Outcome of bone marrow transplantation in acquired and inherited aplastic anaemia in the Republic of Ireland

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ABSTRACT

Background Severe Aplastic Anaemia (SAA) and Fanconi Anaemia (FA) are rare haematological disorders characterised by pancytopenia and bone marrow hypoplasia.

Aims We performed a retrospective study of all patients who underwent BMT for SAA and FA at St James's Hospital, Dublin, and at OLHSC, Crumlin, between 1985 and 2002.

Methods The medical records of 63 patients, 50 with acquired SAA and 13 with FA, were reviewed.

Results The median age at the time of transplant was 14 years (range 3-43 years). The actuarial survival (OS) (n=63) was 76% at 17 years. The transplant related mortality (TRM) was 22% (n=14). The most common cause of death was infection (46%). The survival was significantly better in patients receiving their transplant after 1995 (p=0.002). Outcome was superior in those receiving less than 20 red cell transfusions prior to transplant: OS 91% (<20 Units) versus 62% (≥20 Units).

Conclusions These national results are comparable to those of published international series and support the use of BMT in the treatment of SAA and FA. The known adverse effect of prior transfusion was confirmed.

INTRODUCTION

The term aplastic anaemia (AA) refers to a group of rare stem cells disorders characterized by pancytopenia and a hypocellular bone marrow in the absence of an abnormal infiltrate and with no increase in reticulin.[1]

Severe Aplastic Anaemia (SAA) is defined by a marrow cellularity of <25% and two of the following: neutrophil count < 0.5 x 10^9/l; platelet count < 20 x 10^9/l and a reticulocyte count < 20 x 10^9/l (SAA EBMT Working Party consensus conference). The incidence of SAA varies worldwide between 1.5 - 7.8 per million per year. A higher incidence has been described in the elderly population and in Thailand.[2] The aetiology of SAA is still poorly understood, however a large volume of evidence suggests an immune-mediated pathogenesis in the majority of patients.[3] Acquired Aplastic Anemia, has occasionally been associated with exposure to drugs, environmental agents, industrial chemicals and viral infection. Chloramphenicol, other antibiotics, anticonvulsant drugs, anti-inflammatory, anti-arthritic, anti-psychotic agents have been associated as causative agents in some patients. In the majority of cases no clear aetiology is evident and the disease is referred to as idiopathic acquired aplastic anaemia.

Congenital Aplastic Anaemia is a rare condition with an incidence of two per million per year worldwide. Different types of inherited Aplastic Anaemia have been described: Fanconi Anaemia, Blackfan Diamond and Dyskeratosis Congenita.[4] Fanconi Anaemia (FA) is the most common of the inherited aplasias. It is associated with increased chromosomal fragility, in metaphase preparations (more evident following addition of clastogenic agents). Auerbach[5] described at least eight genes responsible for this autosomal recessive condition. At least four distinct complementation groups have been recognized, each group representing distinct genes on the basis of their separate positions in the human genetic map. The disease becomes clinically evident in the majority of children between the ages of 5-10 years although occasionally the diagnosis may be made in adults. The first manifestation is thrombocytopenia, followed by anemia and

neutropenia as evidence of bone marrow failure The bone marrow aspirate and biopsy appear markedly hypocellular. The clinical phenotype is characterized by multiple somatic abnormalities: low birth weight, skeletal abnormalities e.g. short stature, absence or hypoplasia of the thumb and/or radius, hypo and hyper-pigmentation of the skin, kidney malformations (horseshoe kidney), microcephaly, mental retardation, hypogonadism and an increased risk of developing secondary malignancies including acute myeloid leukaemia.[6] Because of the DNA repair defect, treatment of FA is problematical. The only curative therapy is allogeneic stem cell transplantation, however the ideal conditioning therapy remains controversial. In the absence of definitive therapy, red cell and platelet transfusions together with androgens, have been the main-stay of treatment. Immunosuppression (IS) does not have a role in the treatment of FA but may play some part in the management of Diamond Blackfan Syndrome.[7,8]

