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Prepublished online August 8, 2002;
doi:10.1182/blood-2002-02-0583

Treatment of chronic granulomatous disease with myeloablative conditioning and an unmodified hematopoietic allograft: a survey of the European experience 1985 - 2000

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Blood (print ISSN 0006-4971, online ISSN 1528-0020), is published weekly by the American Society of Hematology, 2021 L St, NW, Suite 900, Washington DC 20036.

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**TREATMENT OF CHRONIC GRANULOMATOUS DISEASE
WITH MYELOABLATIVE CONDITIONING
AND AN UNMODIFIED HEMOPOIETIC ALLOGRAFT:
A SURVEY OF THE EUROPEAN EXPERIENCE 1985 - 2000**

Short title: BMT FOR CHRONIC GRANULOMATOUS DISEASE

Section Heading: CLINICAL OBSERVATIONS, INTERVENTION AND THERAPEUTIC TRIALS

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Total text word count: 3534

Abstract word count: 232

ABSTRACT

Treatment of chronic granulomatous disease (CGD) with myeloablative bone marrow transplantation is considered risky. This study investigated complications and survival according to different risk factors present at transplantation. The outcomes of 27 transplantations for CGD, from 1985 to 2000, reported to the European Bone Marrow Transplant Registry for primary immunodeficiencies were assessed. Most transplant recipients were children (n=25), received a myeloablative busulphan-based regimen (n=23) and had unmodified marrow allografts (n=23) from HLA-identical sibling donors (n=25). After myeloablative conditioning all patients fully engrafted with donor cells, after myelosuppressive regimens 2 out of 4 patients. Severe (grades III or IV) GvHD disease developed in 4 patients: 3 of 9 with preexisting overt infection, 1 of 2 with acute inflammatory disease. Exacerbation of infection during aplasia was observed in 3 patients, inflammatory flare at the infection site during neutrophil engraftment in 2: all 5 patients belonging to the subgroup of 9 with preexisting infection. Overall survival was 23/27, with 22/23 cured of CGD (median follow up 2 years). Survival was especially good in patients without infection at the moment of transplantation (18/18). Preexisting infections and inflammatory lesions have cleared in all survivors (except in one with autologous reconstitution). Myeloablative conditioning followed by transplantation of unmodified hemopoietic stem cells is a valid therapeutic option for children with CGD having an HLA-identical donor, if performed at the first signs of a severe course of the disease. rseger@kispi.unizh.ch

INTRODUCTION

Chronic granulomatous disease (CGD) is an inherited disorder of phagocyte function, characterised by recurrent, often life-threatening bacterial and fungal infections and by granuloma formation in vital organs. Neutrophils, monocytes/macrophages and eosinophils cannot generate microbicidal oxygen metabolites due to a defect in one of the four subunits of the NADPH oxidase of phagocytes (gp91 phox, p47 phox, p67 phox and p22 phox). Conventional treatment consists of lifelong antiinfectious prophylaxis with antibiotics such as cotrimoxazole (1), antimycotics such as itraconazole (2) and/or with interferon gamma (3). Despite these measures the annual mortality is still between 2 (autosomal recessive CGD) to 5 (x-linked CGD) percent (4). Therefore there is a need for more effective therapies. The alternative to conventional treatment, hemopoietic stem cell transplantation (HSCT), considered to carry a high risk of complications and death, is usually postponed until the patient is chronically ill.

In this retrospective study, 27 patients (25 children, 2 adults) with CGD underwent transplantation with an unmodified hemopoietic allograft from an HLA-identical donor. Transplant-associated complications and survival were analysed according to the presence or absence of risk factors at transplantation, e.g. therapy-refractory infection, acute inflammation, sequelae due to chronic inflammation.

PATIENTS, MATERIALS AND METHODS

Data collection

Between 1985 and 2000, 27 patients received a hemopoetic stem cell transplant (HSCT) for CGD in 14 cooperating centres in Europe. Data of the allografts performed during this period were reported to the HSCT Registry for primary immunodeficiencies of the Working Party on Inborn Errors of the European Group for Blood and Marrow Transplantation (EBMT) and the European Society for Immunodeficiencies (ESID). A retrospective analysis was made of data reported up to March 2001, with a follow-up period of 4 months to 12 years (median of 2 years). Data of 7 patients were published previously (5-11).

Patients

The characteristics of the 27 patients (23 male, 4 female; 25 children, 2 adults) are shown in tables 1, 2 and 3.

CGD was confirmed by the absence of NADPH oxidase activity in stimulated neutrophils by one or more of the following tests: nitroblue tetrazolium (NBT) reduction, dihydrorhodamine oxidation, chemiluminescence, superoxide generation. Twenty-two patients had x-CGD (by identification of a carrier-mother and/or by gp91 phox mutation analysis), 2 autosomal recessive CGD (one p47 phox deficiency, one p22 phox deficiency), 2 were uncharacterised and one was an x-CGD carrier with extreme Lyonisation (patient 4).

