

* CHAPTER 42

HSCT for hereditary bone marrow failure syndromes

E. Gluckman, J.E. Wagner

1. Introduction

Aplastic anaemia is a rare disease in children. It is most commonly idiopathic and less often due to a hereditary disorder. However, hereditary bone marrow failure (BMF) syndromes should be considered in both children and adults before the institution of any therapeutic treatment plan.

While new genetic tests are being developed, these are not widely available. Genomic instability in the presence of clastogenic agents is the hallmark of Fanconi anaemia (FA). In contrast, marked telomere dysregulation is characteristic of dyskeratosis congenita (DKC). Mutations affecting ribosome assembly and function are associated with DKC, Shwachman-Diamond syndrome, and Diamond-Blackfan anaemia (DBA) (1).

The principal characteristics of these diseases are detailed in [Table 1](#).

2. Fanconi anaemia (FA)

FA is a rare autosomal recessive disease characterised by congenital abnormalities, progressive BMF, chromosome breakage, and cancer susceptibility. At least 13 genes have been involved in the disease; abnormalities in any of these gene products that normally interact disrupt the FA/BRCA biochemical pathway (2) (see [Table 1](#)).

FA patients often have skeletal, thumb or limb abnormalities and abnormal skin pigmentation (café au lait spots). Other commonly involved organ systems include cardiac, renal system and auditory. Low birth weight and growth retardation are frequent (see [Table 2](#)).

The haematological consequences of FA often develop in the first decade of life but absence of malformations and reversion due to somatic mosaicism can result in delayed or failed diagnosis in a small proportion of patients. Death, however, often results from the complications of BMF or occurrence of malignancy. The most frequent malignancy is AML with clonal cytogenetic abnormalities in the bone marrow; older patients are at high risk of squamous cell carcinomas of the oesophagus, head and neck and urogenital tract.

2.1. Disease-specific pre-HSCT work-up

FA being a heterogeneous disease, clinical diagnosis is not always sufficient to assess the correct diagnosis in children or young adults with AA. Other constitutional AA may have similar congenital abnormalities and FA patients may have no abnormalities.

Table 1: Principal characteristics of hereditary BMF syndromes

Disease	Mutations	Diagnostic tests	Clinical expression
Fanconi anaemia	<i>FANCA</i> <i>FANCB</i> <i>FANCC</i> <i>FANCD1/BRCA2</i> <i>FANCD2</i> <i>FANCD3</i> <i>FANCD4</i> <i>FANCD5</i> <i>FANCD6</i> <i>FANCD7</i> <i>FANCD8</i> <i>FANCD9</i> <i>FANCI</i> <i>FANCD10/BACH1/BRIP1</i> <i>FANCL/PHF9/POG</i> <i>FANCM/HeF</i> <i>FANCN/PALB2</i>	Mitomycin (MMC) or di-expoxybutane (DEB)-induced chromosomal breakage in blood lymphocytes Flow cytometry evidence of cycle arrest on exposure to MMC FANCD2 monoubiquitination for 90% of patients with abnormalities in the FA/BRCA2 pathway upstream of FANCD2	Aplastic anaemia Malformations: thumbs, cafe au lait spots, microcephaly, short stature, kidney, heart, etc. Malignancies: acute leukaemia (AML), squamous cell carcinoma head and neck, anogenital, liver
Dyskeratosis congenita	<i>DCK1</i> <i>TERC</i> <i>TERT</i>	Telomere length	Aplastic anaemia Malformations Pigmentation, nail dystrophy, oral leukoplakia Malignancies: AML, solid tumours: oesophagus, gastro intestinal tract, bladder
Seckel syndrome	<i>SCKL1</i> <i>SCKL2</i> <i>SCKL3</i>	None	Aplastic anaemia Malformations: bird-head, dwarfism, mental retardation
Shwachman-Diamond	<i>SBDS</i>	Decreased serum trypsinogen and/or pancreatic isoamylase	Aplastic anaemia Pancreatic exocrine deficiency
Kostmann	<i>Neutrophil elastase</i> <i>ELA2</i>	None	Chronic neutropenia, leukaemia
Diamond-Blackfan	<i>RSP19</i>	Elevated adenosine deaminase (ADA)	Erythroblastopenia Malformations: thumb, renal cardiac
Congenital amegakaryocytic thrombocytopenia	<i>c-Mpl</i>	None	Amegakaryocytosis, aplastic anaemia

