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and pure red cell aplasia Judith C.W. Marsh, Shreyans Gandhi, and Ghulam J. Mufti ESSENTIALS Aplastic anaemia Aplastic anaemia (AA) is a rare bone marrow failure (BMF) disorder characterized by pancytopenia and a hypocellular bone marrow. AA is commonly acquired, immune mediated, and idiopathic in nature. Activated autoreactive, cytotoxic CD8+ T cells are present but re- cent work has shown that CD4+ T cells appear to be more important in the

pathogenesis of acquired AA. The immune nature of acquired AA provides the rationale for one of the treatment options, namely immunosuppressive therapy. First-line treatment of acquired AA is either immunosuppressive therapy with antithymocyte globulin and ciclosporin or allogeneic haematopoietic stem cell transplantation (HSCT). Both modal- ities offer excellent survival. Patients treated with immunosuppres- sive therapy (IST) are at later risk of relapse and clonal evolution to myelodysplastic syndrome and acute myeloid leukaemia, so require long-term follow-up. HSCT, if successful, is curative, but risks include graft rejection, infections, and graft-versus-host disease (GVHD); re- cent changes to the transplant conditioning regimen have reduced the GVHD risk. Eltrombopag is now licensed to treat severe AA that is refractory to IST with response seen in 40–50% of patients, and its use with upfront IST is being explored further. There is increasing awareness of inherited AA which may present not only in childhood with somatic anomalies, but also in adulthood. Adults with later-onset inherited AA often lack the somatic anomalies seen in children resulting frequently in delayed diagnosis and/or mis- diagnosis as acquired AA. Pure red cell aplasia Pure red cell aplasia (PRCA) is a form of BMF characterized by severe anaemia with reticulocytopenia and reduced erythroid progenitors in the bone marrow. PRCA most commonly is an acquired disorder and immune mediated, and often occurs in association with a wide range of conditions. Diamond–Blackfan anaemia, an inherited form of PRCA, is an- other example of a ribosomopathy, and is caused by mutations in one of many ribosomal protein genes, resulting in haploinsufficiency (see also Chapter 22.5.1). Introduction—the bone marrow failure disorders Aplastic anaemia (AA) and pure red cell aplasia (PRCA) represent two examples of the bone marrow failure (BMF) disorders illustrated in Fig. 22.5.2.1. BMF in this context implies a reduction in one or more circulating blood cell types (red cells, white cells, and platelets) due to failure of production by the bone marrow (BM), resulting in single cytopenias such as anaemia in PRCA, or pancytopenia as in AA. In AA, there is BMF within the haematopoietic stem cell com- partment and in PRCA either production failure or defective matur- ation of erythroid progenitors. Most cases of AA and PRCA are acquired and have an immune basis, but recently there has been increasing awareness of constitu- tional and often inherited forms of BMF, presenting not only in chil- dren but also later in adulthood. Often these arise in the absence of classical somatic anomalies but instead have atypical clinical features such as pulmonary fibrosis and cirrhosis. Examples of congenital AA include Fanconi’s anaemia and dyskeratosis congenita (DC), and Diamond–Blackfan anaemia—the inherited form of PRCA. There is a marked overlap between AA and paroxysmal nocturnal haemoglobinuria (PNH), which arises from an acquired somatic mu- tation in the PIGA gene in the haematopoietic stem cell. PNH may arise from, or evolve to, AA, and small PNH clones are found in about 40% of AA patients. The BMF disorders, whether acquired or in- herited, but particularly the latter, may undergo later clonal evolution and malignant transformation to myelodysplastic syndrome (MDS) and acute myeloid leukaemia (AML) due to genomic instability. With the development of high-throughput sequencing technolo- gies, it has become apparent that somatic mutations are present in approximately 75% of patients with AA. In addition to the expected PIGA mutations associated with PNH clones and STAT3/STAT5b mutations which likely characterize subclinical T-cell large granular lymphocyte leukaemia (T-LGL) clones within AA, a number of mutations commonly associated with MDS/AML have been de- scribed in the absence of any morphological features of these dis- orders. Approximately 20% of patients with AA have been shown to have such mutations with commonly mutated genes including DNMT3A, ASXL1, and BCOR/BCORL. Similar mutations have also been detected in the peripheral blood of healthy individuals, albeit occurring less frequently and at an older age. This phenom- enon, termed clonal haematopoiesis of indeterminate potential (CHIP), is associated with a higher risk of developing a

haemato- logical malignancy (e.g. MDS/AML). The increased prevalence of CHIP in AA, particularly at younger ages, may reflect the inability of the damaged immune system to either eliminate or at least control

22.5.2 Acquired aplastic anaemia and pure red cell aplasia 5337 the abnormal haematopoietic clones which might normally emerge with increasing years. Assessment of mutational status in AA is of clinical relevance due to evidence of an association between gene disruption and clinical outcomes: mutations in PIGA and BCOR/ BCORL are associated with a better prognosis whereas mutations in ASXL1 and DNMT3A are associated with a poorer response to im- munosuppressive treatment, potentially indicating patients suitable for early allogeneic stem cell transplant. Aplastic anaemia Defining aplastic anaemia The traditional definition of AA is a pancytopenia with a hypocellular BM in the absence of an abnormal infiltrate and with no increase in reticulin. Both a BM aspirate and trephine biopsy are required for the diagnosis. There must be at least two of the following findings: (1) haemoglobin concentration less than 100 g/litre; (2) platelet count less than 50 × 109/litre; (3) neutrophil count less than 1.5 × 109/litre. However, additional aspects of the disease should be evaluated and included in the definition, as summarized in Table 22.5.2.1. A constitutional form of AA should be excluded by taking a de- tailed medical and family history, clinical examination, and labora- tory investigation, as this has important implications for clinical management and screening of family members (Table 22.5.2.2, and Chapter 22.5.1). The severity of the disease is graded as for acquired AA (Table 22.5.2.1). Most cases (80%) of acquired AA are idiopathic, but a careful drug and exposure history should be taken to attempt to exclude a drug- or chemical-induced cause. However, there are often confounding factors and no tests are available to prove causality. Nevertheless, any putative drug should be discontinued at presentation of the AA. See Table 22.5.2.2 for a list of licensed drugs reported as a possible cause of idiopathic AA. All patients should be assessed for the presence of abnormal clones, namely PNH clones and cytogenetic or molecular clonal changes as- sociated with MDS. Occasionally, T-LGL/T-lymphocytosis clones may be present (see ‘Aetiology and incidence’). Using conventional BM karyotyping, abnormal cytogenetic clones are detected in up to 12% of AA patients in the absence of morphological features of MDS/ AML. The most common clones found are trisomy 8, trisomy 6, and monosomy 7, among others. Their prognostic significance varies; trisomy 8 is associated with a good response to immunosuppressive treatment whereas monosomy 7 is associated with a poor prognosis and a high risk of MDS/AML. Because it is often difficult to obtain sufficient metaphases due to the BM hypocellularity, cytology/fluor- escence in situ hybridization (FISH) or targeted next-generation sequencing should be performed in such situations. Flow cytometry is used to detect PNH clones in the blood, with around 40% of pa- tients showing deficient expression of glycosylphosphatidylinositol (GPI) proteins on the cell surface of all blood cells. Small PNH clones (in general affecting <10% blood cells) is termed subclinical PNH as there is no clinical or laboratory evidence of haemolysis, but in 10% of patients the size of the PNH clone is larger, resulting in ‘classical’ haemolytic PNH. The deficient expression of GPI-anchored proteins is due to an acquired mutation in the PIGA gene located on the X chromosome and arising from the haematopoietic stem cells, but genetic mutation analysis of PIGA does not form part of the routine diagnostic investigation. Finally, because of the rarity of AA, it is important to consider pos- sible alternative causes of the pancytopenia and hypocellular BM. RBDS VSAA SAA NSAA PNH Hypocellular MDS AML Low risk High risk MDS TLGL (clonal or polyclonal) GATA2 defic DC AA/PNH FA PIGA STAT3 MDS/AML-associated somatic mutations SBDS, RPS5, RPS11, RPS 19… DKC1, TERC, TERT, TINF2, RTEL1…. FANC GATA2 HLA Hypo AML Germline mutations Somatic (acquired)

