Prevention of pure red cell aplasia after major or bidirectional ABO blood group incompatible hematopoietic stem cell transplantation by pretransplant reduction of host anti-donor isoagglutinins

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ABSTRACT

Background

Persistent anti-donor isoagglutinins after major ABO blood group incompatible hematopoietic stem cell transplantation may cause delayed red blood cell engraftment and post-transplant pure red cell aplasia.

Design and Methods

We investigated the effect of pretransplant anti-donor isoagglutinin reduction by *in vivo* absorption and/or plasmapheresis on the incidence of pure red cell aplasia and the time to red blood cell engraftment in 153 hematopoietic stem cell transplant recipients with major ABO incompatibility.

Results

Twelve patients (8%) developed pure red cell aplasia, 3/98 (3%) with, and 9/55 (16%) without prior isoagglutinin reduction (p=0.009). Red blood cell engraftment was faster in patients with isoagglutinin reduction; in addition, peripheral blood hematopoietic stem cell transplantation, acute graft-versus-host disease, and younger age were associated with faster red blood cell engraftment in Cox regression analysis. In patients with pure red cell aplasia the mean red blood cell engraftment occurred after 225 days (p<0.001) and was associated with a simultaneous decrease of anti-donor isoagglutinins. Patients with pure red cell aplasia had higher pretransplant anti-donor isoagglutinin titers (p=0.001) and received more post-transplant red blood cell transfusions (p<0.001).

Conclusions

Following major ABO incompatible hematopoietic stem cell transplantation, pure red cell aplasia and delayed red blood cell engraftment depend on the levels of anti-donor isoagglutinins and are efficiently prevented by the pretransplant removal of these isoagglutinins. The benefits of reducing the time of transfusion-dependency and transfusion-associated risks must be carefully balanced against the potential side effects of isoagglutinin reduction.

Key words: ABO blood group incompatibility, allogeneic hematopoietic stem cell transplantation, pure red cell aplasia.

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Introduction

Approximately 30-40% of all allogeneic hematopoietic stem cell transplants (HSCT) are ABO incompatible.[1-3] These incompatibilities include major and bidirectional ABO incompatibility, defined by the presence of isoagglutinins in the recipient against donor red blood cells (RBC) and carry the risk of post-transplant hemolysis. Whereas post-transplant hemolysis can be efficiently prevented by the removal of RBC from the graft or by reduction of anti-donor isoagglutinins from the recipient,[3-6] there is no general consensus on the prophylactic approach and the respective effect of pretransplant isoagglutinin removal on RBC engraftment and pure red cell aplasia (PRCA).[7]

Post-transplant PRCA is a hyporegenerative anemia characterized by the absence of RBC precursors in the bone marrow, and often requires RBC transfusions for a prolonged period of time. Presumably, it is caused by the destruction of RBC precursors through anti-donor isoagglutinins produced by persisting host plasma cells. We hypothesized that pretransplant removal of anti-donor isoagglutinins by plasmapheresis or transfusion of donor-type ABO incompatible RBC (*in vivo* adsorption) may facilitate RBC engraftment and consequently reduce the incidence of PRCA. We, therefore, performed a retrospective analysis of patients with major or bidirectional ABO incompatible HSCT undergoing two different regimens: (i) pretransplant reduction of anti-donor isoagglutinin titer and post-transplant transfusion of donor-type RBC or (ii) removal of RBC from the graft followed by post-transplant transfusion of recipient-type RBC. In this cohort, the incidence and the risk factors of post-transplant PRCA and the time to RBC engraftment were determined.

Design and Methods

Patients

A total of 153 consecutive patients receiving major (n=123, 80%) or bidirectional (n=30, 20%) ABO incompatible allogeneic HSCT in two Swiss centers from 1980 to 2002 were retrospectively analyzed. All patients gave written informed consent for retrospective data analyses and the study was approved by the institutional review boards. The majority of the patients had been transplanted for hematologic malignancies including acute myelogenous leukemia (n=55, 36%), acute lymphoblastic leukemia (n=24, 16%), and chronic myelogenous leukemia (n=40, 26%). Early disease was defined as acute leukemia in complete first remission after chemotherapy and chronic myelogenous leukemia in first chronic phase (n=61, 40%). All other stages of these malignancies and all other types of hematologic cancers (n=82, 54%) were considered as advanced disease. Ten patients (6%) were transplanted for non-malignant hematologic disorders. The median follow-up time of the surviving patients was 70 months (range, 7-254). Seventy-four percent of the patients received bone marrow and 26% peripheral blood stem cells. Conventional myeloablative conditioning was used in 130/153 (85%) patients and consisted of cyclophosphamide and total body irradiation, cyclophosphamide/total body irradiation and etoposide, or busulfan/cyclophosphamide. The remaining patients (23, 15%) received reduced-intensity conditioning consisting of fludarabine/total body irradiation, fludarabine/busulfan/antithymocyte globulin or cyclophosphamide with or without antithymocyte globulin. Details of the transplant procedures are listed in Table 1. Cyclosporine A combined with short-term methotrexate (78, 51%) or cyclosporine A alone (n=69, 45%) was administered for graft-versus-host disease (GvHD) prophylaxis, the remaining six patients received other regimens. Tapering of the immunosuppression was started 3-6 months after allogeneic HSCT if no signs of GvHD were present and was reduced over a period of 3 months. First-line therapy for acute and chronic GvHD was corticosteroids. In steroid-resistant cases, daclizumab, methotrexate or OKT3 was administered according to each center's policy. Thirteen patients received T-cell-depleted stem cells. The baseline characteristics of patients who did or did not undergo anti-donor isoagglutinin reduction are outlined in Table 1. The two groups differed significantly with respect to age of the patients, and the incidence and severity of acute GvHD. In particular, patients who underwent isoagglutinin reduction were younger and had a higher incidence of acute GvHD.