All 27 patients had had at least one invasive infection of lung, liver, blood or bone, requiring intravenous antibiotic therapy. Nine out of 27 patients had culture-proven, therapy-refractory, life-threatening infections (8 fungal, one mycobacterial) and received iv antibiotics as well as granulocyte transfusions (7 out of 9) at the time of transplantation (table 1). Eighteen out of 27 patients were free of infection at HSCT. Seven of the 18 patients without overt infection had signs of active ongoing inflammation (colitis) or organ sequelae probably due to chronic inflammation (pulmonary restriction) (table 2), the remaining 11 had no inflammation (table 3). The patients or their legal guardians gave informed consent for stem cell transplantation and collection of data. The informed consent process included advice on the beneficial effects of conventional antibacterial/antifungal prophylaxis/treatment and on the risks of allografting especially in the presence of overt infection or inflammation.

Table 1: HSCT for CGD with therapy-refractory infection

Patient No. (Ref.)	CGD Type	Age (yr)	Sex	Sex of Donor	Marrow MNC Infused/kg ($\times 10^8$)	Conditioning	Risk-factors	Outcome	Myeloid Engraftment (% donor cells)
1	X-CGD	11.5	M	F	1.7	Bu, Cy	Diss. Aspergillosis (lung, heart, bone)	Multiorgan failure (died d+9)	(none)
2	X-CGD	17	M	F	3.5	Bu, Cy	Multifocal Aspergillosis (lung, CNS)	Nodular Pneumonia Skin GvHD IV (died+90)	2 mo (full)
3	X-CGD	20	M	F	4.3	Bu Melphalan Campath	Multifocal fungal infection + (lung, para-spinal)	Interstitial pneumonia Tracheostomy bleeding (died + 73)	2 mo (full)
4	X-CGD carrier	6.8	F	M	5**	Bu 8 Flu 180 ATG	Aspergillosis (lung)	Non-engraftment Asp. Pneumonia VOD (died + 46)	(none)
5	gp91 ^{phox}	5.9	M	M	8****	Cy 120 Flu 125 ATG	Mycobacteriosis ++ (lung, nodes)	Non-engraftment Autologous reconstitution (alive)	(none)
6 (5)	gp91 ^{phox}	8	M	F*	4	Bu, Cy	Multifocal Aspergillosis (lung, rib, psoas)	Alive and well	5 yr (full)
7	p22 ^{phox}	14.4	F	M	3.5	Bu, Cy	Multifocal Aspergillosis (lung, CNS)	Asp. pneumonia Skin GvHD IV → chronic 4 yrs → resolved Alive and well	4 yr (full)
8 (6)	gp91 ^{phox}	3	M	M	PBSC	Bu, Cy ATG	Aspergillosis (lung, rib)	Skin GvHD III → chronic → resolved Alive and well	1.8 yr (full)
9	X-CGD	16.8	M	F	2	Bu 16 Flu 200 ATG	Fungal-Gastritis +++	Alive and well	1 yr (full)

* heterozygous carrier of X-CGD	Abbreviations:	
** 0.9x10 ⁶ CD34 cells/kg	Asp. = Aspergillus	GvHD = Graft-versus-host-disease
*** 11x10 ⁶ CD34 cells/kg	ATG = Antithymocyte globulin	MNC = Mononuclear cells
**** non-myeloablative	Bu = Busulphan	PBSC = Peripheral blood stem cells
+ Scediosporum apiospermum	Cy = Cyclophosphamid	Ref. = Reference
++ Mycobacterium gordonae	Flu = Fludarabine	VOD = Venooclusive disease
+++ Ustilago species		

Table 2: HSCT for CGD with active inflammation/inflammatory sequelae

Patient No. (Ref.)	CGD Type	Age (yr)	Sex	Sex of Donor	Marrow MNC Infused/kg ($\times 10^8$)	Conditioning	Risk factors	Outcome	Myeloid Engraftment (% donor cells)
10	gp91 ^{phox}	17	M	F*	3.2	Bu, Cy	Colitis Pulm. restriction (FVC 60%)	Colitis resolved, GvHD IV → chronic → resolving	1.3 yr (full)
11	gp91 ^{phox}	4.3	M	M	1.9	Bu, Cy	<u>Colitis, steroid-dependent</u> Previous Aspergillus-Pneumonia	Colitis resolved Alive and well	1.2 yr (full)
12 (7)	gp91 ^{phox}	11	M	F*	4.7	Bu 13, Cy	Pulm. restriction (FVC 34%, O ₂ -dependent)	Restriction ↓ (FVC 72%, no O ₂) Alive and well	12 yr (full)
13 (8)	X-CGD	6	M	F	PBSC	Bu 8 ** Flu 240 ATG	Pulm. restriction (FVC 28%, Sa O ₂ 75%, clubbed) + Lung cysts	Lung improved (Sa O ₂ 87%) Skin GvHD II Alive + well	4 yr (full, after DLI)
14	p47 ^{phox}	5	F	F	1.1	Bu, Cy	Pulm. restriction (DLCO 40%, O ₂ -dependent, clubbed)	Restriction ↓ (DLCO 76%, no O ₂ , clubbing ↓) Catch-up-growth Alive and well	3 yr (full)
15	gp91 ^{phox}	4.4	M	F	5.9	Bu, Cy	Pulm. restriction (clubbed) + Local bronchiectases	Restriction ↓ (clubbing ↓) Catch-up-growth Skin GvHD II Alive and well	1.5 yr (88%)
16	X-CGD	38.7	M	M	PBSC	TBI 200 ** Flu 180	Pulm. restriction (FVC 38%, O ₂ -dependent, clubbed, wheel chair) +Bronchiectases	Lung improved (no O ₂ , out of chair) Alive and well	1.6 yr (full)
							Abbreviations: see table 2 DLI = Donor lymphocyte infusion TBI = Total body irradiation		
* heterozygous carrier of X-CGD									
** non-myeloablative									

