Diagnosis, Genetics, and Management of Inherited Bone Marrow Failure Syndromes

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The inherited bone marrow failure syndromes are traditionally considered to be pediatric disorders, but in fact, many of the patients now are diagnosed as adults, and many diagnosed as children now live to reach adulthood. The most common of these rare disorders include Fanconi anemia, dyskeratosis congenita, Shwachman-Diamond syndrome and amegakaryocytic thrombocytopenia, which often develop aplastic anemia and may evolve into myelodysplastic syndrome and acute myeloid leukemia; and Diamond-Blackfan anemia, severe congenital neutropenia, and thrombocytopenia absent radii, single cytopenias that rarely if ever become aplastic but have increased risks of leukemia. In addition, the first three syndromes have high risks of solid tumors: head and neck and anogenital squamous cell carcinoma in Fanconi anemia and dyskeratosis congenita, and osteogenic sarcoma in Diamond-Blackfan anemia. Diagnosis of a marrow failure syndrome requires recognition of characteristic physical abnormalities when present, and consideration of these disorders in the differential diagnosis of patients who present with "acquired" aplastic anemia, myelodysplastic syndrome, acute myeloid leukemia, or atypically early cancers of the types seen in the syndromes. Ultimate proof will come from identification of pathogenic mutations in genes associated with each syndrome.

Introduction

The inherited bone marrow failure syndromes (IBMFS) need to be considered in the management of patients with aplastic anemia, with or without pathognomonic physical findings, as well as patients with atypically young onset of the specific types of cancers seen in the IBMFS. More than 25% of pediatric patients and approximately 10% of young adults who present with aplastic anemia have an inherited etiology. Diagnostic tests are available t hat are highly sensitive and specific, and mutated genes have been identified in many of the clearly inherited disorders as well as in some patients with apparently acquired aplastic anemia.

The data reviewed here are from a tabulation of all patients with an IBMFS reported in the literature through June, 2007 (BP Alter, unpublished data). While summaries of case reports are not an ideal source of epidemiologic data, they are often the major resource. Registries such as the International Fanconi Anemia Registry (IFAR), the United Kingdom Dyskeratosis Congenita Registry (DCR), the Diamond-Blackfan Anemia Registry (DBAR), the Severe Chronic Neutropenia International Registry (SCNIR), and others will be cited when appropriate.[1-7] These sources help to define the clinical and laboratory features of the recognized patients (and thus may not identify nonpenetrant individuals) but are less helpful with regard to the untreated and treated natural history, and of very limited value with regard to evidence-based management guidance. The literature suffers from reporting biases, and registries from volunteerism. There are no population-based prospective cohorts, nor controlled clinical trials, and thus no "gold standards."

The numbers of patients reported in the literature with individual data range from more than 100 to more than 1800 for the seven most "common" of these rare syndromes (**Table 1**). Cytopenias are often but not exclusively the problem that brings the patients to the attention of the hematologist. Pancytopenia is the usual presentation for Fanconi anemia (FA) and dyskeratosis congenita (DC), while anemia is the first sign in Diamond-Blackfan anemia (DBA). Patients with isolated neutropenia may have severe congenital neutropenia (SCN) or Shwachman-Diamond syndrome (SDS). Thrombocytopenia is the first hematologic sign in thrombocytopenia absent radii (TAR), and in amegakaryocytic thrombocytopenia (Amega). Aplastic anemia may be the hematologic endpoint in FA, DC, SDS, and Amega, while DBA, SCN, and TAR usually remain single cytopenias. Evolution to leukemia (primarily acute myelogenous leukemia [AML]) and/or myelodysplastic syndrome (MDS) is part of the natural history of these syndromes; in some patients, AML or MDS may be the first presenting sign. Solid tumors occur at increased frequency in FA, DC, and DBA. Mutated genes have been identified in all syndromes except TAR, although some patients who clearly have a specific syndrome lack mutations in the known genes.

It is important to emphasize that none of these syndromes is restricted to children. Patients with DC and FA may first be diagnosed as adults, and those with SDS, SCN, and DBA may either present or be recognized well beyond childhood (**Table 1**). TAR and Amega are the only syndromes where the initial diagnosis is limited to children. Recognition of an IBMFS is facilitated when the patient has characteristic hematologic and physical findings (**Tables 1** and **2**), but diagnoses are often missed absent phenotypic features. Up to 50% of reported patients were

Table 1. Major features of the inherited bone marrow failure syndromes.[1]

