Aplastic Anemia, Pediatric Aspects

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ABSTRACT

Inherited bone marrow failure syndromes (BMFs) comprise at least one-fourth of children with aplastic anemia, and perhaps up to 10% of adults. The most common syndrome is Fanconi’s anemia (FA), with more than 1,000 reported cases. FA is autosomal recessive, with birth defects in ~75% of patients. It is a DNA repair syndrome, diagnosed by finding chromosomal aberrations in cells treated with clastogenic agents. The major problems in FA are, in order, aplastic anemia, leukemia, and other cancers. There are at least five complementation groups; the gene for Group C has been cloned. Carrier identification and gene therapy are beginning in families at risk for FAC mutations. Dyskeratosis congenita (DC) is primarily X-linked (at Xq28), with autosomal recessive and dominant cases as well. Patients classically have reticulated hyperpigmented skin, dystrophic nails, and mucous membrane leukoplakia. ~50% develop aplastic anemia, sometimes prior to the DC phenotype, and ~10% develop cancer. Shwachman-Diamond syndrome consists of exocrine pancreatic insufficiency with neutropenia; ~25% develop aplastic anemia and 5%-10% develop leukemia. Amegakaryocytic thrombocytopenia presents in infancy, and often evolves into aplastic anemia and/or leukemia. Single cytopenias include Diamond-Blackfan anemia (DBA), which is inherited pure red cell aplasia; transient erythroblastopenia of childhood; Kostmann’s syndrome (KS) or infantile genetic agranulocytosis, and thrombocytopenia with absent radii in which there is neonatal thrombocytopenia and absent radii. DBA and KS, particularly the latter treated with G-CSF, may develop leukemia, and solid tumors have been reported in DBA. Treatment for the various BMFs includes bone marrow transplantation, androgens, and hematopoietic cytokines such as G-CSF. These inherited syndromes thus include various combinations of marrow failure and premalignancy. The Oncologist 1996;1:361-366

INTRODUCTION

The epidemiology, pathophysiology, and treatment of acquired aplastic anemia in children does not differ extensively from that of adults, although children may be more responsive to immunosuppression, growth factors, and bone marrow transplantation. However, in children at least one-fourth of the cases of “bone marrow failure” have an identifiable underlying genetic basis. In fact, perhaps 10% or more of adults with aplastic anemia are also in this category. This article will briefly outline the features of those inherited marrow failure syndromes, with an emphasis on current ideas with regard to pathophysiology and treatment. Detailed summaries and literature citations for the clinical syndromes can be found in recent reviews [1-3], and Table 1 contains a summary of the major features.

PANCYTOPENIAS

Fanconi’s Anemia (FA)

FA is the most common of the marrow failure syndromes, with more than 1,000 cases reported. The inheritance is autosomal recessive, and the majority, but by no means all, of the patients have characteristic birth defects, with short stature in 60%, anomalies of the radial ray in close to half, and renal structural abnormalities in 25%. FA is in the class of disorders with DNA repair defects, and the diagnosis depends on finding increased chromosome breaks, gaps, rearrangements, or quadriradii in blood lymphocytes cultured with a DNA crosslinker such as diepoxybutane (DEB) or mitomycin C (MMC).

Complementation analyses have indicated that there may be at least five FA mutations, A through E [4]. The
gene for FA group C (FAC) was cloned in 1992 and shown to be located on chromosome 9q [5], while the genes for groups A and D appear to be linked to 16q and 3p [6, 7]. The FAC protein is apparently new and is located in the cytoplasm [8, 9], inconsistent with a direct role in DNA repair. It may function to prevent cell death by apoptosis, and/or cell damage by oxygen-free radicals, and thus act indirectly on damaged DNA [10]. Cell cycle kinetics are altered in FA cells, with a G2/M arrest noted. Aplastic anemia occurs in over 90% of FA patients, at a mean of eight to nine years of age. Red cells have features of stress fetal-like erythropoiesis, with macrocytosis and increased

