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Autologous Haematopoietic Stem Cell Transplantation for Refractory Crohn's Disease: Efficacy in a Single-Centre Cohort

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Abstract

Background: Haematopoietic stem cell transplantation [HSCT] is considered a therapeutic option for patients with severe Crohn's disease [CD] unresponsive to currently available therapies.
Methods: Autologous HSCT was considered for CD patients with active disease, unresponsive or intolerant to approved medications and unsuitable for surgery. After HSCT, patients were closely followed up every 6 weeks during the first 2 years and every 6 months thereafter up to 5 years. Colonoscopy and/or magnetic resonance imaging were performed at Months 6, 12, 24, and 48 after HSCT.
Results: From December 1, 2007 to December 31, 2015, 37 CD patients were assessed for HSCT. Of these, 35 patients [13 within the ASTIC trial] underwent mobilisation. Six patients did not complete the transplant for various reasons and 29 patients were finally transplanted. Patients were followed up during a median of 12 months [6–60]. At 6 months, 70% of patients achieved drug-free clinical remission (Crohn's Disease Index of Severity [CDAI] < 150). The proportion of patients in drug-free remission (CDAI < 150, Simple Endoscopic activity Score [SES]-CD < 7] was 61% at 1 year, 52% at 2 years, 47% at 3 years, 39% at 4 years, and 15% at 5 years. Patients who relapsed were re-treated and 80% regained clinical remission. Six out of the 29 [21%] required surgery. One patient died due to systemic cytomegalovirus infection 2 months after transplant.
Conclusions: HSCT is a salvage therapy for patients with extensive and refractory CD. Although relapse occurs in a majority of patients within 5 years after transplant, drug responsiveness is regained and clinical remission achieved in 80% of cases.

Key Words: Autologous haematopoietic stem cell transplantation; cellular therapy; Crohn's disease

1. Introduction

Since 1995, autologous haematopoietic stem cell transplantation [HSCT] has been used worldwide as a salvage therapy for patients with severe immune-mediated inflammatory diseases refractory to available therapies, including Crohn's disease [CD]. The rationale behind this strategy is based on the concept of immunoablation using high-dose chemotherapy, with subsequent regeneration of naive T-lymphocytes derived from re-infused haematopoietic progenitor cells.[1] Allogenic HSCT can correct genetic predisposition to disease by re-infusing non-disease-prone haematopoietic stem cells [HSCs] from a healthy donor. However, allogenic HSCT is associated with a mortality risk as high as 20% for haematologic malignancies[2] and therefore its use in CD is limited to patients with known monogenic diseases (eg mutations in interleukin-10 [IL-10] or IL-10 receptor [IL-10R]).[3,4]

During the past decade, case reports and case series have been published on the efficacy of autologous HSCT for treating refractory CD.[5–11] The largest included 24 patients, 18 of whom were followed during 5 or more years after transplantation.[11] In this study, all patients achieved clinical remission after transplant with a Crohn's Disease Activity Index [CDAI] < 150, although relapse over a period of 5 years occurred in a majority of cases.

More recently, the Autologous Stem Cell Transplantation International Crohn Disease [ASTIC] trial comparing the efficacy of autologous HSCT and conventional therapy in patients with refractory CD has been published.[12] The primary endpoint of drug-free clinical and endoscopic remission was not met, although exploratory analyses suggested that: 1] more patients in the HSCT group were able to discontinue all immunosuppressive therapy; and 2] numerically more patients in the HSCT group were in clinical and endoscopic/radiological remission during the first year of follow-up.

Studies on the efficacy and safety of HSCT for the treatment of immune-mediated inflammatory diseases have shown that centre experience is a major determinant of outcomes, with an improvement in those centres transplanting more than 13 patients per year.[1] Since only two participating centres in the ASTIC study transplanted this number of patients, it is convenient to reassess the outcomes of HSCT in those centres accumulating extensive experience. In the present study, we report the results of the largest series of patients with refractory CD treated with autologous HSCT at a single centre.

2. Methods

2.1. Study design

This is a prospective singl-centre study designed to evaluate the feasibility, efficacy, and safety of autologous HSCT in patients with refractory CD.