Table 2: Evaluating new onset cytopenia in children

1. Family history	6. BM aspiration with cytogenetics, BM biopsy if necessary
2. Malformations	7. Chromosome breaks with DEB or MMC
3. Date of onset	8. Alfa foeto-protein
4. Liver function tests	9. HLA typing
5. Blood counts	10. FANCD2

Diagnosis is suspected with:

- Blood counts: pancytopenia with macrocytic anaemia
- Raised alfa-foetoprotein and haemoglobin F

Diagnosis is confirmed with:

- PB lymphocyte cytogenetics with clastogenic agents: nitrogen mustard, DEB or MMC showing increased chromosome breaks with tri- and quadri-radial figures
- Study of the cell cycle showing a G2/M arrest increased by incubation with clastogenic agents

Other tests

- BM cytogenetic abnormalities for diagnosis of leukaemia or myelodysplastic abnormalities with abnormalities in chromosomes 1, 3, 7, 5, 8 and 11 being the most common

New tests not for routine use

- Ubiquitination of FANCD2: this test is specific and sensitive, if negative skin fibroblasts may be positive for FA and this confirms the existence of mosaicism
- Identification of the complementation group with retroviral or lentiviral vectors
- Sequencing and identification of the mutation. This test is useful for preimplantation diagnosis and perhaps for assessing the prognostic

2.2. Results of allo-HSCT in FA

HSCT is currently the only treatment that definitively restores normal haematopoiesis. FA anaemia cells are hypersensitive to DNA cross-linking agents. Cellular exposure to genotoxic agents including cyclophosphamide (Cy), busulfan (Bu) or irradiation increases chromosome breaks and tissue damage. Graft-versus-host disease (GvHD) induces severe tissue damage and absence of repair (3). Standard conditioning with

high dose Cy and total body irradiation (TBI) as for patients with SAA must not be used.

2.2.1. HLA identical sibling transplant

With the use of reduced intensity conditioning regimens, most reports over the past decade have demonstrated good results. In our series of 117 FA patients, conditioned with low dose CY and total lymphoid irradiation (TLI), 5-year survival was 85%. In general, most series have reported younger patient age, higher pre-transplant platelet counts, absence of previous treatment with androgens, normal pre-transplant liver function tests and limited malformations as factors associated with better survival after transplantation (4).

Unfortunately, a rising risk of cancer has been observed in long-term survivors, particularly cancers of the head and neck (5). In an analysis of 700 patients with FA (n=79) or AA (n=621) treated with allogeneic HSCT in Seattle or Paris, the Kaplan-Meier estimate for developing any malignancy by 20 years after transplantation was 14% (6). Among patients with FA, a single hazard peak for solid tumours occurred between 8 and 9 years after HSCT. The Kaplan-Meier estimate of developing any malignancy by 20 years after HSCT was 42% (95%CI, 10–74%), with all being solid tumours. In the multivariate analysis of the 700 patients with marrow failure (FA and non-FA), the diagnosis of FA (relative risk [RR] 11.2, p=0.0001) and treatment with azathioprine (RR 11.7, p=0.0001) were independent predictors of secondary malignancy. Absence of irradiation in the conditioning regimen did not abolish the risk of secondary tumours. As solid tumours occur in FA patients without prior exposure to chemotherapy and radiation, it is clear that cancer is at least in part related to the specific genetic defect present and environment, as shown by different phenotypic expression of the disease in homozygous twins.

In an attempt to reduce the potential impact of irradiation and GvHD on the risk of late effects, including cancer, newer regimens have replaced TLI with fludarabine in combination with low dose CY as well as used T-cell depletion to reduce the risk of GvHD. While limited in number and follow-up, early results are encouraging (7). It is too early to tell if there is any impact upon cancer risk at this time.

