Long-term follow-up of autologous hematopoietic stem cell transplantation for severe refractory Crohn's disease

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Received 24 December 2010; received in revised form 12 May 2011; accepted 12 May 2011

Abstract

Background: Although new therapeutic strategies have been developed to control Crohn’s disease, medical treatment for refractory cases is not able to prevent extensive and/or repeat surgery. Recently, several cases have been reported of successful remission induction in Crohn’s disease patients by means of hematopoietic stem cell transplantation (HSCT). Here we report our long-term (4 to 6 years) outcome in three patients.

Patients: Three patients (two male, one female) with active severe Crohn’s disease were planned to undergo autologous HSCT. All patients were intolerant or refractory to conventional therapies, including anti-TNFα antibodies. Patients either refused surgery or surgery was considered not to be a feasible alternative due to the extensive disease involvement of the small intestine.

Methods: Peripheral blood stem cells were mobilized using a single infusion of cyclophosphamide 4 g/m², followed on day 4 by subcutaneous injections with G-CSF 5 μg/kg twice daily until

Abbreviations: ATG, antithymocyte globulin; Crohn’s disease, DMSO dimethyl sulfoxide; G-CSF, granulocyte colony-stimulating factor; HSCT, hematopoietic stem cell transplantation; IBD, inflammatory bowel disease; TNFα, anti-tumor necrosis factor-α; RBC, red blood cells.

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doi:10.1016/j.crohns.2011.05.004
1. Introduction

Crohn's disease is an inflammatory disorder of potentially any part of the gastrointestinal tract, leading to various intestinal but also extra-intestinal symptoms. It is thought that dysregulation of the normally controlled immune response to commensal bacteria in genetically susceptible patients drives the disease.1 Although medical therapy of inflammatory bowel disease (IBD) has improved due to an extensive repertoire of immunosuppressive drugs and the introduction of anti-tumor necrosis factor-α (TNFα) compounds, the clinical course of the disease cannot be adequately controlled in a substantial group of patients. These patients may not respond or may become refractory to their medication, or they may develop treatment limiting toxicities.2 The course of the disease is chronic and intermittent and the disease responds less well to medical intervention over time. Half of the patients require surgical resection during the course of the disease.3 Unfortunately, surgery is accompanied by a high recurrence rate and many patients are at risk to develop short bowel syndrome. Therefore, there is an unmet need for more effective therapeutic strategies.

Evidence that hematopoietic stem cell transplantation (HSCT) would be an effective treatment for autoimmune diseases firstly came from several animal models4,5 and from incidental case reports on HSCT recipients with coexistent autoimmune disease.6 Already more than 15 years ago a case report on the positive effect of HSCT on active IBD was published, followed by many others showing improvement of the clinical course of disease after HSCT indicated for other (malignant) diseases.7 In the last couple of years, several cases have been reported of successful remission induction of patients with (therapy refractory) Crohn's disease by means of an autologous HSCT. The most extensive experience on autologous HSCT applied specifically for Crohn's disease has been reported by Oyama and colleagues.8,9 All twelve treated patients went into remission with a Crohn's Disease Activity Index (CDAI) score of <150. There was no inpatient mortality and neutropenic fever was the most important complication. Recently published long-term follow-up data showed a clinical relapse-free survival of 63% at 3 years and 36% at 5 years. The percentage of patients in remission (CDAI<150), steroid free, or medication free at any post transplant evaluation interval over 5 years post transplant was 70%, 80% and 60%, respectively.8

Supported by these reports we have mobilized stem cells in three patients with severe refractory Crohn's disease, for whom surgery was not a feasible option due to extensive disease activity, and performed autologous HSCT in two of these patients. Here, we report on the follow-up period of four to six years.

2. Materials and methods

2.1. Patient selection

Three patients with severe refractory Crohn’s disease were selected for an autologous HSCT procedure. The considerations for selection included longstanding refractory disease and exhaustion of the full range of therapeutic strategies including anti-TNFα antibodies. In addition, surgery was unattractive because of unwillingness of the patients and the likelihood of either developing short bowel syndrome (patient 1) or a definite ileostomy (patients 2 and 3). All cases were reviewed prior to selection by an independent gastroenterologist. The protocol was approved by the Medical Ethics Committee of the Academic Medical Center and all patients gave written informed consent.

