

Autologous stem cell transplantation for progressive systemic sclerosis: a prospective non-interventional study from the European Society for Blood and Marrow Transplantation Autoimmune Disease Working Party



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ABSTRACT

Three randomized controlled trials in early severe systemic sclerosis demonstrated that autologous hematopoietic stem cell transplantation was superior to standard cyclophosphamide therapy. This European Society for Blood and Marrow Transplantation multicenter, prospective, non-interventional study was designed to further decipher efficacy and safety of this procedure for severe systemic sclerosis patients in real-life practice and to search for prognostic factors. All consecutive adult patients with systemic sclerosis undergoing a first autologous hematopoietic stem cell transplant between December 2012 and February 2016 were prospectively included in the study. The primary endpoint was progression-free survival. Secondary endpoints were overall survival, non-relapse mortality, response and incidence of progression. Eighty patients with systemic sclerosis were included. The median dura-

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tion of the follow-up was 24 (range, 6-57) months after stem cell transplantation using cyclophosphamide plus antithymocyte globulin conditioning for all, with CD34⁺ selection in 35 patients. At 2 years, the progression-free survival rate was 81.8%, the overall survival rate was 90%, the response rate was 88.7% and the incidence of progression was 11.9%. The 100-day non-relapse mortality rate was 6.25% (n=5) with four deaths from cardiac events, including three due to cyclophosphamide toxicity. Modified Rodnan skin score and forced vital capacity improved with time ($P<0.001$). By multivariate analysis, baseline skin score >24 and older age at transplantation were associated with lower progression-free survival (hazard ratios 3.32 and 1.77, respectively). CD34⁺-cell selection was associated with better response (hazard ratio 0.46). This study confirms the efficacy of autologous stem cell transplantation, using non-myeloablative conditioning, in real-life practice for severe systemic sclerosis. Careful cardio-pulmonary assessment to identify organ involvement at the time of the patient's referral, reduced cyclophosphamide doses and CD34⁺-cell selection may improve outcomes. The study was registered at *ClinicalTrials.gov*: NCT02516124.

Introduction

Systemic sclerosis (SSc) is a rare systemic autoimmune connective tissue disease characterized by vasculopathy, immune dysregulation and progressive fibrosis, affecting primarily the skin, gastrointestinal tract, lungs, heart and kidneys.¹ It is associated with high disease-related mortality² and the main causes of death are related to cardiac, pulmonary and renal involvement.²⁻⁵ Mortality rates in patients with SSc have remained higher than those in the general population (standardized mortality ratio above 3.5) over the past decades, in contrast to reductions in mortality for other rheumatic diseases or cancer.⁶ After the diagnosis of non-Raynaud phenomenon, the following risk factors for higher mortality related to progressive disease have been identified:² diffuse skin fibrosis measured by the modified Rodnan skin score (mRSS, range 0-51),⁷ elevated C-reactive protein levels, altered left or right ventricular ejection fraction, interstitial lung disease with reduced forced vital capacity (FVC) or diffusion capacity for carbon monoxide (DLCO) on lung function testing, proteinuria and male sex. Earlier studies had found that the presence of anti-topoisomerase II (Scl-70) auto-antibodies, reduced functional status - as measured by the Scleroderma Health Assessment Questionnaire (sHAQ, range from 0 to 3, with lower values indicating a better quality of life)⁸ - or pulmonary arterial hypertension adversely affect survival.³⁻⁵ Treatment with biologic or immunosuppressive drugs did not improve SSc survival.^{2,9,10}

Following early phase I-II trials,¹¹⁻¹³ three randomized controlled trials, namely ASSIST (American scleroderma stem cell *versus* immune suppression trial, phase II),¹⁴ ASTIS (Autologous stem cell transplantation international scleroderma trial, phase III)¹⁵ and SCOT (Scleroderma: cyclophosphamide or transplantation, phase II)¹⁶ demonstrated the superiority of autologous hematopoietic stem cell transplantation (HSCT) compared to the standard cyclophosphamide pulse treatment with regards to overall and event-free survival. In this context, autologous HSCT has become the best treatment option for patients with severe or rapidly progressive SSc, with a grade 1 level of evidence since 2012 according to the Autoimmune Disease Working Party (ADWP) of the European Society for Blood and Marrow Transplantation (EBMT) recommendations.^{17,18} After two decades of progress in HSCT for autoimmune diseases, the use of autologous HSCT for severe or rapidly progressive SSc has also been endorsed by the European League Against Rheumatism (EULAR)¹⁰

and the American Society for Blood and Marrow Transplantation^{10,17,19} and the demand for this therapeutic procedure is the fastest growing indication for HSCT in Europe.^{10,17,19,20}

We therefore designed this ADWP-EBMT non-interventional study, NISSC1, to further evaluate the efficacy and safety of autologous HSCT for early, severe, progressive SSc, when performed in real-life practice, and to identify risk factors for early adverse events and transplant-related mortality, in order to make this approach safer.

Methods

Study design

This study is a prospective, open, multicenter, non-interventional study. Data regarding patients included in this study were prospectively collected using a specific case report form, designed according to EBMT guidelines for HSCT in SSc.¹⁷ All EBMT participating centers agreed to report their consecutive patients aged 18 to 65 years with severe progressive SSc undergoing their first autologous HSCT between December 2012 and February 2016. Inclusion and exclusion criteria are detailed in the *Online Supplementary Appendix*. Participating centers followed their own local protocols for the transplant procedure as well as the patients' evaluation at baseline and during the 2-year follow-up.