2.2. Patient eligibility

Autologous HSCT was considered for patients with CD who fulfilled the following inclusion criteria: active disease at assessment with endoscopic and/or radiological evidence of severe disease activity defined by the presence of ulcers; failure or intolerance to all approved medications, including corticosteroids, azathioprine, methotrexate, and anti-tumour necrosis factor [TNF] agents; unsuitability for surgery due to location and/or extent of the disease or when the surgical option was not accepted by the patient [eg colonic CD resulting in permanent ileostomy]; impaired quality of life; and signed written informed consent. Those patients with severe comorbidities, symptoms nonrelated to CD inflammatory activity [eg stenosis or short-bowel syndrome-related diarrhoea], poor compliance, or pregnancy were excluded. Patients were enrolled and followed up from December 2007 to December 2015.

2.3. Ethical issues

The use of HSCT for the treatment of CD was approved by the regional transplantation organisation OCATT [Organització Catalana deTrasplantaments] on July 25, 2007 and by the local ethics committee on July 22, 2007 [ASTIC-study]. Subsequent approval for study continuation as a single centre was obtained on February 23, 2012. All patients provided written informed consent following extensive counselling.

2.4. Procedure of autologous HSCT: premobilisation assessments, mobilisation and conditioning

HSCT procedures have been described previously.[13] Briefly, patients were extensively assessed before mobilisation, including bone marrow aspiration, left ventricle ejection fraction measurement, pulmonary function test, dental evaluation, infection screening (cytomegalovirus [CMV], herpes simplex virus [HSV], varicella zoster virus [VZV], Epstein–Barr virus [EBV], human T-lymphotropic virus types 1 and 2, hepatitis viruses, human immunodeficiency virus [HIV], *Toxoplasma gondii*, and tuberculosis assessment). CD activity was assessed by clinical evaluation [CDAI], biomarkers, endoscopy, and magnetic resonance imaging [MRI]. Fertility preservation was discussed with all patients. Immunosuppressive treatment discontinuation before mobilisation was compulsory but ongoing steroid therapy was permitted.

Patients were hospitalised during the mobilisation phase in order to maximise safety. Cyclophosphamide [Cy] 2 g/m^2/day on 2 consecutive days and granulocyte colony-stimulating factor [G-CSF] 10 mg/kg/ day, started 5 days after the last Cy infusion, were administered until leukapheresis was performed to mobilise HSCs from the bone marrow to peripheral blood.

HSCs were collected from peripheral blood by apheresis in a Cobe Spectra with Mononuclear Cell [MNC] software [Terumo BCT, Lakewood, CO, USA] using double-strength anticoagulant citrate dextrose solution a [ACD-A] [ACD rate up to 2.4 mL/L/min] with routine prophylactic calcium and magnesium infusion [1 mol calcium per every 10 mol citrate]. Apheresis collection was started when the peripheral CD34+ cell count rose above 20 000 CD34+/mL using a 12.5F double-lumen catheter. A minimum of 3 × 10^6 CD34+/kg had to be obtained to proceed with conditioning and, when possible, a reserve of at least 2 × 10^6 CD34+/kg was stored. Cells were cryopreserved in dimethyl sulphoxide [DMSO] 10% and autologous plasma was collected during apheresis.

The conditioning regimen consisted of intravenous Cy total dose 200 mg/kg [50 mg/kg/day for 4 consecutive days, –5 to –2] and intravenous rabbit anti-thymocyte globulin [rATG] total dose 7.5 mg/kg [2.5 mg/kg/day for 3 days, –3 to –1] before administration of harvested HSCs [Day 0]. High doses of corticosteroids [500 mg daily] were administered over 3 days to prevent adverse reactions to rATG.

In addition to the measures applied during mobilisation, supportive care during this phase included isolation in rooms equipped with high-efficiency particle-arresting filters, antibacterial and antifungal prophylactic treatment, prophylactic medication for HSV in HSV-positive patients, and prophylaxis of *Pneumocystis jirovecii* infection. Irradiated transfusions of red cells or platelet units were administered according to standard practice. G-CSF was not administered unless the patient developed fever associated with prolonged

neutropenia. Parenteral nutrition was established during the aplasia period.

Haematological recovery was defined as absolute neutrophil counts > 0.5 × 10^9/L and platelet counts > 20 × 10^9/L without the need for transfusion[s] for at least 3 consecutive days. The Bearman Toxicity Score was used to assess organ toxicity during HSCT.[14]

2.5. Monitoring and follow-up

After discharge, patients were closely followed-up at both Haematology and Gastroenterology Departments. Clinical activity [CDAI] and laboratory testing were assessed weekly during the first 30 days, every 6 weeks during the first 2 years, and every 6 months thereafter until the end of follow-up [5 years]. Recommendations for infectious prophylaxis have been previously described.[13] Colonoscopy and/or MRI were performed at Week 26 after transplant, and at 1, 2, and 4 years of follow-up or at any time when CD relapse was suspected. Patients were assessed for quality of life (Inflammatory Bowel Disease Questionnaire [IBDQ]) every 6 months after transplantation.