mutations Fig. 22.5.2.1 The association of aplastic anaemia with acquired and constitutional disorders of clonal haematopoiesis. AA, aplastic anaemia; AML, acute myeloid leukaemia; DC, Dyskeratosis congenita; FA, Fanconi anaemia; MDS, myelodysplastic syndrome; NSAA, non-severe AA; PNH, paroxysmal nocturnal haemoglobinuria; RBDS, Ribosomal dysgenesis syndromes; SAA, severe AA; TLGL, T-large granular lymphocytosis; VSAA, very severe AA.

section 22 Haematological disorders 5338 The most difficult condition to distinguish from AA is hypocellular MDS as the latter may share many features of AA. In most cases of MDS the BM is hypercellular, but 10 to 15% of patients have a hypocellular marrow. The findings of dysplastic neutrophils, dys- plastic megakaryocytes, increased BM reticulin, ringed sideroblasts, and the presence of blasts/increased CD34+ cells in the blood or BM, all suggest hypocellular MDS. Even then, the distinction be- tween AA and hypocellular MDS may not always be certain and some patients appear to have an ‘AA/hypocellular MDS’ overlap syndrome. However, a recent integrated scoring system that incorp- orates cytohistological, and genetic mutations highly specific for MDS, enables clear separation of hypocellular MDS cases into two distinct groups, one with clinical and genetic features highly con- sistent with a clonal disease with high risk of leukemic evolution, and the other with features more consistent with a nonmalignant bone marrow failure, very low risk of transformation and better survival. Other conditions that are to be considered in the differ- ential diagnosis of AA include hypocellular AML, or especially in children, acute lymphoblastic leukaemia, hairy cell leukaemia, myelofibrosis, lymphoma, atypical mycobacterial infection, and an- orexia nervosa, which can all sometimes present with pancytopenia and a hypocellular BM. Acquired aplastic anaemia Aetiology and incidence Most cases (around 80%) of acquired AA are considered to be idio- pathic. There is a biphasic age distribution with peaks from 10 to 25 years and over 60 years. There is no significant difference in inci- dence between males and females. Because AA is a rare disease, only large national and international prospective studies will provide meaningful data on the aetiology of this condition. The incidence in Table 22.5.2.1 Defining aplastic anaemia Confirmation of diagnosis 1 Traditional definition Pancytopenia with hypocellular BM, haematopoietic tissue replaced by fat cells, in absence of abnormal infiltrate or increase in reticulin At least 2 of the following required: Hb <100 g/litre; platelet count <50 × 109/litre; and neutrophil count <1.5 × 109/litre FBC, reticulocyte count, blood film examination, BM aspirate and trephine 2 Is the diagnosis really AA? Or is there another cause for pancytopenia and hypocellular BM? Exclude hypocellular MDS/AML, hypocellular ALL especially in children, hairy cell leukaemia, myelofibrosis, lymphoma, atypical mycobacterial infection, anorexia nervosa 3 Is the disease an inherited bone marrow failure syndrome? Clues in medical history, extended family history, and clinical examination Fanconi anaemia Presence of café-au-lait spots, short stature, anomalies of upper extremities, etc. Increased chromosome breakages of peripheral blood lymphocytes with DEB/MMC Inherited telomeropathy/Dyskeratosis congenita Nail dystrophy, leukoplakia and skin pigmentation, pulmonary fibrosis, cirrhosis, premature hair greying, avascular necrosis Shwachman–Diamond syndrome History of pancreatic exocrine insufficiency, neutropenia prior to AA, short stature GATA2 deficiency Monocytopenia, B, NK and dendritic cell deficiency, warts, non-tuberculous mycobacterial infections, lymphoedema 4 What is the aetiology? Idiopathic Around 80% of cases Post-hepatitic Liver function tests, viral studies (hepatitis A, B, C, G, usually negative) Viral infection Screen for HIV, hepatitis A, B, C, EBV Drugs and chemicals; environmental/occupational exposures Careful drug and exposure history, but no tests available to prove association PNH Flow cytometry of GPI-anchored proteins on red cells and granulocytes Rarely: pregnancy, systemic lupus erythematosus, thymoma, eosinophilic fasciitis 5 Are there

abnormal clones present? PNH Flow cytometry as above MDS/CHIP BM metaphase cytogenetics ± FISH/SNP-A karyotyping T-LGL clone Morphology, flow cytometry and TCR gene rearrangement studies 6 How severe is the disease? Severe AA Criteria: BM cellularity <25% or 25–50% with <30% residual haematopoietic cells, with 2 out of 3 of the following: neutrophils <0.5 × 109/litre, platelets <20 × 109/litre, reticulocytes <20 × 109/ litre, reticulocytes <60 × 109/litre Very severe AA Same as for severe AA, except neutrophil count <0.2 × 109/litre AA, ALL, acute lymphoblastic leukaemia; aplastic anaemia; BM, bone marrow; CHIP, clonal haematopoiesis of indeterminate potential; DEB, diepoxybutane; EBV, Epstein–Barr virus; FBC, full blood count; FISH, fluorescence in situ hybridization Hb, haemoglobin; MDS/AML, myelodysplastic syndrome/acute myeloid leukaemia; MMC, mitomycin C; PNH, paroxysmal nocturnal haemoglobinuria; SNP-A, single nucleotide polymorphism array-based; TCR, T-cell receptor; T-LGL, T-large granular lymphocyte.