The study was approved by the EBMT board in 2012 and by local ethics committees in participating countries and registered at *ClinicalTrials.gov* (NCT02516124). All patients gave written informed consent to their participation in the study.

Patients' evaluation

The SSc patients' characteristics were assessed at baseline (within 3 months before autologous HSCT) and at 3, 6, 12, 18 and 24 months after transplantation (day 0 was defined as the time point of reinfusion of stem cells). Data collected included mRSS, erythrocyte sedimentation rate (in mm/h), creatine kinase levels, presence or absence of autoantibodies, left ventricular ejection fraction (LVEF in %) and systolic pulmonary arterial pressure (in mmHg) on echocardiography, resting and 24 h Holter-electrocardiograms, abnormal cardiac magnetic resonance imaging (MRI) or pulmonary arterial hypertension on right heart catheterization (RHC), FVC and DLCO (% of predicted values), high resolution computed tomography of the lungs and kidney function as well as immunosuppressive drugs given before transplantation. The sHAQ was used to evaluate patients' quality of life.

Autologous hematopoietic stem cell transplantation procedure

Data collected included details on the mobilization process with or without CD34⁺-cell selection, type of conditioning regimen; total dose of CD34⁺ cells infused; number of days of hospitalization for mobilization and conditioning, use and duration of granulocyte colony-stimulating factor (G-CSF), and time to engraftment.

Endpoints

The primary endpoint was progression-free survival (PFS), defined as survival after autologous HSCT without death or evidence of progression of SSc (see *Online Supplementary Appendix S1*).

The secondary endpoints were: (i) infectious or non-infectious adverse events during the first 100 days after autologous HSCT; (ii) engraftment, defined as 3 consecutive days of neutrophils $>0.5 \times 10^9/L$ and platelets $>20 \times 10^9/L$; (iii) response to treatment, defined as a $>25\%$ improvement in mRSS and/or $\geq 10\%$ improvement in FVC or DLCO as compared to baseline and without need of further immunosuppression; (iv) incidence of progression; (v) non-relapse mortality (NRM), defined as any death without progression; and (vi) overall survival.

Statistical analysis

Cumulative incidence functions were used to estimate response

to treatment, NRM, and progression to accommodate for competing risks. Probabilities of overall survival and PFS were calculated using the Kaplan-Meier method. Univariate analyses were performed using the Gray test for cumulative incidence and the log rank test for overall survival and PFS. Multivariate analysis was performed using a Cox regression model including variables associated with the outcome with a *P*-value less than 0.10 in univariate analyses. Age was included as a continuous variable. A generalized linear model was used to analyze evolution over time of mRSS, FVC, DLCO and sHAQ. Results are expressed as a hazard ratio (HR) with 95% confidence interval (95% CI).

Results

Eighty patients (71.3% female), who had undergone a first autologous HSCT between December 2012 and February 2016 in 13 EBMT centers from six European countries and Brazil were included in this study. The centers are listed in *Online Supplementary Appendix S2*. The median (range) follow-up after transplantation was 24 (6-57) months. Table 1 summarizes the patients' baseline characteristics. The median disease duration since the diagnosis of SSc was 23.8 (5.3-103.7) months.

At baseline, all 80 patients had skin involvement, and mRSS was above 15 in 64 (80%) of them. Abnormal chest

Table 1. Baseline characteristics of the patients (n=80).

| Parameter | N (%) or median (range) |
|--|-------------------------|
| Patients | 80 |
| Age at aHSCT, years | 43 (20-62) |
| Sex, female | 57 (71.3) |
| Disease duration from SSc diagnosis, months | 23.8 (5.3-103.7) |
| Body mass index (BMI) | |
| BMI <18.5 | 8 (10.3) |
| BMI 18.5-30 | 65 (83.3) |
| BMI >30 | 5 (6.4) |
| Skin involvement | |
| Modified Rodnan skin score (mRSS) | 24 (2 - 49) |
| Lung involvement | |
| Lung crepitations | 41 (51.3) |
| Pulmonary function tests, done | 80 (100) |
| FVC, % of predicted value | 72 (43-132) |
| FEV1, % of predicted value | 78 (52-153.3) |
| DLCO, % of predicted value | 59 (34.3-120) |
| Chest imaging, done | 80 (100) |
| Chest X-rays, abnormal | 31/71 (43.7) |
| Thoracic computed tomography (HRCT), abnormal* | 66/77 (85.7) |
| Cardiac involvement | |
| Systolic blood pressure (mmHg) | 110 (76-145) |
| Diastolic blood pressure (mmHg) | 70 (40-90) |
| Heart rate | 82 (50-105) |
| Resting ECG, abnormal | 4/80 (5) |
| 24h Holter ECG, abnormal | 12/60 (20) |
| Echocardiography, done | 80 (100) |
| LVEF (in %) | 65 (47-84) |
| Pericardial effusion, present | 5/79 (6.3) |
| sPAP by cardiac echo (mmHg) | 29 (8-59) |
| Cardiac MRI, done | 59/80 (74) |
| Cardiac MRI, abnormal** | 11 (18.6) |