<table>
<thead>
<tr>
<th>Feature</th>
<th>Fanconi's Anemia</th>
<th>Dyskeratosis Congenita</th>
<th>Shwachman-Diamond Syndrome</th>
<th>Amegakaryocytic Thrombocytopenia</th>
<th>Diamond-Blackfan Anemia</th>
<th>Kostmann's Syndrome</th>
<th>Thrombocytopenia with Absent Radii</th>
</tr>
</thead>
<tbody>
<tr>
<td>Approximate # of patients</td>
<td>1,000</td>
<td>225</td>
<td>200</td>
<td>45</td>
<td>550</td>
<td>200</td>
<td>200</td>
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<tr>
<td>Male:Female</td>
<td>1:3</td>
<td>4:3</td>
<td>1:7</td>
<td>1:6</td>
<td>1:1</td>
<td>1</td>
<td>0:7</td>
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<tr>
<td>Genetics</td>
<td>AR</td>
<td>X</td>
<td>AR</td>
<td>X, AR</td>
<td>AR, AD, sporadic</td>
<td>AR</td>
<td>AR</td>
</tr>
<tr>
<td>Birth defects (%)</td>
<td>80</td>
<td>100</td>
<td>60</td>
<td>40</td>
<td>25</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Upper limb (%)</td>
<td>48</td>
<td>15</td>
<td>&lt;2</td>
<td>0</td>
<td>8</td>
<td>0</td>
<td>100</td>
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<tr>
<td>Median age at diagnosis</td>
<td>7.5 years</td>
<td>16 years</td>
<td>4 months</td>
<td>7 days</td>
<td>2 months</td>
<td>1 month</td>
<td>Birth eptasia of thrombocytopenia</td>
</tr>
<tr>
<td>First hematologic sign</td>
<td>Pancytopenia</td>
<td>Pancytopenia</td>
<td>Neutropenia</td>
<td>Thrombocytopenia Anemia</td>
<td>Neutropenia</td>
<td>Erythroid hypoplasia</td>
<td>Myeloid arrest</td>
</tr>
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<td>Bone marrow</td>
<td>Aplastic</td>
<td>Aplastic</td>
<td>Hypocellular, myeloid arrest</td>
<td>Absent or small megakaryocytes</td>
<td>Erythroid hypoplasia</td>
<td>Myeloid arrest</td>
<td>Absent or immature megakaryocytes</td>
</tr>
<tr>
<td>Aplastic anemia (%)</td>
<td>95</td>
<td>50</td>
<td>20</td>
<td>40</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Leukemia/MDS (%)</td>
<td>12</td>
<td>0.4</td>
<td>5</td>
<td>5</td>
<td>2</td>
<td>2% pre-G-CSF</td>
<td>13% on G-CSF</td>
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<tr>
<td>Cancer (%)</td>
<td>5</td>
<td>10</td>
<td>0</td>
<td>0.4</td>
<td>0</td>
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<td>0</td>
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<tr>
<td>Liver disease (%)</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0.2</td>
<td>0</td>
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<tr>
<td>Hb F level</td>
<td>Increased</td>
<td>Increased</td>
<td>Increased</td>
<td>Increased</td>
<td>Increased</td>
<td>Normal</td>
<td>Normal</td>
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<tr>
<td>Chromosomes</td>
<td>Breaks with clastogens</td>
<td>Bleomycin sensitive</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
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<tr>
<td>Spontaneous remissions (%)</td>
<td>Very rare</td>
<td>Rare</td>
<td>0</td>
<td>20</td>
<td>0</td>
<td>75</td>
<td>75</td>
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<tr>
<td>Treatment</td>
<td>BMT, androgens</td>
<td>Androgens</td>
<td>G-CSF, BMT</td>
<td>BMT</td>
<td>Steroids, BMT, ?IL-3</td>
<td>G-CSF</td>
<td>Platelets</td>
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<tr>
<td>Response (%)</td>
<td>50, transient</td>
<td>50, transient</td>
<td>80</td>
<td>50</td>
<td>95</td>
<td></td>
<td></td>
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<tr>
<td>Prognosis</td>
<td>Poor</td>
<td>Poor</td>
<td>Fair</td>
<td>Poor</td>
<td>Good</td>
<td>Good</td>
<td>Good</td>
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<tr>
<td>Chromosome</td>
<td>Group C = 9q</td>
<td>Xq</td>
<td>Neutropenia</td>
<td>Thrombocytopenia</td>
<td>ADA, BFU-E</td>
<td>BFM</td>
<td>BFM</td>
</tr>
<tr>
<td>Prenatal diagnosis</td>
<td>Chromosome breaks, FAC</td>
<td>Xq28 RFLP</td>
<td>Neutropenia</td>
<td>Thrombocytopenia</td>
<td>ADA, BFU-E</td>
<td>BFM</td>
<td>BFM</td>
</tr>
<tr>
<td>Projected 50% survival age, years</td>
<td>30</td>
<td>33</td>
<td>35</td>
<td>3</td>
<td>40</td>
<td>&gt;20</td>
<td>&gt;55</td>
</tr>
</tbody>
</table>

