Clinical and immunological outcome after paediatric stem cell transplantation in inborn errors of immunity

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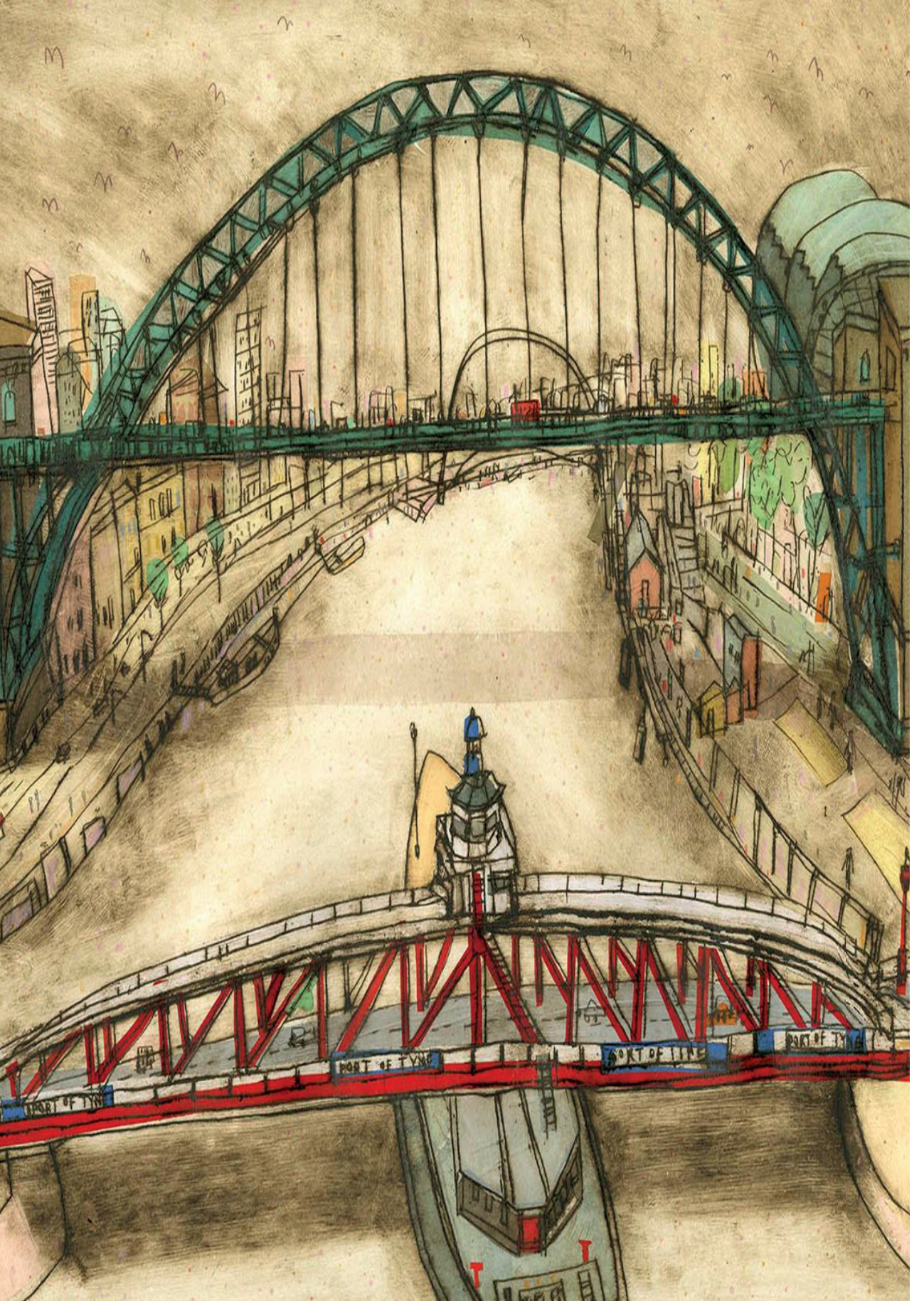
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Chapter 8

Outcome of non-haematological autoimmunity after haematopoietic cell transplantation in primary immunodeficiency

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Abstract

Background: Knowledge of post haematopoietic cell transplantation (HCT) non-haematological autoimmune disease (AD) is far from satisfactory.

Objective: We aimed to study the incidence, risk factors and outcomes of post-HCT AD in children with primary immunodeficiency (PID)

Methods: Multi-centre retrospective study of 596 children with PID who were transplanted from 2009 to 2018.

Results: The indications of HCT were severe combined immunodeficiency (SCID, n=158, 27%) and non-SCID PID (n=438, 73%). The median age at HCT was 2.3 years (range, 0.04 to 18.3 years). The 5-year overall survival for the entire cohort was 79% (95% CIN, 74-83%). The median follow-up of surviving patients was 4.3 years (0.08 to 14.7 years). The cumulative incidence (CIN) of post-HCT AD was 3% (2-5%) at one-year post-HCT, 7% (5-11%) at 5-years post-HCT and 11% (7-17%) at 8 years post-HCT. The median onset of post-HCT AD was 2.2 years (0.12 to 9.6 years). Autoimmune thyroid disorder (n=19, 62%) was the most common post-HCT AD, followed by neuromuscular disorders (n=7, 22%) and rheumatological manifestations (n=5, 16%). All patients but one required treatment for post-HCT AD. After multivariate analysis, age at transplant (p=0.01) and T-depleted graft (p<0.001) were significant predictors of post-HCT AD. None of T-depleted graft recipients developed post-HCT AD. Patients with a lower CD3+ count at 6 months post-HCT had a significant higher incidence of post-HCT AD compared to disease controls. Graft-versus-host disease, viral infection, and donor chimerism had no association with post-HCT AD

Conclusion: The CIN post-HCT AD in PID was 11% at 8 years post-HCT and its occurrence was associated older age at HCT and unmanipulated graft.

Introduction

Autoimmunity is increasingly recognized following haematopoietic cell transplantation (HCT) for malignant and non-malignant diseases in children and adults. Autoimmunity post-HCT can be broadly classified into autoimmune cytopenia (AIC) and non-haematological autoimmune diseases (AD). There is an increasing number of large single and multi-centre reports on post-HCT AIC. In recent single centre reports in children who were transplanted for both malignant and non-malignant disorders, the reported incidence of post-HCT AIC was 2.4% from Ahmed *et al.* (n=500, Kansas City, 2015) and Hwang-Bo *et al.* (n=292, Korea, 2017), 5.0% by Kruizinga *et al.* (n=531, Leiden, 2017), 6.0% by Chang *et al.* (n = 265, Taiwan, 2016), 7.8% by Szanto *et al.* (n = 380, Utrecht, 2020). (1-5). An Italian multicentre study by Faraci *et al.* reported the incidence of 2.1% in 1574 children who were transplanted for all indications between 1998 to 2011. (6) These reports have identified various risk factors for developing post-HCT AIC including non-malignant disorders, chemotherapy naivety before HCT, serotherapy, graft-versus-host disease (GvHD) and cytomegalovirus infection. With regards to post-HCT AIC in specific diseases, the incidence was 2.1% and 4.6% at 1- and 5-years post-HCT in a large European Society for Blood and Marrow Transplant survey of 530 paediatric and adult patients with acquired aplastic anaemia. (7) We have recently reported a higher incidence of post-HCT ACI of 9.4% in the largest series of 502 children with primary immunodeficiency (PID) and the most significant risk factors were the use of alemtuzumab and the presence of acute GvHD. (8)

There are limited data on the incidence and risk factors for non-haematological AD and current data arise mainly from single cases and single centre retrospective studies. These reports have been summarized by Holbro *et al.* and mainly focussed on single organ autoimmunity such as autoimmune thyroid diseases (n=20), myasthenia gravis (n=11), rheumatoid arthritis (n=3), systemic lupus erythematous (SLE, n=1), sarcoidosis (n=6) and psoriasis (n=1). (9) To date, there is no study focusing on non-haematological AD in large cohorts of paediatric patients. Children with PID represent a unique transplant cohort and many experience auto-immunity and autoinflammation prior to transplant. (10)Therefore, we conducted a collaborative retrospective analysis for risk factors and outcomes of the development of non-haematological AD. We included PID patients from three institutions with significant experience in transplanting patients with these conditions.