Clinical response was defined as a decrease of CDAI by ≥ 100 points from baseline and clinical remission as a CDAI < 150. Simple Endoscopic Activity Score [SES-CD] index was used at baseline and during follow-up to assess endoscopic activity. Mucosal healing was defined as a global SES-CD < 7 with no segment score ≥ 4, which should exclude presence of large ulcers.

CD relapse was defined as the presence of endoscopic and/or radiological signs of activity irrespective of the presence or absence [CDAI < 150] of clinical symptoms, or requirement for surgery. Clinical relapse was defined as the presence of a CDAI > 150 concomitantly with endoscopic and/or radiological signs of activity. Patients with colonic location of disease who underwent colectomy were censored for analysis at the time of surgery.

2.6. Statistics

Sample-size calculation was not performed for this study. All patients who underwent transplantation were analysed. Statistical analysis was performed using the statistical package SPSS V.23. Numerical data with normal distribution were analysed with a Student's t test and nonparametric tests [Mann–Whitney or Kruskal–Wallis] were applied for those variables lacking a normal distribution. The results are expressed as the median [range] or mean (standard deviation [SD]).

Graphical LASSO [GLASSO][15] was used to graph the interrelations between clinical variables. Data were analysed in Matlab using the GLASSO package[16] to create a graphical model that describes the multivariate association network within the data. The analysis started with an estimation of the sample covariance matrix and a penalty term of 0.5 was used during the iterative regularisation. The set of variables that presented statistical dependence with the variable of interest was used to fit a generalised regression model.

3. Results

3.1. Patients

A total of 37 patients were assessed for HSCT at our centre. One patient was excluded due to myelodisplasic syndrome at bone-marrow aspirate. One patient underwent a syngenic HSCT from her genetically identical twin sister[17] and was not included in the current analysis. Thus 35 patients underwent mobilisation and 29 patients were transplanted. Reasons for not performing the transplant were the following: poor mobilisation [< 3 × 10^6 CD34+/kg] in one patient, mobilisation failure [< 20.000 CD34+/mL in peripheral blood before apheresis] in another patient, sustained clinical remission in one patient after mobilisation, need for urgent colectomy for significant disease worsening in one patient, withdrawal of consent in one patient who suffered septic shock during mobilisation, and one more patient waiting for transplant [Figure 1]; 13 out of 37 evaluated patients were included in the ASTIC study.

One patient, with positive serology for John Cunningham virus [JC virus], was in clinical remission with natalizumab at inclusion, but was considered for transplant due to a previous aggressive course of CD and the indication for stopping natalizumab after 12 months of therapy.[18]

At the times of mobilisation and of conditioning, 14/29 patients and 11/29 patients were on prednisone [5-50 mg/daily], respectively. Steroids were completely discontinued after tapering in all patients.

Table 1 shows the transplanted patients' demographics and baseline clinical characteristics.

3.2. Mobilisation

Patients were hospitalised for HSC mobilisation. Mean hospitalisation time was 22 days [range 14–73]. This wide variability was due to the need of nutritional support in some patients before mobilisation. After administration of Cy, neutropenia [< 0.5 × 10^9/L] occurred in all patients, with a mean duration of 5 days [range 2–7]. Adverse events and blood cell transfusion requirements during mobilisation are shown in Table 2.

Four weeks after mobilisation, 9/35 [26%] patients presented worsening of CDAI [median increase 14.7, range 5–173] leading in one case to urgent colectomy, 15/35 [43%] showed a decrease of CDAI > 100 points from baseline, and 13/35 [37%] achieved clinical remission. This improvement was transient in all but one patient who achieved sustained remission and did not undergo transplant after 5 years of follow-up.

3.3. Conditioning and transplantation

In all 29 patients entered the conditioning phase after a median of 53 days [range 14–458] after apheresis. Eight patients [27.5%] received delayed transplantation [defined as more than 100 days from mobilisation to conditioning] for the following reasons: five patients were randomised to delayed transplant within the ASTIC study, two patients requested postponement of the transplant for personal reasons, and one patient who had been previously treated with natalizumab in whom it was decided, for safety reasons, to complete a wash-out of 6 months following the last dose of the drug.