22.5.2 Acquired aplastic anaemia and pure red cell aplasia 5339 the West is about one to two per million per year, but it occurs more commonly in eastern Asia, with a two- to fourfold higher incidence. Reasons for this difference in incidence are not known, but may in- clude infections and genetic factors. In rural areas of Thailand, the use of nonbottled water, agricultural pesticides, nonmedical needle exposures, and exposure of farmers to ducks and geese are signifi- cant environmental risk factors for developing AA. Many drugs and chemicals have been implicated in the aeti- ology of AA, but for only a few is there strong evidence for an association, and even then it is usually impossible to prove caus- ality (Table 22.5.2.3). A careful drug history must be obtained. Drug exposure in the year preceding presentation should be de- tailed. Earlier exposures should be recorded but are not likely to be relevant unless the particular drug or drug group has been taken again during the presumed critical period. If the patient is taking several drugs which may have been implicated in AA, even if the evidence is based on case report(s) alone, then all the pu- tative drugs should be discontinued and the patient should not be rechallenged with the drugs at a later stage after recovery of the blood count. Drugs most commonly implicated include anti- biotics and nonsteroidal anti-inflammatory drugs. Post-hepatitic AA accounts for about 5% of all cases, in most cases being non-A, Table 22.5.2.2 Indicators of possible inherited AA from the clinical and family history and clinical examination Clinical history Family history (first-degree relatives and extended family) Clinical examination Birth history Unexplained anaemia, thrombocytopenia or neutropenia, or pancytopenia Short stature Failure to thrive as child Thrombocytopenia Skeletal anomalies Malabsorption Neutropenia Abnormal facies Developmental delay, short stature Aplastic anaemia Nail dystrophy, examine hands and feet Skin or nail problems MDS Leukoplakia Premature greying of hair and use of hair dyes AML High-arched palate Eye or ear problems Cancers Abnormal dentition Liver problems (cirrhosis, portal hypertension, splenomegaly) Liver issues, including those attributed to alcohol excess Skin abnormalities: reticulate pigmentation, hyperpigmentation (café-au-lait spots), hypopigmentation Lung fibrosis Cirrhosis Cardiac murmurs and abnormal heart sounds Osteoporosis Lung fibrosis Pulmonary disease Avascular necrosis of bone Early childhood deaths Genitourinary anomalies Joint or bone abnormalities; corrective orthopaedic surgery Osteoporosis Lymphoedema Learning disabilities Learning disabilities Table 22.5.2.3 Currently licensed drugs and occupational exposures reported as a probable cause of aplastic anaemia (a) Currently licensed drugs Antibiotics Chloramphenicola, sulfonamides, co-trimoxazole, linezolid Anti-inflammatories Phenylbutazone, indomethacin, diclofenac, naproxen, piroxicam, gold, penicillamine Anticonvulsants Phenytoin, carbamazepine Antithyroid Carbimazoleb, thiouracil Antidepressants Dothiepin, phenothiazides Antidiabetic Chlorpropamide, tolbutamide Antimalarial Chloroquine Others Mebendazole, thiazidesc, allopurinol

(b) Occupational and environmental exposures Agent Evidence base Benzene Large industrial studies, case–control study from Thailand Pesticides: organochlorines, e.g. lindane, organophosphates, pentachlorophenol Literature review of case reports and UK case–control study Cutting oils and lubricating agents UK case–control study Recreational drugs, e.g. methylenedioxymethamphetamine (MDMA, ecstasy) Case reports a There is no evidence for an association between chloramphenicol eye drops and aplastic anaemia. b More likely to cause neutropenia alone. c From a case–control study in Thailand.

section 22 Haematological disorders 5340 non-B, non-C, non-G. Patients usually present with jaundice and hepatic symptoms, then, on average 6 weeks later, develop pan- cytopenia when the liver function has usually improved. Rarely AA follows Epstein–Barr virus (EBV) infection. A careful occupa- tional history may reveal exposure to chemicals or pesticides that have been associated with AA. The association of AA with PNH was discussed earlier. T-LGL is characterized by chronic proliferation of cytotoxic T-lymphocytes (CTLs) and is commonly associated with single cytopenias such as PRCA and autoimmune neutropenia. T-LGL clones have also been reported in AA and PNH as well as MDS, as shown by T-cell re- ceptor (TCR) gene rearrangement, and more recently using deep sequencing of the TCR. The haematological target of these expanded T-LGL clones is as yet unknown, but the finding of STAT3 mutations in CTLs in some patients with AA (and MDS and PNH) provides further insight into the mechanism of their proliferation and their potential role in immune-mediated AA. Pathogenesis AA is characterized by a severe quantitative defect in the haemato- poietic stem progenitor cell compartment. The primitive long-term culture-initiating cells and more mature haematopoietic progenitors in the BM (colony-forming cells) of all cell lineages are reduced or absent. There is a reduction in the percentage of CD34+ BM cells, and they are more apoptotic than normal CD34+ cells. There is strong evidence for an immune-mediated pathogenesis for idiopathic AA. Most patients (approximately 70%) will respond to immunosuppressive therapy, and there are multiple data from experimental studies. Following an initial BM insult, likely virus infection, there is immune recognition of a neoantigen or aber- rantly expressed autoantigen that is presented in the context of class I HLA molecules on HSC. The immune response is characterised by marked expansion of CD4+ helper Th1 (clonal), Th2 and Th17 cells, and a decrease in T regulatory cells (Tregs) (Fig. 22.5.2.2). AA Tregs, as defined by CD4+, FOXP3, CD127, are also dysfunctional and are unable to suppress autoreactive CD8+ CTLs, resulting in oligoclonal CTL expansion. The Treg immune signature of AA has been defined, with two distinct Treg subsets, Treg subpopulations (Treg A and Treg B) that differ in number and immune-phenotype among AA responders and non-responders to IST. Treg B popula- tion predominates in responders to IST and they have a memory/ activated phenotype (as shown by high expression of CD95, CCR4 and CD45RO). The CD8+ CTL expansion, along with an increase in proinflammatory cytokines interferon-γ (IFN-γ) and tumour ne- crosis factor-α (TNF-α), leads to death of haematopoietic stem cells (HSC) by apoptosis. Some clones, however, are able to escape this Fig. 22.5.2.2 Pathogenesis of acquired aplastic anaemia: immune-mediated BM apoptosis. FasL, Fas ligand; FasR, Fas receptor; NK, NK cells; TCR, T-cell receptor.

