

Two decades of excellent transplant survival in children with chronic granulomatous disease: A report from a supraregional immunology transplant center in Europe

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Article type: Letter to Blood

Running title: CGD and HCT

Word count: 1195

Figure: 1

Table: 1

Supplemental data: 1 table, 2 figures

References: 9

Keywords: CGD, children, hematopoietic cell transplantation

To the Editor:

Chronic granulomatous disease (CGD), an inherited immunodeficiency resulting from defects in the NADPH oxidase complex rendering phagocytes deficient in producing the superoxide anion necessary for normal killing of bacterial and fungal microorganisms, leads to severe and recurrent infections, inflammatory granulomatous complications and autoimmune diseases.¹ It is associated with substantial morbidity and premature death. Hematopoietic cell transplantation (HCT) is the only long-term curative therapy, but utility was historically limited by high rates of graft failure and transplant-related morbidity and mortality. Transplant survival and graft outcome have improved dramatically over the past 10 years, due to reduced toxicity conditioning regimens, detailed graft selection hierarchy, superior HLA matching technology, better cell-dosed grafts, greater availability of grafts, improved supportive care and more effective anti-microbial therapy. We studied transplant survival post-HCT for CGD in a supra-regional immunology transplant center in the United Kingdom.

We report outcomes of patients with CGD who received their first HCT between 1998-2017 at the Great North Children's Hospital, Newcastle upon Tyne, one of two supra-regional transplant centers in the UK for children with primary immunodeficiency. Outcomes of interest were overall survival (OS), event-free survival (EFS), haematopoietic recovery, toxicities, graft-versus-host disease (GvHD), long-term survival, graft function, post-transplant immune reconstitution and autoimmune disease post-transplant. OS was defined as survival from first HCT to last follow-up or death. An event was defined as death or second procedure for slipping chimerism. Cox proportional-hazard modelling was used to analyse predictors of OS and ES. Variables included for predictor analysis were: year of transplant (1998-2007 versus 2008-2017), age at transplant, donor (family versus unrelated donor), conditioning (myeloablative conditioning (MAC) versus reduced toxicity conditioning (RTC)), stem cell source (marrow versus peripheral blood), total nucleated cell dose (TNC) and CD34 cell dose. Multiple linear regression modelling was used to analyse the impact of conditioning, stem cell source and cell doses on long-term graft function after first successful HCT. CD3, CD4, CD8, CD19, CD27/CD45RA and CD16/56 enumeration were measured routinely. All data analyses were performed using STATA version 14.2.

Fifty-five children with CGD were included, none were excluded. A detailed description of transplant characteristics is summarized (Table 1). Median age at transplant was 5.3 years (range, 0.6-18.0). 45 (82%) had X-linked and 10 (18%) autosomal recessive CGD. Twelve (22%) had growth failure (defined as weight less than fifth centile for age and gender) at the time of transplant. Of 34 who underwent colonoscopy, 31 (91%) had biopsy proven colitis. Four (7%) had biopsy proven granulomatous lung inflammation. One had undefined acute necrotising encephalitis. Prior to 2007 various conditioning regimens were used, with 21 (38%) patients receiving pharmacokinetically guided intravenous (IV) busulfan (Bu) and IV cyclophosphamide with or without serotherapy. From 2007 the conditioning regimen was switched to Fludarabine-Treosulfan-Alemtuzumab with post-HCT GvHD

prophylaxis using ciclosporin (CSA) and mycophenolate mofetil (MMF) for family and unrelated donors (n=24, 44%). Fludarabine-Treosulfan-Thiotepa-ATG-Rituximab was used for CD3 TCR $\alpha\beta$ CD19 depleted haploidentical grafts (n=4, 7%). Of these 4 patients, one received CSA/MMF and the remaining 3 did not receive any post-HSCT GvHD prophylaxis. The median day to neutrophil and platelet engraftment was 16 days (range, 9-37), and 18 days (range, 10-139) respectively. Ten (20%) patients had grade II-IV acute GvHD, 5 (9%) had grade III-IV acute GvHD. None had chronic GvHD. Only one patient who received Busulfan-based conditioning had veno-occlusive disease. Eleven (20%) had CMV viraemia, 5 (9%) adenoviraemia and 5 (9%) EBV viraemia.

