

Stem Cell Disorders

CHAPTER 8

Inherited Bone Marrow Failure Syndromes

Blanche P. Alter and Alan D. D'Andrea

Constitutional aplastic anemia was defined by O'Gorman Hughes (1) as "chronic bone marrow failure associated with other features, such as congenital anomalies, a familial incidence, or thrombocytopenia at birth." In fact, he was referring to *inherited aplastic anemia*. Patients with constitutional aplastic anemia are genetically at risk for bone marrow failure, which may be expressed at birth (and therefore congenital), but it often develops later. O'Gorman Hughes further divided constitutional aplastic anemia into three types by using terminology that is confusing and no longer relevant. We refer to each disorder by its eponym, until more specific information becomes available, and to the entire group as the *inherited bone marrow failure syndromes*, so as to distinguish them from acquired aplastic anemia.

We can sometimes identify homozygotes for autosomal-recessive types of inherited bone marrow failure syndromes (or hemizygotes for X-linked disorders) by phenotype, although we cannot easily identify heterozygotes except by inference from family studies or by mutation analysis in disorders and in families in which the mutation is known. Inherited bone marrow failure is probably more common than published reports indicate, because the phenotype may range from severely abnormal to entirely normal (see the section Inheritance and Environment). In this section, we discuss the classic phenotypes in the major inherited bone marrow failure syndromes and emphasize the variation within each category. Some of this variation may result from the inadvertent inclusion of patients with congenital but not inherited phenotypes that resemble the genetic conditions (phenotypes), because until recently there was no specific diagnostic test for most of the disorders.

The incidence of inherited marrow failure is difficult to ascertain from the literature. Among 134 patients with all types of aplastic anemia who were seen at the Children's Hospital Medical Center in Boston from 1958 to 1977, 40 patients appeared to have inherited disorders (2) (Fig. 8-1). Twenty-six patients had Fanconi's anemia (all diagnosed in the era before testing of chromosome breakage), four patients developed aplastic anemia after amegakaryocytic thrombocytopenia, and ten patients had familial aplastic anemia without physical or cytogenetic evidence for Fanconi's anemia. In 21 years at the Prince of Wales Hospital in Australia (1964 to 1984), 12 of 34 patients were found to have inherited syndromes, including eight patients with Fanconi's anemia (3).

In our earlier analysis of the literature, we found that approximately 25% of cases of childhood aplastic anemia were diagnosed as inherited, which is probably an underestimate (4). The genetic syndromes must be considered carefully in patients of any age with aplastic anemia, because the prognosis, treatment, and approach to bone marrow transplantation (BMT) and potential gene therapy are different when the hematologic disorder is inherited rather than acquired. More detailed reviews of the inherited bone marrow failure syndromes can be found elsewhere (5,6).

PANCYTOPENIAS

Fanconi's Anemia

Fanconi's anemia was first described by Fanconi (7) in 1927 in three brothers with pancytopenia combined with physical

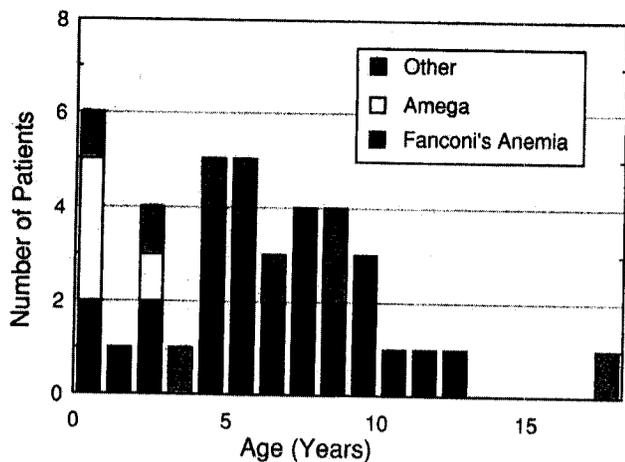


Figure 8-1. Age at diagnosis of inherited aplastic anemia in 40 patients who were seen at the Children's Hospital Medical Center, Boston, from 1958 to 1970. Twenty-six patients had Fanconi's anemia, four patients had amegakaryocytic thrombocytopenia (Amega) and developed aplastic anemia, and ten patients (in six families) had other familial bone marrow failure syndromes. (Adapted from Alter BP, Potter NU, Li FP. Classification and aetiology of the aplastic anaemias. *Clin Haematol* 1978;7:431-465.)

abnormalities. The anemia was macrocytic and thus was called *pernizioisiforme*, despite no further evidence for megaloblastic anemia. Uehlinger (8) then reported a similar patient with aplastic anemia and abnormalities of the thumb and kidney, and Fanconi (9) indicated that Naegeli had suggested in 1931 that familial aplastic anemia plus congenital anomalies should be called *Fanconi's anemia*.

The diagnosis of Fanconi's anemia is based on the finding of characteristic chromosomal breaks in cells that are cultured with a clastogenic agent and is confirmed by complementation or mutation analysis (see the section Cellular Phenotype). The patient's physical appearance may be normal, and he or she may or may not have aplastic anemia. More than 1200 cases of Fanconi's anemia have been mentioned in the literature with sufficient detail for many of the analyses that are described in this section (Table 8-1). The male to female ratio is 1.2:1.0, which is consistent with autosomal-recessive inheritance. Patients have been reported from more than 60 countries and represent all ethnic and racial groups, including whites, blacks, Asians, Native Americans, and persons from India. Figure 8-2 shows the distribution of ages at diagnosis.

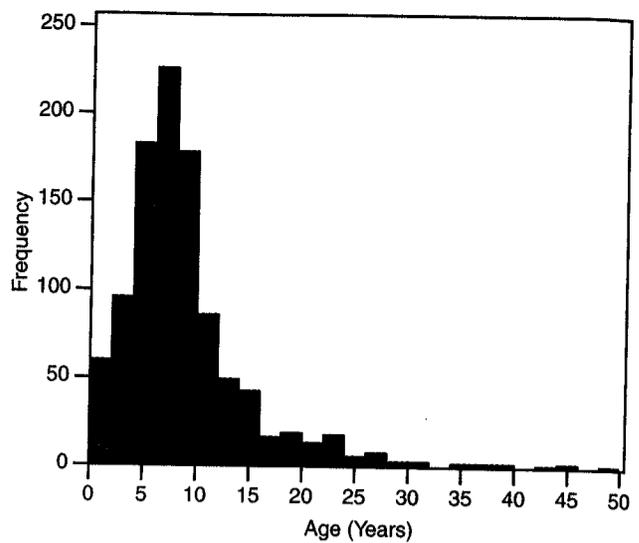


Figure 8-2. Age at diagnosis in approximately 1200 cases of Fanconi's anemia that were published from 1927 to 2000.

Although diagnoses were usually made when aplastic anemia was detected, some diagnoses were made in nonanemic siblings. More recently, diagnoses have also been made after cytogenetic studies in patients with physical anomalies and normal blood counts. The median age at diagnosis in males and females was 6.5 and 8 years of age, respectively. Although the majority of the patients were younger than 15 years of age at the time of the diagnosis of Fanconi's anemia, 4% were diagnosed between birth and 1 year of age, and less than half of those patients had hematologic manifestations at the time of diagnosis. Twenty-seven patients were male, and 16 patients were female. Thus, Fanconi's anemia cannot be excluded as the cause of aplastic anemia in the first year of life, because "the patient is too young." At the other end of the age spectrum, 9% of patients were diagnosed at 16 years of age or older. Fifty-seven patients were male, and 47 patients were female. The proportion of patients with Fanconi's anemia who are adults is undoubtedly underestimated. Inherited aplastic anemia is not usually considered in an adult with aplastic anemia, with or without characteristic physical findings. Testing for chromosome breakage is required to identify Fanconi's anemia as the cause of the aplasia, but it is usually not performed for adult patients.

TABLE 8-1. Fanconi's Anemia Literature

	All Patients
Number of cases	1206
Male to female ratio	1.24:1.00
Male age at diagnosis (in yr)	
Mean	8.1
Median	6.5
Range	0-48
Female age at diagnosis (in yr)	
Mean	9
Median	8
Range	0-48
Number of males ≤ 1 yr of age	27 (4%)
Number of females ≤ 1 yr of age	16 (3%)
Number of males ≥ 16 yr of age	57 (9%)
Number of females ≥ 16 yr of age	47 (9%)
Percent of patients who were reported deceased	38
Projected median survival (in yr)	20

PHYSICAL EXAMINATION

The first patients were diagnosed with Fanconi's anemia because of the combination of aplastic anemia and physical anomalies or because of other family members with aplastic anemia or anomalies, or both. This bias in the literature may have contributed to the perception that patients must have physical anomalies for this diagnosis. The more recent tests for chromosome breakage and for specific genotypes have led to diagnoses in older patients and in those without overt birth defects. Patients with characteristic anomalies are often diagnosed without hematologic involvement.

Overall, approximately 25% of the literature cases had no anomalies, whereas 11% had only short stature or skin pigmentary changes, or both. The frequencies of the more common birth defects are summarized in Table 8-2. Physical abnormalities occurred more frequently in the patients who were diagnosed in infancy than in those who were diagnosed as adults, with the exception of short stature and café-au-lait spots. Figure

TABLE 8-2. Physical Abnormalities in Fanconi's Anemia

Abnormality	All Patients	Age at Diagnosis	
		≤1 Yr of Age	≥16 Yr of Age
Number of cases	1206	43 (4%)	104 (9%)
Skin pigment or café-au-lait spots, or both	55	37	61
Short stature	51	47	57
Upper limbs	43	63	39
Abnormal gonads, male	32	37	44
Abnormal gonads, female	3	50	6
Head	26	37	18
Eyes	23	33	24
Renal	21	42	19
Birth weight ≤ 2500 g	11	47	8
Developmental disability	11	5	8
Ears, hearing decreased	9	23	11
Legs	8	16	7
Cardiopulmonary	6	16	5
Gastrointestinal	5	28	6
No anomalies	25	16	23
Short stature or skin pigment, or both	11	5	19

NOTE: All values, except those in the "Number of cases" row, are percentages.

8-3 shows a boy with almost all the classic anomalies that are seen in patients with Fanconi's anemia. The breadth of anomalies is wide, and some patients clearly have none.

The abnormalities in Fanconi's anemia are listed in detail in Table 8-3. The most common finding is skin hyperpigmentation,

a generalized brown melanin-like darkening that is most prominent on the trunk, neck, and intertriginous areas and that becomes more obvious with age. Children who are affected are often thought to have a permanent suntan. Café-au-lait spots are common, alone or combined with hyperpigmentation, and

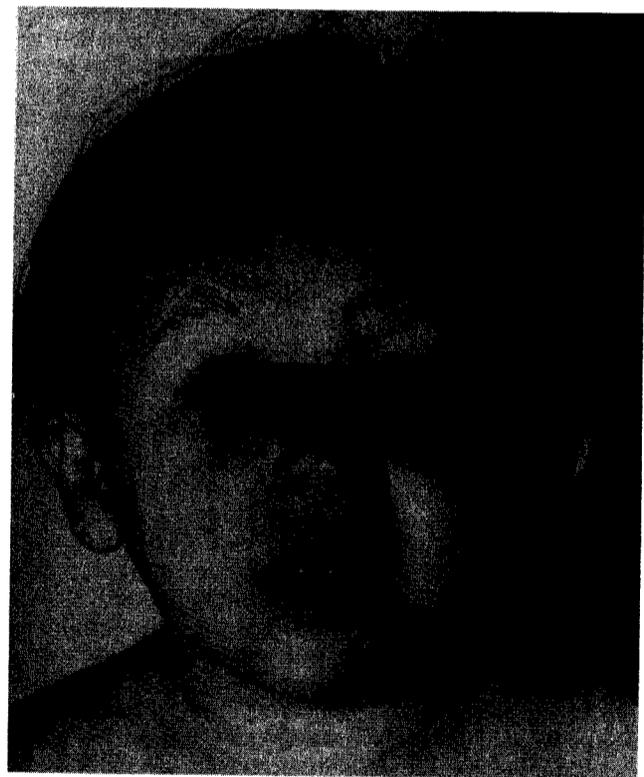
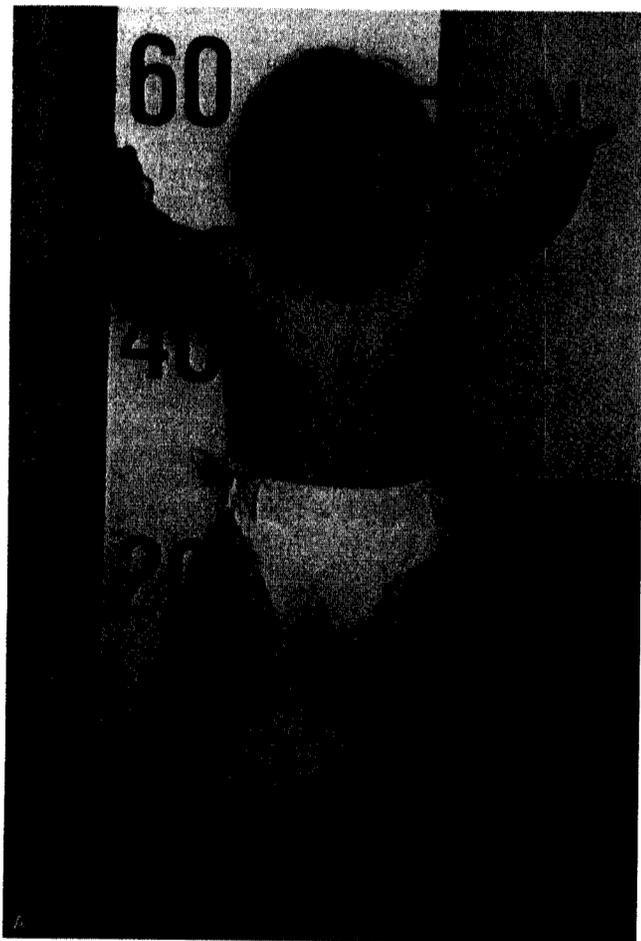


Figure 8-3. Three-year-old boy with Fanconi's anemia, with several phenotypic features. A: Front view. B: Face. (continued)

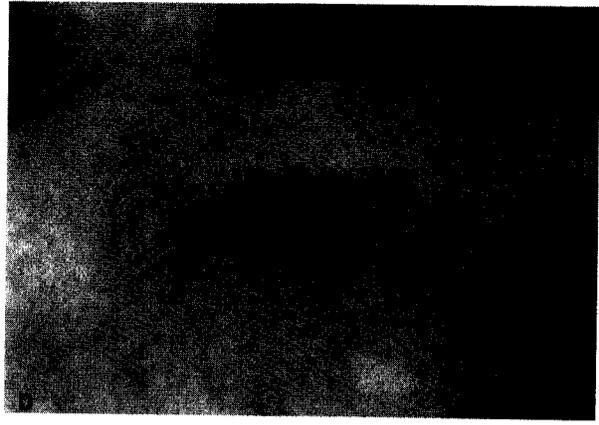


Figure 8-3. (continued) C: Hands. **D:** The back of the right shoulder. Features to be noted are short stature, thumbs attached by threads, microcephaly, broad nasal base, epicanthal folds, micrognathia, and café-au-lait spot with hypopigmented areas beneath.

hypopigmented areas are also seen. The number and size of these pigment changes increases with age (10). Café-au-lait spots may actually be more common than hyperpigmentation, but many case reports do not differentiate between them (11).

Microsomia, which is manifested by short stature and small delicate features, was often the presenting complaint before the development of hematologic problems. Many patients did not eat or grow well in the early years. Upper limb abnormalities, particularly absence or hypoplasia of thumbs, were reported in more than one-half of patients. Absent or hypoplastic radii were always associated with absent or hypoplastic thumbs [unlike thrombocytopenia-absent radius (TAR) syndrome, in which the thumbs are always present despite the absence of the radii]. Supernumerary, bifid thumbs and thumbs that were attached only by threads were also common. Flattening of the thenar eminence and weakness of radial pulses are subtle but common findings in our own experience, and are more common than the literature reflects.

The next most common physical problem involves the genitalia in one-third of male patients, with underdevelopment or undescended testes, or both. Abnormal gonadal development was commented on in only 3% of females, although abnormalities in menses and early menopause were also mentioned in a few instances.

Abnormally shaped heads were reported in 25% of patients with Fanconi's anemia, with most having microcephaly. The facies are often characteristic (Fig. 8-3). Neck anomalies lead to the perception of short or webbed necks, and spine anomalies also occur. The most common abnormalities of the eyes are microphthalmia and strabismus, as well as epicanthal folds, hypertelorism, and ptosis. Ear problems, including deafness as well as structural abnormalities of the external or middle ear, occur in approximately 10% of patients.

Renal defects, most often ectopic, pelvic, or horseshoe kidneys, as well as absent or hypoplastic kidneys, are found in more than 20% of Fanconi's anemia patients. Most of the renal problems are structural, although functional defects that result in reflux or infections can occur. The incidence of renal structural abnormalities may actually be higher than is cited here, because many patients do not have imaging studies performed.

Several abnormalities were described in fewer than 10% of the reports. These include low birth weight (particularly in those who were diagnosed in infancy), developmental delay, defects that involve the lower limbs, congenital heart disease, and gastrointestinal anomalies. Some of the feeding difficulties of infants with Fanconi's anemia might be due to undocu-

mented gastrointestinal abnormalities. The incidence and types of anomalies are similar in both sexes.

Auerbach et al. (12) used a stepwise multivariate analysis of the first 162 patients in the International Fanconi Anemia Registry (IFAR) to develop a scoring system that discriminated between patients with clastogenic stress-induced chromosome breakage [using diepoxybutane (DEB)] and those without chromosome breakage. One point each is added for microphthalmia, birthmarks, genitourinary abnormalities, growth retardation, thrombocytopenia, and the absence of radius or thumb, or both. One point is subtracted for learning disabilities, and one point is subtracted for other skeletal abnormalities. Higher scores mean that the probability of Fanconi's anemia is increased. This system was developed before the use of complementation or mutation analysis to confirm the diagnosis of Fanconi's anemia.

The terms *constitutional aplastic anemia type II* and *Estren-Dameshek aplastic anemia* were used to describe patients who have familial aplastic anemia but lack anomalies. The original paper by Estren and Dameshek (13) described two such families. In fact, two of the patients did have undescended testes. Li and Potter (14) reinvestigated one of these families and discovered that a second cousin, whose parents were both cousins of the original parents, had typical Fanconi's anemia. Now that chromosome breakage and molecular testing are available, patients whose anemia might have been called *Estren-Dameshek* can be correctly diagnosed as Fanconi's anemia. Many patients who were reported in the literature were entirely normal or had only short stature or changes in skin pigment.

Further evidence that the Estren-Dameshek patients belong to the Fanconi's anemia spectrum is provided by the IFAR data (12). In that registry, the diagnosis of Fanconi's anemia was made only when clastogenic stress-induced chromosome breaks were found, independent of physical appearance and family history (although many of the physically and hematologically normal children were tested only because of a positive family history). Thirty percent of the Fanconi's anemia group had aplastic anemia without anomalies, and 7% had neither. Two smaller studies of affected siblings of probands showed that at least 25% of those who were affected lacked anomalies (15,16). Fanconi's anemia is considered to include a spectrum of physical findings, which range from totally normal to the extreme of all the problems that were previously listed.

INHERITANCE AND ENVIRONMENT

Fanconi's anemia is clearly inherited in an autosomal-recessive pattern, despite the apparent slight preponderance in the

TABLE 8-3. Specific Types of Anomalies in Fanconi's Anemia Patients

Skin
Generalized hyperpigmentation on trunk, neck, and intertriginous areas; café-au-lait spots; hypopigmented areas
Body
Short stature, delicate features
Upper limbs
Thumbs—absent or hypoplastic, supernumerary, bifid, rudimentary, short, low set, attached by a thread, triphalangeal, tubular, stiff, and hyperextensible
Radii—absent or hypoplastic (only with abnormal thumbs); absent or weak pulse
Hands—clinodactyly; hypoplastic thenar eminence; six fingers; absent first metacarpal; enlarged, abnormal fingers; short fingers
Ulnae—dysplastic
Gonads
Males—hypogonitalia; undescended testes; hypospadias; abnormal, absent testis; atrophic testes; azoospermia; phimosis; abnormal urethra; micropenis; delayed development
Females—hypogonitalia; bicornuate uterus; abnormality, aplasia of uterus and vagina; atresia of uterus, vagina, and ovary
Other skeletal anomalies
Head and face—microcephaly, hydrocephalus, micrognathia, peculiar face, bird face, flat head, frontal bossing, scaphocephaly, sloped forehead, choanal atresia
Neck—Sprengel's deformity, short, low hairline, webbed
Spine—spina bifida (thoracic, lumbar, cervical, occult sacral), scoliosis, abnormal ribs, sacrococcygeal sinus, Klippel-Feil syndrome, vertebral anomalies, extra vertebrae
Eyes
Small, strabismus, epicanthal folds, hypertelorism, ptosis, slanted, cataracts, astigmatism, blindness, epiphora, nystagmus, proptosis, small iris
Ears
Deaf (usually conductive), abnormal shape, atresia, dysplasia, low set, large, small, infections, abnormal middle ear, absent drum, dimples, rotated, canal stenosis
Kidneys
Ectopic or pelvic, abnormality, horseshoe, hypoplastic or dysplastic, absent, hydronephrosis or hydroureter, infections, duplicated, rotated, reflux, hyperplasia, no function, abnormal artery
Gastrointestinal system
High arch palate, atresia (esophagus, duodenum, jejunum), imperforate anus, tracheoesophageal fistula, Meckel's diverticulum, umbilical hernia, hypoplastic uvula, abnormal biliary ducts, megacolon, abdominal diastasis, Budd-Chiari syndrome
Lower limbs
Feet—toe syndactyly, abnormal toes, flat feet, short toes, clubfoot, six toes, supernumerary toe
Legs—congenital hip dislocation, Perthes' disease, coxa vara, abnormal femur, thigh osteoma, abnormal legs
Cardiopulmonary system
Patent ductus arteriosus, ventricular septal defect, abnormality, peripheral pulmonic stenosis, aortic stenosis, coarctation, absent lung lobes, vascular malformation, aortic atheromas, atrial septal defect, tetralogy of Fallot, pseudotruncus, hypoplastic aorta, abnormal pulmonary drainage, double aortic arch, cardiac myopathy
Other anomalies
Slow development, hyperreflexia, Bell's palsy, central nervous system arterial malformation, stenosis of the internal carotid, small pituitary

NOTE: Abnormalities are listed in approximate order of occurrence within each category.

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literature of males who are affected (Table 8-1). In a large study, 30% of families had two children who were affected, and consanguinity was found in 10% (17). The literature reports include 90 families with consanguinity, 325 families with affected siblings, and ten families with affected cousins. Mothers of Fanconi's anemia patients appear to have an unusual number of miscarriages (30 noted in the literature), and some of those fetuses were found to have significant phys-

ical anomalies. Rogatko and Auerbach (18) confirmed a monogenic autosomal-recessive pattern by segregation analysis of the 86 cases in the report of Schroeder et al. (17) and of 88 affected persons in the IFAR.

The heterozygote incidence may be 1 in 300 persons in the United States and approximately 1 in 100 Afrikaans in South Africa and Ashkenazi Jews, owing to separate founder effects (17,19,20). Physical abnormalities were reported in a few parents, suggesting phenotypic changes in heterozygotes (21-23). Others have suggested that relatives of persons with Fanconi's anemia (possible heterozygotes) have an increased incidence of congenital malformations, particularly genitourinary and hand malformations (24). Some siblings had characteristic physical abnormalities without hematologic disease (25-28). (These children may subsequently have developed aplastic anemia.) Some families have children with pancytopenia and normal physical examinations in addition to children with classic malformations (29-31). A family was described in 1944 with one child who had typical Fanconi's anemia and his brother who had paroxysmal nocturnal hemoglobinuria, developed lung cancer, and was proven to have Fanconi's anemia in 1987 by DEB-induced chromosome breakage (32) (E. C. Gordon-Smith, *personal communication*). A cousin, who was related maternally and paternally to the family, died of leukemia. These variations may reflect incomplete expression of the homozygous state or may occur in heterozygotes. Most of the reports predated stress-induced chromosome breakage studies. The varied expression may indicate allelic but different genetic mutations, interacting mutant genes, or the influence of the environment on the Fanconi's anemia phenotype.

Patients with Fanconi's anemia can now be diagnosed by chromosome breakage or genetic studies before the onset of hematologic or malignant complications [preanemic phase (33-37)]. There are several advantages to presymptomatic diagnoses. Patients can be told to avoid drugs and other agents that have been implicated in the development of acquired aplastic anemia. Prospective analysis leads to a determination of the actual incidences of aplastic anemia, leukemia, and other malignancies. Factors that lead to the development of these complications (second hits) may be identified. Early recognition of Fanconi's anemia in a family may be used for choices regarding family planning.

The environment has been invoked to explain the aplastic anemia in some cases. In one family with three Fanconi's anemia homozygotes (38), one sister died of aplastic anemia at 16 years of age (according to another sister, she had been treating skin infections with coal tar). The other two sisters, who were proven to be Fanconi's anemia homozygotes by DEB-induced chromosome breakage, despite only mild hypoplastic anemia more than 20 years later, developed myelodysplastic syndrome (MDS) and oral cancers in their 30s and 40s, respectively (B. P. Alter, *unpublished data*, 1997). Aplastic anemia developed in some Fanconi's anemia patients after viral illnesses, hepatitis, and tuberculosis (30,31,39,40). Cases of Fanconi's anemia have been reported in which the patient received chloramphenicol before the onset of aplastic anemia (21,41-44). These infectious or drug-related cases suggest a role for the environment in the development of bone marrow failure. Reports of other families in whom siblings had the onset of pancytopenia at the same age suggest additional genetic components (17,39,45,46). Thus, the exact roles of genetics and environment are not yet clarified.

LABORATORY FINDINGS

The blood counts of patients often reveal thrombocytopenia or leukopenia before pancytopenia, which is usually mild or mod-

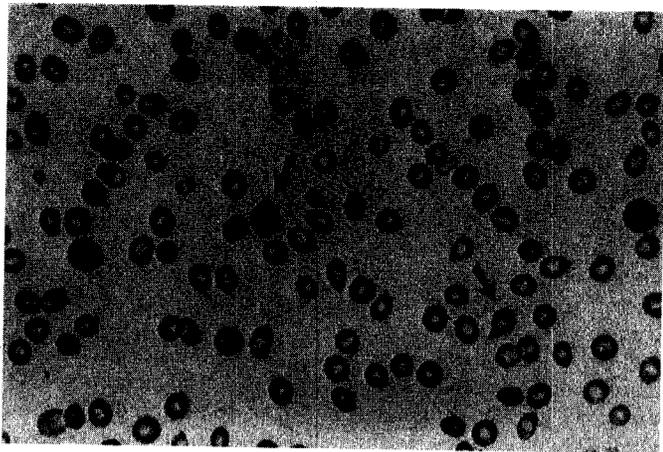


Figure 8-4. Peripheral blood from a patient with Fanconi's anemia. Note anisocytosis, macrocytes (arrows), thrombocytopenia, and neutropenia. (Courtesy of Dr. Gail Wolfe. From Alter BP. The bone marrow failure syndromes. In: Nathan DG, Oski FA, eds. *Hematology of infancy and childhood*, 3rd ed. Philadelphia: WB Saunders, 1987:159-241, with permission.)

erate initially; severe aplasia eventually develops in most cases. Even in the Fanconi's anemia patient with normal blood counts (the preanemic, or the treatment-responsive patient), erythrocytes are macrocytic, with mean cell volumes (MCVs) that are usually greater than 100 fL. The blood smear shows large red blood cells (RBCs) with mild poikilocytosis and anisocytosis, as well as a paucity of platelets and leukocytes, if the numbers of these components are reduced (Fig. 8-4). Eventually, all three cell lines may fail.

At the aplastic stage, the bone marrow is hypocellular and fatty, with few hematopoietic elements and a relative increase, which is identical with that found in the marrow of patients with acquired aplasia, in lymphocytes, reticulum cells, mast cells, and plasma cells. In early Fanconi's anemia, areas of hypercellular marrow may be evident, but these disappear as aplasia progresses (9).

Erythropoiesis in the Fanconi's anemia patient is characterized as stress erythropoiesis and is associated with the production of erythrocytes with fetal characteristics, as in patients who have acquired aplastic anemia—spontaneously or after marrow transplantation—during recovery (47,48). The features of this fetal-like erythropoiesis, which occurs during stress and is present in preanemic, anemic, and remission-stage Fanconi's anemia patients, include macrocytosis, increased fetal hemoglobin (HbF) (by alkali denaturation and Kleihauer-Betke acid elution), and presence of the i antigen. These features have also helped to identify nonanemic, affected siblings of known patients. The HbF is distributed unevenly, not clonally, and no concordance of the various fetal-like features exists at the level of single cells (49). The level of HbF or degree of macrocytosis does not provide any prognostic information.

RBC lifespan may be slightly short, but hemolysis is not a major component of the anemia. Some patients with apparently short RBC lifespan may have had blood loss that was associated with thrombocytopenia. Ferrokinetic studies suggest that most patients have a component of ineffective erythropoiesis in addition to relative marrow failure. Dyserythropoiesis was noted in the marrow erythroblasts in some cases, with fragmentation and multinuclearity (21,40,50). Bone marrow imaging with technetium-99m sulfur colloid showed paradoxical and irregular tracer distribution in Fanconi's anemia that was distinct from the uniform reduction seen in acquired aplastic anemia

(51). This may be related to the varied and irregular onset of aplasia in Fanconi's anemia patients. RBC enzymes have been decreased, increased, or normal in several contradictory studies (37,52-54); the variable results may reflect the heterogeneity of Fanconi's anemia.

The small stature of Fanconi's anemia patients was ascribed to growth hormone (GH) deficiency in 22 patients in whom GH was reported to be measured (39,55-71), whereas six patients were found not to be GH deficient (59,72-74). Treatment with GH led to increased growth without hematologic improvement in 12 of 15 GH-deficient patients. In some families, GH deficiency and Fanconi's anemia segregated independently (64,65). In a study of patients in the IFAR (self-selected for participation, and perhaps biased toward short stature), 44% of patients had a subnormal GH response to stimulation, and 36% of patients were hypothyroid (75). Three patients were treated with recombinant GH, one of whom died from acute myelogenous leukemia. It is thought that the frequency of leukemia in patients with other risk factors that predispose them to leukemia is probably not increased further by GH treatment (76,77), although the use of GH replacement in Fanconi's anemia still warrants careful consideration.

Chromosome Breakage. The characteristic laboratory finding consists of chromosome aberrations, which are seen most easily in metaphase preparations of phytohemagglutinin-stimulated, cultured, peripheral blood lymphocytes. These reveal breaks, gaps, rearrangements, exchanges, and endoreduplications (Fig. 8-5), which are seen in less than 10% of the cells from normal persons but in much higher proportions in cells from Fanconi's anemia homozygotes (78-84). These features are seen infrequently in direct preparations of bone marrow cells, perhaps because cells with significant abnormalities may divide slowly or may not survive *in vivo* (81,85-87). Cultured skin fibroblasts also have abnormal chromosomes (79,80,82). It is thought that the spontaneous aberrations that are seen in cultured blood lymphocytes or fibroblasts may be artifacts of culture that are induced by unknown factors in the medium. Because marrow studies are usually direct or cultured only briefly, these artifacts may not appear. The abnormal lymphocyte chromosomes have no relation to any hematologic findings, and variations in the proportions of abnormal cells or in the number of breaks per cell do not correlate with the clinical course. Furthermore, spontaneous breaks are sometimes absent in bona fide Fanconi's anemia cases (12). Similar spontaneous chromosomal changes have been reported in Bloom's syndrome and ataxia-telangiectasia (88).

Cells from Fanconi's anemia patients are sensitive to oncogenic agents, such as simian virus 40 viral transformation (89), ionizing radiation (90), and alkylating agents (91). These agents damage DNA and produce significantly increased numbers of chromosomal aberrations in Fanconi's anemia cells. The chemicals that are used in several laboratories include DEB, nitrogen mustard, mitomycin C (MMC), cyclophosphamide, and platinum compounds (91-96). Homozygotes are diagnosed based on an amplification of several times the rate of chromosomal aberrations, compared to the baseline spontaneous rate. The cited agents do not result in increased breakage in the cells of patients with non-Fanconi's anemia chromosome breakage syndromes. In addition, cells of patients with Bloom's syndrome show increased sister chromatid exchange after treatment with 5-bromodeoxyuridine, whereas Fanconi's anemia patients' cells do not (97). The chromosome breakage rate in Fanconi's anemia heterozygotes overlaps with the normal range and is not diagnostic of the carrier state.

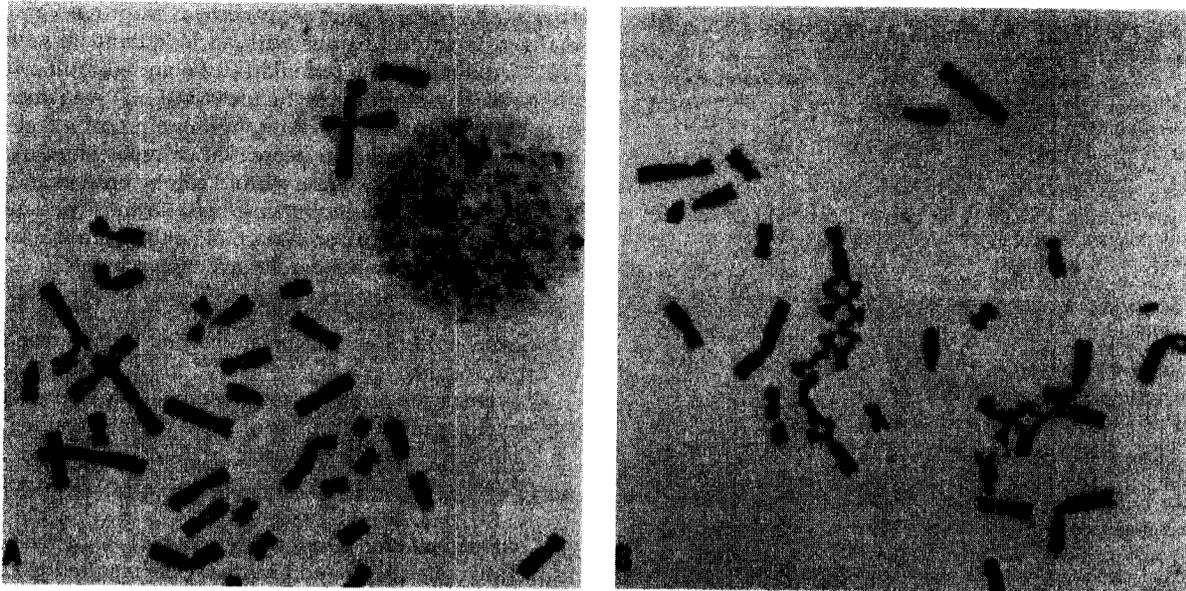


Figure 8-5. Cytogenetic findings in a patient with Fanconi's anemia. **A:** No clastogen. **B:** After culture with diepoxybutane. (From Auerbach AD, Adler B, Chaganti RS. Prenatal and postnatal diagnosis and carrier detection of Fanconi anemia by a cytogenetic method. *Pediatrics* 1981;67:128-135, with permission.)