Prior to the 1970 the outcome for patients with SAA was extremely poor with mortality rates of over 80% in the first 3 months[9]. Improvements in support care, such as antibiotics, antifungals, platelet and red cells transfusion together with definitive therapies such as stem cell transplantation and immunosuppression (IS) have radically improved the outcome for many patients. The age of the patient, severity of disease and the availability of a HLA compatible donor influence the outcome.[10] Patients with SAA under the age of 35 years, clearly benefit from SCT whereas older patients are treated preferentially with IS.[11] Results of SCT using donors other then sibling have been inferior to those obtained when a sibling donor is used. Recent protocols, however, indicate the possibility of improved survival using unrelated donors.[12] The outcome of newer treatment protocols needs to be tested in larger numbers of patients. An algorithm for treatment of SAA has been suggested by Bacigalupo.[11]

PATIENTS

The group consists of patients treated between 1985-2002 in the National Center for Stem Cell Transplantation, St James's Hospital and Our Lady's Hospital for Sick Children Crumlin. The demographics details of the patients with SAA and inherited AA, conditioning regimens and GvHD prophylaxis are shown in Table 1. A sibling donor was available for 78% of all patients. Eleven received marrow from a phenotypically matched family member and three

Table 1
DEMOGRAPHICS OF ALL PATIENTS RECEIVING SCT

TOTAL NUMBER = 63		
Median age at the SCT time	14	(range 3-43)
Gender M/F	39M;	24F
Diagnosis	50 SAA	
	13 FA	
*CMV Status	45 -/- 6 +/+ 4 +/- 5 -/+ 3 na	
CONDITIONING		
TBI+ Cy	3	
TBI +Cy+Campath	1	
TBI+Cy+ATG	1	
Cy+ Campath	3	
Cy + ATG*	24	
Cy	30	
Cy+ Flu+ Campath"	1	
na	1	
GVHD PROPHYLAXIS		
CSA	34 (52.3%)	
CSA+MTX	29(44.6%)	
na	2	
DONOR	**NUMBER**	
Matched sibling	53	
Phenotypically identical	9	
Family member		
MUD	3	
PRE SCT TRANSFUSION		
RCC +PLT	<20	≥ 20
Children	15/21(71%)	7/21(29%)
Adults	15/24(62%)	9/24(37%)

Legenda:

AA=Aplastic Anaemia; FA= Fanconi Anaemia; na=not available; CMV=cytomegalovirus, + = prior infection;Cy=cyclophosphamide; ATG= antitymoglobulin; Flu=Fludarabine; CSA=cyclosporine; MTX=methotrexate; MUD=matched unrelated donor; RCC = red cell transfusion; PLT = platelet transfusion; Campath = anti CD52 monoclonal antibody.

Two patients received a second SCT from the same sibling donor. Data were available for 24 adults patients and for 21 pediatric patients.

from a matched unrelated donor. Unrelated donor bone marrow transplantation was performed where a sibling donor was not available. Three patients developed graft failure, see Table 2.

METHODS

Patients with SAA fulfilled the Camitta Criteria[1] Complementation tests with diepoxybutane or mitomicin C were performed to confirm a diagnosis of Fanconi Anaemia. Investigations included cytogenetic analysis, Ham's test/ flow cytometry to exclude/confirm the presence of paroxysmal nocturnal haemoglobinuria (PNH). Bone marrow was the source of stem cells in all the patients. All blood products were irradiated prior to transfusion (dose 30 Gy of irradiation) to reduce the risk of GvHD. All patients received oral acyclovir as prophylaxis against Herpes Simplex. CMV sero-negative blood products were always given. Prophylaxis against Pneumocystis Carinii was with pentamidine nebulisation or oral cotrimoxazole.