The 5-year OS was 89% (95% CI, 67-95%) (Figure 1), rising to 100% for children transplanted at ≤ 5 years of age (n=28) versus 81% (95%CI, 60-92%) for the children >5 years of age (n=27) ($p=0.04$) (Figure 1). The OS was comparable between matched family (88%, 95% CI, 61-97%) and unrelated donor transplants (89%, 71-95%) (Figure 3). The 4 haploidentical transplants were successful. Year of transplant ($p=0.13$), conditioning ($p=0.58$), stem cell source ($p=0.57$), TNC ($p=0.69$) and CD34 cell dose ($p=0.52$) were not associated with OS. All five deaths were due to transplant-related complications. The median age at transplant of deceased patients was 10.0 years (range 8.4-18 years). The 5-year EFS for the cohort was 77% (95% CI 62-87%). No variables were associated with EFS. All 7 patients with slipping chimerism received successful second procedures (3 had unconditioned stem cell boost infusion and 4 had fully conditioned second transplant).

Median age of long-term survivors was 14 years (range, 2-36 years) with median duration of follow-up of 6.5 years (range, 0.32-19.5). There were no late deaths. **None had clinical evidence of colitis. Of the 11 survivors who were older than 21 years of age, 6 (55%) had unassisted successful pregnancy themselves or with their partners (4 received Busulfan-Cyclophosphamide, 1 Busulfan-Fludarabine and 1 had Fludarabine-Melphalan).** Median donor myeloid chimerism after first successful HCT (n=43) was 92% (range 30-100%). MAC was the only variable associated with higher donor myeloid chimerism ($p=0.036$). MAC ($p=0.002$) and TNC ($p=0.03$) were associated with higher donor T-lymphocyte chimerism (supplemental figure 1 and supplemental table 1). For patients who received unconditioned stem cell infusion (n=3), the median myeloid chimerism was 52% (ranges, 30-100%) and the median T-lymphocyte chimerism was 26% (range 26% to 100%). For patients who received conditioned second transplant (n=4), the median myeloid chimerism was higher at 89% (ranges, 30-100%) and the median T-lymphocyte chimerism was 65% (range 26-100%). Longitudinal immune-reconstitution results were available for 42 patients of survivors after first successful HCT (supplemental figure 2).

The 1- and 5-year cumulative incidence of autoimmune diseases was 9% and 12% respectively, significantly less than that reported in a recent study.² Three (5%) had immune cytopenia in first year post-HCT. whilst 3 (5%) had autoimmune endocrinopathy (2 thyroid dysfunction; 1 type 1 diabetes mellitus). Two patients with immune haemolysis achieved remission with intravenous immunoglobulin and steroid while 1 with immune haemolysis and thrombocytopenia needed additional Rituximab. None

had evidence of immune cytopenia at last follow-up. Autoimmune endocrinopathy occurred one year post-HCT (median 3.9 years; range, 1.4-7.1). One patient with hypothyroidism received thyroxine replacement, 1 with Grave's disease was treated with radioiodine and 1 with type 1 diabetes mellitus received insulin.

The ability of donor-derived neutrophils to replace recipient's defective neutrophils makes HCT a superior therapy compared to conventional standard of care using antimicrobial therapy. Previous studies demonstrated that non-transplanted children have more serious infections, episodes of surgery, poorer growth and reduced quality of life compared to transplanted children.³⁻⁵ Estimated survival of non-transplanted patients was 88% at 10 years and 55% at the age of 30 years.⁶ In this study, our findings emphasize that HCT performed in an experienced immunology transplant center is safe and provides long-term cure for children with CGD. Alternative donors using unrelated and parental haploidentical donors are associated with excellent survival comparable to family donors. X-linked carrier family donors should be avoided as they have inflammatory and autoimmune symptoms and excessive fatigue.⁷⁻⁹ As young age at HCT is associated with favourable outcome, HCT should be performed as early as possible before the onset of disease-related organ damage. **In our center, all newly diagnosed CGD and newborn CGD are recommended for HCT with either matched donor or haploidentical donor if no suitable matched donor available.** Family screening ~~and newborn screening~~ plays an important role in advancing the transplant care for children with CGD.

