22.5 Bone marrow failure 5325 22.5.1 Inherited bon

22.5 Bone marrow failure 5325 22.5.1 Inherited bone marrow failure syndromes 5325 Irene Roberts and Inderjeet S. Dokal

CONTENTS 22.5.1 Inherited bone marrow failure syndromes 5325 Irene Roberts and Inderjeet S. Dokal 22.5.2 Acquired aplastic anaemia and pure red cell aplasia 5336 Judith C.W. Marsh, Shreyans Gandhi, and Ghulam J. Mufti 22.5.3 Paroxysmal nocturnal haemoglobinuria 5348 Lucio Luzzatto 22.5.1 Inherited bone marrow failure syndromes Irene Roberts and Inderjeet S. Dokal ESSENTIALS Inherited forms of bone marrow failure may involve all haematopoi- etic lineages or a single lineage. They are rare, but collectively account for 20 to 30% of patients presenting with aplastic anaemia. They may present at birth or in infancy or childhood, but also sometimes in adults. Associated somatic abnormalities may be helpful in diagnosis. Two of the best characterized syndromes are Fanconi anaemia and dyskeratosis congenita, both frequently associated with generalized bone marrow failure. Other well-recognized disorders lead to much more specific abnormalities affecting a single cell type, e.g. impaired red cell production in Diamond–Blackfan anaemia and impaired neu- trophil production in Shwachman–Diamond syndrome, and reduced platelet production in thrombocytopenia with absent radii syndrome. Advances in understanding the genetics of inherited bone marrow failure syndromes have provided valuable insight into their patho- physiology, and also into normal haematopoiesis. Introduction Bone marrow failure (BMF) is the commonly used term to describe impaired production of normal peripheral blood cells as a result of reduced numbers of haematopoietic stem and progenitor cells in the bone marrow (BM).

Inherited forms of BMF may involve all haem- atopoietic lineages or a single lineage. In some cases, patients may present with a single cytopenia (e.g. thrombocytopenia or anaemia) before progressing to pancytopenia later in the course of the disease. Where all haematopoietic lineages are involved, the term ‘aplastic anaemia’ (AA) is sometimes used since BM biopsies from affected patients show striking hypocellularity, although this term is rather misleading since such patients also have thrombocytopenia and neutropenia. The inherited BMF syndromes are rare. Although their precise incidence and prevalence is not known, collectively they rep- resent approximately 20 to 30% of patients presenting with AA and constitute a significant clinical burden, as many are associated with premature mortality. The main types of inherited BMF are listed in Box 22.5.1.1. Many have associated somatic abnormalities which in some cases are very helpful in making a presumptive diagnosis at an early stage (Table 22.5.1.1). Inherited BMF syndromes may present at birth or at a variable time thereafter, including in adulthood in some cases. The genetic basis for an increasing proportion of cases of inherited 22.5 Bone marrow failure Box 22.5.1.1 Inherited BMF syndromes Pancytopenia (usually associated with a global haematopoietic defect) • Fanconi anaemia • Dyskeratosis congenita • Shwachman–Diamond syndrome • Reticular dysgenesis • Pearson syndrome • Familial aplastic anaemia (autosomal and X-linked forms) • Myelodysplasia (MDS) • Nonhaematological syndromes (Down syndrome, Dubowitz’s syndrome) Single cytopenia (usually) • Anaemia:

— Diamond–Blackfan anaemia

— Congenital dyserythropoietic anaemia • Neutropenia:

— Severe congenital neutropenia

— Cyclic neutropenia • Thrombocytopenia:

— Thrombocytopenia with absent radii (TAR) syndrome

— Congenital amegakaryocytic thrombocytopenia (CAMT)

section 22 Haematological disorders 5326 BMF is now known (Table 22.5.1.1). Advances in understanding the genetics have provided valuable insight not only into the patho- physiology of these syndromes, but also into normal haematopoi- esis. Two of the best characterized syndromes are Fanconi’s anaemia (FA) and dyskeratosis congenita (DC). These two syndromes are frequently associated with generalized BMF/AA and are discussed in some detail in the following sections. By contrast, there are sev- eral well-recognized disorders where the majority of patients have much more specific abnormalities affecting a single cell type, such as impaired red cell production in Diamond–Blackfan anaemia (DBA) and congenital dyserythropoietic anaemia (CDA); impaired neutrophil production in Shwachman–Diamond syndrome (SDS); and reduced platelet production in thrombocytopenia with absent radii (TAR) syndrome and congenital amegakaryocytic thrombo- cytopenia (CAMT). Each of these conditions is reviewed in this chapter. Fanconi anaemia Aetiology The clinical syndrome of FA (Fig. 22.5.1.1) was first described by Guido Fanconi in 1927. The vast majority of cases are inherited in an autosomal recessive manner although occasional families dem- onstrate an X-linked mode of inheritance. FA is characterized by progressive BMF and an increased predisposition to malignancy, es- pecially acute myeloid leukaemia (AML). Pathogenesis