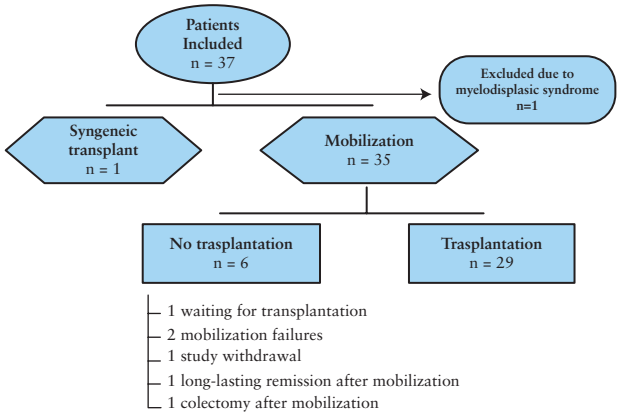

Figure 1. Flow chart of enrolment and outcomes.

Table 1. Demographic and baseline clinical characteristics of transplanted patients [*n* = 29].

Gender F/M, *n* [%]	21 [72]/8 [28]
Age at inclusion mean, years [range]	28.8 [16.5–49.3]
Duration of disease, years mean [range]	10.0 [1.8–25.7]
Location [Montreal], *n* [%]	
L1 [ileal]	1 [3]
L2 [colonic]	7 [24]
L3 [ileocolonic]	14 [48]
L1 + L4 [ileal + upper]	1 [3]
L3 + L4 [ileocolonic + upper]	6 [21]
Behaviour, *n* [%]	
Inflammatory	21 [72]
Stenosing	3 [10]
Penetrating	5 [17]
Extraintestinal manifestations, *n* [%]	21 [72]
Perianal disease, *n* [%]	16 [55]
Active at inclusion	6 [21]
Smoking history, *n* [%]	
Smoker	2 [7]
Non-smoker	17 [59]
Previous smoker	10 [34]
Previous therapies, *n* [%]	
Infliximab	29 [100]
Adalimumab	29 [100]
Thiopurines	28 [97]
Methotrexate	27 [93]
Other immunomodulators[a]	17 [59]
Other biologics[b]	13 [45]
Study medication[c]	2 [7]
Previous CD surgeries, *n* [%]	13 [45]
Ostomy at inclusion	6 [21]
NOD-2 mutation, *n* [%]	4 [14]
Body mass index, mean [range]	22.8 [14–39.7]
CDAI, mean [range]	288.4 [123–456]
SES-CD, mean [range]	19.6 [2–36]
IBDQ, mean [range]	124.3 [66–201]
CRP mg/dL, mean [range]	2.7 [0.02–12.12]
ESR, mean [range]	38.8 [3–126]
Haemoglobin g/L, mean [range]	115.3 [81–144]
Leukocytes x 10^9, mean [range]	9.2 [2.9–16.4]
Platelets x 10^9, mean [range]	409.9 [225–706]
Albumin g/L, mean [range]	36.9 [27–44]

F/M, female/male; CD, Crohn's disease; CDAI, Crohn's disease activity index; SES, simple endoscopic activity score; IBDQ, inflammatory bowel disease questionnaire; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate.

[a]Mycophenolate, tacrolimus, cyclosporine.

[b]Certolizumab, ustekinumab, natalizumab.

[c]Vedolizumab, anti-MadCam.

Hospitalisation mean stay for this period was 27 days [range 22–43]. The mean number of infused CD34+ cells was 10.0 cells/10^6 [SD 4.5]. There were no engraftment failures: neutrophil recovery was reached after a median time of 11 days [range 7–17] and platelet recovery after a median time of 3 days [range 0–8]. Transfusion was required in almost all patients after the conditioning regimen: 26/29 required red blood cell units [3.7 ± 2.3] and 13/29 [45%] patients required platelet units [1 ± 1.4]. Adverse events and transfusion requirements during conditioning and transplantation are summarised in Table 3.

3.4. Long-term safety

The most frequent long-term adverse events were related to viral infections. Eleven patients [38%] presented infections caused by the herpes virus family [CMV, EBV, HSV, and VZV]. One case of CMV systemic infection led to multiorgan failure and death despite early antiviral treatment with ganciclovir 2 months after transplant. Postmortem examination showed no evidence of active CD. Another

Table 2. Adverse events during mobilisation [*n* = 35].