2.2. HSCT protocol

2.2.1. Mobilization

Prior to cyclophosphamide treatment, semen was cryopreserved of the male patients. The female patient was offered treatment with the LHRH analagon gosereline, in an attempt to prevent chemotherapy-induced gonadal toxicity and premature ovarian failure.10 Peripheral blood stem cells were mobilized using a regimen consisting of a single intravenous dose of cyclophosphamide 4 g/m² followed on day 4 by granulocyte colony-stimulating factor (G-CSF) twice
daily, 5 μg/kg/day. Hyperhydration and mesna prophylaxis were given in order to prevent hemorrhagic cystitis. Partial bowel decontamination was obtained using oral ciprofloxacin and fluconazol. In addition, all patients received antibiotic prophylaxis consisting of clarithromycin. Peripheral blood stem cells (target >8 × 10^6 CD34+ cells/kg body weight) were collected by large volume leukapheresis using a COBE Spectra leukapheresis machine (COBE BCT, Lakewood CO) as soon as the white blood cell count was >2 × 10^9/l and CD34+ cells were detectable in the peripheral blood. CD34+ cells were isolated within 48 h after leukapheresis by magnetic cell sorting (CliniMACS CD34 selection procedure, Miltenyi Biotec, Bergisch Gladbach, Germany) according to the manufacturer’s instructions. The CD34+ cells were cryopreserved in dimethyl sulfoxide (DMSO) and stored in the vapor phase of liquid nitrogen until transplantation.

2.2.2. Conditioning
Prior to transplantation, immune ablation was achieved using an immunosuppressive conditioning regimen consisting of cyclophosphamide 50 mg/kg/day intravenously (four consecutive days starting on day −5, with hyperhydration and mesna prophylaxis) and equine antithymocyte globulin (Lymphoglobulin, hATG, Genzyme Corporation, Boston, MA, USA) at a dosage of 30 mg/kg/day (on days −4, −3, −2). Premedication with methylprednisolone at a dose of 500 mg/day was administered intravenously for three consecutive days from day 4 and prednisolone was subsequently tapered over a period of 14 days. Hematopoietic stem cells were thawed and infused intravenously on day 0.

2.2.3. Transplantation
Patients were hospitalized and nursed in clean rooms. Following transplantation, partial bowel decontamination was obtained using oral ciprofloxacin and fluconazol, and patients received antibiotic prophylaxis consisting of metronidazol and intravenous penicillin starting on day 5 until recovery of neutropenia. Supportive care (anti-emetics, broad spectrum antibiotics, parenteral nutrition and transfusions) were given when indicated. The hemoglobin level was maintained above 9.7 g/dl and platelet counts above 10 × 10^9/l. All blood products, excluding the peripheral blood progenitor cells, were irradiated prior to infusion.

2.3. Evaluation of clinical response
Clinical assessment during HSCT and follow-up included medical history, physical examination and standard biochemical tests (including CRP). Endoscopy and both barium small bowel enteroclysis and MRI enterography were performed at baseline and after 3 and 6 months where indicated.

3. Results
HSCT was offered to 3 patients (2 male, 1 female) with a median age of 28 (range 27–51). Patients were resistant or

| Table 1 | Basal demographic, medical history and clinical characteristics at time of. |
|----------|------------------|-------------------|
| Case     | 1                | 2                | 3                |
| Basal demographic and clinical characteristics | | | |
| Sex      | Male             | Male             | Female           |
| Year of birth | 1953           | 1978             | 1978             |
| Diagnosis | 2003            | 2004             | 1999             |
| Disease extension | Stomach, duodenum, jejunum, ileum | Colon including rectum | Colon including rectum |
| Montreal disease pattern | Stricturesing and penetrating | Penetrating | Penetrating |
| Extraintestinal disease manifestations | No              | Stomatitis       | Artralgia        |
| Risk factors | None            | Previous smoker  | Previous smoker  |
| Previous treatments | | | |
| 5-asa | Yes              | Yes              | Yes, intolerant  |
| Corticosteroids | Yes            | Yes              | Yes, intolerant  |
| Antibiotics | Yes            | Yes              | Yes              |
| Azathioprine | Yes             | Yes              | Yes, leucopenia  |
| Infliximab | Yes             | Yes              | Yes              |
| MTX | Yes              | Yes              | Yes              |
| Visilizumab | No              | Yes              | Yes              |
| Adalimumab | No              | No               | No               |
| Surgery | No              | No               | No               |
| HSCT | | | |
| Age at HSCT | 51              | 27               | 28               |
| Year of HSCT | 2004            | 2005             | 2006             |
| Crohn’s disease medication | | | |
| At time of mobilization | Prednisone 15 mg | Prednisone 30 mg | None |
| At time of transplantation | Prednisone 60 mg | Prednisone 40 mg | Not applicable |
intolerant to conventional medication, including anti-TNF-α therapy. All drugs, including steroids, were tapered after the mobilization procedure. Patient disease characteristics are described in Table 1.