Feature	Fanconi anemia	Dyskeratosis congenita	Diamond-Blackfan anemia	Shwachman-Diamond syndrome	Severe congenital neutropenia[2]	Amegakaryocytic thrombocytopenia	Thrombocytopenia absent radii
~N Lit Cases	>1850	425	825	500	374	100	280
Male:Female	1.2:1	4:1	1.1:1	1.5:1	1.2	0.8:1	0.7:1
Age at diagnosis, median (range)[3]	6.6 (0–49)	15 (0–75)	0.25 (0–64)	1 (0–41)	3 (0–70)[2]	0.1 (0–11)	0 -0.6
% diagnosed ≥16 y of age[2]	9	46	1	5	13[2]	0	0
Physical findings[4]	Yes	Yes	Yes	Yes	No	No	Yes
Usual hematology	Pancytopenia	Pancytopenia	Anemia	Neutropenia	Neutropenia	Thrombocytopenia	Thrombocytopenia
Aplastic anemia	Yes	Yes	Rare	Yes	No	Yes	No
Screening test	Chromosome breakage	Telomere length	Adenosine deaminase	Pancreatic insufficiency	Promyelocyte arrest	Absent/abnormal megakaryocytes	Absent megakaryocytes
Leukemia or MDS	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Solid tumors	HNSCC, Gyn, brain	HNSCC	Osteosarcoma	No	No	No	No
Age at cancer, median (range)	15 (0.1–48)	28 (1.5–68)	23 (1.2–44)	18 (2–43)	14 (2–26)	12 (1.6–17)	5.3 (0–67)
Cumulative probability (%) of cancer by age 40-50 y[5]	85	35	52	71	55	53 (by age 17)	14
Projected median survival age[5]	23	45	39	35	50	16	>70
% alive or dead ≥16 y of age	27	58	22	22	32	5	16
Genetics	AR, XLR	XLR, AD, AR	AD	AR	AD, AR	AR	AR?
Genes[6]	>13	>3	>2	>1?	>3	1	?

Abbreviations: AR, autosomal recessive; XLR, X-linked recessive; AD, autosomal dominant; MDS, myelodysplastic syndrome; HNSCC, head and neck squamous cell carcinoma; Gyn, gynecological.
?, data not available or unclear. Cancer means leukemia or solid tumor, probability assuming no other adverse event occurs.

[1]Data derived from cases reported in the literature.
[2]Rosenberg et al[5]
[3]Age at hematologic diagnosis in most cases, except in SCN, where it is the age of starting treatment with granulocyte colony-stimulating factor (G-CSF).
[4]Physical findings are outlined in Table 2.
[5]Kaplan-Meier survival analysis, censored at death.
[6]Genes are described in Table 3.

Table 2. Characteristic physical findings in the inherited bone marrow failure syndromes.

System	Fanconi anemia	Dyskeratosis congenita	Diamond-Blackfan anemia	Shwachman-Diamond syndrome	Severe congenital neutropenia[2]	Amega	Thrombocytopenia absent radii
Skin	Café au lait, hyperpigmentation	Reticulated lacey pigmentation[1]	—	—	—	—	—
Short	Yes	Some; IUGR[2]	Yes	Yes	—	—	Some
Upper limbs	Abnormal thumbs, radii	Dysplastic fingernails[1]	Abnormal or triphalangeal thumbs, flat thenars	—	—	—	Absent radii, abnormal ulnae, humeri; thumbs present
Male gonads	Hypogonadism, cryptorchidism	Hypogonadism, urethral stricture	—	—	—	—	Occasional cryptorchidism
Head, face	Microcephaly, triangular face	Microcephaly[2]	—	Some dysmorphic facies	—	—	Hemangiomas
Eyes	Microphthalmia	Lacrimal duct stenosis, exudative retinopathy[3]	Hypertelorism, epicanthal folds	—	—	—	Hypertelorism, strabismus
Renal	Ectopic, horseshoe		Rare	—	—	—	Horseshoe
Ears, hearing	Small canals, deafness	Rare deafness	Rare microtia	—	—	—	Low set
Lower limbs	Congenital dislocation of hips	Dysplastic toenails	—	Metaphyseal dysostosis	—	—	Dislocated hips, abnormal knees
Cardiopulmonary	Rare	Pulmonary fibrosis	ASD, VSD	—	—	—	Cardiac anomalies
Gastrointestinal	Structural anomalies (atresias, imperforate anus)	Esophageal strictures, liver fibrosis	—	Malabsorption	—	—	Cow's milk allergy
Hair	—	Sparse, early grey	—	—	—	—	—
Oral	—	Leukoplakia,[1] caries	Cleft lip, palate	—	—	—	Micrognathia
Skeletal	Bony deformities	Osteoporosis, aseptic necroses	Short neck, Sprengel, Klippel-Feil	Metaphyseal dysostosis, thoracic dystrophy	—	—	Arms, legs
Developmental delay	Some	Some[2]	Rare	Some	—	—	—
Central nervous system	Small pituitary, PSIS	Cerebellar hypoplasia[2]	—	—	—	—	—
Normal	~25%	~10%?	~70%	?	All	All	None

Abbreviations: Amega, amegakaryocytic thrombocytopenia; UGR, intrauterine growth retardation; ASD, atrial septal defect; VSD, ventricular septal defect.

[1]The DC diagnostic triad includes pigmentation, dysplastic nails, and oral leukoplakia.
[2]Hoyeraal-Hreidarsson syndome.
[3]Revesz syndrome.

diagnosed as "adults" (age 16 years or older). From 5% to 60% of the cases survived to age 16, and the projected cumulative median survival ages range from 16 to 72 years. Thus, adult hematologists/oncologists may encounter patients with an IBMFS not yet diagnosed or who have outgrown the care of pediatric specialists. Literature reports, which date from the earliest cases, also underestimate the future impact that survival after hematopoietic stem cell transplantation (SCT) will have on adult hematology/oncology services.