Although most patients had multiple chromatid breaks and exchanges in most of their peripheral blood lymphocytes, 10% of the patients reported by Auerbach and Alter (98) had breaks in only 10% to 40% of their DEB-treated lymphocytes. Clonal results have been reported by others as well (99). These patients can be diagnosed, because the number of breaks per cell with breaks is still high. In addition, fibroblasts show breaks in a consistent and nonclonal manner. Molecular explanations have now been provided for some of these cases of somatic mosaicism (see the section Somatic Mosaicism in Fanconi's Anemia).

Another approach to the diagnosis of Fanconi's anemia through the use of alkylating agents involves flow cytometry rather than a count of chromosomal aberrations. Treated cells fail to divide, but undergo DNA replication and accumulate in the G₂ phase of the cell cycle, where they are detected because of the increased amount of DNA per cell (100-104).

Prenatal diagnosis has been performed by examination of fetal amniotic fluid cells or chorionic villus specimens (CVS) for increased chromosome breaks (98,105,106). In one series, 14 samples were obtained by CVS, three of which had increased spontaneous and DEB-induced breaks (98). In the same series, 7 of 46 fetuses that were studied by amniocentesis also had increased breaks by both assays. One false-negative was obtained by CVS, with no confirmatory amniocentesis sample. In the positive cases, the cultured cells also grew more slowly. Three cases were also examined using fetal blood that was obtained prenatally, primarily (107) or to confirm an abnormal CVS or amniotic fluid result (108,109). Of the affected cases, only three had physical anomalies, which further supports the suggestion that a large proportion of Fanconi's anemia homozygotes do not have malformations. Spontaneous and clastogenic stress tests also suggested Fanconi's anemia prenatally in two cases in which the fetuses were not known to be at risk for Fanconi's anemia but in which cytogenetic studies were performed for other reasons (108,110). Prenatal testing can be done by mutation analysis in appropriate families (see the section Cellular Phenotype).

The laboratory evaluation of patients in whom Fanconi's anemia is suspected should include complete blood counts, RBC size analysis, and HbF measurement. Skeletal radio-

graphs and renal ultrasonography are useful. The usual diagnostic test ascertains chromosome breakage rates after clastogenic stress. This test has identified Fanconi's anemia homozygosity as the reason for physical anomalies in patients who were not anemic at the time (33-35) and led to the diagnosis of Fanconi's anemia in patients with aplastic anemia who lacked malformations (105,111).

PATHOPHYSIOLOGY

The relationship between birth defects, hematopoietic failure, increased risk of malignancies, chromosome breakage, and DNA repair remains to be elucidated. *In utero*, the development of hematopoiesis and the organs that are most frequently abnormal in Fanconi's anemia occurs at approximately the same time (at 25 to 34 days of gestation), and a common toxic insult has been invoked (46). The Fanconi's anemia genotype may make homozygotes more susceptible to agents that can cause acquired aplastic anemia in normal persons. The oncogenic compounds that damage DNA *in vitro* may also be toxic *in vivo*.

Cellular Phenotype. Fanconi's anemia cells have several other cellular phenotypic abnormalities, which are summarized in Table 8-4, in addition to cross-link sensitivity; a detailed description of these studies is beyond the scope of this chapter. Many of these cellular assays have been performed on cells from multiple complementation groups (see section on Complementation Groups). Accordingly, it remains unclear whether these cellular abnormalities correspond to all Fanconi's anemia complementation groups or to only a subset. Most of the abnormalities that are described for Fanconi's anemia cells may be epiphenomena and may not relate directly to the primary cellular defect in each complementation group. A true understanding of the primary cellular defect in Fanconi's anemia, such as DNA repair, cell cycle regulation, or prevention of apoptosis, may ultimately result from studies of cloned proteins (see the section Fanconi's Anemia Genes).

Several lines of evidence suggest that Fanconi's anemia cells have an underlying molecular defect in cell cycle regulation. First, the cells display a cell cycle arrest with 4N DNA content that is enhanced by treatment with chemical cross-

TABLE 8-4. Cellular Abnormalities in Fanconi's Anemia

Feature	References
Spontaneous chromosome breaks	78-84
Sensitivity to cross-linking agents	91-96
Prolongation of G ₂ phase of cell cycle	100,112
Sensitivity to O ₂	
Poor growth at ambient O ₂	113
Overproduction of O ₂ radicals	114
Deficient O ₂ radical defense	115
Deficiency in superoxide dismutase	116,117
Sensitivity to ionizing radiation during G ₂	118
Overproduction of tumor necrosis factor- α	119
Direct defects in DNA repair	
Accumulation of DNA adducts	120
Defective repair of DNA cross-links	121
Hypermutability (by deletion)	122
Increased apoptosis	123-125
Abnormal induction of p53	124,126
Intrinsic stem cell defect	
Decreased colony growth <i>in vitro</i>	127-133
Decreased gonadal stem cell survival	134

Adapted from D'Andrea AD, Grompe M. Molecular biology of Fanconi anemia: implications for diagnosis and therapy. *Blood* 1997;90:1725-1736.

linking agents (100,112). Second, the cell cycle arrest and reduced proliferation of Fanconi's anemia cells can be partially corrected by overexpression of a protein called *SPHAR*, a member of the cyclin family of proteins (136). Third, caffeine abrogates the G₂ arrest of Fanconi's anemia cells (124). Consistent with these results, caffeine constitutively activates the cyclin-dependent kinase *cdc2* and overrides a normal G₂ cell cycle checkpoint in Fanconi's anemia cells. Finally, the FANCC protein (see below) binds to *cdc2*, suggesting that the Fanconi's anemia complex may be a substrate or modulator of the cyclin B-*cdc2* complex (137).

Fanconi's anemia cells also have an underlying defect in DNA repair. The cells are sensitive to DNA cross-linking agents and ionizing radiation, which suggests a specific defect in the repair of cross-linked DNA or double-strand breaks (138). DNA damage results in a hyperactive p53 response, which suggests the presence of defective repair yet intact checkpoint activities (124). Fanconi's anemia cells also have a defect in the fidelity of nonhomologous end joining and an increased rate of homologous recombination (139-141). Based on these extensive phenotypic defects, it has been hypothesized that Fanconi's anemia results from an underlying molecular defect in cell cycle regulation or DNA repair.

Hematopoietic Defects. Decreased bone marrow short-term and long-term hematopoietic growth was observed by several

groups (127-133). Colony growth was improved but not normalized in some cultures with added stem cell factor (SCF) (131). Production of interleukin (IL)-6 and granulocyte-macrophage colony-stimulating factor (GM-CSF) was decreased in long-term cultures (142). Although one report suggested that nonanemic patients had decreased colonies *in vitro* (129), we found a relation between erythroid colony growth and hematologic status *in vivo*, with better growth in those patients whose blood counts were closer to normal (130). From this, we developed a clinical hematologic classification scheme:

1. Severe aplastic anemia on transfusions.
2. Severe aplastic anemia on androgens, but not responsive on transfusion.
3. Severe or moderate aplasia, responsive to androgens.
4. Severe or moderate aplasia, about to start treatment.
5. Stable, with mild cytopenias, high MCV, and high HbF.
6. Normal hematology.

Complementation Groups. Hybrid cells that were formed from Fanconi's anemia and normal cells resulted in correction of the Fanconi's anemia breakage (143-147). Other cell fusion studies that used cell lines from a variety of Fanconi's anemia patients led to the demonstration of at least two complementation groups, which involved at least nine patients (145-147), although only a single group was found in five Japanese patients (148). Duckworth-Rysiecki et al. also found two complementation groups (144).

At least eight distinct complementation groups (A, B, C, D1, D2, E, F, G) have now been identified using somatic cell fusion techniques and complementation of MMC sensitivity in fused hybrid cells (149-151). These complementation groups are shown in Table 8-5. Several approaches are now available for the rapid diagnosis and assignment of complementation groups to patients (see the section Implications for Diagnosis and Complementation Group). Some differences in the clinical severity of Fanconi's anemia are also now apparent, when comparing different complementation groups or specific mutations within a complementation group. In particular, null mutations appear to be more severe than those mutations that lead to an altered protein (152).

Fanconi's Anemia Genes. Using functional complementation of the eight Fanconi's anemia groups, six genes have now been cloned: FANCA, FANCC, FANCD2, FANCE, FANCF, and FANCG (153-158). The FANCA and FANCD2 genes were also cloned by a positional approach (155,159). The FANCG gene is homologous to the previously described XRCC9 gene (160). The FANCD complementation group is genetically heterogeneous, with at least two genes (FANCD1 and FANCD2) in this group (155). Characteristics of the six cloned genes are summarized in

TABLE 8-5. Fanconi's Anemia Complementation Groups and Genes

Gene	Locus	Genomic DNA (kb)	Complementary DNA (kb)	Exons	Protein (kd)	Amino Acids	Percent of Patients
FANCA	16q24.3	80	5.5	43	163	1455	Approximately 70
FANCB	N/A	—	—	—	—	—	Rare
FANCC	9q22.3	80	1.8	14	63	558	Approximately 10
FANCD1	13q12.3	70	11.4	27	384	3418	Rare
FANCD2	3p25.3	80	4.4	44	162	1451	Rare
FANCE	6p21-22	15	1.6	10	60	536	Approximately 10
FANCF	11p15	—	1.1	1	42	374	Rare
FANCG	9p13	—	2.5	14	70	622	Approximately 10

N/A, not available, not mapped or cloned.

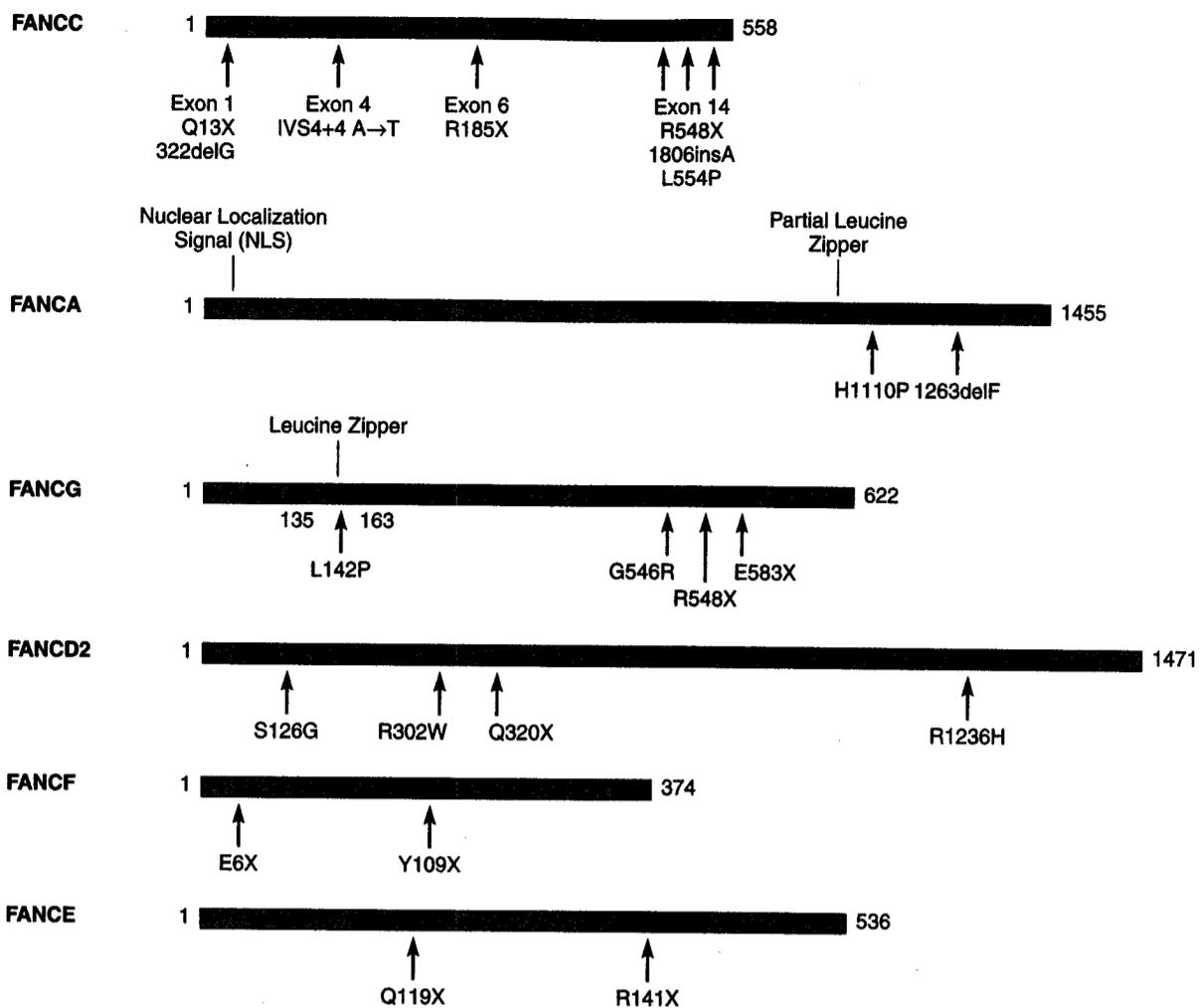


Figure 8-6. Schematic representation of the six cloned Fanconi's anemia proteins. A few patient-derived mutations are indicated. These mutations were identified by systematic mutational analysis of the FANCC (154,161,162), FANCA (163,164), and FANCG genes (165). Fewer patient-derived mutations have been detected for the FANCE (156), FANCF (157), and FANCD2 (155) genes.

Table 8-5. The availability of these gene sequences and their encoded proteins has substantially altered the diagnostic and subtyping approach to the disease.

Five of the seven cloned Fanconi's anemia proteins have little or no homology to other known proteins in genetic databases (Fig. 8-6). However, absence of any one of these proteins, by biallelic mutation of the corresponding gene, results in the common clinical and cellular abnormalities in Fanconi's anemia. Accordingly, it was suggested that these six proteins interact in a common biochemical pathway (166), and recent studies have demonstrated that this hypothesis may be correct (Fig. 8-7). Several of the proteins (including FANCA, FANCC, FANCE, FANCF, and FANCG) appear to be subunits of a large multisubunit protein complex in the nucleus of normal cells (157,167-173). This protein complex appears to be a ubiquitin ligase, which is capable of modifying the downstream, Fanconi's anemia protein, FANCD2 (174). Alternatively, the FA complex may regulate the activity of a ubiquitin ligase. When a normal cell is exposed to DNA damage, the Fanconi's anemia protein complex modifies the FANCD2 protein by monoubiquitination, thereby targeting this protein to DNA repair foci within the nucleus. Ubiquitin is a 76 amino-acid peptide that is added posttranslationally to

regulated proteins. Monoubiquitination of the FANCD2 protein does not alter the stability of the protein but instead appears to direct its translocation to the DNA repair foci in the nucleus. Interestingly, these DNA repair foci contain other proteins that are known to be involved in DNA repair, such as BRCA1, RAD51, and NBS (174-176). The recent discovery (176a) that FANCD1 is caused by biallelic mutations on BRCA2 links the Fanconi genes, BRCA1, and BRCA2 in a common pathway. Moreover, other recent evidence (176b) links the Fanconi anemia and ataxia telangiectasia pathways. The ATM (ataxia telangiectasia mutated) gene encodes an ionizing radiation-activated kinase that phosphorylates the FANCD2 protein, which is monoubiquitinated by the FANCA (A, C, E, F, and G) protein complex. Loss of any of the proteins in the Fanconi pathway leads to spontaneous chromosome breakage, which is increased by cellular exposure to MMC or DEB. These studies suggest that the six cloned Fanconi's anemia proteins interact in a novel biochemical pathway that is activated in response to DNA damage. Disruption of this pathway leads to the characteristic clinical and cellular abnormalities that are observed in Fanconi's anemia. Whether there are other functions of the Fanconi's anemia proteins outside the context of this biochemical pathway remains unknown.

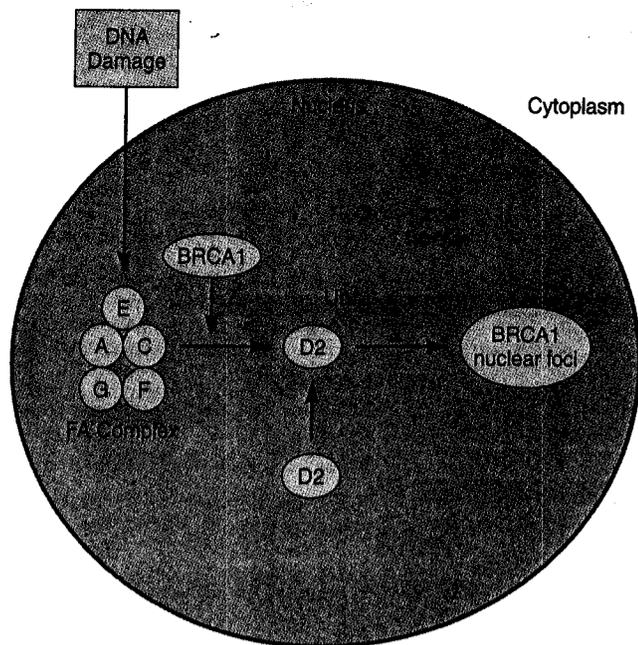


Figure 8-7. Interaction of the FA proteins in a cellular pathway. The FA proteins (A, C, E, F, and G) bind in a functional nuclear complex. This multisubunit complex appears to function as an enzyme. On activation of this complex by DNA damage, the complex enzymatically modifies (monoubiquitinates) the downstream D2 protein. The activated D2 protein is thereby targeted to nuclear foci that are required for DNA repair. These foci contain many proteins (i.e., BRCA1, NBS, RAD51) that are known to play a role in DNA repair and in the maintenance of chromosome stability. Defects in BRCA2 are observed in FANCD1. The role of BRCA2 in this pathway is uncertain. Disruption of the FA pathway leads to the characteristic cellular and clinical abnormalities that are observed in Fanconi's anemia. (Adapted from Garcia-Higuera I, Taniguchi T, Ganesan S, et al. Interaction of the Fanconi anemia proteins and BRCA1 in a common pathway. *Mol Cell* 2001;7:1-20, with permission.)

Implications for Diagnosis and Complementation Group.

Several studies have provided mutational analysis of patients, including those patients in group C (162,177), group A (163,178), and group G (165). While FANCC is a relatively rare group (Table 8-5), there is a common mutant allele for FANCC, IVS4+4 A to T, which is prevalent in Ashkenazi Jews (162), with a carrier frequency of approximately 0.9% in this population (20). FANCA is the most common group. The FANCA gene is large, encompassing 43 exons, and there are a wide range of mutant alleles, thereby making direct mutational screening of FANCA patients costly and inefficient. There is an increased frequency of group G in the German population, as it is associated with a German mutant allele (165). In some ethnic backgrounds, Fanconi's anemia can be diagnosed by direct mutational screening of specific founder mutations.

Knowledge of the specific genotype of a patient is often informative. For instance, for FANCC, the IVS4+4 A to T mutant allele is associated with a more severe phenotype, whereas the delG322 mutation may have a relatively mild phenotype (179). Disease severity may also depend on genetic background, however, as Japanese patients with the IVS4+4 A to T mutation have a comparatively mild phenotype (180).

Although direct mutational screening is not always practical, complementation group assignment is relatively easy and cost effective. Cell lines and primary cells from known Fanconi's anemia patients can be subtyped by using a combination of retroviral complementation with the six cloned Fanconi's anemia complementary DNAs (cDNAs) and by immunoblotting with

antisera that are specific to the six proteins (181). Complementation analysis suggests that FANCA patients have a relatively mild phenotype, compared to patients in group C or group G (152). Group typing may be useful in predicting disease severity or in guiding the relative urgency of risky clinical interventions, such as unrelated BMT. Group assignment is a necessary precondition for the use of gene therapy (135).

Because the six cloned FA proteins appear to interact in a common pathway (Fig. 8-7), it is theoretically possible to screen the downstream events in this pathway (i.e., the monoubiquitination of the FANCD2 protein) as a measure of the integrity of the pathway. Whether such a screening test will replace the DEB test for Fanconi's anemia diagnosis remains unknown.

Somatic Mosaicism in Fanconi's Anemia. The phenomenon of somatic reversion for Fanconi's anemia has recently been described. Somatic reversion results when a mutant gene reverts to a wild-type gene (or to a functionally wild-type gene), thus encoding a functional Fanconi's anemia protein. Reverted cells have a selective growth advantage over mutant cells. Approximately 15% of Fanconi's anemia patients have somatic mosaicism in their peripheral blood (182,183). A mixture of MMC-sensitive and MMC-resistant cells is found in these samples. Mitotic recombination or compensatory frame-shifts were shown to be molecular mechanisms of somatic reversion (184). Somatic mosaicism may make the diagnosis of Fanconi's anemia difficult, because of false-negative chromosome breakage studies. If Fanconi's anemia is strongly suspected in a patient, despite inconclusive breakage studies in peripheral blood, a definitive test can be performed by chromosome breakage studies of primary skin fibroblasts or by direct Fanconi's anemia gene mutational screening.

The high percentage of patients with somatic mosaicism indicates a strong selective advantage for cells that have lost the Fanconi's anemia phenotype. In principle, if the somatic reversion occurs in a pluripotent hematopoietic progenitor cell, then these corrected cells could give rise to clonal repopulation of the bone marrow. This raises the possibility that gene therapy in Fanconi's anemia may be aided by *in vivo* selection. At this time it is unclear whether any special physiologic circumstances are required for this selection to occur. The high incidence of somatic reversion also suggests that many mutant Fanconi's anemia genes have frameshift mutations. These mutations may be "corrected" by new mutations that correct the open reading frame of the Fanconi's anemia gene.

Somatic mosaicism may also cause some complications for Fanconi's anemia patients, especially if the reversion occurs in a more differentiated T lymphocyte. A high incidence of graft rejection has been noted in BMT of mosaic patients (185). Because of their increased sensitivity to bifunctional alkylating agents, Fanconi's anemia patients typically receive a much less aggressive ablative treatment before BMT. If the recipient patient has somatic mosaicism (particularly in T cells), some endogenous cells may be resistant to the ablative regimen and may cause graft rejection. Further studies are required to confirm this hypothesis.

Gene Therapy for Fanconi's Anemia. For gene therapy, bone marrow from a Fanconi's anemia patient of known group can be harvested, transduced *ex vivo* with a retroviral or adenoviral construct that contains the corresponding wild-type cDNA, and reinfused into the recipient. In principle, the genetically corrected stem cells and early progenitor cells should have a selective advantage *in vivo*, which allows for a clonal or oligoclonal reengraftment of the bone marrow and a reconstitution of normal hematopoiesis. The restoration of the Fanconi's ane-

nia pathway by retroviral transduction of the missing functional Fanconi's anemia protein may provide a convenient screening test for the efficacy of gene therapy *in vitro* (Fig. 8-7). Studies that evaluated clinical gene therapy protocols for Fanconi's anemia have been described (186).

Mouse models have provided a useful, albeit nonideal, model for Fanconi's anemia gene therapy. At present, there are FANCC and FANCA knock-out mouse models for Fanconi's anemia (134,187,188), all of which exhibit a "partial" phenotype. These mice have normal development and organogenesis and no obvious cancer predisposition, but they all have decreased fertility. Although the baseline hematopoiesis of the mouse models is relatively normal (189,190), the primary FANCC (-/-) cells undergo enhanced chromosome breakage and decreased survival on exposure to MMC. Systematic comparisons of primary bone marrow cells from FANCC (-/-) and FANCC (+/+) mice were performed (191). In a competitive repopulation assay in an irradiated, normal mouse model, FANCC (+/+) cells selectively outgrew FANCC (-/-) cells, especially on serial transplantation. When wild-type bone marrow cells were transplanted into an unconditioned FANCC (-/-) recipient, the wild-type cells displayed a growth advantage that was enhanced with MMC conditioning *in vivo*. In other studies, FANCC (-/-) mice were shown to have decreased numbers of CD34⁺ cells, which suggests a defect in the differentiation of CD34⁻ to CD34⁺ cells (191,192).

Gene therapy for Fanconi's anemia has several theoretical advantages over conventional therapies. Fanconi's anemia cells that have been corrected by retroviral transduction have a survival advantage over untransduced cells. Retroviral transduction of FANCC or FANCA cDNA improves the clonogenic survival of human FANCC or FANCA mutant bone marrow cells (193,194) or murine FANCC (-/-) bone marrow cells (186). Taken together, these studies suggest that a gene therapy approach to Fanconi's anemia could potentially result in a competitive advantage of corrected cells and an *in vivo* correction of hematopoiesis. Moreover, this competitive engraftment could be enhanced by MMC administration *in vivo* (191). This is also supported by the observation that the FANCC transgene was only detectable in a patient who was given transduced CD34⁺ cells after radiation therapy for a concurrent malignancy (186). The frequent finding of somatic mosaicism of peripheral blood lymphocytes from Fanconi's anemia patients further supports a model of *in vivo* selection of corrected cells. In addition, constitutive overexpression of the Fanconi's anemia proteins does not have deleterious effects on hematopoietic cell growth or colony formation. In fact, a transgenic mouse expressing FANCC constitutively has a slight increase in colony-forming unit-erythrocyte (CFU-E) colony cells. Also, Fanconi's anemia cell lines that are complemented with the FANCA, FANCG, and FANCC cDNA and express high levels of the corresponding Fanconi's anemia protein have normal growth in culture.

Despite these theoretical advantages, gene therapy for Fanconi's anemia also carries various disadvantages and risks. Gene therapy is limited by the relatively poor retroviral transduction efficiency of hematopoietic stem cells with existing retroviral and lentiviral supernatants. Functional complementation with the FANCC cDNA could theoretically "rescue" a premalignant cell and thereby enhance leukemic transformation for the Fanconi's anemia patient. This may be a higher risk for a patient with a stable chromosomal (clonal) abnormality of the bone marrow cells. Expression of the FANCC cDNA in a differentiated lymphocyte could potentially create T-cell mosaicism of the recipient. T-cell mosaicism may decrease the success of bone

marrow engraftment, if a patient requires a subsequent unrelated donor bone marrow transplant. Expression of an exogenous Fanconi's anemia protein after gene therapy could theoretically result in an immune response to the foreign antigen and, subsequently, in graft rejection.

Preimplantation Genetic Diagnosis for Fanconi's Anemia.

Through preimplantation genetic diagnosis (PGD), parents of a known Fanconi's anemia patient with a known group and genotype can use *in vitro* fertilization to generate multiple sibling embryos. By polymerase chain reaction amplification analysis of cells that are obtained from these embryos, the embryos can be tested and screened for HLA type (to ensure that the sibling embryo is a match for the affected child) and for the Fanconi's anemia mutations that are carried in the family (to ensure that the sibling embryo does not have disease). PGD has been successfully performed for other genetic diseases, including cystic fibrosis (58) and Lesch-Nyhan syndrome (195,196). More recently, PGD was used successfully for a family with a known FANCC mutation. After the birth of a normal sibling, the cord blood can be used for transplantation of the older child who is affected. PGD has been limited by the reduced viability of reimplanted embryos after genetic analysis *ex vivo* and by the absolute requirement for accurate detection of the two mutant Fanconi's anemia alleles that are carried in the family. As stated previously, it is often difficult to detect the precise mutation in the Fanconi's anemia gene by direct mutational analysis, and it is difficult to distinguish true pathogenic missense mutations from benign base pair polymorphisms.

Implications for Cancer Diagnostics. Because biallelic germline mutations in a Fanconi's anemia gene result in cancer susceptibility in Fanconi's anemia patients, it is possible that acquired (somatic) mutations in Fanconi's anemia genes may also be oncogenic. A systematic screening of the Fanconi's anemia pathway in tumors from cancer patients from the general (non-Fanconi's anemia) population is therefore warranted (Fig. 8-7). For instance, somatic mutation of an upstream Fanconi's anemia gene may result in loss of a functional Fanconi's anemia pathway and subsequent chromosome instability. Chromosome instability is a common feature of cancer progression. Whether specific Fanconi's anemia groups or mutant alleles within a specific group predispose to a specific cancer remains untested.

PROGNOSIS

In the past, when patients were diagnosed because they had already developed aplastic anemia, and when the only treatment was RBC transfusions, 80% of patients were reported to die within 2 years (197). Almost all patients died within 4 years, with only rare long-term survivals (198,199). Because diagnoses can now be made before the onset of clinically significant hematologic or malignant symptoms, survival from the time of diagnosis is longer. More reliable information should eventually emerge from prospective data, in which a large proportion of patients are identified who have no symptoms.

The cumulative survival of Fanconi's anemia patients in the literature is shown in Figure 8-8. In the entire group, the median predicted cumulative survival is 20 years of age. However, cases reported in the 1990s had a predicted median survival of 30 years of age. Those patients who were 1 year of age or younger had a median survival of 5 years of age, and those who were 16 years of age or older when Fanconi's anemia was diagnosed had a median predicted survival of 30 years of age.

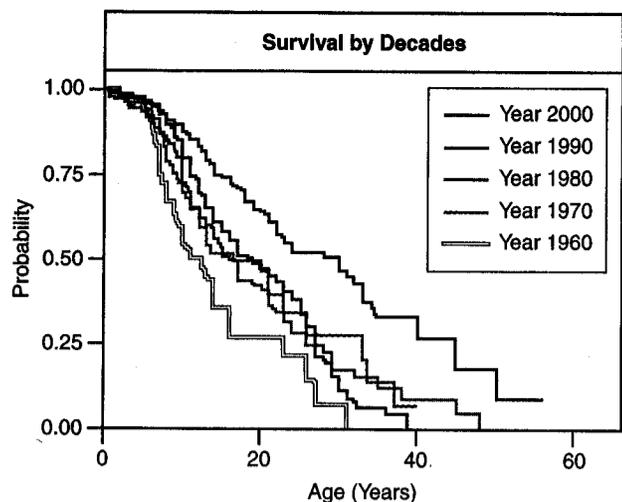


Figure 8-8. Kaplan-Meier plot of cumulative survival in Fanconi's anemia patients. Time is shown as age in years. Lines represent 118 cases that were reported from 1927 to 1960, 117 cases from 1961 to 1970, 254 cases from 1971 to 1980, 314 cases from 1981 to 1990, and 331 cases from 1991 to 2000. The differences are significant.

Older Patients. Older Fanconi's anemia men may be small, with underdeveloped genitalia and abnormalities in spermatogenesis (200). There are four Fanconi's anemia men who are reported to have fathered children (17,201,202); they represent less than 5% of men who have reached 16 years of age. This percentage may be due to underreporting, as well as decreased fertility, which has been noted by several patients (B. P. Alter, unpublished data).

Older women with Fanconi's anemia have irregular menses and early menopause (72,79,201,203). At least 20 Fanconi's anemia patients were reported to have been pregnant; 30 pregnancies have resulted in 21 living infants (36,79,201,203-213). Pregnancies occurred at a median age of 23 years (with a range from 18 to 34 years of age). Transfusions were often necessary because of worsening of maternal anemia, and cesarean sections were performed in six cases because of failure of labor to progress. There were eight miscarriages and four cases of preeclampsia. Nine of the women required RBC transfusions during their pregnancies, and six women required platelets. None of the mothers died during pregnancy, but ten women died at a median age of 32 years (with a range from 26 to 45 years of age), with seven women dying from cancer (see the section Complications) and three women dying from complications of pancytopenia.

TREATMENT

Androgen Therapy. Shahidi and Diamond (214) reported the use of androgens in 1959, with improvement in the first six patients. The response rate was noted to be 75% in the series by Sanchez-Medal (215) and Najean (216). As with any new therapy, initial enthusiasm must give way to reality, and the response rate is now estimated at closer to 50%. The first sign of response is a rise in the reticulocyte count, followed by a rise in hemoglobin (Hgb) within 1 to 2 months. The white blood cell count is somewhat slower, and the platelet response, usually incomplete, may take 6 to 12 months.

Only a few patients were reported to successfully discontinue androgen therapy (often at the time of puberty) and maintain their blood counts (37,128,205,217-224). Many patients eventually become refractory to the androgen with which

they are treated, and changing to another androgen only occasionally succeeds in buying more time for the patient. Some of the complications that are described in the following discussion develop in older patients, in whom androgens may have prolonged life sufficiently for these complications to appear, or they may have contributed to these developments.

Although some reports suggest that androgens alone are as effective as androgens that are combined with corticosteroids, the general recommendation is for a combination of androgens and corticosteroids (216). The growth acceleration of androgens may be counterbalanced by the growth retardation of the corticosteroids (225). In addition, corticosteroids may decrease bleeding at a given platelet count, perhaps by promoting vascular stability (226). The most frequently used androgen is oxymetholone, an oral 17-alkylated androgen, at 2 to 5 mg/kg/day. When prednisone is also given, it is at 5 to 10 mg every other day. If an injectable androgen is desired because of decreased risk of hepatotoxicity, the usual form is nandrolone decanoate, 1 to 2 mg/kg/week, injected intramuscularly, with ice packs and pressure applied to prevent hematomas.

Potential side effects of androgens are obstructive liver disease, peliosis hepatis, and liver tumors. Patients who receive androgens should be monitored frequently with liver chemistries and ultrasonography. If a response occurs, the drug should be tapered slowly but probably not discontinued entirely. The only group of patients in whom discontinuation has been considered routinely are those from South Africa, who may be a genetically distinct Fanconi's anemia variant (227). In most experiences elsewhere, relapses ensue when androgens are stopped, and subsequent remissions on the same or different preparations are sometimes elusive. Although an attenuated androgen, such as danazol, has theoretical appeal because of reduced side effects, there is some concern about its hematopoietic effectiveness. There are only two reports of its use in Fanconi's anemia, with a response in one patient (228,229).

Indications for androgens depend on the degree of cytopenia, not solely on the knowledge that the patient has Fanconi's anemia. One or more of Hgb below 8 g/dL, platelets less than 30,000/ μ L, or a neutrophil count of less than 500/ μ L may warrant treatment.

Hematopoietic Stem Cell Transplantation. Reconstitution of Fanconi's anemia patients with allogeneic hematopoietic stem cells offers the potential of a cure for the aplastic anemia and perhaps cure or prevention of leukemia. However, it does not prevent and may even accelerate the appearance of other malignancies (see section Solid Tumors). More than 200 transplants have been performed worldwide, with survival from HLA-matched siblings almost double that of alternative donors (Fig. 8-9). Although the majority of the reported transplants were from bone marrow, the use of cord blood is increasing, and mobilized peripheral blood may be considered.