AR: autosomal recessive; AD: autosomal dominant; X: X-linked recessive; RFLP: restriction fragment length polymorphism. Reprinted with permission [1].
fetal hemoglobin (Hb F). Although very few cases are identified in infancy (except from clinical phenotype), at least 10% are adults when diagnosed. Drugs, chemicals, toxins, and viruses which cause acquired aplastic anemia may accelerate or unveil aplasia in FA. Hematopoietic cultures demonstrate decreased progenitor cell numbers which correlate to some degree with the clinical severity of in vivo hematopoiesis. Progenitor-derived colony growth may improve with the in vitro addition of some cytokines, such as stem cell factor (SCF). Addition of an antisense oligonucleotide to normal hematopoietic cells produces the FA phenotype (increased chromosome breakage, decreased colony formation) [11].

Treatment for aplastic anemia in FA involves androgens (~50% response), cytokines such as GM-CSF (~80% myeloid response) and G-CSF (100% myeloid response, ~30% erythroid and/or platelet), and stem and progenitor cell transplantation from bone marrow or cord blood (>70% survival). The median survival for FA in recent years is approximately 30 years of age. Only transplant is potentially curative for aplastic anemia or leukemia, although there may be an increased risk of solid tumors (particularly tongue cancer), perhaps related to immunosuppression.

Leukemia has been reported in ~10% of FA cases, both following and without aplastic anemia, at an average age of 14 years. The leukemia is usually myeloid, and treatment (chemotherapy or transplant) is complicated by the increased sensitivity of all tissues to damage from alkylators or radiation. Several FA patients have had myelodysplastic syndromes (MDS), based on marrow morphology and/or cytogenetic clonal abnormalities. Data are not yet available to decide whether those findings are preludes to leukemia or occur independently from true malignant transformation [12].

Older patients (mean age of 23 years), especially females, are at risk for development of cancers such as squamous carcinomas of the oropharyngeal, gastrointestinal, or gynecologic areas. These tumors were reported in ~5% of FA patients. Surgical treatment may be helpful, but chemotherapy and radiation present the same problems as for treatment of leukemia.

Gene therapy trials are now beginning for patients in the C group. This has the potential for prevention or treatment of aplastic anemia, myelodysplasia, or leukemia, but obviously will not impact birth defects and may not prevent development of solid tumors. Efforts must continue to identify the basic defect in FA in order to determine definitive therapies.

Prenatal diagnosis is possible in families with an index case using amniotic fluid cells or fetal blood, and testing for chromosome breakage with clastogenic stress or molecular analysis for mutations in the FAC gene. In addition, 1% of Ashkenazi Jews are heterozygotes for the IVS4 mutation in FAC, and thus prenatal diagnosis can be offered in families identified to be at risk by heterozygote testing.