Fanconi Anemia

FA is an autosomal recessive disorder associated with genomic instability. This diagnosis should be *considered* in patients with aplastic anemia, MDS, or AML, as well as head and neck squamous cell carcinoma (HNSCC) or gynecologic (especially vulvar/vaginal) cancer at younger than the usual age (e.g., less than 50 years old). The median age at diagnosis of FA in literature cases is under age 7 years, the reported range is up to age 49 years, and I am aware of patients diagnosed in their fifth decade (**Table 1**). Nine percent of the published cases were diagnosed as "adults." Characteristic birth defects are helpful when present, but their absence by no means excludes FA; 25% of the reported patients had no obvious physical anomalies (**Table 2**). The most frequent physical findings include short stature, café au lait spots, hyper- and hypopigmentation, microcephaly, microphthalmia, and abnormal thumbs with or without hypoplastic radii. Adult patients may have hypogonadism and infertility, and women have late menarche and early menopause; however, more than two dozen pregnancies and a half dozen fathers have been reported.

Our North American Survey (NAS) found that patients with FA are at exceedingly high risks of aplastic anemia, leukemia and solid tumors, with an observed/expected ratio of approximately 800 for AML and 50 for all cancers; the relative risk is in the thousands for esophageal and vulvar cancers, and the hundreds for HNSCC and liver tumors.[8] In a competing risk analysis (first adverse outcome is bone marrow failure, solid tumor, or leukemia) of 145 participants in the NAS, 55% developed severe bone marrow failure leading to death or transplantation, 29% developed a solid tumor, and 10% developed leukemia (excluding MDS) by age 48 years.[8] Data from 754 individuals in the IFAR were reported using death as the competing event; by age 50 the cumulative incidence of marrow failure was ~90%, hematologic malignancy (leukemia or MDS) 40%, and solid tumor 35%.[2] In similar analyses of the literature and the NAS, the probability of a solid tumor was 75% by age 45 years, and of leukemia was approximately 30% by age 30 years.[8,9] The cumulative probability of any malignancy in the literature cases was 85% by age 45, and the median age was 15 years (**Table 1**). Thus, data from the literature, the IFAR, and the NAS concur. SCT cured bone marrow failure but increased the risk of solid tumor more than 4-fold, and shifted the median age of tumors to 16 years earlier than in those who did not undergo transplantation.[10] The diagnosis of a malignancy preceded the diagnosis of FA in more than one-third of the literature cases with cancer, and the concern is for those in whom FA was never diagnosed and who succumbed to their cancer or its treatment.[9] It has been suggested that bone marrow clones involving monosomy 7 or particularly add(3) (partial trisomies or tetrasomies of 3q) may have a bad prognosis (evolution to AML),[11] but further data are required to confirm this.

The diagnosis of FA requires performance of a test of chromosomal breakage. Peripheral blood T-lymphocytes are stimulated to divide with a mitogen and then exposed to a DNA cross-linking agent such as diexpoxybutane (DEB) or mitomycin C (MMC). Metaphase spreads of FA cells have increased numbers of chromosomal breaks per cell and an increased fraction of cells with breaks; with MMC, there are also increased quadriradial forms. This test may be normal in cells from some patients with FA because of somatic mosaicism, where a hematopoietic stem cell has undergone gene correction, and its progeny have repopulated the bone marrow. There may be a normal chromosomal stability response in lymphocytes, which are derived from the corrected stem cell. The corrected cells have a growth advantage (see below). If mosaicism is suspected, skin fibroblasts should be used for the breakage assay.[12]

Definitive proof of FA is accomplished by identification of the gene complementation group, followed by demonstration of the disease-causing mutation. Thirteen FA genes have been cloned to date; affected patients have mutations in both alleles (with the exception of the X-linked recessive gene for *FANCB*), and unclassifiable patients presumably have mutations in FA genes that have not yet been identified. The known genes are *FANCA*, *FANCB*, *FANCC*, *FANCD1/BRCA2*, *FANCD2*, *FANCE*, *FANCF*, *FANCG*, *FANCI*, *FANCJ/BACH1/BRIP1*, *FANCL*, *FANCM*, and *FANCN/PALB2* (**Table 3**). Following DNA damage, the products of *FANCA*, *FANCB*, *FANCC*, *FANCE*, *FANCF*, *FANCG*, *FANCI*, *FANCL*, and *FANCM* form a multimeric nuclear complex that facilitates FANCD2 monoubiquitination either in S phase or in response to DNA damage. Monoubiquitination of FANCD2 then results in a complex with other FA proteins, including FANCD1 and with non-FA proteins, including BRCA1, RAD51, MRE11, ATM, BLM, RAD50, NBS, and others, which leads to formation of DNA damage repair foci (**Figure 1A**; see Color Figures, page 520).[13] Hematopoietic failure in FA is associated with excess apoptosis of stem and progenitor cells, and may be independent of the defective FA complex. Non-FA proteins that bind to FA proteins are involved in prevention of oxidative stress and programmed cell death and include the STAT pathway, heat shock proteins (hsp70), and GSTP1. Other FA-interacting non-FA proteins are interferon-γ, tumor necrosis factor-α, mip-1α, fas ligand, and double-stranded RNA.[14] FA cells are also defective in intra-S phase cell-cycle checkpoints. The FA pathway is inactivated in up to 25% of "sporadic" cancers, including ovarian, breast,

Table 3. Mutant genes in the inherited bone marrow failure syndromes, as of 2007.