Anti-donor isoagglutinin reduction and transfusion policies

Isoagglutinin reduction was performed by transfusion of ABO incompatible, donor-type RBC (n=70, 46%), plasmapheresis (n=6, 4%), or a combination of both methods (n=22, 14%), as previously described.[6,8] Most of these patients were treated in one center (96/98, 98%), and received a median of three donor-type RBC transfusions on 3 consecutive days, generally administered during total body irradiation immediately before HSCT. The aim was to lower the pretransplant isoagglutinin titer to less than or equal to 1:2 and isoagglutinin titers were measured daily during the procedure. To prevent adverse reactions, the incompatible RBC were transfused slowly over a period of 12 hours after administration of corticosteroids, prehydration with crystalloids followed by forced diuresis with furosemide and mannitol. In general, the procedure was well tolerated, but transfusion reactions including hemolysis, fever, rigors, hematuria and lumbar pain were noted.

Plasmapheresis was performed on 3 consecutive days directly prior to HSCT using a Cobe Spectra® cell separator (Gambro BCT, Lakewood, CO, USA) by exchanging one or two plasma volumes. Replacement fluids were human albumin or fresh-frozen plasma in patients with a history of bleeding. Patients with pretransplant isoagglutinin reduction received post-transplant donor-type RBC transfusions. In cases with post-transplant hemolysis, transfusions were partially switched to recipient-type RBC according to the physicians' judgments. All patients with clinically significant hemolysis received recipient-type RBC transfusion as long as donor-specific

Table 1A. Baseline characteristics of the patients.

	Pretransplant anti-donor isoagglutinin titer reduction			
	Yes (n=98)	No (n=55)	Total (n=153)	p value[1]
Patient age [years (range)]	28 (4-61)	34 (3-61)	30 (7-61)	0.006
Gender [number of patients (percent)]				0.926
Male	56 (57.1)	31 (56.4)	87 (56.9)	
Female	42 (42.9)	24 (43.6)	66 (43.1)	
Donor-recipient gender-match				0.573
Male-male	39 (39.8)	23 (41.8)	62 (40.5)	
Male-female	14 (14.3)	12 (21.8)	26 (17.0)	
Female-female	28 (28.6)	12 (21.8)	40 (26.1	
Female-male	17 (17.3)	8 (14.5)	25 (16.3)	
Matched related/unrelated donor				0.458
Matched related donor	75 (76.5)	42 (76.4)	117 (76.5)	
Matched unrelated donor	15 (15.3)	11 (20.0)	26 (17.0)	
HLA-mismatch	8 (8.2)	2 (3.6)	10 (6.5)	
ABO match				0.347
Major	81 (82.7)	42 (76.4)	123 (80.4)	
Bidirectional	17 (17.3)	13 (23.6)	30 (19.6)	
Diagnosis				0.517
AML	36 (36.7)	19 (34.5)	55 (35.9)	
ALL	16 (16.3)	8 (14.5)	24 (15.7)	
CML	21 (21.4)	19 (34.5)	40 (26.1)	
Lymphoma	7 (7.1)	4 (7.3)	11 (7.2)	
MDS	7 (7.1)	3 (5.5)	10 (6.5)	
SAA	8 (8.2)	1 (1.8)	9 (5.9)	
Other	3 (3.1)	1 (1.8)	4 (2.6)	
Disease stage				0.549
Non-malignant	8 (8.2)	2 (3.6)	10 (6.5)	
Early disease	38 (38.8)	23 (41.8)	61 (39.9)	
Advanced disease	52 (53.1)	30 (54.5)	82 (53.6)	

Table 1B. Baseline characteristics of the patients.

