone marrow failure syndrome that initially presents with severe thrombocytopenia and can evolve into aplastic anemia and leukemia. The disorder presents in infancy and is usually not associated with physical anomalies. It is often recognized early in life on day 1 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.

8.6.1 Genetics and Pathophysiology

CAMT is an autosomal recessively inherited disorder associated with mutations in the c-Mpl gene, which encodes the receptor for TPO (thrombopoietin). High levels of TPO are characteristic of CAMT. The normal function of TPO is to bind to its receptor and increase the number, size and ploidy of megakaryocytes and expression of platelet-specific markers. It is also essential to maintain the number of hematopoietic stem cells, and therefore their mutation can cause pancytopenia too, as seen in CAMT.

A recent classification was proposed in 2005 supported by several other reports based on the course on outcome of the disease as follows [30]:

(a) Type I—early onset of severe thrombocytopenia and pancytopenia. In this group, there is complete loss of functional c-Mpl due to nonsense mutations.
(b) Type II—milder form with transient increases of platelet counts up to nearly normal values during the first year of life and a late onset of bone marrow failure around the age of 3 to 6 years or later. In this group, there are partially functional receptors for the c-Mpl gene (missense mutations).
(c) Type III—there is ineffective production of megakaryocytes with no defects in the c-Mpl gene.

8.6.2 Clinical Features

Bleeding is the primary clinical manifestation: it could be cutaneous, gastrointestinal, pulmonary, and intracranial. Some patients may present with petechial purpura, cranial hematoma, or recurrent per rectal bleeding. Platelet counts among neonates are usually in the level of 150×10^9/L. Mental retardation, renal failure, high tone hearing loss, cataracts, or the development of leukemia are other associated features that may be seen in some patients though most of them lack these features.

8.6.3 Diagnosis

The primary manifestations are thrombocytopenia and megakaryocytopenia or low numbers of platelets and megakaryocytes. Mean platelet volume is typically normal. Abnormal size platelets, absence of platelet alpha granules, Dohle-like bodies or microcytosis have also been observed. There is an absence of megakaryocytes in the bone marrow. However, normal megakaryocytes in the bone marrow cannot rule out CAMT in the first year of life [31]. TPO levels are high. Genetic sequencing of c-Mpl gene is carried out for the diagnosis of probands.

8.6.4 Treatment

Currently, the only curative therapy for CAMT is a hematopoietic stem cell transplant. Platelet

transfusions are reserved for patients experiencing bleeding symptoms. Prophylactic platelet transfusions may be considered for patients posing a high bleeding risk. Antifibrinolytic agents such as tranexamic acid and aminocaproic acid may be useful in mucous membrane bleeds such as oral or nasal bleeding.

8.7 Diamond–Blackfan Anemia

Diamond–Blackfan anemia (DBA) is an inherited bone marrow failure syndrome characterized by pure red cell aplasia, birth defects, and predisposition to cancer. It was first defined as a clinical syndrome by Diamond and Blackfan in 1938. However, the first case report was from Josephs in 1936.

8.7.1 Genetic Defects

RPS19, located at chromosome 19q13.2, was the first gene found to be mutated in this disorder and is essential for maturation of the 40S ribosomal subunit. It is involved in ribosomal biogenesis and is mutated in about 25% of patients with DBA leading to haplo-insufficiency of rps19 protein. Other recently identified genes include RPS24 at chromosome 10q22-q23, RPS17 at chromosome 15q25.2 and large ribosomal subunit-associated proteins such as rpl5, rpl11, and rpl35a [32, 33]. These mutations together are seen in 70% of cases. All the mutations to date have been found in one allele, resulting in severe loss of function or protein haplo-insufficiency. Mutation in GATA-1 is seen in <1% of cases and no gene disorder is identified in 30% cases [34].

8.7.2 Inheritance

Approximately 40–45% of DBA cases are familial with autosomal dominant with variable penetrance and the remainder being sporadic or familial with seemingly different patterns of inheritance. Both mild and severe forms co-exist within a pedigree (variable expressivity). It is yet to be ascertained whether the rest are recessive forms or dominant inheritance with a reduced penetrance, or rarely gonadal mosaicism. It is important to identify family members likely to have silent forms of the disease to exclude them as stem cell transplant donors and for reproductive counseling [34].

8.7.3 Clinical Features

The incidence of DBA is estimated to be between 1/100000 and 1/200000 without ethnic predilection, with both sexes being equally affected. The birth defects associated with DBA are mainly craniofacial (50%), skeletal (39%), genitourinary (38%), and cardiac (30%) cases. The classic DBA facies described by Cathie in 1950 include hypertelorism and broad flat nasal bridge. Thumb anomalies ranging from hypoplasia of the thenar eminence to absence of the radius or forearm, duplicated, bifid or the classic triphalangeal thumb (Aase syndrome) are seen in 9–19% of the patients. A low birth weight and growth retardation are seen in about 25–30% of patients. The true prevalence of constitutional short stature is not known as it can be secondary to chronic anemia, iron overload, and corticosteroid use [35].