| | |
|--|--------------|
| Right heart catheterization, done | 17/80 (21.3) |
| PAP (mmHg) | 17 (8-23) |
| PAP, systolic | 26 (16-33) |
| PAP, diastolic | 10 (6-16) |
| Fluid challenge, done | 7 (41) |
| Musculo-skeletal involvement | |
| Joints involvement | 42 (52.5) |
| Muscle weakness | 22 (27.5) |
| CK levels, U/L | 80 (16-1368) |
| Patients with CK level ≥ 250 U/mL | 9 (12.5) |
| Kidney function | |
| Creatinine clearance, mL/min*** | 111 (58-190) |
| Proteinuria on labstix | 9 (12) |
| Haematuria on labstix | 7 (9.2) |
| SSc auto-antibodies | |
| Anti-nuclear antibody positive | 74 (92.7) |
| Anti-topoisomerase I (Scl70) positive | 57 (71.3) |
| Anti-centromere positive | 3 (4.2) |
| Previous immunosuppressive SSc medication, yes**** | 76 (95.0) |
| Cyclophosphamide (i.v./oral) | 48 (60) |
| Dose, g | 6 (1-17) |
| Methotrexate | 46 (58.2) |
| Mycophenolate mofetil | 16 (20.3) |
| Prednisone or equivalent | 64 (81.0) |
| Azathioprine | 8 (10.0) |
| ATG rabbit | 2 (2.5) |
| Cyclosporine | 3 (3.8) |

If not otherwise stated all data are n (%) or median (range) *Abnormal computed tomography scan includes fibrosis, honeycombing or ground-glass pattern. **Abnormal cardiac magnetic resonance imaging includes reduced left or right ventricular function or late enhancement. ***The Cockcroft-Gault formula was recommended for estimating creatinine clearance, i.e., creatinine clearance = $(140 - \text{age in years}) / \text{serum creatinine in mg/dL} \times \text{body weight in kg} / 72$. ****Previous treatment ever before, multiple answers possible. aHSCT: autologous hematopoietic stem cell transplantation; SSc: systemic sclerosis; BMI: body mass index; FVC: forced vital capacity; FEV1: forced expiratory volume in 1 s; DLCO: diffusing capacity of lung for carbon monoxide; HRCT high-resolution computed tomography; ECG: electrocardiography; LVEF: left ventricular ejection fraction; sPAP: systolic pulmonary artery pressure measured by echo; echo: echocardiography; MRI: magnetic resonance imaging; PAP: pulmonary artery pressure; CK: creatine kinase; SSc: systemic sclerosis; ATG: antithymocyte globulins;

x-rays or abnormal high-resolution computed tomography was reported in 70 (87%) patients. Heart involvement with an abnormal 24 h electrocardiogram was reported in 12/60 (20%) or with abnormal cardiac MRI in 11/59 (19%) patients. Among the 11 patients with abnormal MRI at baseline, echocardiography showed normal LVEF in all cases and pericardial effusion in one patient, the resting ECG was abnormal in one patient and the 24 h ECG was abnormal in two patients; baseline RHC performed in six patients (with fluid challenge in 2 cases) was normal. Overall, 21 patients (26%) were transplanted without any prior cardiac MRI or RHC at baseline; among these, two had pericardial effusion and seven had a systolic pulmonary artery pressure above 25 mmHg on echocardiography, one had ventricular disturbances on resting ECG (n=20), and 2/8 had an abnormal 24 h ECG.

Mobilization and conditioning procedures

All patients were mobilized using cyclophosphamide with a median dose of 2 g/m² (range, 1-4 g/m²) plus G-CSF. The median duration of hospitalization was 4 (1-30) days for mobilization with large variation according to centers. CD34⁺-cell selection was performed in 35 (44%) patients (Table 2).

The median time from mobilization to the start of the conditioning regimen for autologous HSCT was 52 (22-254) days. The conditioning regimen included cyclophosphamide in all 80 patients, alone in 76 (95%) patients, with a median total dose of 200 (50-240) mg/kg. Four patients (5%) received thiotepa 10 mg/kg with a total cyclophosphamide dose of 100 mg/kg as a single-center treatment for patients with cardiac abnormalities at the pre-transplant evaluation. Additional rabbit antithymocyte globulin (ATG), with different doses according to the brand, was also included in the conditioning regimen for all patients and administered over 4 to 5 days. Sixty-seven patients (82%) received methylprednisolone during the ATG treatment. No patient received total body irradiation. A median number of 5.2 x 10⁶/kg (2.53-23.31) CD34⁺ cells were reinfused at transplantation. Following autologous HSCT, G-CSF was administered to 34 (43%) patients for a median duration of 5 (1-13) days. Following autologous HSCT, the median time to neutrophil engraftment was 11 (8-24) days and the median time to platelet engraftment was 9 (1-25) days. In 13 (16.9%) patients, the platelet counts were never below 20x10⁹/L. There was an inverse correlation between the number of CD34⁺ cells infused and the time to engraftment (Spearman: r²= -0.31; P<0.01), while the number of infused CD34⁺ cells and addition of G-CSF until recovery of aplasia were not associated with a reduced number of infectious complications (P=0.32). The median duration of inpatient hospitalization for conditioning, autologous peripheral blood stem cell re-infusion and subsequent supportive care until engraftment and discharge was 24 (8-128) days (Table 2).

