

Fanconi anemia - learning from children

Johanna Svahn, Carlo Dufour

Department of Pediatric Hematology
Oncology and Bone Marrow
Transplantation, Gaslini Childrens
Hospital, Genova, Italy

Abstract

Fanconi Anemia (FA) is a rare autosomic recessive and X-linked disease with chromosomal instability after exposure to cross-linking agents as the hallmark. Clinical features of FA are somatic malformations, progressive bone marrow failure and cancer proneness, however there is wide clinical heterogeneity. The symptom most frequently and early associated with morbidity and mortality is progressive pancytopenia in the first decade of life although acute myelogenous leukemia (AML) or myelodysplastic syndrome (MDS) can appear before aplastic anemia. Squamous cell carcinoma (SCC) of the head-neck, intestinal or genital tract has a very high incidence in FA and can appear at young age. This paper will focus on treatment of bone marrow failure in FA.

Introduction

Fanconi Anemia (FA) is a rare autosomic recessive and X-linked disease with wide clinical and biological heterogeneity. The gold standard diagnostic criteria of FA is increased chromosome fragility after exposure to alkylating agents such as diepoxybutane (DEB) or mitomycin C (MMC). The most frequent clinical features are progressive bone marrow failure, somatic abnormalities and cancer susceptibility. Altered skin pigmentation and/or café au lait spots, short stature, thumb or thumb and radial anomalies, abnormal male gonads, microcephaly, eye anomalies, structural renal defects, low birth weight, developmental delay, and abnormal ears or hearing are the most frequent birth defects. However about 25-40% of FA patients have a normal or nearly normal somatic phenotype.¹ The reported incidence of FA, probably underestimated, is 3 cases/million newborn/year and the disease is slightly more frequent in males than females, the ratio being 1.3/1. The carrier frequency is 1/300 in USA and western Europe, whereas among some populations such as South African Afrikaansers, Ashkenazi Jews and Spanish Gypsies the frequency is higher, about 1/90-1/100, due to genetic founder effects.¹⁻³

To date there are 14 FA complementation

groups corresponding to 14 different cloned genes: *FANCA*, *B*, *C*, *D1*, *D2*, *E*, *F*, *G*, *I*, *J*, *L*, *M*, *N* and *P*. More than 2/3 of Fanconi patients belong to FA-A, FA-C or FA-G group with FA-A accounting for more than 50%. Although most are unique genes, several were previously known, including *FANCD1* (*BRCA2*), *FANCG* (*XRCC9*), *FANCI* (*KIAA1794*), *FANCI* (*BRP11/BACH1*), *FANCL* (*PHF9/POG*), *FANCM* (*Hef*), *FANCN* (*PALB2*) and *FANCP* (*SLX4*). Heterozygotes for *BRCA2* and possibly *BACH1* and *PALB2* are at increased risk of breast cancer and other cancers. In addition there are 3 other genes involved in the pathway but not yet found in any patient and therefore not formally designated as FA genes: *RAD51C*, *DDX11*, *FAAP20*. The Fanconi anemia proteins A, B, C, E, F, G, L and M appear to form a nuclear complex, which leads to ubiquitination of the I and D2 proteins; the latter is involved in DNA damage response mechanisms in cooperation with *FANCD1*, *FANCI*, *FANCN* and *FANCP*, as well as *BRCA1*, *RAD51*, *Mre11*, and other proteins.

The widely variant FA phenotype may depend on whether the mutation in the involved FA gene is null or leads to a partially functional gene product rather than the specific gene that is involved. Attempts to do genotype-phenotype correlations in FA have shown contrasting results between different studies and high-light the genetic and clinical heterogeneity in the population. In addition, somatic reversion of the germ-line mutation in a FA gene leading to mosaicism, defined by the presence of two genetically different cell populations in the organism, one normal and one Fanconi, furthermore increases the difficulty in genotype-phenotype correlations. Hence, the specific role of the Fanconi Anemia proteins in the pathogenesis of somatic abnormalities, bone marrow failure, or oncogenesis remain to be clarified.

Other than birth defects the most frequent presentation of FA leading to diagnosis is progressive bone marrow failure appearing during the first decade of life. Early signs before peripheral blood cytopenia becomes evident may be progressively increasing mean corpuscular volume (MCV) and HbF. Actual aplastic anemia may be preceded by myelodysplastic syndrome (MDS) or acute myelogenous leukemia (AML) and chromosomal aberrations involving chromosome 3 and 7 lead to increased risk of developing MDS/AML.⁴ As cancer can be the only manifestation of FA, it should be suspected in cases of early onset head-neck squamous cell carcinoma (SCC), esophageal or vulvar cancer. This paper will focus on management and treatment of bone marrow failure in FA.

Marrow failure in FA prompts a comprehensive monitoring plan consisting of clinical examination and at least yearly bone marrow studies, including morphology, cytogenetics, FISH, colony studies and immunophenotyping.