Disorder	Gene	Locus	Genomic DNA kb	cDNA kb	Exons	Amino acids	Genetics	~% of pts
Fanconi anemia	*FANCA*	16q24.3	80	5.5	43	1455	AR	~70
	FANCB	Xp22.31	30	2.8	10	859	XLR	Rare
	FANCC	9q22.3	219	4.6	14	558	AR	~10
	FANCD1/(BRCA2)	13q12.3	70	11.4	27	3418	AR	Rare
	FANCD2	3p25.3	75	5	44	1451	AR	Rare
	FANCE	6p21.3	15	2.5	10	536	AR	~10
	FANCF	11p15	3	1.3	1	374	AR	Rare
	FANCG (*XRCC9*)	9p13	6	2.5	14	622	AR	~10
	FANCI (KIAA1794)	15q25-26	73	4.5	38	1328	AR	Rare
	FANCJ (BACH1/BRIP1)	17q22.3	180	4.5	20	1249	AR	Rare
	FANCL (PHF9/POG)	2p16.1	82	1.7	14	375	AR	Rare
	FANCM (Hef)	14q21.3	250	6.5	22	2014	AR	Rare
	FANCN (PALB2)	16p12.1	38	3.5	13	1186	AR	Rare
Dyskeratosis congenita	*DKC1*	Xq28	15	2.6	15	514	XLR	36
	TERC	3q26	1.25	0.45	—	RNA	AD	6
	TERT	5p15.53	43	4.02	16	1132	AD	1
	NOP10	15q14-q15	2.25	0.195	2	64	AR	<1
Diamond-Blackfan anemia	*RPS19*	19q13.3	11	0.6	6	145	AD	25
	RPS24	10q22-23	7	0.6	7	133,131,130	AD	2
	?	8p	—	—	—	—	—	—
Shwachman-Diamond syndrome	*SBDS*	7 centromere	7.9	1.6	5	250	AR	95
Severe congenital neutropenia*	*ELA2*	19p13.3	5.3	0.9	5	240	AD	70-90
	GFI1	1p22	12.9	1.27	9	422	AD	2
	WAS	Xp11.33-.22	7.6	1.8	12	502	XLR	—
Kostmann syndrome*	*HAX1*	1q21.3	4.1	1.1	7	279	AR	—
Amegakaryotic thrombocytopenia	*MPL*	1p34	17	3.7	12	635	AR	~100
Thrombocytopenia absent radii	? bigenic	1q21.1	—	—	—	—	AR	100

***KS is autosomal recessive, while most patients with SCN have autosomal dominant inheritance; *WAS* is X-linked.

non–small cell lung, HNSCC, and cervical (by methylation of *FANCF*), adult AML (deletion or mutation of *FANCA*), and pancreatic cancer (mutation of *FANCC* or *FANCG*).[13,15]

Although data on genotype-phenotype correlations are still early, some genotypes are more severe, such as *FANCC* IVS4+4A>T (founder effect in Ashkenazi Jews), *FANCD2*, *FANCD1/BRCA2* and *FANCN/PALB2*; the latter two have very high rates of severe birth defects and early childhood-onset brain tumors, Wilms tumor, and AML. We reported a predictive model for the association between radial ray and other specific anomalies and early bone marrow failure; patients with FA who lack birth defects and do not have early hematologic manifestations are more likely to develop solid tumors as adults.[12,16]

Management of patients with FA begins with a thorough history and physical examination, laboratory tests and cancer screening. FA is truly a multisystem disease, and clinical assessment involves every organ. Non-hematologic subspecialties should be involved, including cardiology, dentistry, endocrinology, gynecology, hand surgery, head and neck oncology, ophthalmology, otolaryngology, plastic surgery, neurology, and radiology. Recommended hematologic monitoring involves blood counts every 3 to 4 months, and annual bone marrow aspirates and biopsies (including cytogenetics). Solid tumor surveillance is best done by dentists, head and neck oncology teams, and gynecologists. The impact on HNSCC and gynecologic cancers of the vaccine for human papilloma virus (HPV) is awaited with interest.

According to consensus guidelines developed by a panel of experts, treatment of bone marrow failure should be initiated when the hemoglobin (Hb) is less than 8 g/dL, platelets less than 30,000/μL, and absolute neutrophil count less than 500/μL (or there are symptoms from anemia, bleeding, or infection).[17] An SCT is recommended if there is an HLA-matched sibling donor, and *consideration* of transplantation with an unrelated or mismatched donor; andro-

gen therapy may improve but not cure aplastic anemia (**Table 4**). Granulocyte colony-stimulating factor (G-CSF) alone or combined with erythropoietin and/or androgens may be helpful. Preparative regimens for SCT in FA are more specialized than for acquired aplastic anemia due to the chromosomal instability present in every cell in FA, and a sibling donor must be proven to not have FA. Treatment of cancer requires close communication between the primary hematologist and the oncologist, because chemotherapy needs to be modified to use agents that do not damage DNA, and radiation needs to be considered carefully.[18]

