Consequences of ABO incompatibility in multiple myeloma patients undergoing peripheral blood stem cell transplantation after reduced intensity conditioning

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Background. Although the ABO blood group is one of two major antigen systems of relevance for transplantation in humans, there are still conflicting data concerning the influence of ABO-incompatibility on transplant outcome. This study investigated the effect of ABO incompatibility in recipients of haematopoietic progenitor cell transplants from related donors after reduced intensity conditioning (RIC) regimens.

Materials and Methods. We retrospectively analysed data from 19 multiple myeloma patients included in a prospective RIC allogeneic haematopoietic progenitor cell transplantation protocol, focusing on engraftment, transfusion requirement, Graft-versus-Host Disease, transplant-related mortality and survival.

Results. Five out of the 19 patients (26%) received an ABO-incompatible transplant, with minor ABO-mismatch in two patients (10%), major ABO-mismatch in one case (5%), and bidirectional incompatibility in two cases. Neutrophil recovery was not significantly different between the ABO-compatible and ABO-incompatible groups (p=0.85). At 30 days after transplantation, 12 of 19 patients tested (63%) had engraftment with all cells of donor origin (100% chimeric), and continued to be fully chimeric on day 100+ evaluations. Patients with major/bidirectional ABO incompatibility required more red blood cell and platelet units after transplantation and were transfused for longer periods of time, as compared with patients with minor or no ABO incompatibility. Transient, mild haemolysis was noted in one patient between days 10 and 30. Graft-versus-Host Disease, disease progression and transplant-related mortality were not affected by ABO matching.

Discussion. Although delayed red blood cell engraftment and increased transfusion requirements were documented, in this study ABO incompatibility after the RIC protocol used did not impair the clinical outcome.

Keywords: multiple myeloma, haematopoietic progenitor cell transplant, ABO incompatibility, reduced intensity conditioning regimen.

Introduction

ABO blood group incompatibility is present in up to 40% of all haematopoietic progenitor cell (HPC) transplants. Although some studies showed that ABO incompatibility may be associated with an increased risk of Graft-versus-Host-Disease (GvHD), the available data suggest that ABO incompatibility has no adverse effect on the ultimate outcome of allogenic transplants[1-4]. Processing of the component or preparation of the recipient may, however, be

necessary to prevent haemolysis of donor or recipient red blood cells as a result of the infusion of ABO-incompatible red blood cells or plasma contained within the HPC component.

ABO incompatibility is classified according to whether donor isoagglutinins, isoantigens, or both are incompatible with those of the recipient. Major incompatibility occurs when a patient has antibodies directed against the donor's red blood cell (RBC) antigens; minor incompatibility occurs when the donor's plasma contains antibodies directed against the patient's RBC. In addition, bidirectional ABO incompatibility may be present in some transplants[5].

The immediate risk of major ABO-incompatible transplants is haemolysis of large quantities of RBC in the inoculum, a risk that is much lower for recipients of HPC collected by apheresis because of the lower volume of RBC in these components. Delayed haemolysis of the infused red cells may occur in patients conditioned with plasma exchange if recipient isoagglutinins rebound after the apheresis procedures. In contrast patients receiving minor ABO-incompatible transplants do not face a risk of immediate haemolysis from the infusion of incompatible RBC, but may experience delayed haemolysis mediated by the greater number of lymphocytes contained in the HPC component[6]. This delayed haemolysis after minor ABO-incompatible HPC transplants can be severe and fatal[7,8]. The incidence of haemolysis after minor ABO-incompatible transplantation has not been established and may be related to the post-transplant immunosuppression used to suppress GvHD[9,10].

Several groups of investigators have recently developed non-myeloablative or reduced intensity conditioning regimens for allogeneic transplantation; such regimens enable successful engraftment without the extra-haematological toxicities of conventional myeloablative transplant protocols[11].

Here we investigated the impact of ABO incompatibility on the outcome of 19 patients with multiple myeloma who received an allograft following a non-myeloablative conditioning regimen, focusing on kinetics of engraftment, transfusion requirements, incidence of GVDH, transplant-related mortality and survival.

Materials and methods

Patients

This is a retrospective study of a sequential series of 19 MM patients who received allogeneic HPC from related donors between September 2001 and October 2008. All patients had previously undergone autologous transplantation. They had HLA-identical donors as determined serologically and molecularly for class I (*A, B*, and *C* loci) and molecularly for class II (*DRB1, DQB1* loci) and all were evaluable for at least 100 days after transplantation. In total, five out of the 19 patients (26%) received an ABO-incompatible transplant: there was minor ABO-mismatch in two patients (10.5%), major ABO mismatch in one case (5%), and bidirectional incompatibility in two cases. The median age of the patients was 56 years and their ages ranged from 44 to 65 years. Patients and donors gave written informed consent for inclusion in an Italian multicentre RIC allogeneic protocol approved by our Ethical Committee. The patients' characteristics are summarised in Table I.

