Biol Blood Marrow Transplant 20 (2014) 272–278

Autoimmune Hematological Diseases after Allogeneic Hematopoietic Stem Cell Transplantation in Children: An Italian Multicenter Experience

Maura Faraci [1,*], Marco Zecca [2], Marta Pillon [3], Attilio Rovelli [4], Maria Cristina Menconi [5], Mimmo Ripaldi [6], Franca Fagioli [7], Marco Rabusin [8], Ottavio Ziino [9], Edoardo Lanino [1], Franco Locatelli [10], Thomas Daikeler [11], Arcangelo Prete [12] on behalf of the Italian Association of Paediatric Haematology and Oncology

[1] *Haematopoietic Stem Cell Transplantation Unit, Haematology-Oncology Department, G. Gaslini Children's Research Institute, Genova, Italy*
[2] *Paediatric Haematology/Oncology, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy*
[3] *Paediatric Oncology-Haematology Unit, University Hospital of Padova, Padova, Italy*
[4] *Paediatric Haematopoietic Stem Cell Transplantation Unit, MBBM Foundation, University of Milano-Bicocca, Monza, Italy*
[5] *Haematopoietic Stem Cell Transplantation Unit, Paediatric Clinic of University of Pisa, Pisa, Italy*
[6] *Haematopoietic Stem Cell Transplantation Unit, Haematology-Oncology Department, Pausilipon Hospital, Napoli, Italy*
[7] *Haematopoietic Stem Cell Transplantation Unit, Department of Paediatrics, Regina Margherita Children's Hospital, University of Turin, Turin, Italy*
[8] *Haematopoietic Stem Cell Transplantation Unit, Paediatric Clinic, Burlo Garofolo Research Institute, Trieste, Italy*
[9] *Paediatric Haematology Oncology, Ospedale dei Bambini "G. Di Cristina, Palermo", Palermo, Italy*
[10] *Department of Paediatric Haematology-Oncology, IRCCS Bambino Gesù Children's Hospital, Rome, University of Pavia, Pavia, Italy*
[11] *Department of Rheumatology, University Hospital Basel, Basel, Switzerland*
[12] *Paediatric Oncology and Haematology Unit Lalla Seràgnoli, Department of Paediatrics, University of Bologna Sant'Orsola-Malpighi Hospital, Bologna, Italy*

Article history:
Received 1 June 2013
Accepted 19 November 2013

Key Words:
Autoimmune hematological disease
Hematopoietic stem cell transplantation
Children
Rituximab

A B S T R A C T

Autoimmune hematological diseases (AHDs) may occur after allogeneic hematopoietic stem cell transplantation (HSCT), but reports on these complications in large cohorts of pediatric patients are lacking. Between 1998 and 2011, 1574 consecutive children underwent allogeneic HSCT in 9 Italian centers. Thirty-three children (2.1%) developed AHDs: 15 autoimmune hemolytic anemia (45%), 10 immune thrombocytopenia (30%), 5 Evans' syndrome (15%), 2 pure red cell aplasia (6%), and 1 immune neutropenia (3%). The 10-year cumulative incidence of AHDs was 2.5% (95% confidence interval, 1.7 to 3.6). In a multivariate analysis, the use of alternative donor and nonmalignant disease was statistically associated with AHDs. Most patients with AHDs (64%) did not respond to steroids. Sustained complete remission was achieved in 87% of cases with the anti-CD20 monoclonal antibody (rituximab). Four patients (9%) (1 autoimmune hemolytic anemia, 1 Evans' syndrome, 2 immune thrombocytopenia) died at a median of 87 days after AHD diagnosis as a direct or indirect consequence of their disorder. Our data suggest that AHDs are a relatively rare complication occurring after HSCT that usually respond to treatment with rituximab.

INTRODUCTION

Autoimmune disorders, including autoimmune hematological diseases (AHDs), have been reported to occur more frequently than other autoimmune complications after both autologous and allogeneic hematopoietic stem cell transplantation (HSCT) [1-7]. AHDs may involve a single lineage of blood cells, such as autoimmune hemolytic anemia (AIHA), immune thrombocytopenia (ITP), autoimmune neutropenia (AIN), or 2 and/or 3 lineages (such as Evans' syndrome in which AIHA is associated with ITP and/or AIN). The causes of AHDs after HSCT have been the object of several studies, but the association of these complications with graft-versus-host disease (GVHD), T cell depletion, HSCT from an unrelated donor, and the use of serotherapy, in particular alemtuzumab, suggest that immune dysregulation or incomplete immune reconstitution may be the pathogenetic mechanism leading to the development of these complications [1-5].

The management of post-transplantation AHDs is complex, and the response to immunosuppressive therapies is often either incomplete or transient. There are reports on the incidence of AIHA cases in pediatric HSCT recipients [3,7], and an analysis of autoimmune diseases occurring after cord blood transplantation has recently been published [6], but the description of post-transplantation AHDs in a large cohort of children is lacking. The aims of this Italian retrospective, observational, multicenter pediatric study are to report the cumulative incidence, to analyze the risk factors, and to describe the clinical features, treatment, and outcome of AHDs occurring in a series of children who underwent allogeneic HSCT.

METHODS

This study involved all consecutive allogeneic HSCT recipients reported to the Italian Association of Paediatric Haematology and Oncology (AIEOP)-HSCT Registry and treated between 1998 and December 2011 at any AIEOP

Financial disclosure: See Acknowledgments on page 278.

* Correspondence and reprint requests: Maura Faraci, MD, Stem Cell Transplantation Unit, Department of Haematology/Oncology, G. Gaslini Children's Hospital, Largo G. Gaslini 5, 16147 Genova, Italy.
E-mail address: maurafaraci@ospedale-gaslini.ge.it (M. Faraci).