Dyskeratosis Congenita

DC is the one IBMFS in which many patients reach adulthood prior to the diagnosis, and most patients with DC are cared for by adult hematologists (**Table 1**). In fact, because most of the physical findings appear with increasing age (**Table 2**), this is the one IBMFS that is under-recognized by pediatric hematologists. Patients often do not have hematologic signs in childhood or possibly ever (determined by the presence of silent carriers in DC families). The median reported age at diagnosis is around 15 years, with more than half older than 15 years (and up to 70 years or older) when reported. The diagnostic triad of lacey reticulated pigmentation, dysplastic nails, and oral leukoplakia is frequently absent, and the DCR includes those with at least one triad feature plus at least two other somatic features (**Table 2**).[3,19] Early childhood variants of DC are the Hoyeraal-Hreidarsson syndrome (characterized by intrauterine growth retardation, developmental delay, microcephaly, cerebellar hypoplasia, immunodeficiency, and bone marrow failure) and Revesz syndrome (identified by similar features plus exudative retinopathy). The reported cumulative incidence of marrow failure in the DCR is 94% by age 40 years, but this may reflect biased ascertainment of patients with DC by a registry maintained by a hematologist.[3]

The crude frequency of malignancy in patients with DC is 9% to 10% in the literature cases and in the DCR; the majority of the cancers are carcinomas, particularly head and neck and esophageal in 60% of the patients, and colon and anus in 15%. Three patients were reported who had MDS and one AML, but MDS may be underreported (based on my own clinical experience). However, unlike FA, where the frequency of AML and solid tumors is equivalent, solid tumors are much more frequent in DC. The median age for cancer in the literature cases is 28 years, and the cumulative incidence by age 50 years is 35% for all malignancies, substantially lower than in FA, but still striking.

The diagnosis of DC is easily made if the patient has the diagnostic triad, even without any hematologic signs. The converse is the problem: to diagnose DC in a patient with thrombocytopenia or pancytopenia, with minimal or no physical findings. We recently demonstrated that this diagnosis is facilitated by examination of the length of telomeres in leukocyte subsets using flow cytometry with fluorescence in situ hybridization (flow-FISH).[20] Individuals with telomeres less than the first percentile of age-matched control cells had DC, with mutations in known genes where available; this included silent carriers, who did not have physical or hematologic abnormalities. Although patients with acquired aplastic anemia may have short telomeres in granulocytes,[21] patients with DC had short telomeres in all leukocyte subsets. Lymphocytes alone, or the combination of lymphocytes, naïve T cells, and B cells, had both sensitivity and specificity above 90% in distinguishing DC from unaffected relatives and from

Table 4. Management of hematologic complications in the inherited bone marrow failure syndromes and responses.[1]

Treatment	Fanconi anemia	Dyskeratosis congenita	Diamond-Blackfan anemia	Shwachman-Diamond syndrome	Severe congenital neutropenia	Amega	Thrombocytopenia absent radii
Androgens	Yes	Yes	—	—	No	Yes	No
Prednisone	—	—	Yes	—	No	—	No
G-CSF	Rare	Rare	No	Yes	Yes	—	No
Erythropoietin	Rare	Rare	—				
Red cell transfusions	As needed	As needed	First year; as needed	Rare	No	As needed	—
Platelet transfusions	As needed	As needed	No	Rare	No	As needed	First year; as needed
Stem cell transplantation[2]	Yes	Yes	Yes	Yes	Yes	Yes	Yes
"Spontaneous" remission	Mosaicism	Rare	~25%	Pancreatic function may improve with age	No	No	Yes

Abbreviations: Amega, amegakaryocytic thrombocytopenia; G-CSF, granulocyte colony-stimulating factor.
Yes, proven role; —, used rarely, role uncertain; No, role not proven and may be detrimental.
[1]Response rates and durations vary according to the disorder.
[2]Transplantation regimens and successes depend on the disorder and the donor type.

FA, SDS, or DBA. This test may be the equivalent for DC of the chromosome breakage test for FA.

Four genes have now been identified in DC: one X-linked, *DKC1*, which codes for dyskerin (involved in ribosome biogenesis and telomere maintenance), two autosomal dominant, *TERC* and *TERT*, which code for the mRNA for telomerase and the telomerase enzyme, respectively, and one autosomal recessive, *NOP10* (**Table 3**).[19,22] These gene products are part of the telomere maintenance pathway by which the ends of chromosomes are prevented from shortening substantially with each cell replication.[23] Many genes are involved in this pathway, and future research will probably indict others among them as causal in DC (**Figure 1B**; see Color Figures, page 520); very short telomeres may provide a marker for genetic linkage studies. Bone marrow failure may be related to premature shortening of telomeres, reducing the proliferative potential of the hematopoietic stem cells.[4] Approximately 60% of patients with DC in the DCR[19] and in our own experience lack mutations in the four known genes.

Genotype/phenotype associations are complicated: patients with mutations in *DKC1*, *TERC* or *TERT* may have severe phenotypes and early onset aplastic anemia, or be identified because of aplastic anemia in the absence of the characteristic physical findings, or even as silent carriers in families with individuals with classical features and/or aplastic anemia.[4,20] The labeling of a patient with aplastic anemia as someone with DC, rather than an acquired disorder, is more than semantic, since it has implications for treatment, genetic counseling, and other family members.

Treatment guidelines for DC are similar to those for FA with regard to when to treat, and to consider SCT when there is an unaffected HLA-matched sibling donor. Since siblings may be silent carriers, we suggest examination of telomeres in leukocyte subsets to avoid inadvertent use of a donor whose stem cells may fail to engraft.[24] The immunosuppression used to treat acquired aplastic anemia is unlikely to be successful.[25] As in FA, many patients will respond to androgens (**Table 4**). A rare patient has improved with G-CSF plus erythropoietin. However, we recently learned that androgens plus G-CSF may lead to splenic peliosis and rupture, and thus do not recommend this combination in DC.[26] As in FA, SCT for DC requires modified protocols, particularly since patients with DC are at risk of pulmonary and liver fibrosis as part of their underlying genetic disorder. Monitoring of patients with DC and their "unaffected" family members with very short telomeres is similar to the protocol for FA, i.e., blood counts, bone marrow examinations, and cancer surveillance as described above.