Commented [Ag1]: There is no newborn scening for CGD

Acknowledgements: We would like to thanks all patients, their families and the treating multidisciplinary team.

Authorship Contributions: SHL collected the data, performed the statistical analysis, interpreted the data and prepared the manuscript. PM, HW and NW collected the data. MS, ARG contributed equally to the conceptualization of the research, statistical analysis, and interpretation of the data, manuscript writing and critical review at every level of the research stages. SO, TF, SH, AC and MA critically reviewed the manuscript.

Disclosures of potential conflict of interest: All the authors declare no competing financial interests.

References:

1. Gennery A. Recent advances in understanding and treating chronic granulomatous disease. *F1000Res*. 2017;6:1427.
2. Yanir AD, Hanson IC, Shearer WT, Noroski LM, Forbes LR, Seeborg FO, Nicholas S, Chinn I, Orange JS, Rider NL, Leung KS, Naik S, Carrum G, Sasa G, Hegde M, Omer BA, Ahmed N, Allen CE, Khaled Y, Wu MF, Liu H, Gottschalk SM, Heslop HE, Brenner MK, Krance RA and Martinez CA. High Incidence of Autoimmune Disease after Hematopoietic Stem Cell Transplantation for Chronic Granulomatous Disease. *Biol Blood Marrow Transplant*. 2018;24:1643-1650.
3. Cole T, Pearce MS, Cant AJ, Cale CM, Goldblatt D and Gennery AR. Clinical outcome in children with chronic granulomatous disease managed conservatively or with hematopoietic stem cell transplantation. *J Allergy Clin Immunol*. 2013;132:1150-5.
4. Cole T, McKendrick F, Titman P, Cant AJ, Pearce MS, Cale CM, Goldblatt D and Gennery AR. Health related quality of life and emotional health in children with chronic granulomatous disease: a

comparison of those managed conservatively with those that have undergone haematopoietic stem cell transplant. *J Clin Immunol.* 2013;33:8-13.

5. Jones LB, McGrogan P, Flood TJ, Gennery AR, Morton L, Thrasher A, Goldblatt D, Parker L and Cant AJ. Special article: chronic granulomatous disease in the United Kingdom and Ireland: a comprehensive national patient-based registry. *Clin Exp Immunol.* 2008;152:211-8.

6. Horwitz ME, Barrett AJ, Brown MR, Carter CS, Childs R, Gallin JI, Holland SM, Linton GF, Miller JA, Leitman SF, Read EJ and Malech HL. Treatment of chronic granulomatous disease with nonmyeloablative conditioning and a T-cell-depleted hematopoietic allograft. *N Engl J Med.* 2001;344:881-8.

7. Battersby AC, Martin AJ, Tarn J, Ng FW, Cale CM, Goldblatt D and Gennery AR. Raised Serum IL-8 Levels Are Associated with Excessive Fatigue in Female Carriers of X-Linked Chronic Granulomatous Disease in the UK. *J Clin Immunol.* 2017;37:279-281.

8. Battersby AC, Braggins H, Pearce MS, Cale CM, Burns SO, Hackett S, Hughes S, Barge D, Goldblatt D and Gennery AR. Inflammatory and autoimmune manifestations in X-linked carriers of chronic granulomatous disease in the United Kingdom. *J Allergy Clin Immunol.* 2017;140:628-630 e6.