Methods

Patients and Methods

Between January 2009 to December 2018, 596 PID patients who underwent first allogeneic HCT for PID at the Leiden University Medical Center, Netherlands, Great Ormond Street Children's Hospital, London or Great North Children's Hospital, Newcastle, UK were included in the study. Patients with PID who received chemotherapy for malignancy prior to HCT were excluded. Clinical and laboratory data were retrieved from the transplantation database, patients' medical files and laboratory records. Written informed consent was obtained from the patients and/or parents or legal guardians of the patients as per institutional practice for HCT.

The following patients were excluded from analysis: 1) patients who developed recurrent autoimmune diseases due to underlying primary disease (systemic lupus erythematous (SLE)-like in mendelian susceptibility to mycobacterial disease [MSMD, n=1]; vasculitis in Wiskott Aldrich syndrome [WAS, n=1]; nephrotic syndrome in X-linked immunodysregulation polyendocrinopathy enteropathy [IPEX, n=1]) and 7 patients whose autoimmune manifestations were due to chronic GVHD (scleroderma, n=3; vitiligo, n=3; SLE-like, n=1) (supplemental table 1).

Definition and endpoints

The primary endpoint was cumulative incidence (CIN) of non-haematological AD. The intensity of the conditioning regimens for the purpose of this manuscript was classified as myeloablative conditioning (MAC) and reduced intensity conditioning (RIC). MAC referred to Busulfan (16mg/kg)-Cyclophosphamide (200mg/kg) (Bu16-Cy), pharmacokinetic targeted Busulfan (area under the curve >70mg/Lxh)-Fludarabine (Bu-Flu), treosulfan-cyclophosphamide (Treo-Cy) and Fludarabine-Treosulfan-Thiotepa. RIC regimens include Treosulfan-Fludarabine (Treo-Flu), Fludarabine-Melphalan (Flu-Melp), pharmacokinetic targeted Busulfan (area under the curve <70mg/Lxh)-Fludarabine (Bu-Flu), Fludarabine-Cyclosphosphamide (30mg/kg) (Flu-Cy) and other RIC regimens. None received total body irradiation.

Statistical analysis

Quantitative variables were described with median and range while categorical variables were reported with counts and percentages. The association between variables was assessed with the use of Wilcoxon rank-sum test for continuous variables and the chi-square test for categorical variables. CIN was calculated using a competing risk analysis, considering

death and graft failure as competing events. Gray's test was used for univariate comparison. The selected variables were: gender, age at transplant, indication for HCT (SCID versus non-SCID PID), pre-HCT autoimmunity, donor type (matched family donor (MFD) versus matched unrelated donor (MUD) versus mismatched family/unrelated donor (MMFD/MMUD) (HLA-matching £ 9/10 at HLA-A, B, C, DQ and DR) versus haploidentical donor), stem cell source (marrow versus unmanipulated peripheral blood stem cell (PBSC) versus T-cell depleted PBSC versus cord blood), conditioning regimen (none versus MAC versus RIC), serotherapy (none versus alemtuzumab versus anti-thymocyte globulin, ATG), GvHD prophylaxis, acute GvHD, chronic GvHD and viraemia. All factors associated with a *p*-value <0.10 by univariate analysis were included in a multivariate analysis using subdistribution hazard model of Fine-and-Gray. For donor chimerism at 12 months post-HCT and immune reconstitution kinetics for first 12 months, a nested matched case-control study was performed in which each patient with AD was matched with 4 disease controls for the following variables: age (difference < 3 years at transplant), disease category, stem cell source, conditioning regimen, serotherapy. Disease controls (n=124) were: severe combined immunodeficiency (SCID) (n=32), activated PIDK delta syndrome (APDS, n=4), cartilage hair hypoplasia (n=4), CD40 ligand deficiency (n=7), WAS (n=5) and chronic granulomatous disease (n=21), hemophagocytic lymphohistiocytosis (HLH, n=16) and other PID (n=35). Multilevel mixed effects modelling was performed for the longitudinal analysis of $CD3^+$, $CD4^+$, $CD8^+$ and $CD19^+$, NK cells. Kruskal Wallis test was used to compare CD15+ myeloid, CD3+ T-lymphocyte and CD19+ B-lymphocyte donor chimerism between disease controls at 12 months post-HCT and patients with AD at 12 months post-HCT and at onset of AD. All *p*-values quoted are two-sided, with a level of significance of 0.05. Statistical analyses were performed using STATA 14.2.

Results

Patient characteristics

Patient and transplantation characteristics are summarized in Table 1 and supplementary table 2. The median age at HCT was 2.3 years (range, 0.04 to 18.3 years). The 5-year overall survival for the entire cohort was 79% (95% CI, 74-83%). The median follow-up of surviving patients was 4.3 years (range, 0.08 to 14.7 years). Of 596 consecutive first HCT at three centres, 31 were complicated by development of post-HCT AD with the cumulative incidence of post-HCT AD was 3% (95% CI, 2-5%) at one-year post-HCT, 7% (95% CI, 5-11%) at 5-years post-HCT and 11% (95%CI, 7-17%) at 8 years post-HCT (figure 1A) The median onset of post-HCT AD was 2.2 years (range, 0.12 to 9.6 years). Post-HCT AD occurred within the first year post-HCT in 9 (29%) patients and within 5 years post-HCT in 26 (84%) patients. Five (16%)

patients developed the first episode of AD more than 5 years after HCT. Six (19%) patients also developed autoimmune haemolytic anaemia (AIHA); 3 prior to AD and 3 after AD onset. In patients with both post-HCT AIHA and post-HCT AD, the median onset of AD was 1.7 years (range 0.4 – 7.8 years) post-HCT whilst the median onset of AD was 2.3 years (range 0.1-9.6 years) post-HCT in patients with isolated post-HCT AD (p=0.55).

Table 1: Patient and transplantation characteristics and post-HCT AD risk factors

	All patients	Post-HCT AD	No post-HCT AD	5-year CNI of AD (95% CI)	8-year CNI of AD (95% CI)	*p-value*
No. patients	596	31	565			
Patient characteristics						
Gender, n (%)						0.39
Female	200	12 (6)	188 (94)	8 (4-16)	15 (7-30)	
Male	396	19 (5)	377 (95)	7 (4-11)	10 (6-17)	
Age group at HCT, n (%)						0.001
< 10 years of age	505	20 (4)	485 (96)	5 (2-13)	8 (2-21)	
≥ 10 years of age	91	11 (12)	80 (88)	8 (5-13)	12 (7-20)	
Indication of HCT, n (%)						0.38
SCID	158	7 (4)	153 (96)	6 (03-13)	19 (6-58)	
Non-SCID	438	24 (5)	412 (95)	9 (5-12)	12 (7-20)	
Pre-HCT autoimmunity, n (%)						0.24
No	541	26 (5)	511 (95)	7 (4-11)	9 (6-15)	
Yes	55	5 (9)	50 (91)	11 (4-29)	23 (7-73)	
Donor characteristics, n (%)						0.37
MFD	166[1]	7 (4)	159 (96)	7 (3-16)	10 (5-25)	
MUD	255[2]	17 (7)	237 (93)	10 (6-17)	11 (9-19)	
MMUD/MMFD	107[3]	7 (6)	107 (94)	5 (2-14)	14 (5-41)	
Haploidentical donor	61[4]	0	61 (100)	0	0	
Stem cell source, (%)						0.72
Marrow	222[5]	15 (7)	207 (93)	7 (3-16)	16 (8-29)	
PBSC	213	11 (5)	202 (95)	6 (3-11)	8 (4-15)	
CB	79	5 (6)	74 (94)	8 (3-22)	8 (3-23)	
Ex vivo T-cell depletion						<0.0001
No	514	31 (6)	483 (94)	8 (6-12)	12 (8-19)	
Yes	82	0	82 (100)	0	0	
Conditioning regimen						0.47
MAC	123	7 (6)	116 (94)	11 (5-25)	11 (5-25)	
RIC	409[6]	21 (5)	388 (85)	6 (4-10)	12 (7-21)	
None	64	6 (5)	61 (95)	7 (2-23)	7 (2-23)	

