Bone Marrow Transplantation for Fanconi Anaemia Using a Fludarabine-based Preparative Regimen with CD34+ Cell Selection

SY HA, GCF CHAN, AKS CHIANG, ACW LEE, TL LEE, YL LAU

Department of Paediatrics and Adolescent Medicine, The University of Hong Kong, Queen Mary Hospital, Pokfulam Road, Hong Kong, China

SY HA (夏張賢) MBBS, FRCP(Edin), FHKAM(Paed)
GCF CHAN (陳志雄) MD, FHKAM(Paed)
AKS CHIANG (趙國強) MBChB(Manc), PhD, FHKAM(Paed)
TL LEE (李子良) MBBS, FHKAM(Paed)
YL LAU (劉宇隆) MD, FHKAM(Paed)

Department of Paediatrics and Adolescent Medicine, Tuen Mun Hospital, Tsing Chung Koon Road, Tuen Mun, N.T., Hong Kong, China

ACW LEE (李志輝) MBBS, FHKAM(Paed)

Correspondence to: Dr SY HA

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Abstract

Fanconi anaemia (FA) is an important cause of inherited aplastic anaemia in childhood because of its relatively high frequency of occurrence, the implication for different management, and the need for genetic counselling. The common manifestations are congenital physical abnormalities, marrow failure, and predisposition to development of cancers. Bone marrow transplantation has been used to treat marrow failure for FA patients but they are at increased risks of transplant related toxicities and graft versus host disease. We report two children with FA who were treated successfully with matched sibling bone marrow transplantation using a new fludarabine-based conditioning regimen without irradiation. The stem cell source was from marrow which was infused after positive selection of CD34+ cells. Both patients had haematological recovery and no major post-transplant complications occurred. One achieved full donor chimerism and stable mixed chimerism was present in another. This regimen appears to be effective and can prevent FA patients from major transplant related complications.

Key words
Aplastic anaemia; Bone marrow transplantation; Fanconi anaemia

Introduction

Inherited marrow failure syndromes comprise 30-35% of cases of paediatric marrow failure while Fanconi anaemia (FA) represents about two-third of the total.1 Other than Fanconi anaemia, the inherited marrow failure syndromes also include rare conditions such as Shwachman-Diamond syndrome, dyskeratosis congenita, Diamond-Blackfan anaemia, Kostmann’s syndrome etc. FA is an autosomal recessive disorder and there are currently at least 11 known FA genes with diverse mutations.2 The manifestations in FA are heterogeneous with variable phenotypic expression. These include various congenital physical abnormalities such as skin hyper-pigmentation, café au lait spots, short stature, abnormal thumbs and radii, and organ malformations. Marrow failure occurs in most FA patients and approximately 3 quarters of them develop evidence of marrow failure within the first decade of life.3,4 FA patients are also predisposed to develop haemic malignancies and solid tumours.5 It should be noted that 25% or more of the known FA patients have few or none of the physical abnormalities. The diagnosis of FA therefore relies on alertness of clinicians and should then be confirmed by demonstration of cellular hypersensitivity to DNA clastogenic (cross-linking) agent, such as the diethylnitrosamine (DEB) test which should induce excessive chromosome breakage or aberrations in FA lymphocytes.

FA is an important cause of aplastic anaemia in childhood because of the relatively high frequency of occurrence, the
implication to different treatment and the need for genetic counselling. Bone marrow transplantation (BMT) can reverse the marrow failure but the major problems are non-engraftment of graft, transplant related toxicities in particular graft versus host disease (GVHD) and also increased risk of cancer development after the transplant. Different preparative or conditioning regimens have been tried to reduce transplant related complications. Regimen with reduced cyclophosphamide at a dose of 20 mg/kg and low dose thoraco-abdominal irradiation were applied in transplant with reasonable results. In recent years, more and more transplant centres tried to exclude irradiation from the preparative regimen. We report two cases of severe FA treated with matched sibling allogeneic BMT with a new fludarabine-based preparative regimen without irradiation while the stem cell source was processed by positive selection CD34+ cells before infusion to the FA patients.