22.5.2 Acquired aplastic anaemia and pure red cell aplasia 5341 immune attack and preferentially expand, for example, PNH clones, +8, del13q clones and somatic loss of class I HLA alleles through CN-LOH 6p or loss of function somatic mutations. The presence of such ‘immune escape clones’ is associated with good response to IST and low risk of clonal evolution to MDS/AML in adult patients. In AA, the increased proliferative pressure on a reduced HSC pool, results in telomere loss, leading

to genomic instability, with ac- quisition of somatic mutations and risk of transformation to MDS/AML. Short telomeres, in the absence of an inherited telomeropathy, are detected in 30% of patients with acquired AA. In contrast to the immune response in AA, in low risk MDS there are features of early, low grade/smouldering inflammation with increased Th17 and normal Tregs, followed by subsequent reduction in Th17 and expansion of Tregs in high risk MDS. Treg expansion is associated with expansion of the innate immune effector cells, namely myeloid derived suppressor cells (MDSCs) that have a potent immunosup- pressive effect, so there is suppression of immune mediated tumour surveillance, immune subversion and immune escape that facilitates progression towards AML. Clinical features Taking a detailed and extended family history and careful clinical examination will increase the chance of early detection of constitu- tional AA (Table 22.5.2.2). Most cases of AA are acquired and have an immune basis, but in recent years, there has been increasing aware- ness of constitutional and often inherited forms of BMF, presenting not only in children, but also later in adulthood. Furthermore, with the advent of next generation sequencing, an increasing number of genetically defined BMF syndromes have been described, aside from the previously known examples such as Fanconi anaemia, classical X-linked dyskeratosis congenita (DC) and Schwachman-Diamond syndrome. These syndromes presenting in adulthood often lack the classical clinical features, and instead have atypical features such as pulmonary fibrosis and cirrhosis, premature hair greying, avascular necrosis, as seen in autosomal dominant DC, now termed ‘inherited telomeropathies’, due to germline mutations in the telomere gene complex, of which 12 have so far been described, including TERT, TERC, RTEL1, among others. GATA2 deficiency arises as a de novo mutation and then is subsequently inherited as autosomal dominant disorder. It may present with BMF and progress to MDS/AML. It is characterised by monocytopenia, B, MK and dendritic cell defi- ciency, and other features include lymphoedema, non-tuberculous mycobacterial disease, viral warts, pulmonary alveolar proteinosis. These and other types of inherited BMF disorders are discussed in Chapter 22.5.1. Missing a diagnosis of constitutional AA will result in wrong treatment, potential risk of fatal outcome after haemopoietic stem cell transplantation (HSCT) since the HSCT conditioning regimen is different, inappropriate use of an asymptomatic undiagnosed sib- ling donor with the same genetic mutation, and failure to refer for genetic counselling and for long term cancer surveillance. Patients with AA most commonly present with symptoms of an- aemia and skin or mucosal haemorrhage (ecchymoses or petechiae), or visual disturbance due to retinal haemorrhage. Infection may be a presenting feature, but is less common. There is no lymphaden- opathy or hepatosplenomegaly (in the absence of infection) and these findings strongly suggest another diagnosis. A preceding history of jaundice, usually 2 to 3 months before, may also indi- cate a post-hepatitic AA. There may be specific clinical features to indicate a possible inherited bone marrow failure disorder, but some affected patients may have none of these clinical features. Adults with late onset inherited AA often lack the classical features of in- herited disorders such as Fanconi anaemia or DC. The autosomal dominant forms of DC usually lack the classical mucocutaneous features of DC but instead may present with features that include premature greying of the hair, pulmonary fibrosis, and cirrhosis or non-cirrhotic portal hypertension. A detailed family history should be taken as this may reveal similar findings in family members and/or a history of low blood counts or macrocytosis and cancer (Table 22.5.2.2). Clinical investigations The following investigations are required in the evaluation of pa- tients with suspected AA. Full blood count This typically shows pancytopenia. In the early stages, isolated cytopenia, particularly thrombocytopenia, may occur. Anaemia is accompanied by reticulocytopenia, and macrocytosis is common. Careful examination of the blood film is essential to exclude the presence of dysplastic neutrophils and platelets, blasts, and other

abnormal cells such as hairy cells. Bone marrow aspirate and trephine biopsy The BM is hypocellular with prominent fat spaces and variable amounts of residual haematopoietic cells. Erythroid precursors, megakaryocytes, and granulocytic precursors are reduced or ab- sent. Lymphocytes, macrophages, plasma cells, and mast cells ap- pear prominent. A trephine is essential to assess overall cellularity, to exclude an abnormal infiltrate and other disorders. Sometimes the BM is patchy, with hypocellular and cellular areas (Fig. 22.5.2.3). Immunostaining on the trephine may be particularly helpful, for ex- ample, CD34 positive cells to identify blasts in AML and CD61 to identify dysmegakaryopoiesis in MDS. A reticulin stain should be routinely performed because an increase in BM reticulin is not in keeping with a diagnosis of AA and may instead indicate a diagnosis of MDS or hairy cell leukaemia, for example. Liver function tests and virology In post-hepatitic AA, the serology is usually negative for all the known hepatitis viruses. Abnormal liver function tests may occur in DC secondary to cirrhosis or noncirrhotic portal hypertension. Blood should be sent to test for hepatitis A antibody, hepatitis B sur- face antigen, hepatitis C antibody, HIV antibody I and II, and EBV, as these are rare causes of AA; parvovirus may causes red cell aplasia, but not typically AA. Vitamin B12 and folate levels These should be tested to exclude megaloblastic anaemia, which when severe may present with pancytopenia. Antinuclear antibody and anti-DNA antibody These should be tested to exclude systemic lupus erythematosus which is a rare cause of AA. PNH screen Multiparametric flow cytometry is used to identify clones of cells that lack GPI-anchored proteins, such as CD55 and CD59. This is a