	All patients	Post-HCT AD	No post-HCT AD	5-year CNI of AD (95% CI)	8-year CNI of AD (95% CI)	*p-value*
Serotherapy						0.71
None	141	7 (5)	134 (95)	6 (2-14)	9 (4-21)	
Alemtuzumab	366	23 (6)	343 (93)	9 (5-14)	13 (8-22)	
ATG	89	1 (1)	88 (99)	3 (0-18)	3 (0-18)	
GVHD prophylaxis, n (%)						0.83
CNI alone	42[7]	3 (7)	39 (93)	8 (2-33)	20 (5-18)	
CNI/MMF	458[8]	26 (6)	419 (94)	8 (5-12)	17 (8-36)	
Others/none	109[9]	2 (2)	107 (98)	3 (1-11)	4 (1-11)	
Acute GvHD						
No aGvHD	324	16 (6)	308 (94)	7 (4-13)	10 (7-23)	
Grade I-II aGvHD	227	11 (5)	216 (95)	6 (3-12)	9 (5-18)	0.92
Grade III-IV aGvHD	45	4 (9)	41 (92)	15 (5-48)	15 (5-48)	0.32
Chronic GvHD	32	3 (9)	29 (91)	8 (2-35)	18 (5-60)	0.25
Viraemia						
No viraemia	275	16 (6)	275 (95)	6 (3-11)	20 (8-52)	
Any viraemia	305	15 (5)	290 (95)	8 (5-15)	11 (6-21)	0.85
CMV viraemia	136	5 (4)	131 (96)	7 (3-16)	7 (3-16)	0.50
Adenoviraemia	129	4 (3)	125 (97)	4 (2-11)	4 (1-11)	0.23
EBV viraemia	138	7 (5)	131 (95)	11 (5-25)	16 (7-39)	0.98

[4]One had post-cyclosphosphamide for haploidentical donor transplant; 6 CD3/CD19 depletion; 8 CD34 selection; 46 TCR αβ/CD19 depletion
[5]One had marrow and cord
[6]7 had MIC (5 FluCy, 1 Flu, 1 VP16)
[37]41 CSA; 1 tacrolimus
[48]435 CSA/MMF; 7 Tacrolimus/MMF; 3 Sirolimus/MMF;
[9]36 CSA/MTX; 21 CSA/steroid; 4 CSA/MMF/steroid; 1 CSA/OKT; 2 cyclophosphamide/steroid; 1 MTX/MMF; 44 no GvHD prophylaxis
Abbreviation: CB: cord blood; CNI: calcineurin inhibitors; CSA: ciclosporin; GvHD: graft-versus-host disease; HCT: haematopoietic cell transplantation; MAC: myeloablative conditioning; MFD: matched family donor; MMF: mycophenolate mofetil; MMFD: mismatched family donor; MTX: Methotrexate; MMUD: mismatched unrelated donor; MUD: matched unrelated donor; PID: primary immunodeficiency; RIC; reduced intensity conditioning; SCID: severe combined immunodeficiency

The type of AD is shown in Table 2 and detailed information of patients with post-HCT AD is shown in Table 3. Autoimmune thyroid disorder (n=19, 62%) was the most common post-HCT AD, followed by neuromuscular disorders (n=7, 22%) and rheumatogical manifestations (n=5, 16%). The median onset was 2.2 years (range 0.14-9.6 years) post-HCT for autoimmune thyroid disorders, 2.2 years (range 0.4 to 7.8 years) post-HCT for neurological disorders and 3.0 years (range 0.12-5.94 years) post-HCT for rheumatogical manifestations (p=0.98). One patient had overlap syndrome and thyroiditis. Age at HCT had no impact of type of AD (table 5S)

Table 2: Table of non-haematological autoimmune disease

	N (%)
Endocrine system	**19 (61)**
Autoimmune hypothyroidism	10 (32)
Autoimmune thyroiditis	7 (23)
Grave's disease	2 (7)
Rheumatology	
Polyarthritis	3 (10)
SLE	1 (3)
Nephritic/nephrotic syndrome	1 (3)
Neurology	
GBS	2 (7)
Myositis	2 (7)
Stiff person syndrome	1 (3)
Transverse myelitis	1 (3)
Optic neuritis	1 (3)

Risk factors of post-HCT AD

On univariate analysis, age at transplant (SHR 1.09, 95%CI 1.02-1.16) was significantly associated with post-HCT AD (p=0.01). The 5-year cumulative incidence of post-HCT AD was significantly higher in patients aged >10 years at HCT (18%, 95% CI, 10-36%) compared to patients aged <10 years at HCT (24%, 95%CI, 12-48%) (p=0.001) (Figure 1B). The indications for HCT of patients aged > 10 years at HCT who developed AD were: chronic granulomatous disease (CGD, n=23, AD=1), A20 deficiency (n=1, AD=1), APDS (n=3, AD=1), combined immunodeficiency (CID, n=10, AD=1), complex autoimmune diseases (n=2, AD=1), congenital neutropenia (n=2, AD=1), HLH (n=3, n=1), hypomorphic SCID (n=5, AD=1), juvenile idiopathic arthritis (JA, n=2, AD=2) and WAS (n=3, AD=1) (supplemental table 2).

None of 82 patients who received ex-vivo T-cell depleted graft developed post-HCT AD, compared to 5-year CIN of 8% (95%CI 5-12%) in patients who received unmanipulated graft (p<0.001) (Figure 1C).

Gender, diagnosis, conditioning, serotherapy, GvHD prophylaxis, stem cell source, acute GvHD (Figure 1D) and viraemia had no association with post-HCT AD. After multivariate analysis, age at transplant (p=0.01) and T-depleted graft (p<0.001) were significant predictors of post-HCT AD.

In patients with post-AD, a greater proportion of patients with early onset post-HCT AD (< 1 year post-HCT) had viraemia (n=7/9, 36%, 2 CMV, 4 adenovirus and 1 EBV) compared to late onset post HCT AD (n=8/22, 2 CMV, 5 EBV and 1 CMV+EBV) (p=0.05).

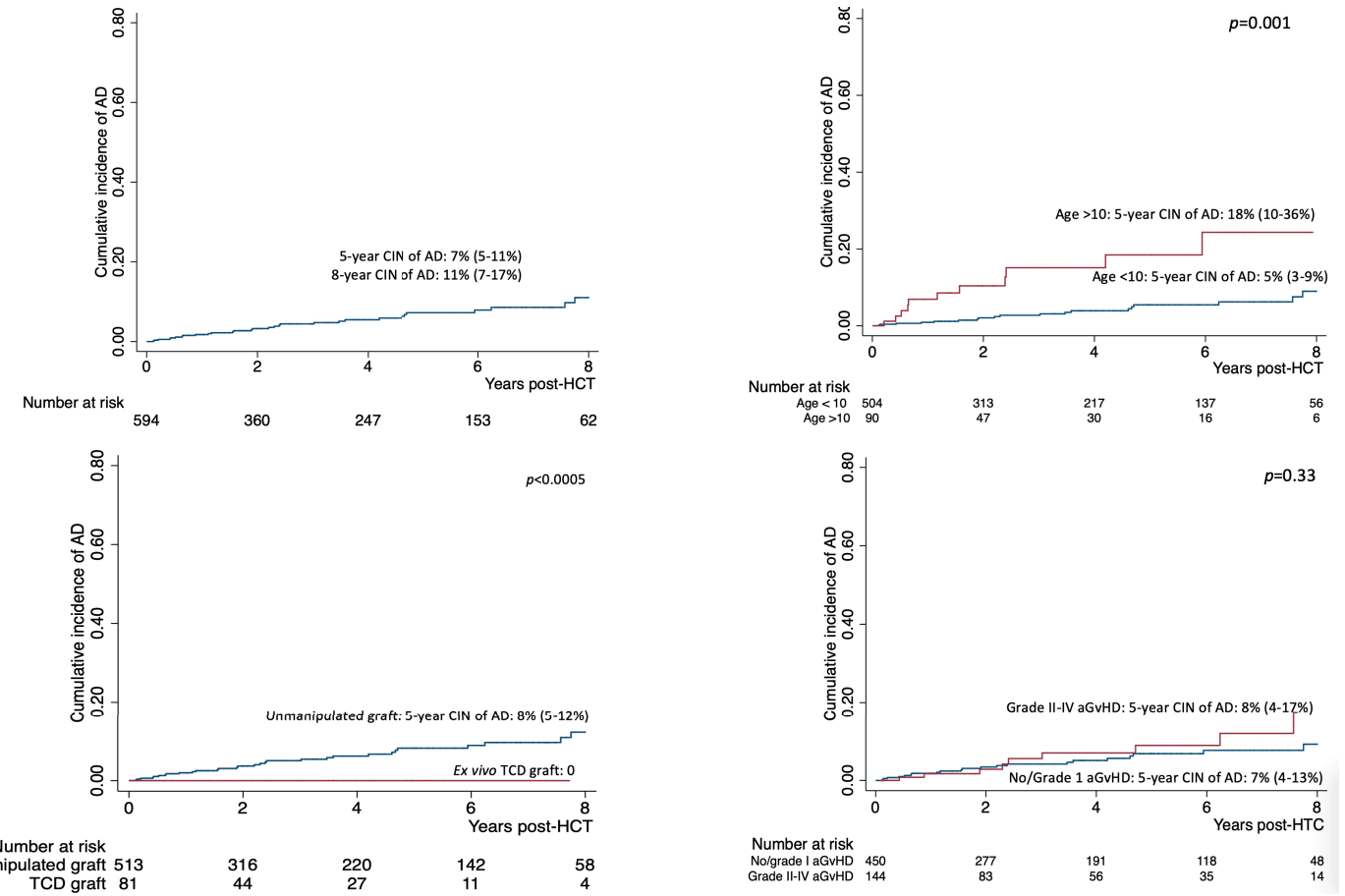

Figure 1: Non-haematological autoimmune disease (AD) post-HCT for children with primary immunodeficiency. A: cumulative incidence (CIN) of AD at 5 years and 8 years post HCT; B) CIN of AD according to age at HCT; C) CIN of AD according to ex-vivo T-cell depletion (TCD); D) CIN of AD according to acute graft-versus-host disease