	Pretransplant anti-donor isoagglutinin titer reduction			
	Yes (n=98)	No (n=55)	Total (n=153)	p value[1]
Conditioning[2]				0.124
Cy/TBI ± others	80 (81.6)	37 (67.3)	117 (76.5)	
Bu/Cy	6 (6.1)	7 (12.7)	13 (8.5)	
Reduced intensity	12 (12.2)	11 (20.0)	23 (15.0)	
Stem cell source				0.534
Bone marrow	74 (75.5)	39 (70.9)	113 (73.9)	
Peripheral blood	24 (24.5)	16 (29.1)	40 (26.1)	
Total nucleated cells[3]	3.7 (0.3-30.7)	3.2 (0.4-15.9)	3.7 (0.3-30.7)	0.258
Hemolysis				0.090
No	54 (55.1)	38 (69.1)	92 (60.1	
Yes	44 (44.9)	17 (30.9)	61 (39.9)	
GvHD prophylaxis				0.606
CsA	47 (48.0)	22 (40.0)	69 (45.1)	
CsA/MTX	47 (48.0)	31 (56.4)	78 (51.0)	
Others	4 (4.1)	2 (3.6)	6 (3.9)	
T-cell depletion				0.771
No	89 (90.8)	51 (92.7)	140 (91.5)	
Yes	9 (9.2)	4 (7.3)	13 (8.5)	
Acute GvHD				0.012
No	24 (24.5)	24 (43.6)	48 (31.4)	
Grade I	20 (20.4)	17 (30.9)	37 (24.2)	
Grade II	29 (29.6)	7 (12.7)	36 (23.5)	
Grade III	12 (12.2)	4 (7.3)	16 (10.5)	
Grade IV	13 (13.3)	3 (5.5)	16 (10.5)	

[1]Differences among the groups were analyzed by χ^2, Mann-Whitney U, or Student's t tests, as appropriate, [2]Myeloablative conditioning: Cy/TBI, Cy/VP16/TBI, Cy/VP16/ATG/TBI, Reduced intensity conditioning: Flu/Bu/ATG, Flu/TBI, Cy±ATG. [3]Median total nucleated cell x10^8/kg body weight. AML: acute myelogenous leukemia; ALL: acute lymphoblastic leukemia; CML: chronic myelogenous leukemia; MDS: myelodysplastic syndrome; SAA: severe aplastic anemia; Cy: cyclophosphamide; TBI: total body irradiation; Bu: busulfan; VP16: etoposide; ATG: antithymocyte globulin; Flu: fludarabine; CsA: cyclosporine A; MTX: methotrexate.

antibodies were measurable or any signs of hemolysis were present.

In the remaining 55 patients who did not undergo isoagglutinin reduction, post-transplant hemolysis was prevented by removal of RBC from the graft. These patients were treated predominantly in the second center (44/55, 80%) and received exclusively recipient- or O-type RBC transfusions as long as anti-donor isoagglutinins were detectable or direct antiglobulin testing was positive. All patients received RBC transfusions if their hemoglobin concentration was less than 60-80 g/L or if clinical signs of anemia were present. Donor-type RBC chimerism was defined as the occurrence of a complete ABO donor blood group type without any transfusional support for at least 2 months.

Laboratory evaluations

During hospitalization, leukocyte and platelet counts and hemoglobin concentration were determined daily, while reticulocyte and differential leukocyte counts were measured at least twice a week. After discharge, these values were assessed on a weekly basis until day 100 and at least four times a year during long-term follow-up. Isoagglutinin titers were determined by serial dilution saline agglutination.[9] Further laboratory evaluations included measurements of creatinine, liver enzymes, bilirubin, and lactate dehydrogenase. Post-transplant PRCA was defined as anemia with low or absent reticulocytes counts (<1%) in the peripheral blood for more than 100 days in association with normal white blood cell and platelet engraftment. Bone marrow examinations were routinely performed 3 months after HSCT, showing a lack of erythropoiesis in the presence of normal myelo- and megakaryopoiesis in all patients with PRCA. RBC engraftment was documented by the appearance of more than 1% reticulocytes in the peripheral blood, platelet engraftment by platelet counts greater than 50×10^9/L for 3 days without transfusion therapy, and neutrophil engraftment by absolute neutrophil counts of more than 0.5×10^9/L for 3 days.

Statistical analysis and literature review

Means and proportions of baseline characteristics were compared by Mann-Whitney U, Student's t, and χ^2 tests, as appropriate. Univariate risk factors for PRCA were analyzed by logistic regression. The survival functions were estimated with the method of Kaplan and Meier and compared by the log rank test. The cumula-