Febrile neutropenia, *n* [%]	22 [63]
Infectious complications, *n* [%]	
Bacteraemia with fever due to piperacilin-tazobactam-resistance	1 [3]
Escherichia coli	
Bacteraemia with septic shock	2[6]
Piperacilin-tazobactam-resistant *Klebsiella pneumoniae*	1[3]
Extended-spectrum ß-lactamase-producing *K. pneumoniae*	1 [3]
Urinary tract infection [*Enterococcus faecium*]	1 [3]
Labial herpes	1 [3]
Influenza group B	1 [3]
Non-infectious complications, *n* [%]	
Renal failure[a]	1 [3]
Tonic-clonic seizure of metabolic origin	1 [3]
Iatrogenic pneumothorax	1 [3]
Adverse reaction to vancomycin	1 [3]
Red blood cell transfusion requirements, *n* [%]	15 [43]

[a]Sepsis and nephrotoxicity related.

Table 3. Adverse events during conditioning and transplantation [*n* = 29].

Febrile neutropenia, *n* [%]	23 [79]
Infectious complications, *n* [%]	
Septic shock	1 [3.4]
Worsening of perianal disease	3 [10.3]
Acute bronchitis	1 [3.4]
Sinusitis	1 [3.4]
Labial herpes	2 [6.8]
Isolated microorganisms	
Gram-negative bacilli	
Pseudomonas aeruginosa	1
Klebsiella oxytoca	1
Gram-positive coccus	
Enteroccus	
Enterococcus faecium [UC, perianal smear, BC]	5
Staphylococcus	
Staphylococcus aureus MRSA	1
Staphylococcus haemolyticus [CtC]	2
Staphylococcus epidermidis [BC, CtC]	2
Non-infectious complications, *n* [%]	
Mucositis I-II [Bearman Scale]	14 [48.2]
Haemorrhagic I-II [Bearman Scale]	4 [13.7]
Renal failure [related to septic shock]	1 [3.4]
Drug toxicity	
Allergic reaction to vancomycin	1 [3.4]
Antithymocyte globulin reaction	
Mild	6 [21]
Severe	3 [10.3]
Corticosteroid-related adverse effects	
Adrenal insufficiency	6 [20.7]
Hyperglycaemia	1 [3.4]
Psychotic disorder	1 [3.4]
Liver dysfunction [parenteral nutrition, antifungals]	3 [10.3]
Red blood cell transfusion requirements	
Patients, *n* [%]	26 [90]
Units, mean [range]	4 [2–9]

UC, urine culture; BC, blood culture; MRSA, methicillin-resistant *Staphylococcus aureus*; CtC, central catheter.

patient with a refractory CMV and EBV colitis ultimately required a colectomy. All other patients recovered without sequelae. Regarding non-infectious complications, symptomatic adrenal insufficiency was observed in eight patients [28%] during corticosteroid tapering. Whereas up to 52% of patients were receiving chronic corticosteroid treatment for refractory CD before mobilisation and/or conditioning, adrenal insufficiency occurred mainly in patients with lower basal cortisol levels before conditioning [< 5 µg/dL]. Substitutive hydrocortisone was initiated in patients with adrenal insufficiency, but only two patients [25%] required substitutive treatment for more than 2 years. Thyroid dysfunction [hyper- or hypothyroidism] was observed in four patients [14%].

3.5. Efficacy

Patients were followed up for a median of 12 months [range 6–60] after transplant. The cumulative probability of clinical and endoscopic relapse-free survival [defined as CDAI < 150 and SES-CD < 7 without treatment] after HSCT was 61% at 1 year, 52% at 2 years, 47% at 3 years, 39% at 4 years and 15% at 5 years [Figure 2]. IBDQ increased a median of 43 points [-52 to 164] from baseline to Week 26. Fifteen patients [52%] presented clinical and/or endoscopic relapse after a median of 53.1 weeks [range 27–227] after transplant. In one-third of these patients [5/15], CD relapse was detected during endoscopic/radiological scheduled assessment while patients remained in clinical remission. All but one patients were treated with anti-TNF, with or without immunomodulators, achieving clinical remission in 80% of cases [12/15]. Three patients did not respond to anti-TNF therapy and required colectomy.

During follow-up, two patients [7%] required ileocaecal resection due to stenosis at the terminal ileum with predominant fibrotic component assessed by MRI [6 and 18 months after HSCT, respectively]. In both cases, inflammatory activity and fibrosis were found in the resection specimens.

Thus, the proportion of patients who were in clinical remission, irrespective of requirements of medical therapy and surgery, was over 70% at every main time point of follow-up: 70% at 6 months, 73% at 1 year, 93% at 3 years, 70% at 4 years and 100% at 5 years [Figure 3]. Despite relapse, an important and statistically significant improvement in clinical and endoscopic scores at 1 year compared with baseline was observed [Table 4]. Thirteen out of 20 patients [65%] who underwent colonoscopy at Month 12 achieved mucosal healing, of whom eight had a SES-CD of 0, with only one patient receiving anti-TNF therapy reintroduced upon relapse. Endoscopic improvement was seen in both extension and severity of lesions, noticing in most cases a decreased number of segments with ulcers [47 segments at baseline compared with 17 segments at Week 52] or complete disappearance.