Table 3: Detailed characteristics of patients with non-haematological autoimmune disease (n=31)

No/year	Diagnosis	Pre-HCT AD	Age at HCT (years)	Conditioning/ Serotherapy/ GvHD prophylaxis	Donor/ stem cell source	aGvHD	cGvHD	Post-HCT viraemia	Post-HCT AIC	Type of post-HCT AD	Onset (years post-HCT)	LSS at onset of AD	Donor chimerism at onset of AD	Treatment	Outcome
1/2009	CD40L def	no	1.1	Treo-Cy Alemtuzumab CSA/MMF	MUD marrow	no	no	EBV	No	SLE/dermato-myositis (ANA+, anti-chromatin+, anti-GAG+)	4.6	CD3 1099 CD19 200 Naïve 1160	CD15 18% CD3 46%	steroid, HD IVIg, hydroxy-chloroquine, infliximab, MTX	In remission with on-going treatment with MTX and infliximab
										Thyroiditis (ATPO+)	5.2	CD3 1099 CD19 200 Naïve 1160	CD15 18% CD3 47%	HD IVIg	resolved
2/2009	CID with LPD	ITP/AIN, splenecto-mised	13.9	Flu-Melph Alemtuzumab CSA/MMF	MUD PBSC	Grade I skin	no	adeno	AIHA	GBS	0.4	CD3 1450 CD19 1540 Naïve 50	WB 100%	HD IVIg	2 relapses, treated with HD IVIg, steroid, and infliximab
3/2009	CGD, X-linked	no	9.0	BuCy Alemtuzumab CSA/MMF	MUD marrow	no	no	adeno	no	Autoimmune thyroiditis (ATPO+)	0.2	CD3 851 CD19 639 Naïve 264	CD15 100% CD3 100%	Carbimazole Radioiodine	Remained on thyroxine replacement
4/2009	HLH, XLP-like	no	3.6	FluTreo Alemtuzumab CSA/MMF	MUD marrow	no	no	CMV	no	Polyarthritis (no autoantibody)	0.2	CD3 2008 CD19 845 Naïve 783	CD15 62% CD 3 63%	MTX Adalimumab Steroid Fusion of right hip	Remained on treatment Wheelchair dependent
5/2010	HLH MUNC 13-4	no	0.6	FluTreo Alemtuzumab CSA/MMF	MUD PBSC	Grade II, skin	no	no	no	Autoimmune thyroiditis (ATPO+)	6.2	NA	NA	L-thyroxine	Remained on thyroxine replacement
6/2010	Cartilage hair hypoplasia	Immune RCA, steroid & rituximab	2.6	FluTreo Alemtuzumab CSA/MMF	MMUD marrow	no	no	no	AIHA	Optic neuritis (anti-MOG+)	7.8	NA	NA	Steroid	Resolved

No/year	Diagnosis	Pre-HCT AD	Age at HCT (years)	Conditioning/ Serotherapy/ GvHD prophylaxis	Donor/ stem cell source	aGvHD	cGvHD	Post-HCT viraemia	Post-HCT AIC	Type of post-HCT AD	Onset (years post-HCT)	LSS at onset of AD	Donor chimerism at onset of AD	Treatment	Outcome
7/2010	SCID	no	1.0	FluTreo No serotherapy CSA/MMF	MMUD cord	no	no	no	no	Autoimmune thyroiditis (ATPO+)	1.7	CD3 7420 CD19 1560 Naïve X	WB 100%	L-thyroxine	Remained on thyroxine replacement
8/2010	SCID	no	1	FluTreo No serotherapy CSA/MMF	MMUD cord	Grade II, skin	no	no	no	Autoimmune hypothyroid-ism (ATPO+)	0.9	CD3 364 CD19 332 Naïve 21	WB 100%	L-thyroxine	Died of infection 4 years post-HCT
9/2010	SCID, T-B+NK+	no	11	BuFlu Alemtuzumab CSA	MUD marrow	no	no	EBV	no	Autoimmune hypothyroid-ism (ATPO+)	0.6	CD3 485 CD19 34 Naïve 54	WB 100%	L-thyroxine	Remained on thyroxine replacement
10/2010	SCID, ADA	no	0.1	Unconditioned No serotherapy CSA/MMF	MMUD cord	no	no	no	no	Juvenile idiopathic arthritis (ANA+; RF-)	3.0	CD3 1918 CD19 41 Naïve 805	CD15 0 CD3 100%	Steroid MTX	Second BMT for poor immune system
11/2010	IL-10 def Intractable colitis	no	1.1	Bu-Flu No serotherapy CSA/MMF	MSD marrow	no	no	no	no	Autoimmune hypothyroid-ism (ATPO+)	3.5	NA	WB 100%	L-thyroxine	Remained on thyroxine replacement
12/2010	SCID, ADA	no	0.4	Unconditioned No serotherapy CSA/MMF	MSD marrow	no	limited	no	no	Autoimmune hypothyroid-ism	4.7	CD3 2440 CD19 780 Naïve 360	WB 100%	L-thyroxine	Remained on thyroxine replacement
13/2011	SCID CD3e def	no	0.8	BuFlu No serotherapy CSA	MSD marrow	Grade III, skin	no	EBV	no	Autoimmune hypothyroid-ism (ATPO+)	7.6	CD3 4270 CD19 780 Naïve 360	CD15 59% CD3 100%	L-thyroxine	Remained on thyroxine replacement
14/2011	LAD	no	8.8	BuFlu ATG CSA/MTX	MUD PBSC	Grade III	exten-sive	CMV, EBV	no	Myositis (no autoantibody)	1.90	CD3 2721 CD19 519 Naïve 81	WB 100	Intensifica-tion of IST	Complete remission

No/year	Diagnosis	Pre-HCT AD	Age at HCT (years)	Conditioning/ Serotherapy/ GvHD prophylaxis	Donor/ stem cell source	aGvHD	cGvHD	Post-HCT viraemia	Post-HCT AIC	Type of post-HCT AD	Onset (years post-HCT)	LSS at onset of AD	Donor chimerism at onset of AD	Treatment	Outcome
15/2011	Complex autoimmune disease	AI hepatitis, hypothyroidism, polyarthritis	18.3	Flu-Cy Alemtuzumab CSA/MMF	MSD PBSC	no	no	no	no	Autoimmune thyroiditis (ATPO+)	1.6	CD3 258 CD19 318 Naïve 28	CD 15 60% CD3 65%	Thyroidectomy	Remained on thyroxine replacement
16/2011	JIA	no	13	Flu-Treo Alemtuzumab CSA/MMF	MUD PBSC	no	no	no	no	GBS (Ganglioside antibody+)	0.7	CD3 392 CD19 136 Naïve 84	WB 100%	Steroid, HD IVIg	Resolved
17/2012	DOCK8 deficiency	no	9.7	Flu-Melp Alemtuzumab CSA/MMF	MSD PBSC	Grade II, skin & gut	no	EBV	no	Autoimmune hypothyroidism (ATPO+)	4.7	CD3 2650 CD19 480 Naïve XX	CD15 54 CD3 100	L-thyroxine	Remained on thyroxine replacement
18/2013	WAS	no	14.9	Flu-Treo Alemtuzumab CSA/MMF	MMUD marrow	Grade I, skin	no	no	no	Polyarthritis (anti-chromatin+)	5.9	NA	CD15 92 CD3 98	Steroid, MTX	Remained on treatment
19/2013	SCID, ADA	no	0.1	Unconditioned No serotherapy CSA/MMF	MUD cord	no	no	no	no	Transverse myelitis (positive echovirus 9 in stools)	1.9	NA	CD 15 0 CD3 95	Steroid, HD IVIg,	Residual weakness
20/2013	CDG, X-linked	no	12	Bu-Flu Alemtuzumab CSA/MMF	MUD marrow	Grade 1, skin	no	no	no	Autoimmune hypothyroidism	4.2	CD3 770 CD19 190 Naïve 360	CD15 100 CD3 53	L-thyroxine	Remained on thyroxine replacement
21/2014	HLH Perforin deficiency	no	0.5	Flu-Treo Alemtuzumab CSA/MMF	MMUD marrow	no	no	CMV	no	Myositis	3.6	CD3 3940 CD19 190 Naïve 360	CD15 16% CD3 96%	Steroid MTX	Remission
22/2014	SCID, RAG 1	no	0.3	Flu-Treo Alemtuzumab CSA/MMF	MUD cord	no	no	no	no	Autoimmune hypothyroidism (ATPO+)	2.3	CD3 5184 CD19 712 Naïve 1852	WB 100%	L-thyroxine	Remained on thyroxine replacement