and pathology The hallmark of the cellular abnormality in FA is genomic in- stability. This is manifest as a high frequency of spontaneous chromosomal breakage and hypersensitivity to DNA cross-linking agents such as diepoxybutane and mitomycin C (MMC) (Fig. 22.5.1.2). Indeed, the most useful screening test for FA remains the demonstration of increased chromosomal breakage in FA cells compared with normal controls after exposure to diepoxybutane/ mitomycin C. This 'FA cell phenotype' has facilitated the identifica- tion of the complex genetics of this disease. There are currently 18 subtypes (or complementation groups) each caused by a mutation in one of the following genes: FANCA, FANCB, FANCC, FANCD1, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCJ, FANCL, FANCM, FANCN, FANCO, FANCP, FANCQ, FANCS, and FANCT. In vitro gene transfer studies confirm the important role played by these genes: introduction of the relevant wild-type FA gene into FA human lymphoid and haematopoietic cells normalizes their re- sponse to mitomycin C and chromosomal breakage and restores their growth to normal. The proteins encoded by the FA genes participate in a complex network essential for normal DNA repair. Nine of the proteins (FANCA, FANCB, FANCC, FANCE, FANCF, FANCG, FANCL, FANCM, and FANCT) form the FA core complex which is necessary for the activation of a second complex, the FANCI–FANCD2 pro- tein complex, to a monoubiquitinated form (FANCI–FANCD2-Ub) (Fig. 22.5.1.3). This FANCI–FANCD2-Ub complex then interacts with DNA repair proteins (including BRCA2, BRCA1, and RAD51) to bring about the repair of the DNA damage. These observations have linked the FA proteins with BRCA1 and BRCA2 (FANCD1) in a DNA damage response pathway 'The FA/BRCA pathway'. Indeed, FA-D1 patients have biallelic mutations in BRCA2. In cells lacking BRCA2, repair of DNA damage by homologous recombination is inaccurate and such cells are hypersensitive to DNA cross-linking agents. More recently, it has been shown that two other FA genes (FANCJ and FANCN) encode BRCA1 and BRCA2 partners, BRIP1 and PALB2, respectively. Mutations in these two FA genes have a specific link to cancer predisposition. Biallelic BRCA2 (FA-D1) and PALB2 (FA-N) mutations, in contrast to mutations in the other FA genes, are associated with high risks of solid childhood malignancies (e.g. Wilms' tumour and medulloblastoma) while heterozygous mu- tations specifically in BRCA1 (FA-S), (FA-D1), PALB2 (FA-N), and BRIP1 (FA-J) confer an increased risk of breast cancer. These differ- ences highlight the complex relationship between the FA proteins and their interactions with other molecules both at the molecular and clinical level. It is now clear that the clinical and biological defects in FA overlap with those of several other chromosomal breakage syndromes, including Seckel's syndrome and the Nijmegen breakage syndrome (NBS). Consistent with this, considerable evidence indicates that the causative genes are involved in some of the same pathways. For example, activation of the FA–BRCA pathway in response to DNA damage (e.g. replication fork arrest) involves ATR (ataxia telangi- ectasia), the gene for which is mutated in a subset of patients with Seckel's syndrome (Fig. 22.5.1.3). ATR appears to directly regulate the Table 22.5.1.1 Characteristics of the BMF syndromes FA DC SDS DBA CDA TAR SCN IAA Other# Inheritance pattern AR, XLR XLR, AR, AD AR AD, XLR AR, AD AR AD, AR ? AD and AR Somatic abnormalities Yes Yes Yes Yes Rare Yes Rare ?None Yes Bone marrow failure AA (>90%) AA (c.80%) AA (20%) RCA Eryth Megs Neutropenia Yes (100%) Yes Short telomeres Yes Yes Yes ? ? ? ? Yes No Malignancy Yes Yes Yes Yes ?No ?No Yes Yes ? Chromosome instability Yes Yes Yes ? ? ? ? Yes ? Genes identified 18 12 1 13 4 1 5+ a 3 AA, aplastic anaemia; AD, autosomal dominant; AR, autosomal recessive; CDA, congenital dyserythropoietic anaemia; DBA, Diamond–Blackfan anaemia; DC, dyskeratosis congenita; FA, Fanconi's anaemia; IAA, idiopathic aplastic anaemia; Eryth, ineffective erythropoiesis; Megs, low megakaryocytes; RCA, red cell aplasia; SCN, severe congenital neutropenia; SDS, Shwachman–Diamond syndrome; TAR, thrombocytopenia with absent radii; XLR, X-linked recessive. Other#, patients in whom genetic defects have been identified (e

g. in SRP72, ERCC6L2) but who do not fit into previously recognized categories. a Heterozygous mutations in TERC and TERT are risk factors for some cases of AA.

22.5.1 Inherited bone marrow failure syndromes 5327 FA pathway as it is required for the monoubiquitination of FANCD2 and FANCI. It is interesting to note that ATR-Seckel cells also exhibit defects in FANCD2 monoubiquitination and that NBS cells with (mutations in NBN) show defects in FANCD2 monoubiquitination. Although there has been considerable progress in elucidating the molecular pathogenesis of FA in recent decades, our understanding remains incomplete. Studies in animal models and human samples show that FA cells display other abnormalities as well as DNA repair defects, including hypersensitivity to oxygen, accelerated telomere shortening, abnormal cell cycle kinetics, upregulation of p53, and overactivation of the mitogen-activated protein kinase (MAPK) pathways leading to overproduction of tumour necrosis factor- α (TNFα). Mouse models of FA have shown that haematopoietic progenitors are hypersensitive to TNFα and interferon-γ (IFNγ). There is some evidence that this effect is mediated by fas-induced apoptosis and this, perhaps together with aldehyde-induced genotoxicity affecting haematopoietic stem cells, may explain the development of progressive BMF in FA. There is also evidence that some of the features of the pathophysiology of BMF are common to both FA and idiopathic AA. Patients with idiopathic AA often have increased IFNγ levels and short telomeres are a feature in both conditions and in DC. (a1) (b1) (c1) (a2) (b2) (c2) (a3) (b3) (c3) Fig. 22.5.1.1 Fanconi anaemia (FA). (a) Photographs of patients with FA (a1–a3) with small mouth and chin (‘Fanconi facies’). (b) Abnormalities of pigmentation (hyper- and hypopigmentation) on the abdomen (b1) with a close up (b2) of a café-au-lait spot and a hypopigmented patch. The bottom photograph (b3) shows the back of an FA patient demonstrating lumbar scoliosis. (c) Hands/forearms of FA children showing hypoplastic thumbs (c1), rudimentary (‘dangling’) thumbs (c2), and a radiograph (c3) showing rudimentary thumb (skeletal) development.