Case Reports

Case 1

Case 1 was a 13-year-old girl who developed insidious onset of anaemic symptoms in late 2002 while she was living in Mainland China. She also had recurrent nose bleeding which was difficult to stop. Investigation at a hospital in Mainland China showed Hb 3.9 g/dl, WBC 1.7 x 10^9/L, and platelet 22 x 10^9/L. She had no febrile episode before presenting to the hospital. She did not have any exposure to chemicals or drugs before disease onset. She was managed as a case of paroxysmal nocturnal haemoglobinuria based on a positive acid lysis test. She was treated with prednisolone for about 8 months but there was no obvious improvement in the haematological parameters. She had 3 episodes of red cell transfusion and 1 episode of platelet transfusion before moving to Hong Kong in the mid 2003. After admission to Queen Mary Hospital, she was noted to have abnormal physical features including multiple areas of hyper-pigmentation, a number of café au lait spots and a surgical scar over lateral aspect of right thumb base which was the result of surgical resection of extra thumb in infancy. Body height was at 75th percentile and body weight at 10-25th percentile. It was also noted that the parents are cousins. Full blood count showed: Hb 5.9 g/dl, MCV 110 fl, neutrophil 1.7 x 10^9/L and platelet 33 x 10^9/L. Ham's test was negative. Trephine biopsy confirmed severe hypoplastic marrow. DEB test on peripheral blood lymphocytes confirmed abnormal increase of chromosome breakage. Her 16-year-old elder sister has been all along healthy and is HLA-matched with the index patient. Blood count of this potential marrow donor was normal and DEB test did not show increased number of chromosome breakage or aberrations. ABO blood group is the same for both the patient and the donor.

In November 2003, we proceeded with bone marrow transplantation with a new fludarabine-based regimen adopted from the University of Minnesota. The regimen consisted of fludarabine (total 175 mg/Kg divided in 5 doses, D-6 to D-2), anti-thymocyte globulin (total 150 mg/Kg divided in 5 doses, D-6 to D-2, ATG Upjohn), and cyclophosphamide (total 20 mg/Kg divided in 4 doses, D-6 to D-3) as the preparative regimen. No irradiation was given. Stem cell source was from the donor’s bone marrow which was infused after processing by Isolex system (300i, v 2.5 device) which employs immunomagnetic selection of CD34+ stem cells. Through the system, CD34+ stem cells are concentrated and used for transplant while T-cells which are responsible for GVHD are depleted. The number

| Table 1 | The nucleated cell dose, CD34+ cell dose and CFU-GM unit of the pre-selection marrow and post-selection fraction after CD34+ stem cell selection by Isolex system |
|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| Case 1                        | Case 2                        | Pre-selection marrow          | Post-selection fraction       | Pre-selection marrow          | Post-selection fraction       |
| Nucleated cells x 10^9         | 67.0                          | 10.8                          | 58.2                          | 1.44                          |
| Nucleated cells / BW x 10^9/Kg | 1.29                          | 0.02                          | 1.37                          | 0.034                         |
| CD34+ cells x 10^6             | 77.7                          | 77.4                          | 150                           | 54.5                          |
| CD34+ cells / BW x 10^9/Kg     | 1.49                          | 1.49                          | 3.54                          | 1.28                          |
| % CD34+ cells                  | 1.16                          | 71.7                          | 2.58                          | 37.8                          |
| Total CFU-GM x 10^6            | 11.1                          | 3.35                          | 13.9                          | 3.21                          |
| Total CFU-GM / BW x 10^9/Kg    | 21.3                          | 6.44                          | 32.8                          | 7.57                          |
| CD3+ cells x 10^6              | 7.17                          | 0.0038                        | 31.8                          | 5.89                          |
| CD3+ cells / BW x 10^9/Kg      | 1380                          | 0.73                          | 7510                          | 13.9                          |
of nucleated cells, CD34+ stem cells, T-cells before and after the selection process was tabulated in Table 1. The CD34+ cell dose was 1.49 x 10^6/Kg body weight while the T-cell dose was 0.73 x 10^4/Kg. The stem cell dose harvested appeared to be satisfactory with reference to usual cell dose requirement. T-cell depletion was adequate as 3-log reduction in T-cell number was achieved with the selection procedure. Only cyclosporine A was used for GVHD prophylaxis. In the last few days of conditioning, she developed macroscopic haematuria which was presumably due to the use of cyclophosphamide. She was treated with hydration and platelet transfusion. Haematuria finally subsided at around 4 weeks post-transplant. She developed an episode of neutropenic fever on Day +5 post-transplant and was treated with antibiotics. Haematological recovery was speedy. Neutrophil engraftment (>0.5 x 10^9/L) occurred on Day +14 with granulocyte colony-stimulating factor (GCSF) augmentation and platelet engraftment (>50 x 10^9/L) on Day +28. Chimerism study by semi-quantitative polymerase chain reaction for highly polymorphic microsatellite markers (same sex for patient and donor) using DNA extracted from peripheral blood showed 100% donor cells on Day +15 and this remained so at 3 months and 6 months post-transplant. She had no acute or chronic GVHD. Her blood count completely normalised at 3 months post-transplant and she remained well 2 years after transplant.