1083-8791/$ – see front matter © 2014 American Society for Blood and Marrow Transplantation.
http://dx.doi.org/10.1016/j.bbmt.2013.11.014

center. Data regarding patients with a minimum follow-up of at least 6 months were collected and analyzed. The AIEOP centers that agreed to participate in the study were asked to identify patients affected by AHD and to answer a specific questionnaire, including queries addressing clinical features of AHDs, laboratory characteristics, therapies used, and outcome. Data concerning demographics, type of primary diagnosis, date and type of transplantation, conditioning regimen, type of stem cell source, and GVHD (maximum grade, duration, and treatment) were retrieved from the AIEOP-HSCT Registry for each study subject. HSCT was classified as (1) matched related donor, including patients who received geno-/phenotypically HLA identical or with a single locus mismatch, and (2) alternative donor, including patients who received HSCT from an unrelated volunteer or from an HLA partially matched/haploidentical related donor.

We analyzed the response to first- and second-line treatment and categorized it as (1) complete response (CR) in cases of normalization of the clinical signs and laboratory tests for AHD, (2) partial response when an improvement in clinical symptoms or laboratory analyses and/or steroid dependence was observed despite the presence of autoantibodies, or (3) nonresponse when the clinical signs/symptoms and laboratory findings were either unchanged or worsened despite therapy.

This retrospective study was approved by the AIEOP-HSCT board. The procedures we followed were in accordance with our institution's ethical standards and with the Helsinki Declaration. As per Italian guidelines, no other specific informed consent was required other than a general agreement to clinical and laboratory data collection for scientific purposes expressed at the time of transplantation.

Statistical Analysis

Patients' data were collected using patient-oriented forms, filled in by the physician in charge at each center and sent to the AIEOP Operations Office in Bologna where data were stored in an electronic database (AIEOP-HSCT Registry) for quality control and statistical analysis by Venus, a facilities-integrated software system running on an IBM mainframe at the Italian Inter-University Computing Centre (CINECA).

Quantitative variables were reported as median and range, whereas categorical variables were expressed as absolute number and percentage. The incidence of AHD was defined as the probability of having AHD at time *t*, death in remission or disease relapse considered the competing event [8]. AHD was calculated as a cumulative incidence curve to adjust the estimate for competing risks [9,10]. All results were expressed as 10-year cumulative incidences and 95% confidence intervals (CIs) [8].

The following variables were included in the univariate analysis of factors predicting the development of AHD: patient gender, median age at diagnosis, type of original disease (malignant versus nonmalignant), median age at HSCT, type of conditioning regimen (total body irradiation–based versus chemotherapy-based), type of stem cell source, type of GVHD prophylaxis, and acute and chronic GVHD occurrence. Logistic regression was used to perform the multivariate analysis, and the model included all variables with $P < .05$ in univariate analysis. The chi-square test was used to compare percentage differences. All *P* values were 2-sided, and $P < .05$ were considered statistically significant. $P > .1$ was reported as not significant (NS), whereas *P* between .05 and .1 were reported in detail.

Statistical analysis was performed using Number Cruncher Statistical System 2007 (Kaysville, UT) and R 2.5.0 software package (http://www.R-project.org) [11-13]. Data were analyzed as of December 15, 2012.

RESULTS

Nine of 20 AIEOP centers performing allogeneic HSCT (45%) agreed to participate in this study. Overall, they performed 41% (n = 1574) of the 3830 transplants reported to the AIEOP-HSCT Registry during the study period. These centers provided the data concerning the children who were eligible for this study, and among them a total of 33 AHDs (2.1%) were reported. The median follow-up after transplantation of AHD patients was 43.5 months (range, 6 to 163.6). AHD was diagnosed after a first transplant in 29 patients, whereas in 4 cases it occurred after a second HSCT performed for either relapse (n = 2) or graft failure (n = 2).

Cumulative Incidence

The cumulative incidence of AHDs was 1.53% (95% CI, 1.02 to 2.30) 1 year after HSCT; it was 2.05% (95% CI, 1.44 to 2.92) 2 years after HSCT, 2.13% (95% CI, 1.50 to 3.02) 3 and 5 years after transplantation, and 2.50% (95% CI, 1.74 to 3.57) 10 years after HSCT. The cumulative incidence was significantly different between groups of patients who received matched related (.74%) and alternative donor (3.62%) stem cells ($P < .001$) (Figure 1).

Risk Factors

The main characteristics of patients who did or did not develop AHDs are reported in Table 1. Univariate analysis (Table 1) showed no significant correlation between the development of AHD and patient gender, type of conditioning regimen (total body irradiation versus chemotherapy based), type of GVHD prophylaxis, or acute and chronic GVHD occurrence. On the contrary, the following factors were found to be significantly associated with an increased risk of AHD: younger age at HSCT (3.1 years [range, .7 to 18.9] for patients developing AHD versus 8.8 years [range, .3 to 22] for patients not developing these disorders; $P = .0005$), transplantation from an alternative donor ($P = .004$), primary

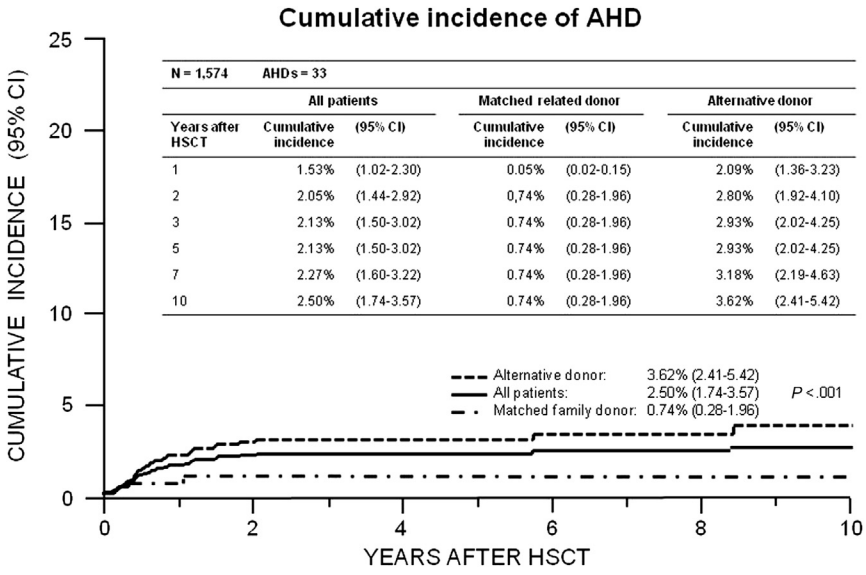

Figure 1. Cumulative incidence of AHDs.