 sensitive and quantitative test for analysis of PNH populations. The old test for PNH was Ham’s test, but this was relatively insensitive and only assessed red blood cells for lysis in the presence of added complement. Additionally, recent blood transfusion may mask and/ or underestimate the size of the red cell PNH clone. In contrast, flow cytometry analyses expression of GPI-anchored proteins not only on the surface of red cells but also granulocytes and mono- cytes, and can detect much smaller PNH clones than Ham’s test. Bacterial aerolysin selectively binds to the GPI anchor and causes lysis of normal cells but not PNH cells that lack the GPI anchor. FLAER (fluorescein-labelled aerolysin) is a labelled, inactive variant of aerolysin that does not cause lysis of cells, but provides a more ac- curate and more sensitive assay than flow cytometry alone to detect PNH cells as it binds to normal but not PNH cells. FLAER is often combined with flow cytometry. Flow cytometry is more sensitive at diagnosing small PNH clones than molecular testing for PIGA gene mutations, hence detection of PIGA gene mutations does not form part of routine diagnostic investigations. Small PNH clones (in general affecting < 10% blood cells) is termed sub-clinical PNH as there is no clinical or laboratory evidence of haemolysis, but in 10% of patients the size of the PNH clone is larger resulting in ‘classical’ haemolytic PNH. Marrow cytogenetics and molecular genetics Abnormal cytogenetic clones may be present in up to 12% of pa- tients with otherwise typical AA, and do not necessarily indicate MDS or leukaemia, but do require following carefully. If insuffi- cient metaphases are obtained, cytology/FISH should be performed, particularly for deletions of 5 and 7 and for trisomy 8, and/or single nucleotide polymorphism array-based (SNP-A) karyotyping. Somatic mutations Many major centres offer testing for acquired somatic mutations in myeloid specific genes, such as ASXL1, DNMT3A using customised diagnostic gene panels. Targeted next-generation sequencing of mu- tation hotspots in key genes such as STAT3, DNMT3a, and ASXL1 may also be of utility. Screen for inherited disorders For all new patients up to middle age and older patients who are potential candidates for BM transplantation, or in whom in- herited AA is suspected, peripheral blood lymphocytes should be tested for chromosomal breakages to exclude Fanconi’s anaemia. Certain

specialist centres provide testing for telomere length and NGS constitutional BMF gene panels. Following the 100 K Genome Project, future testing is planned for all patients by NHS England. Chest radiograph and abdominal ultrasonography Radiological investigations, including chest radiograph and an abdominal ultrasound scan should be performed to exclude in- fection, and an enlarged spleen and/or enlarged lymph nodes, respectively. In younger patients, abnormal or anatomically dis- placed kidneys are features of Fanconi's anaemia. Radiography of the hands and forearms may be indicated for specific inherited forms of BMF. (b) (a) (d) (c) Fig. 22.5.2.3 Bone marrow trephine biopsy sections. (a) Normal bone, showing normal distribution of haematopoietic cells and fat cells within the bone trabeculum; (b) severe AA showing replacement of haematopoietic cells by fat cells; (c) nonsevere AA showing patchy distribution of remaining haematopoietic cells; (d) high-power view of severe AA showing fat cells interspersed by lymphocytes and macrophages.

22.5.2 Acquired aplastic anaemia and pure red cell aplasia 5343 Treatment and prognosis Once the diagnosis is firmly established and the disease has been carefully defined as described previously, the treatment plan for the patient should be mapped out and discussed. Treatment is influ- enced by the age of the patient, disease severity, and the availability of a suitable stem cell donor. Supportive care Transfusions Red cell and platelet transfusions are given to help maintain safe blood counts. Current national and European guide- lines recommend to give prophylactic platelet transfusions when the platelet count is below 10 × 109/litre (or higher in the presence of fever or bleeding). Purpura, menorrhagia, and spontaneous bleeding, mostly from the gums and buccal mucosa, usually develop below this level, but there is also a risk of life-threatening haemor- rhage, particularly in the presence of infection. Transfusions may induce alloimmunization to leucocytes present in red cell and platelet transfusions by generating human leucocyte antigen (HLA) or non-HLA (minor histocompatibility) antibodies or platelet-specific antibodies, which cause refractoriness to platelet transfusions as well as increasingly the risk of graft rejection fol- lowing allogeneic haematopoietic stem cell transplantation (HSCT). The risk of alloimmunization to leucocytes has diminished since the widespread introduction of leucodepletion and irradiation of blood products, but still remains a problem. If patients develop HLA anti- bodies, HLA-compatible platelets may be needed. Other important practical and are refractory to random donor platelets, measures to help prevent bleeding include good dental hygiene, the use of oral tranexamic acid, and control of menorrhagia with appropriate hor- mone therapy. Transfusional haemosiderosis occurs with prolonged red cell transfusions. Iron chelation therapy with desferrioxamine or deferasirox may be indicated, especially in patients who are trans- plant candidates. For patients with haematological disorders under- going HSCT, transplant-related mortality is increased if the serum ferritin is greater than 2000 μg/litre, and many centres commence iron chelation when the serum ferritin is greater than 1000 μg/litre. Infections Patients with AA are at risk of bacterial and fungal infec- tions, with the level of risk depending on the degree of neutropenia. Severe neutropenia (neutrophils <0.5 × 109/litre, and especially <0.2 × 109/litre) carries a high risk of systemic infection arising from endogenous organisms, especially Gram-negative but also Gram- positive bacteria. Fungal infections, particularly aspergillus, occur in severe neutropenia. Patients with neutrophil count <0.5 × 109/litre should receive antibiotic and antifungal prophylaxis as recom- mended by NICE and national AA guidelines. Oral hygiene is important. Entry sites for venous access are potential sources of systemic infection. Fever should be treated with broad-spectrum antibiotics, without waiting for laboratory identification of or- ganisms. Systemic antifungal drugs should be commenced early if fevers fail to resolve with intravenous antibiotics. Granulocyte

colony-stimulating factor (G-CSF) is usually ineffective in severe AA because of a severe reduction or absence of myeloid progenitor cells. Granulocyte transfusions are sometimes administered in life- threatening infections. Psychological support Psychological support for the patient, family, and close friends is of great importance. AA is a rare dis- ease and requires careful explanation of its nature and prognosis. Patients should be given the opportunity to be referred to a centre that specializes in the management of AA. The chronic nature and slow response to treatment should be stressed early in the disease. There is an excellent patient support group based in the United Kingdom (http://www.theaat.org.uk). Specific treatment The treatment choice is essentially either allogeneic HSCT or im- munosuppressive therapy using horse ATG and ciclosporin. Horse ATG has been found to be superior to rabbit ATG and is therefore the initial treatment of choice if available (see ‘Immunosuppressive Age of patient Co-morbidities ≤ 35 y

> 50–60 y HLA identical sibling donor No Yes Unrelated donor HSCT Children 35–50 or 60y? Or Or Horse ATG with ciclosporin HLA matched sibling HSCT If no response 2nd ATG, danazol, haplo/cord HCT Eltrombopag Fig. 22.5.2.4 Algorithm for treatment of severe aplastic anaemia. HSCT, haematopoietic stem cell transplantation.

section 22 Haematological disorders 5344 therapy (ATG and ciclosporin)’). An algorithm summarizing treat- ment options for severe AA is shown in Fig. 22.5.2.4. Haematopoietic stem cell transplantation First-line treatment for younger severe AA patients is allogeneic HSCT from an HLA-identical sibling donor, with 70 to 90% long-term survival (Fig. 22.5.2.5). Results of HSCT in older patients are less successful so older pa- tients should receive immunosuppressive therapy with ATG and ciclosporin as first-line treatment. One possible immunosuppres- sive, nonmyeloablative conditioning regimen is employed for HSCT in AA. Cyclophosphamide (CY) 200 mg/kg with ATG or alemtuzumab is used for patients aged <30 years, and fludarabine, low dose CY with ATG or alemtuzumab (‘FCC’) for patients aged