The outcome was poor in the first marrow transplants from HLA-matched siblings using the standard aplastic anemia cyclophosphamide regimen of 100 to 200 mg/kg given over 3 to 4 days (230,231). Several studies showed that a metabolite of cyclophosphamide is toxic to DNA, which explains the clinical symptoms of severe mucositis with intestinal malabsorption and hemorrhages, fluid retention, cardiac failure, and hemorrhagic cystitis (92,232,233). Gluckman then introduced a modified protocol, using a total cyclophosphamide dose of 20 mg/kg, divided over 4 days, plus 5 Gy of thoracoabdominal radiation (138,233). This protocol became standard and has a cumulative survival probability of approximately 70% (234,235). The

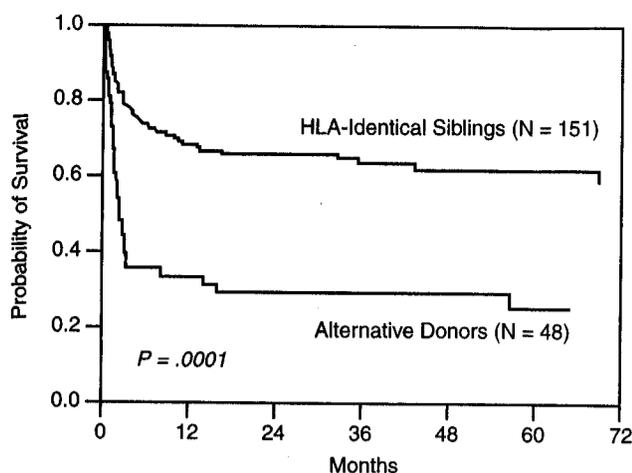


Figure 8-9. Kaplan-Meier plot of cumulative survival after bone marrow transplantation for patients with Fanconi's anemia. Time is shown as months from transplantation. One-hundred and fifty-one patients were transplanted from HLA-identical siblings. Forty-eight patients had alternative donors. (From Gluckman E, Auerbach AD, Horowitz MM, et al. Bone marrow transplantation for Fanconi anemia. *Blood* 1995;86:2856-2862, with permission.)

best predictor of survival was a low number of transfusions before transplant. Recently, nonmyeloablative regimens have shown promise, using fludarabine combined with low-dose cyclophosphamide, for related and unrelated donor transplants (236,237). One concern is that there may be incomplete mixed chimerism, with residual Fanconi's anemia cells that might have a malignant potential.

The preferred donors for Fanconi's anemia transplantations are HLA-matched siblings, who need to be screened with thorough physical examinations, complete blood counts (including examination of the RBC, MCV, and HbF), and cytogenetic studies for chromosome breakage, baseline and after culture, with a clastogenic stress agent, such as DEB. Mutation analysis should be offered in families with known mutations. In more than one case, the donor turned out to have Fanconi's anemia that had not been diagnosed before transplantation (238). Parents have an unexpectedly high rate of HLA identity with Fanconi's anemia patients, only partly accounted for by consanguinity, and may serve as marrow donors (239,240).

In many instances, no HLA-compatible sibling is found who can be a marrow donor. Alternative donors include parents (who may be only haploidentical), other relatives, or unrelated adult donors; the cumulative survival is approximately 30% (234,241). Persons with such transplants are particularly at risk for rejection or graft-versus-host disease. A review from the European group registry suggested that T-cell depletion reduced acute graft-versus-host disease but increased the risk of graft failure, thus leading to no net improvement in survival (241). They reported predictors of worse outcome, including extensive malformations, positive cytomegalovirus serology, prior treatment with androgens (which may have been correlated with abnormal liver function), and female donors. Matched unrelated donors were better than mismatched related donors (235).

Because umbilical cord blood contains hematopoietic progenitor cells, investigators proposed cryopreservation of these cells from a non-Fanconi's anemia sibling who is diagnosed *in utero*, to use for later transplantation (242). Fetal cells are obtained by chorionic villus sampling or amniocentesis, tested for Fanconi's anemia homozygosity by using clastogen-induced chromosome breakage tests or mutation analysis (see the section Cellular Phe-

notype), and HLA typed by serologic and molecular methods. Several cases have now been done, with good results when the donor was related to the patient (228,243). Results from cases that use unrelated cord donors are not nearly as good as those obtained in cases in which the donors are siblings (235,244). An extreme example of the use of placental blood for transplant is the pregnancy that results from PGD and implantation of one or more unaffected, HLA-matched blastocysts (see the section Pre-implantation Genetic Diagnosis for Fanconi's Anemia).

The incidence of malignancies is increased in Fanconi's anemia patients (see the section Complications). It is clear that BMT does not reduce this risk (except for hematologic malignancies); in fact, the cytoreductive therapy and radiation may increase it. Cancer of the tongue has been reported in several transplanted patients (see the section Solid Tumors).

Indications for transplant depend on the type of donor. Those with an HLA-matched sibling donor might be considered for transplant when their cytopenias require intervention (e.g., Hgb <8 g/dL, platelets <30,000/ μ L, and absolute neutrophil count <500/ μ L). Those for whom an alternative donor is the only option might benefit from androgens, granulocyte colony-stimulating factor (G-CSF), and even supportive care. Only those with leukemia or unmanageable cytopenias from aplastic anemia or MDS might be candidates for unrelated or mismatched transplants.

Other Treatment. Supportive care must be provided, as it should for any patient with aplastic anemia. *e*-Aminocaproic acid may be used for symptomatic bleeding, at a dose of 0.1 g/kg every 6 hours orally (245). No family member should be used as a blood product donor, until it is decided that a transplant will not be done (even from an unrelated donor) to decrease the chance of sensitization. Washed or leukofiltered RBCs should be used to reduce the risk of reactions and HLA sensitization from white cells. The possibility of marrow transplantation must be considered early in the course of the patient's anemia. Although the use of androgens and transfusions does not preclude transplantation, the best results are seen in those whose medical complications are minimal. Drugs and chemicals that may be implicated as causal in acquired aplastic anemia should be avoided. In addition, medications or substances that interfere with platelet function (such as aspirin, some antihistamines, non-steroidal antiinflammatory drugs, glycerol guaiacolate, vitamin E, and cod liver oil) should not be given to thrombocytopenic patients. If a severe allergic reaction occurs during a blood transfusion, diphenhydramine (Benadryl) can be used acutely.

Splenectomy has no apparent role in the management of Fanconi's anemia. More than 40 cases were reported to have had this procedure with no apparent long-term benefit. In some patients, transient improvement of pancytopenia occurred, but it was at a time when the bone marrow was not yet hypocellular.

Immunotherapy has no theoretical or factual basis. Although use of high-dose methylprednisolone (246) was reported rarely in Fanconi's anemia, there are unreported instances of several patients in whom this agent or antithymocyte globulin as well as cyclosporin A was used without success (B. P. Alter, unpublished data). In fact, approximately 10% of adults who failed to respond to any of these approaches were shown subsequently by clastogenic stress-induced chromosome breakage studies to have previously undiagnosed Fanconi's anemia (A. D. Auerbach and N. S. Young, unpublished data).

Lithium was reported to improve the blood counts in two of five Fanconi's anemia patients, presumably those whose marrow reserve was still present (248).

Hematopoietic growth factors may have a limited role in the future management of Fanconi's anemia patients. GM-CSF was

TABLE 8-6. Complications in Fanconi's Anemia

	All Patients	Leukemia	Myelodysplastic Syndrome	Solid Tumor	Liver Tumor
Number of cases	1206	103	74	59	34
Percent of total	100	8.5	6.1	4.9	2.8
Male to female ratio	1.24	1.51	1.06	0.37	1.43
Age at diagnosis of Fanconi's anemia (yr)					
Mean	8.4	10.4	11.5	13.2	9.4
Median	7	9	9.5	9.5	7
Range	0-48	0.13-28	0.3-43	0.1-44	3-48
Age at complication (yr)					
Mean	—	14.5	15.9	23.4	16.1
Median	—	13.8	14	26	13
Range	—	0.13-29	1.8-43	0.3-45	6-48
Number of reported deceased	455	73	37	35	27
Percent of reported deceased	38%	71%	50%	59%	79%
Projected median survival (yr)	20	16	22	31	14

found to produce transient increases in neutrophil counts without effects on Hgb or platelets and without induction of acute leukemia or cytogenetic clones at up to 15 months (249,250). Results were similar with G-CSF, which also increased neutrophils but did not impact on Hgb or platelets (251,252). However, clonal cytogenetics (including monosomy 7 in three cases) or increased myeloblasts, or both, were observed in 4 out of 16 patients during or after G-CSF treatment. These complications may be manifestations of the natural history of Fanconi's anemia (see the section Complications). The use of G-CSF or GM-CSF should be restricted to patients with severe neutropenia and risk of serious infections and should be monitored with frequent blood counts, bone marrow examinations, and bone marrow cytogenetic studies.

COMPLICATIONS

The *in vitro* data regarding defects in DNA repair and cellular damage in Fanconi's anemia suggest that it might be a premalignant condition, which is borne out by the *in vivo* observations. Approximately 200 cases have been reported with leukemia or solid tumors—an overall incidence of approximately 15%. Because this incidence reflects biased reporting of interesting cases, the true figure may differ from this estimate. More than 100 patients were reported with leukemia, more than 30 were reported with liver tumors, and approximately 60 were reported with other cancers (Table 8-6). German observed that cancer in Fanconi's anemia patients has been reported only since the mid 1960s and suggested that androgen therapy,

which began in the early 1960s, allowed patients to survive long enough to develop the malignancy for which they were at risk (253). He also suggested that androgens might be implicated in the cause of these malignancies. Many patients who never received androgens developed leukemia or cancer, however, and, thus, the role of androgens is not relevant except, perhaps, for liver tumors.

The finding of a single gene for Fanconi's anemia (heterozygosity) was thought to be sufficient to confer a risk of malignancy. Garriga and Crosby (254) reported an increased incidence of leukemia in Fanconi's anemia families, and Swift (239,255) found a predisposition to cancer in heterozygotes. Subsequently, these analyses were extended from the original eight families to 25 families by Swift et al. (256) and to nine families by Potter et al. (257); neither study found an increase in cancer in Fanconi's anemia families. [Another disorder that was thought to be present at increased incidence in Fanconi's anemia heterozygotes is diabetes mellitus (258,259).] The earlier and perhaps erroneous conclusions regarding cancer were attributed to small numbers, incorrect assignment of Fanconi's anemia heterozygotes, and biased selection. The cancer risk of heterozygotes should be clarified in the near future, because heterozygote status can be confirmed with mutation analysis.

Leukemia. Leukemia has been reported in more than 100 cases, representing almost 10% of Fanconi's anemia patients in the literature (Table 8-7 and Fig. 8-10). In a single series of 44 patients, nine patients developed leukemia (20%), and five

TABLE 8-7. Leukemia in Fanconi's Anemia

Leukemia	Male	Female	All Patients	References
Acute lymphocytic leukemia	3	2	5	67,262-265
AML, unspecified	23	14	37	21,217,224,236,260,266-286
AML M1, acute myelocytic, without maturation	1	1	2	287,288
AML M2, acute myelocytic, with maturation	2	2	4	279,289,290
AML M3, acute promyelocytic	0	0	0	—
AML M4, acute myelomonocytic	12	8	20	17,81,84,291-305
AML M5, acute monocytic	6	4	10	40,284,306-312
AML M6, erythroleukemia	5	2	7	59,87,217,309,313,314
AML M7, acute megakaryocytic	1	0	1	315
AML, acute nonlymphocytic	1	5	6	243,312,316-318
Other acute leukemia	8	3	11	17,43,86,260,311,319-322
Total	62	41	103	—

AML, acute myeloid leukemia.

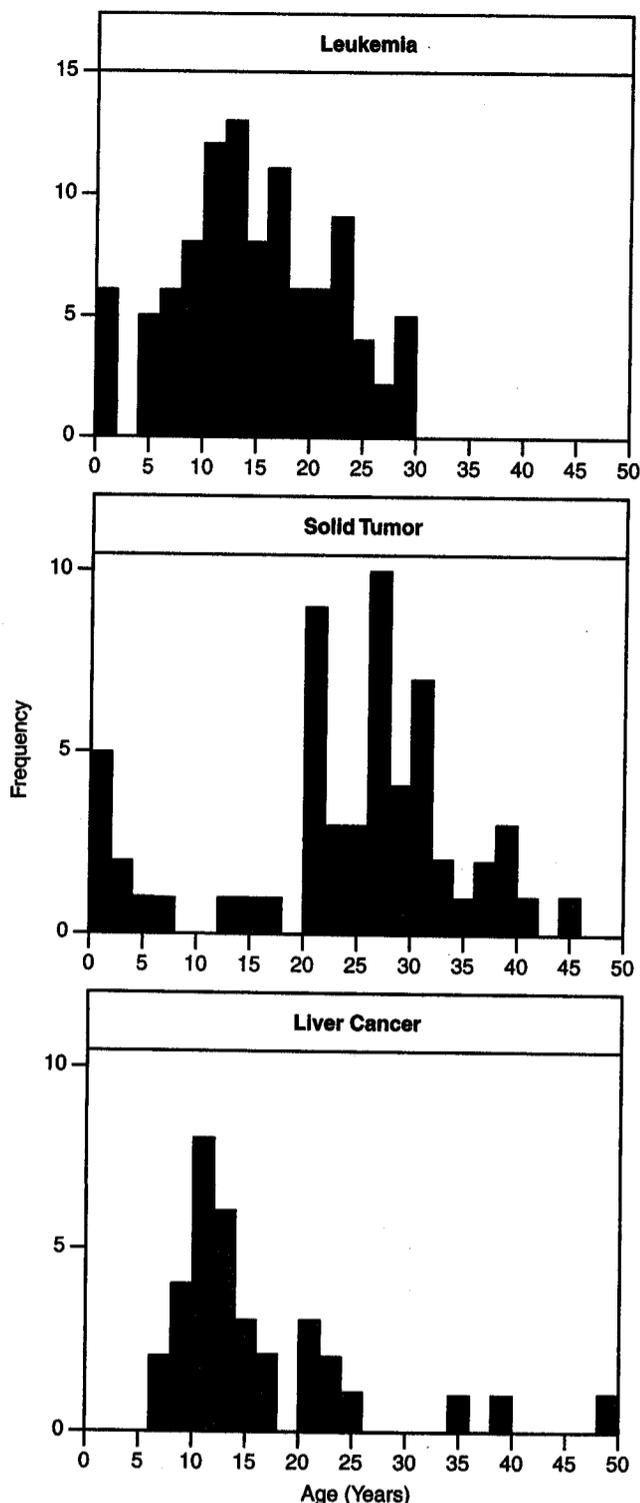


Figure 8-10. Age at diagnosis of cancer in Fanconi's anemia in 94 patients with leukemia, 60 patients with solid tumors, and 28 patients with liver tumors. Age at complication was not reported for all patients with those diagnoses.

more patients were preleukemic (260). In 25% of those patients with leukemia, the diagnosis of Fanconi's anemia was made only during the evaluation for leukemia. Approximately one-third of the patients had received androgens, which indicates the severity of preexisting aplastic anemia. Because two-thirds of those with leukemia had not received androgens, androgens

are an unlikely cause of leukemia. At least two patients presented with acute myelomonocytic leukemia, and they were treated with bone marrow transplants. They developed toxicity from the preparation, and the diagnosis of Fanconi's anemia was made retrospectively, after they were found to have increased chromosome breakage (261).

The characteristics of patients with Fanconi's anemia and leukemia are compared to the characteristics of the total group and those with other malignancies in Table 8-6. The male to female ratio is 1.5:1.0. The diagnosis of Fanconi's anemia was made at a median age of 9 years in those who eventually developed leukemia, which is significantly older than the diagnosis at a median of 7 years of age in those who did not develop leukemia. Leukemia was diagnosed at a median of 14 years of age (with a range from 0.1 to 29.0 years of age). Sixteen patients were over 16 years of age at the time of diagnosis of Fanconi's anemia, and, in ten of these patients, Fanconi's anemia was first diagnosed when the patients presented with leukemia.

Because the most common leukemia in children is lymphocytic, it is noteworthy that, until 1989, all leukemias reported in Fanconi's anemia were myeloid. Five cases of acute lymphocytic leukemia (ALL) have now been reported, although most of the leukemias are myeloid (Table 8-7). Because several patients were discussed in more than one publication, the number of literature reports of patients with Fanconi's anemia and leukemia probably exceeds the actual number of cases. Five patients with leukemia had coincidental hepatic tumors, which were usually discovered at postmortem examination (221,271,280,293,319,323).

Treatment of the leukemia was less than satisfactory, and deaths usually occurred within the first 2 months after diagnosis. Fanconi's anemia patients with leukemia are exquisitely sensitive to the toxic effects of chemotherapy, as predicted by the previous discussion regarding agents that increase damage to DNA. The combination of the forms of leukemia that are difficult to treat in anyone (e.g., myeloid), abnormal DNA repair, and a lack of marrow reserve does not afford a good prognosis. The only apparent long-term remission that has been reported is one of 9 years in duration (304,305,315; K. J. Roozendaal, *personal communication*, 1989). One 2-year survivor after reduced-dosage chemotherapy subsequently succumbed to varicella (92; A. D. Auerbach, *personal communication*). The median survival age for patients with leukemia is 16 years of age, which is younger than the survival age for Fanconi's anemia patients without leukemia. Seventy-five percent of patients with leukemia died at the time of the reports, and further follow-up was not available for most of the other patients. Although bone marrow transplant offers a theoretical cure for the leukemia of patients with Fanconi's anemia, these patients are usually very ill, and only a few have survived (243,290).

Will all patients develop leukemia if they do not succumb to aplastic anemia first? Probably not, because the development of leukemia appears to reach a plateau by age 30, whereas the older patients have the additional risk of solid tumors. Only long-term prospective studies can answer this question definitively.

Myelodysplastic Syndrome. Patients with Fanconi's anemia may develop syndromes that are variably called *myelodysplastic*, *refractory anemia*, or *preleukemia*. Some conditions evolve into full-blown leukemia; other patients die in their preleukemic phase, or their conditions are reported before further developments ensue. The risk of MDS was between 11% and 34% in cross-sectional studies (251,260,324,325). Although more than one-half of the cases of leukemia in Fanconi's anemia have cytogenetic clones, it has not been shown that the presence of a clone in the absence of leukemia means that leukemia is inevitable. A cytogenetic clone is included in the French-American-British classification of MDS

(326), and specific clones impact on the prognosis of MDS in the general population (327). However, application of these criteria to MDS in Fanconi's anemia may not be appropriate. Until recently, most of the reported cases had MDS diagnosed because of dysplastic marrow morphology or the presence of a clone, or both. In a systematic analysis in which clonality was considered independently of morphologic MDS, we found that one-half of the patients with adequate cytogenetic preparations had a clone, and clonal variation that included disappearance was common (325). One-third of the patients had morphologic MDS, and, thus, there were patients with cytogenetic clones who did not have MDS by other criteria. Poor outcome correlated with MDS, not with the presence of a clone. Patients with clones have survived for more than 12 years without the development of leukemia or died from cytopenic but nonmalignant complications of MDS. No consistent pattern was found in the involved chromosomes, although chromosomes 1 and 7 were more common than others; partial or complete deletions, translocations, and marker chromosomes were found. Details of the cytogenetic findings can be found elsewhere (279,283,325,328). Less than 10% of the Fanconi's anemia patients reported with leukemia had documented MDS; in those with prior MDS, the emergence of leukemia was within 1.5 years. The relevance of clonal cytogenetics may become more apparent when methods that are more sensitive than classic banding are widely used, such as fluorescence *in situ* hybridization (329).

The relation between morphologic MDS, cytogenetic clones, and acute myelogenous leukemia (AML) is not entirely clear, and remains the topic of active investigation. Because the age of the patient at diagnosis of Fanconi's anemia, complication (MDS or AML), and death is older in those with MDS than it is those with AML, it is not immediately apparent that all MDS become AML (Table 8-6). For this reason, it is not recommended that a bone marrow transplant be offered to the Fanconi's anemia patient who has a clone without other clinical indications of a need for transplant.

Three Fanconi's anemia patients were reported with Sweet's syndrome [acute neutrophilic infiltration of skin, which is asso-

ciated with malignancy 20% of the time (330)]. All three patients had myelodysplastic bone marrow, and two had clonal chromosomal abnormalities. The skin infiltrates responded to prednisone, but sustained treatment was required. This differs from responses in non-Fanconi's anemia patients with Sweet's syndrome, in which permanent resolution of symptoms occurs.

Solid Tumors. Fifty-nine patients (5%) were reported with a total of 70 cancers other than leukemia or liver tumors (Tables 8-6 and 8-8). The group with cancer is different in several features from the entire Fanconi's anemia population (Table 8-6 and Fig. 8-10). The preponderance of women with cancer (the male to female ratio is 0.4) is owing to the prevalence of gynecologic malignancies. The median age at diagnosis of Fanconi's anemia was 9.5 years of age in the cancer group, with one-third of the patients diagnosed at 16 years of age or older. In approximately 20% of patients, it appears that the diagnosis of Fanconi's anemia had not been made before the development of cancer. Eight tumors were diagnosed before 10 years of age, but the median age was 26 years of age, and 80% of patients were at least 20 years of age when cancer was detected. The usual median age for the types of cancers that are seen in Fanconi's anemia is approximately 65 (331), and, thus, their occurrence at a median age of 26 years of age is highly unusual.

The types of tumors are listed in Table 8-8. The largest number of tumors was in the head and neck region, including the tongue, gingiva, pharynx, larynx, epiglottis, and mandible, as well as the esophagus. Gynecologic cancers, particularly vulvar and cervical, were also common in women. The other areas that are listed in the table occurred less frequently. Most of the tumors were squamous cell carcinomas. Three patients had solid tumors as well as AML (269,287,289).

The median survivals that are shown in Table 8-6 suggest that survival is longer in those patients with cancer than in the overall group of Fanconi's anemia patients. This can be interpreted to indicate that cancer is clearly a disease of the older Fanconi's anemia patient. Thus, if these patients do not die from aplastic anemia, leukemia, or liver disease, they are at a high

TABLE 8-8. Solid Tumors in Fanconi's Anemia

Type	Male	Female	All Patients	References
Nonhepatic				
Oropharynx	10	12	22	201,207,221,268,293,307,332-347
Esophagus	1	8	9	44,204,332,348-354
Vulva and anus	—	4	4	79,299,334,355
Vulva	—	5	5	338,356-358
Anus	—	2	2	201,359
Cervix	—	3	3	269,355,356
Brain	1	3	4	287,360,361
Skin (nonmelanoma)	0	5	5	201,279,284,359,362
Breast	—	4	4	201,206,222,229
Lung	2	0	2	17,182
Lymphoma	1	1	2	363,364
Gastric	2	0	2	365,366
Renal	0	3	3	201,209,360,367
Colon	0	1	1	299
Osteogenic sarcoma	0	1	1	368
Retinoblastoma	0	1	1	289
Total cancers	17	53	70	—
Total patients	16	43	59	—
Hepatic				
Adenoma	6	5	11	211,319,369-375
Hepatoma	14	8	22	73,205,221,268,271,280,293,323,344,350,376-390
Not stated	0	1	1	211,372
Total liver tumors	20	14	34	—

risk for a solid tumor. The predicted median survival age for patients with solid tumors is 31 years of age, at which age more than 75% of the total group of Fanconi's anemia patients have already died. Survival is short after the diagnosis of a malignancy. Treatment of most of these tumors is difficult because of increased toxicity from chemotherapy or radiation therapy, and surgery is recommended whenever possible. More than one-half of the patients had died by the time that they were reported.

Tumors that occurred after bone marrow transplant were not included in the previous analyses, because transplant itself may be a risk factor for solid tumors. At least eight patients (four men, four women) were reported (some of them more than once) with tongue cancer after marrow transplant (188,211,391-398); five had died at the time of the reports. The tumors were diagnosed at 3 to 15 years after transplant in patients between 11 and 33 years of age. The incidence of solid tumors after transplant, specifically in Fanconi's anemia, is not known, because there is no clear denominator. Among more than 1000 long-term survivors who were transplanted for all hematologic indications in the European Blood and Marrow Transplantation Registry, the actuarial incidence of malignant neoplasms was 13% at 15 years, and oral or esophageal cancers were increased tenfold compared to the general population in the Danish and German Cancer Registries (399). Thus, it would appear that the combination of Fanconi's anemia and transplant might have an even higher risk.

Liver Tumors. Hepatic tumors were reported in 34 patients (3%). The male to female ratio of 1.4:1.0 and median age at diagnosis of Fanconi's anemia of 7 years (Table 8-6) indicate that this group is not different from the overall group. The median age at which the liver tumors were detected was 13 years of age, although the range was wide and encompassed the oldest patients (Fig. 8-10). Because only one patient did not have antecedent androgen treatment, it might be argued that androgen treatment increases the risk of liver tumors in Fanconi's anemia patients. Hepatocellular carcinomas were twice as common as adenomas (Table 8-8), although the former were not overtly malignant in that they did not metastasize or invade, and they were often not associated with an increase in serum α -fetoprotein. One patient also had tongue cancer (344), one had esophageal cancer (350), and five had leukemia (221,271,280,293,319,323). The liver tumors were often found at postmortem examination. In general, patients with liver tumors, whether adenomas or hepatomas, did not die from their liver tumors but from other malignan-

cies or complications of bone marrow failure. Discontinuation of androgens, alone or combined with BMT, often led to resolution of the tumors (205,372). Peliosis hepatis also reversed when androgens were stopped. Surgical removal of tumors was undertaken occasionally.

Summary of Malignancy in Fanconi's Anemia. The risk of the development of leukemia, a liver tumor, or a solid tumor in Fanconi's anemia patients totals greater than 15% in the literature, although the true frequency may be obscured by overreporting. It is an important risk, particularly in older patients, because the average age at which malignancies are diagnosed is beyond the age of survival of many Fanconi's anemia patients. Prolongation of survival by a combination of androgens, better supportive care, and stem cell transplantation (and gene therapy, in the future) may lead to more time for malignancies to appear and may even increase the risk of cancer (399a,399b). In addition, Fanconi's anemia may now be diagnosed by chromosome breakage or mutation analysis in patients with characteristic cancers but without any other stigmata of Fanconi's anemia. Concerns about the development of cancer in older patients cannot be used as contraindications for aggressive management, such as stem cell transplantation. However, cytoreductive chemotherapy and irradiation may themselves increase the risk of malignancies. To some degree, aplastic anemia may soon be considered to be the least of the problems of the Fanconi's anemia patient.

Dyskeratosis Congenita

Dyskeratosis congenita, also known as *Zinsser-Cole-Engman syndrome*, which is named after the first physicians who described these patients, is a rare form of ectodermal dysplasia, with a 40% to 50% frequency of aplastic anemia and a 10% to 15% frequency of cancer. The diagnostic triad consists of dermatologic manifestations and nail dystrophies that usually begin in the first decade of life, and leukoplakia that begins in the second decade of life; all of these conditions become more extreme with increasing age (Fig. 8-11). Aplastic anemia usually develops in the second decade of life, and cancer develops in the third and fourth decades of life.

INHERITANCE AND ENVIRONMENT

More than 275 cases of dyskeratosis congenita have been reported as case reports with data that could be analyzed on an individual basis, many of which are summarized elsewhere (400,401). Dokal reviewed the 148 members of the Dyskeratosis

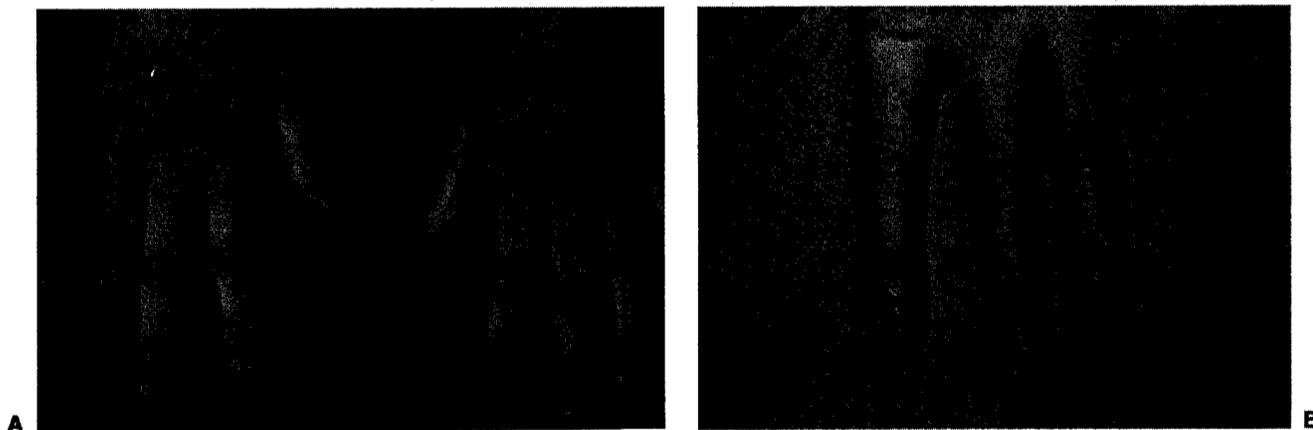


Figure 8-11. A-D: Dystrophic fingernails in dyskeratosis congenita. (From Alter BP, Drachtman RA. Dyskeratosis congenita: nails and hands. *Am J Hematol* 1998;58:298, with permission.) (continued)

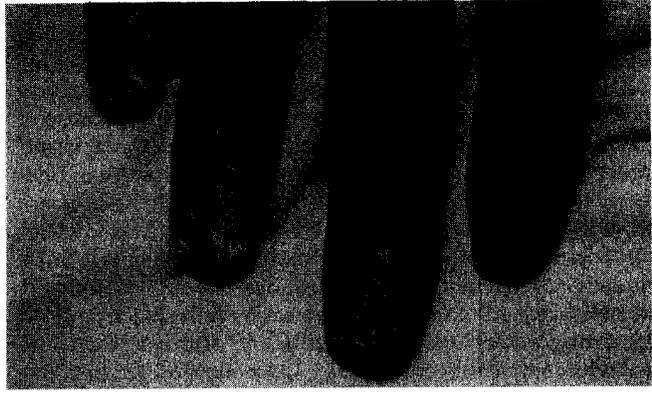
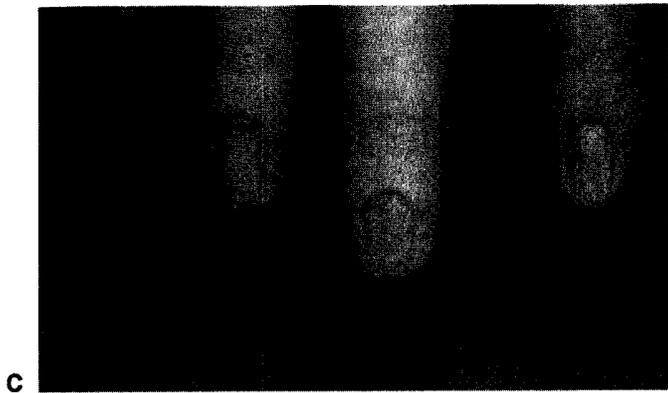


Figure 8-11. (continued)

Congenita Registry (DCR) that was established at Hammsmith Hospital (402). Although the impression is that dyskeratosis congenita is an X-linked disorder, the male to female ratio is 4.5:1.0 in the literature cases and 3.9:1.0 in the DCR (Table 8-9).

Presumed X-linked recessive: More than 200 males have been reported as single cases or from families with males only. More than 30 males were in sibships, with an additional seven families with uncles and nephews and five families with maternal cousins.

Possible autosomal recessive: Forty-four patients were sporadic females or males and females in sibships. Seven families had affected children who were the products of consanguineous marriages. There is an excess of females in this category, because sporadic males could not be distinguished from X-linked males.

Possible autosomal dominant: Thirty cases were in families in which the transmission appeared to be dominant, with two or more generations involved. The sex ratio is approximately 1 in this group.

The families that appear to be autosomal recessive or dominant might be X-linked with inactivation of the normal X chromosome and variable levels of expression, but it is more likely that the dyskeratosis congenita phenotype may be due to more

than one gene. Similarly, the presumably X-linked male group may contain autosomal-recessive patients who happen to be male, as well as new mutation dominants. These possibilities may explain the apparent preponderance of females in the autosomal groups. The X-linked recessive group can be defined more specifically using Xq28 restriction fragment-length polymorphisms (403) and mutation analyses (see the section Pathophysiology) (402). No ethnic and sex association was found; blacks have been reported in all groups, and Asians have been reported in the X-linked and autosomal-recessive groups.

Tables 8-9 through 8-12 compare the findings in the three groups. In comparison to the X-linked recessive males, the autosomal-dominant patients appear to be milder, with a lower frequency of all the components in the diagnostic triad and a lower rate of serious complications. The autosomal-recessive group resembles the X-linked males, although there may be a higher frequency of aplastic anemia in the autosomal-recessive patients.

The age at diagnosis of dyskeratosis congenita in the three groups is depicted in Figure 8-12. The ages may be inappropriately skewed to the high side, because, in many cases, the only age available was that given at the time of the report. The

TABLE 8-9. Dyskeratosis Congenita Literature

Characteristic	X-linked and Sporadic Male	Autosomal Recessive	Autosomal Dominant
Number of cases	200	44	30
Male/female	200/0	10/34	14/16
Ratio	—	0.29	0.88
Age at diagnosis (or report) (yr)			
Mean	18.2	14.9	30
Median	15	13	25
Range	0.3-68	1.2-42	7-58
Age at presentation of nail changes (yr)			
Mean	8.5	5.8	9.5
Median	8	5	7
Range	0-34	0-15	7-17
Age at presentation of skin pigmentation (yr)			
Mean	9.4	7.1	13.1
Median	9	4	13
Range	0-30	0-29	7-18
Age at presentation of leukoplakia (yr)			
Mean	12.6	9	15.3
Median	10	7	17
Range	0.7-43	2-25	12-17

NOTE: Compiled from individual case reports, not including the Dyskeratosis Congenita Registry summary (402). Physical features were not always reported.

TABLE 8-10. Physical Abnormalities in Dyskeratosis Congenita

Characteristic	X-linked and Sporadic Male	Autosomal Recessive	Autosomal Dominant
Number of cases	200	44	30
Skin pigmentation	92	86	67
Nail dystrophy	90	39	13
Leukoplakia	68	68	33
Eye abnormalities	40	43	13
Mouth abnormalities	19	30	10
Developmental delay	15	16	0
Skeletal anomalies	11	25	7
Short stature	13	23	3
Hyperhidrosis	11	9	7
Hair loss	14	30	23
Urinary tract abnormalities	8	5	3
Gastrointestinal abnormalities	13	23	7
Other conditions	10	23	10
Gonadal anomalies	4	9	3

NOTE: Physical features were not always reported. Numbers are percent of cases except in top row, where they are total numbers.