Table 1
Clinical Risk Factors for AHD after Allogeneic HSCT and Univariate Analysis

	Patients with AHD	Patients without AHD	*P*
Gender, n (%)			
Male	16 (48)	922 (60)	.1887
Female	17 (52)	619 (40)	
Median age at diagnosis, yr (range)	2.2 (.1-16.6)	6.1 (.1-20)	.04
Type of underlying disease, n (%)			
Malignant	11 (34)	1208 (78)	<.0001
Nonmalignant	22 (66)	333 (22)	
Median age at HSCT, yr (range)	3.1 (.7-18.9)	8.8 (.3-22)	.0005
Type of HSCT, n (%)			
Alternative donor	29 (88)	978 (63)	.004
Matched related donor	4 (12)	563 (37)	
Type of conditioning regimen, n (%)			
TBI based	6 (18)	654 (58)	.015
Chemotherapy based	27 (81)	887 (42)	
Source of stem cell, n (%)			
Bone marrow	18 (55)	1154 (73)	.003
PBSC	5 (15)	237 (15)	
Cord blood	10 (30)	183 (12)	
Type of GVHD prophylaxis, n (%)			
CSA +ATG ± MTXs	28 (85)	617 (40)	.01
CSA ± MTXs	3 (9)	805 (51)	
T depletion ± other	1 (3)	115 (7)	
Other or none	1 (3)	4 (2)	
Acute GVHD, n (%)			
No	10 (30)	456 (30)	.794
Yes	23 (69)	947 (61)	
Grades I-II	17 (74)	740 (78)	
Grades III-IV	6 (26)	207 (22)	
Unknown	0	138 (9)	
Chronic GVHD, n (%)			
No	18 (55)	894 (58)	.042
Yes	13 (39)	357 (23)	
Not applicable/unknown	2 (6)	290 (19)	
Limited	5 (39)	200 (56)	
Extensive	8 (61)	157 (44)	
Total, n (%)	33 (2.1)	1541 (97.9)	

BM indicates bone marrow; PBSC, peripheral blood stem cells; TBI, total body irradiation; ATG, antithymocyte globulin; CSA, cyclosporine; MTXs, short course of methotrexate.

diagnosis of a nonmalignant disease ($P < .0001$), and the use of cord blood as stem cell source ($P = .003$). In multivariate analysis, primary diagnosis of a nonmalignant disease ($P = .00017$) and the use of an alternative donor ($P = .019$) remained factors significantly associated with the development of AHD (Table 2).

Clinical Characteristics and Treatments

Table 3 reports the transplant characteristics of patients experiencing AHD. Table 4 shows details on type of AHD treatment (first- and second-line treatment) and on final outcome. First- and second-line treatment included steroids (methylprednisolone or other type of steroids) at dosages ranging from 1 to 40 mg/kg, intravenous immunoglobulin (IVIG) administered from 400 mg/kg/day to 1 g/kg/day for several courses, and rituximab (RTX) given at 375 mg/m^2 weekly for 2 to 6 courses.

AIHA as a single manifestation of AHD was diagnosed in 15 children (46%) at a median of 5.2 months (range, 1.3 to 100.9) after HSCT. The 10-year cumulative incidence of AIHA was 1.5% (95% CI, .95 to 2.41). The median hemoglobin level at AIHA diagnosis was 7.4 g/dL (range, 4.5 to 9.4); 11 of 15 children received a median of 3 RBC transfusions (range, 1 to 8). Six children (40%) affected by AIHA achieved CR after

Table 2
Multivariate Analysis of Risk Factors Associated with the Occurrence of AHD

Risk Factors	Odds Ratio	95% CI	*P*
Chronic GVHD			
Extensive vs. absent	2.29	.85-6.18	.102
Limited vs. absent	1.39	.48-3.97	.544
Age at HSCT (per yr)	.94	.86-1.02	.138
Stem cell source			
CB vs. BM	1.76	.69-4.49	.235
PBSC vs. BM	1.08	.34-3.48	.894
Diagnosis			
Nonmalignant vs. malignant	6.87	2.51-8.78	.00017
TBI: yes vs. no	1.36	.43-4.37	.601
Donor			
MUD vs. MFD	3.73	1.23-1.27	.019
PMFD vs. MFD	2.14	.35-3.21	.412

BM indicates bone marrow; CB, cord blood; PBSC, peripheral blood stem cells; TBI, total body irradiation; MUD, matched unrelated donor; MFD, matched family donor; PMFD, partially matched family donor.

first-line therapy including methylprednisolone alone (n = 3) or associated with IVIG (n = 2) and RTX (n = 1), whereas 7 of 8 children who did not respond to first-line treatment obtained CR after a median of 1.8 months (range, 1 to 3) after RTX was administered as second- or third-line therapy (Table 4).

Ten patients presented ITP (30%) at a median of 40.2 months (range, 2.5 to 101.68) after HSCT. The 10-year cumulative incidence of ITP was .9% (95% CI, .05 to 1.80). The median number of platelets at the time of ITP diagnosis was 16,500/μL (range, 3000 to 48,000), and antibodies against platelets were found to be positive in 6 of 8 evaluated patients (75%). Four children with ITP achieved CR after first-line treatment including IVIG (n = 3) and methylprednisolone (n = 1). Of the remaining 6 patients, 2 were responsive to RTX and 1 to an infusion of allogeneic CD34, whereas 2 died of transplant-related complications and 1 of relapse of the underlying disease. Four children developed an infection before or during ITP, and the origin was viral (cytomegalovirus) in 2 cases and mycotic (1 candida, 1 aspergillus) in 2 cases.