> 30 years. Alemtuzumab is associated with a lower incidence of chronic graft-versus-host disease (GVHD). Ciclosporin (usu- ally with methotrexate, unless using alemtuzumab) is given to suppress GVHD and to aid engraftment. Irradiation should not be used in AA patients receiving transplants from matched sib- ling donors. Children grow and develop normally and fertility is well preserved post transplantation. Chronic GVHD and infection are the main causes of transplant-related morbidity and mortality. Graft rejection may be early, with failure of engraftment of host cells, or late, after initial engraftment. It occurs in 10 to 15% of pa- tients. Analysis of chimerism on myeloid cells (CD15) and T cells (CD3) in patients receiving alemtuzumab-based conditioning demonstrates that stable mixed T-cell chimerism in the presence of full donor myeloid chimerism is associated with excellent sur- vival and a low incidence of chronic GVHD. Using an ATG-based conditioning regimen, BM stem cells are used instead of periph- eral blood stem cells, but either can be used with

alemtuzumab- based conditioning. It is important to give at least 3 × 108 nucleated marrow cells/kg body weight (or >2 × 106 CD34+ cells/kg) to reduce the risk of graft rejection. Matched unrelated donor (MUD) HSCT is considered for patients who have no HLA-compatible donor and who have failed treatment with one course of immunosuppressive therapy. However, for children who lack a matched sibling donor, first line MUD HSCT may be considered. Because of recent improvements in outcomes after MUD HSCT in adults with SAA, there is currently active debate for consid- ering first line MUD HSCT as an option for adults who need urgent HSCT. Patients undergoing MUD HSCT should receive a fludarabine, low dose CY based conditioning with ATG or alemtuzumab. Low dose total body irradiation (2Gy) is also needed when using ATG but not with alemtuzumab conditioning. Survival Days following Transplantation (OS) 0,00 0.0 0.2 0.4 0.6 0.8 1.0 1000,00 2000,00 3000,00 Age >50yrs (n = 12) 72.9% 86.5% Effect of age < 50 or >50 years (d) Age <50yrs (n = 33) 4000,00 P = 0.02 MSD (n = 21) UD (n = 29) P = 0.34 83% Matched sibling and UD HSCT (c) Survival Days following Transplantation (OS) 0,00 0.0 0.2 0.4 0.6 0.8 1.0 1000,00 2000,00 3000,00 4000,00 95% 100 75 50 25 0 0 1000 2000 3000 4000 Days from transplant FCA; n–52 FCA- TBI; n = 48 79% 73% (b) Unrelated donor HSCT Survival (%) 1,000 0,750 0,500 0,250 0,000 0,0 1000,0 Matched sibling donor HSCT (a) 2000,0 3000,0 4000,0 Age ≥ 20, n = 890 P<0.0001 70% 87% Age ≥ 20, n = 996 Fig. 22.5.2.5 Overall survival after matched sibling donor and unrelated donor HSCT for severe AA. (a, b) Data from the European Group for Blood and Marrow Transplantation (EBMT) Registry. Conditioning regimens used for matched sibling transplants comprised high-dose cyclophosphamide with ATG, and for unrelated donor transplants fludarabine, low-dose cyclophosphamide and ATG, with or without low-dose total body irradiation. (c, d) Results from a multicentre study from the United Kingdom and Toronto, Canada, using alemtuzumab-based conditioning instead of ATG. (a, b) Bacigalupo A et al., 2012, with permission.

22.5.2 Acquired aplastic anaemia and pure red cell aplasia 5345 For patients who lack both a suitable matched sibling and unrelated donor, alternative transplant approaches currently being explored are cord blood HSCT and haploidentical HSCT. A haploidentical donor, whether a parent or sibling or child, is usually readily avail- able for most patients. In contrast, cord blood transplant often requires 2 cord units for adult HSCT and is more expensive than haploidentical HSCT. Haploidentical HSCT performed during the 1980s was usually unsuccessful due to a very high incidence of GVHD and graft rejection, but the more recent use of high-dose cyclophosphamide given in the immediate post-transplant phase to eliminate alloreactive donor T cells is showing promising potential. Immunosuppressive therapy (ATG and ciclosporin) ATG is a polyclonal IgG antibody preparation prepared by immunizing horses, rabbits, or pigs with human thymocytes. Its mechanism of action in AA is not entirely clear, but may be partly due to depletion of autoreactive, cytotoxic T cells, in addition to direct stimulation of residual BM CD34+ cells or a reduction in their degree of apoptosis, or other mechanisms. The response rate to ATG in AA is increased when it is combined with ciclosporin. Horse ATG is preferred to rabbit ATG as it results in a higher response

rate (68% at 6 months compared to 37% for rabbit ATG) and better survival, despite rabbit ATG being more immunosuppressive than horse ATG in terms of the degree and duration of lymphodepletion, indicating that different mechanisms are important in the mode of action of the two agents. ATG and ciclosporin are indicated for patients who are ineligible for HSCT or whose disease is not severe enough to warrant a transplant. This includes older patients with nonsevere AA, and younger patients who have severe disease but who lack an HLA-identical sibling donor. ATG is highly immunosuppressive and must be given as an in- patient treatment, preferably with patients nursed in isolation fa- cilities. Response is delayed, rarely occurring before 3 to 4 months. Around 70% will respond and achieve normal or near-normal blood counts, with a 5-year overall survival of 80%. Survival is better in younger patients compared to older patients, and better in patients with severe AA compared to those with very severe AA (Fig. 22.5.2.6). Patients require long-term follow-up for later events, such as relapse of AA which occurs in up to 30%, necessitating fur- ther treatment with ATG or consideration for HSCT. Later clonal evolution to MDS/AML occurs in 15% of patients. Retreatment with a second course of ATG results in response rates of about 35% for nonresponse and 50 to 60% for relapse after a first course of ATG. Patients with nonsevere AA who are not dependent on red cell or platelet transfusions may remain stable for months or years without definitive treatment. They should have their blood count monitored regularly, and if it worsens such that they require transfusions, they may then be treated with ATG and ciclosporin. Oral ciclosporin may be used on its own, although the response rate is lower than with the combination of ATG and ciclosporin. Patients with inherited AA more commonly present with nonsevere AA, so a high degree of suspicion should be maintained and such patients investigated as thoroughly as possible for a potential inherited BMF disorder. In addition, among the nonresponders to ATG, there may be some who have an unsuspected, underlying inherited BMF syndrome. Other immunosuppressive agents In an attempt to improve the response to ATG and ciclosporin, the additional use of either mycophenolate or sirolimus has been assessed in clinical trials, but nei- ther agent improves the response rate or survival. Alemtuzumab has been used to treat AA, and the response rate is comparable to ATG for relapsed AA (56%) and refractory AA (37%) but lower for untreated AA (19% response), so its use is not recommended as first-line therapy. High-dose cyclophosphamide (200 mg/kg body weight) without haematopoietic stem cell support had been proposed as an alter- native treatment for refractory AA. However, a prospective ran- domized study comparing cyclophosphamide and ciclosporin with ATG and ciclosporin was terminated prematurely because of a high incidence of systemic fungal infections and early deaths Fig. 22.5.2.6 Overall survival (OS) of patients with severe aplastic anaemia transplanted with ‘FCC’ conditioning regimen. (a) OS for patients transplanted from matched sibling donors (MSD) and matched unrelated donors (MUD). (b) OS according to co-morbidity index. (c) OS comparing patients aged <50 years with those aged ≥50 years. GVHD, graft versus host disease; CI, cumulative incidence. Reproduced with permission from Marsh JCW, et al. (2011). Alemtuzumab with fludarabine and cyclophosphamide reduces chronic graft versus host disease after allogeneic stem cell transplantation for acquired aplastic anemia. Blood, 118, 2351–7.