Diamond-Blackfan Anemia

Patients with DBA usually present in infancy (90% younger than 1 year in literature cases), although older adults have been reported. Many patients will do well and eventually need care by adult hematologists (**Table 1**). Physical abnormalities may be as subtle as flattened thenar muscles; short stature is common, while triphalangeal thumbs, microtia and cleft palates are rare (**Table 2**). The diagnosis is commonly based on macrocytic anemia with erythroid hypoplasia, with usually normal platelets and white blood cells; evolution to full blown aplastic anemia is sufficiently rare to lead to questioning the initial diagnosis. Leukemia and MDS have been reported, as have a handful of solid tumors, including a half-dozen cases of osteogenic sarcoma.[6] The median age in the literature for cancer is 23 years, and the cumulative probability is 50%.

There is no "chromosome breakage" or "telomere length" test for DBA. The majority of patients have an increased level of red cell adenosine deaminase (ADA), which is a specific but not entirely sensitive test (there are very few false positives, but approximately 10% of patients with classical DBA have normal ADA levels). Most patients have macrocytic red cells, with increased fetal hemoglobin (Hb F), but these nonspecific findings are common to any of the IBMFS.

Most cases of DBA are associated with autosomal dominant inheritance (including new mutation sporadic cases). Two genes represent about 25% of known patients (**Table 3**). *RPS19* and *RPS24* code for ribosomal proteins; haploinsufficiency leads to inadequate formation of 40s ribosomal subunits.[27] The precise mechanism whereby this results in erythroid hypoplasia is not yet fully elucidated, but may include decreased formation of ribosomes, altered polysome recruitment of mRNA, altered initiation of translation of transcription factors, or disturbed phosphorylation of RPS19.[28] Since the majority of patients do not have mutations in *RPS19* or *RPS24*, further gene hunting is ongoing.

Most patients with DBA respond to corticosteroids, which are used initially in high doses and then tapered to the lowest and preferably alternate day dose that can maintain an adequate hemoglobin level (usually > 8-10 g/dL) (**Table 4**). Those who are not steroid responsive, or whose dose is unacceptably high, can receive transfusions of packed red cells. These patients will eventually need iron chelation, however. Approximately 25% of patients with DBA improve and no longer require treatment ("spontaneous remission"), although they may relapse as they move through adulthood. Some hematologists tend to transfuse patients with DBA during the first year and in the adolescent growth spurt, due to concern about growth impairment from steroids; others are more concerned about iron overload and transfusion-related viruses, and attempt to find rational steroid doses for these children; there is no consensus here. SCT has been used successfully from both related and unrelated donors, and to date there are no reports of post-SCT malignancies.

Close monitoring of blood counts is required, even in patients who have had a remission, because they may relapse. In treated patients, the response to steroids may fluctuate. Very rarely do patients improve on cyclosporine, and even fewer on androgens. There is no consensus on the

clinical applicability of annual bone marrow examinations, although some do recommend this in anticipation of evolution to MDS or leukemia.

Shwachman-Diamond Syndrome

SDS is an autosomal-recessive disorder, primarily diagnosed in childhood, where the major symptom is malabsorption, with excessive fatty stools and failure to thrive (**Table 1**); patients rarely are identified as adults. The usual physical findings include short stature, with metaphyseal dysostosis particularly at the hips and femurs in about half the patients. Some patients have learning disabilities (**Table 2**). Neutropenia is usually identified during the general evaluation. Other cytopenias and macrocytosis may be observed, and many patients evolve to aplastic anemia, MDS, or leukemia. The median age for the latter in the literature reports is 18 years, and the age-dependent cumulative probability of leukemia is greater than 70%. Unlike FA or DC, there have not been reports of patients with SDS and a solid tumor.

While there are many causes of malabsorption, the gastrointestinal component of the diagnosis of SDS depends on proof of exocrine pancreatic insufficiency. This is currently done by demonstration of reduced levels of serum trypsinogen (which may improve with age) and isoamylase (which fails to increase in the normal manner after age 3 years in SDS), and/or detection of a fatty pancreas by ultrasound, computerized tomography, or magnetic resonance imaging.[29] In addition, neutropenia below 1500/μL must be documented on more than one occasion.[7] There are several patients with neutropenia and histories of diarrhea who do not fulfill these strict criteria, whom we now call "Shwachman-like" for want of a better term.

Patients with SDS quite often have bone marrow cytogenetic clones, particularly involving chromosome 7 [monosomy 7, der(7), and i(7q)], as well as del(20q). No patients with i(7q) were reported to develop AML, although half of the clones were associated with dysmyelopoietic bone marrow morphology, and almost half had hypoplastic but otherwise normal bone marrows. The prognostic significance of clonal cytogenetics is confusing in any of the IBMFS, and particularly so in SDS, where much longer longitudinal follow-up is needed.