3.1. Safety and efficacy of stem cell mobilization

In general, the stem cell mobilization procedure with cyclophosphamide and G-CSF was safe. Besides minor expected side-effects such as nausea which were successfully managed, there were two major complications: in patient 1 a transient deterioration of kidney function was observed due to decreased oral intake because of nausea, which responded rapidly to intravenous fluids and patient 2 had an episode of culture-negative neutropenic fever, which was empirically treated with broad-spectrum antibiotics. The blood cultures remained negative and the fever resolved promptly.

In patient 1, a sufficient number of stem cells were harvested (28.9 × 10^6 CD34+ cells/kg) in only one leukapheresis procedure. Despite a rather low recovery after CD34+ selection of 27%, a large enough amount of stem cells for reinfusion (7.87 × 10^6/kg) with a purity of 97% was obtained. In patient 2 and 3 however, a second stem cell mobilization procedure was required. In patient 2 the initial yield was sufficient (10.6 × 10^6 CD34+ cells/kg), but due to low recovery the yield after CD34 selection was borderline (2.5 × 10^6 CD34+ cells/kg). Therefore, a second mobilization procedure using only G-CSF 5 µg/kg twice daily for 5 days was performed two months later. The second mobilization yielded a total 2.1 × 10^6 CD34+ cells/kg, with 50% recovery after CD34 selection and a purity of 94%. In patient 3 the first attempt at stem cell mobilization with cyclophosphamide priming failed with a total yield after two leukapheresis procedures of 1.85 × 10^6 CD34+ cells/kg. Because of the low yield, CD34 selection was not performed. A second attempt at stem cell mobilization was done three months later using only G-CSF 5 µg/kg twice daily for 5 days. This resulted after two days in a yield of 5.84 × 10^6 CD34+ cells/kg, with a recovery of 53% and a purity of 89.7%. A third leukapheresis yielded 1.7 × 10^6 CD34+ cells/kg with a recovery of 66.5% and a purity of 79.7% after CD34 selection.

During the stem cell mobilization phase patient 1 required one transfusion of Red Blood Cells (RBC) (3 units), patient 2 required two transfusions (in total 7 units of RBC) and patient 3 received one platelet transfusion (pooled platelets from five donors).

3.2. Safety of hematopoietic stem cell pretreatment and transfusion

During the HSCT conditioning, patient 1 developed an allergic reaction to ATG (fever and hypotension), that resolved with fluid challenge and patient 2 had a quick and transient deterioration of kidney function due to hypotension after the first dose of ATG. In patients 1 and 2 respectively, a total of 5.9 × 10^6 and 3.5 × 10^6 CD34+ cells/kg were rein fused. The posttransplantation course was uneventful with an episode of rotavirus-associated diarrhea in both patients, Clostridium difficile-associated diarrhea and an episode of fever caused by a central venous catheter-associated coagulase-negative staphylococci (CNS) bacteremia in patient 2. Recovery of neutrophils to > 0.5 × 10^9/l was reached at day 12 and 14 in patient 1 and 2, and recovery of platelets > 50 × 10^9/l was seen at day 14 and 9 respectively. Patient 1 required transfusion with six units of RBC and 3 platelet transfusions; patient 2 required six units of RBC and 1 platelet pool. Both patients discontinued their immunosuppressive therapies post HSCT and regained normal appetite and oral intake.