***SAA conditioning** (See Table 1)*

The preparative regimens for SAA patients, consisted of cyclophosphamide 50mg /kg per day on four successive days (22 patients) or combined with ATG (24 patients). Two patient were treated with single fraction thoraco-abdominal irradiation (TAI) 500cGy followed by cyclophosphamide 200 mg/kg, GvHD prophylaxis was carried out using cyclosporin A and short course methotrexate.[13]

***FA conditioning** (See Table 1)*

The conditioning regimen adopted in the majority of our FA patients (8 patients) was cyclophosphamide without ATG. The dose was reduced to 100-140mg/kg as reported by Flowers[14] and by Zanis-Neto.[15] In three cases the modified Gluckman protocol[16] was used (cyclophosphamide 20mg/kg intravenously over 4 days and a 5 Gy TAI).[17]

GvHD prophylaxis

Forty nine per cent (n=31, 13 adults and 18 children) received cyclosporin A alone. Forty eight per cent 48% (n=30, 9 adults and 21 children) received cyclosporin A and short course methotrexate.

Statistical methods

Kaplan Meier actuarial method was used for analysis of overall survival**.** Proportions were compared using Fisher's exact test. The log-rank test was used for the equality of survival curves. Statistical significance was defined as p value<0.05.

Table 2
PATIENTS WHO DEVELOPED GRAFT FAILURE (N=3)

PATIENTS	A	B	C
Gender M/F	M	M	M
Age at diagnosis	3	15	7
Diagnosis FA/AA	AA	AA	AA
Type of SCT (Sibling/MUD) **Cell dose (mnc x10^8/kg)**	sib 1.47	sib 1.5	mud na
TAI yes/no	yes	no	no
Second SCT yes/no	yes	no	no
CMV	+/+	-/-	na
Status alive/†	†	†	†

†= patient died; MUD=matched unrelated donor; CMV=cytomegalovirus; TAI=thoracoabdominal irradiation

RESULTS

SAA patients

The median time to reach a neutrophil count >0.5 x 10^9/ was 19 days (range 12-48); and a platelet count >20 x10^9/l was 21.6 days (range 7-63). A median of 1.89 x10^8 mononuclear cells/kg was infused (range 0.57-3.8 x10^8 cells/kg). Two paediatric patients received total body irradiation as conditioning for a second transplant, because graft failure. In one case anti-CD52+monoclonal antibody for T cell depletion to prevent GvHD was given. Three pediatric patients died of graft failure. Two had been infused with a dose of stem cell in the lower range, in the third case the cell dose number was not available (see Table 2).

FA patients

The median time to reach a neutrophil count >0.5 x 10^9/l was 14 days (range10-30); and a platelet count >20 x10^9/l was 19.6 days (range 10-38). A median of 1.25 x10^8/kg mononuclear cells/kg was infused (range 0.81-4.5 x10^8 cells/kg).

SAA patients and FA

A cumulative analysis of FA and SAA was performed. The median time to reach a neutrophil count >0.5 x 10^9/l was 18 days(range lo-48); for a platelet count >20 x10^9/l was 20.6 days (range 7-63). A median of 1.84 x10^8/kg mononuclear cells/kg was infused (range 0.57-4.5 x10^8 cells/kg). Twenty six patients

received ATG to prevent graft rejection. All patients receiving immunosuppressive treatment with ATG, were treated with steroids (methylprednisolone 2-5 mg/kg/day) to prevent serum sickness. Cyclosporin A was continued for one year post transplant. The overall actuarial survival of the group was 76% at 17 years (Figure 1). The median follow up was 4.7 years, with 25% of the patients followed for less than 1.7 years, 25% for more than 9.4 years and a maximum follow-up time of 17 years. Fifteen deaths occurred in total with a mortality rate of 3.7 deaths per 100 person years. Eight deaths occurred in the first year. Fourteen of the 15 deaths occurred in the first five years representing a mortality rate of 6.3 per 100 person years over this period. There was no significant difference in the survival rates between adults and children (Figure 2), with overall survival of 81% for children and 68% for adults at 5 years (p= 0.29). There was no significant difference in the survival rates in those with a FA (78%) or SAA (76%)