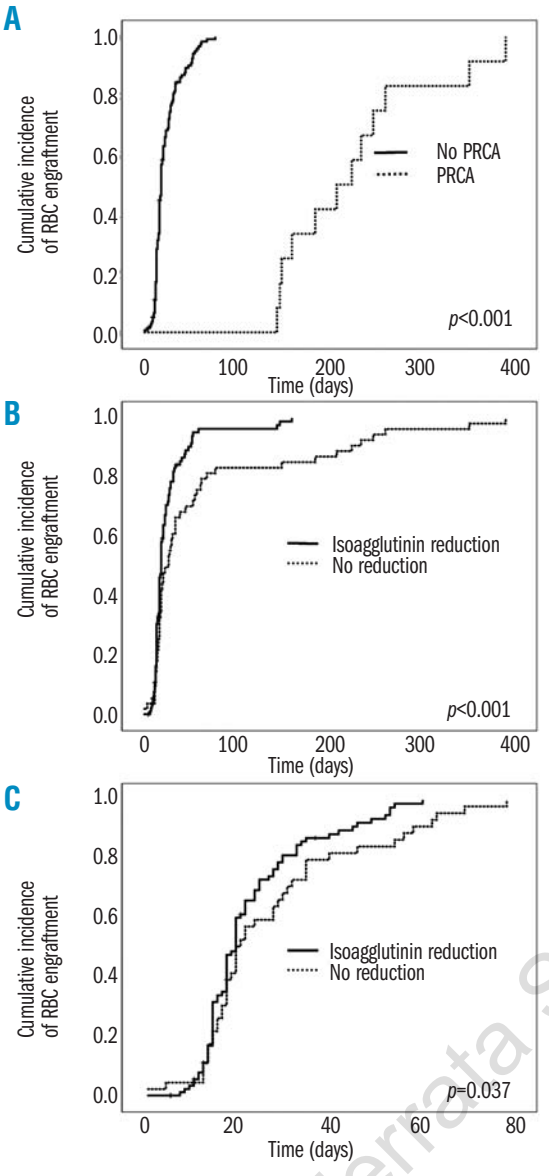

Figure 1. Cumulative incidence of PRCA and RBC engraftment (A) Incidence of PRCA. Mean time to RBC engraftment was later in patients with PRCA (dotted lines, n=12) (225 days; 95%-CI 180-270) than in patients without PRCA (solid lines, n=141) (25 days; 95%-CI 22-27) (p<0.001). (B) RBC engraftment in all patients with major or bidirectional ABO incompatible HSCT (n=153). Patients who had undergone anti-donor isoagglutinin reduction (solid lines, n=55) had faster RBC engraftment (p<0.001) than patients who had not undergone such reduction (dotted lines, n=98). (C) RBC engraftment after exclusion of the 12 patients with PRCA. The delayed in RBC engraftment was still evident after exclusion of all patients with PRCA (p=0.037).

tive incidence of PRCA was calculated using death without RBC engraftment as a competing risk. Cox proportional hazards regression models were used for the multivariate analysis of risk factors for RBC engraftment. To account for onset times of acute GvHD a proportional hazards regression model was built using a time-dependent covariate for acute GvHD in such a way that patients were in the group without acute GvHD at the time of transplant and switched to the group with acute GvHD at the time of onset of this complication.

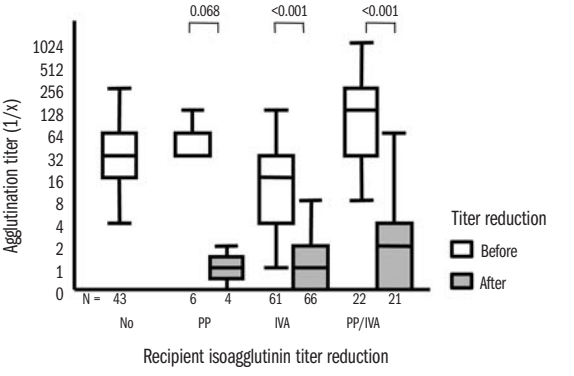

Figure 2. Pretransplant anti-donor isoagglutinin reduction. Box plots represent median values of agglutination titers before (white) and after (gray) isoagglutinin reduction. Isoagglutinins were not reduced (No) or reduced by plasmapheresis (PP), *in vivo* adsorption (IVA) or a combination of both techniques (IVA/PP). The median anti-donor isoagglutinin titer in recipients of major ABO incompatible HSCT was 1:32 before and 1:1 after the reduction of anti-donor isoagglutinins (p<0.001). The adsorption capacity of the different procedures was similar, but the combination of IVA and PP was used more often in patients with higher titers.

Covariates (age, disease stage, HLA match, year of transplantation, gender mismatch, conditioning, and GvHD prophylaxis) were entered in a forward stepwise fashion. All reported p values are two-sided, and p value less than 0.05 were assumed to be statistically significant.

To identify previously published case reports and case series with post-transplant PRCA a Pubmed search was carried out using the MESH terms *red cell aplasia* or *PRCA*, *HSCT* or *stem cell transplantation*, and *ABO blood group*. Only articles written in English were considered for the analysis. Case series were defined as reports on PRCA patients with an appropriate control group (major ABO incompatible patients without PRCA) allowing incidence calculation.