 after cyclophosphamide. Furthermore, when the dose was reduced to 120 mg/kg there were still unacceptable toxicities and mortality on account of the predictably prolonged period of neutropenia and thrombocytopenia. Consequently, the use of cyclophosphamide alone to treat AA is not now generally recommended. Oxymetholone and corticosteroids Oxymetholone is sometimes used in AA, most often in inherited AA such as FA and DC when HSCT is not possible. Up to 25% of patients with severe acquired, refractory AA will

respond to this drug. A response to androgens should raise the possibility of an inherited BMF disorder. The hepato- toxicity and virilizing side effects restrict their use. Corticosteroids have no role in the treatment of AA, other than helping to prevent the complication of serum sickness following treatment with ATG. Corticosteroids are not effective in treating AA and increase the risk of infections and later complications such as avascular necrosis. Haematopoietic growth factors As serum levels of endogenous haematopoietic growth factors such as G-CSF, IL-3, erythropoietin (EPO), thrombopoietin, and stem cell factor are invariably markedly elevated in AA, clinical treatment with any of these agents is inef- fective in most cases. There is no indication for using G-CSF or EPO to treat the underlying disease. Eltrombopag The thrombopoietin receptor agonist, eltrombopag, has been licensed for treatment of refractory severe AA patients who have failed to respond to a course of IST. Response rates are 40–50%, but there is a 20% risk of early cytogenetic clones including monosomy 7, so patients must be monitored with regular BM cytogenetic analysis. More recently, a phase II study of horse ATG, ciclosporin and eltrombopag for first line treatment of severe AA reported mark- edly high response rate compared to historical data. A European prospective randomised study (‘RACE’ study) comparing ATG and ciclosporin with or without eltrombopag as first line therapy, has recently been completed and data analysis is in progress. Inherited aplastic anaemia (See Chapter 22.5.1.) Early diagnosis is important to (1) initiate a management plan and to avoid inappropriate treatment that would be given for acquired AA, (2) help prevent multiorgan complications from treatment, (3) institute genetic counselling, (4) ensure selection of appropriate donors for fu- ture HSCT, and (5) to ensure long term cancer surveillance programme. Bone marrow failure affecting the erythroid cell lineage: pure red-cell aplasia PRCA is an acquired or inherited disorder, characterized haemato logically by severe normocytic, normochromic anaemia (the an- aemia in inherited PRCA is macrocytic), reticulocytopenia, and reduced or absent erythroid progenitors in the BM. The white cell, neutrophil, and platelet counts are normal. The aetiology and patho- physiology are heterogeneous. Acquired PRCA Aetiology Acquired PRCA can present in different forms. It may present in children with transient anaemia following a viral infection (tran- sient erythroblastopenia of childhood (TEC)). In a proportion of cases of TEC, there may also be neutropenia. Occasionally, cases occur in clusters, suggesting exposure to a virus or toxin, but a common virus has not been proven. The natural history is of spon- taneous recovery over a few weeks. The most important differen- tial diagnosis is inherited PRCA, Diamond–Blackfan anaemia, but in contrast to Diamond–Blackfan anaemia, the presentation of TEC is later (most cases occur after 1 year of age), the anaemia is normocytic and normochromic, levels of fetal haemoglobin and red cell adenine deaminase are normal and patients lack somatic anom- alies of Diamond–Blackfan anaemia. Another acquired condition to exclude in children is Pearson’s syndrome, a rare sporadic mito-chondrial cytopathy due to mutations in mitochondrial DNA and characterized by sideroblastic anaemia with vacuolization of haem- atological precursors, neutropenia and thrombocytopenia, exocrine pancreatic insufficiency, and metabolic acidosis. Acquired PRCA may be immune mediated and idiopathic, but all cases of acquired PRCA should be investigated thoroughly for a possible secondary cause, as PRCA may occur in association with a wide range of disorders or conditions (Table 22.5.2.4). A thymoma occurs in up to 10% of patients with PRCA and up to 5% of patients with thymoma have PRCA. PRCA may precede, Table 22.5.2.4 Secondary causes of pure red cell aplasia Associations Examples/comments Thymoma May be associated with hypogammaglobulinaemia (Good’s syndrome) and myasthenia gravis Systemic autoimmune disorders Rheumatoid arthritis, systemic lupus erythematosus, Sjögren’s syndrome, mixed connective tissue disease B-lymphoproliferative and plasma cell disorders Chronic lymphocytic

leukaemia, lymphoma, Waldenstroms macroglobulinaemia, MGUS T-LGL Myeloid blood disorders MDS, myeloproliferative disorder, e.g. myelofibrosis Solid tumours Gastric carcinoma, renal cell carcinoma Infections B19 parvovirus, HIV, EBV, CMV, hepatitis B, C Drugs Phenytoin, isoniazid, rifampicin, azathioprine, procainamide, EPO in CKD (anti-EPO antibodies) ABO incompatible haematopoietic stem cell transplantation Recipient antibody against incompatible donor ABO blood group. Recent reports of response to daratumumab (anti CD38 monoclonal antibody) Pregnancy CKD, chronic kidney disease; CMV, cytomegalovirus; EPO, erythropoietin; MDS, myelodysplastic syndrome; T-LGL, T-cell large granular lymphocytic leukaemia.