Nonetheless, the good results of HLA-identical sibling HSCT raise several questions regarding the optimal timing of BMT and the best conditioning regimen. Concerning the former, there is a general agreement that HLA-identical sibling HSCT should be performed as first-line therapy, without first using androgens or corticosteroids, which have considerable side effects. When blood counts fulfil the criteria of severe AA (Hb <8 g/dL, neutrophils <0.5 × 10⁹/L, or platelets <20 × 10⁹/L), transfusions and infections are more likely, making this a suitable time to perform HSCT. During the

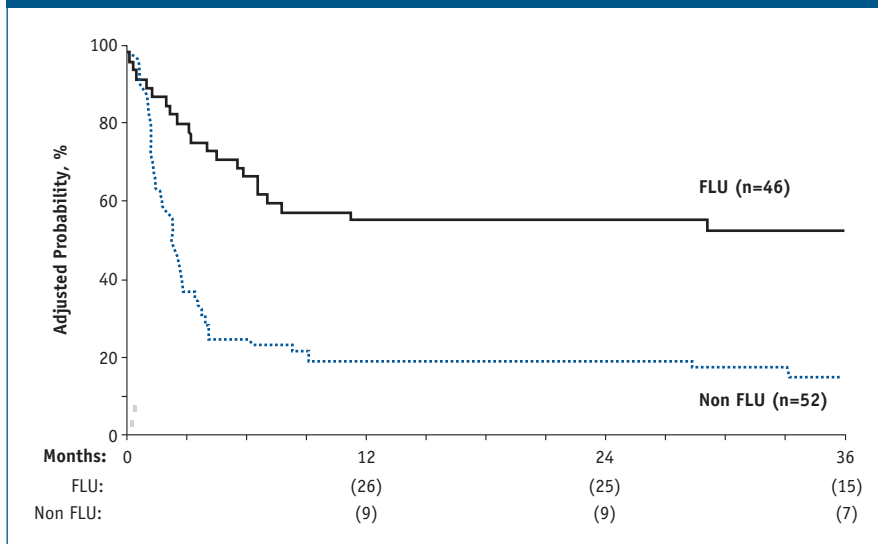
waiting period, it is important to regularly perform bone marrow aspiration and cytogenetic analysis for detection of clonal abnormalities or leukaemic transformation. Results show that transplants performed late (after a long period of aplasia or during leukaemic transformation) are associated with markedly poorer results. Most patients treated for acute leukaemia do not tolerate standard dose chemotherapy and have a very poor prognosis, although some long-term survivors have been reported after HSCT. In terms of the best conditioning regimen, the aims are to 1) avoid rejection in a population of patients who have received multiple transfusions, 2) limit early and late toxicities and 3) minimise risk of GvHD. Several combinations have been used in a limited number of patients. Fludarabine containing regimens, in combination with low dose CY or Bu and ATG, appear to be well tolerated. GvHD must be prevented, as it is more likely to be severe in FA patients because of the underlying DNA repair defect and because lichen planus lesions associated with chronic GvHD may be a precursor of squamous cell carcinoma. Cyclosporin A (CsA) and mycophenolate mofetil (MMF) are being used more frequently as methods of GvHD prophylaxis with some groups incorporating T-cell depletion of sibling donor marrow.

2.2.2. Results of unrelated donor adult volunteer HSCT

The EBMT working party on AA has analysed the outcome of alternative donor HSCT in 67 FA patients. The median 2-year survival was $28\pm 8\%$. Causes of death included infection, haemorrhage, acute GvHD, chronic GvHD, hepatic veno-occlusive disease (VOD), and multi organ failure (MOF) (8).

The CIBMTR analysed data from 98 patients transplanted with unrelated donor marrow (excluding those with peripheral blood or umbilical cord blood [CB] grafts) between 1990 and 2003 (9). Probabilities of neutrophil (89 vs. 69%, $p=0.02$) and platelet recovery (74 vs. 23%, $p<0.001$) were higher after fludarabine (Flu) than non-Flu containing regimens. Risks of acute (RR 2.95, $p=0.003$) and chronic GvHD (RR 3.30, $p=0.03$) were significantly higher in recipients of non T-cell depleted than T-cell depleted grafts. Day-100 mortality rate was significantly higher after non-Flu than Flu containing regimens (65 vs. 24%, respectively $p<0.001$). Corresponding 3-year adjusted overall survival rates were 13% vs. 52% ($p<0.001$) with best survival in those treated a Flu-based regimen (Figure 1). In addition, mortality was higher in recipients who were older (>10 years), CMV seropositive and who had received >20 blood product transfusions pre-transplant. Based on these results significant changes in practice were suggested: use of a Flu-containing conditioning regimen in the context of T-cell depleted marrow allografts and earlier referral with transplantation prior to excessive transfusions.