Table 3: HSCT for CGD with no overt infection/inflammation

Patient No.	CGD Type	Age (yr)	Sex	Sex of Donor	Marrow MNC Infused/kg (x10 ⁸)	Conditioning	Risk-factors	Outcome	Myeloid Engraftment (% donor cells)
17-27 (9-11)	5 x X-CGD 4 x gp91 ^{phox} 2 x unknown	0.8-14	10x M 1x F	8x F* 3x M**	2.1-9.5	9x Bu, Cy 1x Bu, Cy, TNI 1x Bu, Cy, TT, ATG	7x none 4x Asp. pneumonia (history)	1x GvHD II All alive and well	0.3-4 yr (all full)
median		7			4.7				2 yr
* 2 heterozygous carriers of X-CGD ** 2 matched unrelated donors (one MLC negative, one molecularly matched)						Abbreviations: see table 3 TNI = Total node irradiation TT = Thiohepa			

Transplantation

Donor and recipient HLA-matching was confirmed by serotyping and/or molecular typing of the HLA class I and II loci, respectively. Twenty-five of the 27 patients were transplanted from a sibling with HLA-identical genotype (5 of the 25 donors being heterozygous carriers for CGD). Only 2 patients (with no overt infection or inflammation) received an HLA phenotypically identical graft from an unrelated donor (patients 19 and 25).

The majority of patients (23 out of 27) received a busulphan-based myeloablative conditioning regimen, mostly combined with cyclophosphamide (21 out of 27). Busulphan (Bu) was used at a total dose of 16 mg/kg or 20 mg/kg (in children below 5 years of age) at days -9 to -6 before transplantation and cyclophosphamide (Cy) at a total dose of 200 mg/kg at days -5 to -2. Only in 4 debilitated patients, unable to tolerate a Bu/Cy regimen, lower-intensity conditioning regimens were applied: One patient with active therapy-refractory infection (patient 4) and 2 patients with very compromised lung function (patient 13 and patient 16) were conditioned according to published myelosuppressive protocols (using either low-dose busulphan (8mg/kg;12) or low-dose total body irradiation (200 cGy;13)), another patient with active infection (patient 5) according to an immunosuppressive protocol (using cyclophosphamide (120mg/kg), fludarabine (125mg/m²) and antithymocyte globulin;14).

Twenty-four of the 27 patients received full, T-cell replete marrow grafts with a median cell dose of 4.3×10^8 /kg mononucleated cells (MNC) (range 1.1 to 9.5×10^8 /kg). Three patients with a lower intensity conditioning regimen obtained G-CSF-stimulated, T-cell-replete peripheral blood stem cells (PBSC) with cell doses of 17.6×10^8 MNC/kg (patient 16), 27×10^8 MNC/kg (patient 8), and 27.8×10^8 MNC/kg (patient 13), respectively. As prophylaxis for graft-versus-host disease (GvHD) all patients received cyclosporine A, 13 short-term methotrexate and 4 prednisone. All patients were nursed in a high-efficiency, particulate air-filtered protected environment and 24 were given oral gut decontamination. In addition all patients received intravenous immunoglobulin therapy (except two) and *Pneumocystis carinii* prophylaxis by co-trimoxazole after HSCT (except one given pentamidine). Chimerism was studied by karyotyping and/or analysis of informative microsatellite DNA sequences. The presence of oxidase-positive neutrophils was detected by cytochemical nitroblue tetrazolium (NBT) tests and/or by flow cytometry with the use of a dihydrorhodamine oxidation assay.

Disease-free survival was defined as survival with adequate neutrophil killing function, reflected by 1. clearing of preexisting (fungal) infection and/or preexisting (pulmonary or intestinal) inflammation, 2. absence of new bacterial or fungal infection after withdrawal of antibiotic prophylaxis and 3. demonstration of neutrophils with an NADPH-oxidase activity similar to the level of the respective stem cell donor.