More than 95% of patients who meet the diagnostic criteria for SDS have mutations in one gene, *SBDS* (Shwachman-Bodian-Diamond syndrome) (**Table 3**).[30] Most mutations are due to gene conversion between the *SBDS* gene and an adjacent pseudogene. There are two common alleles, and some SBDS protein is apparently required, because no patients are homozygous for the null allele, 183-184TA>CT (if heterozygous they have another, hypomorphic allele), while many are homozygous for the hypomorphic allele, 258+2T>C. Similar to the RPS genes in DBA, SBDS also has a function in ribosome formation, in this case involving the 60s subunit.[31] However, the reason for neutropenia or bone marrow failure in patients with *SBDS* mutations remains unclear.

Malabsorption is treated by administration of pancreatic enzyme supplements with food, with the addition of the fat soluble vitamins (A, D, E, K). Pancreatic function often improves with age, and fewer or no supplements may be needed by adult patients. Neutropenia is infrequently of clinical significance and usually improves on low doses of G-CSF (**Table 4**). There is no evidence that such treatment is associated with an increase in the already high baseline risk of AML.[5] Bone marrow function does not improve with age, and clonal cytogenetics, MDS, and leukemia may develop. Patients should have frequent blood counts, and annual bone marrow examinations with cytogenetics may not be strictly "clinically" indicated, but may be helpful in interpretation of findings in evolving marrows. SCT has been used successfully in many patients with SDS, although there is increased cardiac toxicity from preparative regimens that include cyclophosphamide.

Severe Congenital Neutropenia

SCN is the term for a group of disorders characterized by early onset neutropenia (< 500/μL), pyogenic infections, and a marrow maturation arrest. The diagnosis is usually made in infancy (**Table 1**). The inheritance was autosomal recessive in the family first described by Kostmann, but most cases are sporadic or autosomal dominant (**Table 3**). There are no characteristic physical abnormalities, and the diagnosis depends only on demonstration of severe neutropenia on at least three blood counts separated by more than a month. Cyclic neutropenia needs to be excluded, ideally by obtaining blood counts twice weekly for 6 weeks, since the usual cycle is approximately 21 days. Other causes of neutropenia should also be investigated, such as immune-mediated or hypersplenism. A bone marrow examination is useful to demonstrate the promyelocyte arrest, rule out early MDS or leukemia, and examine bone marrow cytogenetics for a clonal disorder.

Most patients with SCN have a dominant mutation in the gene for neutrophil elastase, *ELA2*, while approximately 2% have a mutation in GFI1, and a small proportion have X-linked disease due to mutations in *WAS* (**Table 3**).[32] A few families, including the original Kostmann family, have homozygous mutations in *HAX1*.[33] Patients with cyclic neutropenia also have mutations in *ELA2*, and the cyclic and SCN mutations are generally separated at the amino- and carboxy-terminal parts of the gene.[34] The function of the normal SCN genes may be to prevent apoptosis of myeloid precursor cells. Several patients with SCN (particularly those with AML) have had somatic mutations in *G-CSFR*, but a prospective study has not been done to determine whether this is predictive of leukemic transformation.

The treatment of neutropenia in patients with SCN is G-CSF, to which most respond with improved neutrophil counts (**Table 4**). The aim is to raise the count to above

1500/μL, usually using 5 to 10 μg/kg/day or every other day. There were 3 patients with SCN who developed AML in the pre–G-CSF era, suggesting that SCN is a preleukemic disorder. Following more than 20 years of use of G-CSF, it is clear that MDS/AML is a part of the syndrome. The role of growth factor treatment in the malignant evolution is not clear. Analysis of 374 patients in the SCNIR demonstrated that the cumulative incidence of MDS/AML increased with time on treatment, reaching 21% after 10 years, while death from sepsis was 8%.[5] However, rather than indict G-CSF as causal in the malignant evolution, it was suggested that patients with SCN who had "healthier" stem cells required lower doses of G-CSF (below the median of 8 μg/kg/day) in order to attain the target neutrophil count, and had a cumulative incidence of MDS/AML of 11%. In contrast, those patients whose stem cells did not respond to lower doses, and who despite higher G-CSF doses (above the median) still did not achieve an adequate neutrophil count, had a cumulative incidence of MDS/AML of 40%. These findings suggest that at least the unresponsive patients should be considered for SCT, particularly if they have a matched sibling donor. No solid tumors were reported.

Amegakaryocytic Thrombocytopenia

Unlike most of the IBMFS, patients with Amega (and those with SCN, above) do not have a characteristic physical appearance, except perhaps for bruises due to thrombocytopenia (**Table 2**). They usually present in infancy with thrombocytopenia, which often progresses to aplastic anemia and/or AML. Since mild thrombocytopenia may be missed, some patients are only diagnosed retrospectively (**Table 1**). However, all reported patients were diagnosed and reported before adulthood; this could be biased reporting. Since there are no physical features that specifically suggest Amega, the hematologist must look for a history of early bruising, and must consider this diagnosis in any young patient with nonimmune thrombocytopenia, or pancytopenia, particularly with macrocytic erythrocytes and/or increased Hb F.

The cumulative incidence of aplastic anemia in 47 patients reported in the literature was 91% by age 13 years, and the cumulative incidence of AML in the 4 such cases was 55% by age 17 years. The projected median age for aplastic anemia was 5 years, and for AML 17. Amega is classified among the IBMFS as preaplastic and preleukemic. No solid tumors have been reported.