Results

Post-transplant pure red cell aplasia

Twelve out of 153 (8%) patients who received ABO incompatible transplants developed post-transplant PRCA. PRCA was self-limiting and all patients ultimately became transfusion-independent. Figure 1A shows the time to RBC engraftment of all patients with PRCA (mean 225 days, 95%-CI 180-270) compared to that in the 141 patients without PRCA (mean 25 days, 95%-CI 22-27) (p<0.001). The median anti-donor isoagglutinin titer in patients with PRCA was 1:64 (range, 1:32-1:1024) compared to 1:16 (range, 1:1-1:1024) in patients without PRCA (p=0.001). PRCA was observed exclusively in patients with anti-donor isoagglutinin titers greater than 1:16. It occurred in 3/98 (3%) patients who had undergone anti-donor isoagglutinin reduction and in 9/55 (16%) who had not (p=0.009). Figure 2 outlines the reduction of isoagglutinins by *in vivo* adsorption, plasmapheresis or a combination of both methods. Isoagglutinins were reduced by a median of five titer steps from 1:32 to 1:1 (p<0.001). The adsorption capaci-

Table 2. Clinical course of patients with PRCA.

Patient - UPN	39	40	49	106	149	153	177	231	252	684	808	887
ABO (D/R)	A/O	A/O	A/O	A/O	A/O	A/O	A/O	A/O	A/O	A/O	AB/O	B/O
Age at HSCT	37	36	14	34	26	41	51	43	39	53	26	40
Gender (D/R)	F/F	M/F	M/M	M/M	F/M	M/M	M/M	M/M	F/M	M/M	F/F	M/M
Year of HSCT	86	86	87	92	96	96	98	00	01	98	00	01
Disease	AML	CML	ALL	CML	CML	CML	CML	AML	NHL	CIMF	CML	AML
Disease stage[1]	II	II	II	I	I	I	I	II	II	II	II	I
Conditioning	Cy/TBI	Cy/TBI	Cy/VP16 /TBI	Bu/Cy	Cy/TBI	Bu/Cy	Bu/Cy	Cy/TBI	Flu/Bu /ATG	Cy/VP16 /TBI	Cy/VP16 /TBI	Cy/TBI
HLA match	MRD	MRD	MRD	MRD	MRD	MRD	MRD	MUD	MRD	MRD	MRD	MRD
Stem cell source	BM	BM	BM	BM	BM	BM	BM	BM	PB	PB	BM	PB
GvHD prophylaxis	CsA	CsA	CsA	CsA/MTX	CsA/MTX	CsA/MTX	CsA/MTX	CsA/MTX	CsA/MTX	CsA/MTX	CsA/MTX	CsA/MTX
Acute GvHD[2]	0	I (12)	I (10)	I (36)	0	I (24)	0	IV (708)	IV (193)	0	I (33)	0
Isoagglutinin reduction	No	No	No	No	No	No	No	No	No	PP/IVA	PP/IVA	IVA
Isoagglutinins[3]	1:1024	1:32	1:64	1:64	1:128	ND	ND	1:64	1:64	1:256/1:2	1:256/1:4	1:32/1:1
IAT decrease (days)	229	180	148	NR	213	188	346	173	166	139	125	157
Transfusions (n/days)	29/250	22/190	12/118	55/370	52/259	32/175	52/353	60/228	36/350	27/179	43/213	16/74
RBC take/chimerism[4]	245/Yes	181/Yes	148/Yes	382/No	256/Yes	222/Yes	346/Yes	214/Yes	203/No	147/Yes	143/Yes	157/Yes
Outcome (months)	CR	Rel (18)	CR	Rel (17)	CR	CR	CR	Rel (17)	GvHD	Rel (40)	CR	CR
Follow up (months)	122	34	129	167	36	24	30	25/Death	12/Death	41/Death	12	36

AML: acute myelogenous leukemia; ALL: acute lymphatic leukemia; ATG: antithymocyte globulin; B: busulfan; CIMF: chronic idiopathic myelofibrosis; CML: chronic myelogenous leukemia; CR: complete remission; CsA: cyclosporine A; Cy: cyclophosphamide; Flu: fludarabine; IVA: in vivo adsorption; MRD: matched related donor; MTX: methotrexate; MUD: matched unrelated donor; ND: not determined; NHL: non-Hodgkin lymphoma; PP: plasmapheresis; Rel: relapse; TBI: total body irradiation; VP16: etoposide. [1]non malignant disease, 0; early disease stage, I; advanced disease stage II, [2]Grade (day of onset). [3]Pretransplant anti-donor isoagglutinin titer before and after titer reduction. [4]Donor-type RBC chimerism was defined as the occurrence of a complete donor blood group type without any transfusional support for at least 2 months.

ty was similar with the different procedures, but the combination of *in vivo* adsorption and plasmapheresis was used more often in patients with higher titers.