Efficacy

At 1 and 2 years after autologous HSCT, the PFS rate was 87.5% (95% CI: 80.2-94.7) and 81.8% (95% CI: 73.1-90.5), respectively, and the rate of response to treatment was 75% (95% CI: 63.7-83.2) and 88.7% (95% CI: 79-94.1), respectively. At the 1- and 2-year time-points, the incidence of progression was 6.3% (95% CI: 2.3-13.1) and 11.9% (95% CI: 5.8-20.5), respectively, and the overall survival rate was 91.2% (95% CI: 85.1-97.4) and 90% (95% CI 83.4 - 96.6) at 1 and 2 years, respectively (Figure 1). Three of the four

Table 2. Treatment regimen used for mobilization and conditioning.

| Parameter | N (%) or median (range) |
|--|-------------------------|
| Mobilization | |
| CYC 2 g/m ² | 45 (57.7%) |
| CYC 4 g/m ² | 23 (29.5%) |
| Other dose | 10 (12.8%) |
| Missing = 2 | |
| G-CSF, yes | 80 (100%) |
| Leukapheresis | |
| 1 | 61 (77.2%) |
| 2 | 11 (13.9%) |
| 3 | 7 (8.9%) |
| CD34⁺ selection | |
| Yes | 35 (43.8%) |
| None | 45 (56.3%) |
| Conditioning regimen | |
| CYC 200 mg/kg | 72 (90.0%) |
| CYC other dose mg/kg | 4 (5.0%) |
| CYC 100 mg/kg + Thiotepa 10 mg/kg | 4 (5.0%) |
| Rabbit ATG, yes | 80 (100%) |
| Thymoglobulin (Sanofi/Genzyme), mg/kg | 7.5 (2.5-7.5) |
| Grafalon (Neovi/Fresenius), mg/kg | 40 (30-41) |
| Growth factors | |
| G-CSF administration after conditioning | 34 (43.0%) |
| N. of days of administration | 5 (1-13) |
| Cells infused | |
| CD34 ⁺ cells reinfused, 10 ⁶ /kg | 5.2 (2.53-23.31) |
| Duration of hospitalization | |
| Mobilization, days | 4 (1-30) |
| Conditioning, days | 24 (7-128) |

CYC: cyclophosphamide; G-CSF: granulocyte – colony-stimulating factor; ATG: antithymocyte globulin;

patients transplanted with thiotepa and a reduced-dose cyclophosphamide regimen responded; one with worsening skin and lung involvement at day 350 was then treated with prednisone, mycophenolate mofetil and rituximab. By multivariate analysis, mRSS >24 at baseline and older age at transplantation were significantly associated with a lower PFS with hazard ratios of 3.33 (95% CI: 1.04-10.62) and 1.77 (95% CI: 1.07-2.94), respectively. CD34⁺-cell selection was associated with a better response to therapy (HR: 0.46; 95% CI: 0.27-0.76).

Figure 2 illustrates the evolution of the parameters over time. The mean mRSS decreased from 23.9 (standard deviation [SD] 9.7) at baseline to 14.2 (SD 9.2) at 1 year and 12.6 (SD 8.3) at 2 years (n=55, P<0.001). Regarding lung function, the mean FVC increased from 73.6% (SD 16.9) of predicted value at baseline to 79.5% (SD 16.9) at 1 year and 80.6% (SD 19.1) at 2 years (n=37, P<0.001); the mean DLCO was 60.2% (SD 19.3) at baseline, 59.7% (SD 17.7) at 1 year and 60.4% (SD 19.1) at 2 years (n=35). The sHAQ score was available in 15 patients and decreased significantly from 0.96 (SD 0.8) at baseline to 0.73 (SD 0.6) at 1 year and 0.70 (SD 0.6) at 2 years (P<0.001). The mean erythrocyte sedimentation rate decreased from 23.2 mm/h (SD 19.8) at baseline to 16.1 mm/h (SD 11.3) at 1 year and 18.7 mm/h (SD 23.6) at 2 years (n=38, P<0.001). Anti-Scl-70 and anti-centromere autoantibodies did not change after autologous HSCT.

Safety

The NRM rate at 100 days and 2 years was 6.25% (95% CI 2.3-13) (Figure 1). Four of the five deaths not due to

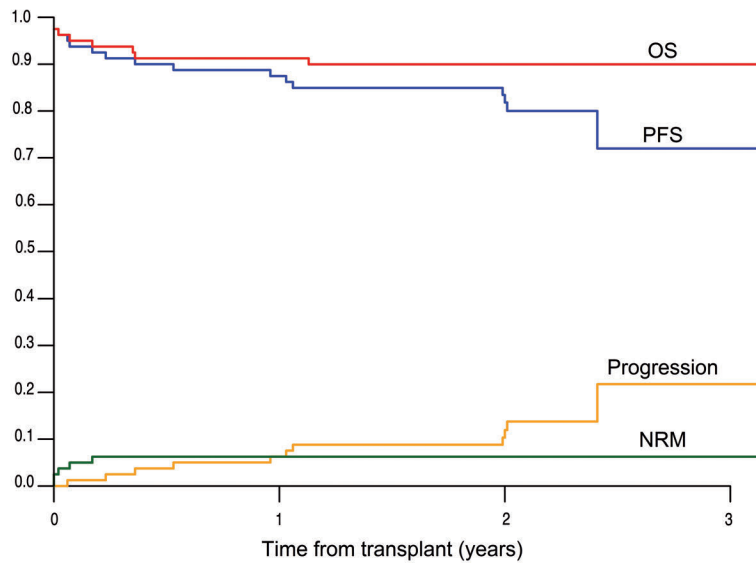


Figure 1. Kaplan-Meier curves of outcomes of patients undergoing autologous hematopoietic stem cell transplantation for severe systemic sclerosis. Overall survival (OS), progression-free survival (PFS), progression and non-relapse (treatment)-related mortality (NRM) over time.