TABLE 8-11. Complications in Dyskeratosis Congenita

	X-linked and Sporadic Male	Autosomal Recessive	Autosomal Dominant
Number of cases	200	44	30
Male to female ratio	—	0.29	0.88
Age at time of diagnosis (or report) (yr)			
Mean	18.2	14.9	30
Median	15	13	25
Range	0.3-68	1.2-42	7-58
Aplastic anemia			
Number of cases (%)	72 (36)	26 (59)	1 (3)
Age at diagnosis (yr)			
Mean	13.3	13.7	16
Median	10.5	11	16
Range	1-41	2-45	16
Cancer			
Number of cases (%)	35 (13)	6 (14)	2 (7)
Age at diagnosis (yr)			
Mean	30.4	27.8	30
Median	29	24.5	30
Range	13-68	21-42	17, 43
Deceased			
Number of cases (%)	60 (30)	12 (27)	1 (3)
Age at diagnosis (yr)			
Mean	21.2	21.2	39
Median	19.5	22.5	39
Range	2-70	5-34	39
Projected median	33	34	—

TABLE 8-12. Cancer in Dyskeratosis Congenita

Type	X-linked and Sporadic Male	Autosomal Recessive		Autosomal Dominant		All Patients	References ^a
		Male	Female	Male	Female		
Oropharyngeal	12	0	4	0	1	17	402,419,435-445
Gastrointestinal	12	0	0	0	0	12	402,410,420,435,439,446-455
Myelodysplastic syndrome	4	0	0	0	0	4	402,452
Skin	3	1	0	1	0	5	402,456-460
Hodgkin's disease	2	0	0	0	0	2	461,462
Bronchial	0	2	0	0	0	2	402,463
Pancreatic	0	1	0	0	0	1	402,461
Liver	1	0	0	0	0	1	464
Cervical and vaginal	0	0	1	0	0	1	465
Total cancers	37	1	5	1	1	45	—
Total patients	35	1	5	1	1	43	—

^aSome cases were cited in more than one reference.

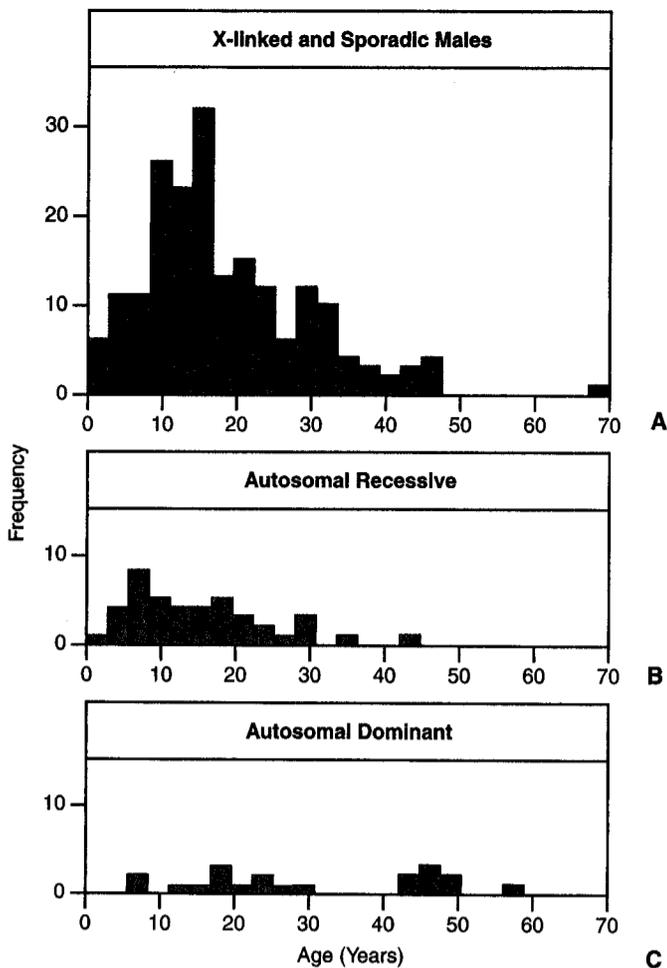


Figure 8-12. Age at diagnosis of dyskeratosis congenita in more than 250 published cases from 1910 to 2000. **A:** Two hundred X-linked and sporadic males. **B:** Forty-four autosomal-recessive patients. **C:** Thirty autosomal-dominant patients.

median age was 15 years of age for X-linked and sporadic males, 13 years of age for autosomal recessives, and 25 years of age for autosomal dominants, thus distinguishing the latter group again. The ages at the development of the components of the diagnostic triad are summarized in Table 8-9. All findings occurred earlier in the autosomal-recessive patients than in the other categories. All three components of the diagnostic triad were reported in more than 70% of the X-linked and autosomal-recessive patients and in 50% of the dominant cases.

PHYSICAL EXAMINATION

The major skin finding in patients with dyskeratosis congenita is lacy, reticulated hyperpigmentation, with dark, grayish macules on an atrophic and sometimes hypopigmented background, which involves the face, neck, shoulders, and trunk. These changes become more dramatic with increasing age. The dystrophic nail changes (hands and feet) include small nail plates, which may develop longitudinal ridging and then may become hypoplastic and eventually disappear. Leukoplakia involves the oral and other mucous membrane surfaces.

Several other systems are also involved (Table 8-10). The eyes are affected in approximately 40% of the patients, most often with epiphora (excessive tearing) due to blocked lacrimal ducts or blepharitis, as well as cataracts, a lack of eyelashes, conjunctivitis, ectropion, abnormal fundi, glaucoma, strabis-

mus, and ulcers. Many patients have poor dentition, with multiple caries and early loss of all teeth. Skeletal abnormalities include osteoporosis, frequent fractures, aseptic necroses (all but one of the latter eight patients had received prednisone), and scoliosis. Several patients have intracranial calcifications. Approximately 15% of the patients are short, slender, delicate, or asthenic in appearance. Hyperhidrosis of palms and soles is common. Early hair thinning or loss and premature graying were also reported. Urinary tract involvement was primarily mucosal, with meatal and urethral stenosis, phimosis, hypospadias, pyelonephritis, penile leukoplakia, and one horseshoe kidney reported. Gastrointestinal problems include esophageal stenosis, diverticula, spasm, duodenal ulcer, anal leukoplakia, bifid uvula, and umbilical hernia. Male hypogonadism with hypoplastic testes was reported in a few cases, similar to Fanconi's anemia. One woman had a vaginal constriction, one had vulvar leukoplakia, and one had a hysterectomy. Four women apparently had successful pregnancies. Other rare reports include deafness, absent eardrum, bird face, cardiac disease, Dandy-Walker deformity, cholesteatoma, and microcephaly.

Because of the coincidence of skin abnormalities, aplastic anemia, and malignancies, dyskeratosis congenita has sometimes been confused or compared with Fanconi's anemia (404-409). In fact, in one series of five Fanconi's anemia patients, one patient probably had dyskeratosis congenita and not Fanconi's anemia (199). The genetics and physical abnormalities of patients with Fanconi's anemia and dyskeratosis congenita are actually quite different; although the same systems may be involved, the types of anomalies are characteristic. In addition, aplastic anemia usually occurs earlier in Fanconi's anemia than in dyskeratosis congenita. These disorders should not be confused clinically and can be distinguished definitively by analysis of chromosome breakage after clastogenic stress or by mutation analyses.

APLASTIC ANEMIA

Thirty-six percent of the X-linked male dyskeratosis congenita patients and 59% of the autosomal-recessive patients were reported to develop aplastic anemia at a median age of 11 years (Table 8-11). Aplastic anemia was rare in the autosomal-dominant patients. In many cases, hematologic symptoms preceded the diagnosis of dyskeratosis congenita, although, in retrospect, the physical abnormalities of dyskeratosis congenita had been present for several years. In the younger patients, hematologic changes may have occurred before the appearance of the dyskeratosis congenita triad. The frequency of bone marrow failure was much higher in the Hammersmith DCR cases, in which it occurred in 86% of the 118 men, with an actuarial probability of 94% by 40 years of age (402). This difference may be related to biased referrals of patients with severe hematologic involvement.

LABORATORY FINDINGS

Blood Counts. Thrombocytopenia or anemia, or both, are the initial signs in most patients who ultimately develop aplastic anemia. Macrocytosis and elevated HbF are common manifestations of stress erythropoiesis, even in patients without pancytopenia. Bone marrow aspirates may be hypercellular at first, and several patients were initially thought to have hypersplenism. Decreased megakaryocytes, hypocellularity, and aplasia eventually ensue. Ferrokinetic studies are consistent with aplastic anemia (406,407,410). A few patients had decreased immunoglobulins (Igs) or decreased cellular immunity, but this has been inconsistent (411).

Chromosome Breakage. Chromosome breakage was studied in several patients. In a few patients, baseline breakage was

apparently increased, but the data from many patients and, particularly, from controls were often not cited. Breakage studies were normal in most patients, including those who were examined with DEB, MMC, or nitrogen mustard. Patients with dyskeratosis congenita probably do not have increased breakage in lymphocytes, particularly with clastogenic stress, and can be differentiated from those with Fanconi's anemia on this basis. However, cultured fibroblasts develop chromosomal rearrangements, which suggests that dyskeratosis congenita may be considered a chromosomal instability disorder (412).

Hematopoietic Cultures. Hematopoietic cultures have been performed in a few instances of patients with dyskeratosis congenita. In all patients, the numbers of progenitors were reduced or there were none. All these patients were studied when they already had hematologic symptoms (127,413-419). Addition of GM-CSF increased colony numbers (418), as did SCF (131). Long-term cultures were also defective in dyskeratosis congenita (420).

PATHOPHYSIOLOGY

Dyskeratosis congenita is inherited in a predominantly X-linked pattern, but there are families with apparently autosomal-recessive inheritance, as well as others with dominant inheritance. The autosomal recessives might have X-linked inheritance with lyonization and variable expression in males and females. The more compelling explanation is that there are at least three dyskeratosis congenita genes. Patients with dyskeratosis congenita have a genetic risk for bone marrow failure, but an environmental factor may be required for its manifestation. At least one patient received chloramphenicol before the development of pancytopenia (421).

The X-linked gene was localized to Xq28 with restriction fragment-length polymorphisms (403,422). Subsequently, genetic linkage and XCIP (X chromosome inactivation pattern) analysis narrowed the region to only 1.4 Mb (423). One of the 28 positional gene candidates in this region was found to have a 3'

deletion in one patient, and missense mutations were found in others, thus allowing identification of the dyskeratosis congenita gene, DKC1 (424,425). Verification of DC as the causative gene in the disease came from the identification of multiple missense mutations in the open reading frame (402,426,427). Because the gene is highly conserved with genes in other lower eukaryotes, the distinction between true missense mutations and benign polymorphisms is readily apparent.

The DKC1 gene is composed of 15 exons that span 15 kilobases (kb), and the cDNA is 2.5 kb. The corresponding protein, dyskerin, is 514 amino acids in size, with a predicted molecular weight of 57 kd. A mutational screen of dyskeratosis congenita patients and patients with a related syndrome, Hoyeraal-Hreidarsson, led to the recognition of a wide range of mutations (Fig. 8-13). Because most of these are missense mutations, it is likely that they encode mutant proteins with partial activity. True null mutations in the DC gene may be lethal (402).

The cellular function of dyskerin remains unclear. The protein contains multiple phosphorylation sites and a carboxy-terminal lysine-rich repeat domain. Dyskerin is the ortholog of rat NAP57 and yeast CBF5, suggesting that this protein may function in ribosomal RNA biogenesis and in the assembly of ribosomes (424,428). Consistent with this hypothesis are studies that showed that dyskerin localizes to the nucleolus of mammalian cells. Taken together, these data suggest that dyskerin plays a role in ribosomal assembly and, therefore, indirectly in protein translation and in cell survival. The two tissues that are most highly affected in dyskeratosis congenita (skin epithelium and bone marrow) have a high turnover in adults, suggesting that dyskerin plays a role in survival of cells with a high proliferative capacity.

Although Fanconi's anemia cells have a characteristic cellular phenotype (i.e., sensitivity to DNA cross-linkers), dyskeratosis congenita cells have no consistent phenotype, which makes complementation studies and functional assessment of mutations more difficult. One study found that dyskerin binds to

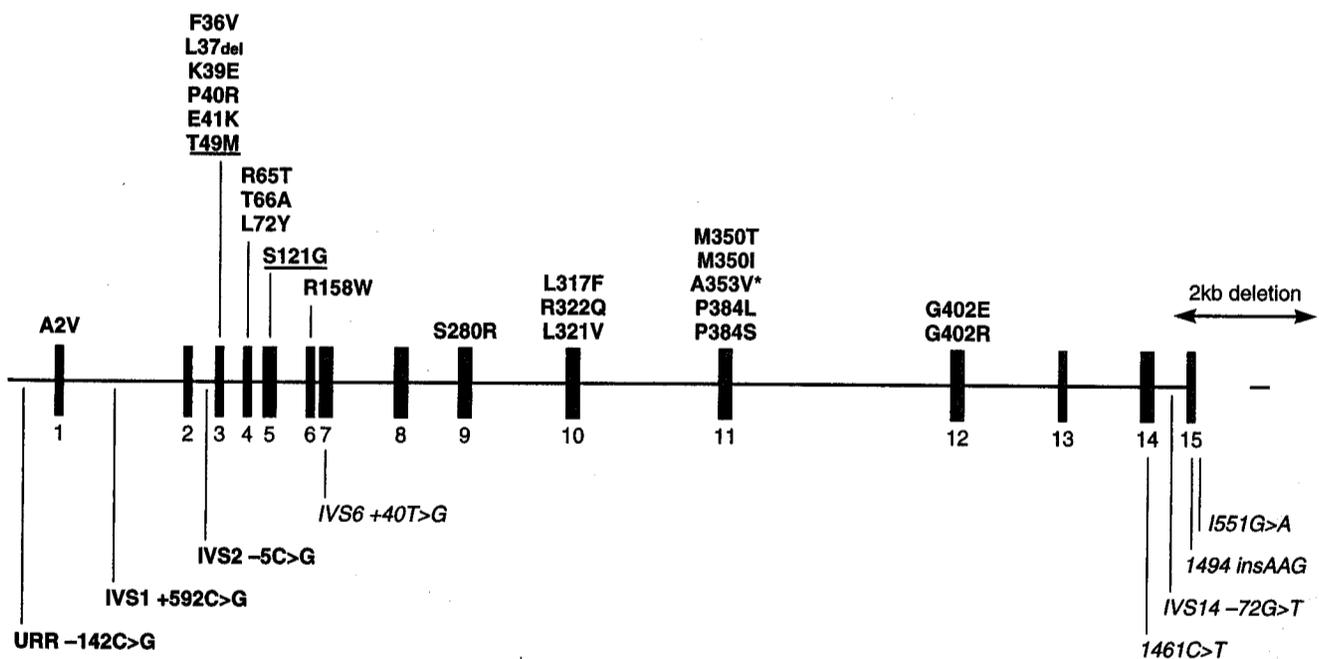


Figure 8-13. Mutations in the DKC1 gene. Schematic representation of the 15 exons with patient-derived mutations (*bold*) and polymorphisms (*italics*). Asterisk indicates the A353V mutation that has recurred in 17 different families. Underlined mutations were found in patients with the Hoyeraal-Hreidarsson syndrome. kb, kilobase. (Modified from Dokal I. Dyskeratosis congenita in all its forms. *Br J Haematol* 2000;110:768-779, with permission.)

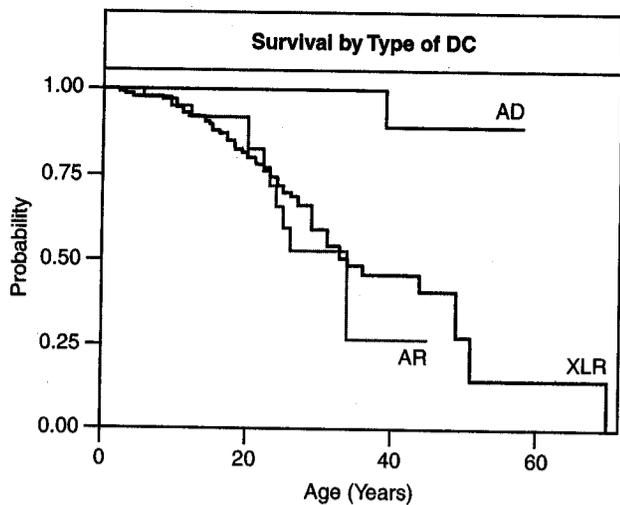


Figure 8-14. Kaplan-Meier plot of cumulative survival in dyskeratosis congenita (DC). Time is shown as age in years. AD, 20 autosomal-dominant patients; AR, 42 autosomal-recessive patients; XLR, 195 X-linked and sporadic males. The differences are significant.

telomerase and therefore may play a role in telomere length maintenance (429). Peripheral blood cells of patients with X-linked dyskeratosis congenita have much shorter telomeres than those in normal cells (430). This is especially interesting, because accelerated telomere shortening is associated with murine carcinomas (431) and may therefore be relevant to the development of squamous cell carcinomas in dyskeratosis congenita (see the section Cancer).

The cloning of the DKC1 gene has important implications for the diagnosis of dyskeratosis congenita and related syndromes. The clinical abnormalities are variable from patient to patient in severity and age of onset. However, pulmonary disease, myelodysplasia, and malignancy may develop in the older patients who survive or avoid the complications of early bone marrow failure.

Mutational screening of the DKC1 gene is warranted in any patient with clinical signs that are consistent with the disease, although considerable disease heterogeneity is evident. In the related syndrome, the *Hoyeraal-Hreidarsson syndrome*, patients have a severe multisystem disorder that is characterized by microcephaly, cerebellar hypoplasia, growth retardation, immunodeficiency, and aplastic anemia. This syndrome was found to result from unique missense mutations in the DKC1 gene (432-434). Based on these new findings, mutational screening of the DKC1 gene should be considered in any patient with severe clinical phenotypes who has some of the features of dyskeratosis congenita or *Hoyeraal-Hreidarsson syndrome*, even if he or she does not have the more classic signs of dyskeratosis congenita, such as skin and nail changes.

PROGNOSIS

The prognosis is poor in dyskeratosis congenita. One-third of the X-linked and sporadic male patients and of the autosomal-recessive patients died by the time of the reports, at an actuarial median age of 34 and 26 years of age, respectively (Fig. 8-14). More than one-half of the deaths were due to complications of aplastic anemia, such as infection or hemorrhage. Unsuccessful bone marrow transplants and cancer were responsible for most of the rest of the poor outcomes (see the section Cancer). Dokal suggested that patients with dyskeratosis congenita have a predisposition to endothelial activation and damage, based on the observation of increased levels of von Willebrand's factor and

pulmonary complications in several patients, including some complications that occurred posttransplant (402).

CANCER

Cancers were reported in 35 X-linked males, six autosomal-recessive patients (one male, five females), and one male and one female who were in the autosomal-dominant group (Tables 8-11 and 8-12). This summary includes the cases that were reported by Dokal (402), and, thus, there may be inadvertent duplicate reporting. At least two patients had two or more tumors. The majority of the tumors were squamous cell carcinomas. The sites were similar to those reported in patients with Fanconi's anemia (Table 8-8) and involve areas that are known to be abnormal in dyskeratosis congenita, such as mucous membranes and the gastrointestinal tract. The median age for cancer was similar in all three types of dyskeratosis congenita and was approximately 30 years of age, with a range from 13 to 68 years of age, which is substantially higher than the median age of 11 years for the development of aplastic anemia (Table 8-11). Among those who developed cancer, the predicted median survival is 36 years of age. In contrast to Fanconi's anemia patients, only a rare patient with dyskeratosis congenita has been reported to have leukemia or MDS.

TREATMENT

Treatment for the aplastic anemia of dyskeratosis congenita is similar to that for Fanconi's anemia. Almost 40 patients were reported to receive *androgens*, usually combined with prednisone, as described earlier for Fanconi's anemia, and approximately 50% of patients responded, a similar response rate to that seen in Fanconi's anemia. As in Fanconi's anemia, responses are not cures, and androgens must be maintained; subsequent failures do ensue. *Splenectomies* were reported in at least eight male patients, with only temporary improvements. *Supportive care* with RBC and platelet transfusions, antibiotics, and ϵ -aminocaproic acid (466) should all be provided when indicated clinically. There are no reports on the use of antilymphocyte globulin or cyclosporin A, but these agents would not be expected to work (one of our patients received these at another institution without response) (400).

Hematopoietic growth factors were used in a small number of cases, mostly for brief intervals in which only neutrophil responses were documented. GM-CSF was effective in two patients (467,468), IL-3 was effective for one of three patients (469), and G-CSF was effective for three patients (470-472). All of those were X-linked or sporadic male cases. We treated one man from an autosomal-recessive family with G-CSF for more than 1 year [with erythropoietin (EPO) for the last 10 months] with an excellent neutrophil response and a 6-month improvement in Hgb and platelets (473). Unfortunately, severe aplastic anemia recurred despite continuation of both cytokines. Nevertheless, the combination of G-CSF and EPO might warrant additional long-term trials.

BMT was reported in 20 X-linked or sporadic males (452,460,470,474-483), with six survivors (30%) when reported. Four of seven autosomal-recessive patients (57%) were alive when reported (460,475,484-488). Causes of deaths included acute and chronic graft-versus-host disease, infections, venoocclusive disease of the liver at as long as 7 years after transplantation, and pulmonary fibrosis at up to 20 years. Some patients developed a mucositis syndrome that was similar to that seen in Fanconi's anemia patients who received standard levels of cyclophosphamide preparation. Most of the dyskeratosis congenita patients were prepared for transplant with standard cyclophosphamide plus irradiation. The long-term prognosis for most patients after transplant is poor, with actuarial median

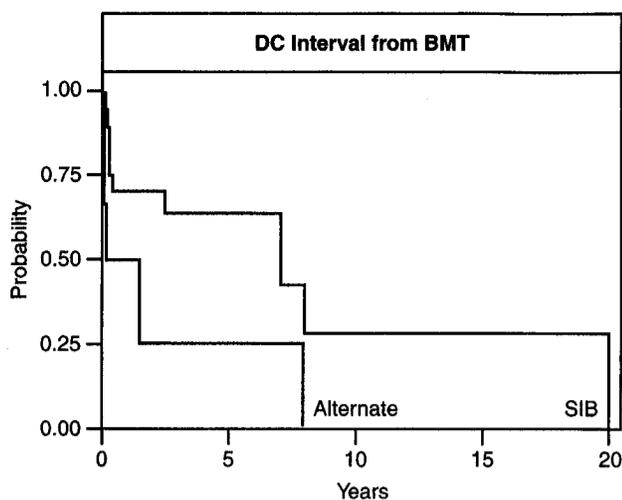


Figure 8-15. Kaplan-Meier plot of cumulative survival after bone marrow transplantation (BMT) for patients with dyskeratosis congenita (DC). Time is shown as years from transplantation. One patient was unsuccessfully transplanted from a brother with DC (not shown). The difference between sibling and alternate donors is not significant. Alternate, seven patients were transplanted from alternate donors; SIB, 20 patients were transplanted from HLA-identical siblings.

survivals of 7 years for those with HLA-matched sibling donors, and 1.5 years for the five cases in which alternate donors were used (Fig. 8-15). The median survivals were 8 years for the autosomal-recessive patients, and 1.5 years for the X-linked and sporadic men.

Transplantation in dyskeratosis congenita patients must thus be approached with caution. In addition, because the physical features of dyskeratosis congenita may appear late or may be subtle, potential donors who may in fact be affected with dyskeratosis congenita may be difficult to detect unless they have an identifiable mutation. Finally, we can speculate that, as in Fanconi's anemia, marrow transplantation does not reduce (and may increase) the risk of development of tumors. None have been reported so far, perhaps because the long-term survival after transplant has been short, and the number of patients who have undergone transplants is small.

Disorders That Are Related to Dyskeratosis Congenita

As mentioned previously in the discussion of the *DKC1* gene (see the section Pathophysiology), a small number of male patients has been described under the rubric of *Hoyeraal-Hreidarsson syndrome*. The patients are small, with intrauterine growth retardation, microcephaly, cerebellar hypoplasia, developmental delay, progressive pancytopenia, and immunodeficiencies, and the inheritance appears to be X-linked. Recent studies of three patients identified mutations in the *DKC1* gene (432,489). Seven of the 11 patients died from infectious or hemorrhagic complications of aplastic anemia, and the oldest survivor was 5 years of age and was 3 years of age after an unrelated bone marrow transplant.

An even more rare disorder, *Revesz syndrome*, has been reported in four cases, and one of the authors is aware of two more (486,490,491; B. P. Alter, unpublished data, 1999). There were three males and three females, and the characteristic findings include intrauterine growth retardation, cerebellar hypoplasia, and microcephaly. The patients have features of dyskeratosis congenita, such as dystrophic nails, oral leukoplakia, sparse

hair, and reticular skin pigmentation, as well as bone marrow failure in four of the six patients. However, the unique feature of these patients is bilateral exudative retinopathy, which was called *Coats' retinopathy*, but which resembles Norrie's disease (492). No germline mutations were found in the *DKC1* gene or the Norrie's gene in one of our patients, however, and the genetic basis of this syndrome remains unclear. A major concern is the combination of thrombocytopenia and hemorrhagic retinopathy, and it is recommended that platelet transfusions be provided for unresponsive thrombocytopenia to decrease further retinal hemorrhages. All patients, who were younger than 4 years of age when reported, were alive, although Revesz's original patient subsequently died (T. Revesz, personal communication, 1999).

Still another disorder with overlap with those disorders that were previously mentioned is the *ataxia-pancytopenia syndrome*, which was first described by Li et al. (493,494) in a family with ataxia in the father and all five children. Two brothers died with aplastic anemia, one with acute myeloblastic leukemia, and one with acute myelomonocytic leukemia. The only surviving sibling was a 19-year-old girl with mild anemia. A second family was reported by Daghistani et al. (495) in which the mother, her son, and her daughter had ataxia; the son had pancytopenia and monosomy 7 and developed acute myeloblastic leukemia. There are a small number of additional case reports of families or sporadic cases with men and women with ataxia, cerebellar atrophy, microcephaly, tongue ulcers, immunodeficiencies, and aplastic anemia or leukemia (496-499); none of these cases had monosomy 7. Eight of the 15 patients died between 3 and 10 years of age; four died from leukemia, and four died from complications of aplastic anemia. Treatment of pancytopenia with prednisone was effective in one case (497), whereas antithymocyte globulin, cyclosporine, and G-CSF were ineffective on another (496), and prednisone plus danazol were also ineffective (498).

Shwachman-Diamond Syndrome

The Shwachman-Diamond (Bodian Shwachman) syndrome consists of exocrine pancreatic insufficiency plus neutropenia (500-502). More than 300 cases have been reported (Table 8-13). Signs of pancreatic insufficiency, usually apparent in infancy, are diarrhea, malabsorption, steatorrhea, and failure to thrive. Neutropenia is identified early as part of the general workup or because of skin infections or pneumonia. Additional hematologic problems develop in 40% of patients, such as anemia, thrombocytopenia, and pancytopenia. Occasionally anemia or thrombocytopenia is the initial hematologic problem. The ratio of males to females is 1.6:1.0. Segregation analysis provides evidence that the inheritance is autosomal recessive, although reports of consanguinity are rare (503). Shwachman-Diamond syndrome has been reported in all racial groups, with no ethnic propensity. Pregnancies and birth histories of patients are uneventful, although more than 10% of patients had low birth weight. One Shwachman-Diamond patient was reported who had a successful pregnancy, during which her white blood cell count rose slightly, her absolute neutrophil count doubled from her usual range of less than 2000/ μ L to approximately 4000/ μ L, and her platelets dropped from more than 120,000/ μ L to less than 110,000/ μ L. She required a caesarean section for cephalopelvic disproportion, which perhaps was related to her small stature. However, there were no major complications from her Shwachman-Diamond syndrome (504).

The most prominent physical findings are related to malnourishment, including short stature in more than one-half of patients, protuberant abdomen, and low weight. Forty percent of patients had radiographic evidence for metaphyseal dysosto-

TABLE 8-13. Shwachman-Diamond Syndrome Literature

	All Patients	Cytopenias	No Anemia or Thrombocytopenia
Number of cases (%)	336	134 (40)	202 (60)
Male/female	196/121	79/53	117/68
Ratio	1.6	1.5	1.7
Number with metaphyseal dys- ostosis (%)	124 (37)	51 (38)	73 (36)
Age at malabsorption			
Mean	1.1	1.0	1.1
Median	0.3	0.3	0.3
Range	0-16	0-16	0-16
Age at marrow failure			
Mean	—	7.5	—
Median	—	3	—
Range	—	0-35	—
Number with mental retarda- tion (%)	33 (10)	20 (15)	13 (6)
Number with abnormal physi- cal examination (%)	43 (13)	18 (13)	25 (12)
Leukemia			
Number of cases (%)	23 (7)	10 (7)	13 (6)
Male/female	19/1	9/1	10/0
Age at diagnosis (yr)			
Mean	17.6	11.5	24.3
Median	14	7.8	23.5
Range	1.8-43	1.8-38	6-43
MDS			
Number of cases (%)	30 (9)	5 (4)	25 (12)
Male/female	15/11	3/2	12/9
Age at diagnosis (yr)			
Mean	10.3	7.6	10.8
Median	8.1	7.5	8.1
Range	2-42	3.5-12	2-42
MDS clone alone	5	—	—
Died	2	—	—
MDS morphology alone	6	—	—
Died	4	—	—
Deceased			
Number of cases (%)	68 (20)	32 (24)	36 (18)
Male/female	37/25	20/12	17/13
Age at death (yr)			
Mean	7.6	8.2	7.1
Median	3.3	5.3	0.9
Range	0.3-43	0.4-35	0.3-43
Projected median age for all patients (yr)	35	25	37
Leukemia	14	—	—
MDS	16	—	—

MDS, myelodysplastic syndrome.

sis. Ten percent had mental retardation, and 1.5% had microcephaly. Several had an ichthyotic skin rash. Rare physical anomalies include hypertelorism, retinitis pigmentosa, toe or finger syndactyly, cleft palate, dental dysplasia, ptosis, strabismus, short neck, coxa valga, and skin pigmentation.

The combination of pancreatic dysfunction plus bone marrow failure was noted by Ozsoylu and Argun (505), who found decreased duodenal trypsin in patients with acquired aplastic anemia or Fanconi's anemia. Those patients did not have symptomatic malabsorption. In addition, patients with Shwachman-Diamond syndrome have decreased amylase and lipase, as well as trypsin.

LABORATORY FINDINGS

By definition, all patients with Shwachman-Diamond syndrome have neutropenia (neutrophils below 1500/ μ L on more than one occasion). It may be chronic, intermittent, or cyclic and is usually noted early in childhood. Thirty percent of patients were reported to have two involved lineages, and 10% had all three lineages

involved. Pancytopenia occurred at a median age of 3 years (with a range from 0 to 35 years of age). There are a few reports of defects in neutrophil mobility, but this is an inconsistent finding (506-509).

Bone marrow examination shows myeloid hypocellularity or maturation arrest. The erythroid series is normal or hyperplastic. HbF levels are often elevated, even without anemia, which suggests hematopoietic stress (510). Igs are occasionally decreased. Hepatic dysfunction and fibrosis have also been noted. Chromosomes are normal, and no increased breakage is found after clastogenic stress.

Pancreatic insufficiency is documented by the demonstration of low or absent duodenal trypsin, amylase, and lipase. Less invasive than duodenal incubation, serum trypsinogen was shown to be low in young patients, although it does increase with age and is associated with improvement in absorption (511). Other methods for demonstration of pancreatic insufficiency include ultrasound or imaging studies that demonstrate a fatty pancreas. Patients do not have cystic fibrosis, and sweat chloride levels are normal.

PATHOPHYSIOLOGY

The inheritance of Shwachman-Diamond syndrome is autosomal recessive (503). Although the exocrine pancreas and bone marrow hematopoiesis develop at approximately the same time during gestation, familial cases, as well as Shwachman-Diamond syndrome in only one of a pair of twins argue against an intrauterine insult as the cause (512). Culture of bone marrow progenitors shows decreased colony-forming units granulocyte-macrophage (CFU-GM) and CFU-E in most patients, which suggests a stem cell deficit. No evidence is found for humoral or cellular inhibitors of granulopoiesis. Shwachman-Diamond syndrome thus resembles other inherited bone marrow failure disorders, with reduced numbers of hematopoietic progenitor cells.

The gene for Shwachman-Diamond syndrome has been mapped to the centromere of chromosome 7, and, so far, the data are consistent with a single locus with several different mutations (513).

THERAPY AND OUTCOME

Malabsorption responds to treatment with oral pancreatic enzymes. Infections are treated with the appropriate antibiotics and may decrease with age. Supportive care should be provided, with transfusions for anemia and platelets for thrombocytopenia. Corticosteroids or androgens, or both, were used in approximately a dozen patients, with hematologic improvement in one-half. The neutropenia does respond to G-CSF (514). Among 12 patients who were reported to receive G-CSF, four developed mildly dysplastic bone marrows with clonal cytogenetics (see the following section, Myelodysplastic Syndrome).

Evolution to pancytopenia or leukemia are the major hematologic complications. Deaths were reported in 24% of the group with cytopenias, 18% of those with only neutropenia, and 70% of those with leukemia (Table 8-13). The projected median survival age for the entire group is 35 years of age; the median survival is 25 years of age for those with cytopenias, 14 years of age for those with leukemia, and 37 years of age for those without hematologic complications. Those without these complications reach a plateau of almost 80% survival by the late teenage years (Fig. 8-16). The reported deaths were usually due to infection, bleeding, or leukemia.

More than 20 patients had a bone marrow transplant; one-half received bone marrow from sibling donors, and one-half received

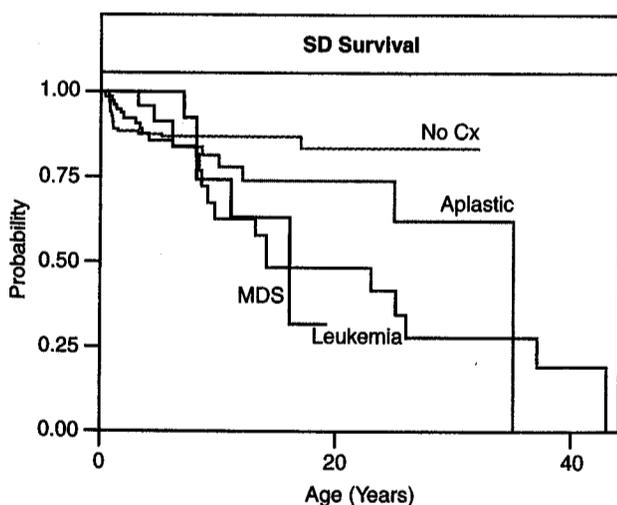


Figure 8-16. Kaplan-Meier plot of cumulative survival in Shwachman-Diamond (SD) syndrome. The differences between curves are significant. Aplastic, 94 patients with aplastic anemia; Leukemia, 18 patients with leukemia; MDS, 16 patients with myelodysplastic syndrome; No Cx, 156 patients with no hematologic complications.