RESULTS

Engraftment

Full donor-derived hemopoietic chimerism was observed in 22 out of 23 patients who received an HLA-identical unmodified stem cell graft (bone marrow 22, PBSC 1) after myeloablative busulphan-based conditioning (one patient (#1) died at d+9 and could not be evaluated). Hematopoietic recovery in this group occurred with a median time to neutrophil count $>500/\text{ul}$ of 18.5 days (range 9 to 40 days). Donor-derived hematopoiesis was stable with a median follow-up time of 2 years (range 0,3 to 12 years). In 4 patients, who received an HLA identical unmodified stem cell graft after lower intensity conditioning (bone marrow 2, PBSC 2), full donor-derived hematopoiesis was achieved in only 2 patients (in # 13 only at 9 months after a donor lymphocyte infusion), while the other two patients did not engraft. One patient (#4) received a very low CD34-cell number ($0.9 \times 10^6/\text{kg}$) in a T-cell replete graft and died of aspergillosis despite a stem cell boost and peritransplant granulocyte transfusions. The other patient (#5) received an adequate CD34-cell number ($11 \times 10^6/\text{kg}$) in a T-cell replete graft after an immunosuppressive conditioning protocol (Cy 120 mg/kg, Fludarabine 125 mg/m², antithymocyte globulin (ATG)), but developed autologous reconstitution. He had not received granulocyte transfusions.

Neutrophil function

An NADPH-oxidase activity in the donor range could be documented in all 24 patients with donor-derived hematopoiesis. In the 5 patients transplanted with heterozygous carriers of x-CGD a mosaicism of oxidase-active/non-active neutrophils was demonstrated. The degree of Lyonisation of the x-linked gp91-phox gene in the recipient was identical to the one in the carrier donor in all 5 cases (results not shown).

Clinical outcome and adverse events

As a result of the development of oxidase-activity, therapy-refractory preexisting infections (3 episodes of life-threatening aspergillosis, one episode of severe gastritis due to *Ustilago*), were eradicated in 4 out of 4 evaluable patients. In patient 6 healing of a fistula over the rib and normalisation of C-reactive protein was observed within the first month after HSCT; resolution of active (hypermetabolic) lesions was complete after 3 months as evidenced by repeat whole-body positron-emission-tomography (PET) using F18-fluorodeoxyglucose (FDG) (fig. 1). An exacerbation of the preexisting *Aspergillus pneumonia* during aplasia was seen in 3 out of 9 patients (# 1, 4 and 7) and a diffuse inflammatory pulmonary reaction at the time of neutrophil engraftment in 2 out of 6 evaluable patients (# 2 and 3). In 4 out of the 5 patients the pneumonia was bilateral, progressed to white lungs, required ventilation and was not survived. Lung biopsies were considered too invasive to be performed.