Regarding perianal disease, only one of six patients with active fistula drainage at the time of inclusion had sustained remission after HSCT. The remaining five patients had persistent active disease requiring surgery [abscess drainage and seton placement] and/or medical treatment. None of the patients with inactive perianal disease at the time of HSCT [n = 10] relapsed during follow-up.

Peripheral arthropathy was the most prevalent extraintestinal manifestation before HSCT in our cohort [19/29; 66%]. Symptoms disappeared in 90% of cases after HSCT, in parallel with CD remission. One year after HSCT, three patients [10%] had persistent articular pain despite CD being in clinical and endoscopic remission. Regarding cutaneous extraintestinal manifestations, one patient presented pyoderma gangrenosum after mobilisation, which was treated with corticosteroids and cyclosporin with partial response. Complete remission was observed after transplant, with no further relapse during follow-up.

3.6. Predictive factors of relapse after autologous HSCT

The GLASSO analysis demonstrated statistical dependence between CDAI, C-reactive protein [CRP], haematocrit, platelets, total days with neutrophils < 0.5 × 10^9, CD3, CD4CD45RO, CD4CD45RA, and remission at Week 52.

The generalised linear regression model showed that the CDAI and CD4CD45RO were the most statistically significant variables, with p-values of 0.074 and 0.0275, respectively. The regression coefficient for CDAI was 0.21 and for CD4CD45RO -0.4217. These coefficients show that the higher the CDAI, the higher the chances of remission. The opposite behaviour was observed for CD4CD45RO [Table 5].

4. Discussion

To our knowledge, this is the largest single-centre experience in autologous HSCT as a rescue therapy for refractory CD. Our data show that this therapy affords significant and durable benefit in the

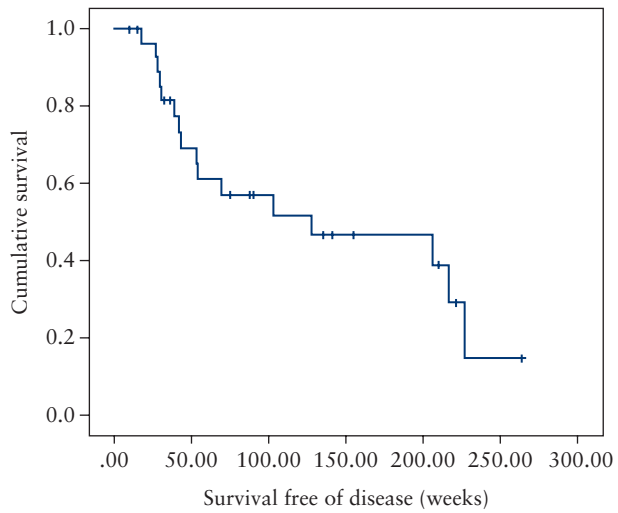

Figure 2. Cumulative probability of clinical and endoscopic relapse-free survival.

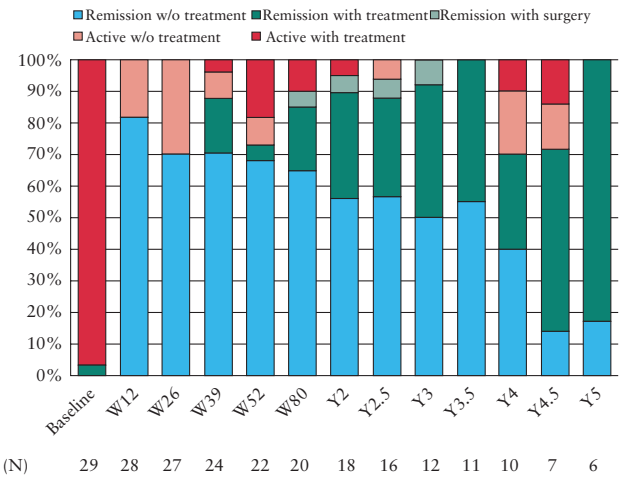

Figure 3. Proportion of patients and disease status during follow-up.

Table 4. Mean CDAI and mean SES-CD at baseline and at 1-year follow-up.