No/year	Diagnosis	Pre-HCT AD	Age at HCT (years)	Conditioning/ Serotherapy/ GvHD prophylaxis	Donor/ stem cell source	aGvHD	cGvHD	Post-HCT viraemia	Post-HCT AIC	Type of post-HCT AD	Onset (years post-HCT)	LSS at onset of AD	Donor chimerism at onset of AD	Treatment	Outcome
23/2015	ADPS	no	16.2	Flu-Treo Alemtuzumab CSA/MMF	MUD PBSC	Grade II, skin	no	no	AIHA	Autoimmune thyroiditis (ATPO+; TBII+)	2.4	CD3 637 CD19 185 Naïve 210	CD15 100% CD3 71%	L-thyroxine	Remained on thyroxine replacement
24/2015	A20 deficiency	IDDM	14.6	Flu-Treo Alemtuzumab CSA/MMF	MUD PBSC	Grade I, skin	no	CMV	no	Stiff person syndrome (anti GAG+)	2.4	CD3 1765 CD19 116 Naïve 813	CD15 100% CD3 77%	HD IVIg, plasmapheresis daratumumab	Still active disease, on treatment
25/2016	JIA	no	11	Flu-Treo Alemtuzumab CSA/MMF	MSD marrow	Grade 1, skin	no	no	no	Autoimmune thyroiditis (ATPO+)	0.5	CD3 514 CD19 274 Naïve 161	CD15 97% CD3 74%	L-thyroxine	Remained on thyroxine replacement
26/2016	XIAP	no	1.1	Flu-Treo Alemtuzumab CSA/MMF	MUD PBSC	Grade 3, skin & gut	no	no	no	Autoimmune thyroiditis (ATPO+)	2.3	CD3 514 CD19 274 Naïve 161	WB 100%	L-thyroxine	Remained on thyroxine replacement
27/2016	CGD, X-linked	no	2.1	Bu-Flu Alemtuzumab CSA/MMF	MMUD marrow	Grade 2, skin	no	adeno	AIHA	Nephritic/ nephrotic syndrome	0.4	CD3 479 CD19 100 Naïve 130	CD15 94 CD3 87	Steroid	Remission
28/2016	RALD	AI cytopenia, SLE	4.9	Bu-Flu Alemtuzumab CSA/MMF	MMUD marrow	no	no	adeno	AIHA	Grave's disease (ATPO+, TBII+, TRA+)	1.55	CD3 515 CD19 168 Naïve 252	CD15 100 CD3 87	No treatment	Remission
29/2017	Congenital neutropenia, ELA mutation	no	10.2	Flu-Treo Alemtuzumab CSA/MMF	MSD marrow	no	no	no	no	Grave's disease (ATPO-, TBII+)	3.1	CD3 747 CD19 316 Naïve 389	CD15 100 CD3 82	Thyroidectomy	Remained on thyroxine replacement
30/2018	HLH Perforin def	no	10.3	Flu-Treo-Thiotepa Alemtuzumab CSA/TMX	MUD marrow	no	limited	CMV	no	Autoimmune hypothyroidism (ATPO+)	0.2	CD3 1028 CD19 519 Naïve NA	WB 100%	L-thyroxine	Remained on thyroxine replacement

No/year	Diagnosis	Pre-HCT AD	Age at HCT (years)	Conditioning/ Serotherapy/ GvHD prophylaxis	Donor/ stem cell source	aGvHD	cGvHD	Post-HCT viraemia	Post-HCT AIC	Type of post-HCT AD	Onset (years post-HCT)	LSS at onset of AD	Donor chimerism at onset of AD	Treatment	Outcome
31/2018	CGD, AR	no	2.8	Flu-Treo Alemtuzumab CSA/MMF	MUD marrow	Grade I, skin	no	no	no	Autoimmune hypothyroid-ism (ATPO+, TRA+)	1.1	CD3 3100 CD19 500 Naïve NA	WB 100%	L-thyroxine	Remained on thyroxine replacement

ADA: adenosine deaminase deficiency; adeno: adenovirus SCID; AI: autoimmune; AIHA: autoimmune haemolytic anaemia; AIN: autoimmune neutropenia; APDS: activated protein kinase delta syndrome; AR, autosomal recessive, Bu: Busulfan; CID: combined immunodeficiency; CMV: cytomegalovirus; CSA: ciclosporin; Cy: cyclophosphamide; Def: deficiency; EBV: Epstein Barr virus; Flu: fludarabine; GBS: Guillain-Barre syndrome; GvHD: graft-versus-host disease; HD-IVIg: high dose intravenous immunoglobulin; HLH: haemophagocytic lymphohistiocytosis; IDDM: insulin dependent diabetes mellitus; IPEX: immunodysregulation polyendocrinopathy enteropathy X-linked; IST: immunosuppressive therapy; ITP: immune thrombocytopenia; JIA: Juvenile idiopathic arthritis; LAD: leukocyte adhesion deficiency; LPD: lymphoproliferation: LSS:: lymphocyte subset; Melph: melphalan; MMFD: mismatched family donor; MUD: mismatched unrelated donor; MTX: methotrexate; NA: not available; PBSC: peripheral blood stem cell; PNP: purine nucleoside phosphorylase (PNP); RALD: RAS associated autoimmune lymphoproliferative disorder; RCA: red cell aplasia; RF: rheumatoid factor; SCID: severe combined immunodeficiency; TBII: Thyroid binding inhibitory immunoglobulin; Thio: Thiotepa; TRA: thyroglobulin antibody; Treo: treosulfan; WAS: Wiskott Aldrich syndrome; WB: whole blood

Impact of donor chimerism on post-HCT AD

Myeloid chimerism data were available for 122 disease controls at 12 months post-HCT, 31 patients with AD at 12 months post-HCT and 29 patients at the onset of AD. The median myeloid donor chimerism was 100% (range 0 - 100%; 88/122 (72%) had myeloid chimerism ≥90%) in disease controls at 12 months post-HCT, 100% (range 0-100%; 24/31 (77%) had myeloid chimerism ≥90%) in patients with AD at 12 months post-HCT and 100% (range 0-100%; 20/29, 69% myeloid chimerism ≥90%) at onset of AD (*p*.0.91) (Figure 2A). B-lymphocyte chimerism data were available for 91 disease controls at 12 months post-HCT, 21 patients with AD at 12 months post-HCT and 19 patients at the onset of AD. There was no significant difference in B-lymphocyte donor chimerism between patents with AD and disease controls (*p*=0.38) (Figure 2B). T-lymphocyte chimerism data were available for 124 disease controls at 12 months post-HCT, 30 patients with AD at 12 months post-HCT and 29 patients at the onset of AD. The median T-lymphocyte donor chimerism was 100% (range 7 - 100%; 95/122 (79%) had T-lymphocyte chimerism ≥90%) in disease controls at 12 months post-HCT, 100% (range 12-100%; 19/30 (63%) had T-lymphocyte chimerism ≥90%) in patients with AD at 12 months post-HCT and 95% (range 46-100%; 17/29 (59%) had T-lymphocyte chimerism ≥90%) at onset of AD (*p*.0.24) (Figure 2C).