Correspondence: Carlo Dufour, Haematology Unit, Department of Pediatric Haemato-Oncology, G. Gaslini Children's Hospital, Genova, Italy.
E-mail: carlodufour@ospedale-gaslini.ge.it

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At diagnosis parents and siblings should be evaluated by DEB test and by molecular genetic testing if the patient has a known mutation. At diagnosis, even if the marrow failure is at a very early stage, the patient and family should be HLA typed, because 1st choice treatment is hematopoietic stem cell transplantation (HSCT) if an HLA matched family donor is available. If no family donor is available an unrelated donor search should be initiated as soon as possible. The reason for the urgency to identify a donor is because the earlier transplant is done, to a younger patient who has not received transfusions, the better is the outcome.

Due to the chromosome fragility inherent to the FA cells, FA patients display extraordinary toxicity when treated with chemo- or radiotherapy. Therefore the conditioning regimen prior to HSCT must be reduced compared to other patient groups. Using irradiation in the preparative regimen has been debated over the past decades. Several groups have reported results after transplant with or without total body irradiation (TBI), the results are summarized in Table 1. A registry analysis that compared irradiation vs non irradiation in 148 FA patients registered to the Center for International Blood and Marrow Transplant Research (CIBMTR) database receiving an HLA-matched sibling transplant between 1991 and 2001 showed no differences in engraftment, acute and chronic GVHD, transplant related mortality (TRM) or OS in the two groups.⁵ The message was therefore in support to the use of non-irradiation preparative regimen. The follow-up was too short to evaluate if irradiation was associated with increased risk of tumours.

Regarding timing of HSCT from a matched family donor, published experience leads us to suggest that transplant should be performed in early marrow failure, as manifested by mild cytopenia and hypoplasia of marrow hematopoietic tissue but before cytopenia becomes se-

Table 1. Matched family donor transplant: conditioning regimen and outcome.

	Dufour <i>et al.</i> (BJH 2001) ¹⁸	Farzin <i>et al.</i> (BJH 2007) ¹⁹	Tan <i>et al.</i> (PBC 2006) ²⁰	Ayas <i>et al.</i> (BMT 2008) ²¹	Bonfim <i>et al.</i> (BBMT 2007) ⁹
Patient nr	27	35	11	34	67
Conditioning	TAI/TBI 500cGy, LDCY or HDCY	TAI/TBI 400cGy, LDCY, ATG	LD CY + FLU 125 mg/m ² , ATG, TCD	CY 60 mg/kg, ATG	CY 60 mg/kg
Graft failure	8%	5,7%	9%	0	10,5%
Ac GVHD	36%	14,2%	0		17%
Cr GVHD	12,5%	12%	0		28%
TRM	18,5%	8,5%	9%		9%
Late tumours	0	5,7%			3%
Follow-up	3 yrs	10 yrs	2 yrs	2,9 yrs	3,7 yrs
OS	81,5%	83%	100%	97%	97%
EFS			82%	82%	

LDCY: low dose cyclophosphamide, 20 mg/kg; HD CY: high dose cyclophosphamide, 100-200 mg/kg; TCD: T Cell depleted graft; ATG: anti lymphocyte globuline.

vere, i.e. before platelets decrease below $40 \times 10^9/L$, before transfusion requirement, and before appearance of cytogenetic aberrations involving chromosome 3 and 7 and/or AML. Age at transplant is consistently a prognostic factor with better outcome if performed before 10 years. The evidence suggest that conditioning regimen for a matched family donor transplant should be without irradiation, using fludarabine and cyclophosphamide and with T cell depletion (TCD), either of the graft or *in vivo* with Mabcampath for example, to prevent GVHD.

In case a matched family donor is not available, a donor search in international registries should be started as soon as possible and transplant should be performed essentially with the same criteria as a matched family donor transplant, i.e. before severe cytopenia requiring transfusions and before appearance of a cytogenetic clone and/or AML. Regarding the conditioning regimen in alternative donor transplants, there is no doubt that fludarabine has changed the outcome relevantly, from previously very poor, improving survival and engraftment and reducing early mortality and transplant related mortality (TRM).⁶ The use of irradiation varies between countries and transplant centres, as summarized in Table 2. In three consecutive phase II clinical trials assessing the optimal approach to alternative donor transplants, MacMillan *et al.* confirmed in multivariate analysis that age >10 years and any transfusion prior to transplant was associated with higher 3-year mortality. Among these trials, a TBI dose de-escalation from 300 to 150 cGy led to secondary graft failure in 2/2 pts and therefore dose de-escalation was stopped and returned to 300 cGy.⁷ A single-center report of 7 patients transplanted with alternative donors without irradiation resulted in 22% rejection rate which however could be recuperated with a second transplant and all patients were alive at median 3 years follow-up.⁸ Bonfim *et al.* re-

ported 60 patients receiving alternative donor transplants without irradiation obtaining OS at 5 years 92% for patients below 10 years of age and 53% for patients above 10 years. Noteworthy in this report, 10 patients developed tongue carcinoma, 7 of whom had had GVHD.⁹ (C. Bonfim, update of ref 9 as oral communication, FARE Scientific Symposium 2010) This highlights the importance of reducing GVHD, by TCD of the graft or *in vivo* TCD using Mabcampath and/or ATG.