section 22 Haematological disorders 5328 ctb ctb (a) (b) mci ctg ctb ctb ring/tri qr tri tri
Fig. 22.5.1.2 (a, b) Chromosomal abnormalities seen in FA lymphocytes following incubation with diepoxybutane. ctb, chromatid break; ctg, chromatid gap; mci, multiple chromatid interchanges (complex rearrangement); tri, triradial; qr, quadriradial. Courtesy of Nicola Foot, Hammersmith Hospital. Constitutional biallelic mutations in Fanconi anaemia genes MAPKs Altered oxidative stress response Other aberrations e.g. defective telomere maintenance Enviromental factors Sunlight Smoking Infections Alcohol FA phenotype Genomic instability Altered cell checkpoints and survival DNA repair foci FA core Complex ABCE FGLM I I UB BRCA2 (D1) BRCA1 ERCC4 (Q) RAD51 (0) Altered DNA damage response ‘FA-BRCA pathway’ ATR DNA damage TNF-α D2 D2 BRIP1 (J) PALB2 (N) SLX4 (P) Fig. 22.5.1.3 Schematic representation of the FA–BRCA pathway and related networks. The diagram shows that the constitutional mutations in FA cells lead to aberration of the FA–BRCA pathway, abnormal handling of oxidative stress, aberrant activation of mitogen-activated protein kinases (MAPKs), defective telomere maintenance, as well as other biological aberrations. The net impact of these is increased genomic instability and altered cell survival/ checkpoints. The diagram also highlights the potential role of environmental factors such as smoking in adding to the effect of the FA mutations. Within the FA–BRCA pathway, the proteins shown in yellow are those mutated in different FA patients. The FA core complex consists of nine FA-proteins (A, B, C, E, F, G, L, M, and T) and this, together with ATR (ataxia telangiectasia and RAD3-related protein), is essential for activation (ubiquitination) of the I–D2 complex after DNA damage. Activated I–D2-Ub translocates to DNA repair foci where it associates with other DNA damage response proteins

including BRCA2, RAD51, and SLX4 and participates in DNA repair. TNF, tumour necrosis factor; Ub, ubiquitination.

22.5.1 Inherited bone marrow failure syndromes 5329 Clinical features As well as progressive BMF, most patients with FA have at least one somatic abnormality (Table 22.5.1.2). The most common clinical signs are skeletal abnormalities, particularly absent thumbs and/or radial hypoplasia; skin lesions, particularly café-au-lait spots; short stature; and microphthalmia (Fig. 22.5.1.1). However the devel- opment of almost all organs and tissues may be affected, including genitourinary abnormalities, such as underdeveloped gonads and horseshoe kidneys; and gastrointestinal, cardiac, and neurological anomalies (Table 22.5.1.2). There is marked variation between pa- tients both in the pattern of somatic abnormalities and in the course of the disease. It is important to note that almost one-third of pa- tients have no physical abnormalities, making the diagnosis of FA on clinical grounds difficult and unreliable. Although the somatic abnormalities of FA will be evident at birth, the blood count in neonates is usually normal. Pancytopenia develops insidiously as BM cellularity falls and presents in most cases between the ages of 5 and 10 years (median age 7 years), al- though late presentation in adolescents or adults sometimes occurs. Typically the first haematological signs of BMF in FA are thrombo- cytopenia and anaemia and the neutrophil count is often maintained in the early stages. As BMF progresses, the main causes of death are fatal haemorrhage or infection due to progressive BMF. As patients survive longer, the increased risk of leukaemia and other malignancies in FA is becoming more evident. By the age of 40 years, the cumulative incidence of haematological malignancy is 33% and of nonhaematological malignancies is 28%. The most frequent haematological malignancy is acute myeloid leukaemia. Amongst the nonhaematological cancers the most frequent are hep- atic tumours and squamous cell carcinoma of the vulva, oesophagus, head, and neck. Anecdotal evidence, supported by recent data from the German FA registry, suggests that malignancies occur mainly in patients with late-onset BMF and longer survival, with a me- dian age of 13 years for leukaemia and 25 years for solid tumours. Long-term follow-up of FA patients treated by haematopoietic stem cell transplantation (HSCT) also shows a higher incidence of nonhaematological malignancies in patients with FA than in pa- tients with other types of BMF who have undergone HSCT. An additional feature of FA is the occurrence, in a small pro- portion of patients of somatic mosaicism. This occurs when the ‘pathogenic’ allele reverts to ‘wild type’ in a single haematopoietic (somatic) cell, that is, the reverted cell effectively becomes a ‘het- erozygous cell’, which would be expected to have a growth/survival advantage in the background of FA cells. Such FA mosaic patients may then experience an improvement in their blood count and a return of the diepoxybutane/mitomycin C test to normal indi- cating that a single haematopoietic stem cell may be sufficient to restore adequate haematopoiesis. This process can be viewed as a natural form of haematopoietic stem cell gene therapy suggesting that therapeutic gene therapy might be a valuable approach for BMF in FA. Treatment The most common indication for treatment in FA is BMF. Although HSCT is the only curative treatment, anabolic steroids, such as oxymetholone and danazol, achieve useful haematological responses in 50 to 70% of patients by delaying the need for red cell or platelet transfusions or HSCT. Unfortunately, anabolic steroids often have serious side effects, such as liver dysfunction, and so patients need to be monitored closely and many patients become refractory. HSCT protocols have to be adapted specifically for FA patients because of their hypersensitivity to irradiation and alkylating agents. Reduced doses of cyclophosphamide (20–40 mg/kg) are typically used as well as protocols that avoid the need for irradiation or use lower doses than usual for HSCT by using immunosuppressive drugs such as fludarabine. Gene therapy protocols are also being investigated in FA as a means of ameliorating