9. Marciano BE, Zerbe CS, Falcone EL, Ding L, DeRavin SS, Daub J, Kreuzburg S, Yockey L, Hunsberger S, Foruraghi L, Barnhart LA, Matharu K, Anderson V, Darnell DN, Frein C, Fink DL, Lau KP, Long Priel DA, Gallin JI, Malech HL, Uzel G, Freeman AF, Kuhns DB, Rosenzweig SD and Holland SM. X-linked carriers of chronic granulomatous disease: Illness, lyonization, and stability. *J Allergy Clin Immunol.* 2018;141:365-371.

Table 1: Patient and transplantation characteristics and outcome after first HCT in children with CGD (N=55)

Patient characteristics	
Year of transplant	
1998-2007, n (%)	17 (31)
2008-2017, n (%)	38 (69)
Male, n (%)	51 (93)
Inheritance	
X-linked CGD, n (%)	45 (82)
Autosomal recessive CGD, n (%)	10 (18)
Age at transplant, months, median (range)	5.3 (0.6-18.0)
Donor characteristics	
Type of donor	
Matched family donor, n (%)	20 (36)
Unrelated donor, n (%)	31 (56)
Haploidentical donor, n (%)	4 (8)
Stem cell source	
Marrow, n (%)	29 (53)
PB, n (%)	24 (43)
CB, n (%)	2 (4)
Stem cell dose	
Marrow	
TNC, x 10 ⁸ /kg, median (range)	5.0 (1.6-24.4)
CD34, x 10 ⁶ /kg, median (range)	4.3 (0.75-53.0)
PB	
TNC, x 10 ⁸ /kg, median (range)	10.3 (1.2-22.8)
CD34, x 10 ⁶ /kg, median (range)	8.0 (3.0-29.2)
Transplant characteristics	
Conditioning regimen	
<i>Myeloablative conditioning (MAC)</i>	
Busulfan-Cyclophosphamide, n (%)	21 (38)
Fludarabine-Treosulfan-Thiotepa, n (%)	4 (7)
<i>Reduced toxicity conditioning (RTC)</i>	
Treosulfan-fludarabine, n (%)	24 (45)

Busulfan-fludarabine, n (%)	4 (7)
Treosulfan-cyclophosphamide, n (%)	1 (2)
Fludarabine-melphalan, n (%)	1 (2)
Serotherapy	
None, n (%)	9 (16)
ATG, n (%)	4 (8)
Alemtuzumab, n (%)	42 (76)
GVHD prophylaxis	
None, n (%)	3 (5)
CSA alone, n (%)	2 (4)
CSA + MTX, n (%)	16 (29)
CSA + MMF, n (%)	33 (60)
CSA + steroid (for CB), n (%)	1 (2)
Haematopoietic recovery	
Days to neutrophil recovery, median (range)	16 (9-37)
Days to platelet recovery, media (range)	18 (10 - 139)
Transplant-related complications	
Acute GvHD	
Grade II-IV, n (%)	10 (20)
Grade III-IV, n (%)	5 (9)
Chronic GvHD ²	
Veno-occlusive disease, n (%)	1 (2)
Number of patient received second procedures for slipping chimerism, n (%)	7 (13)
Number of patients received unconditioned stem cell boost infusion	3
Myeloid chimerism prior to unconditioned stem cell boost infusion, %, median (range)	17 (15-23)
Number of patients received fully conditioned second transplant	4
Myeloid chimerism prior to unconditioned stem cell boost infusion, %, median (range)	11 (0-13)
Cause of death (n=5)	
Multi-organ failure	2
Grade IV acute GvHD	1
Pulmonary haemorrhage	1
Post-transplant lymphoproliferative disease	1
Long-term outcome	

ATG: anti-thymocyte globulin (Grafalon) for haploidentical transplant; CB: cord blood; CGD: chronic granulomatous disease; CSA: ciclosporin; MMF: mycophenolate mofetil; MTX: methotrexate; PB: peripheral blood; TNC: total nucleated cell dose