Five children developed Evans' syndrome (15%) (4 AIHA + ITP, 1 AIHA + ITP + AIN) at a median of 9.2 months after HSCT (range, 2.4 to 24.4). Two patients with AIHA + ITP obtained CR 3 and 6 months after RTX as first- or second-line treatment, and the third achieved CR after steroids administered as first-line treatment. One child died of multiple organ failure 6 months after Evans' syndrome diagnosis despite several immunosuppressive therapies (steroids, IVIG, 6 doses of RTX, vincristine, cyclophosphamide, plasma exchange, splenectomy, alemtuzumab, and rapamycin) (Table 4). The child with Evans' syndrome characterized by AIHA + ITP + AIN was affected by type 1 mucopolysaccharidosis and developed autoimmune pancytopenia in the context of chronic extensive GVHD 23 months after a second unrelated donor bone marrow HSCT had to be performed due to primary graft failure of the unrelated donor cord blood HSCT. Although CR of AIHA and ITP was obtained after 4 doses of RTX, AIN persisted in the contest of chronic GVHD treated with dexamethasone, 2 courses of monoclonal antibodies against anti-CD25, rapamycin, and finally a CD34+ positively selected boost of peripheral blood stem cells from the original donor. Fifteen months after the diagnosis of Evans' syndrome, hematological values returned within normal ranges even though low direct antiglobulin test positivity persisted.

Table 3
Characteristics of Patients with AHD, Transplantation, Procedures, Outcome, and Development of AHD

Patient No.	Primary Disease	Sex	Yr of HSCT	Age at HSCT (yr/mo)	Conditioning Regimen	Donor	Cell Origin	GVHD Prophylaxis	aGVHD Maximum Grade	cGVHD	Survival Status	AHD
1	HLH	M	1998	3 mo	Bus,VP16,CY	AD	CB	ATG,CSA,MPD	Absent	Absent	5.6 mo (D)	AIHA
2	MPS1	F	1999	1.5 yr	Bus,CY	AD	BM	ATG,CSA,MPD	Absent	Absent	145 mo (A)	AIHA
3	Langh. His	M	2002	2.1 yr	Bus,Vp16,CY	AD	CB	ATG,CSA,MPD	I	Absent	114 mo (A)	AIHA
4	ALL	M	2003	11.8 yr	TBI,TT,CY	AD	BM	ATG,CSA,MTXs	II	Limited	103 mo (A)	AIHA
5	HLH	M	2005	1.7 yr	Bus,TT,Fluda	AD	BM	ATG,CSA,MTXs	Absent	Absent	80.1 mo (A)	AIHA
6	MPS1	F	2006	1.5 yr	Bus,CY	AD	CB	ATG,CSA,MPD	II	Extensive	69.1 mo (A)	AIHA
7	AML	F	2007	9 mo	Bus,CY,L-PAM	AD	BM	ATG,CSA,MTXs	I	Absent	53 mo (A)	AIHA
8	Fanconi A	F	2008	5.2 yr	Fluda,CY,ATG	AD	CB	CSA,MPD	II	Extensive	44.7 mo (A)	AIHA
9	C. Anemia	M	2008	4.4 yr	TT, Treo,Fluda	AD	PBSC	ATG,CSA,MTXs	Absent	Absent	36.4 mo (A)	AIHA
10	AML	M	2009	17.2 yr	Bus,CY,L-PAM	AD	BM	ATG,CSA,MTXs	II	Limited	25.6 mo (A)	AIHA
11	ALL	F	2010	3.2 yr	TBI,TT,CY	AD	BM	ATG,CSA,MTXs	I	Absent	20.1 mo (A)	AIHA
12	ALL	F	2010	1.9 yr	Bus,TT,CY	AD	PBSC	ATG,CSA,MTXs	I	Absent	18.2 mo	AIHA
13	SAA	M	2010	6.5 yr	Fluda,CY,ATG	AD	BM	CSA,MTXs	Absent	Absent	17.7 mo (A)	AIHA
14	SAA	M	2010	8.4 yr	Fluda,CY,ATG	AD	BM	CSA,MTXs	II	Absent	14.5 mo (A)	AIHA
15	ALL	M	2011	2.9 yr	TT,Treo,Fluda	AD	BM	ATG,CSA,MTXs	II	Absent	4.7 mo (A)	AIHA
16	SAA	F	2003	12.1 yr	Fluda,CY,ATG	AD	BM	CSA+MTXs	II	Extensive	102 mo (A)	ITP
17	Fanconi A	F	2005	9.25 yr	Fluda,CY,400TBI,ATG	AD	BM	CSA	III	Extensive	72.5 mo (A)	ITP
18	Osteopetrosis	M	2007	7 mo	Bus,CY,TT	AD	CB	ATG,CSA,MPD	II	Limited	52 mo (A)	ITP
19	Fanconi A	F	2008	6.2 yr	Fluda,CY,ATG	RD	BM	CSA,MTXs	IV	Extensive	42.3 mo (A)	ITP
20	SCID	M	2008	9 mo	Bus,CY	AD	CB	ATG,CSA,MPD	Absent	Absent	42.9 mo (A)	ITP
21	SAA	F	2008	2.2 yr	Fluda,CY,ATG	RD*	BM	CSA,MTXs	Absent	Absent	38.5 mo A)	ITP
22	NB	F	2009	12,6 yr	TT,L-PAM	AD†	PBSC	ATG,CSA,MTXs	IV	Extensive	6.45 mo (D)	ITP
23	MPS 1	F	2009	9 mo	Bus,CY	AD	CB	ATG,CSA,MPD	II	Limited	24.2 mo (A)	ITP
24	ALL	F	2010	19 yr	TBI,TT,CY	RD	BM	CSA	III	NE	2.5 mo (D)	ITP
25	AML	F	2011	11.4 yr	Bus,TT,Fluda	AD‡	BM	ATG,Basil,CSA,MMF	Absent	Absent	5.7 mo (D)	ITP
26	MPS1	F	2001	1.1 yr	Bus,CY	AD	BM	ATG,CSA,MPD	Absent	Absent	131 mo (A)	Evans
27	SCID	M	2007	10 d	No CR	AD	BM	No GVHD proph.	III	Absent	57.5 mo (A)	Evans
28	ALL	M	2008	16.5 yr	TBI,TT,CY	AD	PBSC	ATG,CSA,MTXs	IV	Extensive	38.4 mo (A)	Evans
29	MPS1	M	2010	2.7 yr	Bus,CY	AD	CB	ATG,CSA,MPD	II	Absent	9 mo (D)	Evans
30	MPS1	F	2010	3.7 yr	TT,Treo,Fluda	AD§	BM	Campath,Tac,MTXs	II	Extensive	15.5 mo (A)	Evans
31	ThalassemiaM	M	1998	7.7 yr	Bus,CY	RD	BM	CSA,ATG	Absent	Absent	164 mo (D)	PRCA
32	WAS	M	2010	9 mo	Treo,Fluda	AD	BM	ATG,CSA	Absent	Absent	14.5 mo (A)	PRCA
33	ALL	F	2007	16.8 yr	TBI,TT,CY	AD	PBSC	ATG,CSA,MTXs	I	Limited	53.9 mo (A)	AIN