	Baselinemean [SD]	Week 52mean [SD]	Baseline—Week 52mean [SD]	*p*-Value [bilateral]
CDAI [*n* = 22]	301,4 [84,6]	114,8 [112,0]	156,5 [127,6]	< 0.001
SES-CD [*n* = 20][a]	18,7 [9,5]	7,2 [9,3]	11,5 [10,4]	< 0.001

CD, Crohn's disease; CDAI, Crohn's disease activity index; SES, simple endoscopic activity score; SD, standard deviation.
[a]Two patients with upper disease were assessed only by magnetic resonance imaging.

Table 5. Predictors of haematopoietic stem cell transplantation [HSCT] efficacy in CD.

Variable	Regression coefficient[remission at 1 year]
CD4CD45RO [memory T cells; baseline]	-0.42 [*p* = 0.03]
CDAI [baseline]	0.21 [*p* = 0.07]
CRP [baseline]	0.71 [*p* = 0.12]
Haematocrit [baseline]	-0.14 [*p* = 0.38]
Platelets [baseline]	-0.03 [*p* = 0.95]
Total days of neutrophil count < 5 × 10^9 during conditioning	-0.06 [*p* = 0.15]
CD4CD45RA [naïve T cells; baseline]	0.004 [*p* = 0.17]

CDAI, Crohn's disease activity index; CRP, C-reactive protein.

majority of these patients who were unresponsive to all available medical options, although it carries a significant toxicity.

The proportion of relapse in our cohort throughout follow-up [52%] indicates that although autologous HCST is not a cure for CD, it can change the course of the disease by 'allegedly resetting' the immune system, allowing a great majority of very refractory patients [up to 80%] to regain response to conventional therapy upon relapse after HSCT.

The main goal of therapy in CD is to achieve durable long-standing remission. In our series, the percentage of clinical and endoscopic relapse-free survival without restarting CD medication or surgical requirement after HSCT was 61% at 1 year, 47% at 3 years and 15% at 5 years. The largest series published so far regarding HSCT for refractory CD from a single centre[11] included 24 patients who presented clinical relapse-free survival rates of 91% at 1 year, 57% at 3 years, and 19% at 5 years. However, those patients did not undergo endoscopic assessment systematically; only clinical outcomes of CD activity based on measures every 6 months of CDAI and Crohn Severity Index [CSI: a non-validated index which includes clinical symptoms, treatment use, biological parameters. and presence of abnormalities at endoscopy or radiology] were provided. Clinical indices alone [eg CDAI] have a poor correlation with the presence of inflammatory lesions, especially when the disease involves the upper gastrointestinal tract, or the patient has undergone previous surgeries, or extraintestinal manifestations are predominant.[19] Since endoscopic assessment has become an important endpoint in clinical trials of new drugs for CD, as it considerably reduces the placebo response rates,[20] a close endoscopic and/or radiological follow-up was established in our cohort of patients in order to identify early relapse. Those patients were started on medical therapy, in one-third of cases in the absence of clinical symptoms, and therefore the proportion of relapse-free survival proved lower in our cohort of patients compared with previous series.

The ASTIC trial compared the outcomes at 1 year of 45 patients who received cyclophosphamide mobilisation and were then randomised to HSCT, with outcomes of conventional care. The primary endpoint chosen for the ASTIC trial was clinical remission off all medication for 3 months with no evidence of mucosal disease ulceration based on radiological or endoscopic assessment of the entire intestine. This endpoint was much more stringent than those used in clinical trials of conventional drugs in CD, which may have been hampered by the absence of statistically significant differences between conventional treatment and HSCT after the mobilisation phase. However, significant improvements in clinical disease activity, patient-reported outcomes, quality of life, and endoscopic mucosal healing at 1 year were recorded in the 40 patients who ultimately underwent HSCT during the trial programme.[21] Similar gains were observed in the patient cohort described in the current study.

In another series that included nine patients[10] who underwent autologous HSCT with a conditioning regimen based on high-dose Cy but without rATG, early relapse was observed in almost 80% of patients [7/9]. In contrast, the safety profile and tolerability during conditioning and follow-up were better, with no cases of transplant-related mortality or serious infectious complications. The use of rATG in the conditioning regimen might lead to prolonged immunosuppression by removing peripheral antigen-reactive T cells, with reduced relapse rates but a higher risk of infections.[22]

The close endoscopic and radiological follow-up of patients was established to identify early disease, leading to a prompt reintroduction of conventional treatment in order to avoid clinical recurrence, based on the assumption that treating early lesions could achieve better outcomes.