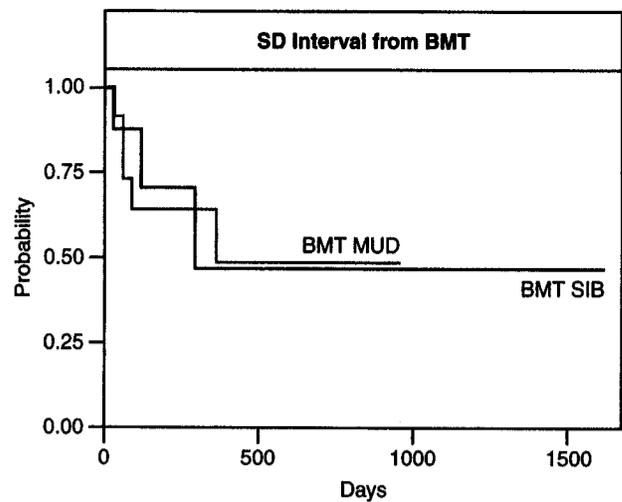


Figure 8-17. Kaplan-Meier plot of cumulative survival after bone marrow transplantation (BMT) for patients with Shwachman-Diamond (SD) syndrome. Time is shown as days from transplantation. The differences are not significant. MUD, 12 patients had matched, unrelated donors; SIB, seven patients were transplanted from HLA-identical siblings.

marrow from alternative donors (515-531). The outcomes were similar, with an absolute mortality of approximately 50% from either type of donor, projected median survivals of approximately 1 year, and plateaus at 47% survival (Fig. 8-17). Deaths after transplant were from complications of marrow transplant, including cyclophosphamide cardiotoxicity, or from leukemia. As with other inherited bone marrow failure disorders, cases may have been reported because of severity or complications (e.g., leukemia), and Shwachman-Diamond syndrome may be milder than it would appear from the literature.

Leukemia. Twenty-three patients, all male except one, developed leukemia (7%) at a median of 14 years of age (with a range from 2 to 43 years of age). Five patients had acute lymphoblastic leukemia, 17 had acute megaloblastic leukemia (four M1, two M2, three M4, three M5, five M6) and one had juvenile chronic myelocytic leukemia (500,502,519-521,524,526,529,532-541). Ten patients had prior histories of cytopenias, whereas 13 patients did not. Seventy percent died at a median age of 14 years for the total leukemic group; the median age of death was 8 years of age for those with prior cytopenias and 24 years of age for those without cytopenias.

Myelodysplastic Syndrome. Thirty patients had MDS, of whom eight developed leukemia and are included in the analyses that were previously mentioned (519-522,524-528,530,533,540,542-546). Unlike leukemia, the male to female ratio in MDS was similar to the ratio in all Shwachman-Diamond patients. MDS was more frequent in those without cytopenias. The median age was 8 years (with a range from 2 to 42 years of age). The projected median age at death for those with MDS was 16 years of age (Fig. 8-16).

Five of the 30 patients had marrow cytogenetic clones without morphologic evidence of MDS. Clones included monosomy 7 with $t(6;13)$, $t(4;7)$ and deletion 7 in patients who had received G-CSF, as well as monosomy 7 in three patients, isochromosome 7q in 11 patients, $der(7)$ in three, and other clones in five other patients, all of whom did not receive G-CSF. Chromosome 7 was involved in a total of 22 patients.

Shwachman-Diamond syndrome thus resembles many of the other inherited bone marrow failure syndromes in that it has a malignant propensity. Although it is not clear that MDS

inevitably progresses to leukemia, leukemia does occur in the syndrome. To date, no solid tumors have been reported.

Other Disorders That Involve Pancreas and Bone Marrow

A disorder with exocrine pancreatic insufficiency and *refractory sideroblastic anemia with vacuolization of bone marrow precursors* was identified in four patients by Pearson et al. (547) in 1979 and is called *Pearson's syndrome*. Anemia was more significant than neutropenia, myeloid and erythroid precursors had vacuoles, and there were ringed sideroblasts. Since then, there have been more than 70 cases reported. The male to female ratio is 0.7, and the median age at detection of anemia is 2 months (with a range from birth to 7 years of age). One-third of patients have low birth weight, and metabolic acidosis is a frequent presenting symptom. Approximately 30% of patients have exocrine pancreatic insufficiency, and insulin-dependent diabetes often develops. Liver and renal failure ensue, and contribute to the metabolic problems. The anemia requires transfusions, although there may be some response to EPO and to G-CSF for neutropenia. The anemia may improve in more than one-third of the patients, at a median of 2 years of age (with a range from 3 months to 10 years of age). One-half of the cases died from acidosis, renal or liver failure, sepsis, and heart block when they were reported; the patients usually did not die from bone marrow failure.

The molecular basis for this syndrome was found to be large deletions of mitochondrial DNA (548). The features of the syndrome that are consistent with a mitochondrial disorder include the involvement of multiple tissues, the paradox of ringed sideroblasts (a problem of iron loading rather than of heme synthesis) and macrocytic anemia, and severe and usually fatal metabolic acidosis (549). Mitochondrial DNA is cytoplasmic, inherited maternally, and heteroplasmic at the mitochondrial, cellular, and organ levels. Each cell has many mitochondria, which may have normal and mutant DNA within the mitochondrion and within the cell, and the proportion of cells with mutant mitochondria varies from organ to organ. Thus, the clinical problems are highly variable from organ to organ, patient to patient, and time to time. The size of the DNA deletion and the presence or absence of duplications and rearrangements does not correlate with the clinical course (550). Those who survive often develop Kearns-Sayre syndrome (progressive ophthalmoplegia, pigmentary retinopathy, cardiac conduction defect, hearing loss, and endocrinopathies), in which the same mitochondrial deletions have been found; patients whose first diagnosis is Kearns-Sayre syndrome usually do not have a preceding marrow failure phase (551).

Patients with *cartilage hair hypoplasia* have an autosomal-recessive disorder with metaphyseal dysostosis, short stature, and characteristic fine hair (552). Approximately 80% of 108 Finnish patients had mild macrocytic anemia, which was severe in 16% of those patients. Lymphopenia was detected in 65% of patients, and neutropenia was detected in 25%, but pancreatic insufficiency was rare. The incidence of malignancy was increased by sevenfold and included Hodgkin's disease in two patients, non-Hodgkin's lymphoma in three patients, melanosis progonoma (retinoblastic teratoma) of the testis in one patient, one vocal cord squamous cell carcinoma, and three cases of basal cell carcinoma (552-555). The gene was mapped to 9p13 in 1994, and mutations were recently found in the RNA component of RNase MRP (556,557). Disruption of the MRP gene may interfere with cell cycle control, but this mechanism is currently speculative.

Amegakaryocytic Thrombocytopenia

A small number of patients present with thrombocytopenia, usually in infancy, and subsequently develop aplastic anemia. This was called type III constitutional aplastic anemia by O'Gorman Hughes (295), but the term *megakaryocytic thrombocytopenia* is more descriptive. Although the differential diagnosis of neonatal thrombocytopenia is lengthy, the entity to be discussed here excludes those conditions with increased bone marrow megakaryocytes as well as those that are due to congenital infection (particularly viral, such as rubella). Immune thrombocytopenias are also not included, despite the occasional development of amegakaryocytosis, which is presumably due to the reactivity of antiplatelet antibodies with megakaryocytes (558). In addition, TAR syndrome is discussed later, because it represents a pure single cytopenia. Fanconi's anemia, which can begin with thrombocytopenia, was already discussed. Finally, children with associated trisomies, such as trisomy 13 and 18, have also been excluded. Various syndromes that are associated with inherited thrombocytopenias are listed in Table 8-14. The accepted cases of amegakaryocytic thrombocytopenia are summarized in Table 8-15, with separate analyses of those with normal appearances and those with congenital anomalies.

More than 50 patients were reported with normal physical appearance, in whom thrombocytopenia occurred primarily early in the first year of life, with absent or reduced bone marrow megakaryocytes (1,659-561). The male to female ratio was approximately 1. One-half as many children were described with amegakaryocytic thrombocytopenia in the first year of life who had physical abnormalities that fit no other specific syndrome; the sex ratio was equal in this group as well (6,562,563). The birth defects included microcephaly, micrognathia, intracranial struc-

TABLE 8-14. Inherited Thrombocytopenia Syndromes

Disorder	Genetics	Chromosome	Gene
Amegakaryocytic thrombocytopenia, no birth defects	AR	1p34	c-mpl
Amegakaryocytic thrombocytopenia, with birth defects	AR	N/A	N/A
Thrombocytopenia—absent radii syndrome	AR	N/A	N/A
X-linked macrothrombocytopenia	X-linked	Xp11.23	GATA-1
Hoyeraal-Hreidarsson syndrome	X-linked	Xq28	DC1
Familial platelet disorder—acute myelocytic leukemia	AD	21q22.1-2	CBFA2
Familial dominant thrombocytopenia	AD	10p11.2-12	THC2
Amegakaryocytic thrombocytopenia, with radioulnar synostosis	AD	7p15-p14.2	HOXA11
Trisomy 13	Nondisjunction	Trisomy 13	—
Trisomy 18	Nondisjunction	Trisomy 18	—
Jacobsen syndrome	11q monosomy, partial deletion	11q23	N/A

AD, autosomal dominant; AR, autosomal recessive; N/A, not available.

TABLE 8-15. Amegakaryocytic Thrombocytopenia Literature

	No Anomalies	Anomalies	Patients
Number of cases	52	20	72
Male/female	27/24	11/8	38/32
Ratio	1.1	1.4	1.1
Age at diagnosis (d)			
Mean	301	49	237
Median	40	0	6
Range	0-3285	0-365	0-3285
Aplastic anemia			
Number of cases (%)	25 (48)	3 (15)	28 (39)
Age at diagnosis (yr)			
Mean	3.4	3.5	3.4
Median	3	2.3	2.9
Range	0.4-12.5	2.2-6	0.4-12.5
Leukemia and preleukemia, number of cases (%)	2 (4)	0	2 (3)
Deceased			
Number of cases (%)	17 (33)	13 (65)	30 (42)
Age at death (yr)			
Mean	5.5	2.4	4.2
Median	4	0.5	2.8
Range	0.01-21.00	0-10	0-21
Projected median age	9	2.7	7

tural anomalies, congenital heart disease, failure to thrive, and developmental delays. Some of the specific cases with cerebellar atrophy were recently shown to belong to the Hoyeraal-Hreidarsson syndrome, with mutations in the DKC1 gene that is mutant in dyskeratosis congenita (see the section Dyskeratosis Congenita). The physical findings in some patients resembled those that are seen in Fanconi's anemia, and the diagnosis of Fanconi's anemia cannot be excluded retrospectively from the reports. The inheritance may be autosomal recessive, because some families had affected siblings or consanguinity, or both.

In both groups, the pregnancies and deliveries were essentially unremarkable, although the frequency of spontaneous abortions was 10%. Low birth weight was recorded in 25% of patients with normal appearance and in almost one-half of those with birth defects. The presentation was usually due to bleeding in the skin, mucous membranes, or gastrointestinal tract. Evolution to aplastic anemia was reported in almost one-half of the patients, primarily in those without birth defects.

LABORATORY FINDINGS

The initial abnormality is thrombocytopenia, with normal white blood cell counts and Hgb levels. Reported platelet counts ranged from 0/ μ L to 88,000/ μ L at diagnosis, white blood cell counts were normal, and Hgbs were low only after bleeding had occurred. Macrocytosis, as well as increased HbF and i antigen, suggested a broader level of marrow failure. One 5-year-old boy with amegakaryocytic thrombocytopenia had increased MCV and HbF but had not yet developed aplastic anemia (564). Bone marrow cellularity is normal until aplastic anemia appears, except for markedly decreased or absent megakaryocytes; those that are present are small and apparently inactive. Homologous platelet survival is normal, because the defect is underproduction not increased destruction (565). Peripheral blood chromosomes do not show the increased breaks that are characteristic of Fanconi's anemia.

Prenatal diagnosis is possible by means of platelet counts in fetal blood that is obtained during the midtrimester. Mibashan and Millar (566) examined three fetuses who were at risk and detected thrombocytopenia in one fetus. Because the gene has now been cloned (see Pathophysiology), molecular diagnosis should be possible.

PATHOPHYSIOLOGY

The thrombocytopenia is apparently due to a defect in production, because megakaryocytes are decreased or absent. After pancytopenia develops, hematopoietic progenitor cells are decreased or absent (127,561,567,568). Thrombocytopenia may be the first sign of aplastic anemia in many conditions, although this group is distinguished by the relatively long duration before pancytopenia. Serum levels of thrombopoietin (TPO), IL-11, and IL-6 were elevated, which is related to megakaryocytic deficiency (569,570). Cultures did not respond to added TPO, and the expression of c-mpl RNA was impaired (571).

The gene that is involved in amegakaryocytic thrombocytopenia is the receptor for TPO, which is encoded by the c-mpl gene. Mutations in c-mpl have been reported in at least 15 patients, all of whom are without birth defects (560,561,572,573).

OUTCOME

Aplastic anemia developed in almost one-half the patients, at a median of 3 years of age, the oldest was 12 years of age. One-half of the patients in whom aplastic anemia developed died from bleeding or infection. One-half of those without aplastic anemia died also from central nervous system or gastrointestinal hemorrhages. Two-thirds of the deaths were in cases that were described before 1980, and systematic platelet support was not reported. The predicted median survival is 9 years of age in those without birth defects and younger than 3 years of age in those with physical anomalies, and the actual median ages at reported deaths were 4 and 0.5 years of age. The oldest patient in the former group died at 21 years of age, and the oldest patient in the latter group died at 10 years of age.

One male with normal physical appearance had amegakaryocytic thrombocytopenia from birth, developed aplastic anemia at 5 years of age, responded poorly to androgens plus steroids, and evolved further into acute myelomonocytic leukemia at 16 years of age, with death at 17 years of age (295; N. Potter and B. P. Alter, unpublished data). A female patient had thrombocytopenia at 2 months of age, pancytopenia at 5 months of age, and a preleukemic picture with abnormalities that involved chromosome 19 (M. B. Harris, V. Najfeld, M. A. Weiner, et al., unpublished data, 1984). Thus, amegakaryocytic thrombocytopenia

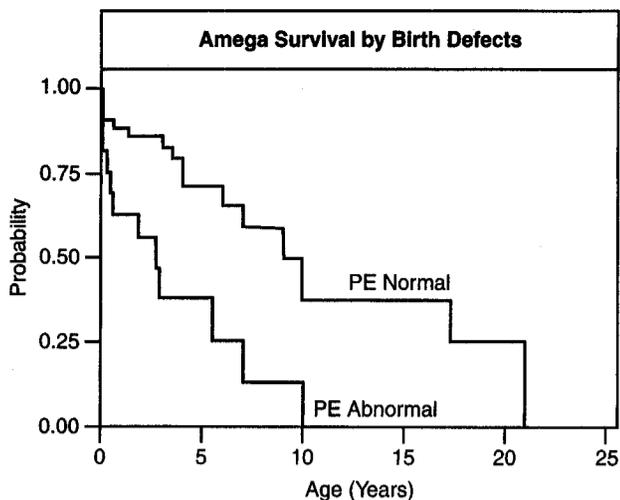


Figure 8-18. Kaplan-Meier plot of cumulative survival in amegakaryocytic thrombocytopenia (Amega). Patients with only thrombocytopenia and those with aplastic anemia are pooled, because the numbers are small. Outcomes were not available for all patients. The differences are significant. PE abnormal, 16 patients with birth defects; PE normal, 42 patients without birth defects.

may also be one of the marrow failure syndromes with a propensity to malignancy.

TREATMENT

Steroids alone were not effective in the treatment of amegakaryocytic thrombocytopenia. Steroids plus androgens resulted in temporary or partial responses in a few patients. Splenectomies were performed in four patients without effect. Figure 8-18 shows the survival curves for patients without and patients with anomalies. We can speculate that platelet support might prevent early deaths from thrombocytopenia, but the rate of evolution to aplastic anemia might then be higher. Hematopoietic growth factors that stimulate platelet production might be considered when they become available. Guinan et al. (559) did show a platelet response to IL-3 but not to GM-CSF in five patients in a phase I and II trial, and Taylor et al. (563) reported slight responses in two out of six patients with PIXY321 (GM-CSF and IL-3 fusion protein), but neither of these agents is currently available.

Fifteen patients were treated with BMT; ten of the transplants were from HLA-matched siblings, and five were from unrelated donors, two of which were cord bloods. All of these patients were in the group without birth defects, and all but one patient survived (4,243,561,574-576). Thus, stem cell transplant has the potential to cure thrombocytopenia and prevent the development of aplastic anemia or leukemia.

Familial Marrow Dysfunction

Several families have been reported with various combinations of physical and immunologic abnormalities, in which hematopoietic defects and often leukemia have occurred. The ages at the development of hematologic manifestations vary from childhood to adulthood, and the inheritance patterns have been of all types. Some of the families have anomalies that resemble but in fact differ from those described in Fanconi's anemia. One group, the Estren-Dameshek familial aplastic patients, has been reclassified as Fanconi's anemia. Another group of patients has anomalies that resemble those that are seen in Fanconi's anemia patients, but the group does not have Fanconi's anemia. Other

patients fit into known genetic syndromes, such as Brachmann-de Lange, Dubowitz's, Seckel's, and Down's syndromes, and are discussed separately. Several patients with physical anomalies and aplasia are sporadic.

Some patients inherited their disease in an autosomal-dominant manner. The *IVIC syndrome*, which is named for the initials of the institution that first reported it (Instituto Venezolano de Investigaciones Cientificas), is characterized by radial ray hypoplasia, with absent thumbs or hypoplastic radial carpal bones, hearing impairment, strabismus, imperforate anus, and thrombocytopenia. The physical anomalies were noted in 24 members of five generations in the first family, in two children and their father in the second family, and in a mother and son in the third family that was reported (577-579). Mild thrombocytopenia and leukocytosis appeared before 50 years of age in 13 persons in the first family. The incidence of hematologic abnormalities is unknown, because many patients are still young. Baseline chromosome breakage was normal. Despite the lack of complete aplastic anemia, the physical findings resemble those that are seen in Fanconi's anemia. It has been suggested that this syndrome be renamed *oculooradial syndrome* (580).

The *WT syndrome* was named after the initials of the first (and, so far, the only) two families who have been reported (581). Twelve patients were described, with three generations in one family and five generations in the other family. They had radial-ulnar hypoplasia, abnormal thumbs, short fingers, fifth-finger clinodactyly, pancytopenia or thrombocytopenia, and leukemia. Baseline chromosomes were normal without increased breakage. As in the *IVIC syndrome*, the physical findings in the *WT syndrome* were subtly different from those in Fanconi's anemia. The authors suggested that several cases of atypical Fanconi's anemia might be *WT syndrome* instead. Chromosome studies after clastogenic stress should help to sort out this confusion.

A family was described with dominant bone marrow failure, acute nonlymphocytic leukemia, hyperpigmented skin, warts, immune dysfunction, and multiple spontaneous abortions (582). DEB-induced chromosome breakage was not increased. This disorder resembles Fanconi's anemia clinically but not genetically.

Aufderheide (583) reported a five-generation family with 14 members who developed mild to profound single cytopenias or pancytopenia by the third decade of life. Vascular occlusions were also present in nine members of this family. One patient had chromosome breaks in 20% of his cells, but his father, who also had aplastic anemia, had normal chromosomes. Kato et al. (584) reported a mother with aplastic anemia and her son with adult-onset neutropenia and thrombocytopenia.

Dokal et al. (585) reported two families with dominantly inherited proximal fusion of the radius and ulna. Among the 16 cases with this anomaly, four cases had adult-onset aplastic anemia or leukemia. Two families were noted recently in which the fathers had radioulnar synostosis and their children had synostosis plus amegakaryocytic thrombocytopenia, which was cured by stem cell transplantation; germline mutations were identified in *HOXA11*, a gene that is involved in bone morphogenesis (586).

The syndrome of cerebellar ataxia and pancytopenia was discussed previously in the section *Dyskeratosis Congenita*.

In a report on 19 members of eight families with acquired aplastic anemia, four families with nine patients had a vertical pattern, with a parent or aunt or uncle who also had aplasia (587). These could be due to common environmental factors or to a genetic propensity for bone marrow failure.

Autosomal-recessive inheritance is the apparent pattern in several other families. Abels and Reed (588) reported two brothers who had short stature and macrocytosis and who developed

pancytopenia at approximately 10 years of age. One brother had immune deficiency and multiple cutaneous squamous and basal cell carcinomas. He also had oral telangiectasias and neck and chest poikiloderma, but these findings were insufficient to diagnose dyskeratosis congenita. Because the patients were both male, the inheritance might also have been X-linked recessive.

Another family with associated immune disorders and hematopoietic failures was described by Linsk et al. (589). Four of six siblings from a consanguineous marriage had pure red cell aplasia or neutropenia, or both, as well as unusual crystalloid structures that were demonstrated in the neutrophils by electron microscopy. Chitambar et al. (590) described 8 of 14 members in one generation of a large maternal kindred in which aplastic anemia, acute nonlymphocytic leukemia, and monosomy 7 were found. This association of aplasia and leukemia was also noted in families with possible X-linked recessive inheritance. In the Scandinavian report on acquired aplastic anemia with multiple family members (587), ten patients in four families belonged to sibships.

Monosomy 7 was also a feature in the family that was discussed by Chitambar et al. (590). A sporadic patient with ataxia and hypoplastic anemia was described by Samad et al. (591). This 16-year-old man had Friedreich's ataxia, short stature, hypogonadism, and hyperreflexia. His macrocytic anemia responded to testosterone. Peripheral blood but not marrow chromosomes showed baseline increased breakage.

A clearly X-linked family was presented by Li et al. (592), in which eight males in three generations had adult-onset pancytopenia, acute myelogenous leukemia, light chain disease, or ALL (one case).

Another X-linked syndrome with hematopoietic complications is the *X-linked lymphoproliferative syndrome*, to which more than 25 kindreds belong (593). At least 17 of these boys developed fatal aplastic anemia during or before malignant infectious mononucleosis. Other components of this syndrome include hypoproliferative disorders, agranulocytosis, and hypogammaglobulinemia, as well as proliferative disorders that are associated with the Epstein-Barr virus, including American Burkitt lymphoma, immunoblastic B-cell sarcoma, plasmacytoma, and fatal mononucleosis. The disease is caused by a defect in the SH2DIA (SH2-domain 1A) gene at Xq25 (594,594a,594b). This gene, also called SAP for SLAM (signaling lymphocyte activation molecule)-associated protein, helps regulate T-cell activation during immune responses.

There are also reports of sporadic cases with aplastic anemia and physical abnormalities. One patient and a set of twins had anomalies that were similar to those that are seen in patients with Fanconi's anemia, and increased baseline chromosome breaks were seen in one patient (595,596). This case did not have increased breakage after clastogenic stress (A. D. Auerbach, *personal communication*). In a larger series of cases that were reported to the IFAR, 11 children had aplastic anemia and anomalies, and their chromosome breakage was not increased by DEB (597). It is probable that many cases that were called *Fanconi's anemia* in the older literature and are included in our own analyses did not have Fanconi's anemia. Only modern testing with DEB or MMC can help to properly categorize all of these cases.

Also, there are cases that do not fit into any categories. Two adult siblings had thrombocytopenia (not pancytopenia) and a robertsonian translocation (t13;14), although six other patients with the translocation did not have hematologic problems (598). In three of the eight families with 19 members with aplastic anemia, who were previously cited (587), anemia might have been related to drugs. In other reports, four families had more than one patient with chloramphenicol-related aplastic anemia (599-601). Two families had siblings with aplastic anemia after hepa-

titis (602,603). Gold, methyprylon (piperidine), and idiopathic aplastic anemia also have each been reported in sets of siblings (604-606). A mother and child pair with idiopathic aplastic anemia was also reported (607).

Thus, aplastic anemia might be associated with familial (genetic) predisposition to specific adverse environments. In some cases, physical abnormalities may call attention to the possibly inherited nature of the condition. The familial and inherited marrow failure syndromes are clearly heterogeneous, with a large variety of phenotypes and all the possible inheritance patterns. It remains for future investigations to elucidate the relevant genetic and environmental factors.

LABORATORY FINDINGS

The patients in this heterogeneous group with familial marrow dysfunction have variable degrees of pancytopenia, macrocytosis, elevated HbF, and hypocellular bone marrow. Families with additional findings, such as immune deficiencies, novel chromosomes, or monosomy 7, may be distinguishable from those with nonfamilial disorders, but those with familial disease without characteristic findings are more difficult to diagnose. Baseline chromosome breakage is usually normal in the non-Fanconi's anemia familial cases, but examination with clastogenic agents is required for definitive distinction. The numbers of hematopoietic stem cells are also reduced, but this too is nondiagnostic (567).

PATHOPHYSIOLOGY

The combination of genetic and environmental factors is essentially unique to each of the types of cases that are outlined in this section on familial marrow dysfunctions. The inheritance patterns are of all types. At the hematopoietic level, the defects may be multiple, because some of the patients have only single cytopenias. Thus, the pluripotent or committed progenitor cells may be defective.

THERAPY AND OUTCOME

Several of the patients that were described previously were treated with transfusions, antilymphocyte globulin, or androgens, with some limited success. Because each case is practically unique, no guidelines can be established, except to suggest that androgens might be more effective than immunosuppression. Because immune dysfunction is part of some of the syndromes, even that statement is overly simplistic. In general, the drug and supportive care should be the same as the treatment that was described previously for patients with Fanconi's anemia or acquired aplastic anemia. Although BMT cannot be dismissed out of hand, it is risky, because related donors may have the same condition. In several families, aplastic anemia is just the first step to preleukemia and leukemia. Overall, the prognosis for familial bone marrow failure is not good.

Down's Syndrome

Trisomy 21, or Down's syndrome, is often associated with a neonatal transient myeloproliferative syndrome and, later, with an increased risk of leukemia (608). A few patients have been reported with aplastic anemia. A 17-year-old boy had idiopathic aplastic anemia and trisomy 21 and apparently responded to androgen treatment (609). Vetrella et al. (610) described a newborn with trisomy 21, cystic fibrosis, and amegakaryocytic thrombocytopenia, who died at 49 days of age, with pancytopenia shortly before death. A 12-year-old boy developed aplastic anemia that was unresponsive to androgens and died within 10 weeks of diagnosis (611); another patient was mentioned with aplastic anemia at 19 months, who appeared to respond to androgens (612). A fifth case was published in which a patient

developed aplastic anemia at 9 months of age and died at 26 months of age with gastroenteritis (613). Although bone marrow was generally hypocellular in these patients, the last case had increased numbers of CFU-GM, apparently with cellular and serum inhibitors of hematopoiesis. The few patients with trisomy 21 who developed aplastic anemia raise the question of whether this is a true association or merely coincidence.

Dubowitz's Syndrome

A rare, apparently autosomal-recessive condition in which aplastic anemia has occurred is Dubowitz's syndrome. In a review of 141 cases, hematologic and malignant complications were noted in 12 (614). The major features are intrauterine and postnatal growth retardation, microcephaly, moderate mental retardation, hyperactivity, eczema, and facial anomalies, such as hypertelorism, epicanthal folds, blepharophimosis, broad nose, and abnormal ears. Six patients had aplastic anemia, two had leukopenia, and one each had acute lymphoblastic leukemia, non-Hodgkin's lymphoma, malignant lymphoma, and neuroblastoma. Thus, approximately 10% of the patients with Dubowitz's syndrome have had hematopoietic or oncologic problems. This is another syndrome with growth defects that are associated with hematopoietic disorders and malignancies.

Seckel's Syndrome

Another rare, autosomal-recessive condition in which aplastic anemia has been reported is Seckel's syndrome. Although more than 60 patients have been published as having this syndrome, it may be an overused term that is applied to a heterogeneous group of microcephalic dwarfs; thus, the true number is not clear. The stringent definition includes severe intrauterine and postnatal growth retardation, severe microcephaly, severe mental retardation, the typical face with receding forehead and chin, antimongoloid slant of palpebral fissures, prominently curved nose, relatively large eyes and teeth, highly arched palate, hirsutism, and clinodactyly (615).

Among the group with Seckel's syndrome, at least 10% of patients developed aplastic anemia (616-620), although two patients also had hypersplenism (617). One-half of those patients died from sepsis during childhood. All received transfusions, and androgen treatment was ineffective. One patient died 2 weeks after BMT, whereas another was cured (620). Fanconi's anemia was considered in all cases, because the patients were small, microcephalic, and retarded and had pancytopenia. In fact, the patients with Seckel's syndrome are much smaller and more severely microcephalic and retarded than those with Fanconi's anemia. Chromosome studies were normal in two of the patients with aplastic anemia. Endogenous breakage was increased in one patient and further increased with MMC in the sibling of that patient, but the diagnosis of Fanconi's anemia could not be firmly established. Another patient with aplastic anemia had increased spontaneous and MMC-induced breakage in fibroblasts and lymphocytes (621). Two patients who did not have aplastic anemia had normal chromosomes, by the criteria of endogenous breakage, as well as SCEs (622). One patient was reported to develop AML at 26 years of age (623). Seckel's syndrome is another autosomal-recessive syndrome with growth retardation, a small but real risk of aplastic anemia, and, perhaps, leukemia. There is probably no association with increased chromosome breakage. The genes that are responsible for Seckel's syndrome have not been identified, but there is no evidence that Seckel's syndrome patients have mutations in FA genes (624).

SINGLE CYTOPENIAS: RED BLOOD CELLS

Diamond-Blackfan Anemia

The first descriptions of red cell aplasia in infancy consisted of two cases that were reported by Josephs (625) in 1936 and four cases that were reported by Diamond and Blackfan (626) in 1938. A variety of synonyms and eponyms have been used: congenital hypoplastic anemia, chronic congenital aregenerative anemia, erythroblastopenia, chronic idiopathic erythroblastopenia with aplastic anemia (type Josephs-Diamond-Blackfan), and Diamond-Blackfan anemia (DBA). Diamond and Blackfan used the term *congenital hypoplastic anemia* because they thought the disorder differed from complete aplastic anemia only in degree. The term *hypoplastic* is now used when marrow depression (pancytopenia) is only partial; thus, the term is not appropriate for a single cytopenia. Erythroblastopenia is probably the most descriptive appellation, but now the commonly used term is *DBA*.

The following are diagnostic criteria for DBA:

- Normochromic, usually macrocytic but occasionally normocytic, anemia that develops early in childhood
- Reticulocytopenia
- Normocellular bone marrow with selective deficiency of red-cell precursors
- Normal or slightly decreased leukocyte counts
- Normal or often increased platelet counts

These criteria clearly differentiate DBA from aplastic anemia, but may not always distinguish it from transient erythroblastopenia of childhood (TEC) (see the section Transient Erythroblastopenia of Childhood).

More than 700 cases of DBA have been reported in case reports, and many of the references are cited in previous reviews (4,627-629). Additionally, there are almost 500 cases that are summarized in large series, although many of these patients may also have been subjects in individual case reports (131,630-633). The ages at which DBA was diagnosed (or the first transfusion or treatment initiated) are shown in Figure 8-19 and summarized in Table 8-16. Boys were slightly younger than girls (with medians of 2 and 3 months of age). Ten percent of patients were severely anemic at birth, with severe anemia presenting in 25% by 1 month of age, 50% by 3 months of age, 75% by 6 months of age, and 90% by 18 months of age. Five percent of patients were diagnosed between 1.5 and 2.5 years of age, 3% before 6 years of age, and another 2% between 6 and 68 years of age. The male to female ratio is 1.1:1. Most of the reports from almost 50 countries are of white patients, but DBA has been described in blacks, Asians, and Indians.

A dozen cases presented in patients who were older than 6 years of age. A 34-year-old man with anemia had an anemic daughter who was diagnosed at 6 years of age, and, subsequently, a grandson was diagnosed with classic DBA (634,635). A female who was diagnosed as anemic at 16 years of age had a son who was diagnosed at 9 months of age (636). The paternal grandfather in a family in which four males were diagnosed in three generations was diagnosed at 20 years of age (637). One male was diagnosed at 9 years of age in a large consanguineous family in which seven members were affected in one generation, of whom five were male siblings and two were male and female cousins (638). Two women, who were 25 and 64 years of age, had long-standing anemia that responded to prednisone, were of short stature, and had other findings that are typical of DBA, including webbed necks and thenar atrophy (639). Two additional males were diagnosed at 7 and 22 years of age (640), and three females were diagnosed at 11, 13, and 35 years of age (630,640-642).

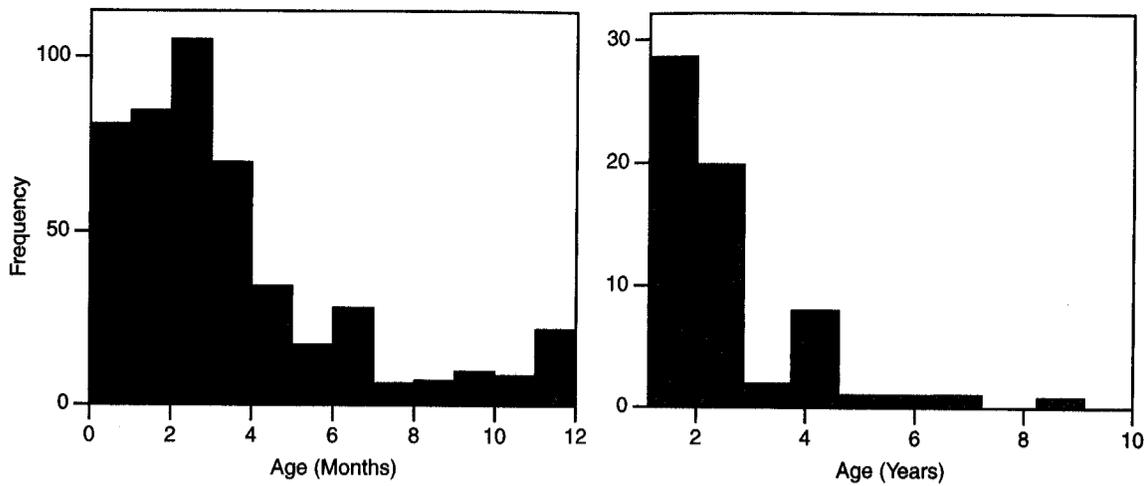


Figure 8-19. Age at onset of anemia in approximately 550 published cases with Diamond-Blackfan anemia. Note the scale on the abscissa is in months on the left, for those between 0 and 12 months of age, and in years on the right, for those between 1 and 10 years of age. Not shown are nine patients who were between 11 and 64 years of age at diagnosis.

INHERITANCE AND ENVIRONMENT

The inheritance of DBA appears to have more than a single pattern. There were more than 30 families with *dominant* disease that involved parent-child transmission. The carrier parent often had a history of anemia, although, in some cases, the parent only had increased HbF or macrocytosis, or both, without significant anemia. The ratio of males to females in the dominant group was approximately 1. The incidence of physical abnormalities was lower, and the clinical course was generally milder than it was in the overall DBA population. A large series that was reported by Willig et al. (643,644) had 33 families with classic DBA in at least two generations. Twenty-one of 154 sporadic cases had a first-degree relative with anemia, increased MCV, or increased red-cell adenosine deaminase (ADA) (see the section Laboratory Findings), and these relatives had the same DBA gene mutation as the proband in their family, which suggests that some "sporadic" cases may be autosomal dominant.