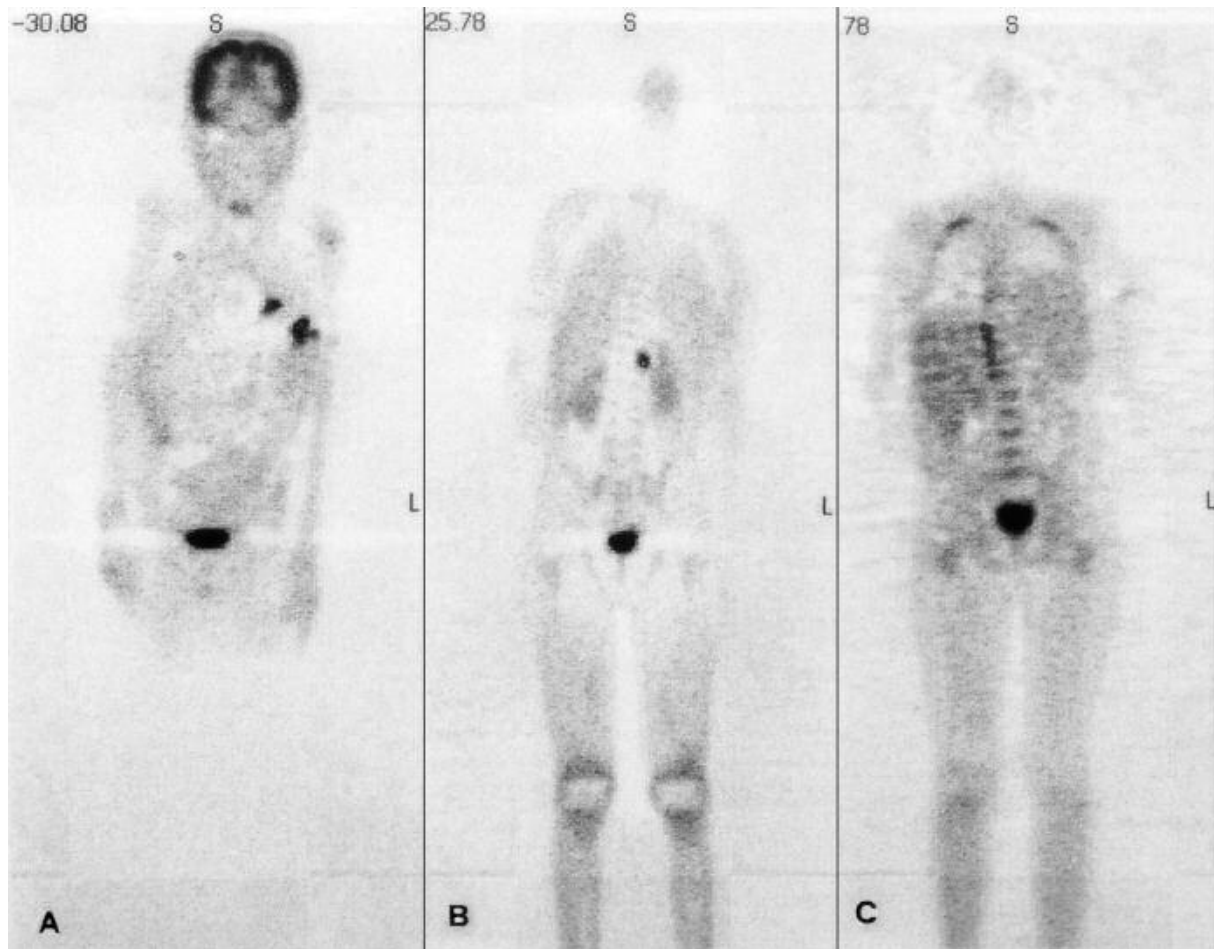


Figure 1: Coronal emission fluorodeoxyglucose-positron emission tomography (FDG-PET) before (A and B) and 3 months after HSCT (C) in patient 6 with multifocal aspergillosis. PET shows multiple lesions in the lungs and a focus in the upper left psoas with intense FDG uptake before HSCT, which have disappeared 3 months after HSCT. Physiologic high FDG uptake is seen in the brain and bladder.

The development of oxidase-activity also led to the resolution of serious preexisting inflammatory disease and the improvement of inflammatory sequelae. Two out of 2 patients with severe biopsy-proven granulomatous colitis (one steroid-dependent) lost their symptoms within 2 months of HSCT and had no recurrence during a follow-up period of 1.2 and 1.3 years respectively. Surprisingly, improvement of preexisting pulmonary inflammatory sequelae was also seen, albeit at a slower pace. Before HSCT 2 patients were carefully documented to have severe pulmonary restriction as evidenced by a forced-vital-capacity (FVC) of 34 % (patient 12) and a diffusing capacity for CO (DLCO) of 40 % (patient 14) as well as decreased oxygen saturations (SaO₂ of 83 % (patient 12) and of 85 % (patient 14). After HSCT there was normalisation of oxygen transport within 1 year and near normalisation of lung function within 2½ years (fig. 2).

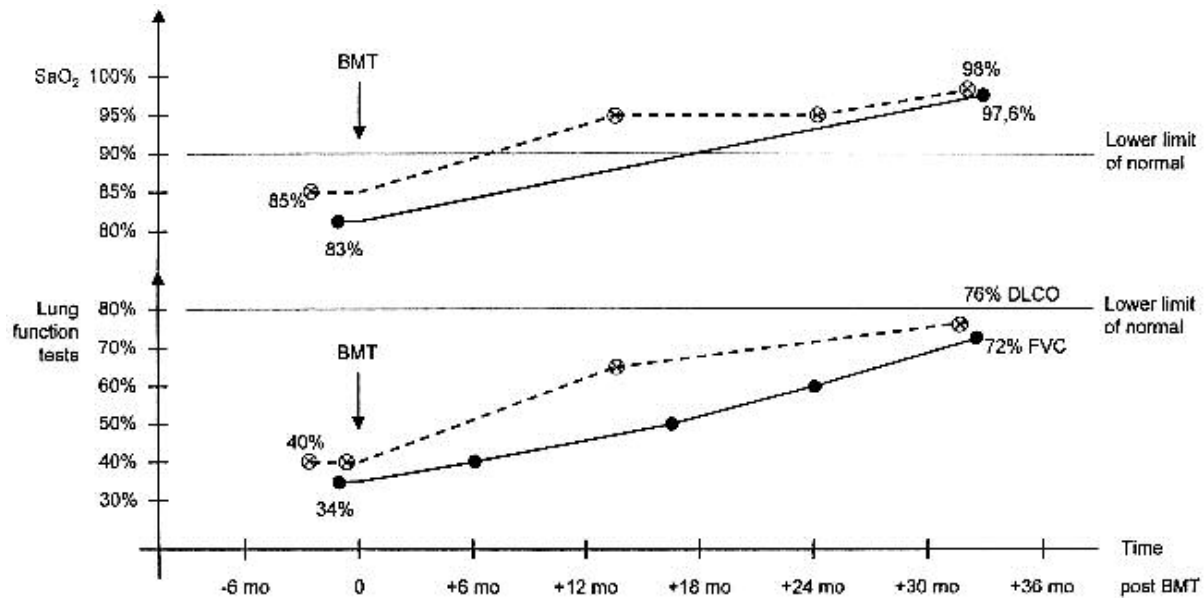


Figure 2: Oxygen saturation (SaO₂) and pulmonary function test results before and after HSCT in patients 12 and 14 with severe pulmonary restriction. DLCO = Diffusing capacity for CO, FVC = Forced vital capacity.