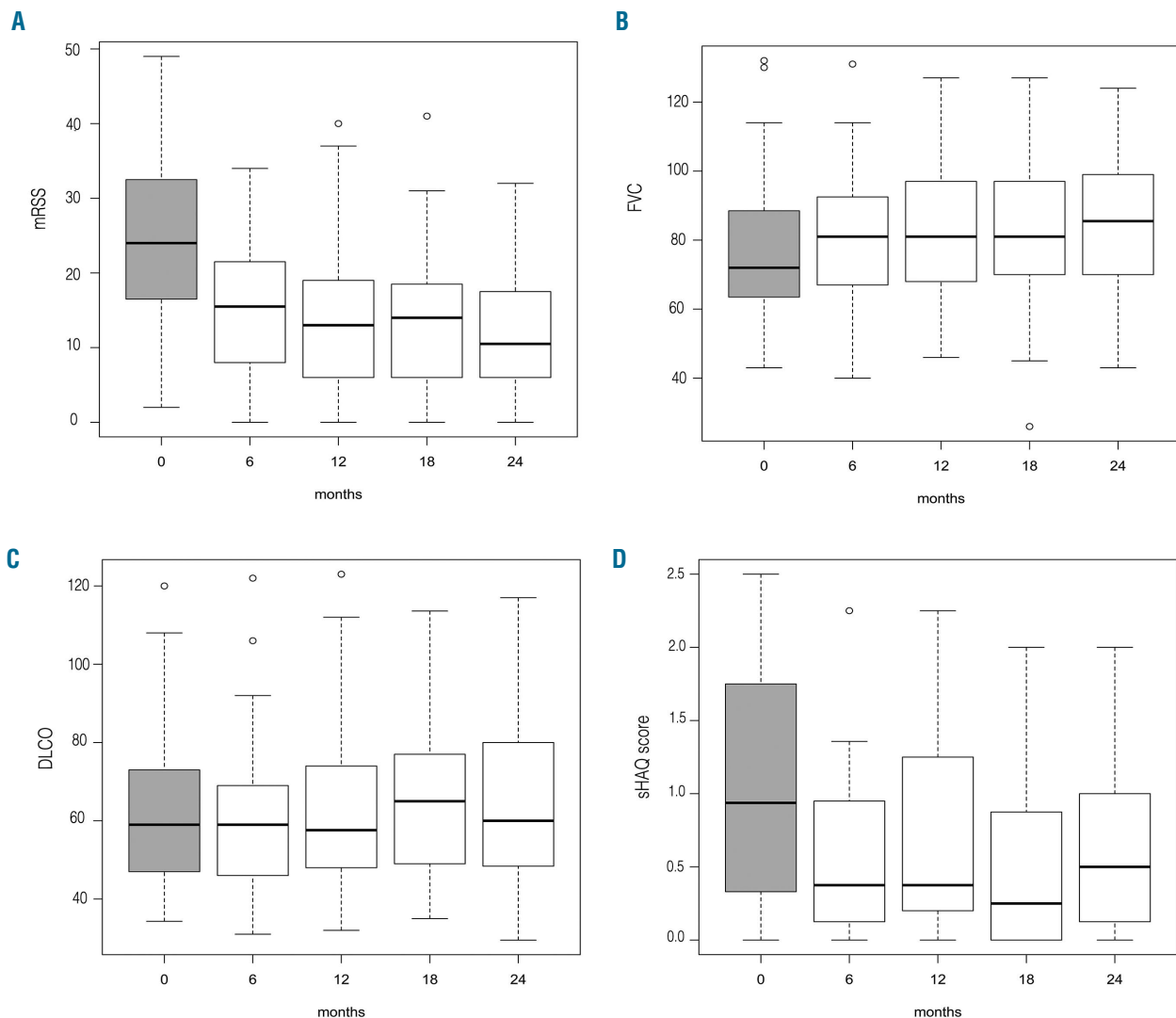


Figure 2. Boxplots of the four most important efficacy parameters in the 24 months following autologous hematopoietic stem cell transplantation for severe systemic sclerosis. (A) Improvement of the modified Rodnan skin score (mRSS) ($n=55$; $P<0.001$). (B) Improvement of forced vital capacity (FVC) ($n=37$; $P=0.001$). (C) Stabilization of diffusion capacity of carbon monoxide (DLCO) ($n=35$; $P=0.01$). (D) Improvement of Scleroderma Health Assessment Questionnaire (sHAQ) score ($n=15$, $P=0.001$).