p= 0.66 (Figure 3). There was no significant difference in the survival rates in those treated with ATG 87% versus those without ATG 71%, p= 0.13. Survival rates were significantly lower in those treated before 1995 (55%) compared to those treated after 1995 (91%) (Figure 4). There was a significant (p=0.007) difference in the outcome of those who received more than 20 units of red cells and/or platelets prior to transplant (Figure 5). Infection was the major cause of death (see Figure 6). One patient died from donor leukaemia following a SCT from his phenotypically identical cousin.[18]

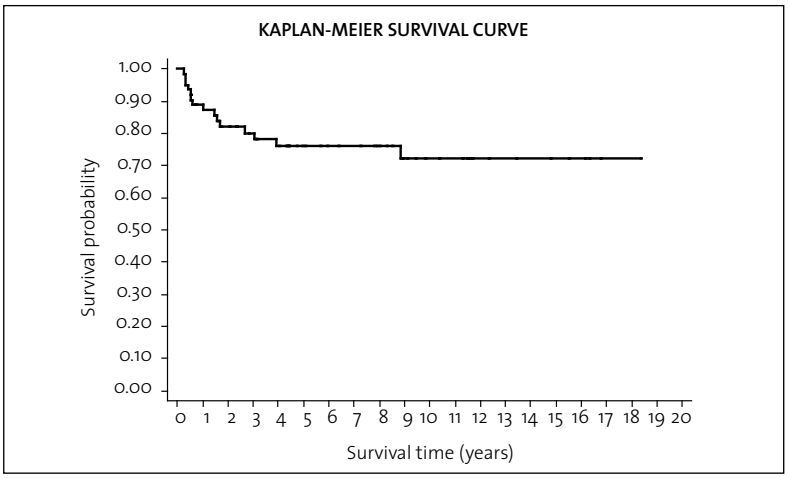

Figure 1 — PROBABILITY OF SURVIVAL AFTER ALLOGENEIC BMT FOR APLASTIC ANAEMIA IN PATIENTS COHORT TRANSPLANTED IN 1985-2002. THE OVERALL SURVIVAL AT 17 YEARS OF FOLLOW UP IS 76%.

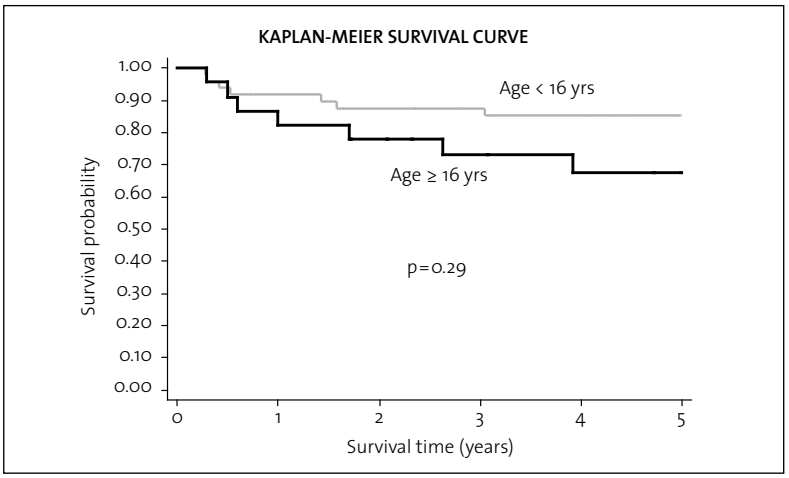

Figure 2 — PROBABILITY OF SURVIVAL AFTER ALLOGENEIC BMT FOR APLASTIC ANAEMIA IN ADULTS AND CHILDREN. NO SIGNIFICANT DIFFERENCE (P= 0.29) WAS FOUND IN THE SURVIVAL RATES. OS=81% (CHILDREN) AND OS=68% (ADULTS) RESPECTIVELY AT 5 YEARS.