the BMF. Prognosis Despite improvements in supportive care and HSCT and a dramatic increase in our understanding of the pathogenesis of FA, the prog- nosis remains poor. By the age of 40 years, patients with FA have a cumulative incidence of BMF of approximately 90% as well as a cumulative incidence of haematological malignancy of 33% and of nonhaematological malignancies of 28%. Median survival in the largest reported series to date (International Fanconi Anaemia Registry) was 24 years. Major challenges in the future include developing better treatment of malignancies and the management of complications (e.g. pulmonary disease) in adulthood. Dyskeratosis congenita Aetiology The classical DC clinical triad of abnormal skin pigmentation, nail dystrophy, and mucosal leucoplakia (Fig. 22.5.1.4) was first de- scribed by Jacobi in 1906 and Zinsser in 1910. DC is now known to affect almost all tissues but the principal cause of early mortality is BMF. Like FA, patients with DC also have a predisposition to ma- lignancy. DC is now regarded as one of a group of disorders known as 'telomeropathies', characterized by abnormal telomere function. It is a very heterogeneous disorder, both clinically and genetically; Table 22.5.1.2 Somatic abnormalities in FA Abnormality Percentage of patients Skeletal (radial ray, vertebral, scoliosis, rib) 71 Skin pigmentation (café-au-lait, hyper- and hypopigmentation) 64 Short stature 63 Eyes (microphthalmia) 38 Renal and urinary tract 34 Male genital 20 Mental retardation 16 Gastrointestinal (e.g. anorectal, duodenal atresia) 14 Heart 13 Hearing 11 Central nervous system (e.g. hydrocephalus, septum pellucidum) 8 No abnormalities 30 Source data from Auerbach, A., Buchwald, M. & Joenie, H. (2001) In: The metabolic and molecular bases of inherited diseases (eds. C. Scriver, W. Sly, B. Childs, A. Beaudet, D. Valle, K. Kinzler and B. Vogelstein). McGraw Hill, New York.

section 22 Haematological disorders 5330 X-linked recessive, autosomal dominant, and autosomal recessive forms of the disease are recognized. Pathogenesis and pathology The genes responsible for approximately 70% of cases have now been identified. The DKC1 gene, which is responsible for all cases of X- linked DC, was the first to be identified, in 1998. This gene is highly conserved and encodes the protein dyskerin which is a core compo- nent of telomerase. Mutations in DKC1 also underlie some cases of a severe, multisystem form of DC, known as Hoyeraal–Hreidarsson syndrome (HH). Autosomal dominant DC is heterogeneous and to date, heterozy- gous mutations in three genes (TERC, TERT, and TIN2) have been characterized. Like dyskerin, TERC (telomerase RNA component) and TERT (telomerase reverse transcriptase) are key core compo- nents of telomerase, a ribonucleoprotein essential for maintaining telomere length in rapidly dividing cells such as haematopoietic cells (Fig. 22.5.1.5). TERT catalyses the addition of repeats and TERC acts as the template. In cells which lack telomerase, the telo- meres shorten with each successive round of replication until they reach a critical length and the cells enter senescence. Accordingly, patients with DKC1 and TERC gene mutations have very short telomeres compared to age-matched controls. Families with het- erozygous TERC and TERT gene mutations frequently exhibit the phenomenon of genetic anticipation whereby the disease mani- fests at a much younger age and with greater severity in the off- spring of an affected parent despite the same pathogenic telomerase mutation. Autosomal dominant DC can also be caused by mutations in the TIN2 protein, a component of the shelterin complex which has at least three effects on telomeres. Firstly, the complex determines the structure of the telomeric terminus; secondly, it is implicated in the generation of t-loops; and thirdly, it controls the synthesis of telo- meric DNA by telomerase. Heterozygous TIN2 mutations have now been identified in a subset of patients with DC, HH, AA, and Revesz's syndrome (bilateral exudative retinopathy, BMF, nail dystrophy, fine hair, cerebellar hypoplasia, and growth retardation). Patients with TIN2 mutations tend to have very short

telomeres and severe dis- ease. Almost all affected patients have de novo TIN2 mutations in contrast to those with TERC or TERT mutations. Autosomal recessive DC is also a heterogeneous disease. To date, mutations in eight genes have been identified: NOP10, TERT, NHP2, TCAB1, RTEL1, CTC1, PARN, and USB1, all of which re- sult in reduced telomere length apart from USB1. Homozygous NOP10 mutations are associated with reduced telomere length and reduced TERC levels. Biallelic TERT mutations, in contrast to heterozygous TERT mutations, also have greatly reduced telomere length and telomerase activity, as do patients with biallelic mu- tations in NHP2. Both NOP10 and NHP2 are components of H/ ACA ribonucleoprotein complex (H/ACA RNP). This complex is comprised of a RNA molecule and four highly conserved proteins, dyskerin, GAR1, NOP10, and NHP2, involved in ribosome biogen- esis, pre-mRNA splicing, and telomere maintenance. To date, mu- tations have been identified in all components of this H/ACA RNP complex in patients with DC except for GAR1. Compound hetero- zygous mutations in the TCAB1 gene, which encodes a telomerase holoenzyme, cause short telomeres by disrupting telomere localiza- tion to Cajal bodies, resulting in misdirection of telomerase RNA to nucleoli. Biallelic mutations in the RTEL1 (regulator of telomere length 1) gene result in telomeres which are not only very short, but also have qualitative defects and are associated with severe DC presumably because RTEL1 is a helicase with an important role in homologous recombination and telomere maintenance. Biallelic mutations in the CTC1 (conserved telomere maintenance compo- nent 1) gene were initially identified in the pleotropic syndrome Coats plus (characterized by retinopathy, intracranial calcifications and cysts, osteopenia, and gastrointestinal abnormalities) but are now known to also be a rare cause of DC. Finally, patients with USB1 mutations appear to be a different biological subtype of DC; clinic- ally, affected individuals have poikiloderma with neutropenia and (a) (b) (c) (d) (e) (f) Fig. 22.5.1.4 Photographs of patients with DC showing abnormal skin pigmentation (a, b, c), nail dystrophy (d, e), and leucoplakia of the tongue (f).