3.3. Clinical evaluation of HSCT treatment and follow-up

At a median follow-up of 62 (58–75) months all patients are alive. The two patients (cases 1 and 2) who underwent the complete HSCT protocol are in clinical remission under immunosuppressive therapy. The patient (case 3) who underwent stem cell mobilization only, remained in clinical remission and off of all immunosuppressive drugs for more than 2 years. A detailed description of the follow-up on these patients is described below:

Case 1. Case 1 is a 51-year old male with diagnosed Crohn’s disease of the stomach and almost the entire small bowel, dependent on parenteral nutrition. This patient was offered autologous HSCT because he was unresponsive to immunosuppressive drugs (steroids in combination with azathioprine/methotrexate) and biologic therapy (infliximab). Surgery would have put him at great risk of developing a short bowel syndrome as the whole small intestine was affected by the disease. After mobilization, an initial clinical response was observed. However, the patient experienced a clinical and endoscopic relapse within 10 weeks. After immune ablation a total of 5.9 × 10^6/kg CD34+ cells/kg was rein fused and the patient could be discharged on day 15 after HSCT. In week 8 following transplantation, the patient was clinically in a partial remission, gaining weight (4 kg) without the need for supplementary parenteral nutrition.

Follow-up: At 8 weeks clear improvement of the inflammatory lesions was observed endoscopically (Fig. 1A). Also, barium enteroclysis (Fig. 1B) and MRI enterography (Fig. 1C) showed regression of small bowel stenosis. A sharp drop in CRP levels was seen after the mobilization phase, which further decreased during follow-up (Fig. 1D). Two months after transplant he unexpectedly developed leukopenia (1.6 × 10^9/l), which resolved spontaneously within 2 months. Six months post-transplant the patient developed abdominal pain and gastric ulcers were detected during gastroscopy. Biopsies did not reveal H. pylori, CMV, EBV or other infectious agents and the ulcers had vanished 2 months later. MRI performed at 10 weeks showed no improvement compared to the baseline MRI, which showed bowel wall thickening, stenosis and inflammation of the horizontal part of the duodenum and jejunum. At the MRI performed at 6 months persistence of stenotic segments of the...
Duodenum was seen. However, inflammatory activity evidently decreased and the stenosis in the jejunum disappeared (Fig. 1D). As expected, residual strictures in the duodenum persisted which were dilated three (1×), twelve (3×) and 36 (1×) months after transplantation.

Twelve months post-transplantation azathioprine was started (150 mg/day) and switched to certolizumab 2 years post-transplantation because of leukopenia and thrombocytopenia. Repeat gastroscopy with enteral balloon dilatation of his proximal strictures was last performed 3 years ago. In 2007, he was admitted for unexplained ascites. Extensive work-up excluded common causes including primary sclerosing cholangitis, portal hypertension, and hepatitis. The ascites resolved spontaneously without continued use of diuretics.

At present, the patient is doing relatively well. His weight has improved over the years and he now only sporadically vomits following meals. During his six years of follow up he did not have a luminal relapse of his Crohn’s disease and certolizumab treatment will be continued.

Case 2. The next case is a 27-year old male patient with a short but complicated history of refractory Crohn’s disease of the colon unresponsive to immuno-suppressive drugs (azathioprine and methotrexate), failing on infliximab and experimental anti-CD3 (visilizumab) therapy. In July of 2005 he underwent stem cell mobilization followed by HSCT in October of the same year. After HSCT the stool frequency dropped from bloody and watery >10/day to semisolid without blood 6–7 times daily before hospital discharge. Dramatic improvement of the colonic mucosa was observed during colonoscopy at 10 weeks. The ulcerated areas had regained epithelium and no active inflammation was seen.

Follow-up: Six months after HSCT 6-MP (mercaptopurine 100 mg) was started combined with infliximab. After having experienced no clinical complaints and having a high quality of life for 5 years, he relapsed with a mildly active distal colitis in the beginning of 2010. He was switched from infliximab to adalimumab, continuing 6-MP, and three months following his change of therapy was again in complete clinical remission, with normalized CRP levels.

Case 3. Our third patient (28 years of age) is a female with a similar history. She has a completely refractory (including failure of anti-TNFα therapy) Crohn’s disease of her colon complicated by arthritis and perianal abscesses, needing multiple hospital admissions yearly. Patient refused colectomy and was therefore planned to undergo HSCT. In this case, complete
clinical remission was achieved after the stem cell mobilization procedure. For this reason the stem cell transplantation itself was deferred.