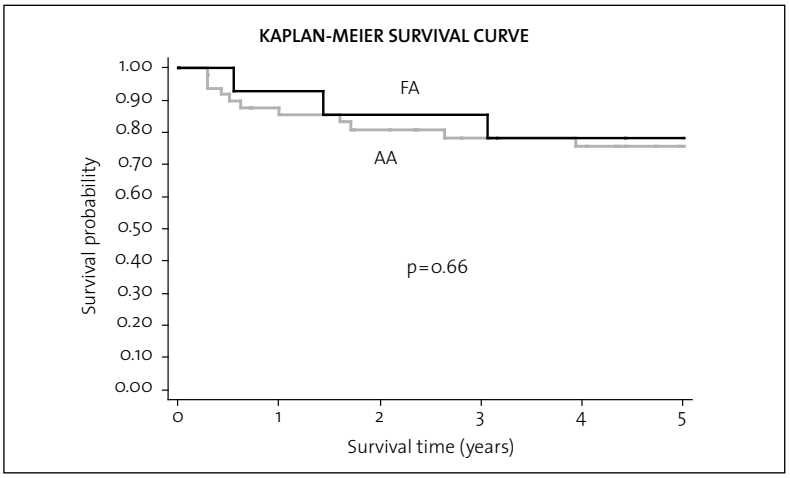

Figure 3 — THERE WAS NO SIGNIFICANT DIFFERENCE (P= 0.66) IN THE SURVIVAL RATES IN PATIENTS WITH FA (78%) VERSUS PATIENTS WITH SAA (76%).

DISCUSSION

SAA

SCT and IS are the current treatments of choice for SAA. IS with ATG and/or cyclosporine is recommended for the treatment of elderly patients not eligible for SCT (above 40 years of age).[19] Allogeneic hematopoietic SCTs are potentially curative, but may be associated with transplant related mortality (TRM). TRM is mainly related to toxicity of the conditioning regimen, infections and GvHD. If an HLA-identical sibling donor is available, survival varies from 75-90%.[9,10] The broadly adopted conditioning regimen for SAA consists of cyclophosphamide and ATG.[13]

FA

The conditioning regimen for patients with FA undergoing SCT remains problematical in the view of severe toxicity experienced by patients with

conventional preparative regimens. In this series patients with FA were conditioned with low dose cyclophosphamide (100-140mg/kg) as reported by Flowers et al.[14] The dose of cyclophosphamide was further reduced if there was concomitant use of TAI.[20]

Graft failure was not found in FA patients. It was not possible to establish if it was a significant difference in graft failure incidence between FA and AA group because of the small sample. FA patients are more sensitive to cytotoxic chemotherapy and low dose chemotherapy conditioning regimen without the use of total body irradiation (TBI) or TAI are recommended.[21]

SAA and FA

Although radiation is useful in reducing the rejection rate, it should not be used because of the risk of secondary malignancies.[22] Bone marrow cells rather than mobilized peripheral blood stem cells (PBSC) should be used as source material because of the increased risk of Graft versus Host Disease (GvHD) with PBSC, as reported by Schrezenmeier.[23] The number of mononuclear cells infused has been shown to be inversely correlated with the incidence of graft failure. A dose of ≥ 3.0 x10^8 cells/kg has been shown to reduce this risk. A median of 1.84 x10^8/kg mononuclear marrow cells (range 0.57-4.5 x10^8 cells/kg) was given and the graft failure rate was 5%. Three paediatric patients experienced graft failure and in two the cell dose infused was low (table1). A large body of evidences exists indicating that the number of red cell and platelet transfusion prior to transplantation has a deleterious effect on the outcome.[24] Graft rejection may be influenced by immunization of the recipients following exposure to HLA antigens. Contaminating white cells in platelet transfusions may be more efficient at immunizing recipients than those present in red cell transfusions because of their increased number. The early referral of patients for SCT and the use of universal leukodepletion for red cells may decrease the sensitization to HLA antigens and may have contributed to improvements in survival in recent years.[25,26] Dendritic cells contained in transfusion products may also contribute to sensitization[27].