M indicates male; F, female; HLH, hemophagocytic lymphohistiocytosis; Thalassemia M, thalassemia major; MPS1, mucopolysaccaridosis type 1; Langh His, Langherans cell histiocytosis; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; SAA, severe aplastic anemia; Fanconi A, Fanconi anemia; SCID, severe combined immunodeficiency disease; WAS, Wiskott-Aldrich syndrome; C. Anemia, congenital anemia; NB, neuroblastoma; Proph, prophylaxis; Bus, busulfan; CY, cyclophosphamide; AD, alternative donor; Fluda, fludarabine; L-PAM, melphalan; TT, thiotepa; TBI, total body irradiation; Treo, treosulphan; VP16, etoposide; ATG, antithymocyte globulin; UD, unrelated donor; RD, related donor; PMRD, partially matched related donor; Haplo, haploidentical donor; BM, bone marrow; PBSC, peripheral blood stem cells; CB, cord blood; CSA, cyclosporine A; MPD, methylprednisolone; MTXs, short course of methotrexate; Tac, tacrolimus; Basil, basiliximab; MMF, mycophenolate mofetil; GF, graft failure; A, alive; D, dead; NE, not assessable; AHD, autoimmune disease; mo(s), month(s); d(s), day(s); yr(s), year(s); Infect, infections; aGVHD, acute GVHD; cGVHD, chronic GVHD; AIHA, autoimmune hemolytic anemia; Evans, Evans' syndrome; ITP, immune trombocytopenia; PRCA, pure red cell aplasia.

* Patient received a second HSCT due to primary graft failure after a first RD HSCT conditioned with Flu, Cy, ATG (GVHD prophylaxis with MTX+CSA).

† Patient received a second HSCT due to a relapse that occurred 2 yr after the first autologous HSCT (conditioned with Bus+LPAM).

‡ Patient received a second HSCT due to a relapse that occurred 1 yr after a first CB HSCT conditioned with Bus, Cy, LPAM (GVHD prophylaxis with ATG, CSA, MPD).

§ Patient received a second HSCT due to graft failure that occurred after a first cord blood HSCT conditioned with Bus, CY (GVHD prophylaxis with ATG, CSA, MPD).

Table 4
Characteristics of Patients with AHD, Treatments, Responses, and Outcome

Patient No.	AHD	Mo from HSCT	Age at AHD (yr/mo)	Infect	aGVHD cGVHD	First-Line Therapy	Response (Time)	Second- to Third-Line Therapy	Response (Time)	Survival Status	Cause of Death	Follow-up of AHD (days, mo)
1	AIHA	3.3	6 mo	NO	NO	MPD: 5 mg/kg+ IVIG: 400 mg/kg × 5	CR (1 mo)			Dead	Traumatic Hemothorax	10 days
2	AIHA	4.7	2	NO	NO	MPD (40 mg/kg)	PR	IvIg→ RTX (3)	CR (1 mo)	Alive		140 mo
3	AIHA	4.3	2.5	EBV	NO	MPD (2 mg/kg)	PR	RTX (4)	CR (1 mo)	Alive		109 mo
4	AIHA	100.9	20.6	NO	NO	MPD (2 mg/kg)	PR	Rapa	PR	Alive		3.5 mo
5	AIHA	13.6	2.8	NO	NO	MPD (2 mg/kg) + IVIG (400 mg/kg × 5)	PR	RTX (4)	CR (1 mo)	Alive		66 mo
6	AIHA	5.9	2	NO	Extes	MPD (2 mg/kg)	PR	RTX (2)	CR (1 mo)	Alive		64 mo
7	AIHA	9.4	1.7	NO	NO	MPD (2 mg/kg)	CR (1 mo)			Alive		44 mo
8	AIHA	8.1	6	CMV	NO	IVIG (800 mg/kg × 1)	NR	RTX (2)	CR (3 mo)	Alive		37 mo
9	AIHA	10.1	5.2	NO	NO	MPD (5 mg/kg)	CR (1 mo)			Alive		26 mo
10	AIHA	1.3	17.3	BK/EBV	II	RTX (2)	CR (3 mo)			Alive		25 mo
11	AIHA	4.9	3.6	CMV	NO	MPD(10 mg/kg)	NR	RTX (5)	CR (3 mo)	Alive		15.1 mo
12	AIHA	18.1	3.4	CMV	NO	MPD (2 mg/kg) + IVIG (800 mg/kg × 2)	CR (1 mo)			Alive		1 mo
13	AIHA	7.3	7.1	NO	NO	MPD (5 mg/kg)	PR			Alive		10.4 mo
14	AIHA	5	8.9	Adeno	NO	MPD (1 mg/kg)	CR (1 mo)			Alive		10 mo
15	AIHA	3.8	3.3	EBV	NO	MPD (2 mg/kg)	PR	RTX (3)	CR (3 mo)	Alive		4.7 mo
16	ITP	68.8	17.9	NO	NO	IVIG (400 mg/kg × 2)	CR (1 mo)			Alive		33 mo
17	ITP	7.3	9.8	CMV	Exten	IVIG (1 g/kg)	CR (7 days)			Alive		65 mo
18	ITP	9.2	1.5	NO	NO	MPD (30 mg/kg) + IVIG (800 mg/kg × 3)	PR	RTX (4)	CR (1 mo)	Alive		43 mo
19	ITP	12.8	7.3	NO	Lim	PDN (.5 mg/kg) + IVIG (800 mg/kg × 3)	PR	$CD34^{+}$ for PT	CR (2 mo)	Alive		30 mo
20	ITP	6.7	1.5	NO	NO	Beta (.4 mg/kg) + IVIG (800 mg/kg × 2)	CR (15 days)			Alive		35 mo
21	ITP	4.2	2.5	NO	NO	PDN (1 mg/kg) + IVIG (800 mg/kg × 2)	NR	RTX (3)	CR (1 mo)	Alive		34 mo
22	ITP	2.9	12.9	Asper	IV	DEXA (.9 mg/kg) + IVIG (1 g/kg × 1)	NR			Dead	Hemorrhage, Asper, cGVHD	3.5 mo
23	ITP	6.1	1.5	NO	NO	MPD (30 mg/kg)	CR (1 mo)			Alive		18.1 mo
24	ITP	1.7	19.9	CMV	III	MPD (1.5 mg/kg)	NR			Dead	MOF, sepsis	23 days
25	ITP	2	11.5	Cand	NO	MPD (1 mg/kg)	NR	RTX (3)	NR	Dead	Relapse	3.7 mo
26	Evans	21.8	2	V.Resp	NO	MPD (2 mg/kg)	CR (1 mo)			Alive		108 mo
27	Evans	24.4	2.8	NO	NO	IVIG (1 g/kg × 6)	NR	RTX (3)	CR (6 mo)	Alive		33 mo
28	Evans	5.4	16.9	NO	Exten	RTX (4)	CR (3 mo)			Alive		33 mo
29	Evans	2.5	2.9	CMV	NO	MPD (2.4 mg/kg) + IVIG (1 g/kg × 14)	NR	RTX (6)→ VCR,CY,PX….	NR	Dead	MOF, anemia, hemorrhage	6.5 mo
30	Evans	9.2	4.5	Pseud	Exten	MPD (4 mg/kg) + IVIG (1 g/kg × 2)	NR	RTX (4)→ Dexa, Anti-CD25, CD34+, Rapa	CR: AIHA/ITP PR: AIN	Alive		15.5 mo
31	PRCA	2.3	8	NO	NO	EPO (150 UI/kg)	PR	MPD	PR	Alive		161 mo
32	PRCA	1.6	10	Klebs	NO	EPO (500 UI/kg)	NR	PDN (1 mg/kg)	CR (1 mo)	Alive		13 mo
33	AIN	14.3	18	NO	NO	PDN (1 mg/kg) + IVIG (1 g/kg × 7)	CR (1 mo)			Alive		40 mo