Table I - Patients' characteristics (n=19).

	Number	(range or %)
Age, median	56	(44-65)
Sex (M/F)	11/8	
Donor or recipient seropositive for cytomegalovirus	19	(100)
Sex mismatch: female donor to male recipient	8	(42)
$CD34^+$ cells received ($x10^6$/kg recipient's weight), median	5	(2.1-11.4)
ABO compatible	14	(74)
ABO incompatible		
Major ABO mismatch	1	(5)
Minor ABO mismatch	2	(10)
Bidirectional incompatibility	2	(10)

Conditioning regimen and Graft-versus-Host Disease prophylaxis

The conditioning regimen consisted of fludarabine 30 mg/m^2 (on days -4, -3, and -2) and/or total-body irradiation (200 cGy) (on day 0). GvHD prophylaxis consisted of cyclosporine A and mycophenolate. The doses of cyclosporine A were adjusted to maintain levels in the therapeutic range and were gradually tapered down and discontinued if no GvHD of greater than grade I appeared. Decisions about additional donor lymphocyte infusions were based on the chimeric status of the patients, as well as on their disease status.

Haematopoietic stem cell collection

The HPC of all donors (4 males and 15 females; median age of 58 years, range 38-72 years) were mobilised with granulocyte colony-stimulating factor at a dose of 10 µg/Kg administered by subcutaneous injection every 12 hours for 5 or 6 consecutive days. The HPC were collected from the 5th day using a continuous-flow blood cell separator (Spectra, Caridian BCT, Lakewood, CO, USA). Acid citrate dextrose-A was used as anticoagulant. The blood volume processed ranged from two to three times the donor's total blood volume according to the CD34$^+$ cell concentration in the donor's peripheral blood. The target was to collect a CD34$^+$ cell dose of at least 4x10^6/Kg of recipient's weight. Prophylactic plasma exchange to reduce alloantibody levels before HPC infusion was performed in one case.

Supportive care and transfusion practices

Antimicrobial prophylaxis consisted of trimethoprim-sulfamethoxazole, acyclovir and fluconazole during the neutropenic period and until day +180. Cytomegalovirus antigenaemia was monitored weekly during the first 3 months after the transplant and then as clinically indicated. Patients who tested positive were treated with gancyclovir or foscarnet.

Red blood cells were transfused to maintain haemoglobin levels of 8 g/dL or more, or when patients were symptomatic. Platelet support was given when platelet counts were less than 10x10^9/L, or less than 20x10^9/L when patients had signs of bleeding or when clinically indicated. All blood products were leucoreduced before storage and irradiated (2,500 cGy) to prevent transfusion-associated GvHD.

Patients with major ABO incompatibility received platelet and plasma transfusions of donor type and RBC transfusions of recipient type during the first period after the transplant and switched over to donor blood type when conversion of the ABO blood group was observed and antidonor alloantibody titres were undetectable. Platelets and plasma of recipient type and RBC of donor blood type were transfused after the transplant in patients with minor ABO incompatibility.

For patients with bidirectional incompatibility, the first choice of blood groups was AB for platelet and plasma infusions, and type O for RBC transfusions. In the case of ABO-incompatible platelet transfusions, pooled random platelets suspended in additive solution were used to minimise the amount of alloantibodies contained in the plasma.

Laboratory evaluations

After transplantation, complete blood counts including reticulocyte count and white blood cell differential, lactate dehydrogenase concentration, liver function tests and total bilirubin were performed.

Myeloid engraftment was defined as neutrophil recovery to more than 0.5x10^9/L for 3 consecutive days and as a platelet count over 20x10^9/L in the absence of platelet transfusions.

A rise in indirect bilirubin levels (to twice the upper limit of normal) and/or an increase in lactate dehydrogenase values to over 700 U/L (normal values, 313-618 U/L) in the presence of normal liver function tests and reticulocytosis were considered signs of haemolysis.

ABO and Rh typing, native ABO antibody titres, direct antiglobulin tests and RBC indirect antiglobulin tests were performed by the blood bank before the allogeneic HPC transplant and whenever clinically indicated. A patient was considered to have pure red cell aplasia when bone marrow biopsy demonstrated the absence of RBC precursors with adequate myeloid, lymphoid and megakaryocytic populations.

Chimerism was documented by short tandem repeat studies using polymerase chain reaction analysis. These analyses were performed with a diagnostic Kit (AmpFISTR®Identifiler® PCR Amplification Kit) that amplifies 15 short tandem repeat loci plus the gender marker amelogenin.

Products were identified by an automatic electrophoresis system (ABI Prism, 3130 Avant Genetic Analyzer Applied Biosystems).