8.7.4 Diagnosis

The diagnostic criteria of DBA, revised at the sixth Annual Diamond Blackfan Anemia International Consensus Conference in 2005, are as below:

Diagnostic criteria

- Age less than 1 year
- Macrocytic anemia with no other significant cytopenias
- Reticulocytopenia
- Normal marrow cellularity with a paucity of erythroid precursors

Supporting criteria

Major

- Gene mutation described in “classical” DBA
- Positive family history

Minor

- Elevated erythrocyte adenosine deaminase (eADA) activity
- Congenital anomalies described in "classical" DBA
- Elevated HbF
- No evidence of another inherited bone marrow failure syndrome

Non-classical DBA—Otherwise normal individual with positive family history having a mutation shared by affected family members.

Non-classical, sporadic—Individual suspected of having DBA, but diagnostic criteria is insufficient, and reported mutation is present.

Probable DBA

1. Three diagnostic criteria are present along with a positive family history.
2. Two diagnostic criteria and three minor supporting criteria are present.
3. Positive family history and three minor supporting criteria are evident, even in the absence of diagnostic criteria.

In case anemia and reticulocytopenia are present and the bone marrow is normal, bone marrow should be repeated at a later date. The presence of additional cytopenias does not preclude the diagnosis of DBA and may be severe enough to warrant treatment [34].

8.7.4.1 Family Screening

In a family in which classical DBA is present in the parent and offspring, or in two or more siblings, the risk of recurrence in the subsequent generation is up to 50%. If the mutation of the proband is excluded in both parents, this is likely to be a new sporadic mutation with a recurrence risk related to the possibility of gonadal mosaicism. If no mutation is identified in the proband and if elevated eADA activity, Hb F and/or MCV (highly suggestive of DBA) are found in asymptomatic first-degree relatives, the recurrence risk should be considered as 50%. However the possibility of false positive results in these tests should be kept in mind. If, however, these values are normal in first-degree relatives, the recurrence risk is only 5–10% [34].

An evaluation of the family of the first identified case is necessary. All immediate family members should be evaluated with a thorough relevant history (anemia, cancer, birth defects, etc.), complete blood count including red cell indices, eADA activity, and HbF. If the proband has an identifiable mutation, then the parents and siblings need to have appropriate mutation analysis if available. The nature of any other positive findings will determine the extent of the family evaluation. Elevated eADA, increased HbF and MCV are not very strong independent criteria, but should be evaluated in the sibling donor when HSCT is planned, and if positive, mutational analysis should be carried out.

Prenatal diagnosis is possible for DBA if a mutation is identified in the family. More recently, preimplantation genetic diagnosis (PGD) is an option to greatly reduce the risk of a second affected child. This can be performed in order to select and implant embryos without the parental mutation, hence eliminating the risk for DBA. This method can also be combined with PGD for human leukocyte antigen (HLA) typing for families with an affected child in need of an HLA-matched stem cell transplant [35] after due ethical considerations.

8.7.5 Management and Follow-Up

A periodic history to assess any new complaints and physical examination with blood count monitoring is done at 4–6 month intervals in stable DBA patients. If any of these are abnormal, bone marrow aspirate, biopsy, cytogenetics, and FISH should be performed to determine early signs of evolution to MDS and AML. Radiation exposure from diagnostic tests should be minimized in these patients to minimize the risk of malignancies [34].

Corticosteroids form the cornerstone of treatment in DBA. Approximately 80% of DBA patients respond to an initial course of steroids. After starting steroids, an increase in hemoglobin is usually seen within 2–4 weeks. The dose is then tapered and maintained at the minimum

dosage required for continued transfusion independence. Steroids should be discontinued in the absence of response after 4 weeks. In order to minimize their adverse effects, steroids are avoided before 1 year of age. Chelation may be required in case of iron overload secondary to frequent transfusions. In around 20% of DBA patients, steroids (or red cell transfusions) may eventually be stopped completely with continued maintenance of adequate hemoglobin levels (remission) [34, 35].

8.8 Severe Congenital Neutropenia

Severe congenital neutropenia (SCN), Kostmann syndrome, is an inherited bone marrow failure syndrome characterized by severe chronic neutropenia with maturation arrest of neutrophil precursors at the promyelocyte stage in the bone marrow. This entity was first described by Kostmann in 1956 in Swedish kindred.