Unrelated Cord blood (UCB) as an alternative stem cell source to bone marrow or peripheral blood has been reported in a registry based analysis of 93 UCB in FA where OS was 53% with Fludarabine in the conditioning regimen (vs 19% non Fludarabine). There were relatively high figures of GVHD and TRM, 32% and 59% respectively.¹⁰ As of today, UCB transplant should be considered only when other alternative donor stem cell source cannot be found. Preliminary results from a retrospective analysis of the EBMT database show that mismatched or haploidentical family donors may

be a valid option if no matched unrelated donor can be found (E. Korthof, oral communication FARE Scientific Symposium 2010). In summary, alternative donor transplants are improving in survival, getting close to that of matched sibling donor transplants, whereas TRM and GVHD are still a problem. It is still an unresolved issue whether to perform irradiation or not. Furthermore, the intrinsic cancer proneness in FA significantly increases with acute severe and chronic GVHD and in general a 4.4-fold higher risk for SCC was found in patients who received transplants vs who did not. The cumulative incidence of SCC was 24% at 15 years after transplant and SCC manifested at younger age in transplanted FA patients compared to non transplanted.¹¹

Other treatment alternatives for marrow failure in FA are only temporary options awaiting HSCT which is the only treatment that can cure bone marrow failure and prevent the risk of hematologic malignancy. The use of androgens, oxymetholone at the dose of 2-5 mg/kg/day, can bridge the gap to HSCT, but should be avoided long-term due to side-effects including masculinization, final short stature, peliosis hepatis, liver adenomas and hepatocellular carcinomas. Androgens were reported to adversely affect the outcome of subsequent HSCT in some studies¹² but not in others.¹³ Hematopoietic growth factors such as EPO and G-CSF have no demonstrated effect. G-CSF has the potential risk of stimulating clonal escape and should be used only in rare cases of severe infections in neutropenia. Anti-fungal agent voriconazole should be used with caution due to increased risk of SCC¹⁴ (AIFA nota informativa 22/9/10). Experimental trials studying the safety and efficacy of anti-TNF alpha agent etanercept in FA, based on the *in vitro* beneficial effect of etanercept on colony formation in FA marrow mononuclear cells¹⁵ are currently being evaluated.

There is limited experience on treatment of

Table 2. Matched unrelated donor transplants: conditioning regimen and outcome.

	MacMillan <i>et al.</i> (BBMT 2009) ⁹	Chaudhury <i>et al.</i> (BJH 2008) ²²	Yabe <i>et al.</i> (BJH 2006) ²³	Bonfim <i>et al.</i> (oral communication 2010) ⁹	Motwani <i>et al.</i> (BMT 2005) ⁸
Patient nr	24	21	27	60	7
Conditioning	TBI 300cGy CY 40 mg/kg, FLU 140 mg/m ² , ATG, CsA	TBI 450cGy, CY 40 mg/kg, FLU 150 mg/m ² , ATG, TCD, tacrolimus	TBI 300-450 cGy, CY 40 mg/kg, FLU 150-180 mg/m ² , ATG, tacrolimus	CY 60 mg/kg, FLU 125 mg/m ² , ATG, CsA	CY 20-30 mg/kg, FLU 125 mg/m ² , ATG
Ac GVHD	23%	5,5%	11%		71%
Cr GVHD		5,5%	30%		28%
Follow-up	short	5 yrs	3,1 yrs	5 yrs	3 yrs
OS	86%	72%	96%	92% pt<10 yrs age, 53% pt>10 yrs age	100%

CsA: Cyclosporine A.

advanced MDS or AML in FA patients. A report of 4 patients treated with a reduced intensity chemotherapy course (mini-FLAG) showed not more than expected haematological toxicity and no organ toxicity. Three of 4 patients had a marrow response and could be transplanted in aplasia, one patient required other chemotherapy to clear blasts pre-HSCT.¹⁶ The results do not answer whether chemotherapy given before HSCT may improve the poor survival. An alternative approach is to proceed to HSCT without prior cytoreduction, using TBI 300 cGy, cyclophosphamide 40 mg, Flu 140 mg, ATG, and TCD in conditioning regimen.¹⁷

Finally, taking care of a FA patient and his/her family is a great challenge due to the delicate balance between treating to cure on one hand and limiting the damage and side effects that the treatment causes to a cross-linker sensitive patient on the other hand.

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