Statistical analysis

Descriptive statistics are presented as median values and ranges. One way analysis of variance, chi-square test and Fisher's exact test were used to compare the means of the number of RBC and platelet transfusions and days to neutrophil and platelet engraftment among the ABO-matched and ABO-mismatched groups.

Results

Patients' characteristics

The patients' characteristics, nature of ABO mismatching, number of $CD34^+$ cells transfused and myeloid engraftment are summarised in Tables II and III.

Five out of the 19 patients (26%) received an ABO-incompatible transplant; in two cases (10%) there was a minor ABO mismatch, in one case (5%) a major ABO mismatch and in two cases (10%) bidirectional incompatibility. Patients in these groups did not significantly differ for age, sex, disease phase at transplant, pretransplant cytomegalovirus status, or the number of $CD34^+$ cells/kg infused.

Haematological recovery and chimerism

Patients received a median of $5x10^6$ $CD34^+$ cells/kg (range, 2.1-11.4x10^6), with no difference between the matched and mismatched ABO groups. Data on engraftment according to donor-recipient ABO compatibility are shown in Table III. Neutrophils decreased to less than $0.5x10^9$/L in 10 cases (53%) and recovered in these patients at a median of 6 days after transplantation (range, 8-14 days). Neutrophil recovery was not significantly different between the ABO-compatible and ABO-incompatible groups (p=0.85). The median time to an unsupported platelet count above $20x10^9$/L was 4 and 7 days for ABO-compatible/minor ABO-mismatched and major/bidirectional ABO-mismatched patients, respectively (p=0.33). Fifteen patients (79%) did not require any platelet support.

At 30 days after transplantation, 12 of 19 patients tested (63%) had engraftment with all cells of donor origin (100% chimeric), and continued to be fully chimeric on day 100+ evaluations. ABO compatibility did not influence the kinetics of donor-recipient molecular chimerism after transplantation.

Transfusion requirements

The median number of RBC and platelet units transfused within the first 100 days after transplantation was compared among ABO-compatible/minor ABO-mismatched and major/

Table II - Clinical and laboratory data of the 19 multiple myeloma patients receiving ABO-mismatched HPC from related donors following non-myeloablative conditioning.

Patient	Age (years)	Sex[a]	RBC group	$CD34^+$ cells/Kg received	DLI	GvHD grade[b]	Engraftment ANC[c] (day)	Engraftment Platelets[c] (day)	Chimerism Day 30	Chimerism Day 100	N RBC transf.	Outcome[d]
Minor ABO mismatch												
1	54	F/F	A/AB	5.8	0	I S	11	0	50	95	0	NCR, alive Day 547
2	50	F/M	0/A	5.1	0	0	0	0	100	100	0	CR, alive Day 1300
Major ABO mismatch												
1	55	M/F	0/A	5.1	0	I S	12	9	97	97	2	CR, alive Day 425
Bidirectional incompatibility												
1	62	F/M	B/A	7.9	0	0	0	0	94	95	8	NCR, died Day 100
2	54	M/M	B/A	3	0	I S	8	13	93	97	8	CR, alive Day 310

[a]Recipient/donor; F=female, M=male; DLI, donor lymphocyte infusions; [b]GvHD grades 0-IV; S=skin, [c]Absolute neutrophil count >$0.5x10^9$/L for 3 consecutive days and unsupported platelet count >$20x10^9$/L; [d]NCR=near complete remission, CR=complete remission.

Table III - Patients' characteristics and engraftment data

Patient characteristics	ABO compatible (n=14)	Minor ABO mismatch (n=2)	Major ABO mismatch (n=1)	Bidirectional incompatibility (n=2)	p value[a]
Males/females	8/6	1/1	1/0	1/1	
Age, median (range), years	56 (44-65)	53 (51-55)	55	58 (54-62)	
GvHD prophylaxis[b]	14	2	1	2	
$CD34^+$ cells received$x10^6$/kg	4.9 (2.1-11.4)	5.4 (5.1-5.8)	5.1	5.4 (3-7.9)	
Engraftment data					
Days to ANC[c] >$0.5x10^9$/L	6 (0-14)	5.5 (0-11)	124	4 (0-8)	0.85
Days to platelets >$20x10^9$/L	4 (0-18)	0	9	6.5 (0-13)	0.33
N. of RBC transfusions	1 (0-4)	0	2	8 (6-10)	<0.01
N. of platelet transfusions	0.1 (0-1)	0	0	3 (2-4)	<0.01
Mixed chimerism[d] (day 30)	4/14	1/2	0/1	2/0	0.52

[a]Major/bidirectional ABO mismatched versus others; [b]Cyclosporine and mycophenolate; [c]Absolute neutrophil count; [d]Presence of more than 5% donor cells and host-derived cells on more than one occasion in whole blood or bone marrow.

bidirectional ABO-mismatched allogeneic HPC recipients; the median numbers of RBC transfusions were 0.87 (range, 0-4) and 6 (range, 6-10), respectively ($p < 0.01$). Mild haemolysis was noted in one patient between days 10 and 30, with an increased bilirubin value of 2.6 mg/dL (normal range, 0.2-1.3 mg/dL). The median numbers of platelet transfusions were 0.12 (range, 0-1) and 2 (range, 2-4), for the remaining cases ($p < 0.01$). As shown in Table III, patients with major/bidirectional ABO incompatibility required more RBC and platelet units after transplantation and were transfused for longer periods of time, as compared with patients with minor or no ABO incompatibility.