**Case 2**

Case 2 is a boy who presented to Tuen Mun Hospital in early 2004 at the age of 7.5 years with a febrile illness. Fever subsided soon after admission and no major infection was found. However he was noted to be pale. Blood count revealed pancytopenia with Hb 7.5 g/dL, MCV103 fl, WBC 4.45 x 10^9/L, neutrophil 0.71 x 10^9/L and platelet 12 x 10^9/L. Bone marrow aspiration and trephine biopsy confirmed hypoplastic marrow with 10% cellularity. DEB test showed 7.32 breaks per cell confirming FA. He has short fifth digits of hands and feet. Otherwise he has no other somatic features of FA. His parents are non-consanguineous. He had blood transfusion twice in the following one year. His sister who was 4 years younger is HLA-matched and DEB test showed normal result (0.01 break per cell). Case 2 has blood group O+ while the donor has blood group A+. Transplantation using the same regimen as for Case 1 was done in January 2005, i.e. one year after diagnosis. Despite relatively small size of the donor, the CD34+ stem cell dose was 1.28 x 10^6/kg which should be regarded as satisfactory. The T-cell dose was 13.9 x 10^4/Kg which implied inadequate T-cell depletion. He had a transient episode of fever on Day +1 after infusion of the selected marrow product. He also developed serum sickness to ATG in form of fever, erythematous skin rash and arthritis on Day +4. Symptoms subsided after the use of naprosyn. Haematological recovery was as quick as in Case 1. Platelet engrafted on Day +6 (Platelet > 20 x 10^9/L) and on Day +21 (platelet > 50 x 10^9/L). Neutrophil engrafted on Day +21. The subsequent post-transplant course was smooth without development of GVHD despite inadequate T-cell depletion.

Mixed chimeric state (sex mismatched for patient and donor) was noted by fluorescence in-situ hybridization (FISH) on sex chromosome at early phase of post-transplant course. On Day +17, chimerism study showed 33% donor cells and it went up to 73% on Day +32. Approaching Day +80, we noted that there was a downward trend in all three cell lineages. Haemoglobin level dropped from 10.7 g/dl on Day +55 to 6.9 g/dl on Day +81, neutrophil count from 1.29 x 10^9/L to 1.1 x 10^9/L and platelet count from 209 x 10^9/L to 140 x 10^9/L. FISH study showed mild decline of donor cell proportion in peripheral blood from 67% to 62%. Though the more significant decline in Hb level could be related to blood group incompatibility (patient group O+, donor group A+) but graft rejection could not be excluded at that time. We therefore decided to give donor lymphocyte infusion to the patient. The first DLI with T-cell number of 4.5 x 10^4/Kg was infused on Day +81 and the second and the third DLI with T-cell number of 5 x 10^5/Kg was given at 2 weeks' interval. The haematological parameters gradually improved though the chimerism study showed that the donor cell proportion was stabilised around 60-70%. The blood count completely normalised by Day +150 and remained so thereafter. The latest blood count at 10 months post-transplant showed Hb 15.1 g/dl, WBC 9.86 x 10^9/L, neutrophil 5 x 10^9/L and platelet 158 x 10^9/L. It is planned that chimerism study will be repeated again at 1 year post-transplant. The temporal profile of neutrophil and platelet counts, the DLI and chimerism study results are outlined in Figure 1.

**Discussion**

FA accounts for two-third of inherited marrow failure syndromes in childhood. During the past 15 years, a total of 29 children were seen at our department for marrow failure and 8 (27.6%) out of these 29 were confirmed to be...
one of these inherited syndromes. The relative proportion is close to what was reported in the literature though some patients were referred from other hospitals for transplantation. These inherited syndromes include 5 patients with FA, two of whom had bone marrow transplantation and were reported here, 2 patients with dyskeratosis congenita and 1 with Diamond-Blackfan anaemia. The last 3 patients underwent matched sibling marrow transplantation successfully.

FA is an autosomal recessive disorder with genetic and clinical heterogeneity. A lot of progress has been made regarding different complementation groups, genetic mutations and the correlation with clinical manifestations in FA. The phenotypic expressions of FA patients are very diverse and these include a variety of congenital physical abnormalities, marrow aplasia, and a predisposition to cancers. The diagnosis is critical as the treatment approach is very different from acquired aplastic anaemia and genetic counselling is necessary. The diagnosis often relies on alertness of the clinicians who should get the hints from the clinical history and physical signs. In Case 1, extra digit was removed in infancy and the parents are consanguineous. Omission of these hints might have delayed a proper diagnosis when the patient was seen again at late childhood for other complications of FA. For Case 2, the cytopenia was a part of marrow failure and was only picked up incidentally during a minor febrile episode. Chromosome breakage analysis by DEB test was essential to confirm the diagnosis in both cases. The test is particularly valuable in Case 2 as he does not have obvious physical manifestations. It is important to note that 25% of FA patients do not have any physical abnormalities. This implies that DEB test should be done as an integral part of investigatory workup for children with aplastic anaemia.