 Rothmund–Thomson syndrome and, unlike other subtypes of DC, telomere length is normal. Clinical features In addition to the typical mucocutaneous triad of abnormal skin pigmentation, nail dystrophy, and leucoplakia, patients with DC may also have a variety of noncutaneous (dental, gastrointestinal, genitourinary, neurological, ophthalmic, pulmonary, and skeletal) abnormalities (Fig. 22.5.1.4 and Table 22.5.1.3). Changes in skin pigmentation and in the nails generally appear first, usually by the age of 10 years. In most cases, these changes are followed by BMF which typically develops below the age of 20 years and 80 to 90% of patients will have BM abnormalities by the age of 30 years. The minimal clinical criteria for diagnosis of DC are the presence of at least two out of the four major features (abnormal skin pigmentation, nail dystrophy, leucoplakia, and BMF) and two or more of the other somatic features known to occur in DC. The main causes of death in DC are BMF/immunodeficiency (c.60–70%), pulmonary complications (c.10–15%), and malig- nancy (c.10%). It is important to note that the diagnosis of DC on clinical grounds can be very difficult because of the heterogeneity of the condition. BM abnormalities may appear before the mucocutaneous manifest- ations, leading to patients being misdiagnosed as having ‘idiopathic aplastic anaemia’. The clinical presentation in patients with TERT mutations is highly variable ranging from near DC phenotype to just AA. Heterozygous TERC mutations may occur in patients with AA and in patients with myelodysplastic syndrome (MDS) as well as in DC. Furthermore, heterozygous mutations in TERT and TERC have been identified in some patients with idiopathic pulmonary fibrosis, liver disease, and leukaemia. Treatment and prognosis As with FA, anabolic steroids (oxymetholone and danazol) produce a valuable haematological response in around two-thirds of patients by delaying the need for red cell

or platelet transfusions or HSCT for several years. Patients with DC may respond to a dose as low as 0.25 mg oxymetholone/kg per day and this can be increased, if neces- sary to 2 to 5 mg/kg per day, although close monitoring is essential in view of the side effects, particularly liver toxicity. However, the only long-term cure for the BMF is HSCT. Transplant-related mortality is higher in DC than in other BMF syndromes, mainly because of pul- monary and vascular complications, probably due to the underlying telomere defect. HSCT protocols have been adapted to try to re- duce HSCT-related toxicity, for example, by using nonmyeloablative fludarabine-based protocols as for FA, although the long-term bene- fits of this approach are not yet clear. The main causes of death in DC are BMF and HSCT-related toxicity. Shelterin Tankyrase TRF2 RAP1 USB1 snRNA processing TCAB1 Cajal body Telomerase Genes mutated in dyskeratosis congenita and related bone marrow failure syndromes—“the telomereopathies” Helicases RTEL1 POT1 TPP1 TERT TERC Dyskerin NOP10 GAR1 NHP2 1 Capping complex TEN1 CTC1 STN1 TIN2 TRF1 Fig. 22.5.1.5 Schematic representation of complexes involved in telomere maintenance. The telomerase complex includes TERC, TERT, dyskerin, NOP10, NHP2, and GAR1. The shelterin complex includes the six proteins TRF1, TRF2, TPP1, POT1, RAP1, and TIN2. The telomere capping (CST) complex is composed of CTC1, STN1, and TEN1. Protein/RNA names indicated by red arrows are mutated in DC and related disorders: Hemizygous DKC1 (dyskerin) mutations are observed in X-linked DC and HH. Heterozygous TERC mutations are associated with DC, AA, MDS, AML, and pulmonary fibrosis. Heterozygous TERT mutations are responsible for some cases of AA, DC, MDS, AML, and pulmonary/liver fibrosis. Biallelic mutations in TERT can cause classic DC and HH. Heterozygous TIN2 mutations have been observed in DC, AA, HH, and Revesz’s syndrome. Biallelic NOP10, NHP2, TCAB1, USB1, and CTC1 mutations have been seen in autosomal recessive DC. Biallelic RTEL1 and PARN mutations are observed in autosomal recessive HH.

section 22 Haematological disorders 5332 Shwachman–Diamond syndrome Aetiology SDS is an autosomal recessive disorder characterized by exocrine pancreatic insufficiency, BMF and, in some patients, a variety of other somatic abnormalities, particularly involving the skeletal system. SDS, which was first reported independently by Shwachman and colleagues and Bodian and colleagues in 1964, is an example of a group of ribosome biogenesis disorders known as ‘ribosomopathies’. The risk of leukaemia, as in FA and DC, is increased. Pathology and pathogenesis More than 90% of SDS patients have biallelic mutations in the SBDS gene which encodes for a protein which plays an important role in the maturation of the large (60S) ribosomal protein (RP) subunit (Fig. 22.5.1.6). Clinical features Exocrine pancreatic insufficiency and BMF are the hallmarks of SDS, occurring in all patients. Signs of pancreatic insufficiency, typ- ically failure to thrive and malabsorption, are usually apparent early in infancy, although improvement in pancreatic function with age has been reported in a subset of SDS patients. Associated skeletal abnormalities are common in SDS, including short stature (c.70%), metaphyseal dysostosis (75%), rib and thoracic cage abnormalities, hypertelorism, syndactyly, cleft palate, and dental dysplasia. Other abnormalities can include an ichthyotic skin rash (c.60%) or skin Table 22.5.1.3 Somatic abnormalities in dyskeratosis congenita Abnormality Percentage of patients Abnormal skin pigmentation 89 Nail dystrophy 88 BMF 85.5 Leucoplakia 78 Epiphora 30.5 Learning difficulties/developmental delay/mental retardation 25.4 Pulmonary disease 20.3 Short stature 19.5 Extensive dental caries/loss 16.9 Oesophageal stricture 16.9 Premature hair loss/greying/sparse eyelashes 16.1 Hyperhidrosis 15.3 Malignancy 9.8 Intrauterine growth retardation 7.6 Liver disease/peptic ulceration/enteropathy 7.3 Ataxia/cerebellar hypoplasia 6.8 Hypogonadism/undescended testes 5.9 Microcephaly 5.9 Urethral stricture/phimosis 5.1 Osteoporosis/aseptic necrosis/scoliosis 5.1

Deafness 0.8 Ribosomal DNA 45S rRNA 30S 18S Nucleus Cytoplasm 40S subunit 80S ribosome 60S subunit DBA RPS7,RPS10, RPS17, RPS19, RPS24, RPS26, RPS29 DBA RPL5,RPL11, RPL15, RPL26, RPL35a 5q- syndrome RPS14 SDS SBDS 5.8S 28S 5S 32S Fig. 22.5.1.6 Schematic diagram showing scheme of ribosomal (r)RNA processing in human cells and the points at which this is possibly disrupted in the different BMF syndromes. The rRNAs are transcribed by RNA polymerase I as a single precursor transcript (45S rRNA). The 45S rRNA is then processed to 18S, 5.8S, and 28S rRNAs. The 18S is a component of the 40S ribosomal subunit. The 5.8S and 28S together with 5S (synthesized independently) are components of the 60S ribosomal subunit. The 40S and 60S subunits are assembled to form the 80S ribosome. The processing steps affected in DBA (heterozygous mutations in RPS7, RPS10, RPS17, RPS24, RPS26, RPS29, RPL5, RPL11, RPL26, and RPL35a), 5q– syndrome (haploinsufficiency of RPS14) and SDS (biallelic mutations in SBDS) are indicated by the different coloured stars.