Clinical outcome after transplantation

Acute GvHD occurred in 11 out of 19 evaluable patients (58%) within the first 100 days after their transplant. The GvHD was grade I in eight patients (42%), grade II in two patients (10%) and grade III in one patient (5%). Thirteen of 19 patients (68%) developed chronic GvHD up to day 100 after transplantation; the GvHD was extensive in six cases (31%) and limited in seven patients (37%). ABO compatibility did not influence the incidence of GvHD. At a median follow-up of 39 months (range, 3-89 months), six patients (32%) died: 5 of 14 (36%) in the ABO-compatible group, 1 of 3 (33%) in the major/bidirectional ABO-mismatched group and none in the minor ABO-mismatched group. The causes of death were infectious complications either alone or with coexisting acute or chronic GvHD.

Discussion and conclusions

It has been shown that a non-myeloablative conditioning regimen in heavily treated patients with multiple myeloma receiving allografts from their HLA-matched siblings permits durable engraftment[12]. Most of the patients were not candidates for conventional myeloablative conditioning because of age, co-morbid conditions, and/or extent of prior therapy. Following reduced intensity conditioning regimens, haematopoietic and immune function are both host and donor in origin for variable periods after transplantation, with a delayed disappearance of host alloantibody-producing plasma cells. Thus, the risks of haemolytic reactions and delayed RBC engraftment may be higher in cases of donor-recipient ABO-incompatibility when non-myeloablative regimens are used. Recent studies suggest that late recovery of neutrophils may occur after major ABO-mismatched transplantation in recipients of HPC from both related and unrelated donors[13-16], although this finding were not confirmed in the present study. As expected, we found that a major/bidirectional ABO-mismatch was associated with more platelet and RBC transfusions

within the first 100 days after transplantation and an increased incidence of mixed chimerism. Patients who had received a minor ABO-mismatched transplant, as well as most of those who had received an ABO-compatible one, did not require any transfusional support. Although transient, mild haemolysis was documented in one patient after an ABO-incompatible transplant in our study, a clinical diagnosis of severe haemolysis was not made in any of the patients.

Few studies have analysed the impact of ABO incompatibility on the development of GvHD after allogeneic transplants following reduced intensity conditioning protocols. In a study by Mielcarek *et al.*[17] accelerated disappearance of donor-directed alloantibodies was observed in patients with GvHD, suggesting that donor T cells lead to more rapid elimination of residual antibody-producing host cells. Another approach that has been successful in some cases of pure red cell aplasia after transplantation is the administration of a single dose of the humanised anti-CD20 rituximab[18,19]. This seems an interesting option to be considered to eliminate residual host B cells after major ABO-incompatible allogeneic HPC transplants when a reduced intensity conditioning regimen has been used. In our study, the incidence and time of onset of acute and chronic GvHD were not affected by ABO-mismatching, as reported by other series[18]. The impact of ABO incompatibility on transplant-related mortality after allogeneic transplantation is still controversial. Most studies conducted in patients treated with conventional myeloablative conditioning did not report an increased mortality after ABO-incompatible transplants but only few have analysed the impact of ABO-mismatching after reduced intensity conditioning regimens[5,20]. Kanda *et al.* recently demonstrated that higher transplant-related mortality observed in the early period after bidirectional ABO-mismatched HPC transplants may be due to the combination of major and minor ABO-mismatching with additive or synergistic enhancement of single adverse effects[21]. Theoretically, major ABO-mismatching leads to antidonor cell damage and release of cytokines soon after transplantation. This may enhance the subsequent activation of antihost donor-derived lymphocytes in the minor mismatch direction. Fatal transplant complications such as severe acute GvHD may, therefore, occur more often among the bidirectional mismatched group. In the present study, transplant-related mortality was not affected by ABO-incompatibility.

In conclusion, although delayed RBC engraftment and increased transfusion requirements were documented after major/bidirectional ABO-incompatible transplants, the reduced intensity conditioning regimen used in our protocol enabled successful clinical outcomes without major immunohaematological complications, reflecting that a sufficient degree of B-cell suppression was achieved by the treatment scheme.

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