A regression Cox model analysis carried out retrospectively by the EBMT working party for Severe Aplastic Anaemia in order to find differences in outcome over time clearly showed an improved survival after 1995.[28] Similarly we found improved survival rates following SCT for SAA after 1995 (Figure 4).

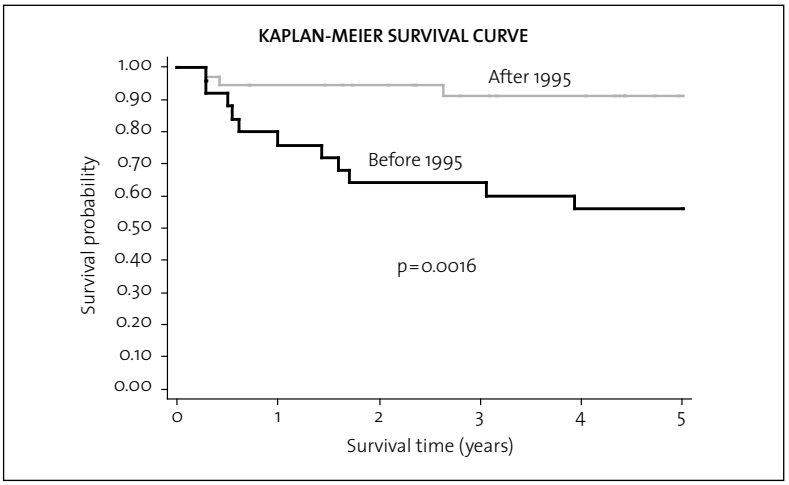

Figure 4 — SURVIVAL RATES WERE SIGNIFICANTLY LOWER IN PATIENTS TREATED BEFORE 1995 (OS=55%) COMPARED TO THOSE TREATED AFTER 1995 (OS=91%). P=0.0016.

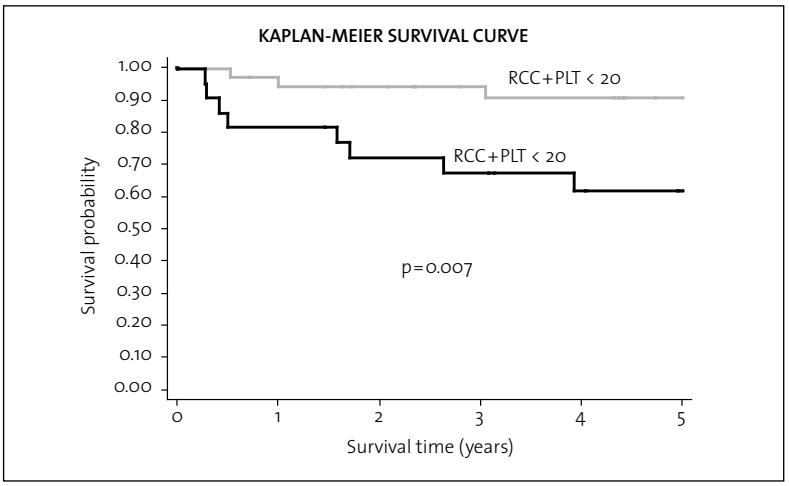

Figure 5 — PATIENTS TRANSFUSED PRE BMT WITH (PLT+RCC) < 20, VERSUS PATIENTS TRANSFUSED WITH (PLT+RCC) ≥ 20. THERE WAS SIGNIFICANT DIFFERENCE IN OS, RESPECTIVELY 62% VS. 91% AT 5 YEARS, P=0.007.