8.8.1 Pathogenesis

It is proposed that Kostmann syndrome represents a defect in the regulation or production of granulocyte colony-stimulating factor (GCSF). Neutrophils from these patients have markedly increased levels of 2 cytosolic protein tyrosine phosphatases (PTPs) that contain Src-homology 2 domain (SH2 domain), Anti-Src Homology Phosphatase-1 (SHP-1) and Src Homology Phosphatase 2 (SHP2). Over-expression of these proteins alters intracellular signal transduction. A selective deficiency of anti-apoptotic BCL-2 expression in myeloid cells leads to release of mitochondrial cytochrome c, thus activating intracellular apoptotic caspase pathway [36]. Previous hypothesis that the underlying defect in Kostmann syndrome is decreased G-CSF production or its diminished binding to GCSF-R no longer holds true as studies show that there are increased GCSF-R on myeloid cells with normal binding affinity for G-CSF. The autosomal dominant (60–80% cases) form of SCN is associated with inherited or spontaneous point mutation in one copy of gene encoding neutrophil elastase ELA2 [37]. A subset of SCN patients harbor acquired somatic mutations in the CSF3R gene encoding GCSF-R and have shown a strong predisposition to AML. These mutations truncate the intracellular leading to defective internalization and loss of binding sites for negative regulators leading to extended signaling of STAT5 (Signal transducer and activator of transcription 5). A subset of SCN patients are reported to have constitutive mutations in the extracellular domain of the GCSF-R that act in dominant-negative manner leading to hypo-responsiveness to G-CSF [38].

Recent studies point to recurrent homozygous germline mutation in HAX1 in SCN. The mitochondrial protein HAX1 has critical role in signal transduction and cytoskeletal control. It maintains the inner mitochondrial membrane potential and thus protects neutrophils from apoptosis. HAXI deficiency causes AR form of Kostmann disease [39].

8.8.2 Clinical Features

The classic Kostmann syndrome presents in early infancy with an equal incidence in males and females. Severe neutropenia (ANC < 200/cu mm) is brought to clinical attention after an initial infection which typically occurs shortly after birth. Common indicators to this disorder are temperature instability in neonatal period, fever, irritability and recurrent oral ulcers, gingivitis, localized infections such as pharyngitis, sinusitis, otitis media, bronchitis, pneumonia, cellulitis, cutaneous abscess, perianal abscess, lung or liver abscess, enteritis with chronic diarrhea and vomiting. Streptococcal or staphylococcal sepsis commonly occur though Pseudomonas, fungi, and Clostridium may rarely be causative. The mortality rate in the absence of appropriate medical management is around 70%. The progression to MDS and AML is seen in 7% of cases [36].

8.8.3 Diagnosis

Patients have ANCs generally below 0.2×10^9/L and neutrophils may be completely absent from peripheral blood. There may be transient rise of neutrophils during an episode of acute infection, but normal ANC is seldom reached. There may be co-existent mild anemia and thrombocytosis. A two- to four-fold increase in blood monocytes and an eosinophilia is usually common. Majority of the patients have elevated IgG levels with a normal immune response after vaccinations. Testing for anti-neutrophil antibodies is helpful to exclude autoimmune neutropenia of infancy. The bone marrow usually shows maturation arrest of myeloid precursors at stage of promyelocytes and myelocytes with few neutrophils. The promyelocytes show atypical nuclei with cytoplasmic vacuolization. Megakaryocytes are normal in number and morphology. Cytogenetics at the time of diagnosis is almost always normal. Monosomy 7 is the most frequent aberration acquired in 50% of cases with progression of disease and usually coincides with the development of MDS and AML [36].

8.8.4 Management

Management is usually supportive in the form of antibiotics and recombinant human G-CSF (rHuG-CSF) whose dosages depend on the patient's clinical course and ANC. Careful monitoring for cytogenetic abnormalities and G-CSF-R mutation is necessary to initiate rHuG-CSF. Adverse events of rHuG-CSF therapy include mild splenomegaly, osteoporosis, and malignant transformation into MDS/leukemia. HSCT remains the only currently available treatment for patients who are refractory to rHuG-CSF [36].

8.9 Thrombocytopenia with Absent Radii (TAR)

Thrombocytopenia with absent radii (TAR) syndrome is a rare autosomal recessive disease characterized by hypomegakaryocytic thrombocytopenia and bilaterally absent radii [40].

8.9.1 Genetics

Mutations in c-mpl gene coding for TPO receptor were earlier attributed to TAR, and these patients generally have elevated TPO levels. However, recent studies indicate that mutations in multifunctional transforming growth factor (TGF)-β2 gene and lack of expression of CD105 antigen on bone marrow stromal cells which is a part of the receptor complex for TGF-β1 and TGF-β3 have been implicated in this disease. The deletion on chromosome 1q21.1 described by Klopocki et al. though found to be associated is not considered sufficient to cause TAR. TAR is characterized by a defect in megakaryocyte proliferation and differentiation and the inability to form proplatelets [41].