The clinical details of all patients with PRCA are listed in Table 2. All 12 patients with PRCA had blood group O and had received a major ABO incompatible HSCT from a blood group A (n=10), AB (n=1), or B (n=1) donor. No case of PRCA was observed after bidirectional ABO incompatible HSCT. Presumably, this difference can be attributed to the lower pretransplant isoagglutinin titers in the group of patients with bidirectional ABO incompatibility (bidirectional incompatibility: median 1:16; range 1:1-1:256 *vs.* major incompatibility: median 1:32; range 1:1-1:1024; p=0.012). The three patients with PRCA despite anti-donor isoagglutinin reduction had a shorter time to RBC engraftment (143, 147 and 157 days) than the nine patients without titer reduction. Patients with PRCA received more RBC transfusions (mean 36, range 12-60 *vs.* 12, range 0-59; p<0.001). In 11 patients, resolution of PRCA was preceded by a spontaneous decrease of anti-donor isoagglutinins to titers less than 1:8, whereas one patient recovered from PRCA despite persistently elevated anti-donor isoagglutinins. In this patient, a relapse of the underlying disease was diagnosed shortly after recovery from PRCA and the increase of hemoglobin was caused by recipient-type RBC. Ten patients with PRCA eventually achieved complete donor-type RBC chimerism. Two patients never had a complete switch to donor-type RBC in the peripheral blood, one because of relapse and the other because of death due to acute grade IV GvHD. Two patients had late-onset acute GvHD. One patient received reduced intensity conditioning and HSCT and developed grade IV acute GvHD 1 month after tapering off immunosuppression. In this patient, the resolution of PRCA was associated with the onset of acute GvHD. The second patient experienced a late relapse after HSCT which was treated with reinduction chemotherapy followed by donor-lymphocyte infusion. Eighty-seven days after donor lymphocyte infusion he developed a therapy-refractory grade IV acute GvHD of the liver and the skin and died 142 days after infusion of the donor lymphocytes.

The risk factors for PRCA were analyzed by univariate logistic regression (Table 3). Patients who underwent isoagglutinin reduction were less likely to develop PRCA (odds ratio 0.16, 95%-CI 0.04-0.63, p=0.008), whereas HSCT recipients from A or AB donors were at greater risk (odds ratio 9.96, 95%-CI 1.25-79.21, p=0.039). Patients with acute GvHD grades II-IV showed a trend toward a lower incidence of PRCA (odds ratio 0.23, 95%-CI 0.05-1.07, p=0.062) and patients older than the mean age at HSCT showed a trend toward a higher incidence of PRCA (odds ratio 3.61, 0.94-13.89, p=0.062). However, neither of these tendencies was statistically significant.

Hematopoietic engraftment

Eight patients did not achieve RBC engraftment because of early transplant-related death (5%) with a median survival time of 12 days (range, 6-37 days). The mean time to RBC engraftment in the remaining

patients was 42 days (95%-CI 32-53). Neutrophil engraftment was reached after 17 days (95%-CI 16-19) and platelet engraftment after 32 days (95%-CI 27-38) without differences between the groups (p=0.379 and p=0.364, respectively). As shown in Figure 1B, patients who underwent anti-donor isoagglutinin reduction had faster RBC engraftment (p<0.001). This difference also persisted after exclusion of all patients with PRCA (mean 23 days, 95%-CI 21-26 *vs.* 28 days; 95%-CI 23-33; p=0.037; Figure 1C). Isoagglutinin reduction, bidirectional ABO incompatibility, HSCT using peripheral blood stem cells, younger age of the recipient at transplantation, reduced-intensity conditioning regimens, and the occurrence of acute grade II-IV GvHD significantly reduced the time to RBC engraftment according to the results of a Cox regression analysis (Table 4). In contrast, the number of stem cells in the graft, the year of HSCT, the conditioning regimen, gender mismatch, or GvHD prophylaxis did not influence RBC engraftment. Cyclosporine A as a single agent for GvHD prophylaxis has been described as a risk factor for the development of PRCA and delayed RBC engraftment. However, in this study, the distribution of the two GvHD prophylaxis regimens was similar among patients with and without PRCA and GvHD prophylaxis did not influence the time to RBC engraftment.

Discussion

The present study documents a protective effect of pretransplant anti-donor isoagglutinin reduction on the development of delayed RBC engraftment and post-transplant PRCA following major or bidirectional ABO incompatible HSCT. PRCA occurred in 3% of patients who underwent isoagglutinin reduction and in 16% who did not. As previously reported the importance of anti-donor isoagglutinins for the occurrence of post-transplant PRCA was supported by a clear association between the recovery of reticulocytes and the decrease of anti-donor isoagglutinins in 11/12 patients with PRCA.[10] In the remaining patient re-emergence of RBC hematopoiesis occurred despite persistently elevated anti-donor isoagglutinins and relapse was later diagnosed. Thus, RBC engraftment represented host rather than donor RBC erythropoiesis and was the first sign of relapse.