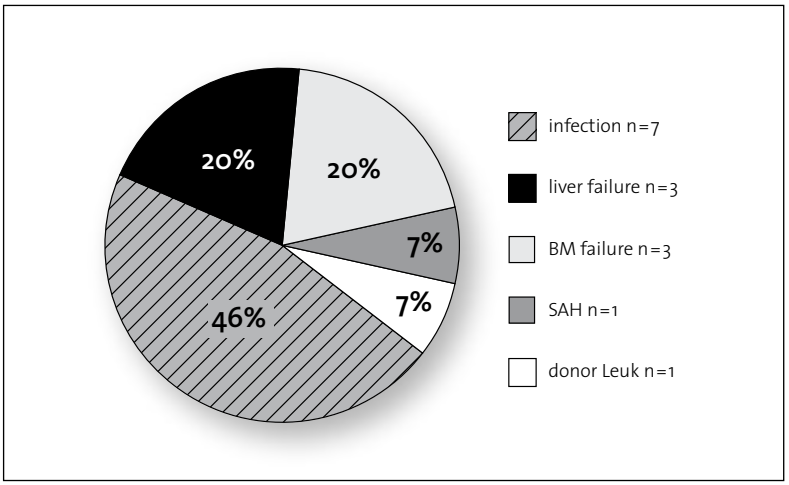

Figure 6 — CAUSE OF DEATH.

Larger series shows a relatively poor outcome for FA treated with SCT, as the FA patients are more sensitive to cytotoxic chemotherapy,[29] therefore low dose conditioning without TBI is recommended.

In this retrospective study, patients with a diagnosis of FA did not show a significance difference in OS, when compared with patients with SAA probably because of the small numbers.

The relative use of SCT or IS in SAA patients, has been reported by Bacigalupo et al.[10] They demonstrated that neutrophil count and patient age, at the time of treatment, are important predictors of outcome. SCT was shown to be advantageous in young patients (≤10 years of age) and in adults (≥50 years of age) with neutrophils of $<0.1x10^9$/l. In those with a neutrophil count of $>0.5x\ 10^9$/l, IS was more efficient in those aged ≥50 years of age only. Patients aged 10 – 40 years with a neutrophil count ≤ $0.3x10^9$/l, appeared to have the same outcome whether treated with either SCT or IS. In the absence of a matched sibling donor the use of unrelated matched donors has been evaluated. Results are significantly worse,[30] than with sibling donors in part because of the late referral pattern to transplant units and the fact that patients are heavily pre-transfused at the time of SCT. Newer protocols using powerful immunosuppression with purine analogues may alter the outcome favorably.[11]

SCT for haematological malignancies is associated with the definitive risk of GvHD even in fully matched sibling pairs. In patients receiving SCT for leukaemia the so-called Graft versus Leukaemia (GvL) effect is closely associated with GvHD and a reduced risk of leukaemia relapse. The use of mobilized peripheral blood stem cells has been shown in randomised studies to increase the risk of chronic GvHD but reduce the relapse rate and therefore may be the stem cell sources of choice in patients with high-risk leukaemias. In SAA, however, the occurrence of GvHD offers no benefits and therefore the recommendation of the EBMT Working Party is that bone marrow should be the source of stem cell for patients undergoing SCT for SAA.[31]

Deaths from infections have decreased since the introduction of CMV antigen testing and pre-emptive treatment with ganciclovir.[32] The introduction of antifungal prophylaxis and early treatment in conjunction with early diagnosis of invasive fungal pneumonia with CT scanning may have also contributed to reduced mortality post SCT.[33]

In the last 20 years the outcome for patients with SAA has improved significantly. Results obtained in the National Transplant Centre at St James's and OLHSC Crumlin are in keeping with the international experience. The optimum treatment for FA remains problematical.

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