Another 30 families might be construed as *recessive*, because, apparently, there were affected siblings and normal parents, consanguinity, or affected cousins. In addition to one set of male twins in a sibship with three affected males (645), a set of affected identical twins has also been reported (646). The incidence of anomalies was actually slightly higher in the recessives than in the overall group, but the anemia was milder and more responsive. The inheritance in the apparently recessive families might be dominant with variable expression, but that is less likely, because there are several families with consanguinity. Removal of all of the patients in families leaves several hundreds of cases that are apparently sporadic, which suggests that DBA occurs as a new mutation or as an acquired disease or that penetrance is extremely variable.

Several patients were the products of problem pregnancies, which suggests that they had congenitally acquired marrow failure. The pregnancy problems include preeclampsia, toxemia, rashes, premature placental separation, hemorrhage, spotting, and positive tests for syphilis. Exposures during pregnancy include diethylstilbestrol, x-rays, chlorothiazide, reserpine, thyroid hormone, prednisone, phenylbutazone, chloramphenicol, and anagryne. A few mothers had previous spontaneous abortions or miscarriages. Seven percent of the patients weighed 2500 g or less at birth, but only 14 patients were born at less than 36 weeks gestation, and, thus, most

were small for gestational age (intrauterine growth retardation). The low birth weight might reflect pregnancy problems or poor growth that is intrinsic to DBA itself.

The anemia in patients with DBA was often noted at birth or during infancy. Jaundice due to hemolytic disease of the newborn from Rh or ABO blood group incompatibility occurred occasionally and led to prolonged anemia that became chronic (647) or that sometimes resolved after a few months (648). A few patients had antecedent illnesses such as diarrhea, respiratory infections, urinary tract infections, measles, or mumps, or a smallpox vaccination. One child was treated with chloramphenicol (649). In most patients, the illness was more likely to be due to the anemia or to be unrelated than to be the cause of the anemia. The signs of anemia were usually pallor, lethargy, irritability, and heart failure.

PHYSICAL EXAMINATION

Physical abnormalities were described in more than 160 patients (25%) (Table 8-17). Abnormalities of the head and

TABLE 8-16. Diamond-Blackfan Anemia Literature

	All Patients
Number of cases	705
Male/female	332/313
Ratio	1.1
Male age at diagnosis (mo)	
Mean	9.6
Median	2.0
Range	0-408
Female age at diagnosis (mo)	
Mean	14.2
Median	3.0
Range	0-768
Number of male patients older than 1 year of age	29 (9%)
Number of female patients older than 1 year of age	39 (12%)
Deceased	
Number of cases	90 (13%)
Age at death (yr)	
Mean	11.5
Median	8.8
Range	0-65
Projected median age	43

TABLE 8-17. Physical Abnormalities in Diamond-Blackfan Anemia (Percent of Cases)

Abnormality	Percent of Cases
Birthweight \leq 2500 g	7
Head, face, palate	7
Upper limbs	8
Short	12
Eyes	5
Renal	4
Neck	3
Hypogonads	2
Retardation	2
Cardiopulmonary	2
Nose	1
Other skeletal	4
Other	10
At least one anomaly ^a	25
Short stature alone	3

^aNot including low birth weight or short stature. Many patients had more than one abnormality. Physical descriptions were available for 650 patients.

face were common. The typical face was described by Cathie (650) as "tow-colored hair, snub nose, thick upper lips, rather wide set eyes, and an intelligent expression" and was observed in many of the children, who resemble each other more than they do their own family members. Cleft lip and palate were noted. Other findings include micrognathia, microcephaly or macrocephaly, macroglossia, wide fontanelle, and dysmorphic features.

Upper limbs, particularly thumbs, are often abnormal. In our experience, the most common features are subtle flattening of the thenar eminences and weakness of radial pulses. Other radial hand anomalies are also common. Triphalangeal thumbs were noted in 22 patients who were otherwise similar to the total group of patients. Although this association was initially separated by some into the *Aase syndrome* (651), it is probably an inappropriate example of splitting rather than lumping patients into groups according to their abnormalities (652). Fourteen patients had duplicated thumbs, and 13 had otherwise abnormal thumbs, such as absent or subluxed thumbs. Thumb anomalies may be unilateral or bilateral. The presence of abnormal thumbs does not predict the course of the anemia.

Another common finding in DBA patients is short stature. Because many patients have received corticosteroids, it is often difficult to identify genetic short stature, but it was seen in more than 10% of the patients. There were four reports of dwarfism, including achondroplasia (653,654), metaphyseal dysostosis (655), and cartilage hair hypoplasia (655). Short or webbed necks were reported in 3% of patients, including both Klippel-Feil syndrome (fused cervical vertebrae) and Sprengel's deformity (elevation of the scapula). A Turnerlike phenotype was sometimes mentioned.

Five percent of patients had eye anomalies, most frequently hypertelorism, as well as blue sclerae, glaucoma, epicanthal folds, microphthalmos, cataracts, and strabismus. Kidney abnormalities, including horseshoe, duplicated, and absent kidney, were also noted in a few patients. Male hypogonadism was noted in 2%, and 2% of patients were retarded. Lower limb problems included dislocated hips, achondroplasia, and clubfoot. Congenital heart disease of all kinds was seen occasionally. The presence of anomalies serves more to confirm the diagnosis of DBA than to provide any prognosis regarding the course of the disease.

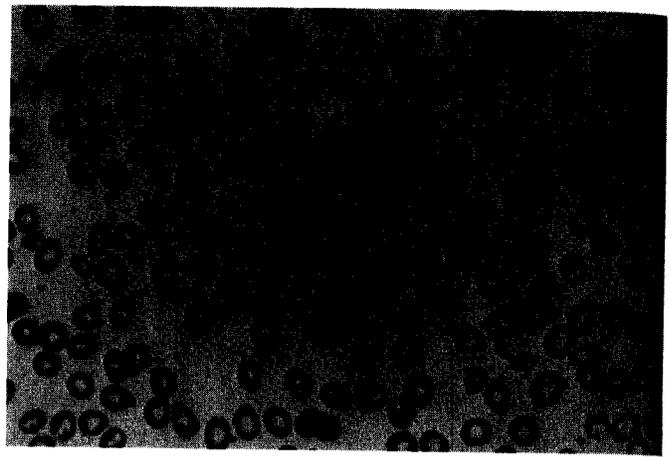


Figure 8-20. Peripheral blood from a patient with Diamond-Blackfan anemia. Note anisocytosis with microcytosis and macrocytosis, as well as teardrop erythrocytes (arrows). (Courtesy of Dr. Gail Wolfe. From Alter BP. The bone marrow failure syndromes. In: Nathan DG, Oski FA, eds. *Hematology of infancy and childhood*, 3rd ed. Philadelphia: WB Saunders, 1987:159-241, with permission.)

LABORATORY FINDINGS

All patients with DBA are anemic by definition. Hgb levels at birth show a range of 2.6 to 14.8 g/dL, with a median of 7 g/dL. In approximately 60 infants who were diagnosed with DBA between birth and 2 months of age, the median Hgb was 4 g/dL, with a range from 1.5 to 10.0 g/dL. In those who were diagnosed later, the Hgb was also usually in the 4 g/dL range. Macrocytosis was almost uniform, and reticulocytes were usually absent. Figure 8-20 shows a representative blood film, with anisocytosis, macrocytosis, and an occasional teardrop.

White-cell counts are generally normal in DBA patients, although counts sometimes decrease with the person's age. Values of 5000/ μ L or less were found at some time in 20% of patients, and values of less than 3000/ μ L were found in 5%. Two heavily transfused older patients developed significant neutropenia (N. S. Young, unpublished data). Platelet counts are also usually normal, although 25% of patients had at least one count that was less than 150,000/ μ L, and 20% had one count that was greater than 400,000/ μ L. Buchanan et al. (656) noted elevated platelets in 50% of 38 patients and decreased platelets in 25% on at least one occasion. They found that platelet function was normal.

HbF is usually increased and is distributed heterogeneously (Fig. 8-21), which indicates that the patients do not have a single clone of completely fetal cells. The proportion of G γ to A γ plus G γ is greater than 50%, which is similar to that found in fetal RBCs. The titer of red cell-membrane i antigen is also increased, as in fetuses, whereas the adult counterpart, I antigen, remains at adult levels. These fetal-like erythrocyte features are seen in newly diagnosed patients, patients who respond to corticosteroids, and patients in spontaneous remissions. They are not unique to patients who are affected with DBA, but they are characteristic of the stress erythropoiesis that is seen in any type of bone marrow failure (47,48).

Bone marrow examination by aspiration or biopsy shows normal cellularity, myeloid cells, and megakaryocytes. Lymphocytes are often increased (this is seen even in normal infants; most of the marrow tests are done in infants with DBA) and were initially thought to be hematogones (657). Eosinophilia is occasional (658). Three patterns of erythroid development were described by Bernard et al. (659):

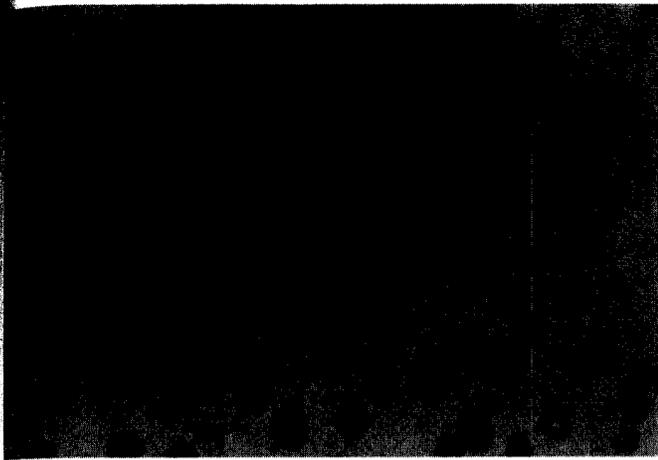


Figure 8-21. Kleihauer-Betke acid elution study of blood from a patient with Diamond-Blackfan anemia, showing the heterogeneous distribution of the fetal hemoglobin. (Courtesy of Dr. Gail Wolfe. From Alter BP. The bone marrow failure syndromes. In: Nathan DG, Oski FA, eds. *Hematology of infancy and childhood*, 3rd ed. Philadelphia: WB Saunders, 1987:159-241, with permission.)

- Erythroid hypoplasia or total aplasia is seen in 90% of patients with DBA (Fig. 8-22). The only erythroid precursors in some patients are immature proerythroblasts.
- Five percent of cases have normal numbers and maturation of erythroblasts.
- The remaining 5% of patients have erythroid hyperplasia, but this is present with a maturation arrest and increased numbers of immature precursors.

Despite the variable bone marrow picture, all DBA patients have reticulocytopenia. Thus, a few patients must have ineffective erythropoiesis. Dyserythropoietic morphology has been seen occasionally (see section Congenital Dyserythropoietic Anemia). Ringed sideroblasts were noted rarely, then disappeared (660,661). Patients who receive many transfusions accumulate iron in the marrow (Fig. 8-22) and other organs.

Serum levels of iron, ferritin, folic acid, vitamin B₁₂, and EPO all are elevated, and DBA is not due to a deficiency of any of the normal hematinic agents (662). DBA patients do

not have antibodies to EPO. Routine urinalysis is normal. It was once thought that an abnormality in tryptophan metabolism occurs (663,664), but other investigators failed to confirm this (665). Hypocalcemia was reported only once (666), and mild hypogammaglobulinemia was noted in several patients (627,659,667,668). These parameters are normal in many other patients. Low numbers of T lymphocytes and a reduction in the ratio of helper to suppressor T cells were reported by Finlay et al. (669), but abnormalities of T-cell function were not observed. Ferrokinetic studies showed the delay in plasma iron clearance and low RBC utilization that were expected in aplastic anemia (659,666,670). Autologous RBC survival times were slightly shortened (627,671), and haptoglobins were low in three patients (627,672), which suggests a mild hemolytic anemia.

Patients with DBA have negative direct antiglobulin (Coombs') tests, and their disease is not due to RBC autoantibodies, although alloantibodies may develop after many transfusions. Results of bone marrow cultures and erythropoietic inhibitor assays are described in the section Pathophysiology.

Abnormalities were observed in RBC enzymes and involved purine or pyrimidine metabolism. Giblett et al. (673) described a patient with atypical DBA who also had lymphopenia and nucleoside phosphorylase deficiency; other patients had normal nucleoside phosphorylase levels (674,675). Increased levels of the pyrimidine enzymes orotate phosphoribosyl transferase and orotidine monophosphate decarboxylase (ODC) were reported in five patients in one study (675), and increased ODC was reported in five of ten patients in another study (676). ODC is an age-dependent enzyme that is increased in cord blood cells, and the elevation is consistent with the presence of young, fetal-like erythrocytes.

Glader et al. (674) and Glader and Backer (676,677) have observed increased RBC ADA in 26 of 29 DBA patients. ADA, a critical enzyme in the purine salvage pathway, is not elevated in erythrocytes from cord blood or in patients with hemolytic or other aplastic anemias. ADA was also elevated in 2 of 12 DBA parents (674). Whitehouse et al. (678) found an increase in ADA in 9 of 19 patients and in 2 of 15 relatives. The significance of ADA is not clear, because it is also increased in some children with acute lymphoblastic leukemia, and thus may indicate disordered erythropoiesis that is distinct from fetal-like erythropoiesis (677). Increased ADA does help to distinguish DBA from TEC (see the section Transient Erythro-

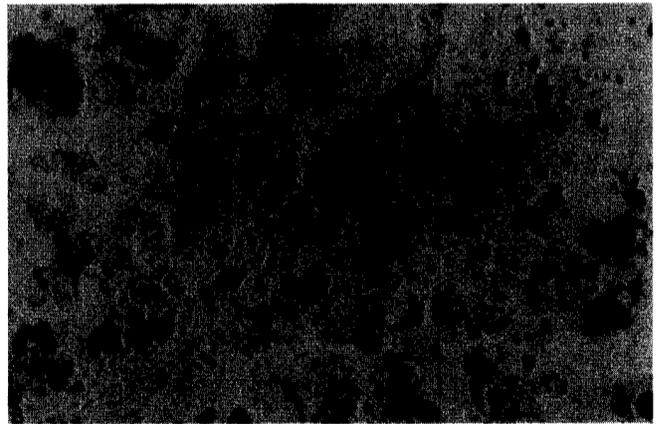
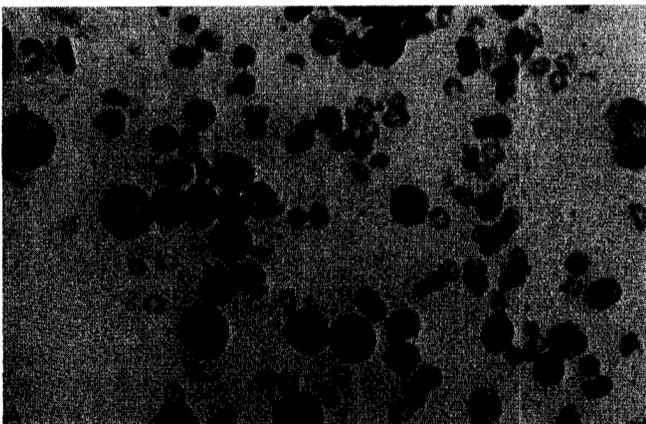


Figure 8-22. A: Bone marrow aspirate from a patient with Diamond-Blackfan anemia, showing normal cellularity with erythroid hypoplasia. B: Iron stain of bone marrow aspirate from a 2-year-old child with transfusion-dependent Diamond-Blackfan anemia. (Courtesy of Dr. Gail Wolfe. From Alter BP. The bone marrow failure syndromes. In: Nathan DG, Oski FA, eds. *Hematology of infancy and childhood*, 3rd ed. Philadelphia: WB Saunders, 1987:159-241, with permission.)

blastopenia of Childhood). As mentioned previously, some otherwise normal parents of classic DBA patients have elevated ADA and a mutant DBA gene, which is supportive of a dominant inheritance pattern in those families (643,644).

Chromosomes are normal in most patients with DBA (627). Rare abnormalities include an achromatic area in chromosome 1 (666), a pericentric inversion in one patient (679), enlargement of chromosome 16 in three of six patients (680), and breaks and endoreduplication of chromosome 16 (681). One patient had increased spontaneous and x-ray-induced chromosome breakage, without increased breakage due to MMC (682). Others showed no increased breakage to DEB (683,684). Sister chromatid exchange is normal. Chromosome breakage studies are not informative, except to distinguish DBA from Fanconi's anemia. However, several patients were identified with translocations or rearrangements that permitted mapping and cloning of the first DBA gene (see the section Diamond-Blackfan Anemia Genes).

A small number of patients with clinical DBA were found to have parvovirus DNA in their marrow. Three patients who had documented, second-trimester, intrauterine infections did not respond to intravenous Ig therapy (685). One patient died from respiratory failure at 9 months, whereas the other two patients remained transfusion-dependent. Three others whose marrows contained parvovirus DNA at the time of their diagnosis of DBA underwent spontaneous remissions after steroid treatment for 2 months, 3 years, and 9 years (686).

Prenatal Diagnosis. One fetus in a family with two DBA children had an apparently high-output cardiac failure when it was studied with two-dimensional fetal Doppler echocardiography, (687) although the reliability of this test has been questioned (681). Ultrasound may be used to detect cardiomegaly and effusions from hydropic anemic fetuses, and intrauterine transfusions might be offered (688,689). The possibilities of increased fetal erythrocyte ADA or decreased fetal blood erythroid burst-forming units (BFU-E) have not been examined. Prenatal gene mutation analyses might also be considered (see the section Diamond-Blackfan Anemia Genes).

PATHOPHYSIOLOGY

Erythropoiesis. DBA may have more than a single basis, because the genetics and the phenotypes appear to be multiple. Most *in vitro* studies of the erythropoietic defect have been limited to small numbers of patients. Different results may be related to true variability of the disease. The general consensus is that the erythroid progenitor cell is intrinsically abnormal. The major erythropoietic hormone, EPO, is increased in DBA patients to levels that are higher than expected for a given degree of anemia (662).

A few of the early patients appeared to have RBC alloantibodies (647,648,690,691). These patients had neonatal jaundice and ABO or Rh incompatibility; anemia persisted longer than expected, or bona fide DBA was eventually diagnosed. Antibody specificity may have extended to erythroblasts or progenitors, or blood group sensitization may have been a real but unrelated episode. Blood group incompatibility was not found to be significant in a large number of patients (628).

Cellular inhibitors were proposed by Hoffman et al. (692), but one of those patients was later studied by Nathan et al. (693), who were unable to detect inhibitory lymphocytes when HLA-identical marrow was used as the target population. Another patient was found to have normal erythroid

progenitors and no cellular inhibitors (694). Nathan et al. (695) found no inhibition of normal or autologous marrow CFU-E by the lymphocytes in four transfusion-dependent patients and no inhibition of normal or patient blood BFU-E by the lymphocytes of eight patients, who ranged from transfusion-dependent to those with steroid-independent remissions. They demonstrated that patient T cells were actually stimulatory to normal-blood null-cell BFU-E, as are normal T cells (696).

Cumulative evidence indicates that the erythroid stem cell is abnormal in DBA. Cultures of bone marrow and blood mononuclear cells in plasma clot or methylcellulose showed reduced numbers of CFU-E or BFU-E in more than 50 patients, and normal numbers in approximately 12 patients (697). Patients with normal quantities of progenitors may have been younger and untreated. It was suggested that those patients who subsequently responded to prednisone had better erythroid growth *in vitro*. Addition of steroids *in vitro* was also found to increase erythropoiesis in a few studies (695,698).

Several studies suggested that DBA erythroid progenitor cells required unusually high concentrations of EPO (695,699-702). These EPO studies used crude EPO, which may have contained other erythroid growth-promoting factors. Lipton et al. (701) demonstrated increased EPO sensitivity in DBA bone marrow cultures to which a source of burst-promoting activity (BPA) was added; this suggested that BPA might act directly on the progenitor cell and not through accessory cell mediation (702). Halperin et al. (699) found that IL-3 increased the size and number of marrow BFU-E. The earlier suggestion that DBA erythroid progenitor cells were insensitive to EPO may have reflected a specific insensitivity to BPA or to IL-3. The protective role of EPO was supported by the observation by Perdahl et al. that the hormone was required to prevent accelerated apoptosis (703). Dianzani et al. (704) showed that there were no mutations in the receptor for erythropoietin and also that there was no pathogenetic role for IL-9 or for the 5q hematopoietic cluster (705).

Another candidate ligand-receptor set is SCF and its receptor, c-kit, which are mutant in Steel and W mice that have a macrocytic anemia. *In vitro* addition of SCF to bone marrow BFU-E cultures resulted in increased colony numbers in the majority of DBA studies (131,640,706). However, mutations were not found in SCF or c-kit genes (707-709), and SCF gene expression was normal (710,711). In addition, treatment of both Steel and W mice with prednisone in doses that are comparable to those that are effective in DBA patients did not improve the anemia of the mice (712).

Diamond-Blackfan Anemia Genes. The identification of DBA genes has relied on the mapping of DBA loci through large kindreds. The first clue to localization of a gene at 19q13 was provided by one patient with a reciprocal X;19 translocation (713) and three patients with microdeletion syndromes that involved 19q13.2 (714-717). The translocation breakpoint occurred within the gene for ribosomal protein S19 (718). Approximately 25% of unrelated DBA patients were shown to have mutations in this gene, including nonsense, frameshift, splice site, or missense mutations (Fig. 8-23). Only one of the two RPS19 alleles was mutated in the DBA patients, which suggested that the disease results from haploinsufficiency at this genetic locus. Presumably, biallelic mutation of the RPS19 gene would be lethal. How a 50% reduction in a ribosomal subunit can cause the developmental abnormalities of DBA remains unknown. A decrease in function of this ribosomal subunit may result in impaired protein translation. This

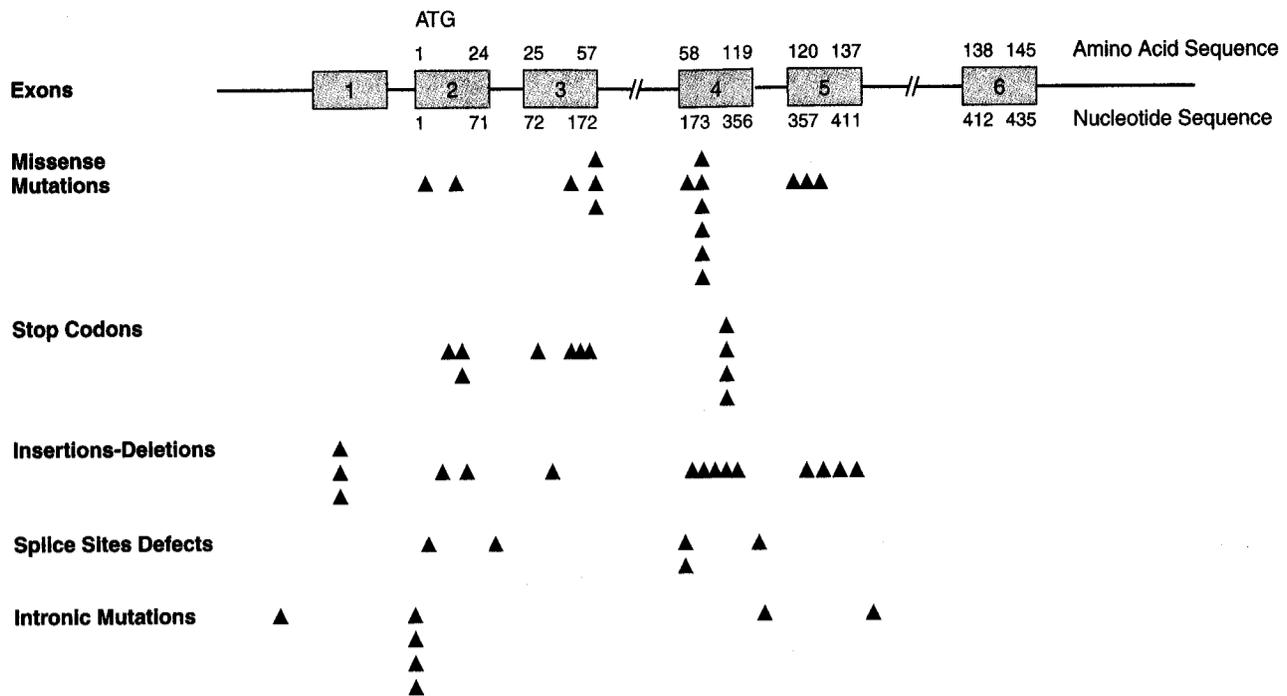


Figure 8-23. Point mutations in RPS19, one of the Diamond-Blackfan anemia genes. The positions of single nucleotide substitutions or small deletions or insertions in the RPS19 gene, which are found in DBA patients, are indicated along the six exons of the RPS19 gene. (From Willig T, Draptchinskaia N, Dianzani U, et al. Mutations in ribosomal protein S19 gene and Diamond Blackfan anemia: wide variations in phenotypic expression. *Blood* 1999;94:4294-4306, with permission.)

impairment may be most evident in specific cell lineages, such as the erythrocyte lineage.

Another DBA genetic locus was recently identified at chromosome 8p23.2-p22, and evidence was obtained for at least a third gene (719). In that study of 38 families, 47% mapped to 8p, 34% to 19q, and the remaining 18% to neither of those loci. No ribosomal subunit genes map to the 8p region, which suggests that the DBA gene at this locus may have an alternative function.

The cloning of DBA genes has diagnostic implications. Some DBA patients have clinical findings that resemble those that are seen in Fanconi's anemia, although DBA patients differ in that the anemia may respond to corticosteroid therapy. Genetic diagnoses may serve to identify the patient as DBA, if the Fanconi's anemia testing is inconclusive. At this time, the only specific test that can be offered to suspected DBA patients is mutational analysis of the RPS19 genetic locus (720). One "common" mutant allele has been identified, which allows for direct screening of this polymorphic site (644).

THERAPY AND OUTCOME

Transfusions. The only treatment that was initially available for DBA patients was transfusions, without which affected children died of anemia (625). This remains the mainstay of the steroid-resistant patient. White-cell free-packed RBCs are given every 3 to 6 weeks, as needed, to keep the Hgb at a level higher than 6 g/dL. Careful cross-matching for minor blood groups is usually done only when alloantibodies develop from sensitization. The major complication from transfusions is hemosiderosis. This was the cause of death in at least 20% of the more than 50 patients for whom the cause of death was stated. The side effects of iron overload in DBA are identical

with those that are seen in thalassemia major, including diabetes, cardiac failure, liver disease, growth failure, and failure to enter puberty. These complications do not develop as rapidly in DBA patients as they do in thalassemia patients, in whom there is the added complication of a hemolytic, rather than an aplastic, anemia. Chelation of iron with subcutaneous deferoxamine should begin soon after the patients have begun a chronic transfusion program, as they are shown to have increased iron stores. Although an oral chelator is under development, its use is not advised in DBA because of the risk of neutropenia (721-723).

Splenectomy. Splenectomy was reported in approximately 40 patients, with no apparent benefit except in those who had hypersplenism that was related to transfusions. Fifty percent of the splenectomized patients were reported to have died, and at least one-half of those deaths were due to infections. Splenectomy is not usually recommended today.

Corticosteroids. Gasser first proposed the treatment of DBA patients with steroids, based on his observations of transient erythroblastopenia in patients with transient allergic disorders and of eosinophils in the bone marrow of DBA patients (658,724). The first drugs were cortisone or adrenocorticotropic hormone (725), but prednisone and prednisolone have become the drugs of choice. Twelve of 22 patients responded in the first series by Allen and Diamond (726). The current recommendation is prednisone, 2 mg/kg/day, in three or four divided doses. Reticulocytes usually appear within 1 to 2 weeks. Some of the erythropoiesis is apparently ineffective, because a sustained rise in Hgb may not occur for several weeks, although it usually appears within 1 month. The high divided-dose protocol is maintained until the Hgb reaches 10

g/dL. It is then tapered slowly, by sequential removal of the divided doses, until the patient is on a single daily dose that maintains the Hgb. This dose is then doubled and administered on alternate days, followed by a slow reduction in the amount. Treatment on alternate days reduces the side effects of the steroids. One group used prednisone daily for a week, followed by 1 to 2 weeks without any drug, to permit better growth (670), but this protocol did not work in our hands. The alternate-day dose depends on the patient and varies from as low as 1 mg to more than 40 mg; some patients are sensitive to small doses, as well as to small changes in dose. Failure to remit with the previously outlined prednisone protocol may be an indication for a trial of 4 to 6 mg/kg/day or a trial of prednisolone or dexamethasone. Any medications that might be marrow suppressive or that affect the metabolism of prednisone, such as phenytoin or phenobarbital, should be discontinued. Steroid remissions have occurred in patients after 10 years of transfusions (627,649,727); thus, a history of transfusions should not preclude an adequate trial of steroids that are combined with iron chelation, as needed. Steroid side effects include growth retardation in more than one-half the patients (628) and osteoporosis, aseptic necroses of femoral or humeral heads, weight gain, cushingoid appearance, hypertension, diabetes, fluid retention, gastric ulcers, cataracts, and glaucoma. These side effects may be sufficiently serious to warrant switching to a transfusion program, despite hematologic response.

There are several patterns of response to steroids:

- Rapid response, followed by steroid-independent remission (less than 5%).
- Intermittent response (approximately 5%).
- Response, followed by steroid dependence (60%). As many as 20% of these patients may eventually be able to maintain their Hgb levels without steroids.
- Steroid response and dependence, followed by later failure to respond to the same or higher doses (5%).
- Requirement for large daily doses, which usually means resuming transfusions because of the steroid side effects (less than 5%).
- No response (30% to 40%). Overall, steroid nonresponders, high-dose responders, or subsequent failures make up almost 50% of cases.

In general, 15% to 25% of patients may eventually have a hematologic remission that is unrelated to their treatment or response to treatment (728).

Several other therapeutic approaches also have been attempted. Androgens (see the section Fanconi's Anemia) were reported in more than 50 patients, with apparent response in only three patients. Immunosuppressive drugs were given to a few patients. 6-Mercaptopurine was used successfully in one patient (729) and unsuccessfully in another (730). Two patients had transient reticulocytosis after administration of cyclophosphamide and antilymphocyte globulin (731) (N. S. Young, unpublished data), whereas others had no response to cyclophosphamide (730,732,733). Vincristine has also been ineffective (731,733,734). Ozsoylu (735) suggested that high doses of intravenous methylprednisolone are effective, as was described previously for severe acquired aplastic anemia.

Cyclosporin A. Cyclosporin A treatment was reported in 29 patients. It permitted the elimination of prednisone in only three patients (736-739), but it did result in lower doses of prednisone in another 13 patients (740-746). However, some of these

responses were only transient. Twelve patients did not respond to cyclosporin A alone or when it was combined with prednisone (742,744,745,747). Responses to immunosuppressive agents suggest that DBA may be an autoimmune disease, but supportive *in vitro* data are lacking.

Cytokines. EPO treatment seemed reasonable, based on the *in vitro* data that suggest response to high doses of the hormone. In a total of ten patients, doses of up to 200 U/kg/day for as long as 5 months had no impact on erythropoiesis (748,749).

IL-3 was also a good candidate based on *in vitro* studies. Approximately 100 DBA patients were treated with IL-3 in five studies, with the achievement of independence from transfusions and steroids in 10% (630,750-753). However, side effects included serious allergic responses, fevers, chills, and deep vein thromboses, and this agent is no longer available.

Hematopoietic Stem Cell Transplant. BMT is an option for those patients who do not respond to reasonable doses of steroids. Almost 40 patients have been transplanted from HLA-matched sibling donors, with a 75% absolute survival. Eight cases had alternative donors, with a 38% absolute survival. Their ages at transplantation ranged from 1 to 31 years of age and 1 to 18 years of age, respectively, and most were steroid nonresponders or had relapsed on steroids and had received transfusions. The survivals plateau at 76% and 43%, respectively, with exclusion of the last death, which was from metastatic osteosarcoma that developed after the transplant, in the group with alternative donors (Fig. 8-24). The results for marrow donors were similar to those for cord donors, with respective actuarial survivals of 59% and 63%. The Diamond-Blackfan Anemia Registry reported an actuarial survival of 88%, when sibling donors were used, and 28% with the alternative donors (728). It should be noted that they include one patient who had late-onset osteogenic sarcoma and who was originally reported by Giri et al. (754). The results suggest that DBA may be cured by hematopoietic stem cell transplant.

Reported deaths after transplant were related to interstitial pneumonitis, graft rejection, graft-versus-host disease, and

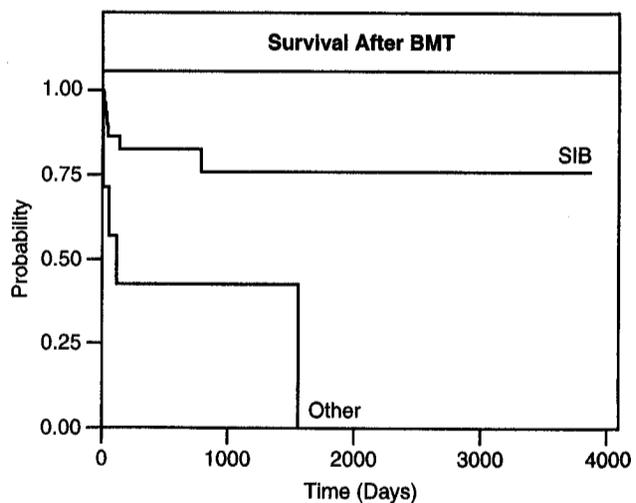


Figure 8-24. Kaplan-Meier plot of cumulative survival in Diamond-Blackfan anemia after bone marrow transplantation (BMT). Time is shown as time after BMT in days. Other, 3 matched unrelated donor marrows, 3 matched unrelated donor cords, 1 mother, 1 grand uncle; SIB, 38 patients with a sibling donor.

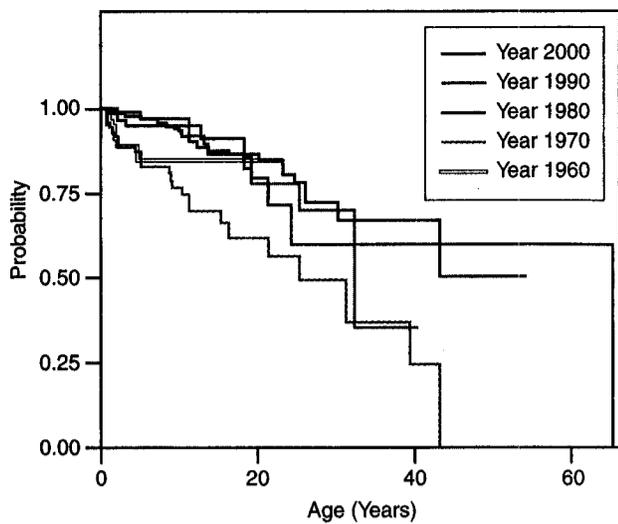


Figure 8-25. Kaplan-Meier plot of cumulative survival in Diamond-Blackfan anemia. Time is shown as age in years, because most cases are diagnosed in infancy. Lines represent 78 cases reported from 1936 to 1960, 113 cases from 1961 to 1970, 90 cases from 1971 to 1980, 115 cases from 1981 to 1990, and 309 cases from 1991 to 2000. The differences are significant.

venoocclusive disease in the sibling group and graft-versus-host disease, sepsis, Epstein-Barr virus lymphoma in donor cells, and venoocclusive disease in the alternative donor group. The decision for transplantation in DBA is not easy, because perhaps 20% of patients may eventually have a remission. In general, stem cell transplants are more successful in young, relatively untransfused (and unsensitized) patients; thus, the decision to undergo transplant sometimes must be made before a potential remission may occur. As in other genetic diseases, DBA must be ruled out in the donors.