22.5.2 Acquired aplastic anaemia and pure red cell aplasia 5347 accompany, or follow the development of a thymoma. Myasthenia gravis is a frequently reported autoimmune association with thymoma. Good's syndrome describes the occurrence of thymoma with im- munodeficiency, characterized by hypogammaglobulinaemia, and B- and T-cell dysfunction. Because thymoma and PRCA is a rare as- sociation, optimal treatment is not known. Historical data report that surgical resection of the thymoma induces remission of the PRCA in about 25% of cases; the PRCA may also relapse later after thymec- tomy. A study from the Mayo Clinic reported on the outcome of 13 patients seen with PRCA and thymoma over a 50-year period. Out of 12 patients who underwent thymectomy, only one achieved a normal haemoglobin level and all other patients required additional treat- ment for the PRCA. At a median follow-up of 26 months, four were in complete remission and eight required continued blood transfusions. Nevertheless, it is proposed that thymectomy should be considered if the patient is clinically fit enough for surgery. PRCA may occur in association with a wide range of systemic auto- immune disorders, including rheumatoid arthritis, systemic lupus erythematosus, and Sjögren's syndrome. The most common viral in- fection associated with PRCA is B19 parvovirus, and rarely following EBV, cytomegalovirus (CMV), or hepatitis B and C. Parvovirus- associated PRCA may occur in (1) patients with haemolytic anaemia such as hereditary spherocytosis, sickle cell disease, pyruvate kinase deficiency, and glucose-6-phosphate dehydrogenase deficiency (pro- ducing a so-called aplastic crisis); and (2) immunocompromised pa- tients, for example, transplant recipients, patients with HIV, or during chemotherapy or monoclonal antibody therapy for lymphoma. The re- ceptor for B19 parvovirus is the blood group P antigen expressed on the CFU-E erythroid progenitors and mature red cells. The virus invades and destroys the cells, resulting in sudden and severe anaemia with reticulocytopenia. PRCA secondary to parvovirus infection responds well to intravenous immunoglobulin. Lymphoproliferative disorders, such as chronic lymphocytic leukaemia and Hodgkin's or non- Hodgkin's lymphoma, may sometimes be complicated by PRCA. In con- trast, PRCA occurs more commonly with T-lymphocytosis (T-LGL). T-LGL may also be associated with other cytopenias, such as auto- immune neutropenia or immune thrombocytopenic purpura. It is a clonal disorder as demonstrated by rearrangement of the TCR. The immunophenotype is typically CD3, CD8, and TCRαβ positive, more rarely CD4 TCRαβ or TCRγδ positive. A long list of drugs has been incriminated in the aetiology of red cell aplasia, but the association is very rare and mostly there is only a single case report for each drug. Exceptions are azathioprine, pheny- toin, procainamide, and isoniazid, for which more cases have been re- ported. Severe and sudden onset of PRCA due to EPO may occur. This is due to anti-EPO antibodies against endogenous EPO, and in most cases it was associated with the use of epoetin-α (Eprex) when given subcutaneously in chronic kidney disease. EPO-induced PRCA had a peak incidence in 2001 to 2002 and was most likely due to changes in the formulation of the drug and the use of uncoated rubber stoppers. Clinical investigations 1. Full blood count: anaemia, reticulocytopenia, normal white cell, neutrophil, and platelet counts. Normal mean cell volume and mean cell haemoglobin in acquired

PRCA; macrocytosis in Diamond–Blackfan anaemia. Examine blood film for T-LGLs, smear cells, and small mature lymphocytes of B-CLL. 2. BM aspirate and trephine: absence or reduction in erythroid pro- genitors; sometimes maturation arrest at proerythroblast stage; giant pronormoblasts in parvovirus-induced PRCA. Examine BM for secondary MDS, myeloproliferative disorder and CLL, lymphoma, and T-LGL. Parvovirus B19 DNA in situ hybridiza- tion study on BM slides. 3. Autoimmune profile to exclude rheumatoid arthritis, systemic lupus erythematosus, and other autoimmune disorders. 4. Anticholinesterase receptor antibodies to exclude myasthenia gravis. 5. Serum immunoglobulins to exclude hypogammaglobulinaemia. 6. Viral screen: parvovirus serology and/or B19 DNA in serum, HIV, EBV, CMV, and hepatitis B and C. 7. Immunophenotyping and gene rearrangement studies for heavy- chain and TCR monoclonal expansions. 8. CT scan of chest to exclude thymoma; CT scan of chest, ab- domen, and pelvis to exclude lymphoma. 9. For Diamond–Blackfan anaemia, elevated erythrocyte adenine deaminase in 80 to 85% of patients; fetal haemoglobin often elevated and i red blood cell antigen expressed. Research testing for mutation in one of the known ribosomal protein genes (see Chapter 22.5.1). Treatment Blood transfusions are required until recovery occurs. Any drugs that have been implicated in the disease should be discontinued. Thymectomy should be considered in patients with a thymoma. Intravenous im- munoglobulin is indicated for parvovirus-induced PRCA. Secondary causes of PRCA may respond to treatment of the underlying condition but specific treatment of the PRCA is often required. Immunosuppression is the mainstay of treatment for idiopathic acquired and secondary PRCA not responding to treatment of the underling disorder, based on the findings of a wide range of im- munological abnormalities involving serum autoantibodies, and T-cell and NK cell-mediated effects on erythropoiesis. The op- timal treatment of PRCA is restricted by the rarity of the condition. Historically, the first-line treatment used to be prednisolone at a dose of 1 mg/kg per day, to which 30 to 60% of patients responded. Issues relating to corticosteroids are (1) relapse which is common and occurs in around 80% of patients; and (2) toxicity, for example, infection, diabetes, weight gain, avascular necrosis and osteoporosis, especially with long-term use. For nonresponders, remission may be induced by cytotoxic im- munosuppressants such as azathioprine and cyclophosphamide, but there are concerns about the long-term risk of malignancies and gonadal toxicity. ATG has sometimes been used, with anecdotal re- ports of response in PRCA, but this requires inpatient treatment and the main side effects include infection and severe allergic reaction. For the above-mentioned reasons, there has been a move away from corticosteroids and cytotoxic immunosuppressants in the treatment of PRCA. Instead, oral ciclosporin (CSA) has become the first-line treatment. CSA was first proposed as a first-line treat- ment for PRCA back in 1990 when review of the literature showed an overall response rate of 65%. Subsequent clinical studies, most from Japanese national surveys, report excellent results with CSA. For primary acquired PRCA, the response rate is 74 to 87% and 60 to 73% for secondary PRCA. On withdrawal of CSA, the risk of relapse is high. It appears that continued CSA, at a lower dose, is required to maintain remission in the long term. It is important to monitor patients’ renal function, blood pressure, and CSA blood levels for potential long-term toxicity of the drug.

Revision #1
Created 2026-01-22 16:42:37 UTC by Omar Ayman
Updated 2026-01-22 16:42:37 UTC by Omar Ayman