Altogether improvement of pulmonary restriction was seen in 5 of 5 evaluable patients with reversal of oxygen-dependence in 3 out of 3, disappearance of clubbing in 2 out of 4 and a wheelchair becoming unnecessary in 1 out of 1. Preexisting bronchiectasis and lung cysts, however, persisted on repeated CT examinations. Catch-up-growth was documented in 2 patients with resolving pulmonary restriction aged 4.4 and 5 years (fig. 3), respectively.

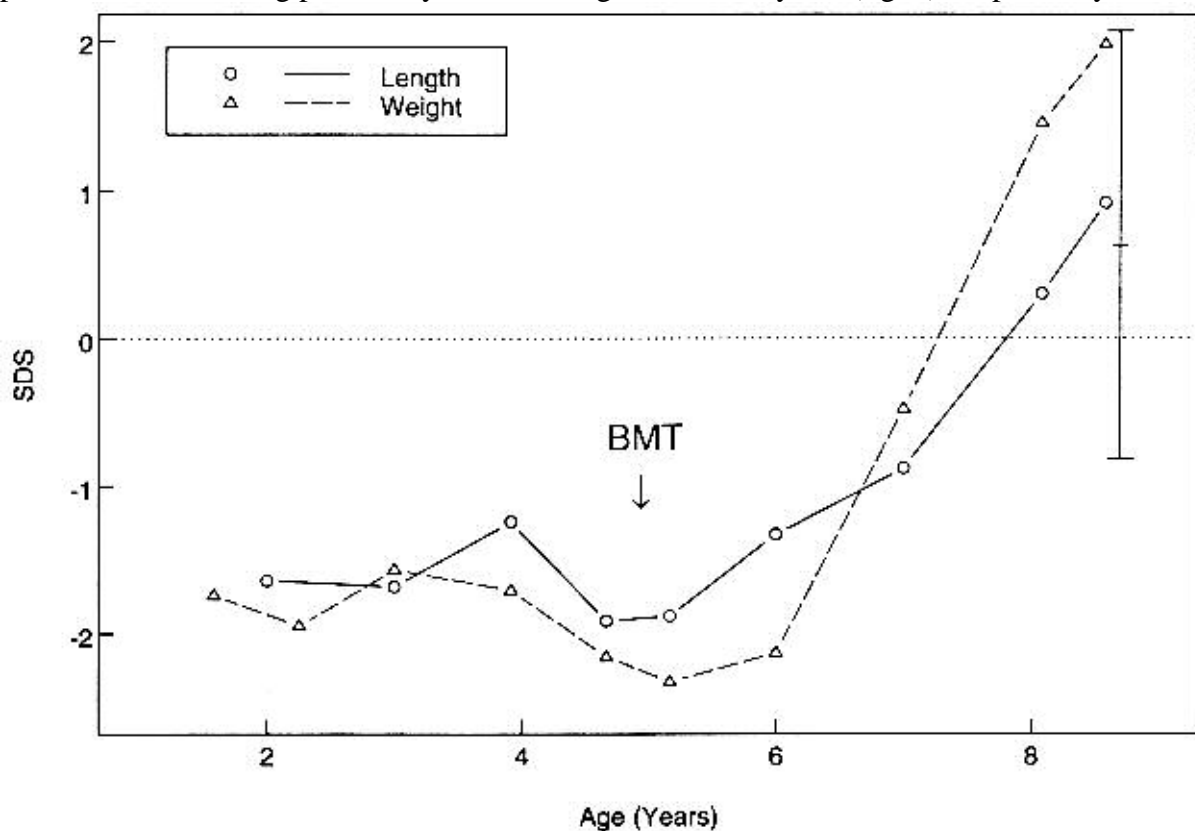


Figure 3: Growth before and after HSCT of patient 14 with severe pulmonary restriction. Standard deviation scores of weight and length are shown and compared with target height.