Inheritance is autosomal recessive in the majority of cases although an autosomal dominant with variable penetrance has also been proposed. TAR syndrome phenotypically overlaps with Roberts syndrome, but while the former is compound heterozygous form of a mild and a severe mutation, Roberts syndrome is the homozygous form with severe mutation [41, 42].

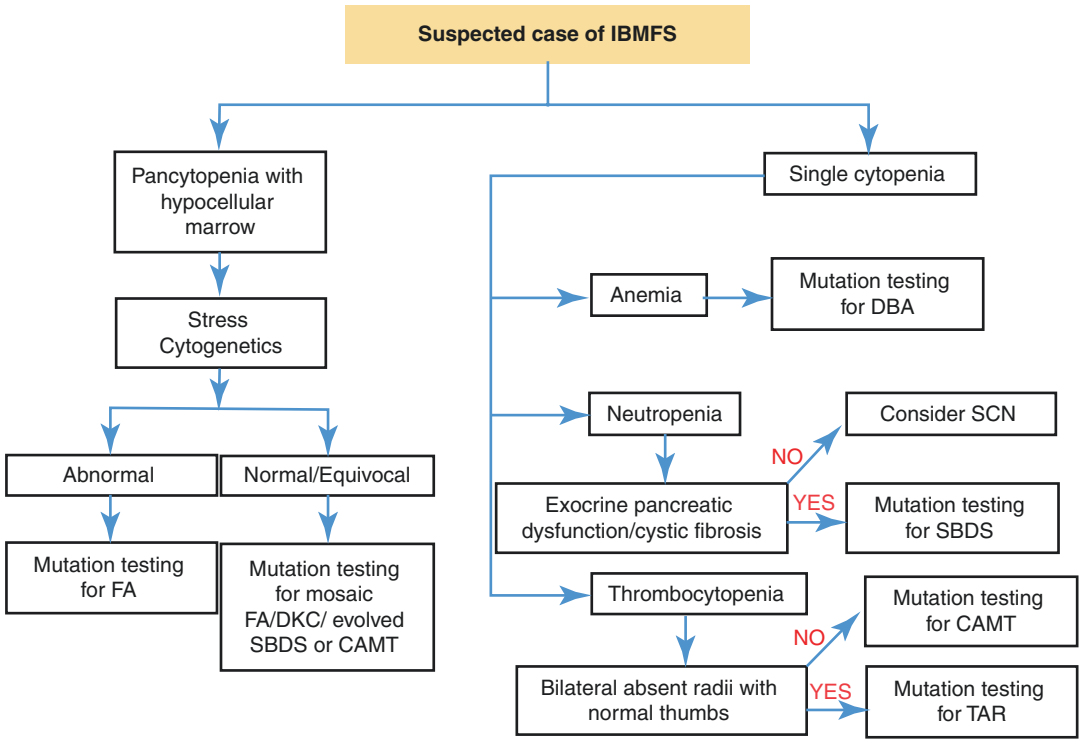
8.9.2 Clinical Features

Classically, the syndrome presents in the neonatal period with severe thrombocytopenia (usually <20,000/cu mm) and bilateral aplasia of the radii, which is the most common skeletal defect, but unlike Fanconi anemia, thumbs are present. The other skeletal abnormalities include anomalies of the lower extremity and short stature. Cardiac and facial anomalies may be found in 15% to 33% and 50% of patients, respectively. Hypoplasia of the cerebellar vermis and corpus callosum and mental retardation are seen in about 7% of all cases of TAR syndrome. Thrombocytopenia and bleeding episodes may improve with age. Survival is significantly longer in patients with TAR syndrome than that of Fanconi anemia or Diamond–Blackfan anemia, with the survival curve reaching a plateau of 75% by 4 years of age. Progression to aplastic anemia is not seen though AML or MDS can occur however at a lower frequency [41].

Patients usually present with symptoms of thrombocytopenia (platelet count <10 × 10^9/L) as early as in the first week of life. Leukocytosis (usually >35 × 10^9/L) may be present and precedes thrombocytopenia, with a left shift and eosinophilia in about 50% of patients. Bone marrow erythropoiesis is normal or may show compensatory erythroid hyperplasia due to anemia following bleeding episodes. Bleeding is most frequent during the first 1 to 2 years of life, with increased mortality due to intracranial hemorrhage [40].

8.9.3 Management

Treatment is usually supportive in the form of platelet support. Prophylactic transfusions are restricted to patients at high risk of clinically significant hemorrhage. Leukocyte-reduced platelet concentrates or random single-donor platelets reduce risk of exposure to foreign human leukocyte antigen and alloimmunization. HSCT is an option for patients whose counts do not improve with age and life-threatening bleeds not adequately controlled with platelets [41, 42].



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