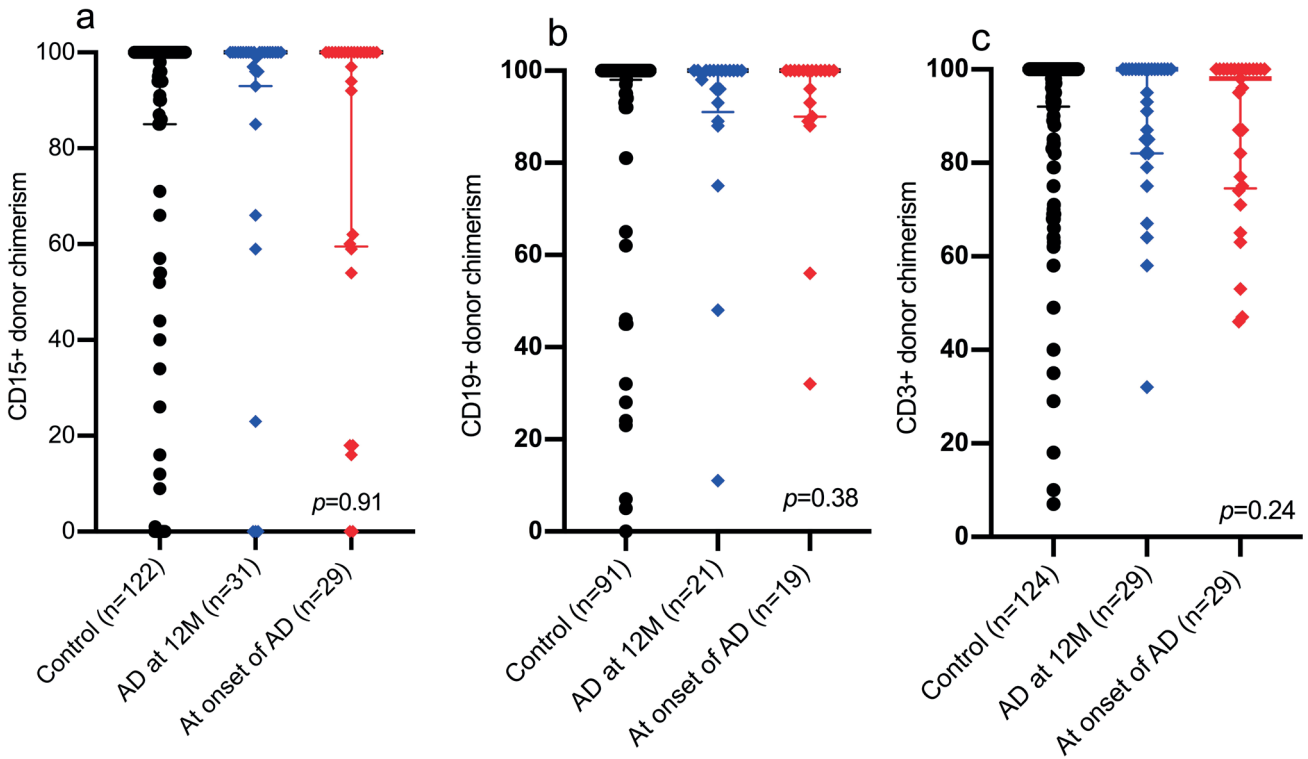

Figure 2: Donor chimerism of controls at 12 months post-HCT and patients with post-HCT AD at 12 months post-HCT at the the onset of AD. A: CD15+ myeloid donor chimerism; B: CD3+ T-lymphocyte donor chimerism; C: CD19+ B-lymphocyte dono chimerism
Abbreviations: AD: autoimmune desease; HCT: haematopoietic cell transplatation; M: months

Impact of lymphocyte reconstitution kinetics on post-HCT AD

$CD3^+$ T-lymphocyte and $CD19^+$ B-lymphocyte immune reconstitution kinetics within the first 12 months are illustrated in Figure 3. Patients with AD had a significantly lower CD+3 T-lymphocyte count (mean ± SE 554±41 cells/μL, median 403 cells/μL, range 40-1732 cells/μL) at 6 months post-HCT, compared to disease controls (mean ± SE 889±155 cells/μL, median 697 cells/μL, range 29-4148 cells/μL) (p = 0.036). There was no significant difference in circulating $CD4^+$, $CD8^+$, $CD19^+$, and NK cells (p=0.22) between patients with AD and controls at any time point post-transplant (Figure 3B and supplemental table 4). Of available data on naïve T cells at 6 months post-HCT, the median total naïve T cells at 6 months post-HCT was 63 cells/μL (range 0-715 cells/μL) in patients with AD (n=15) and 87 cells/μL (range 0-1490 cells/μL) in disease controls (n=74) (p=0.27) (supplemental figure 1).

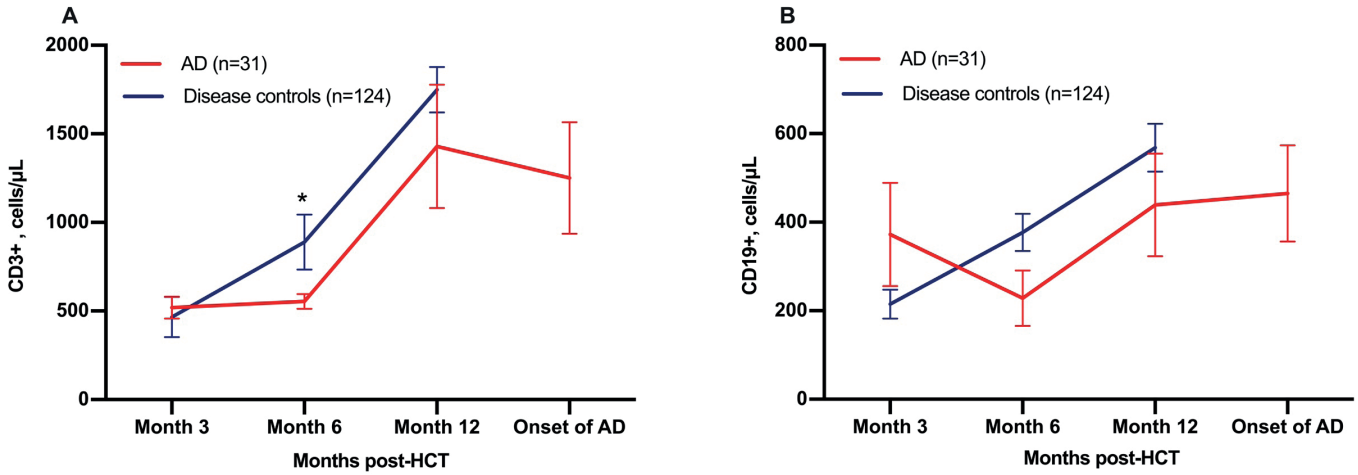

Figure 3: Immune reconstitution kinetics post-HCT. Means and standard error (SE) for CD3+ (A) and CD19+ (B) were meausred at different time points post-HCT for disease controls and at onset of AD for patients who developed AD. * indicates $p<0.05$.
Abbreviations: AD: autoimmune disease; HCT: haematopoietic cell transplantation

Treatment and outcomes

Of 19 patients with autoimmune thyroid disorders, 18 patients required treatment: 2 had thyroidectomy and L-thyroxine replacement, 1 had carbimazole, radioiodine and L-thyroxine replacement, and 16 received L-thyroxine. Patient 28 (table 2) had Grave's disease (positive ATPO, positive thyroglobulin antibody (TRA), positive thyroid binding inhibitory immunoglobulin (TBII), suppressed TSH, high T4 and T3) but remained clinically euthyroid. Biochemical hyperthyroidism resolved after 2 years without treatment. Patient No 8 who was transplanted for SCID and developed autoimmune hypothyroidism at 9 months post-HCT died of infection at 4 years post-HCT. All remaining 17 patients remained on L-thyroxine replacement at last follow-up (median follow-up after onset of AD was 3.1 years, range 0.6-8.0 years) and all immunosuppressive therapy had been discontinued

All 7 patients with neuromuscular disorders received immunomodulators. After a median follow-up of 3.6 years (range 1.9 to 5.0 years) after onset of neuromuscular features, 5 had normal neurological examination, 1 had residual muscular weakness and 1 (A20 deficiency) remained on active treatment for stiff person syndrome 1.9 years after onset of symptoms.

Of 5 patients with rheumatological manifestations, patient No 27 (CGD) with nephritic-nephrotic syndrome achieved remission with steroid treatment after 1.7 months and remained in remission at latest follow-up 1.9 years after the onset of AD. All 3 patients (patients No4, 10 and 18) with polyarthritis required treatment, two (patients No 4 and 10) remained in remission with ongoing therapy and patient No 18 underwent a second transplant for poor immune function and had no recurrence of arthritis after the second transplant. Patient No 1 was transplanted for CD40L deficiency and diagnosed with an overlap syndrome of SLE and dermatomyositis at 4.6 years post-HCT. He was treated with steroids, high dose immunoglobulin, infliximab, hydroxychloroquine and methotrexate. At his latest follow-up 4.6 years after the onset of the overlap syndrome, his disease was in remission with on-going treatment using infliximab and methotrexate.