Survival Data. The survival data on more than 600 patients that were reported in the literature are shown in Figure 8-25. Patients who are reported in more recent years have a cumulative survival that is better than those who were reported initially, reflecting the effect of steroid treatment and better

programs for transfusion and chelation. The projected median survival for all patients is 43 years of age. Almost 100 patients were reported to have died (13%) at a median of 6 years of age in the entire group (Table 8-16). The most common causes of death were complications of iron overload and pneumonia or sepsis. Other deaths were due to BMT complications, leukemia, cancer, renal disease, anesthesia, pulmonary emboli, and undefined central nervous system disorders.

The quality of life is good for most patients. The spontaneous remitters and the steroid responders who can be maintained on low doses live essentially normal lives. Those who must be transfused can receive chelation therapy, as do patients with thalassemia major. The future of hematopoietic stem cell transplants lies in cautious optimism, with improvements needed for those for whom alternative donors are needed. Gene therapy may also become available as that technology is developed and as the genes become identified.

Pregnancy. Many DBA patients have grown to adulthood and had children. Among 25 reported DBA females who had 29 pregnancies, 12 babies were normal, and 16 had DBA; there was one miscarriage (504). In the Boston series of 76 DBA patients, five men and three women had 13 children, three of whom had DBA (631). Pregnancy is undoubtedly underreported, except in the context of dominant inheritance. From the limited information that is available, there appears to be temporary worsening of anemia during pregnancy in almost one-half of the cases, perhaps due to marrow suppression by estrogens. We advised maintenance of maternal Hgb at a level greater than 8 to 9 g/dL to avoid maternal anemia that might lead to intrauterine growth retardation, preterm delivery, or fetal distress (504). Seven of the 28 deliveries required cesarean sections, owing to fetal anemia, toxemia, and failure of labor to progress.

COMPLICATIONS

Leukemia. Leukemia was reported in ten patients (Table 8-18). One girl had a spontaneous remission of DBA at 5 years of age, developed ALL at 13 years of age, and, after complete remission of the ALL, she was free from both conditions at 17 years of age (755; G. Schaison, *personal communication*, 1982). Nine patients

TABLE 8-18. Cancer in Diamond-Blackfan Anemia

Type	Male	Female	Unknown	All Patients	References
Leukemia					
Acute lymphoblastic	0	1	0	1	759
Acute myeloblastic ^a	5	2	2	9	276,631,733,757,758,760-762
MDS ^b	2	0	0	2	760,764
Total leukemia + MDS	7	3	2	12	—
Osteogenic sarcoma	2	3	0	5	632,754,763,764
Sarcoma, soft tissue	1	0	0	1	764
Breast	—	2	0	2	632,765
Hodgkin's disease	2	0	0	2	632,766,767
Non-Hodgkin's lymphoma	0	1	0	1	768
Hepatoma	2	0	0	2	769,770
Colon	0	1	0	1	764
Fibrosarcoma	1	0	0	1	771
Stomach cancer	1	0	0	1	770
Melanoma	0	1	0	1	631
Total solid tumors	9	8	0	17	—

MDS, myelodysplastic syndrome.

^aOne patient had MDS that developed into acute myeloid leukemia within 1 year.

^bMDS is not considered cancer in these analyses. Some cases were reported more than once. No patient had more than one cancer.

had various forms of AML. These included two patients who were from the original series of Diamond et al. (756) and who had intermittent remissions of DBA but died of acute myeloblastic leukemia at 31 and 43 years of age (757; F. H. Gardner, unpublished data, 1984). One patient had received radiation to his thymus and long bones to stimulate the bone marrow. A girl whose DBA treatment included cyclophosphamide died of acute promyelocytic leukemia at 13 years of age (733). One boy developed acute megakaryoblastic leukemia at 14 months of age; it is possible that his anemia from 2 months of age was really a long preleukemic phase (758). Potential extenuating circumstances were less apparent for the other cases. The majority of the patients died from their leukemia. Janov et al. (631) calculated the relative risk of leukemia to be 200-fold.

Myelodysplastic Syndrome. One patient had MDS that developed into AML within one year, whereas two others had MDS at the time of the reports and died from complications of bone marrow failure (760,764).

Solid Tumors. Seventeen patients with DBA developed non-hematologic neoplasms (Table 8-18). The most common were sarcomas, including five osteogenic and one soft-tissue sarcoma. There were two Hodgkin's and one non-Hodgkin's lymphoma, two breast cancers, and one each of colon, fibrohistiocytoma, gastric, and melanoma cancers. Two patients also developed hepatomas, one of whom had transfusional iron overload, and the other had received androgens. Although a formal risk ratio cannot be determined from literature case series, DBA can be classified in the premalignant category in which many of the bone marrow failure syndromes now appear to belong.

Aplastic Anemia. Evolution to complete aplastic anemia has not been convincingly documented in DBA patients, although it was mentioned once without details by Najean (772). One reported case developed cytomegalovirus infection before pancytopenia (773), and another was multiply transfused and thus might also have a viral etiology (774). Pancytopenia has also occurred in patients with severe iron overload or terminal sepsis. In general, DBA remains a single cytopenia, and the overall prognosis is better than in many of the other marrow failure disorders.

Transient Erythroblastopenia of Childhood

Acute erythroblastopenia in previously hematologically normal children was first described by Gasser (724) in 1949 in 12 children in whom erythroblastopenia followed toxic, allergic, or infectious episodes. These children recovered rapidly and did not develop anemia. Baar then studied RBC lifespan in an 8-year-old with "complete transient aplasia of the erythropoietic tissue" (775). The term *erythroblastopenia of childhood* was first used by Wranne in 1970 to describe four cases with temporary red cell aplasia (776). More than 500 cases have been reported since 1970, as detailed case reports and as series of cases without individual data (5,6,697). TEC is defined as peripheral blood reticulocytopenia, usually with anemia and with bone marrow erythroblastopenia and normal white blood cell and platelet counts, and is temporary in duration. Some of the younger patients with TEC were sometimes initially thought to have DBA; Table 8-19 lists several features that distinguish the two conditions.

The male to female ratio is 1.3:1 among those with TEC. It is an acquired condition and occurs at a median of 23 months of

TABLE 8-19. Comparison of Diamond-Blackfan Anemia and Transient Erythroblastopenia of Childhood

	Diamond-Blackfan Anemia	Transient Erythroblastopenia of Childhood
Number of reported cases	>700	>500
Male/female	1.1	1.3
Age at diagnosis (mo)		
Mean	11	26
Median	3	23
Range	0-768	1-192
Patients older than 1 yr of age at diagnosis	12%	84%
Etiology	Genetic	Acquired
Antecedent history	None	Viral illness
Physical examination abnormal	25%	0%
Laboratory		
Hemoglobin g/dL	1.2-14.8	2.2-12.5
White blood cells <5000/ μ L	15%	20%
Platelets >400,000/ μ L	20%	45%
Red blood cell adenosine deaminase	Increased	Normal
Mean cell volume increased at diagnosis	80%	5%
During recovery	100%	90%
In remission	100%	0%
Fetal hemoglobin increased at diagnosis	100%	20%
During recovery	100%	100%
In remission	85%	0%
i Antigen increased	100%	20%
During recovery	100%	60%
In remission	90%	0%

Adapted from Alter BP. The bone marrow failure syndromes. In: Nathan DG, Oski FA, eds. *Hematology of infancy and childhood*, 3rd ed. Philadelphia: WB Saunders, 1987:159-241; and Link MP, Alter BP. Fetal erythropoiesis during recovery from transient erythroblastopenia of childhood (TEC). *Pediatr Res* 1981;15:1036-1039.

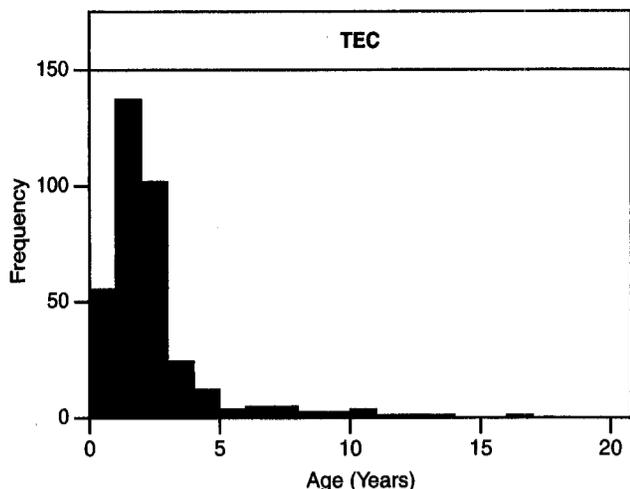


Figure 8-26. Age at diagnosis in more than 500 published cases with transient erythroblastopenia of childhood (TEC). Compare this figure with Figure 8-19 for Diamond-Blackfan anemia.

age (Fig. 8-26). More than 80% of patients who were studied were older than 1 year of age, compared to only approximately 10% of those with DBA in the same age range (compare Figs. 8-19 and 8-26). Only 10% of TEC patients were older than 3 years of age, and only four patients were older than 10 years of age; the oldest was 16 years of age.

The presenting symptom of TEC was pallor in a previously normal child, in whom prior normal blood counts were sometimes available. More than one-half of patients gave a history of a preceding illness (the median time interval between the prior illness and TEC presentation was 1 month, with a range of 0 to 4 months), which was usually viral—upper respiratory or gastrointestinal. Because these illnesses are common in young children, their relevance is difficult to ascertain, although a viral cause of the aplasia is appealing (see the section Pathophysiology). A few children had neurologic manifestations of anemia: seizures, breath holding, or transient ischemic attacks (777-785). Drug or toxin exposure included piperazine, aspirin, sulfonamides, valproic acid, phenytoin, and phenobarbital (782,786-790).

Familial TEC has been recorded rarely. Identical and fraternal twins had simultaneous onset of anemia (787,791,792). Four pairs of siblings had TEC at similar ages, but 2 to 3 years apart in onset (792,793). In these familial cases, TEC might have been caused by the same viral or environmental factor, combined with a genetic propensity. TEC has been reported from 17 countries, and cases have included blacks, Hispanics, and Japanese. Most of the reports have been from temperate climates.

TEC is somewhat seasonal, with the largest numbers of cases from the Northern Hemisphere occurring between October and January. However, the number of cases with this information is sufficiently small that monthly variations may be due to chance alone. At a given center, clusters may be due to specific local viral epidemics. Until the putative etiologic virus is identified, the cause remains speculative.

Physical examinations in TEC patients are normal except for pallor, and findings relate to anemia, such as tachycardia. The anemia is of gradual onset; thus, the pallor often is not noticed by parents until the anemia is profound.

LABORATORY FINDINGS

Hgb levels ranged from 2.2 to 12.5 g/dL, with a median of 5.6 g/dL. Reticulocytes were less than 1% in most of the children.

White blood cell counts were generally normal, but the absolute neutrophil count was less than 1000/ μ L in approximately 6% of the patients, and thrombocytopenia of less than 100,000/ μ L was reported in approximately 5%, some of whom were also neutropenic. It is possible that whatever suppresses the erythroid series may affect all cell lines in a few patients. Twenty percent had white blood counts greater than 10,000/ μ L, which perhaps is associated with intercurrent infections, although one patient did have true leukoerythroblastosis, perhaps due to marrow stimulation by hypoxia (794). Platelet counts greater than 400,000/ μ L were noted in almost one-half the patients, which is more than the proportion of DBA children with increased platelet counts. The MCVs were usually normal for age, with mean and median of 80 fL, although the range was from 62 to 112 fL. HbF levels also were usually normal, although the range was from 0.2% to 9.2%. A suggestion from one small study that blood group A patients predominate (759) was not borne out in another small study (795). Bone marrow examinations showed significant to profound erythroblastopenia in more than 90% of patients. In those in whom erythroblasts were seen, a maturation arrest was often present. Marrow lymphocytes were frequently elevated and led to the incorrect diagnosis of acute leukemia in at least one case (796). The 5% to 10% of patients with reticulocytosis or erythroid hyperplasia at presentation presumably had begun to recover without the benefit of medical attention.

Several erythrocyte characteristics may distinguish TEC from DBA. Wang and Mentzer (797) pointed out that the RBCs in TEC were adult or normal for age in MCV, HbF, i antigen, and RBC enzyme levels. In DBA, these parameters were more fetal-like. This distinction, which is based on normal versus fetal-like features, is only relevant to the reticulocytopenic patient at the time of diagnosis. During recovery from TEC, the RBCs have the features of stress erythropoiesis and are identical with those seen during any bone marrow recovery. This transient cohort of fetal-like erythrocytes can be detected as soon as the first reticulocytes appear, using a sensitive immunologic assay for F-reticulocytes (798,799). As Link and Alter (795) documented, this cohort of fetal-like erythrocytes then evolves again into normal RBCs. Thus, interpretation of fetal-like features depends on the stage of the disorder, and the ultimate distinction between TEC and DBA may be clear only from the outcome. Macrocytosis, elevated HbF, and reticulocytopenia, as well as marrow erythroblastopenia, in a child younger than 1 year of age most likely indicate DBA. Elevated RBC ADA is found in DBA and not in TEC (677).

PATHOPHYSIOLOGY

A viral cause for TEC is most likely. The history of an antecedent (usually viral) illness that presents 2 months before TEC is intriguing. Suppression of erythropoiesis in a normal person would require symptom duration of 1 to 2 months to be manifest as symptomatic anemia. It is not clear why recovery would then occur within another month. If the cause is viral, the time course of the development of specific antibodies would be relevant. The prime suspect is parvovirus. However, almost 50 cases have been analyzed individually or by one laboratory for parvovirus antibody, but only 20% of cases were found to have antibody, and causality remains to be proven. Because parvovirus inhibits the growth of CFU-E, it is logical that this particular virus leads to aplasia in patients with shortened RBC survival due to hemolytic anemia. Its role in those with previously normal erythropoiesis has not yet been proven and requires sensitive assays for antigen or for parvoviral DNA.

Several groups have investigated the erythropoietic defect in TEC. Levels of EPO are high (800). Erythroid progenitor cell cultures have provided a wide variety of results. A consensus of all of the culture studies is difficult to reach, because not all parameters were examined in all patients. Probably one-half of all cases of TEC have reduced erythroid progenitor cell numbers. More than one-half of cases have serum inhibitors in autologous or allogeneic cultures, usually IgG. Inhibitory mononuclear cells may be present in one-fourth of cases. No patients had serum and cellular inhibitors, but few were examined for both (697). TEC may be due to an as yet unknown virus, which may infect CFU-E and not be cleared until specific antibodies develop to the virus. In some cases, a specific IgG may be directed against epitopes on erythroid progenitors themselves, thus inhibiting growth of autologous or allogeneic erythroid colonies. Recovery in these cases might require antiidiotype antibodies.

THERAPY AND OUTCOME

As indicated by the name of this disorder, TEC is transient, and all patients recover. Recurrent TEC was observed only twice and occurred within 1 year (800,801). Most patients show signs of recovery within the first month after diagnosis, and 5% to 10% of patients have already begun to recover when they are first seen. The longest interval to recovery without recurrence was 8 months, and only nine patients took more than 4 months to recover. No treatment was necessary for approximately one-half of the patients for whom the clinical course was described. In most patients, the nadir Hgb was reached by the time they were seen. More than one transfusion was needed in less than 10% of patients. Prednisone was administered to 10% to 20% of patients, but reticulocytes appeared within 1 day in many patients, a phenomenon that is almost certainly unrelated to the treatment.

Our recommendation for TEC is watchful waiting, with transfusion only when anemia leads to cardiovascular compromise. These children tolerate their anemia extremely well, because it has developed gradually, and it is often the cardiovascular status of the physician rather than the patient that leads to transfusion. Prednisone, anabolic steroids, and other immunosuppressive therapies have no apparent role in the management of TEC. The prognosis is excellent, and the distinction from DBA is simple in retrospect (Table 8-19).

Congenital Dyserythropoietic Anemia

Dyserythropoiesis refers to ineffective, morphologically abnormal erythroid production. *Dysplastic* indicates qualitative abnormalities of the stem cell or the microenvironment. *Aplas-*

tic refers to quantitative abnormalities of the same compartments. Although these congenital conditions are not, strictly speaking, bone marrow failure syndromes, they are inherited marrow disorders, which may result in anemia without reticulocytosis. Erythropoiesis is ineffective, because a discrepancy exists between erythroid output from marrow to circulation (anemia) and erythroid marrow content (erythroid hyperplasia), thus implying intramedullary destruction.

In healthy persons, approximately 1 in 1000 bone marrow erythroblasts is abnormal (802). Multinucleated erythroblasts and karyorrhexis are occasionally seen in megaloblastic anemia, iron deficiency, leukemia, and hemolytic anemia, and are indicative of bone marrow stress. The incidence of dyserythropoietic erythroblasts may be substantial in acquired or inherited aplastic anemia. In one study of aplastic anemia, 5% to 90% of erythroblasts were megaloblastic or showed nuclear-cytoplasmic asynchrony, 1% to 3% were binucleate, and as many as 5% had cytoplasmic connections or chromatin bridges (803). During recovery from BMT, all patients transiently had as many as 30% dyserythropoietic erythroblasts (804). These morphologic abnormalities are more extreme in congenital dyserythropoietic anemia (CDA) than in aplastic anemia or in marrow transplantation recovery.

All patients with CDA have anemia with insufficient reticulocytosis and ineffective erythropoiesis, and all ethnic groups are affected. The major types of CDA were described in detail in a book by Lewis and Verwilghen (805) in 1977 and are summarized in a recent review by Wickramasinghe (806). Table 8-20 outlines the major types:

Type I: macrocytosis, with bone marrow megaloblastoid changes and internuclear chromatin bridges between cells.

Type II: normocytosis or macrocytosis, with binucleated and multinucleated erythroblasts, pluripolar mitoses, and karyorrhexis.

Type III: macrocytosis, with erythroblastic multinuclearity of up to 12 nuclei (gigantoblasts).

Types I and III are diagnosed primarily by bone marrow morphology. Type II is characterized by positive reaction to some acidified normal sera [which is called *hereditary erythroblastic multinuclearity with a positive acidified serum (HEMPAS) test* (807)]. More than 50 additional cases have been reported that do not clearly fit into types I to III.

TYPE I

More than 100 cases of type I CDA have been reported [the first 20 cases were reported by Heimpel (808,809)]. The onset of anemia, jaundice, or both, ranges from infancy to old age, with a median age of onset of 10 years of age. The male to female ratio is

TABLE 8-20. Types of Congenital Dyserythropoietic Anemias

Feature	Type I	Type II	Type III
Reported cases	130	200	60
Male/female ratio	1.1	0.9	0.8
Anemia	Mild-moderate	Moderate	Mild-moderate
Red cell size	Macrocytic	Normo- or macrocytic	Macrocytic
Bone marrow erythroblasts	Megaloblastoid, binucleated (2-5%), chromatin bridges	Bi- and multinucleated (10-40%), karyorrhexis	Gigantoblasts (10-40%)
Inheritance	Recessive	Recessive	Dominant
Acid serum hemolysis	Negative	Positive	Negative
Anti-i reaction	Slight	Strong	Slight
Anti-I reaction	Slight	Strong	Slight
Effect of splenectomy (%)	50	96	100

1:1:1.0, and the inheritance is autosomal recessive. Consanguinity was reported in at least 15 families, and affected siblings or cousins were reported in almost 20. Physical examination may show icterus and splenomegaly, as well as brown skin pigmentation; a few patients had toe syndactyly or abnormal fingers.

The anemia is often mild, with a median Hgb of 9 g/dL and a range of 2 to 15 g/dL. Reticulocytes range from 1% to 7%. RBCs are macrocytic, with a median MCV of 100 fL and a range of 66 to 133 fL. The blood film shows anisocytosis, poikilocytosis, punctate basophilia, and occasional Cabot's rings. White blood cells and platelets are normal. Indirect bilirubin is elevated (1 to 4 mg/dL), as is serum lactate dehydrogenase, whereas haptoglobin is low and transferrin is saturated. Plasma iron turnover is as much as ten times the normal rate, and RBC utilization is reduced to less than 30%. RBC survival is slightly shortened, with a chromium-51 (^{51}Cr) half-life of 15 to 28 days (with a mean 21 days). Globin synthesis studies usually show non- α to α ratios of 1, although an imbalance of 0.5 to 0.7 was observed occasionally (810,811). Negative reactions are seen in the acidified serum test, and i antigen titer is usually in the adult low range, in contrast to that seen in type II CDA.

Bone marrow examination shows marked erythroid hyperplasia (812). The abnormalities are confined to the more mature erythroblasts and the polychromatophilic and orthochromatic series (Fig. 8-27). Nuclear maturation and cytoplasmic maturation are dissociated, and nuclei are immature and megaloblastoid. As many as 2% of erythroblasts are large cells with incomplete nuclear division, often with double nuclei in which one component is more mature than the other. As many as 2% of cells show thin chromatin bridges that connect the nuclei of two cells. Electron microscopy demonstrates widening of nuclear membrane pores in mature erythroblasts, with condensation, vacuolization, and disintegration of nuclear chromatin, with cytoplasmic penetrance. Structural changes are found in the nucleolus, microtubules, and siderotic material in the cytoplasm (812-814).

The defect in CDA is at the stem cell level. The numbers of CFU-E and BFU-E are normal, but the colonies contain a mixture of normal and abnormal cells, when they are examined by electron microscopy (815). This suggests that the abnormality is expressed variably in the mature progeny of each stem cell.

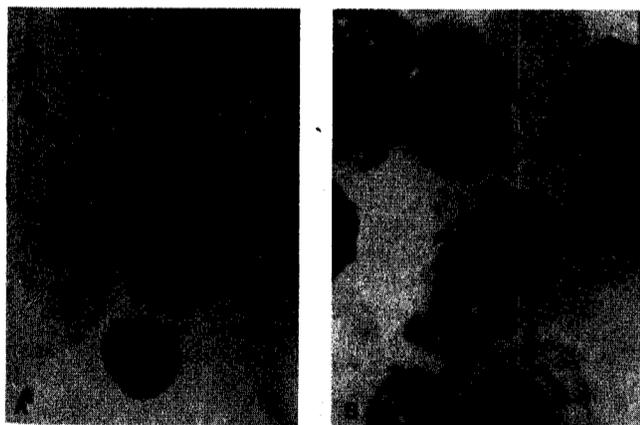


Figure 8-27. Bone marrow from a patient with congenital dyserythropoietic anemia type I. **A:** Dot and leader indicate binucleate erythroblast with nuclei of different sizes and maturity. **B:** Dot and leader indicate internuclear chromatin bridges that connect two erythroblasts. (From Lewis SM, Verwilghen RL, eds. *Dyserythropoiesis*. London: Academic Press, 1977, with permission.)

The gene for CDA I (CDAN1) was mapped in a large Israeli Bedouin kinship to chromosome 15q15.1-15.3 (816). However, several other unrelated patients of Lebanese and English origin did not have haplotypes that linked to this region, thus suggesting that there is genetic heterogeneity in CDA type I (817).

Treatment with the usual hematinics, such as vitamins, metals, and steroids, is without effect. A few transfusions were required in 20% of patients. Splenomegaly is common, but splenectomy (reported in 10% of cases) does not improve the anemia. Some patients develop gallstones from the hemolytic anemia. Hemosiderosis is the most important long-term complication because of increased intestinal absorption of iron, ineffective erythropoiesis, and mild hemolysis; phlebotomy or iron chelation with deferoxamine warrants consideration. Four of the patients were reported to have died, one at 10 years of age from complications of splenectomy, one at 84 years of age from old age, and two from persistent pulmonary hypertension in infancy (818,819).

The beneficial effect of interferon- α was discovered during treatment of a patient with CDA type I for posttransfusion chronic hepatitis C (820) and was confirmed in several other patients (821-823). A potential mechanism was suggested by the observation that Epstein-Barr virus transformed B lymphoblasts from patients with CDA type I produce less interferon- α *in vitro* than do normal cells (823). Addition of interferon- α to erythroid cultures improves the ultrastructural Swiss-cheese appearance of erythroblasts (824).

Successful bone marrow transplant was reported in one case, using a sibling donor (825).

TYPE II

Type II CDA, also known as *HEMPAS*, has been reported in approximately 200 patients. Many of the early cases were summarized by Verwilghen (826,827). More than two dozen cases were in sibships, and 11 cases were in families with consanguinity. The male to female ratio was 0.9:1, and the inheritance is autosomal recessive. Anemia is noted between infancy and adulthood, at a median of 14 years of age, and varies from mild to more severe anemia that requires regular transfusions. The median Hgb was 9.5 g/dL, and the range was from 3 to 15 g/dL. Jaundice, hepatosplenomegaly, and gallstones are more common in patients with type II CDA than in those with type I CDA.

Although anemia may be severe in patients with type II CDA, reticulocytosis is inadequate and averages 4%. RBCs are usually normochromic and normocytic, although macrocytosis has been observed; the median MCV is 94 fL, and the range is from 73 to 114 fL. The smear shows anisocytosis, poikilocytosis, teardrops, and basophilic stippling, all of which are nonspecific findings. The anemia and the RBC lifespan are worse in type II CDA than in type I CDA patients; the ^{51}Cr half-life averages 17 days (with a range from 7 to 31 days).

Electron microscopy shows an excess of endoplasmic reticulum parallel with the cell membrane, which leads to the appearance of a characteristic double membrane, or cistern, in late erythroblasts and some erythrocytes (813,814). Many patients with type II CDA also have bone marrow reticuloendothelial cells that resemble Gaucher cells, with birefringent, paraaminosalicylic acid-positive, needlelike inclusions. These inclusions may be the products of catabolism from the rapidly turning-over marrow erythroblasts. The marrow shows erythroid hyperplasia, with binucleated and multinucleated mature erythroblasts in 10% to 40% of the erythroid precursors (Fig. 8-28). The internuclear chromatin bridges of type I CDA are not seen, nor is the multinuclearity as extreme as is seen in type III CDA.



Figure 8-28. Bone marrow from a patient with congenital dyserythropoietic anemia type II, showing binucleated and multinucleated erythroblasts. (Courtesy of Dr. Gail Wolfe. From Alter BP. The bone marrow failure syndromes. In: Nathan DG, Oski FA, eds. *Hematology of infancy and childhood*, 3rd ed. Philadelphia: WB Saunders, 1987:159-241, with permission.)

The pathognomonic findings in HEMPAS are serologic (807,828,829). HEMPAS RBCs are lysed by approximately 30% of acidified sera from normal persons, but not by the patient's own serum. In contrast, in paroxysmal nocturnal hemoglobinuria, patient cells are lysed by acidified patient serum. In CDA type II, the RBCs have a specific HEMPAS antigen, and many normal sera contain an anti-HEMPAS IgM antibody. In some cases of type II CDA, up to 30 normal sera must be examined until a positive acidified test results; some were thought not to be type II CDA until this was obtained (830). HEMPAS erythrocytes are also distinct in that they are more strongly agglutinated with anti-i antibody than cells of newborn infants or of patients with stress erythropoiesis (831). Fluorescent labels demonstrated i antigen on all RBCs in HEMPAS. Heterozygotes also have increased expression of i antigen (807,832). HEMPAS RBCs also express increased amounts of I antigen. HEMPAS erythrocytes were shown by Rosse et al. (833) to bind a normal amount of complement C1, but more antibody and less C4 than normals. This causes binding of an excess of C3 and hemolysis. The RBC plasma membrane abnormality in HEMPAS is related to decreased N-glycan synthesis near the N-acetylglucosaminylphosphotransferase II and α -mannosidase II steps (834). Bands 3 and 4.5 lack glycosylation with lactosaminoglycans (835).

The number of erythroid progenitors is probably normal in marrow and blood. Although one study found only normal morphology of the erythroblasts that were produced in culture (836), other studies reported multinuclearity that was similar to that seen in the bone marrow (837,838). As in type I CDA, the defect in type II CDA is in the erythroid stem cell and is expressed variably in more mature erythroblasts.

One gene for CDA II (CDAN2) was localized to chromosome 20q11.2 by a genome-wide search using 12 Italian families and one French family (839). Further studies indicated that the majority of the Italian CDA type II patients linked to 20q11.2, but this was not due to a founder effect (840). Two other Italian families were not linked to this locus (841), and other patients were found to have mutations in the genes for α -mannosidase II (834). Thus CDA II has genetic heterogeneity.

Patients whose anemias are severe are supported by blood transfusions. Unlike in type I CDA, splenectomy is effective

in approximately 70% of type II cases and leads to an increase in RBC lifespan and abrogation of transfusions. Iron accumulation, from transfusions and from increased intestinal absorption, is a major complication, even in untransfused patients. Phlebotomy and iron chelation have a definite role in the management of patients with type II CDA (842). Two of the patients died of hemochromatosis at 25 and 30 years of age.

Some of the phenotypic heterogeneity in CDA II could be related to coinherited Gilbert syndrome; serum bilirubin levels and gallstones were increased in patients with the homozygous A(TA)7TAA box variant of the UGT1A gene, which leads to reduced expression of uridine diphosphate glucuronosyl transferase (843). Similarly, iron overload was increased in a patient with CDA type II and hereditary hemochromatosis due to homozygous C282Y mutation in HFE (844).

TYPE III

Approximately 70 patients have been reported in 16 families with type III CDA (845,846). The male to female ratio was 0.8:1.0. Three families had apparently dominant CDA, whereas three had affected siblings, and one had consanguinity. The median age at diagnosis was 24 years of age, with a range from birth to old age, similar to that in types I and II CDA. Anemia is mild to moderate, and MCVs may be normal or increased (with a median 94 fL and a range from 79 to 135 fL). The bone marrow shows erythroid hyperplasia, with multinuclearity in 10% to 40% of erythroblasts, including some large giantoblasts with up to 12 nuclei (Fig. 8-29). Hemolysis does not occur with acidified sera, and reactions with anti-i antigen are similar to those seen in stress erythropoiesis. RBC survival is slightly shortened, with a ^{51}Cr survival half-life of 21 days. The defect is in the stem cell, and *in vitro* culture results in colonies that contain normal and abnormal erythroblasts (847).

The gene for CDA III, CDAN3, was mapped to 15q21-25 in a large Swedish family that was the subject of many reports (848).

The need for transfusions or splenectomy is rare. As in the other CDAs, hemosiderosis is the major problem and resulted in the death of one patient who was 42 years of age. Members of the Swedish family have an increased risk of monoclonal gammopathy, myeloma, and ocular angioid streaks (849).

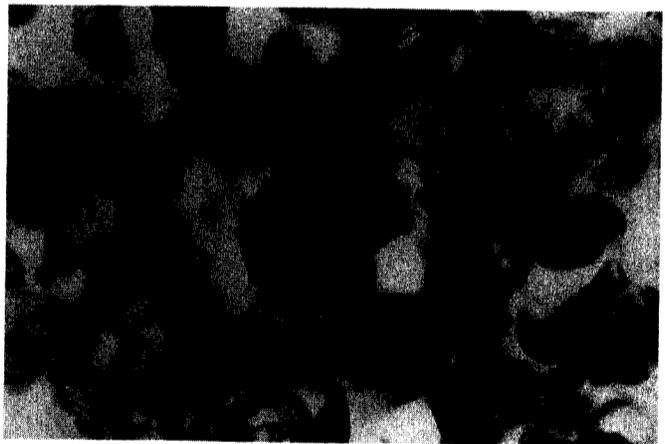


Figure 8-29. Bone marrow from a patient with congenital dyserythropoietic anemia type III, showing multinucleated erythroblast. (Courtesy of Dr. Gail Wolfe. From Alter BP. The bone marrow failure syndromes. In: Nathan DG, Oski FA, eds. *Hematology of infancy and childhood*, 3rd ed. Philadelphia: WB Saunders, 1987:159-241, with permission.)

VARIANTS

More than 50 patients have been reported with apparent CDA that does not conform to the types that were previously described (806). In rare cases, the bone marrow morphology resembled type II CDA, but the cases were classified as type IV CDA, because the acidified serum tests were negative, which might indicate only that an insufficient number of sera were examined. The level of i antigen was not increased. The inheritance was apparently dominant in one family; thus, at least some of these cases might indeed belong to a different type (850-854). Anemia was mild in all but one case and was diagnosed from infancy to adulthood. Binucleated erythroblasts made up 10% to 40% of the marrow erythroblasts. The clinical course was relatively benign in the reported cases, with splenectomy in only one.

Another group of patients was termed *CDA with thalassemia* (855-861). One family had dominant inheritance, and another family had three affected siblings. The age at diagnosis ranged from infancy to old age, the anemia was mild to moderate, MCVs ranged from microcytic to macrocytic, and multinuclearity was present in 25% to 35% of the erythroblasts. Tests with acidified sera were negative, and i antigen was positive in two of the three families. Globin chain synthesis was imbalanced, with β to α ratios of 0.5, which is similar to those seen in β -thalassemia trait and CDA, but two families were from ethnic groups without a high incidence of thalassemia.

Almost 40 other patients were reported with CDAs that are even more difficult to classify. Five families had an affected parent and child, two had consanguinity, and three had affected siblings. These cases all may represent different disorders. The proportion of binucleated or multinucleated erythroblasts was always low, less than 4%. In some, the morphology resembled type I CDA, with rare internuclear

bridges, as well as type II CDA, with symmetric binuclearity, karyorrhexis, and even double membranes by electron microscopy. The ages at diagnosis of anemia ranged from birth to adulthood, and the anemia ranged from mild to severe. MCVs encompassed the entire spectrum. Acidified serum testing was usually negative, i antigen was variable, and RBC ^{51}Cr half-life ranged from 5 to 29 days. Splenectomy was reported in eight patients, with occasional efficacy. Erythroid cultures from one case demonstrated that multinuclearity was present in some cells in each colony, which indicates a stem cell disorder, as in the other CDAs (862). The clinical variability suggests that these cases may represent several types of CDA. Perhaps these patients are double heterozygotes, instead of homozygotes, for recessive CDA genes.