Table 3. Baseline characteristics and results from the pretransplant evaluation in patients who died during the follow-up.

| Age at aHSCT/ gender | SAP/DBP (HR) | Resting ECG | 24h-Holter ECG | Echo LVEF (%) | Echo PASP | Cardiac MRI | RHC | Proteins | Haematuria | Serum creatinine ($\mu\text{mol/L}$) | Mobilization CYC (g/m^2) | Conditioning regimen (mg/kg) | Time to death (days) | Cause of death |
|-------------------------|-----------------|----------------|-------------------|---------------------|--------------|----------------|-----------------------------------|----------|------------|--|---|---|----------------------------|--|
| 28/F | 90/60 (80) | Normal | Normal | 60 | 19 | Normal | Not done | Positive | Positive | 36 | 2 | CYC 200 | 1 | aHSCT related: cardiac toxicity |
| 46/F | 110/75 (90) | Normal | Not done | 67 | 23 | Normal | Not done | Negative | Negative | 48 | 2 | CYC 200 | 1 | aHSCT related: cardiac toxicity and CYC toxicity |
| 55/F | 125/75 (65) | Normal | Abnormal | 68 | 31 | Normal | Not done | Negative | Negative | 56 | 3 | CYC 200 | 9 | aHSCT related: cardiac toxicity and CYC toxicity |
| 35/F | 100/70 (88) | Normal | Not done | 62 | . | Not done | Not done | Negative | Negative | 35 | 2 | CYC 200 | 61 | aHSCT related: cardiac toxicity and CYC toxicity |
| 52/M | 135/85 (72) | Normal | Abnormal | 55 | 20 | Abnormal | Mean PAP=17 No fluid challenge | Positive | Negative | 62 | 3 | CYC 100 + Thiotepa 10 | 26 | aHSCT related: infection + MOF |
| 58/M | 120/80 (82) | Normal | Normal | 66 | 30 | Normal | Not done | Negative | Negative | 115 | 3 | CYC 200 | 128 | Progression: Pulmonary toxicity |
| 43/M | 120/80 (100) | Normal | Normal | 64 | 16 | Normal | Not done | Positive | Positive | 35 | 4 | CYC 200 | 131 | Progression |
| 51/F | 120/70 (70) | Normal | Normal | 72 | 30 | Normal | Not done | Negative | Negative | 37 | 3 | CYC 200 | 414 | Progression |

aHSCT: autologous hematopoietic stem cell transplantation; SAP/DAP: systolic arterial pressure/diastolic blood pressure; HR: heart rate; ECG: electrocardiography; Echo: echocardiographic; LVEF: left ventricular ejection fraction; PASP: pulmonary arterial systolic pressure; MRI: magnetic resonance imaging; RHC: right heart catheterization; CYC: cyclophosphamide; M: male; F: female; MOF: multiorgan failure; NA: not applicable;

relapse were related to a cardiac event, due to cyclophosphamide toxicity in three patients. A 35-year old female, who died from cardiac toxicity and multiorgan failure on day +61 after autologous HSCT, had normal resting ECG and LVEF (62%) on echocardiography, and abnormal LVEF on heart scintigraphy (47%); MRI and RHC were not performed at baseline. Three other patients died from cardiac toxicity at day +1 (n=2) and day +9 (n=1), with normal cardiac MRI and no RHC evaluation at baseline. A fifth patient (conditioning with thiotepa + reduced-dose cyclophosphamide) died from cytomegalovirus reactivation with viral copies detectable also in the lung, with bacterial (*Pseudomonas*) pneumonia and multiorgan failure at day +21. This patient had a normal resting ECG, with an abnormal 24 h ECG, abnormal MRI and a mean pulmonary artery pressure of 17 mmHg on RHC performed without fluid challenge at baseline. During the 24 months of follow-up, three additional patients died, all due to disease progression. Baseline characteristics of all patients who died during the follow-up and the respective causes of death are presented in Table 3.

During the 100 days after transplantation, a total of 119 adverse events were reported among the 80 patients as detailed in Table 4. The percentage of infectious complications was not significantly different between the patients who did or did not receive CD34⁺-selected grafts ($P=0.44$), or according to the cyclophosphamide dose at the time of mobilization (2 g/m^2 vs. 4 g/m^2 , $P=0.16$) or depending on whether they were or were not given G-CSF after their transplant ($P=0.32$) (Online Supplementary Table S3).

Discussion

The NISCC1 study is the largest prospective cohort today analyzing the efficacy and safety of autologous

HSCT in 80 patients with severe SSc in a real-life setting. The PFS rate was 82% and significant improvements were observed in skin score, lung function and quality of life during the 2 years of follow-up, consistent with results of previous randomized controlled trials¹⁴⁻¹⁶ and earlier studies.¹¹⁻¹³ Nevertheless, the NRM rate associated with autologous HSCT was 6.25%, with most (4 out of 5) of the deaths being related to early cardiac events; this is similar to the 6% NRM rate reported retrospectively among 89 SSc patients transplanted between 2002 to 2011 in one USA and one Brazilian expert center using a non-myeloablative conditioning regimen.²¹

We used PFS as the primary endpoint to assess autologous HSCT efficacy rather than event-free survival, as previously used^{14,15} and which was defined as survival without respiratory, renal, or cardiac failure. PFS, which encompasses disease progression and toxic deaths, better reflects safety and efficacy of HSCT.