The management of FA is directed towards the management of complications and genetic counselling. Patients with FA generally develop some degree of marrow dysfunction ranging from mild asymptomatic cytopenias in any lineage to severe aplastic anaemia, myelodysplasia or acute myeloid leukaemia. The time of onset of bone marrow aplasia is highly variable. Androgen has been used for treatment of marrow failure for many years but the effects are unpredictable and often transient and the side effects are significant. There are also worries that the use of androgen may adversely affect the outcome of transplantation. Use of cytokines such as granulocyte colony-stimulating factor has also been tried in circumstances of severe neutropenia and recurrent infections.

BMT is now the treatment of choice provided there is an HLA matched sibling. It will correct the marrow aplasia and will also reduce the risk of future haemic malignancies. The indication for matched sibling marrow transplantation

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**Figure 1** Mixed chimerism and the trend of haematological recovery after transplantation in Case 2.
is the presence of significant cytopenia (platelet < 50 x 10⁹/L, haemoglobin < 8 g/dl or transfusion dependency, or neutrophil < 1 x 10⁹/L) of any of the 3 lineages. However, early study showed that FA patients were particularly sensitive to cyclophosphamide at a dose of 200 mg/Kg which is used routinely for acquired idiopathic aplastic anaemia. Transplant related toxicities were much increased if such dose was used in FA patients. Different regimens have been designed to reduce the toxic effects of standard chemotherapy and to allow a safe transplant. Regimen using much reduced dose of cyclophosphamide at 60 or 80 mg/Kg has showed successful outcome. Several centres around the world have also eliminated radiation from the preparative regimen with encouraging results. A fludarabine-based preparative regimen without irradiation has been used in FA patients with success. Fludarabine is an antimetabolite which is used in chronic lymphoid leukaemia and has potent immunosuppressive effect. Yet it is in general well tolerated and does not have the same toxic effects as cyclophosphamide. The drug is increasingly used as part of the preparative regimen for transplantation, especially in non-myleoablative transplantation. Together with ATG, the patient will be rendered so immunosuppressive that incoming graft will not be rejected. The transplant group at the University of Minnesota employed a preparative therapy of ATG 150 mg/m², cyclophosphamide 20 mg/Kg and fludarabine 175 mg/m² and at the same time the use of T-cell depleted marrow (with Isoplex CD34+ stem cell positive selection). T-cell depletion enables substantial reduction in the risk of GVHD at transplant. Such manoeuvre has been widely practised in unrelated donor transplant but the downsides are increased graft failure and disease relapse. GVHD prophylaxis consists of cyclosporine with or without short course of steroid. The special benefits are from the low dose of cyclophosphamide and omission of irradiation. The outcome has been excellent though mixed chimerism is not infrequently seen. Better prevention of GVHD may reduce the risk of late tumour development. Similarly, non-irradiation conditioning regimens may reduce the tumour risk. Interactions between potential risk factors are complex. The regimen also omitted methotrexate which may cause engraftment problem, more liver toxicities and mucositis.

Our two patients had recovery of marrow function using this bone marrow transplant regimen with no major toxicities. T-cell depletion by the Isoplex system in our hospital achieved good yield of haemopoietic stem cells in both cases but T-cell (CD3+ cells) reduction in Case 2 was only 2.73 log (<3 log) which should be regarded as unsatisfactory. In spite of this, no GVHD was seen in the two patients. Only in larger series, definite conclusion as regards to the benefits of the new regimen can be arrived in comparison with old regimens. Case 2 had mixed chimerism after transplant. It is not infrequent to see such phenomenon after the fludarabine-based regimen but most patients will gradually attain full donor chimerism with time (personal communication with Minnesota Group).

The problem is that many FA patients do not have matched sibling donor. New approach could be the extension of similar fludarabine-based regimen to alternative donor transplant such as in the case of unrelated donor or haplo-identical related donor. The preliminary results are encouraging and this may open up treatment option for FA patients who require a transplant but with no matched sibling donor.

**Conclusion**

FA is an important cause of severe marrow aplasia in childhood. Diagnosis requires clinical alertness and application of confirmative DEB test. Matched sibling bone marrow transplantation is the treatment of choice which results in good long-term survival. The current new regimen protects FA patients from serious transplant related toxicities by sparing the use of radiation and usage of a much reduced dose of cyclophosphamide. CD34+ stem cell selection enables T cell depletion so as to reduce the risk of acute and chronic GVHD. This is particularly important for FA patients since GVHD may predispose them to the development of carcinoma in future. In summary, this fludarabine-based regimen is effective for FA patients who undergo HLA-identical sibling bone marrow transplantation.

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**References**