Discussion

The present study is the largest analysis to document post-HCT AD from a cohort of 596 children with PID who were transplanted between 2009 and 2018. The 5-year cumulative incidence of post-HCT AD is 7% (95% CI, 5-11%) and autoimmune thyroid disorder is the most common post-HCT AD in our cohort. Our analysis indicates age at transplant and CD3+ lymphocyte count at 6 months post-HCT had significant associations with post-HCT AD. Serotherapy, acute GvHD, CMV/EBV/AdV viraemia and donor T and myeloid chimerism were not associated with post-HCT AD. All patients but one required treatment for post-HCT AD.

Whilst there is an increasing number of reports in post-HCT AIC, there are limited studies which examine post-HCT AD. Post-HCT AD has been reported following autologous and allogeneic HCT regardless of the underlying disease for which HCT was indicated. Autoimmune thyroid disorder is the most frequently reported AD after solid organ and stem cell transplantation, with reported incidences ranging from 2.9 to 12% after HCT. (11-13) The lifetime risk of *de novo* autoimmune thyroid disease in the general population varies from 2 to 4% with a female to male ratio of 3:1 (13, 14). The incidence of autoimmune thyroid disease is 3.8% in 493 paediatric transplant survivors for PID within only 5 years after transplant in our cohort, which is higher compared to reported incidence of 1.4% of children between ages of 1 and 16 years. (15) We can exclude the possibility of radiation injury to the thyroid gland because none had total body irradiation. Immune mediated neurological disorders post-HCT are mainly reported in case series: GBS (n=22) (16), transverse myelitis (n=5) (17-20), myasthenia gravis (n=25) (21), optic neuritis (n=2) (22, 23). None in our cohort had myasthenia gravis. Similarly, rheumatological disorders are reported in case series, including juvenile arthritis (n=1) (24), rheumatoid arthritis (n=3) (9), sarcoidosis (n=6) and SLE (n=1). (25)

Autoimmunity post-HCT has been observed over decades but the mechanism of post-HCT autoimmunity is unresolved and likely multi-factorial. Several mechanisms have been postulated, including dysfunctional immune reconstitution, adoptive transfer of autoreactive T cell and autoantibodies from donors to recipients and immune-dysregulation due to infection (molecular mimicry), drugs (conditioning and immunosuppressive therapy) and dysfunctional regulatory T cells. (9, 26, 27) Homeostatic expansion of lymphocytes is the concept of robust T cell expansion after a state of lymphopenia (9). In our cohort, the CD3+ lymphocyte count is significantly lower in patients with post-HCT AD, compared to disease controls. Due to the retrospective nature of this study, there were insufficient data in our cohort to determine the impact of naïve T cells at 6 months post-HCT on development of post-HCT AD. Therefore, this study is unable to determine if dysregulation of T cell reconstitution or impaired thymopoiesis post-HCT play any roles in development of post-HCT AD. Adoptive transfer of autoreactive T cell and autoantibodies from donors to recipients has been suggested as one of the mechanisms of post-HCT AD. Examples include adoptive transfer of psoriasis in a syngeneic HCT (28), thyroid disease (29), coeliac disease (30), insulin-dependent diabetes mellitus (31), myasthenia gravis (32), vitiligo (33), and Crohn's disease (34). In our cohort, none of the 82 patients (60 haploidentical donor, 20 MMUD/MMFD, 1 MUD and 1 MFD) who received *ex vivo* T cell depleted grafts developed post-HCT AD. It could suggest that T cell depletion of the graft eliminaties potential harmful T cells a/o prevents emergence of pathogenic T cells during the early post-HCT period during which immune dysbalance/dysregulation is most likely to occur. Additionally, patients who have a genetic predisposition (such as a vulnerable HLA-type) may 'inherit' AD from adoptive transfer of cells from an HLA-

identical donor, or may develop AD because of the inherent genetic susceptibility.

Different studies have identified risk factors for post-HCT AIC and the results depend on the patient population studied. In paediatric studies, non-malignant disease, unrelated HCT, cord blood or PBSC as stem cell source, no TBI exposure, GvHD, CMV and alemtuzumab were associated with post-AIC. (1-6, 8). There are limited studies which have looked at risk factors for post-HCT AD. For autoimmune thyroid disease, Isshiki *et al.* reported HLA B35 in the recipient was associated with a higher incidence of autoimmune thyroid dysfunction after HCT in 72 adult transplant survivors in Japan (12). Another single retrospective study in China which included 721 autologous and allogeneic HCT recipients reported female donor and HLA DR9 were independently associated with development of post-HCT autoimmune thyroid disorder. (13) In our recent study, the median onset of post-HCT AIC was 6.5 months post-HCT and alemtuzumab and GvHD were associated with post-HCT AIC in children with PID (8) Together with reported studies in children, post-HCT AIC usually occurs within the first year after HCT, a period in which immune reconstitution takes place and disturbances could be due to temporarily use of immunosuppressive agents, viral reactivations and acute GVHD. In contrast, the median onset of post-AD was 2.2 years and both alemtuzumab and GvHD had no association with post-HCT AD. This is interesting, as GvHD may cause direct thymic damage, and thus reduce self-antigen expression on medullary thymic epithelial cells, important in the development of central tolerance. (35) Our study shows that post-HCT AD usually presents at a later stage in the setting of mostly stable chimerism, no viral reactivation, no GvHD and no immunosuppressive agents. Moreover, this paper suggests a slow increasing overall frequency whereas this does not occur with AIC. This points to different immunological mechanisms that may on one hand reflect the natural patterns in non-transplanted controls/healthy individuals and on the other hand be more pronounced because of HCT related aspects including impaired negative thymic selection and dysregulated peripheral tolerance etc. In that context it is remarkable that AD does not appear more prevalent in mismatched donor and haploidentical donor HCT. Only age has a significant association with post-HCT AD. Prolonged immune dysregulation prior to transplant and the use of immunomodulators in older children and adolescents might increase the susceptibility of abnormal T cell haemostatic expansion post-HCT.

Data on treatment and outcomes of patients with post-HCT AD mainly come from case reports and series. (36) Autoimmune thyroid disease in our cohort, which present as “hypo-”, or “hyper-” or “hyper- then switch to hypo-“, was managed according to clinical manifestations of thyroid dysfunction, either with thyroid replacement therapy or with suppression in cases of hyperthyroidism. In our study, post-transplant autoimmune diseases can be adequately managed and controlled in most cases. Neurological outcomes are variable, one patient had residual weakness and one remained on treatment for stiff-person syndrome. In this study

we did not observe patients with distinct and sequential AD. Whether patients presenting with one AD are at increased risk for additional AD manifestations can only be answered with longer follow-up.

Overall, the manifestations of new AD after HCT are diverse and therefore, the diagnosis of post-HCT AD can be difficult and challenging as there are broad differential diagnoses, including disease recurrence, chronic GvHD, infection and drug/conditioning-related side effects. As a result of improved HCT strategies and supportive care transplant survival in PID has significantly increased during recent decades. Therefore, more emphasis is put on quality of long-term graft function, late effects as well as quality of life. Prospective studies are needed to evaluate the incidence, pattern, outcomes and biological mechanisms of AD after HCT. As AD post-HCT may affect the quality of life of transplant survivors, early recognition of post-HCT AD is crucial to reduce preventable morbidity and mortality. A guideline for assessment and management of post-HCT AD in children with PID is needed to ensure its timely identification and appropriate management.