As observed by others, the threshold of anti-donor isoagglutinin titers necessary for successful donor-type RBC engraftment ranged from 1:8 and 1:2.[11,12] In line with these data, nearly all published cases of post-transplant PRCA were reported by centers that did not use pretransplant isoagglutinin reduction.[13-15] In the few studies in which PRCA occurred despite isoagglutinin reduction, a rebound of anti-donor isoagglutinins suggests post-transplant transfusion of recipient-type RBC.[16] If the isoagglutinins are only temporarily removed prior to the HSCT, they may rebound soon after transplantation to an even higher level.[17] In contrast, pretransplant anti-donor isoagglutinin reduction by the above mentioned strategies in combination with post-transplant transfusion of donor-type RBC may provide a durable suppression of anti-donor isoagglutinins and facilitate RBC engraftment. In conclusion, the combination of pretransplant reduction of isoagglutinin titers and the at least partial post-transplant transfusion of donor-type RBC appears to be crucial for the prevention of PRCA.

Table 3. Univariate risk factor analysis for pure red cell aplasia.

Risk factors	Odds ratio	95%-CI	p value[1]
Isoagglutinin reduction			
No	1.00		
Yes	0.16	0.04-0.63	0.008
Acute GvHD			
Grade 0-I	1.00		
Grade II-IV	0.23	0.05-1.07	0.062
GvHD prophylaxis[2]			
CsA/MTX	1.00		
CsA	1.86	0.53-6.46	0.331
Blood group constellation			
A or AB donor to O recipient	9.96	1.25-79.21	0.039
Other combinations	1.00		
Conditioning			
Myeloablative	1.00		
Non-myeloablative	0.49	0.06-4.00	0.507
Stem cell source			
Bone marrow	1.00		
Peripheral blood	0.94	0.24-3.65	0.925
Age at HSCT			
Below mean age	1.00		
above mean age	3.61	0.94-13.89	0.062
Rhesus compatibility			
Compatible	1.00		
Incompatible	0.44	0.05-3.61	0.446
Donor type[3]			
Matched related	1.00		
Matched unrelated	0.38	0.05-3.13	0.372

[1]Univariate risk factors were analyzed by logistic regression. [2]Six patients with alternative GvHD prophylaxis were excluded: [3]Ten patients with HLA-mismatches were excluded.

Table 4. Cox regression analysis of red blood cells engraftment.

Variable	Hazard ratio	95%-CI	p value
ABO incompatibility			
Major	1.00	–	–
Bidirectional	1.97	1.26-3.06	0.003
Isoagglutinin titer reduction[1]			
No	1.00	–	–
Yes	1.57	1.05-2.35	0.029
Acute GvHD[2]			
Grade 0-I	1.00	–	–
Grade II-IV	1.79	1.19-2.70	0.050
Stem cell source			
Bone marrow	1.00	–	–
Peripheral blood	2.18	1.41-3.38	<0.001
Conditioning			
Myeloablative	1.00	–	–
Reduced intensity	1.81	1.08-3.03	0.025
Age at HSCT[3]	0.99	0.97-1.00	0.032

[1]Anti-donor isoagglutinin titer reduction: in vivo *adsorption, plasmapheresis, or both methods. [2]Acute GvHD was analyzed as a time-dependent covariate. [3]Increment=1 year.*

Although the mechanism of post-transplant PRCA is not fully understood, it is believed that the persistence of host B lymphocytes or plasma cells producing anti-donor isoagglutinins is responsible for the delayed engraftment.[12] In support of this hypothesis, autologous plasma derived from patients with PRCA inhibits donor-type erythropoiesis *in vitro*.[18-20] In contrast, early erythroid progenitors can engraft at the same rate as myeloid progenitors measured by erythroid burst forming unit assays, indicating that ABO antigens are acquired at a later stage of erythroid commitment.[21] Moreover, mixed chimerism analyses of hematopoietic cells after HSCT demonstrated temporal differences in the post-transplant eradication of recipient cells with a persistence of plasma cells for up to 9 months.[12]

In the present study the overall incidence of post-transplant PRCA was 8% and this complication occurred after major but not bidirectional ABO incompatible HSCT. The only two risk factors identified were a lack of pretransplant isoagglutinin reduction and the blood group constellation A or AB donor into an O group recipient. A review of the published literature identified 128 patients with PRCA in 18 case series[11–13,15,20-33] and 35 case reports[16,18,19,34-66] with an overall incidence of 15% (range, 2-50%).[12,22] However, this may overestimate the true incidence due to a publication bias. The most frequent blood group constellation was A or AB donor and O group recipient, occurring in 77% of all patients. The higher incidence of PRCA in recipients with anti-A isoagglutinins may be explained by the higher levels and the longer persistence of anti-A antibodies as compared to anti-B antibodies.[25] Major ABO-incompatibility was the most common risk factor described in 120 patients, bidirectional ABO incompatibility was found in 5 patients.[16,25,37] Two of the remaining three patients developed PRCA after ABO compatible HSCT[41,49] and one after autologous HSCT.[39] No clear explanation was found for the PRCA in these patients. Anti-donor isoagglutinin titers were reported in most of the publications. The median titers were 1:128 (1:2-1:4096) for IgM and 1:512 (1:2-1:16000) for IgG, but the titers were evaluated using a variety of different techniques. Most authors suggested high pretransplant isoagglutinin titers as a cause of the observed PRCA, but none of the case series could clearly establish a correlation between the incidence of PRCA and the titer levels. Parvovirus B19 infection has been shown to be responsible for PRCA after allogeneic or autologous HSCT.[67-69] However, in a large series of 201 adult patients who underwent allogeneic HSCT, no parvovirus B19 infections occurred in the first year and only three cases were detected in the second year after HSCT.[70] It, therefore, seems unlikely that parvovirus B19 infections are primarily responsible for post-transplant PRCA, although this was not routinely analyzed in our cohort of patients.