d(s) indicates day(s); Infect, infections; aGVHD, acute GVHD; cGVHD, chronic GVHD; Evans, Evans' syndrome; M, male; F, female; VResp, respiratory virus; CMV, cytomegalovirus; EBV, Epstein-Barr virus; Adeno, adenovirus; Asp, aspergillus; Pseud, pseudomonas; Klebs, klebsiella; Cand, candida; MPD, methylprednisolone; IVIG, intravenous immunoglobulin; EPO, erythropoietin; PDN, prednisone; Beta, betamethasone; DEXA, dexamethasone; PR, partial response; NR, nonresponse; mo, month; RTX(n), number of doses of RTX; PT, poor take; Rapa, rapamycin; VCR, vincristine; CY, cyclophosphamide; PX, plasma exchange; CD34+, infusion of allogeneic CD34+ cells; anti-CD25, monoclonal antibody anti-CD25; MOF, multiple organ failure.

Pure red cell aplasia (PRCA) occurred in 2 patients (6%) with major donor-recipient ABO incompatibility 50 and 71 days after unrelated and related donor HSCT, respectively, that were performed to treat nonmalignant disease. In both patients, recombinant human erythropoietin (150 to 500 U/kg/day, given weekly for 10 and 6 doses, respectively) was unsuccessfully administered. Both patients received steroids, and 1 achieved stable CR. In the second patient, complete correction of PRCA was obtained after a second allogeneic peripheral blood stem cell infusion preceded by cyclophosphamide and antithymocyte globulin.

One patient (3%) developed AIN 14.3 months after unrelated donor HSCT performed for acute lymphoblastic leukemia. The search for indirect antibodies against neutrophils was negative. CR was achieved 1 month after treatment with steroids and IVIG (7 doses).

Rituximab Treatment

In our experience, 15 children received RTX as first- or second-line therapy (8 AIHA,4 Evans' syndrome,3 ITP). The CR was obtained in 13 patients treated with RTX (87%) (100% in AIHA group, 75% in Evans' group, and 66% in ITP patients) after a median of 60 days (range, 30 to 180) from start RTX treatment.

Outcome

Twenty-eight of 33 patients with AHDs were alive at last follow-up (85%). Five patients died after AHD diagnosis: 3 died without obtaining remission from AHD, and causes of death were multiple organ failure in 1 Evans' syndrome, multiple organ failure and sepsis in 1 ITP, and chronic GVHD and aspergillus in 1 ITP. Two patients died after obtaining CR of AIHA and ITP, respectively: 1 of traumatic hemothorax during the placement of a central venous catheter and 1 after relapse of the underlying malignancy.

DISCUSSION

The literature reports the incidence and risk factors of autoimmune-mediated hemolysis in adults [2,14,15] and children [3], but there are only single case reports of other AHDs (Evans' syndrome, ITP, AIN, PRCA) in the pediatric age group [7,16,17]. To the best of our knowledge, this is the first report on AHDs occurring in a large population of consecutive children who received any type of allogeneic HSCT. The study is focused on the homogeneous group of organ-specific alternative donors that most often occurred after HSCT.

Although the incidence of alternative donors after HSCT is reported as the overall incidence of autoimmune manifestations both hematological and nonhematological [6,18], it is difficult to compare the incidence we observed with that of other reports. Moreover, the incidence of AHD in our cohort was lower than that reported in patients receiving cord blood transplantations (41 of 726, 4.6%) [6] and higher than that reported after autologous and allogeneic transplants for autoimmune disease (6 of 363, 1.6%) [18]. The retrospective nature of this study carries the risk of underestimating the true incidence of AHDs (in particular of mild forms), and this possibly explains the lower incidence of AHD we observed in our population.