Figure 1: Impact of fludarabine on survival in patients with FA treated with unrelated donor HSCT



2.2.3. Results of related and unrelated donor CB transplantation (CBT)

In the circumstance where an HLA identical sibling donor is available, CBT and BMT give similar results in terms of survival. However, reports comparing the two demonstrate a reduction in the frequency and severity of acute and chronic GvHD related to the relative immaturity of neonatal T-cells.

However, the majority of patients do not have an HLA matched sibling donor. Eurocord analysed the results of unrelated CBT in 93 FA patients (10). The incidence of neutrophil recovery at 60 days was $60 \pm 5\%$. In addition to high cell dose, Flu-containing regimens (as in marrow recipients) (9) were associated with better neutrophil engraftment. The incidence of acute and chronic GvHD was 32.5% and 16%, respectively. Overall survival was $40 \pm 5\%$. In multivariate analysis factors associated with favourable outcome were use of Flu, high number of cells infused and negative recipient CMV serology.

To date, there has been no formal comparison of outcomes in recipients of unrelated CB and marrow. However, results demonstrate that Flu is associated with better survival regardless of stem cell source in patients with FA. This suggests that Flu, a potent immune suppressive agent, enhances engraftment without paying the price

of extra-medullary toxicity. In the future, studies may help us determine the place of CB. For now, CB is clearly indicated in those FA patients for whom an HLA-A, B, C, DRB1 allele-matched unrelated volunteer donor cannot be identified. Preimplantation genetic diagnosis (PGD) to select an embryo produced by in vitro fertilisation that is both unaffected by a heritable genetic disease and HLA identical to the affected recipient has been performed. Clearly, this approach is controversial (11) with marked differences in its acceptance by different countries worldwide. Globally, the strategy has been most often used for couples at high risk of having children with thalassaemia. However, the first successful use of PGD for a specific HLA type was for a child with FA. With this approach, the couple can avoid the risk of having additional affected children (and the consequent consideration of abortion) and also have a healthy child that will be an HLA identical match with the existing child needing HSCT. In these cases, it is typical for the CB to be collected at birth, eliminating risk to the newborn child. In the U.S., the use of the technology is expanding. To date, 5 transplants have been successfully performed in patients with FA.

2.2.4. Post-HSCT monitoring in FA

Patients with FA require particular attention because of their sensitivity to toxic agents, various organ dysfunctions due to congenital malformations and increased risk of developing malignancies. This should include at least yearly endocrinological and growth follow-up, bone marrow cytogenetic and oral follow-up. Patients with oral lichen planus should be biopsied regularly and lesions removed.

3. Other congenital cytopenias

3.1. Dyskeratosis congenita

Dyskeratosis congenita (DKC), also known as Zinsser-Engman-Cole syndrome, is a rare, progressive bone marrow failure syndrome characterised by the triad of reticulated skin hyperpigmentation, nail dystrophy, and oral leukoplakia. Evidence exists for telomerase dysfunction, ribosome deficiency, and protein synthesis dysfunction in this disorder. Early mortality is often associated with bone marrow failure, infections, fatal pulmonary complications, or malignancy. Results of HSCT are disappointing because of severe late effects including diffuse vasculitis and lung fibrosis (9).

Conditioning with a Flu-containing non-myeloablative regimen may give better short-term results but may not delay fatal outcome due to multi-organ failure related to the underlying genetic defect.

3.2. Seckel syndrome

Seckel syndrome is a rare autosomal recessive disorder with growth retardation, microcephaly with mental retardation and a characteristic bird-headed facial appearance. Two loci have been identified. Very few transplants have been reported in the literature. In one case late pulmonary fibrosis occurring 2 years after transplant was the cause of death despite an early favourable outcome.

3.3. Shwachman-Diamond syndrome

Shwachman-Diamond syndrome is an autosomal recessive disorder with clinical features that include pancreatic exocrine insufficiency, skeletal abnormalities and pancytopenia (10). AML transformation has been observed. Very few patients have been treated by allogeneic HSCT.

3.4. Diamond-Blackfan anaemia

DBA is characterised by chronic constitutional aregenerative anaemia with absent or decreased erythroid precursors in the BM. Both autosomal dominant and recessive inheritance are described.

Most patients present with anaemia in the neonatal period or in infancy. Approximately 30% patients have a variety of physical anomalies including thumb, upper limb, craniofacial, heart and urogenital malformations.