**Dyskeratosis Congenita (DC)**

DC is a form of ectodermal dysplasia in which ~50% develop aplastic anemia. More than 200 cases have been reported. The diagnostic triad includes skin reticulated hyperpigmentation, dystrophic nails (both in the first decade), and mucous membrane leukoplakia in the second decade. The latter is often premalignant. Ninety percent of reported cases are male, and there is linkage to Xq28. Affected females and consanguineous families suggest an autosomal recessive pattern in these cases, while dominant inheritance is also noted [13]. Data are controversial with regard to whether DC is in the category of chromosome breakage disorders [14].

Bone marrow failure often follows but may precede the diagnosis of DC and occurs at an average age of 14 years. Patients with DC and aplastic anemia have reduced numbers of hematopoietic progenitor cells, which may be augmented in vitro with hematopoietic growth factors. Treatment with androgens is moderately useful, while G-CSF or GM-CSF may have potential, at least for myeloid responses. Bone marrow transplant appears initially successful, but the long-term survival is below 40%. The median survival is ~33 years of age for those with aplastic anemia.

Most cancers in DC are squamous or adenocarcinoma, with the majority including oropharyngeal and gastrointestinal tumors. Cancer treatment has not been very successful, and the median survival age is in the early 30s. Leukemia has also been reported.

**Shwachman-Diamond Syndrome (SD)**

More than 200 cases of SD have been reported, with exocrine pancreatic insufficiency plus neutropenia; almost half the cases also have metaphyseal dysostosis. Other cytopenias are common, and ~25% develop full-blown aplastic anemia. SD is an autosomal recessive disorder. An apparent stem cell deficit is suggested by decreased progenitor cells in culture assays. Cytopenias were reported to improve in some patients treated with corticosteroids, alone or combined with androgens. G-CSF led to increased neutrophil counts in several patients.

Early deaths are from infectious complications of the neutropenia. Five percent to 10% develop myelodysplasia and/or leukemia before the end of the second decade. The median survival in SD is more than 35 years of age overall, but less for those with aplastic anemia and/or leukemia. Bone marrow transplant may cure the hematopoietic disease.

A few other conditions bear some resemblance to SD. Patients with cartilage-hair hypoplasia (CHH) have an autosomal recessive condition with short stature, fine sparse hair, some malabsorption, neutropenia, or pure red cell aplasia in 80%, and genetic linkage to a region of chromosome 9p [15]. They are at risk for lymphoid and
skin malignancies. Pearson’s syndrome consists of refractory sideroblastic anemia with marrow vacuoles and exocrine pancreatic dysfunction and is due to a deletion of mitochondrial DNA which includes enzymes of the respiratory chain. Most of the patients succumb to complications of metabolic acidosis and infection. This syndrome needs to be included in the differential diagnosis of many disorders, including non-immune hydrops, cytopenias with liver or renal disease, and refractory anemias, especially myelodysplasia with ringed sideroblasts.

Prenatal diagnosis might be possible for SD if severe neutropenia were found in a fetal blood sample, but fetal neutrophil counts are low even in normal pregnancies, and thus the specificity is questionable. SD with metaphyseal dysostosis, or CHH with short stature, might be identified by prenatal ultrasound. Pearson’s syndrome might be detected by molecular identification of the mitochondrial DNA deletion.

Amegakaryocytic Thrombocytopenia (Amega)

Fewer than 50 children have been described who presented with thrombocytopenia in early infancy, and who were physically normal, or had central nervous system or cardiac anomalies (but normal radii, in contrast to thrombocytopenia—absent radius [TAR], see below). Inheritance appears to be both X-linked and autosomal recessive. The bone marrow has decreased or abnormal megakaryocytes, and the numbers of megakaryocyte progenitors are decreased, but improved with the in vitro addition of interleukin 3 (IL-3) and GM-CSF. Evidence for a stem cell disorder derives from the presence of macrocytic red cells and increased fetal hemoglobin. Approximately half the patients develop pancytopenia, and evolution to leukemia has also been observed. Bone marrow transplant may be curative, and there may be a role for treatment with IL-3 [16]. Prenatal diagnosis can be made if there is thrombocytopenia in a fetal blood sample in an at-risk pregnancy.