22.5.1 Inherited bone marrow failure syndromes 5333 pigmentation, hepatomegaly/protuberant abdomen, and ptosis. BMF usually presents as an isolated neutropenia but approximately 20% of patients have pancytopenia. AML or MDS develops in ap- proximately 25% of patients and appears to be more common in affected males. The age at which leukaemia develops varies widely, from 1 to 43 years. The diagnosis of SDS is made by a combination of clinical features, family history, the clinical combination of exocrine pancreatic insuf- ficiency with BMF, and, in recent years, mutational analysis of the SBDS gene. The main differential diagnosis is Pearson syndrome which also presents with exocrine pancreatic insufficiency and BMF but is distinguished by the presence of a mitochondrial DNA dele- tion and characteristic erythroid abnormalities in the BM. Treatment and prognosis The malabsorption in patients with SDS responds to treatment with oral pancreatic enzymes. Isolated neutropenia is usually man- aged by measures to prevent, or promptly treat, infection together with injections of granulocyte colony-stimulating factor (G-CSF) to boost neutrophil production if required. As for FA and DC, oxymetholone may be useful for delaying the need for red cell and/ or platelet-transfusions or HSCT for patients with severe, symptom- atic pancytopenia. The development of leukaemia usually has a poor prognosis as the response to standard AML chemotherapy is poor. HSCT is the only curative treatment for BMF and often the best op- tion for AML, using conditioning regimens that include fludarabine. It is noteworthy that an increased frequency of nonhaematological malignancies has not been reported in SDS. Diamond–Blackfan anaemia Aetiology DBA is a rare inherited form of red cell aplasia (five cases/million livebirths) which usually presents in early infancy. The vast ma- jority of cases exhibit an autosomal dominant pattern of inherit- ance and at least half of these cases are associated with heterozygous mutations of one of the RP genes. Like SDS, DBA is considered a ribosomopathy and many patients have associated somatic anom- alies, particularly affecting the skeleton. The cause of the remaining autosomal dominant cases remains to be determined. Occasional families with a DBA-like pattern of disease and X-linked inheritance have been found to be due to mutations in the haematopoietic tran- scription factor gene GATA1. Occasional cases of AML, MDS, and progressive BMF have been reported. Pathology and pathogenesis Mutations in at least 12 different RP genes have now been reported in patients with DBA and provide the genetic basis for around two- thirds of cases. The gene mutations affect either the small RP unit (RPS7, RPS10, RPS17, RPS19, RPS24, RPS26, and RPS29) or the large ribosomal subunit (RPL5, RPL11, RPL15, RPL26, and RPL35a) (Fig. 22.5.1.6). This suggests that the primary defect in DBA is defective ribosome biogenesis, which then leads to other bio- logical defects including increased apoptosis, upregulation of p53, and - defective erythroid progenitor development. The exact mech- anism by which RP protein mutations

cause selective impairment of red cell production is unclear. This question is further com- plicated by evidence from some studies that defects in other haematopoietic lineages may be present in some patients with re- fractory DBA. Recently, constitutional hemizygous GATA1 muta- tions have been identified in rare patients with ‘DBA-like’ disease; in these cases the mechanism of disease is likely to be completely different. Clinical features DBA usually presents in early infancy, with features of anaemia such as pallor or failure to thrive. Data from the DBA Registry of North America found a median age at presentation of 8 weeks with 93% of patients presenting in the first year of life. Occasional cases pre- sent before birth (as fetal anaemia) or in adulthood. The hallmark of classical DBA is reduced numbers of BM erythroid precursors with resultant normochromic macrocytic anaemia. Around 50% of pa- tients have associated somatic abnormalities typically affecting the craniofacial bones (high-arched palate, cleft lip, hypertelorism, and flat nasal bridge) and upper limb, especially the thumb. Around 30% of patients with DBA are below the third centile for height. There is also an increased frequency of cardiac and urogenital malforma- tions. The anaemia at presentation is often severe (haemoglobin <50 g/litre). The presence of severe anaemia together with a low reticu- locyte count and normal platelet and leucocyte counts is typical. The diagnosis is made when these features are accompanied by a normocellular BM with a selective loss or severe reduction in im- mature erythroid cells while other cell types are normal. Red cell adenosine deaminase is elevated in most cases and may provide useful supportive evidence for a diagnosis of DBA but is not specific. Where available, mutational analysis of ribosomal protein genes is useful to confirm the diagnosis and establish which family members are affected. The genotype–phenotype relationships between the different mutations and disease manifestations remain to be clarified al- though recent studies suggest that patients with RPL5 mutations tend to have multiple physical abnormalities, including craniofacial, thumb, and heart anomalies, whereas isolated thumb malforma- tions are predominantly seen in patients with heterozygous RPL11 mutations. Further collaborative studies will be needed to validate these findings and identify other associations that are likely to be important both for genetic counselling and providing mechanistic insight into the pathogenesis of DBA. As for the other inherited BMF syndromes, patients with DBA appear to be at increased risk of developing AML and MDS, although relatively few cases have been reported to date. DBA has also been reported to evolve to AA in some patients. Even in patients without AA, BM cellularity is often reduced and such patients are more likely to develop neutropenia and/or thrombocytopenia. Treatment and prognosis Most patients with DBA require treatment in order to maintain a satisfactory haemoglobin concentration compatible with normal growth and development. The mainstay of treatment is oral cortico- steroids to which approximately 80% of patients will respond ini- tially. Steroids should be used in as low a dose as possible, or given on alternate days, due to the toxicity of chronic steroid therapy. Patients with DBA who are steroid refractory, or require very high doses to maintain a satisfactory haemoglobin concentration, are treated