SINGLE CYTOPENIAS: WHITE BLOOD CELLS

Severe Congenital Neutropenia

Inherited isolated neutropenia was first recognized as a distinct entity in 1956 by Kostmann (863,864), who called it *infantile genetic agranulocytosis*. Other names for this condition are *severe chronic neutropenia*, and *severe congenital neutropenia* (SCN) (see Chapter 14 for a more complete discussion of neutropenia). The term *Kostmann's syndrome* might be reserved for patients in whom the inheritance is clearly autosomal recessive; most patients are autosomal dominants or sporadic new mutations. In this section, the term SCN is used for cases in which neutropenia is less than 200/ μL , with severe pyogenic infections in infancy and a bone marrow myeloid arrest at the promyelocyte-myelocyte stage. More than 300 cases in the literature fit this description (Table 8-21). The male to female ratio is 1. Most of Kostmann's original 14 and subsequent ten

TABLE 8-21. Congenital Neutropenia Literature

	Reported on or before 1989	Reported after 1989	All Patients
Number of cases	128	178	306
Male/female	55/68	90/81	145/149
Ratio	0.8	1.1	1
Leukemia			
Number of cases (%)	3 (2)	23 (13)	26 (9)
Male/female	2/1	14/9	16/10
Age at diagnosis (yr)			
Mean	14	11.1	—
Median	14	12	—
Range	14-14	2-23	—
MDS ^a			
Number of cases (%)	0 (0)	13 (7)	13 (5)
Male/female	8/5	8/5	—
Age at diagnosis (yr)			
Mean	11.3	11.8	—
Median	11	11	—
Range	1-22	1-22	—
Deceased			
Number of cases (%)	67 (52)	15 (8)	82 (30)
Male/female	26/37	9/6	35/43
Age at death (yr)			
Mean	2.1	6.9	3
Median	0.7	3.3	0.8
Range	0.05-20	0.1-23	0.05-23
Projected median age for all patients (yr)	3	—	23
Leukemia	14	23	14.8
MDS	—	23	23

MDS, myelodysplastic syndrome.

^aIncludes four patients with bone marrow cytogenetic clones but without morphologic MDS.

cases were members of a large intermarried kinship in northern Sweden, which led to the suggestion that the inheritance is autosomal recessive (865). Cases have been reported in all ethnic groups, including blacks, Native Americans, and Asians. At least a dozen cases were in families with parental consanguinity, and more than 20 families had affected siblings. However, eight families appear to have dominant inheritance (866).

Age at presentation is young, with 50% of the patients symptomatic within the first month after birth and 90% symptomatic by 6 months of age. In fact, the few who were diagnosed later in life may not have had the same diagnosis. Birth weights are generally normal, as are physical examinations, except for signs of infection, such as skin abscesses.

LABORATORY FINDINGS

Neutropenia is extreme in these patients, although several infants had almost normal absolute neutrophil counts in the first week or two of life, which declined rapidly thereafter. The average absolute neutrophil count is less than 200/ μ L; many patients have total neutropenia. Eosinophils and monocytes are frequently high (as many as 50% monocytes in some patients), but these are not as effective as phagocytes as are neutrophils. Ig levels are also frequently increased. Congenital neutropenia is a single cytopenia, because the Hgbs are usually normal (with a mean level of 10 g/dL), and platelet counts are normal or even high. Bone marrow is cellular, with absent or markedly decreased myeloid precursors. When precursors are present, there is an arrest at the myelocyte or promyelocyte stage.

PATHOPHYSIOLOGY

Congenital neutropenia primarily affects the neutrophil series. Bone marrow cultures have decreased, increased, or, most often, normal numbers of colony-forming units (colony-forming unit culture). The colonies, which contain neutrophils in normal persons, that do grow in semisolid media have been reported rarely to contain neutrophils (867) or, more frequently, to contain eosinophils, monocytes, and abnormal or arrested myeloid precursors (868). A block in myeloid differentiation was also seen in long-term cultures from some patients (867). Thus, in some patients the *in vivo* defect in myeloid differentiation is apparent *in vitro*.

Recently linkage analysis in familial cases of autosomal-dominant cyclic neutropenia mapped the locus to 19p13.3 and identified mutations in the neutrophil elastase gene (ELA2) at that locus (869). The same group then showed that 22 of 25 patients with SCN had 18 different heterozygous mutations in ELA2 (870). The mutations in cyclic neutropenia cluster around the active site of the enzyme, whereas the mutations in SCN are on the opposite face. Thorough investigation of the mutant and wild-type proteins led to the conclusion that mutant ELA2 may act as a dominant negative inhibitor of the function of the normal enzyme (871).

THERAPY AND OUTCOME

The prognosis for patients with congenital neutropenia was poor in the era before G-CSF, with more than one-half of patients reported to have died at a median of 7 months of age, with a range of 2 weeks of age to 20 years of age. The Kaplan-Meier survival curve (Fig. 8-30) for this group indicates a projected median survival of 3 years of age, and only 10% of patients are long-term survivors. Most of the deaths were from sepsis or pneumonia. No cases evolved into aplastic anemia, which shows that congenital neutropenia is a true single cytopenia. Infections were treated with antibiotics, and many patients received prophylactic treatment. Lithium

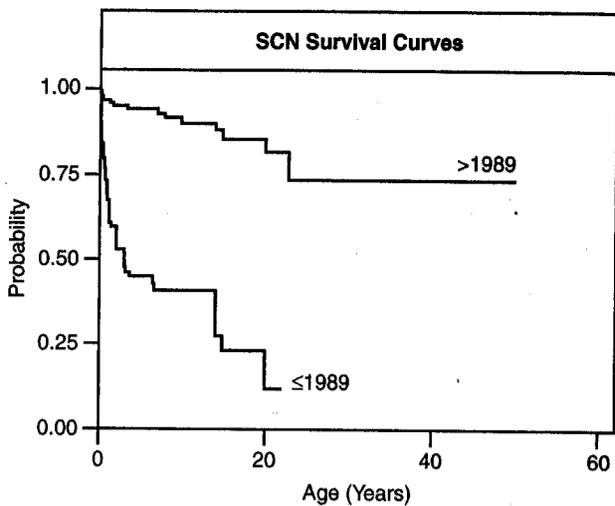


Figure 8-30. Kaplan-Meier plot of cumulative survival in severe congenital neutropenia (SCN). Time is shown as age in years. Lines represent 128 patients who were reported in the era before granulocyte colony-stimulating factor (before 1989) and 178 patients who were reported after granulocyte colony-stimulating factor (after 1989).

therapy was suggested because of its ability to raise white counts in hematologically normal persons, but it was relatively ineffective in congenital neutropenia (872,873). BMT cured one patient (874), but the graft was lost in another (875).

The most exciting therapeutic advance was the use of recombinant growth factors. Subcutaneous G-CSF is effective at raising neutrophil counts in children with congenital neutropenia in a dose-related manner. The factor must be administered chronically, but it seems to be without major short-term side effects (876,877). However, bone loss has been reported and warrants treatment (878,879).

Only 8% of the cases that have been reported since 1989 died, at a median of 3 years of age, with a range of 1 month of age to 23 years of age and a projected plateau of 75% survival at 23 years of age. Deaths still occur from sepsis. Since 1989, bone marrow transplant was reported in 19 patients, of whom five died from complications; most transplants were from alternative donors (880) (Fig. 8-31).

Prenatal diagnosis was considered early for congenital neutropenia, using fetal blood that was obtained in the middle trimester (881). However, the absolute neutrophil count is less than 200/ μ L in normal fetuses (882), and truly absolute neutropenia would be required to diagnose congenital neutropenia *in utero*. At this time, mutations in ELA2 might be sought *in utero*.

Leukemia. Before G-CSF, three patients developed acute monocytic leukemia, all at 14 years of age, and died at 4 to 6 months after diagnosis (883-885). One family was reported to have one child with congenital neutropenia and a sibling with acute lymphoblastic leukemia (886). Since the introduction of G-CSF, leukemia or MDS, or both, was observed in approximately 10% of the treated patients. In a large series reported from the Severe Chronic Neutropenia Registry in which leukemia and MDS were not analyzed separately, the frequency was 9% (887). In individual case reports, leukemia occurred in 13% of patients after 1989 and consisted mainly of AML that was not otherwise defined, acute myelomonocytic leukemia, and acute monocytic leukemia. MDS was reported in 7% of patients, and monosomy 7 clonality was noted in many

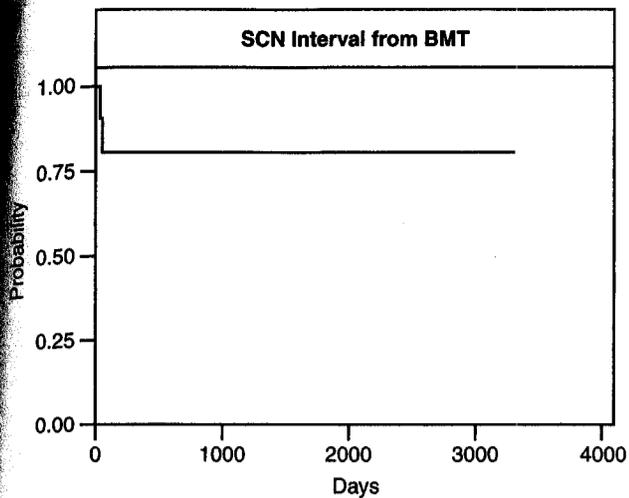


Figure 8-31. Kaplan-Meier plot of cumulative survival in six patients with severe congenital neutropenia (SCN) after bone marrow transplantation (BMT). Time is shown as the interval from BMT in days.

patients with MDS and leukemia (887,888). Although the evolution to MDS or leukemia is worrisome, G-CSF has made a major contribution to the longevity of patients with congenital neutropenia.

Somatic mutations of one of the two alleles of the G-CSF receptor (G-CSF-R) have been described in several patients (889,890). The mutations generally result in loss of the carboxy terminal differentiation domain of the receptor. These truncated forms of the G-CSF-R are thought to have dominant activity, which results in increased cell growth without granulocyte differentiation. Importantly, because only somatic mutations in the G-CSF-R have been described, this gene is not the underlying genetic cause of congenital neutropenia. However, these somatic mutations may contribute to the transformation into acute myeloblastic leukemia (891) and have been identified before development of leukemia (892). The precise mechanism by which the truncated G-CSF-R acts dominantly over the endogenous wild-type allele remains unknown. The mutant receptor may have delayed internalization and down-regulation after ligand binding, resulting in a sustained proliferative signal. Such a strong proliferative drive may block the granulocyte differentiation signal that is generated from the wild-type G-CSF-R that is encoded by the normal allele (893).

A propensity for malignancy (leukemia) is seen in congenital neutropenia, as in many other bone marrow failure syndromes.

SINGLE CYTOPENIAS: PLATELETS

Thrombocytopenia with Absent Radii

TAR syndrome represents a true single cytopenia, with no reports of evolution to pancytopenia or malignancy. Comprehensive reviews are provided elsewhere (5,6,894–896). The patients are often diagnosed at birth, due to the combination of the characteristic physical appearance plus thrombocytopenia. The pathognomonic physical finding is bilateral absence of the radii, with thumbs present (Fig. 8-32). The presence of thumbs differentiates TAR from Fanconi's anemia (and from trisomy 18), in which thumbs are absent if radii are absent. Hemorrhagic manifestations in TAR patients are often also present at birth, with petechiae or bloody diarrhea, or both, apparent in 60% of



Figure 8-32. Newborn infant with thrombocytopenia-absent radius syndrome. Note that thumbs are present. (Courtesy of Dr. Jeffrey Lipton. From Alter BP. The bone marrow failure syndromes. In: Nathan DG, Oski FA, eds. *Hematology of infancy and childhood*, 3rd ed. Philadelphia: WB Saunders, 1987:159–241, with permission.)

patients within the first week of life and in more than 95% of patients by 4 months of age.

Table 8-22 compares several features of TAR with Fanconi's anemia. The inheritance pattern in TAR is autosomal recessive, with several families reported with affected siblings and consanguinity reported in three families (897–899). The male to female ratio is 0.8:1.0. Two sets of identical twins had TAR (900,901), whereas only one of a pair of fraternal twins was affected (902). This finding is consistent with the cause being genetic rather than acquired. In most cases, parents of TAR patients are normal, and normal offspring of affected mothers have been reported (894,903). A few families involved more than one generation (aunts or uncles and nephews or nieces, with one example of parent-child transmission) or cousins (894,903–909), and one family had two affected half-siblings (910), suggesting that dominant (or perhaps pseudodominant) inheritance might be relevant in a few families. Although all ethnic groups are affected, including blacks, reports of Asians are rare; however, this may reflect a reporting, rather than a genetic, bias (911).

All TAR patients have absent radii, with thumbs present (Fig. 8-32). Most are affected bilaterally, with only five apparently bona fide cases in which the absent radius was unilateral, and the hematologic picture was typical (912–916). Short stature is common in TAR and Fanconi's anemia patients, but those with Fanconi's anemia are often smaller. The other hand abnormalities in TAR are shortening of the middle phalanx of the fifth finger, clinodactyly, occasional finger syndactyly, and, sometimes, hypoplasia of the thumbs. Additional abnormalities of the forearms may include absent ulnae or ulnar shortening or bowing in 40% of patients. Approximately one-third of patients have abnormal upper arms, with either short or absent humeri; the ulnar or humeral lesions are usually bilateral. Scapular hypoplasia and web necks further account for abnormal upper body appearances, along with micrognathia and occasional brachycephaly or microcephaly. Hypertelorism,

TABLE 8-22. Comparison of Thrombocytopenia–Absent Radius Syndrome and Fanconi's Anemia

Feature	Thrombocytopenia–Absent Radius Syndrome	Fanconi's Anemia
Number of reported cases	225	1200
Median age at diagnosis (yr)	0	8
Male to female ratio	0.8:1.0	1.2:1.0
Inheritance	Recessive	Recessive
Low birth weight (%)	9	11
Stature	Short	Short
Skeletal deformities		
Absent radii, thumbs present (%)	100	0
Hand anomalies (%)	40	43
Lower limbs (%)	37	8
Cardiac anomalies (%)	8	6
Skin		
Hemangiomas (%)	8	0
Pigmentation (%)	0	55
Blood	Thrombocytopenia	Pancytopenia
Marrow	Absent megakaryocytes	Aplastic
Marrow colonies decreased	Colony-forming unit megakaryocyte	Colony-forming unit granulocyte-macrophage, colony-forming unit erythroid
Fetal hemoglobin	Normal	Increased
Chromosome breaks	Absent	Present
Malignancies (%)	1	16
Reported deaths (%)	20	38
Projected median survival (yr)	—	20
Survival plateau	Approximately 75% at 4 yr	None

epicanthal folds, strabismus, and low-set ears are also seen, as are facial hemangiomas (in 10%).

The lower limbs are abnormal in 40% of patients. Abnormalities include deformed, subluxed, or hypoplastic knees (917); dislocated hips or patellae; and varus or valgus rotation at hips, knees, or feet. Short legs and absent tibiae or fibulae have been observed. Congenital heart disease (in 10% of patients) includes atrial or ventricular septal defects, tetralogy of Fallot, dextrocardia, and ectopia cordis. A few patients had gonadal anomalies, such as undescended testes, hypoplasia, unicornuate uterus, and vaginal atresia. Low birth weight was observed at term in 15% of the babies.

A major distinction of TAR from Fanconi's anemia is that, in Fanconi's anemia, thumbs are absent when radii are normal. In addition, TAR involves only thrombocytopenia, whereas Fanconi's anemia eventually develops pancytopenia. There are four reports of trisomy 18 with absent or hypoplastic radii or thrombocytopenia, or both (918–921), but they are distinguished by other characteristic anomalies, as well as the cytogenetic abnormality. Roberts' syndrome and SC phocomelia may have a similar phenotype (922,923). Other syndromes with radial anomalies are beyond the scope of this analysis.

Almost 20% of patients were reported to have bloody diarrhea in infancy, which was ascribed specifically to an allergy to cow's milk (894,914). Removal of milk from the diet alleviates that symptom and may perhaps lead to improvement of the thrombocytopenia.

LABORATORY FINDINGS

Platelet counts are less than 50,000/ μ L at the time of diagnosis in more than 75% of patients. Anemia is probably secondary to bleeding, and reticulocytosis is usually associated. Leukocytosis is a common finding, with white blood counts greater than 15,000/ μ L in more than 75% of those reported, greater than 20,000/ μ L in two-thirds of patients, and greater than 40,000/ μ L in one-third of infants. Levels of greater than 100,000/ μ L have been reported. More than 12 patients had immature myeloid precursors in the circulation, but none had true leukoerythroblastosis. This leukemoid reaction has been mistaken for congenital leukemia, but it is, in fact, transient and usually subsides during infancy. Splenomegaly

may occur due to extramedullary hematopoiesis, and eosinophilia is not uncommon. Bone marrow examinations show normal cellularity and normal or increased myeloid and erythroid cell lines, with absence of megakaryocytes in most patients and decreased, hypoplastic, or immature megakaryocytes in the rest.

Laboratory tests with normal results include MCV, HbF, and studies of chromosome breakage, spontaneous and with clastogenic stress, which distinguish TAR from Fanconi's anemia. Karyotypic analysis also distinguishes TAR from trisomy 18. Hypogammaglobulinemia was reported in one group of patients from Nigeria but has not been a general problem (912). Platelet size is normal, except in one report (924), and platelet function is generally normal (925–927), although abnormalities of platelet aggregation and storage pool defects were reported (924,928,929). Clinical symptoms are likely due to quantitative rather than qualitative defects.

PATHOPHYSIOLOGY

The inheritance pattern is most likely autosomal recessive, for a single or for more than one genetic defect, with a recurrence risk of one in four. TAR is one of several inherited hematologic conditions that are associated with radial ray anomalies (the others are Fanconi's anemia and DBA). In the case of TAR, only the platelet lineage is significantly affected. Cultures of hematopoietic progenitor cells indicate that the myeloid and erythroid lineages are normal (129,930–933). Although some studies found no growth of megakaryocytic progenitors (931–934), others found essentially normal numbers (935). A unique megakaryocyte colony-stimulating factor was increased in the plasma of one patient (934); this was probably TPO, which is elevated (936). Unlike amegakaryocytic thrombocytopenia (discussed previously in the section Amegakaryocytic Thrombocytopenia), the *c-mpl* gene is normal in TAR (937,938). However, because TAR is a true single cytopenia, without evolution to aplastic anemia or leukemia, the hematopoietic defect presumably involves only the megakaryocytic lineage.

THERAPY AND OUTCOME

Most infants with TAR have hemorrhagic manifestations, which they may outgrow after the first year of life. More than 40

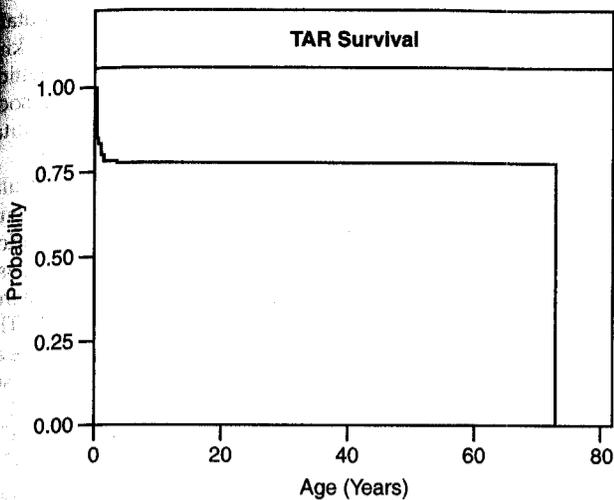


Figure 8-33. Kaplan-Meier plot of cumulative survival in thrombocytopenia-absent radius (TAR) syndrome. The plateau is approximately 75%, with the only death reported in a patient older than 4 years of age occurring in a 73-year-old patient with three different types of cancer.

deaths were reported (20%), 80% of which occurred in the first year of life. The projected survival curve is shown in Figure 8-33 and has a plateau of 75% survival by 4 years of age. Most deaths were from intracranial or gastrointestinal bleeding. The data cited here encompass 50 years of reports of TAR cases, many of whom received limited treatment. Patients who survive the perilous first year of life demonstrate an increase in platelet count to greater than 100,000/ μ L, which is adequate for the orthopedic surgical procedures that are needed for their arms and legs. Thrombocytopenia may recur during illnesses, but it usually is not severe. Dietary change may be helpful for those with milk allergy.

The most important therapy is platelet transfusions. These are provided during bleeding episodes or operations and are clearly indicated as prophylaxis for infants with severe symptomatic thrombocytopenia. Single donors should be used to reduce the risk of sensitization, and HLA-matched platelets can be obtained, if necessary. The platelet count should be maintained at more than 10,000 to 15,000/ μ L. The prediction is that the duration of this support is finite and short (less than 1 year). Other approaches have included splenectomy, corticosteroids, and androgens, all without apparent benefit. One adult showed a transient elevation of the platelet count after splenectomy (925). A small dose of prednisone might decrease the bleeding tendency at a given platelet count, and ϵ -aminocaproic acid may also be useful during bleeding episodes (see the section Treatment, in the section on Fanconi's anemia for details).

The leukemoid reaction and eosinophilia disappear during the first year of life in patients with TAR, although the high white-cell count has led to some infants being diagnosed with *congenital leukemia*. One patient was reported with acute lymphoblastic leukemia (939), one with stage D(S) neuroblastoma at birth who died at 3 months of age (940), and one who developed ileal adenocarcinoma at 67 years of age, ovarian cancer at 70 years of age, and bladder squamous cell carcinoma at 73 years of age (941).

Heroic treatments such as myelotoxic drugs or BMT are generally contraindicated, because spontaneous improvement occurs. One patient who had a life-threatening central nervous system hemorrhage was treated successfully by BMT (942). In general, this approach is excessive, and the prognosis for TAR patients is good.

Prenatal diagnosis was reported in more than two dozen cases. Absent radii may be diagnosed using radiography (943,944), ultrasonography (945), or fetoscopy (946,947). Because a few patients were reported with unilateral radial aplasia, both forearms must be examined. In one case, micrognathia was detected by ultrasonography (897). The diagnosis can be confirmed by a platelet count in fetal blood that is obtained by fetoscopy or cordocentesis (566,947,948). Seventy percent of the 28 fetuses studied so far were affected.

LEUKOERYTHROBLASTOSIS

Leukoerythroblastosis is the term that was suggested by Vaughan (949) in 1936 to describe "an anemia characterized by the presence in the peripheral blood of immature red cells and a few immature white cells of the myeloid series," that is, erythroblasts and leukoblasts. The RBCs are usually normochromic and normocytic, with poikilocytes, fragments, target cells, and teardrops. Giant platelets may also be seen. Leukoerythroblastosis must be distinguished from a *leukemoid reaction*, which is a reactive leukocytosis with an orderly progression of immature through mature myeloid cells. Another related term, *leukemic hiatus*, applies when the myeloid cells are immature and mature but without intermediate forms; thus, there is a gap or hiatus.

The disorders in which leukoerythroblastosis has been seen are outlined in Table 8-23, which is a composite of several large adult and pediatric series (950,951). The initial descriptions focused on the association with bone marrow invasion, particularly from metastatic solid tumors, hematologic malignancies, infections, or other marrow components, in which cells might be crowded out of the marrow prematurely (myelophthysis). Hypoxia from nonhematologic or hematologic causes might

TABLE 8-23. Conditions Associated with Leukoerythroblastosis

Marrow invasion
Tumor
Solid tumor with bone marrow metastases
Lymphoma
Hodgkin's disease
Multiple myeloma
Leukemia
Neuroblastoma
Preleukemia
Infection
Osteomyelitis
Sepsis
Tuberculosis
Congenital
Marrow components
Osteopetrosis
Storage disease
Histiocytosis
Vasculitis, including rheumatoid arthritis
Myeloproliferative disorders
Polycythemia vera
Myelofibrosis, myeloid metaplasia
Down's syndrome, transient myeloproliferative disease
Chronic myelogenous leukemia
Erythroleukemia
Thrombocythemia
Hematologic disease
Erythroblastosis fetalis
Pernicious anemia
Thalassemia major
Other hemolytic anemias
Hypoxia
Cyanotic congenital heart disease
Congestive heart failure
Respiratory disease

also stimulate premature release of marrow cells. In myeloproliferative disorders, the premature release of nucleated cells might be related to the intrinsic abnormality of the cells. Diseases in which leukoerythroblastosis occurs are discussed in many other chapters in this book; this section is restricted to osteopetrosis.

Osteopetrosis

Osteopetrosis is a syndrome with three major forms: (a) infantile malignant autosomal recessive, (b) intermediate autosomal recessive, and (c) autosomal-dominant "marble bone disease," or Albers-Schönberg disease, which was first described in 1904 (952-954). The most severe form is diagnosed in infancy and early childhood, and is characterized by dense bones that fracture easily because of a defect in bone resorption. The patients have large heads, sclerotic bones, and hepatosplenomegaly, and they experience blindness, deafness, cranial nerve palsies, and pancytopenia. Many cases are familial, with a high degree of consanguinity. The disease may be severe *in utero*, because there is often a history of stillbirths and spontaneous abortions.

LABORATORY FINDINGS

The hematologic complications of osteopetrosis are severe, with components of leukoerythroblastosis including macrocytic anemia, reticulocytosis, teardrop RBCs, circulating erythroblasts, and leukocytosis with immature myeloid elements. One manifestation of the stress erythropoiesis that occurs is an increase in HbF (955). The marrow cavity is gradually narrowed by bone, and the diploic spaces are small. Bone marrow aspiration is difficult, and needles often break in attempts to penetrate the sclerotic bone. The marrow that remains is hypocellular and fibrotic. Osteoblasts, as well as osteoclasts, are increased (956). Hepatosplenomegaly develops because of extramedullary hematopoiesis. Hypersplenism follows and leads to thrombocytopenia, leukopenia, and hemolytic anemia due to extracorporeal destruction of intrinsically normal erythrocytes (957).

PATHOPHYSIOLOGY

The osteoclasts are abnormal in osteopetrosis, as they are unable to resorb bone and produce the remodeling that occurs in normal bone. In experiments using osteopetrotic mice, Walker (958) showed that bone marrow or spleen cells from normal mice led to bone remodeling in osteopetrotic litter mates, and spleen cells from affected mice led to osteopetrosis in normal mice (959). Marrow transplantations that cured osteopetrosis and contained a cytoplasmic marker (giant lysosomes in Chédiak-Higashi mice) replaced recipient with donor osteoclasts (959). Similar studies using donors with defective erythropoiesis (W^e/W^v) indicated that the stem cell that gives rise to osteoclasts may be more primordial than the colony-forming unit spleen (960). Although osteopetrosis was cured and donor leukocytes and platelets were sustained, the defective donor erythrocytosis was replaced by the normal erythropoiesis of the recipient. Several laboratories have used *in vitro* studies to suggest that osteoclasts are derived from hematopoietic stem cells that are in the mononuclear light density fraction (961).

Hematopoiesis is intrinsically normal in osteopetrosis. The peripheral blood contains increased numbers of CFU-GM, BFU-E, and even CFU-E (normally found only in marrow), which may migrate from the crowded bone marrow cavity to sites of extramedullary hematopoiesis (962,963). Osteoclasts are numerically normal, morphologically normal or abnormal, and functionally abnormal.

Several genes have been found recently to be responsible for osteopetrosis. The autosomal-dominant form maps to chromo-

some 1p21, although the candidate gene CSF-1, which is mutant in the *op/op* mouse, was excluded in an extended Danish kindred (964). The milder autosomal-recessive disorder, in which there is renal tubular acidosis, was shown in 1985 to be associated with a deficiency of carbonic anhydrase II (965), and mutations in carbonic anhydrase II were identified in 1992 (966).

The severe autosomal-recessive osteopetrosis is due to mutations in more than one gene. One gene maps to 11q13, syntenic with mouse chromosome 19, the site of the murine *oc* mutation (967). The murine gene is *Tcirg-1*, which codes for the osteoclast-specific subunit of the vacuolar proton pump. Five of nine patients were found to have mutations in this gene, *TCIRG1* (968). The gene is also called *OC116*, which is described as encoding the $\alpha 3$ subunit of the vacuolar adenosine triphosphatase from osteoclasts, and was mutant in five of ten patients in another study (969). Another gene that is involved in the same pathway, the *CLC-7* chloride channel, was mutated in 1 of 12 patients and in mice (970). The disruption of the pathway of acidification of extracellular lysosome between osteoclasts and bone leads to a defect in bone degradation and severe osteopetrosis. Thus there are at least two genes that are responsible for the severe form of osteopetrosis.

THERAPY AND OUTCOME

Death usually occurs in infancy or early childhood in osteopetrosis patients; no patients have survived beyond 20 years of age. Most deaths are from the complications of bone marrow failure, infection, and hemorrhage. Circulating phagocytes, which may be derived from the same lineage as the osteoclasts, may function abnormally, leading to reduced host resistance to infection (971,972).

Symptomatic anemia and thrombocytopenia can be treated with transfusions of RBCs and platelets, although hypersplenism decreases the efficacy of such treatment. Splenectomy offers temporary improvement, but the rest of the reticuloendothelial system remains active and the primary bone disorder is not cured. Prednisone therapy was used in several patients, again with transient improvement, from decreased hypersplenism and reticuloendothelial suppression (971,973). Long-term therapy with interferon- γ was found to increase bone resorption and improve hematopoiesis, but did not provide a cure (974).

The only possible cure for osteopetrosis at this time is offered by BMT (975). More than 100 transplants have been done, with a 3-year probability of survival of 50% (976). In those situations in which a sex marker was found, the osteoclasts were of donor origin, whereas the osteoblasts remained host. Short-term follow-up indicated restoration of normal hematopoiesis, improvement of radiographic findings, and stabilization of physical changes. Long-term follow-up of nine Bedouin cases in a single center showed survival of four patients, with hematologic improvement but persistence of visual impairment (977), and, in another patient, progression of neurodegeneration after transplant with a 5/6 unrelated cord (978). Stem cell transplant must be done early, before the bony changes have encroached on hearing and vision.

Prenatal diagnosis by radiography was first performed in 1943 (979), although it was not always successful (980). More recently, increased bone density, fractures, macrocephaly, and hydrocephaly were detected by ultrasonography and confirmed by radiography (981,982). Linkage analysis was used in Bedouin families in which osteopetrosis was linked to 11q13; 3 of 12 patients were affected (983).

SUMMARY

The major inherited bone marrow failure disorders are summarized in Table 8-24. The diagnosis of acquired aplastic anemia

TABLE 8-24. Comparison of Inherited Bone Marrow Failure Syndromes

Feature	Fanconi's Anemia	Dyskeratosis Congenita	Shwachman-Diamond Syndrome	Amegakaryocytic Thrombocytopenia	Diamond-Blackfan Anemia	Severe Congenital Neutropenia	Thrombocytopenia—Absent Radius Syndrome
Number of cases	1200	275	340	75	700	300	225
Male to female ratio	1.2	4.5	1.6	1.1	1.1	1	0.8
Genetics	Autosomal recessive	X-linked, autosomal recessive and dominant	Autosomal recessive	Autosomal recessive	Autosomal recessive, dominant, or sporadic	Autosomal dominant and recessive	Autosomal recessive
Physical abnormalities (%)	~75	100	40	30	25	0	100
Hand and arm anomalies (%)	40	1	1	0	8	0	100
Median age at diagnosis of initial hematologic disease	8 yr	16 yr	<1 yr	~1 mo	2 mo	~1 mo	<1 wk
First hematologic manifestation	Pancytopenia	Pancytopenia	Neutropenia	Thrombocytopenia	Anemia	Neutropenia	Thrombocytopenia
Bone marrow	Aplastic	Aplastic	Hypocellular or myeloid arrest	Absent or small megakaryocytes	Erythroid hypoplasia	Promyelocyte arrest	Megakaryocytes absent or immature
Aplastic anemia (%)	>90	40	40	40	0	0	0
Leukemia (%)	9	0	7	3	1	9	0.4
Myelodysplastic syndrome (%)	6	1	9	0	0.3	5	0
Liver tumor (%)	3	0	0	0	0.3	0	0
Solid tumor (%)	5	14	0	0	2	0	0
Fetal hemoglobin	Increased	Increased	Increased	Increased	Increased	Normal	Normal
Chromosomes	Breaks increased with clastogens	Normal	Normal	Normal	Normal	Normal	Normal
Spontaneous remissions	Very rare	None	Very rare	None	15% to 25%	None	75%
Treatment; responses	Androgens; 50%, transient	Androgens; 50%, transient	Steroids or androgens; 50%, transient	None	Steroids; 60%, some transient	Granulocyte colony-stimulating factor; excellent	Platelets, as needed for <1 yr
Prognosis	Poor	Poor	Fair	Poor	Good	Good	Good
Prenatal diagnosis	Chromosomes, mutations	Mutations	Neutropenia	Thrombocytopenia, mutations	Anemia, adenosine deaminase, erythroid burst-forming unit, mutations	Neutropenia, mutations	Absent radii, thrombocytopenia
Projected median survival age	20 yr	33 yr	35 yr	7 yr	43 yr	23 yr	Older than 50 yr

should be made only after serious consideration of these inherited conditions. Physical anomalies may be absent, but family histories or specific tests (chromosome breakage or mutation analyses) may provide clues. The number of patients with each condition and the proportions with the cited complications cannot be construed as prevalence figures, because they are based on literature reports, not epidemiologic studies. Despite underreporting and underdiagnosis, the numbers do provide some perspective on these entities.

Most of the conditions that are discussed in this chapter and summarized in Table 8-24 are expressed in probable homozygotes (for autosomal recessives) or hemizygotes (X-linked), although a few are dominants. Because heterozygotes for recessive disorders cannot usually be identified (except as parents), patients with multiple bone marrow failure genes or those with apparently acquired diseases that may in fact be inherited cannot be defined at this time.

Treatment depends on diagnosis. Patients with pancytopenia due to Fanconi's anemia, dyskeratosis congenita, amegakaryocytic thrombocytopenia, or Shwachman-Diamond syndrome may respond to androgens. Those with Diamond-Blackfan anemia should receive corticosteroids, patients with SCN should receive G-CSF, and patients with TAR should receive platelets. BMT, particularly for those with Fanconi's anemia, requires modification of current preparative protocols; patients with DBA and TAR, which, respectively, may and do improve spontaneously, may not need transplantation at all.

In all cases in which transplantation is used, the donor must prove to be unaffected by the disease. Immunotherapy and growth factor treatment must be tailored for specific diseases. Risks of evolution of leukemia or other malignancies must be considered for all treatments, and those therapies with higher risk must be considered carefully in patients whose underlying condition is premalignant. Treatment of malignant complications is also difficult in inherited disorders in which abnormalities extend beyond hematopoietic tissues.

Prenatal diagnosis is possible for many of the inherited marrow failure disorders. Families at risk are usually identified through a proband, after which subsequent pregnancies may be monitored. Early diagnosis of an affected fetus may eventually permit treatment *in utero* or at birth. Diagnosis of a fetus that is not affected and is HLA-matched to the proband may provide placental blood for treatment by stem cell transplantation.

Much remains to be understood about the genetics, pathophysiology, and treatment of inherited and acquired aplastic anemia. This requires correct diagnoses, proper treatment, and careful follow-up. The prognoses for most of these disorders have improved with recent therapeutic advances, and it is anticipated that this improvement will accelerate with more knowledge of the specific molecular and cellular defects.

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