Apparent Inherited Marrow Dysfunction

A variety of poorly described familial and/or congenital disorders has been reported, including autosomal dominant or recessive with anomalies (e.g., IVIC, WT, ataxia-pancytopenia with monosomy 7), and dominant, X-linked, or autosomal recessive without anomalies. There are also sporadic cases with physical abnormalities and aplastic anemia which do not fit a known syndrome. In addition, there are several families with multiple generations, or multiple members of a single generation, with apparently “acquired” aplastic anemia, which may reflect the combination of noxious environmental factors with inadequate genetic hematopoietic abilities.

Known Syndromes and Marrow Failure

Although rare, a small proportion of patients with a variety of well-described genetic syndromes has developed aplastic anemia or marrow failure single cytopenias. These include Down’s (five cases), Dubowitz (10% of cases), Seckel (25% with aplastic anemia or malignancy), Noonan’s (three patients with amegakaryocytic thrombocytopenia and one with aplastic anemia), and trisomy 18 (thrombocytopenia). The incidence of marrow failure in any of these syndromes is not clear.

Single Cytopenias

Diamond-Blackfan Anemia (DBA)

Patients with DBA usually present in infancy or early childhood with normochromic, usually macrocytic anemia, reticulocytopenia, and marrow erythroid hypoplasia. More than 500 cases have been published, describing dominant inheritance in 20 families, recessive in 30, and sporadic in the majority. Approximately one-fourth have physical anomalies more subtle than in FA, including triphalangeal or otherwise abnormal thumbs, short stature, and skeletal anomalies. Red cells have fetal-like features, with high MCV, Hb F, and I antigen. More than 90% have increased red cell adenosine deaminase (ADA), which is quite specific [17].

The erythroid progenitor cell is defective in DBA, with very low numbers of colony forming units-erythroid (CFU-E) or BFU-E in standard cultures. Erythroid colony growth is improved with the addition of high concentrations of erythropoietin (EPO) and IL-3 in some studies. We found that SCF increased the numbers of colonies in 90%, and led to normal numbers in 75% of patients [18]. The genetic defect is not known, and the genes for both SCF and its receptor, c-kit, are normal. There appears to be an acceleration in programmed cell death (apoptosis), further indication that the DBA defect is intrinsic to the erythroid progenitor [19].

A small number of patients had intrauterine parvovirus infection, were born with hydrops and severe anemia, and had B19 viral DNA in the marrow (using polymerase chain reaction). However, they failed to respond to intravenous immunoglobulin and thus are clinically indistinguishable from classical DBA.

The first line of treatment for DBA consists of corticosteroids, with more than 60% responding and requiring low- or moderate-dose steroid maintenance. High-dose intravenous methylprednisolone may be helpful in a few patients, particularly those who are young and not iron-overloaded. Transfusions are required for the rest of the patients, with transfusion hemosiderosis requiring iron chelation with desferrioxamine. IL-3
produced a response in fewer than 15% of more than 60 patients, leading to both IL-3-requiring and treatment-free remissions. SCF is not yet available for clinical trials. Bone marrow transplant may cure DBA; the survival rate in ~30 patients transplanted is 65%.

Overall, the median survival age for DBA is 42 years. Malignancies have been reported, including leukemia in 2%, MDS in three cases, hepatoma, and Hodgkin’s disease [20]. There are no clear reports of evolution to aplastic anemia. Thus DBA is a single cytopenia, with a better prognosis than many of the other marrow failure syndromes.

Prenatal diagnosis of DBA has not been reported but might perhaps be done using fetal blood, if there were a macrocytic anemia, the red cell ADA were higher than normal fetal controls, and fetal blood erythroid progenitors were decreased or absent.