 with regular red cell transfusions, usually at 4- to 5- weekly inter- vals. Infants under the age of 12 months are also treated with regular red cell transfusions to avoid steroid toxicity in this age group. The main complication of chronic red cell transfusion therapy is iron overload and all regularly transfused patients with DBA also require treatment with an iron-chelating agent. The only curative option for DBA is HSCT. Patients and families need to weigh up the relative benefits and risks of HSCT versus life-long transfusion/chelation. Data from the DBA of North America Registry show that the actu- arial survival rates at ages over 40 years were 100% for those in sus- tained remission, 87% for steroid-

maintained patients, and 57% for transfusion-dependent patients. Of the 36 deaths they reported, 25 were treatment-related, including 14 patients who died as a result of HSCT-related complications. Congenital dyserythropoietic anaemias Aetiology The CDAs represent a heterogeneous group of disorders of erythro- poiesis characterized by anaemia and ineffective erythropoiesis. First by Crookston and colleagues in 1966, these disorders are clas- sified into three main types (I, II, and III) of which type II is the commonest. Type I and type II CDA are inherited in an autosomal recessive fashion while type III, which is rare, is autosomal dom- inant. The genes responsible for most cases of type II and some cases of type I and type III CDA have been identified. Additional vari- ants of CDA are described and this classification system is likely to be further refined as the genetic basis of these disorders is un- covered. Mutations in the transcription factor genes GATA1 and KLF1 have recently been identified as the molecular basis for some of these cases. CDA type I The majority of cases of CDA type I are due to mutations in the CDAN1 gene which encodes a ubiquitous protein codanin-1 likely to be involved in intracellular transport. Occasional cases due to mutations in the C15ORF41 gene which encodes a protein of un- known function have also been described. Patients usually present with splenomegaly and mild to moderate macrocytic anaemia (c.70–120 g/litre). Some patients also exhibit nonhaematological features such as skeletal abnormalities and/or abnormal skin pig- mentation. The diagnosis is made after expert review of the blood film and BM, including electron microscopy. The presence of inter- nuclear chromatin bridging and binuclearity of the erythroblasts is characteristic but not specific and the defining feature on elec- tron microscopy is the presence of a spongy (‘Swiss cheese’) ap- pearance of the heterochromatin in the majority of erythroblasts. Where possible, the diagnosis should be confirmed by molecular genetic analysis because the morphological abnormalities may be confused with other inherited red cell disorders, such as pyruvate kinase deficiency. Markers of haemolysis may also be present (ele- vated lactate dehydrogenase and bilirubin). Patients with mild anaemia do not require treatment. For those with more severe an- aemia, the options are regular red cell transfusion with chelation or HSCT. A proportion of patients with CDA type I respond to treat- ment with α-interferon, becoming transfusion independent by an unknown mechanism. CDA type II Originally known as HEMPAS (hereditary erythroblastic multi nuclearity with a positive acidified serum lysis test), CDA type II is now known to be caused in the vast majority of patients by mutations in the SEC23B gene. This gene encodes a secretory pathway protein and more than 60 different mutations have now been reported in affected individuals. Most patients present with anaemia with or without a variable degree of jaundice, hepatomegaly, splenomegaly, and cirrhosis. Typically the anaemia is moderate (haemoglobin 80–110 g/litre) but approximately 10% of cases are transfusion de- pendent and some cases present with anaemia at or before birth. Peripheral blood red cell morphology is usually unremarkable but the BM findings are characteristic with more than 10% binucleate or multinucleate erythroblasts and peripheral cisternae (‘double mem- brane’) beneath the plasma membrane of erythroid cells on electron microscopy. The diagnosis is now made by genetic analysis of cases with typical clinical and BM findings; the acidified lysis test (Ham’s test) is no longer used. Most patients with CDA type II are not trans- fusion dependent. For those that are splenectomy may lead to trans- fusion independence. Patients with CDA type II, whether transfused or not, have an increased risk of iron overload and should be moni- tored from the age of 10 years as iron chelation therapy may be re- quired. HSCT is the only cure and may be valuable in severe cases. CDA type III This is extremely rare. Although usually autosomal dominant, oc- casional sporadic cases are reported. The autosomal dominant form of CDA type III is caused by mutations in the KIF23 gene which en- codes a protein essential for cytokinesis. The molecular basis for the sporadic form of CDA type III is

unknown. The diagnosis is usually made as a result of the characteristic giant multinucleate erythro- blasts seen by in the BM by light microscopy. Treatment is with red cell transfusion if required. Congenital and cyclic neutropenias Aetiology Severe congenital neutropenia (SCN) is clinically and genetically heterogeneous. The majority of cases are autosomal dominant and are due to mutations in the ELANE gene which may also present as cyclic neutropenia. Occasional autosomal dominant cases are due to mutations in the GFI1 gene while the genetic basis of other cases remains to be determined. Around 30 to 40% of cases of SCN, including SDS as described earlier, are autosomal recessive. To date, mutations in the HAX1, G6PC3, VPS45, JAGN1, and CLPB genes have been described in these affected families. Kostmann's syn- drome, the first reported form of SCN, is now known to be caused by mutations in the HAX1 gene. Pathology and pathogenesis The most commonly mutated gene in SCN and cyclic neutropenia is the ELANE gene which encodes neutrophil elastase, a serine protease synthesized mainly at the promyelocyte stage of neutrophil differen- tiation. Mutations in ELANE lead to accumulation of nonfunctional neutrophil elastase within the cell which triggers an unfolded pro- tein response leading to maturational arrest. In cyclic neutropenia, the ELANE mutations are usually clustered around the active site in