In our cohort of patients, nonmalignant underlying disease, transplantation from alternative donors, and cord blood as a source of hematopoietic stem cells represented risk factors statistically associated with AHDs in univariate analysis, whereas only the first 2 variables remained significant in multivariate analysis. The importance of nonmalignant disease as a risk factor for AIHA was also reported by O'Brien et al. [3] in a pediatric cohort and by Daikeler et al. [6] in the EUROCORD experience, suggesting that a more competent immune system before HSCT could predispose to the development of autoimmune disorders after the allograft.

The higher frequency of AHD observed in children transplanted from an alternative donor has also been reported in other studies [3,14]. This observation could be explained by the immunological dysregulation due to the use of alemtuzumab or antithymocyte globulin administered before unrelated donor HSCT [4], thus confirming previous observations concerning the development of AIHA after T cell–depleted HSCT [2].

In our study, younger age at HSCT was found to be associated with the risk of AHD, but this effect disappeared in multivariate analysis. The association of AHD in younger patients is reported in some studies [3,6,7], but its pathogenesis is not clear. The most attractive hypothesis is reported by Page et al. [7], who suggested a mechanism of interference between post-transplant immunosuppressive therapies (cyclosporine A, antithymocyte globulin) and the normal immune ontogeny resulting in immune dysregulation.

The association between GVHD and autoimmunity is well known [1], but the role of GVHD in post-transplant AHDs is still under debate [3,6]. In our experience, few patients were suffering from GVHD at the time of AHD (8 of 33 patients, 24%), and neither acute nor chronic GVHD was statistically correlated with the development of AHD.

Infections may be an important trigger for autoimmune events occurring after HSCT [5,14,19,20]. In our study, 33% of patients with AHD had viral infection or reactivation before or during the AHD. Fifty-four percent of these viral events were represented by cytomegalovirus reactivations, thus confirming that cytomegalovirus may contribute to the onset of post-transplantation autoimmune complications [19]. Although the prognosis of patients with post-transplant autoimmune diseases is variable and some spontaneous remissions are possible [21], the incidence of life-threatening events and the resistance to first-line standard treatments are most common in patients affected by AHDs [3,14-20].

Other drugs used on patients with post-transplantation AHDs, either alone or in combination, include RTX [14,22-24], IVIG [20,25], vincristin [26], splenectomy [27], and other immunosuppressive treatments. The efficacy of RTX in the treatment of AIHA [20-24,28,29], as well as the encouraging results reported in ITP [30] and PRCA [31], has led to an increased and consolidated use of this monoclonal antibody even in patients affected by post-transplantation AHDs [3,6,20,22,24]. In our experience, steroid treatment alone was unsuccessful in 64% of AHD pediatric recipients, thus requiring the addition of other immunosuppressive therapies. Our data support the use of RTX for AHDs occurring after allogeneic HSCT (CR in 87% of patients treated with RTX), confirming data reported in cord blood transplantation recipients [6]. The long-term effect of RTX on B cell reconstitution of patients given an allograft remains to be further investigated.

The effectiveness of IVIG administered alone or in association with steroids for the treatment of post-transplantation AHDs was reported only in single cases [20,25]. In the study by Hartert et al. [20], 5 patients (2 AIHA and 3 ITP) treated with IVIG during first-line therapy obtained CR, confirming the reports in the literature stating that IVIG may be a good treatment option for patients with post-HSCT AHD, in particular for patients affected by ITP.

The efficacy of erythropoietin in the treatment of PRCA as a late consequence of major ABO incompatibility was demonstrated by some authors [30-33]. However, new therapeutic strategies represented by the use of RTX [30] and recently by bortezomib [34] have been proposed as a treatment for PRCA. Regarding AIN, we confirmed that unlike other AHDs, most patients with post-transplantation AIN improved without any specific treatment [35] or after standard therapy with steroids and IVIG [36].

In conclusion, our retrospective pediatric study found that (1) AHD is a relatively rare complication of the HSCT course, with an observed 10-year cumulative incidence of 2.5% (95% CI, 1.7 to 3.6); (2) children with nonmalignant diseases or receiving allograft from an alternative donor are at especially high risk of developing AHD; (3) AIHA is the most frequently reported AHD, followed by ITP, Evans' syndrome, PRCA, and AIN; (4) RTX represents an efficacious treatment for patients with steroid-refractory disease (87% of CRs in treated patients); and (5) mortality due to AHD is lower (9%) compared with other pediatric experiences [3,6], suggesting that the new therapeutic approach has led to an improvement in the prognosis of these patients.

ACKNOWLEDGMENTS

Financial disclosure: The authors have nothing to disclose.

Conflict of interest statement: There are no conflicts of interest to report.