Usually, the patients are treated with transfusions and steroids and at least 50% patients respond. Allogeneic HSCT is an option in steroid resistant patients. In a report from the DBA Registry, 354 patients were registered and 20 were transplanted. The 5-year OS for HLA identical sibling transplants was 87.5%. Results were poor with alternative donors. CIBMTR reported 61 patients transplanted from 1984 to 2000. Most patients (67%) were transplanted with HLA identical donors. The 3 year probability of overall survival was 64%. Results were better in HLA identical sibling transplants (11).

3.5. Kostmann syndrome

Kostmann syndrome is an inherited disorder with severe neutropenia and early onset of severe bacterial infections. More than 90% of the patients respond to r-HuG-CSF but approximately 10% will develop MDS/AML, regardless of their treatment or response.

Allo-HSCT is the treatment of choice in patients refractory to G-CSF or with acute leukaemia (12). In the French chronic neutropenia registry including 101 patients, 9 patients were transplanted, 7 with an unrelated donor and 2 with an HLA identical sibling donor. Four patients had acute leukaemia, 4 were refractory to G-CSF and 1 had BMF. The OS at 5 years was 61% indicating that HSCT should be considered in these patients even if there is no HLA identical sibling.

3.6. Inherited thrombocytopenia

3.6.1. Congenital amegakaryocytic thrombocytopenia

Affected infants are identified within days or weeks of birth. Transmission is autosomal recessive. Despite optimal supportive care, AA develops leading to death in the absence of HSCT, which is the only chance of cure in this disease.

3.6.2. Thrombocytopenia with absent radii

TAR syndrome includes shortened or absent forearms due to the absence of development of the bilateral radii, associated with severe thrombocytopenia at birth. Skeletal anomalies are also seen in other bones but do not affect the hands and fingers. Usually the degree of thrombocytopenia is greatest at birth requiring transfusions, however thrombocytopenia becomes less severe during the first year of life and most patients will not require platelet transfusion after infancy. HSCT is not recommended.

3.7. Other rare inherited BMF syndromes

Nijmegen breakage syndrome (NBS) is a rare autosomal recessive condition of chromosomal instability that is clinically characterised by microcephaly, a distinct facial appearance, short stature, immunodeficiency, radiation sensitivity, and a strong predisposition to lymphoid malignancy. Mutations in the *NBS1* gene located in band 8q21 are responsible for NBS.

Pearson syndrome is currently recognised as a rare, multisystemic, mitochondrial cytopathy. Its features are refractory sideroblastic anaemia, pancytopenia, defective oxidative phosphorylation, exocrine pancreatic insufficiency, and variable hepatic, renal, and endocrine failure. Death often occurs in infancy or early childhood due to infection or metabolic crisis. Patients may recover from the refractory anaemia. Older survivors have Kearns-Sayre syndrome (KSS), which is a mitochondriopathy characterised by progressive external ophthalmoplegia and weakness of skeletal muscle.

DNA ligase IV deficiencies due to mutation of LIG4 gene is a rare disease; one case of successful transplant has been described.

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Multiple Choice Questionnaire

To find the correct answer, go to <http://www.esh.org/ebmt-handbook2008answers.htm>

1. Fanconi anaemia is diagnosed by the finding of which one of the following:

- a) Presence of malformations
- b) Aplastic anaemia
- c) Bone marrow biopsy
- d) Cytogenetics in PBL with clastogenic agents

2. Presence of bone marrow failure with malformations, leukoplakia and nail dystrophy is more likely to be which of the following?

- a) Fanconi anaemia
- b) Dyskeratosis congenita

- c) Shwachman syndrome
- d) Diamond-Blackfan syndrome
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3. The best first line therapeutic option in Kostmann disease is:

- a) Supportive care
- b) Antibiotics
- c) G-CSF
- d) Haematopoietic stem cell transplantation
-

4. Prior to initiating treatment of an adult with aplastic anaemia, which tests should be done?

- a) DEB or MMC to rule out FA
- b) Bone marrow aspirate with cytogenetic evaluation
- c) HLA type family members and patient
- d) Perform molecular testing to rule out Shwachman-Diamond syndrome and DBA
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5. In Fanconi anaemia conditioning for HSCT must be reduced because:

- a) Aplastic anaemia is not severe
- b) Hypersensitivity to clastogenic agents
- c) Age of the patients
- d) Absence of leukaemia