In NISCC1, the incidence of progression was 6.3% and 11.9% at 1 and 2 years, respectively, highlighting the need to identify patients who may benefit from early maintenance therapy after autologous HSCT. Several mechanisms contribute to the resetting of the immunological response and disease control after autologous HSCT, and vary according to individual patients.¹² We showed that the immune reconstitution profile and T-cell repertoire diversity together with a decrease in mRSS >25% and in FVC >10% at 1-2 years after transplantation could identify long-term responders and non-responders/relapsing patients at 4-5 years.²² The immune reconstitution process and the balance between reinduction of tolerance and sustained autoreactivity after transplantation will depend on the type of conditioning regimen used to ablate the original immune system, the effect of ATG or *ex vivo* CD34⁺-cell selection to

further deplete/eliminate residual autoreactive cells and the use of post-transplant maintenance immunosuppression.^{15,16,22-24}

All patients in the NISCC1 study received a 'non-myeloablative' cyclophosphamide-based conditioning regimen, which included rabbit-derived ATG in all cases. The degree of T-cell depletion for each patient varied according to the type and total dose of ATG as well as timing of its administration in relation to the peripheral blood stem cell infusion, and whether the infused cells had undergone *ex vivo* CD34⁺ selection. In a previous retrospective EBMT analysis, we failed to demonstrate a benefit of *ex vivo* CD34⁺-cell selection²³ in 138 SSc patients with similar baseline characteristics transplanted between 2000 and 2012. Surprisingly, the NISCC1 study showed a superior response to treatment in patients who received a CD34⁺-selected graft, with no increased rate of infectious complications, consistent with the recent findings of Ayano *et al.* in a retrospective analysis of 19 SSc patients.²⁴ The reason for the difference in outcomes between these studies is unclear, but the population included in the NISCC1 prospective cohort was more homogenous in terms of conditioning, including *in vivo* T-cell depletion with rabbit-derived ATG. All patients in the NISCC1 study received rabbit ATG formulations (although of different brands) while, in the EBMT retrospective study,²³ 64% of patients treated with ATG received a rabbit-derived product and 35% the horse-derived ATG formulation, which were shown to be associated with significantly different clinical outcomes in aplastic anemia.²⁵ This important finding raises the question of whether a randomized, controlled trial should be performed to clarify whether more profound depletion of autoreactive cells from the peripheral blood stem cell graft by CD34⁺-cell selection may translate into sustained clinical benefit in SSc patients after autologous HSCT, without adversely affecting safety and cost-effectiveness.

During the immune reconstitution process, the re-emergence of naïve T and B cells, the renewal of the immune repertoire and reinstatement of synergistic immunoregulatory mechanisms are expected. Therefore maintenance or rapid reintroduction of immunosuppression after autologous HSCT may improve patients' outcome. The ongoing open-label trial (*ClinicalTrials.gov identifier: NCT01413100*) analyzing the addition of mycophenolate mofetil for 2 years after autologous HSCT and the next NISCC2 study (*ClinicalTrials.gov identifier: NCT 03444805*) analyzing post-transplantation management may clarify the potential benefit of post-transplant maintenance immunosuppression.

A critical point for autologous HSCT in autoimmune diseases is safety. The risks of early transplant-related complications and mortality vary according to the type of disease, pre-existing internal organ involvement, the experience of the transplant center and the intensity of the conditioning regimen.^{12,26,27} Major cardiac adverse events and NRM after autologous HSCT in SSc are predominantly related to primary cardiac and lung involvement, which are undervalued in the absence of systematic MRI and RHC at baseline.^{28,29} Our study revealed important variations in clinical practice in the pre-transplant cardio-pulmonary evaluation process. Although all patients who died during the first 100 days had normal resting ECG and LVEF on echocardiography at baseline, they were not all assessed by 24 h ECG (3/5), MRI (4/5) or RHC with fluid

challenge (1/5). Overall, 24 h ECG was only used in 75% of the NISCC1 patients to detect subclinical rhythm or conduction disturbances at baseline, although it has been recommended since 2004.²⁷ Similarly, cardiac MRI and RHC with fluid challenge were only performed in 74% and 21% of the patients, respectively, while their use has been advocated among expert centers since 2012 to mini-

Table 4. Complications reported in the 100 days following autologous hematopoietic stem cell transplantation among the 80 patients treated for severe systemic sclerosis.

| Post-transplant non-infectious complications in 21 (26%) patients | Events (n=24) |
|--|---------------|
| CYC-related acute cardiomyopathy / 4 deaths | 4 |
| Cardiomyopathy with myocardial infarction | 1 |
| ARDS + acute heart failure | 1 |
| Arrhythmias | 2 |
| Pericardial effusion | 1 |
| Atrial flutter/fibrillation | 2 |
| Allergy to ATG with respiratory failure + pulmonary hemorrhagic syndrome | 1 |
| ATG-related fever | 1 |
| Acute pulmonary edema | 1 |
| Renal failure | 3 |
| Psychosis/depression | 2 |
| Epistaxis | 1 |
| Oral mucositis | 1 |
| Anal fissure | 1 |
| Hemorrhagic cystitis | 2 |
| Post-transplant infections reported in 60 (75%) patients | Events (n=95) |
| Neutropenic fever with no documented infection | 25 |
| Catheter infection | 2 |
| Sepsis, including 1 septic shock | 2 |
| Sinusitis | 2 |
| Pneumonia | 8 |
| Endocarditis | 1 |
| Bacterial | |
| Pneumonia | 4 |
| Pleuritis | 1 |
| Sepsis Gram-positive bacillus | 4 |
| Sepsis Gram-negative bacillus | 1 |
| Catheter infection not documented | 3 |
| Cellulitis | 3 |
| Viral | |
| CMV reactivation | 13 |
| EBV reactivation | 13 |
| Pneumonia | 3 |
| Herpes simplex infection | 4 |
| BK virus cystitis | 1 |
| Hemorrhagic cystitis, adenovirus-positive | 1 |
| Fungal | |
| Fungal sinus infection | 1 |
| Skin infection-right foot-- <i>Fusarium spp.</i> | 1 |
| Candida stomatitis | 1 |
| Parasitic | |
| Toxoplasma reactivation | 1 |