**Transient Erythroblastopenia of Childhood (TEC)**

There are more than 600 reports of young children (the majority aged one to three years) with an acquired pure red cell aplasia, possibly associated with a preceding viral illness. The red cells have normal size for age, and Hb F is not increased until patients recover. Bone marrows usually show erythroblastopenia unless recovery has already begun. Depending on the time in the course of the illness, marrow BFU-E and/or CFU-E may be decreased, and serum- (or, less often, cell-) mediated inhibition can be demonstrated. IgG autoantibodies have been documented to erythroid progenitors. Parvovirus is usually not the culprit (possible in less than 10%).

Treatment involves transfusion if clinically indicated, and all patients recover, most within one to two months from diagnosis. The prognosis is excellent, and distinction from DBA is usually simple in retrospect. TEC remains a disease in search of a virus.

**Kostmann’s Syndrome (KS)**

Also known as infantile genetic agranulocytosis (IGA), or severe congenital neutropenia (SCN), KS is an autosomal recessive severe neutropenia with a high early mortality from infections in the approximately 200 cases in the literature. Neutropenia may be total, and is usually below 200µl. Marrows show absent or markedly decreased myelopoiesis with a maturation arrest at the promyelocyte or myelocyte stage. Colony-forming units (CFU-C) may be normal in number, but the colonies contain monocytes, eosinophils, or only early myeloid forms. An abnormal G-CSF receptor has been identified as a somatic mutation in myeloid cells in a small number of patients, although the receptor was normal in the majority of those examined [21].

The median survival was three years of age until the introduction of G-CSF treatment. Bone marrow transplant is effective treatment for those who have a donor. More than 90% of patients respond to daily G-CSF. However, KS is also premalignant. At least three patients who never received G-CSF were reported to have leukemia at 12 to 14 years of age, while 5% to 10% of those on G-CSF have already developed leukemia or MDS. Monosomy 7 and activating Ras mutations were also noted [22]. Some of those with leukemia were found to have a truncation of the C-terminal of the G-CSF receptor, even before the leukemia. The role of G-CSF in the leukemic transformation is unclear (albeit worrisome), since it may have enabled the patients to live longer and not succumb to serious infections before the natural history of the KS was expressed. Only further time on treatment will identify the specific role of G-CSF in the management of KS.

KS might be diagnosed if extreme neutropenia were found in a fetal blood sample, with the caveats mentioned above regarding SD.

**Thrombocytopenia with Absent Radii (TAR)**

Patients with TAR present in the neonatal period with thrombocytopenia and have bilateral (less than 2% are unilateral) absence of the radii, with thumbs present. These features are distinct from the radial anomalies in FA, where thumbs are absent if radii are absent, and where findings are frequently unilateral. Patients with trisomy 18 may have abnormal radii and thrombocytopenia, but there are other characteristic features of trisomy 18 which identify them. Many TAR patients have an apparently benign leukemoid reaction, although it has initially been mistaken for congenital leukemia. Some patients appear to have a milk allergy. Bone marrow megakaryocytes are absent or decreased and hypoplastic. Megakaryocyte progenitors were reduced in some studies but normal in others.

The major risk of serious or even fatal hemorrhage is in the first year, and the survival curve plateaus above 70% by four years of age. The most important therapy currently is platelet transfusion in the first year, using HLA-matched donors if necessary. In most cases, the platelet count improves to over 100,000/µl within a year. Bone marrow transplant may be curative but is not usually needed in this true single cytopenia. The role for possible platelet-stimulatory hematopoietic growth factors such as thrombopoietin has yet to be defined.

Prenatal diagnosis of TAR can be made by the combination of ultrasound evidence for radial aplasia and thrombocytopenia in a fetal blood sample.

**SUMMARY**

The inherited or congenital bone marrow failure syndromes represent a small fraction of the genes for aplastic
REFERENCES


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