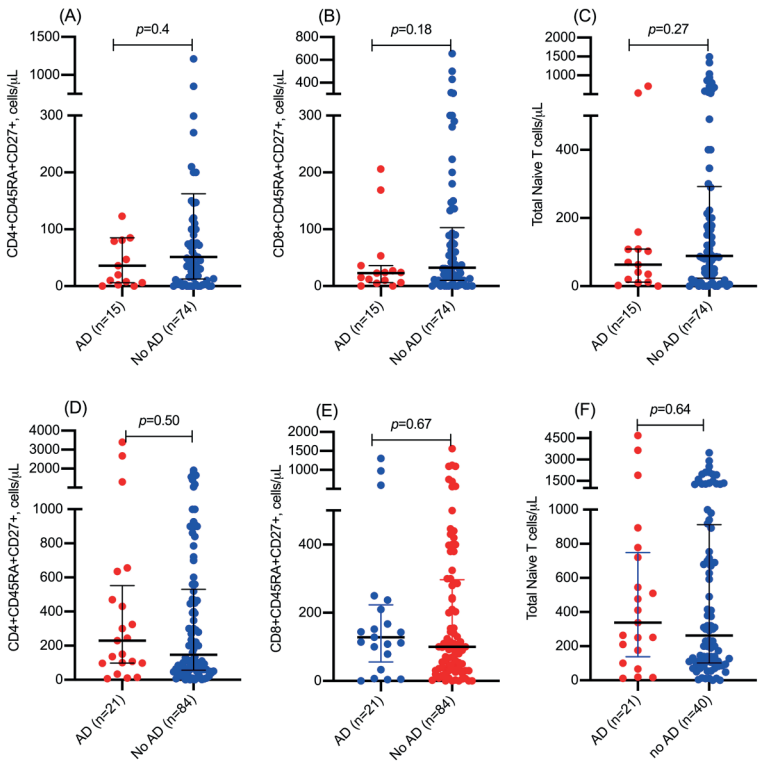

Figure S1: Naive T cells at 6 months and 12 months post-HCT in patients with AD and controls: (A) CD4+CD45RA+CD27+ at 6 months post-HCT (B) CD8+CD45RA+CD27+ at 6 months post-HCT (C) Total naive T cells at 6 months post-HCT (D) CD4+CD45RA+CD27+ at 12 months post-HCT (E) CD8+CD45RA+CD27+ at 12 months post-HCT (F) Total naive T cells at 12 months post-HCT

Table S1: Detailed characteristics of patients who had post-HCT AD likely secondary to underlying disease or recurrent diseases or GvHD. These patients had been excluded for analysis

No/year	Diagnosis	Pre-HCT AD	Age at HCT (years)	Conditioning/ Serotherapy/ GvHD prophylaxis	Donor/ stem cell source	aGvHD	cGvHD	Post-HCT viraemia	Post-HCT AIC	Type of post-HCT AD	Onset (years post-HCT)	LSS at onset of AD	Donor chimerism at onset of AD	Treatment	Outcome
1/2009	IPEX	No	0.5	FluTreo Alemtuzumab Tacrolimus/MMF	MUD CB	no	no	no	AIHA	Nephrotic syndrome (minimal change disease)	0.2	CD3 459 CD19 450 Naïve NA	CD15 17% CD3 68%	Steroid	Remission
2/2010	SCID, RAG	AIHA (treated with ritux-imab)	0.3	Bu No serotherapy No GvHD proph-laxis	MSD marrow	no	Extensive (localized morphea, lung)	no	no	scleroderma	0.6	CD3 950 CD19 1590 Naïve 174	WB 100%	Steroid, CSA	Remission
3/2010	SCID, CGC	No	0.8	Unconditioned No serotherapy CSA/steroid	MUD CB	Grade 3, skin & gut	Limited (skin)	no	no	vitiligo	1.9	CD3 800 CD19 0 Naïve 270	CD15 0 CD3 100%	Topical steroid and topical tacrolimus	Controlled. No new lesion
4/2011	SCID, CGC	No	0.1	Unconditioned No serotherapy CSA	Haplo CD34+ selection	Grade 2, skin & gut	Limited (skin)	adeno	no	scleroderma	1.0	CD3 1820 CD19 310 Naïve ND	CD15 0 CD3 100%	Methotrexate	Still on treat-ment with progressing tiptoeing
5/2011	CGD, X-linked	AIHA (treated with ritux-imab)	14	Bu Flu Alemtuzumab CSA/MMF	9/10 DQ MMUD CD34+ selection	Grade 2, skin	Limited (skin)	no	no	scleroderma	0.5	CD3 180 CD19 310 Naïve ND	WB 100%	Physiotherapy No IST	Remission
6/2011	SCD, PNP	No	0.6	FluTreo Alemtuzumab CSA/MMF	MUD PBSC	no	Limited (skin)	no	no	vitiligo	5.7	CD3 2850 CD19 460 Naïve 1950	CD15 73% CD3 75%	Topical steroid and topical tacrolimus	Spreading vitiligo

No/year	Diagnosis	Pre-HCT AD	Age at HCT (years)	Conditioning/ Serotherapy/ GvHD prophylaxis	Donor/ stem cell source	aGvHD	cGvHD	Post-HCT viraemia	Post-HCT AIC	Type of post-HCT AD	Onset (years post-HCT)	LSS at onset of AD	Donor chimerism at onset of AD	Treatment	Outcome
7/2012	WAS	No	0.9	FluTreo Alemtuzumab CSA/MMF	MUD PBSC	no	no	no	no	Vasculitis (biopsy proven) (ANCA+)	0.14	CD3 33 CD19 0 Naïve 0	WB 100%	Topical steroid and topical tacrolimus	Resolved
8/2012	SCID, Artemis	No	0.6	FluTreo Alemtuzumab CSA/MMF	MUD CB	Grade 2, skin	no	no	AIHA and ITP	Vitiligo after second HCT for refractory aGvHD	2.67	CD3 1715 CD19 444 Naïve 892	WB 100%	No treatment	No new lesion
9/2013	Chediak-Higashi syndrome with EBV driven HLH	No	8	FluTreo No serotherapy Tacolimus/MMF	MUD CB	Grade 2, skin	Extensive (skin)	No	no	Overlap syndrome – SLE/dermatomyositis/ scleroderma (ANA>640; dsDNA+)	0.8	CD3 1884 CD19 2371 Naïve 207	WB 100%	IST and ECP	Improved with IST and ECP but still significant disease
10/2014	Mendelian Susceptibility to Mycobacterial infections (MSMD), IkBalpha gain of function	No	3.2	FluTreo No serotherapy Tacolimus/MMF	MMUD Marrow	no	no	EBV	no	SLE-like, nephrotic syndrome (minimal change disease)	1.6	CD3 708 CD19 174 Naïve 467	CD15 1% CD3 36%	Second HCT using TCR αβ/ CD19 depleted parental graft	Resolved

Adeno: adenovirus; AIHA: autoimmune haemolytic anaemia; Bu: Busulfan; CSA: ciclosporin; EBV: Epstein Barr virus; Flu: fludarabine; GvHD: graft-versus-host disease; HLH: haemophagocytic lymphohistiocytosisIPEX: immunodysregulation polyendocrinopathy enteropathy X-linked; IST: immunosuppressive therapy; ITP: immune thrombocytopenia; LSS: lymphocyte subset; MMF: mycophenolate mofetil; MMFD: mismatched family donor; MMUD: mismatched unrelated donor; MUD: mismatched unrelated donor; MTX: methotrexate; ND: not done; PBSC: peripheral blood stem cell; PNP: purine nucleoside phosphorylase (PNP); SCID: severe combined immunodeficiency; SLE: systemic lupus erythematous; Treo: treosulfan; WAS: Wiskott Aldrich syndrome; WB: whole blood

Table 2S: List of diagnosis of study population

Patient characteristics		Number of patients with AD
Diagnosis		
SCID, n (%)	158	7
Non-SCID PID, n (%)	438	24
A20 deficiency	1	1
ADA2	1	0
ADPS	7	1
Autoimmune enteropathy	6	0
C1q deficiency	1	0
Cartilage hair hypoplasia	9	0
CD27/CD70 deficiency	1	0
CD40 ligand deficiency	18	1
CGD	82	4
Congenital neutropenia	6	1
CTLA4 haploinsufficiency	2	0
CTPS1	2	0
DNA ligase IV	5	0
DOCK 8 deficiency	18	1
FADD defect	2	0
HLH	61	4
Hypomorphic SCID	8	1
ICF syndrome	3	0
ICOS	1	0
IFNGR2	4	0
IRF8 deficiency	3	0
IPEX/IPEX-like	10	0
ITK deficiency	2	0
JIA	6	2
Leukocyte adhesion defect	6	1
LRBA	3	0
MHC class II deficiency	33	0
NEMO	3	0
NK deficiency	2	0
Nigmegen Breakage syndrome	2	0
PNP deficiency	2	0
RALD	2	0
STAT3 gain-of-function	6	1
STAT3 loss-of-function	5	0
STAT1 gain-of-function	3	0
XIAP	9	1
XLP/XLP-like	10	0
WAS	31	1
Zinc transport defect	2	0
Others	58	3

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