CYC: cyclophosphamide; ARDS: acute respiratory distress syndrome; ATG: antithymocyte globulins; CMV: cytomegalovirus; EBV: Epstein-Barr virus.

mize the risk of the transplant procedure.^{21,30,31} From 2013 to 2017, the EBMT and collaborative partners worked collectively to establish common standards for the use and interpretation of cardiac MRI results and RHC with fluid challenge for a rigorous cardio-pulmonary assessment of SSc patients, carefully defining eligibility and selection criteria for transplant referral.³²

Optimized treatment regimens are warranted to improve the safety of autologous HSCT, even in patients without cardiac involvement. Interestingly, the NRM rate in the NISCC1 study was similar to the 6% reported among 32 SSc patients transplanted between 2005 and 2011, using a ‘myeloablative’ conditioning regimen comprising total body irradiation (8 Gy), cyclophosphamide (120 mg/kg) and horse-derived ATG in the prospective SCOT randomized controlled trial.¹⁶ However, in the SCOT trial, NRM was primarily related to late fatal myelodysplastic syndromes, while no cyclophosphamide toxicity was reported. Within the follow-up period of the NISCC1 study, there were no reports of myelodysplastic syndrome or other myeloid malignancies. In addition, there were no reports of serious late infectious complications or secondary autoimmune phenomena that may occasionally present as long-term complications following autologous HSCT in autoimmune diseases. Although further follow-up is required, it is possible that there are long-term advantages, in terms of late effects, of the ‘non-myeloablative’ regimen used in the NISCC1 study.

Early toxicity remains the principal challenge, including a NRM rate of 6%. To reduce toxicity and thus treatment-related mortality of the conditioning, a more individualized regimen with respect to heart, lung or kidney involvement might further improve patients’ safety, without excluding patients with organ manifestations. Two studies addressing patients with heart involvement are ongoing, both using a conditioning regimen with a reduced dose of cyclophosphamide and the addition of either thiotepa, as in four patients in our NISCC1 cohort (NCT01895244), or rituximab and fludarabine followed by reinfusion of autologous hematopoietic stem cells (NCT03593902), although the hematopoietic recovery from this reduced-dose regimen is prompt and reinfusion is not necessary in this setting.

Because mobilization with G-CSF alone may exacerbate autoimmune diseases, there is a clinical rationale for combining G-CSF with cyclophosphamide. The use of 2 g/m² cyclophosphamide for mobilization was as effective as 4 g/m² at achieving the EBMT recommended minimum CD34⁺-cell yield, supporting dose reduction in the mobilization regimen to limit early toxicity as well as long-term risks associated with cumulative doses of cyclophosphamide. During the autologous HSCT procedures, inevitable infectious and non-infectious toxicity occurred especially following the high-dose cytotoxic conditioning and the cytopenic phase. A correlation between the num-

ber of infused CD34⁺ cells and a reduced duration of aplasia after autologous HSCT has been described previously,^{33,34} which was confirmed in the NISCC1 study, although CD34⁺-cell dose and addition of G-CSF until recovery from aplasia were not associated with a reduced number of infectious complications.

The NISCC1 study has several limitations. Since the study was a non-interventional trial, follow-up physical and laboratory evaluations, although recommended by EBMT guidelines,¹⁷ were not mandatory, and were not obtained at all time-points. The non-interventional study design was also the cause of significant variation in data collection practices by the 13 participating sites. Data were prospectively collected and checked for consistency by the EBMT study office; however, when some examinations were not performed, as may occur in routine clinical practice, data were classified as ‘missing’. The NISCC1 results showed significant variation in local practices when evaluating patients at baseline. Similarly, the number of reported days for mobilization varied largely according to centers; indeed, some patients were hospitalized for the whole duration of this procedure, whereas in other cases it was carried in part on an outpatient basis.

We aimed to investigate the factors influencing the safety and efficacy of autologous HSCT. We used validated parameters to assess post-transplant outcome, such as mRSS or lung function tests despite their large intra- and inter-individual variability. The results enable deeper analysis of the benefits and risks of the transplant procedure in relation to both SSc-related factors, including the extent of heart, lung and kidney involvement at the time of patient referral, and transplant-related factors such as the conditioning regimen and graft manipulation. No international consensus has yet validated a unique activity score in SSc and further studies will be necessary in the post-transplant setting to investigate disease activity, the concept of drug-free remission and longer term outcomes, with or without post-transplant interventions.

In conclusion, the NISCC1 is a large prospective real-world data study of importance for patients, clinicians, and healthcare payers, given the poor prognosis and burden of disability of patients with severe SSc. It allowed us to assess multicenter adherence and compliance to good clinical practice and adds significantly to the evidence base supporting the delivery of autologous HSCT for severe SSc in routine clinical practice.

Disclosures

No conflicts of interests to disclose.

Contributions

JH, DF, MB, JS and ML: conception, study design, analyses and interpretation of data. MO, HUS, NDP, TD, MS, RS, TM, GP, BS, DM, EM, BL, JOB, MS, AH, FO, LK and ZM: acquisition of data.

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