Graft-versus-Host Disease

Acute GvHD grade II-IV occurred in 7 of 27 patients, chronic GvHD in 3 of 27 patients (limited in 2, extensive in 1). Acute GvHD of grades III or IV occurred in four patients, one child aged 3 and 3 adolescents aged 14, 17 and 17 years, respectively. Of these, patients 2, 7 and 8 with life-threatening aspergillosis developed severe GvHD of the skin only, resembling Lyell-syndrome. In the 2 survivors GvHD became chronic and slowly resolved under prolonged immunosuppressive therapy. Patient 10 had overt granulomatous colitis and developed severe GvHD of gut, liver and skin. Again chronic GvHD developed and has gradually responded to treatment. In 16 patients without overt infection or active inflammation only three cases of acute skin GvHD grade II developed in children aged 1.2, 4.4 and 6 years respectively.

Survival

Four out of 27 transplanted CGD patients (15 %) died, all in the group of patients with preexisting therapy-refractory fungal infections. The causes of death were progression of aspergillosis before engraftment in 2 (patients 1 and 4), coincidence of inflammatory pulmonary reaction and skin GvHD IV at neutrophil engraftment in 1 (patient 2) and uncontrolled hemorrhage through an eroded carotid artery in 1 (patient 3 with tracheostomy). In 1 out of 23 surviving patients CGD persists. This child did not engraft after an immunosuppressive conditioning protocol and had autologous reconstitution (patient 5). In 22 of the 23 surviving patients CGD is cured (81 % of all transplanted patients). 19 out of 22 cured patients are alive and well at last follow-up (median 2 years, range 0.3-12 years), in 3 of the 22 cured patients quality of life has improved to normal activity without special care (2 with residual pulmonary sequelae of the lung and Karnofsky performance scores of 80 and 90 (patients 13 and 16), respectively, 1 with resolving GvHD grade IV and a score of 80 (patient 7)).

DISCUSSION

Despite data to show that good preventive treatment improves survival and quality of life in childhood (15) CGD is still associated with high morbidity and mortality (4). In spite of itraconazole and γ -interferon prophylaxis, which have been reported to reduce the number of *Aspergillus* infections, invasive aspergillosis is now by far the commonest cause of death in CGD, accounting for one third of the mortality (4). Other invasive infections and inflammatory sequelae are not uncommon. Widespread granuloma formation in vital organs results in granulomatous colitis, lung fibrosis and cor pulmonale (16). Patients surviving multiple *Aspergillus*-pneumonias almost inevitably develop restrictive lung disease. Currently, there are no means to change this course.

Although overt infections refractory to antimicrobial treatment (e.g. multifocal aspergillosis), acute inflammatory disease dependent on high dose steroid therapy (e.g. colitis) and inflammatory sequelae (e.g. severe pulmonary restriction with oxygen dependence) may seem absolute contraindications to HSCT, this view must be as challenged: In our study a total of 16 CGD patients with these risk factors were transplanted, in addition to 11 patients without such risks. Despite this selective enrolment 81 % of all transplanted patients (22 of 27) are now cured of CGD and are alive and well (19 of 22) or have improved quality of life (3 of 22). Infections and inflammatory lesions in these patients have all cleared. The four deaths occurred only in the group of patients with pre-existing fungal infections refractory to all conventional treatments. We can therefore conclude that HLA-genotypical HSCT, hitherto reported only in individual CGD-patients (6-11, 17-22), is a valid alternative to conventional treatment.

In order to achieve maximal engraftment we have (with the exception of 4 debilitated patients) exclusively used myeloablative regimens, mostly Bu 16 mg/kg, Cy 200mg/kg and have refrained from T-cell depletion of the HLA-identical grafts. This technique has resulted in full and stable engraftment of donor-derived hemopoiesis after a median of 18,5 days in all 22 evaluable cases. This contrasts with the results of a recent NIH-trial, using an immunosuppressive, non-myeloablative conditioning followed by a T-cell depleted HLA-genotypical HSCT in 10 CGD patients without active infection (14). This procedure resulted in 2 cases of non-engraftment and necessitated donor lymphocyte infusions (DLIs) in all cases in order to convert to a more favorable donor chimerism. DLIs provoked GvHD in 3 patients, two of grade II, one of grade IV (resulting in infectious death). Use of a T-cell depleted graft was probably the main reason for the relatively high rejection rate utilising the NIH approach. However, use of a T-cell replete graft in patient 5 of our study, with an otherwise identical approach, did not prevent non-engraftment. For the present we would therefore favour an unmodified transplant and a more myelosuppressive conditioning. The classical myeloablative protocol provides excellent disease-free survival and quality of life. Fears of secondary tumours following a simple Bu/Cy course for conditioning remain theoretical and are not substantiated in our European registry of more than 1000 transplants for immunodeficiency diseases since 1975 (unpublished results). Despite the good outcome for the 18 non-infected, low risk patients reported in this study, myeloablative conditioning still has several disadvantages compared with low-intensity regimens, e.g. greater propensity to tissue injury, longer periods of neutropenia and risk of permanent gonadal failure. Further search for an ideal low-intensity conditioning for transplantation of a non-malignant disease such as CGD is warranted and would benefit also the severely debilitated patients with active infection or pulmonary restriction who cannot tolerate a myeloablative regimen.