REFERENCES

1. Tivol E, Komorowski R, Drobyski WR. Emergent autoimmunity in graft-versus-host disease. *Blood*. 2005;105:4885-4891.
2. Drobyski WR, Potluri J, Sauer D, et al. Autoimmune hemolytic anemia following T cell-depleted allogeneic bone marrow transplantation. *Bone Marrow Transplant*. 1996;17:1093-1099.
3. O'Brien TA, Eastlund T, Peters C, et al. Autoimmune haemolytic anaemia complicating haematopoietic cell transplantation in paediatric patients: high incidence and significant mortality in unrelated donor transplants for non-malignant diseases. *Br J Haematol*. 2004;127:67-75.
4. Loh Y, Oyama Y, Statkute L, et al. Development of a secondary autoimmune disorder after hematopoietic stem cell transplantation for autoimmune diseases: role of conditioning regimen used. *Blood*. 2007;109:2643-2648.
5. Daikeler T, Tyndall A. Autoimmunity following haematopoietic stem-cell transplantation. *Best Pract Res Clin Haematol*. 2007;20:349-360.
6. Daikeler T, Labopin M, Ruggeri A, et al. New autoimmune diseases after cord blood transplantation: a retrospective study of EUROCORD and the Autoimmune Disease Working Party of EBMT. *Blood*. 2013;121:1059-1064.
7. Page KM, Mendizabal AM, Prasad VK, et al. Posttransplant autoimmune hemolytic anemia and other autoimmune cytopenias are increased in very young infants undergoing unrelated donor umbilical cord blood transplantation. *Biol Blood Marrow Transplant*. 2008;14:1108-1117.
8. Klein JP, Rizzo JD, Zhang MJ, et al. Statistical methods for the analysis and presentation of the results of bone marrow transplants. Part I. Unadjusted analysis. *Bone Marrow Transplant*. 2001;28:909-915.
9. Pepe MS, Longton G, Pettinger M, et al. Summarizing data on survival, relapse, and chronic graft-versus-host disease after bone marrow transplantation: motivation for and description of new methods. *Br J Haematol*. 1993;83:602-607.
10. Gray RJ. A class of K-sample tests for comparing the cumulative incidence of a competing risk. *Ann Stat*. 1988;16:1141-1154.
11. Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. *J Am Stat Assoc*. 1999;94:496-509.
12. Scrucca L, Santucci A, Aversa F. Competing risk analysis using R: an easy guide for clinicians. *Bone Marrow Transplant*. 2007;40:381-387.
13. Scrucca L, Santucci A, Aversa F. Regression modeling of competing risk using R: an in depth guide for clinicians. *Bone Marrow Transplant*. 2010;45:1388-1395.
14. Sanz J, Arriaga F, Montesinos P, et al. Autoimmune hemolytic anemia following allogeneic hematopoietic stem cell transplantation in adult patients. *Bone Marrow Transplant*. 2007;39:555-561.
15. Chen FE, Owen I, Savage D, et al. Late onset haemolysis and red cell autoimmunisation after allogeneic bone marrow transplant. *Bone Marrow Transplant*. 1997;19:491-495.
16. Urban C, Benesch M, Sovinz P, et al. Fatal Evans' syndrome after matched unrelated donor transplantation for hyper-IgM syndrome. *Eur J Haematol*. 2004;72:444-447.
17. Beck JC, Burke MJ, Tolar J. Response of refractory immune thrombocytopenia after bone marrow transplantation to romiplostim. *Pediatr Blood Cancer*. 2010;54:490-491.
18. Daikeler T, Labopin M, Di Gioia M, et al. Secondary autoimmune diseases occurring after HSCT for an autoimmune disease: a retrospective study of the EBMT Autoimmune Disease Working Party. *Blood*. 2011;118:1693-1698.
19. Sherer Y, Shoenfeld Y. Autoimmune diseases and autoimmunity post-bone marrow transplantation. *Bone Marrow Transplant*. 1998;22:873-881.
20. Hartert A, Willenbacher W, Günzelmann S, et al. Successful treatment of thrombocytopenia and hemolytic anemia with IvIG in a patient with lupus-like syndrome after mismatched related PBSCT. *Bone Marrow Transplant*. 2001;27:337-340.
21. Holbro A, Abinun M, Daikeler T. Management of autoimmune diseases after haematopoietic stem cell transplantation. *Br J Haematol*. 2012;157:281-290.
22. Hongeng S, Tardtong P, Worapongpaiboon S, et al. Successful treatment of refractory autoimmune haemolytic anaemia in a post-unrelated bone marrow transplant paediatric patient with rituximab. *Bone Marrow Transplant*. 2002;29:871-872.
23. Simms-Waldrip T, Ikeda A, Goldfinger D, et al. Dramatic response to rituximab in a child with severe cold autoimmune hemolytic anemia arising after allogeneic hematopoietic SCT. *Bone Marrow Transplant*. 2010;45:201-202.
24. Raj K, Narayanan S, Augustson B, et al. Rituximab is effective in the management of refractory autoimmune cytopenias occurring after allogeneic stem cell transplantation. *Bone Marrow Transplant*. 2005;35:299-301.
25. Lee SJ, Churchill WH, Konugres A, et al. Idiopathic thrombocytopenic purpura following allogeneic bone marrow transplantation—treatment with anti-D immunoglobulin. *Bone Marrow Transplant*. 1997;19:173-174.
26. Dovat S, Roberts RL, Wakim M, et al. Immune thrombocytopenia after umbilical cord progenitor cell transplant: response to vincristine. *Bone Marrow Transplant*. 1999;24:321-323.
27. Proleznik I, Prentice HG, Pretnar J, et al. Splenectomy for severe autoimmune cytopenias after allogenic stem cell transplantation: case report. *Int J Hematol*. 2005;82:75-78.
28. Quartier P, Brethon B, Philippet P, et al. Treatment of childhood autoimmune haemolytic anaemia with rituximab. *Lancet*. 2001;358:1511-1513.
29. Zecca M, Nobili B, Ramenghi U, et al. Rituximab for the treatment of refractory autoimmune hemolytic anemia in children. *Blood*. 2003;101:3857-3861.
30. Delgado J, Bustos JG, Jimenez-Yuste V, et al. Anti-CD20 monoclonal antibody therapy in refractory immune thrombocytopenic purpura. *Haematologica*. 2002;87:215-216.
31. Zecca M, De Stefano P, Nobili B, et al. Anti-CD20 monoclonal antibody for the treatment of severe, immune-mediated, pure red cell aplasia and hemolytic anemia. *Blood*. 2001;97:3995-3997.
32. Heyll A, Aul C, Runde V, et al. Treatment of pure red cell aplasia after major ABO-incompatible bone marrow transplantation with recombinant erythropoietin. *Blood*. 1991;77:906.
33. Santamaría A, Sureda A, Martino R, et al. Successful treatment of pure red cell aplasia after major ABO-incompatible T cell-depleted bone marrow transplantation with erythropoietin. *Bone Marrow Transplant*. 1997;20:1105-1107.
34. Poon LM, Koh LP. Successful treatment of isohemagglutinin-mediated pure red cell aplasia after ABO-mismatched allogeneic hematopoietic cell transplant using bortezomib. *Bone Marrow Transplant*. 2012;47:870-871.
35. Tosi P, Bandini G, Tazzari P, et al. Autoimmune neutropenia after unrelated bone marrow transplantation. *Bone Marrow Transplant*. 1994;14:1003-1004.
36. Klumpp TR, Block CC, Caligiuri MA, et al. Immune-mediated cytopenia following bone marrow transplantation. Case reports and review of the literature. *Medicine (Baltimore)*. 1992;71:73-83.