Due to the risk of CD relapse after autologous HSCT, the systematic reintroduction of conventional therapy immediately after procedure deserves consideration. In a retrospective study involving 20 patients with systemic lupus erythematosus [SLE], cyclosporine or mycophenolate were introduced early after autologous HSCT to prevent relapse in three patients, but all of them relapsed.[23] The potential added risk of adverse events associated with immunosuppressive treatment immediately after conditioning should also be taken into account. Therefore, early reintroduction of conventional therapy after HSCT should be explored in future trials.

When looking at predictive factors of outcome at 1 year, a positive association between CDAI and remission at 1 year and a negative one with CD4CD45RO [memory T cells] were found. We can hypothesise that the less memory cells are circulating, the higher the probability of removing them with chemotherapy, thus leading to sustained remission. In our cohort, a higher CDAI was predictive of remission at 1 year. Similarly in the ASTIC trial, a high SES-CD along with colonic location and inflammatory phenotype were also predictive of steroid-free clinical remission at 1 year.[21] Likewise in multiple sclerosis, disease activity measured by enhanced lesions on MRI was the most significant variable related to better outcomes.[24] These results suggest that greater inflammation at baseline corresponds to better responses to HSCT. However, since the regression coefficients are low [0.21], the presence of high baseline markers of inflammation should not be used as a criterion for patient selection for HSCT.

Safety is a cornerstone of HSCT for the treatment of non-malignant conditions such as immune-mediated diseases. Patients with CD might have higher infectious risks compared with those who undergo transplantation for malignancies or other immune-mediated diseases that do not involve the intestinal tract. In our cohort of patients, most of the severe complications [mainly infections] related to mobilisation and conditioning occurred in the first five transplanted patients. Implementation of a number of measures described in a recent publication significantly improved safety.[13] In our cohort, one patient died due to a disseminated CMV infection 8 weeks after HSCT. In the ASTIC study, one patient died due to sinusoidal obstructive syndrome 20 days after conditioning.[12] Treatment-related mortality of HSCT for immune-mediated diseases is around 5%,[1] but varies depending on the underlying disease [eg higher mortality rates in systemic sclerosis] and the conditioning regimen [myeloablative versus non-myeloablative]. The dose of cyclophosphamide used in the current study was 4 g/m^2; it remains to be seen if lower doses can have a safer profile without compromising efficacy.

Mortality has also been linked to the experience level of a given centre. In a single experienced centre, autologous HSCT for patients with SLE resulted in marked serological, clinical, and organ improvement, with 2% [1/50] treatment-related mortality and 50% probability of maintaining remission after 5 years.[25] In comparison, a multicentre analysis of HSCT for SLE which included 53 patients transplanted in 23 different centres, reported a similar 55% 5-year disease-free survival, but treatment-related mortality was as high as 13%.[26] In an observational study of HSCT for treatment of refractory autoimmune diseases [AD] over 12 years,[1] a multivariate analysis revealed a centre effect, influencing the 100-day transplant-related mortality and the overall survival rate. This centre effect, presumably due to better patient selection and clinical monitoring during and after the procedures, may contribute to heterogeneous perceptions about the risk and benefit ratio of autologous HSCT in immune-mediated inflammatory diseases. Within this context, close cooperation between transplant teams and the referring specialist is a key factor. Such results may be of importance for further health care decision policy, and would support the need for referring centres with significant levels of activity and resources for adapted clinical care in treating immune-mediated inflammatory diseases, including CD.

In conclusion, the results of the current study indicate that autologous HSCT is an effective and feasible rescue therapy for patients with refractory CD, which can effect a change in the course of a refractory process in 80% of patients, though it does not represent a cure since re-treatment is needed in the majority of cases. Extraordinary and specific measures must be implemented, and procedures should be concentrated in experienced centres to reduce the risk of severe adverse events and mortality.

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Conflict of Interest

None.

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Author Contributions

MR, JP, and ER and designed the study; JP, ER, IO, IA, AJ-A, and AL-G carried out patient recruitment; ER performed endoscopic examinations; FF, IE, and DC were in charge of patients during mobilisation; PM, ML, and JC were responsible for apheresis and preservation of HSCs; MR, FF-A, CM, GG, LR, EC, AU, and MS-L were in charge of patients during conditioning and transplantation; RB, MG, AG, MCM, AJ-A, AL-G, and ER worked on acquisition of data and follow-up; MR, ER, JP, AJ-A, AL-G, AS, and VR were in charge of the analysis and interpretation of data. All authors contributed to critical revision of the manuscript and accepted the final version for submission.

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