Among the IBMFS, the genetic basis for Amega is the easiest to understand, since all examined cases had recessive mutations in *MPL*, the gene for the receptor for thrombopoietin, the hematopoietic growth factor that stimulates megakaryopoiesis (**Table 3**). Recent studies suggested that there is a genotype/phenotype correlation, in which null mutations result in severe early thrombocytopenia and rapid progression to pancytopenia ("CAMTI"), while missense mutations have some improvement in platelet counts early in childhood and delayed evolution to aplastic anemia ("CAMTII").[35] The number of patients for whom the mutation is reported is too small to determine whether the risk of aplastic anemia or AML differs between the two groups.

Patients with Amega and pancytopenia may show improved blood counts on androgens, but the recommendation now is to consider SCT. There are no published large series, but it appears that patients with Amega do well with the SCT protocols in current use for acquired aplastic anemia (personal experience). The problems of SCT-associated toxicity that are seen in FA and DC (and cardiotoxicity in SDS) do not appear to be relevant in Amega.

Thrombocytopenia Absent Radii

The easiest clinical diagnosis is the TAR syndrome, usually suspected at birth in a child with absent radii but thumbs present, albeit often malformed (to distinguish from FA, where thumbs are absent if radii are absent). A low platelet count confirms the diagnosis, while a transient leukemoid reaction is also common (ascribed to cows' milk allergy). Patients with TAR generally survive long enough to need care from adult hematologists; 16% of the literature cases had reached age 16 years when reported, and the survival curve reaches a plateau of 78% at age 5 years (**Table 1**). These patients may have additional somatic anomalies, including short or absent ulnae, absent humeri, dislocated hips, abnormal knees, and cardiac defects (**Table 2**). Many of the birth defects resemble those seen in FA, but are less frequent in TAR.

Close to 300 patients have been reported, with 4 cases of leukemia at ages 1 month to 41 years (3 AML, 1 ALL), and 3 patients with 5 solid tumors (a newborn with neuroblastoma, a 21 year old with histiocytosis, and a 67 year old with ileal adenocarcinoma, followed by ovarian cancer and bladder SCC). The solid tumors might be coincidental to the TAR, but the reports of 3 AML suggest that TAR should be classified with the other IBMFS as preleukemic. However, aplastic anemia is not part of TAR.

The genetics of TAR remain unclear. The *MPL* gene is normal, and serum levels of thrombopoietin are elevated, suggesting a block in differentiation to megakaryocytes. Indeed, bone marrow examination shows decreased and/or abnormal megakaryocytes as the only abnormality. A recent study identified an interstitial deletion of 200 kb on chromosome 1q21.1 in all of 30 patients with TAR, and only 25% of the parents (who were all unaffected). These results suggest a bigenic inheritance pattern, in which deletion 1q21.1 is necessary but not sufficient, and a second unknown mutant gene is required for the syndrome.[36]

Ten percent to 20% of infants with TAR require transfusions, but in most cases the platelet count starts to rise by 1 year of age, and may reach a level at which orthopedic surgery can be performed without platelet coverage, although it does not usually become normal. SCT is a very rare requirement.

Conclusions

An IBMFS should be considered for patients with one or more cytopenias due to failure of production. Clues may be obtained from the history of the individual or their family (marrow failure, MDS, leukemia, or solid tumors), as well as from overt or subtle findings on physical examination (e.g., skin, skeletal anomalies) or imaging (e.g., renal or pancreatic ultrasound). Macrocytosis, often with increased Hb F, suggests an insidious onset, consistent with an inherited disorder. A history of failure to respond to immunotherapy is often helpful, although it is preferable to make the diagnosis before such therapy has been used.

Our knowledge of the entire clinical spectrum of each of the IBMFS is still incomplete. Literature case reports are a limited source of epidemiologic data, due to biased reporting of interesting patients (especially those with leukemia or solid tumors), missing data in many fields of interest, lack of proof of diagnosis or adverse outcomes, left truncation (failure to diagnose and report patients who develop relevant complications), unidentified duplicate reporting, and inadequate denominators. Large registries may have biased ascertainment or enrollment, and data from those sources cannot be combined with case reports, since there may be duplication of patients with adverse outcomes. Unbiased disease-specific cohorts must be established, closely followed for a long time, and analyzed with time-dependent models in order to provide insight into the natural history, the true risks of malignancy, and the proper management of each condition. Determination that a person with aplastic anemia or a single cytopenia has a deleterious mutation in a gene for an IBMFS should lead to genetic counseling of that person and their family members, who should also be examined to identify carriers of recessive conditions, and nonpenetrant individuals who have the same dominant mutant gene as the affected patients.

The recommended diagnostic scheme for each IBMFS is to do chromosome breakage for FA, telomere length by flow-FISH in blood leukocyte subsets for DC, red cell ADA for DBA, serum trypsinogen at any age and isoamylase after age 3 years for SDS, rule out cyclic and immune neutropenia for SCN, rule out immune-mediated thrombocytopenia for Amega, and identify the absence of radii for TAR. Where possible, identification of mutant genes in the proband will then confirm the diagnosis and lead to classification of family members. Clinical judgment (including family histories and physical examinations) must be used to determine which tests will be done, and which adult ages to include. There are no data to inform us with regard to the upper age beyond which any of the IBMFS need not to at least be considered, since the penetrance is highly variable within and across syndromes.

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