Follow-up: The patient remained in complete clinical and endoscopic remission without any medication for two years and had an uncomplicated pregnancy 1.5 years after mobilization. In 2008, she relapsed with a severe active Crohn’s colitis for which she was treated with infliximab and methotrexate. After three infusions she experienced an allergic response to infliximab and, despite proper anti-allergic medication, a loss of response. Transplantation of the mobilized and stored CD34+ cells was proposed to the patient, which she declined because of cyclophosphamide associated toxicity. Consequently, she was included in a phase I trial on the safety and feasibility of autologous mesenchymal stromal cell infusion in refractory Crohn’s disease, for which conditioning is not needed.11 No response was achieved and currently she experiences persistent but tolerable activity with weekly adalimumab treatment in combination with steroids. As no medical therapeutic options are left we are now reconsidering colectomy.

4. Discussion

Our first experience of autologous HSCT for Crohn’s disease, employing similar protocols for mobilization, conditioning and supportive care as used in other published articles on HSCT for Crohn’s disease,9 is encouraging, as our patients have, since the onset of their disease, never been in remission for as long as after HSCT. The effect of HSCT was only partially successful in resolving Crohn’s lesions at endoscopy. There was no transplant-related mortality and adverse effects were manageable. In one patient, an unexplained prolonged leukopenia following transplantation was observed which occurred 2 months after initial neutrophil recovery and was not associated with infectious complications.

We did observe some difficulties with stem cell mobilization and CD34+ selection. In 2 of the 3 patients, the initial procedure yielded an insufficient amount of CD34+ cells either before or after selection. This may in part have been due to the prior use of methotrexate in both patients, combined with thalidomide in one of the patients, even though this medication was stopped two months before the cyclophosphamide mobilization treatment. A subsequent second attempt at stem cell mobilization using G-CSF only was successful, although two leukapheresis procedures were required for both patients. The recovery of CD34+ cells after selection using the CliniMACS selection procedure was rather low (median 48.5%, range 23–66.5%). Whether this is related to the underlying disease is unknown, but similar low recovery rates were not reported in the series published by Oyama et al.9

Recently, safety and efficacy of autologous HSCT with unselected peripheral blood stem cells was demonstrated in four previously refractory Crohn’s disease patients.12 In our study peripheral blood cells were used after CD34+ cell selection, in order to deplete activated T cells to reduce the likelihood of relapse related to the reinfusion of autoreactive T cells.13 The last case presented is a good example of the role of the immunosuppressive effects of the stem cell mobilization regime which includes a high dose of cyclophosphamide. In 390 patients undergoing autologous HSCT for various autoimmune diseases a mobilization associated mortality of 1.5% and an overall procedure related mortality of 9% were found.14 To evaluate the efficacy of HSCT mobilization followed by high dose immune ablation and autologous stem cell transplantation versus HSC mobilization only, a multicenter, prospective, randomized phase III study has been initiated by the European Crohn’s and Colitis Organisation (ECCO) in collaboration with the European Group for Blood and Marrow Transplantation (EBMT).

Not only autologous but also allogeneic HSCT has been shown to maintain remission in Crohn’s disease patients.15 Theoretically, due to the additional graft versus autoimmunity effect in allogeneic HSCT this approach could give a longer lasting effect than autologous HSCT in the treatment of Crohn’s disease, even after withdrawal of prophylactic immunosuppression.16 However, allogeneic HSCT is associated with graft-versus-host disease and has a considerably higher morbidity and mortality than autologous HSCT.17 Therefore, allogeneic HSCT at this point does not seem to be a viable option for a nonmalignant disease with very low mortality rates.

In conclusion, autologous HSCT is a relatively well tolerated and safe procedure. Given the considerable mortality rate of HSCT for autoimmune diseases, this treatment should only be considered in highly selected patients with severe and therapy resistant disease, or for patients for whom surgery is not a treatment option. Larger clinical trials are and should be conducted as more information is warranted.

Acknowledgments

The authors thank the staff of the Laboratory for Stem Cell Transplantation at Sanquin Research for their assistance.

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