The roles of reduced intensity conditioning and the number of transplanted cells in the pathogenesis of post-transplant PRCA are still controversial. Although several publications have reported on the occurrence of PRCA after reduced intensity conditioning transplants, with an incidence ranging from 2-50%, it is difficult to draw definitive conclusions as they described very heterogeneous populations.[12,20,21,27,31,71] In theory, conditioning protocols of lower intensity and GvHD prophylaxis regimens could lead to a higher incidence of post-transplant PRCA because of less activity against plasma cells. In our cohort, however, we did not find a higher incidence of PRCA in the small group of patients who received reduced intensity conditioning and the time to RBC engraftment was similar in the groups conditioned with reduced intensity or myeloablative regimens.

The eradication of recipient-type plasma cells after allogeneic HSCT depends on a graft-versus-plasma cell effect. This was demonstrated by Mielcarek *et al*. who showed that donor-specific isoagglutinins disappear more rapidly in the case of matched unrelated donors than matched related donors and patients with acute GvHD had a more rapid clearance of isoagglutinins in both groups.[72] In the present study, 7/12 patients with post-transplant PRCA developed acute GvHD, but only in one patient was the resolution of PRCA related to the onset of GvHD. However, the majority of patients had only mild acute GvHD, which may not have been sufficient to overcome PRCA. Moreover, although several case reports suggested such an association, it was not confirmed by any of the case series.[30, 49] In support of the findings of Mielcarek *et al.*, we observed that both the occurrence of acute GvHD and isoagglutinin reduction were associated with a shorter time to RBC engraftment in patients with major ABO incompatible HSCT. In contrast, faster RBC engraftment in patients who underwent GvHD was not observed after ABO-identical HSCT (*data not shown*). Finally, the use of cyclosporine A alone without methotrexate for GvHD prophylaxis has been associated with delayed RBC engraftment or hemolysis.[23,73] Since methotrexate has a clear immunosuppressive effect on B-lymphocytes and antibody production, it might reduce the isoagglutinin titers after HSCT. However, in our study, we did not observe a difference in the incidence of PRCA and delayed RBC engraftment depending on whether methotrexate was or was not administered.

The first therapeutic approach to overcome post-transplant PRCA is the reduction or withdrawal of immunosuppression to enhance the graft-versus-plasma cell effect.[48,49] If this strategy is not successful, several other strategies have been proposed according to the pathophysiology of the disorder: plasmapheresis,[43,46,64] antithymocyte globulin,[38,41] erythropoietin,[39,42,44,61] corticosteroids,[53,56] rituximab,[47,54] and donor-lymphocyte infusions to induce GvHD.[45,51] Virtually all of these treatments have only been evaluated in a few patients or in single case reports. In our cohort, one patient was treated with plasmapheresis without clinical improvement; all other patients received supportive treatment with RBC transfusions and iron chelation if necessary. Anti-donor isoagglutinins eventually disappeared in all but one patient without specific measures. The spontaneous antibody clearance may reflect the approximate lifetime of recipient plasma cells or the eradication of the remaining plasma cells by the donor immune system. Since the best treatment for patients with PRCA is unknown, the potential side effects of

any strategies used must be carefully balanced against the benefit of reducing the time of transfusion-dependency with the associated risks.

In summary, from the analysis of a large group of patients who underwent major ABO incompatible HSCT, it was found that pretransplant reduction of anti-donor isoagglutinins enhanced RBC engraftment and prevented post-transplant PRCA. In addition our data suggest that post-transplant administration of donor-type RBC may be crucial to circumvent the rebound of anti-donor isoagglutinins. Finally, acute GvHD was associated with earlier RBC engraftment in cases of major and bidirectional ABO incompatibility, but did not prevent PRCA. These data not only shed light on beneficial prophylactic and therapeutic approaches in patients with major ABO incompatible HSCT but also provide some basic immunological information on the lifetime and regulation of anti-A/B producing plasma cells.

Authorship and Disclosures

All authors made substantial intellectual contributions to the manuscript. GS and JDS conducted the study. GS and JRP analyzed the data. GS wrote the manuscript. JH, EB, PVV, LS, JG, AG, US, and JRP collected clinical data and treated the patients. JDS initiated the study, GS coordinated and supervised this study.

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