 contrast to those found in SCN although the mechanism by which this leads to the different manifestations of SCN and cyclic neutro- penia is unclear. The second most commonly mutated gene in SCN is HAX1. Biallelic HAX1 mutations lead to premature cell death suggesting that HAX1 protein, which is a critical regulator of mito- chondrial membrane potential and cellular viability, plays a role in apoptosis. Clinical features SCN usually presents at birth or the first few weeks of life due to the combination of severe neutropenia (typically $<0.2 \times 109$/litre) and bacterial infection. Affected individuals have severe, recurrent bacterial infections throughout life leading to premature death in childhood unless diagnosed and treated promptly. The diagnosis is made by a combination of the clinical history, blood count, and BM abnormalities and should be confirmed by genetic analysis as this is essential for genetic counselling. Despite the low neutrophil count, the haemoglobin and platelet counts are usually normal. The BM in SCN shows maturation arrest at the promyelocyte/myelocyte stage. Cyclical neutropenia is characterized by a neutrophil count that usu- ally reaches a nadir with a 21-day periodicity. Around the nadir, pa- tients may develop fever and mouth ulcers. Treatment and prognosis The mainstay of treatment for SCN is G-CSF. The vast majority of patients respond and will remain on life-long treatment. It is now clear that patients with SCN have a markedly increased risk of developing leukaemia (AML) and MDS which increases with age, approaching 25% by age 25 years. The majority of SCN patients who develop AML have a mutation in the G-CSF receptor gene (CSF3R); however, the precise contribution of G-CSF therapy to the develop- ment of CSF3R mutations remains unclear. The risk of AML appears to be lower in cyclic neutropenia. The only curative treatment for SCN is HSCT which is a valuable option especially for patients who become refractory to G-CSF therapy. Congenital thrombocytopenias Aetiology A number of rare BMF disorders may present with isolated thrombocytopenia due to reduced platelet production, the best recognized being TAR syndrome and CAMT. The inheritance of TAR is interesting as recent data indicate that it is caused by com- pound inheritance of a low-frequency regulatory single nucleotide polymorphism together with a rare null mutation in the RBM8A gene (which encodes a subunit of the exon-junction complex). CAMT is genetically heterogeneous. The majority of reported cases are autosomal recessive and caused by mutations in the MPL gene which encodes the thrombopoietin receptor. A subgroup of pa- tients with CAMT exhibit an X-linked inheritance pattern. An add- itional syndrome with similarities to TAR has also been described,

amegakaryocytic thrombocytopenia with radio-ulnar synostosis (ATRUS or RUSAT syndrome). Mutations in the HOXA11 gene and in the MECOM gene have been identified in different families with ATRUS/RUSAT syndrome. Some patients with mutations in the MECOM gene do progress to BMF or AML. TAR syndrome This syndrome presents in the neonatal period with distinctive skeletal abnormalities, particularly bilateral radial aplasia, to- gether with thrombocytopenia often with associated bleeding. Some affected individuals have additional skeletal abnormalities (absent ulnae, absent humeri, and/or clinodactyly) or somatic abnormalities, such as microcephaly, hypertelorism, strabismus, and/or heart defects, and approximately 50% of patients have cow's milk intolerance. The platelet count is usually less than 50 × 109/litre and the white cell count is elevated in more than 90% of patients, sometimes exceeding 100 × 109/litre and mimicking congenital leukaemia. BM examination shows reduced or absent megakaryocytes. Thrombopoietin receptor and thrombopoietin levels are both normal. The mainstay of treatment for TAR is platelet transfusion. However, although death in the neonatal period due to intracranial haemorrhage may occur, babies that survive the first year of life generally do well since the platelet count spon- taneously improves and is usually maintained at low normal levels thereafter. In contrast to CAMT (see next paragraph), BMF is not a feature but AML has been reported suggesting that TAR syndrome may be a preleukaemic syndrome. CAMT This rare disorder typically presents in infancy and is characterized by isolated thrombocytopenia and absent or reduced megakaryocytes in the BM. Most patients have no associated somatic abnormal- ities, although some patients with MPL gene mutations have cen- tral nervous system abnormalities, such as cerebral and cerebellar hypoplasia. In contrast to TAR syndrome, approximately 50% of pa- tients develop BMF, usually by the age of 5 years, with a hypocellular BM, reduced haemoglobin, and reduced leucocyte count in addition to thrombocytopenia. The treatment of choice for patients with se- vere thrombocytopenia and/or BMF is HSCT. FURTHER READING Alter BP (2014). Fanconi anaemia and the development of leukemia. Best Pract Res Clin Haematol, 27, 214–21. Bessler M, et al. (2015). Inherited bone marrow failure syndromes. In: Orkin SH, et al. (eds) Hematology of infancy and childhood, 8th edition, pp. 182–253. WB Saunders, Philadelphia. Bluteau O, Sebert M, Le Blanc T, et al. (2018). A landscape of germ line mutations in a cohort of inherited bone marrow failure patients. Blood, 131, 717–32. Dokal I (2014). Inherited bone marrow failure syndromes. EHA 19 Education Book, 8, 299–308. Dror Y, et al. (2011). Draft consensus guidelines for diagnosis and treatment of Shwachman–Diamond syndrome. Ann NY Acad Sci, 1242, 40–55. Gerrad G, et al. (2013). Target enrichment and high-throughput sequencing of 80 ribosomal protein genes to identify mutations asso- ciated with Diamond-Blackfan anaemia. Br J Haematol, 162, 530–6. Gramatges MM, Bertuch AA (2013). Short telomeres: from dyskeratosis congenita to sporadic aplastic anemia and malignancy. Transl Res, 162, 353–63. Hauck F, Klein C (2013). Pathogenic mechanisms and clinical implica- tions of congenital neutropenia syndromes. Curr Opin Allergy Clin Immunol, 13, 596–606.

Revision #1
Created 2026-01-22 16:42:36 UTC by Omar Ayman
Updated 2026-01-22 16:42:36 UTC by Omar Ayman