Most practitioners postpone HSCT until the CGD patient is chronically ill. We have transplanted 11 patients after recovery from one or more invasive infections of lung (including *Aspergillus pneumonia*), liver, blood and/or bone before the manifestation of chronic illness. The transplant course was uneventful, without exacerbation of any occult infection, inflammatory reaction or severe GvHD. All 11 transplanted children are now cured from their CGD. The absence of transplantation-related deaths in this limited series is comparable to the excellent results in other non-malignant hematologic disorders such as thalassemia (with a disease-free survival of up to 91%).

HLA-genoidetical HSCT in patients with active inflammation or organ disability due to chronic inflammation is also feasible with excellent survival and increased quality of life. We were surprised by the gradual, but marked regression of pulmonary restriction, previously considered to be irreversible due to lung fibrosis. Although comparative biopsy specimens before and after HSCT were not available, it seems probable that restriction is reversible, because it is mainly caused by cellular infiltrates and granulomas that regress after HSCT. Such infiltrates may be the result of occult smoldering infections, overcome by the new phagocyte system with a normal microbial killing capacity. Alternatively the infiltrates may be sterile and the result of an exaggerated inflammatory response directed against undigested microbial material (23), again overcome by the normal donor-derived phagocytes. Gross anatomic destruction e.g. bronchiectases (patient 16) and lung cysts (patient 13), remained unchanged after HSCT. Active inflammatory disease, e.g. biopsy-proven granulomatous colitis, also responded well to HSCT, on a short term basis probably because of the massive immunosuppression by the conditioning regimen (e.g. by cyclophosphamide) and the GvH-prophylaxis (e.g. cyclosporine A) and on a long term basis probably because of the new donor-derived immune system. Severe GvHD remains a risk, possibly because of TNF α -levels raised in granulomatous colitis (24). Optimal GvHD prevention is thus imperative.

HSCT can even be successful in active infection refractory to conventional treatment, but is more risky. We have encountered two serious complications. One is a severe form of GvHD curiously limited to the skin and resembling Lyell-syndrome. Since TNF α is a critical mediator involved in the induction as well as in the effector phase of acute GvHD (25), raised TNF α -levels in aspergillosis and the propensity of CGD-phagocytes to increased TNF α -production might be responsible for this phenomenon (23). The severe skin GvHD is responsive to immunosuppression by steroids and ATG, but can become chronic before it finally resolves. TNF-antagonist therapy may be beneficial if given as early treatment for GvHD, but one would have to proceed cautiously in order not to compromise the anti-*Aspergillus* effect of granulocyte transfusions given during the aplasia period (26). The second complication encountered during transplantation of CGD patients with active infection is a severe inflammatory reaction at the infected site at the moment of neutrophil engraftment, manifesting as "white lungs". Again raised TNF α -levels may be responsible, resulting in neutrophil extravasation and stimulation (27). This remains speculative, since no biopsy specimens were available to prove this sequence of events.

In future transplantations the considerable risks of HLA-genoidetical HSCT in CGD patients with therapy-refractory fungal infections may be reduced by three precautions: First, all infectious foci must be detected and treated before and during HSCT. CT and combined PET/CT scans will reveal infectious foci (28) which should then be biopsied and cultured for identification of the organism(s) and resistance testing. Antimicrobials with intracellular action have to be combined with granulocyte transfusions, preferably G-CSF primed, since this protects the collected cells against apoptosis and prolongs their half life (5). Second, GVHD has to be prevented by increased immunosuppression, e.g. by a cyclosporine/short term methotrexate/steroid (1 mg/kg/day) regimen. New approaches might include the administration of TNF α -antagonists neutralising circulating TNF α or a selective allo-T-cell-

depletion of the graft (29). Finally inflammatory reactions post-HSCT should be dampened by omitting G-CSF in the recipient and administering heavy immunosuppression (e.g. high dose steroids), if needed.

The time of transplantation is of critical importance. Patients with completely absent NADPH-oxidase activity may follow different clinical courses for reasons not yet fully understood. Some reasons are probably genetic with polymorphisms of host defense molecules and proinflammatory cytokines acting as additional risk factors (16). Some are psychosocial, e.g. involving the availability and adequacy of medical care and the daily compliance with life-long antibiotic prophylaxis, even in periods of well-being, holidays and puberty. If transplantation is delayed to adolescence and beyond, the chances of invasive fungal infections and of inflammatory sequelae increase (30) as does the probability of GvHD. Therefore patients with CGD who have an HLA-identical sibling and a history of recurrent invasive infections and/or inflammatory, steroid-dependent disease and/or inadequate medical care/compliance with antibiotic prophylaxis should be considered prime candidates for HSCT, before irreversible organ damage occurs. Patients with therapy-refractory infections or organ disability due to chronic inflammation may still be eligible, but run a higher risk of complications especially of GvHD. Transplantations other than with perfectly matched donors are presently discouraged.

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