Review

Autologous stem cell transplantation in systemic sclerosis: a systematic review

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Received on January 9, 2017; accepted in revised form on May 29, 2017.

Clin Exp Rheumatol 2017; 35 (Suppl. 106): S198-S207.

Key words: autologous stem cell transplantation, systemic sclerosis, systematic review

Funding: MN is supported by an NHMRC research fellowship (APP1071735).

Competing interests: none declared.

ABSTRACT

Objective. *Haematopoetic autologous stem cell transplantation (ASCT) has emerged as a treatment option for patients with refractory, severe autoimmune disease. This is a systematic review of the current literature on ASCT in adult patients with systemic sclerosis (SSc).*

Methods. *Original articles published between 2005 and 2016 that evaluated the use of ASCT in patients with SSc were reviewed with respect to the primary outcomes of overall and transplant related mortality (TRM) rates, and secondary outcomes of changes in modified Rodnan Skin Score (mRSS), forced vital capacity (FVC), progression/event free survival (P/EFS) and quality of life measures. We also focussed on patient characteristics, the ASCT conditioning and mobilisation regimens used, and their relationship to patient outcome in each study.*

Results. *Of the 155 articles found, only 9 articles were suitable for review. There were 2 placebo-controlled trials (RCTs), ASTIS and ASSIST, and 7 observational and cohort studies. In general, patients undergoing ASCT had diffuse SSc with mRSS >14, and interstitial lung disease. The 2 RCTs showed a benefit in P/EFS (80-81%), FVC and quality of life measures in ASCT compared to monthly cyclophosphamide. All the studies showed an improvement in mRSS. TRM rates varied among studies, from 0 to 23%, with a trend to higher mortality rates in studies using higher doses of cyclophosphamide or myeloablative conditioning regimens.*

Conclusions. *We conclude that ASCT is beneficial in some patients with SSc and that patient selection and conditioning regimens are critical determinants of prognosis and mortality post-ASCT.*

Introduction

Systemic sclerosis (SSc) is a chronic disease characterised by diffuse vasculopathy, inflammation, immune activation and tissue fibrosis (1). It is a heterogeneous disease with clinical manifestations including skin fibrosis, micro vascular changes and internal organ involvement (2, 3). In SSc normal immune regulatory systems fail resulting in abnormal activation and development of auto-reactive immune cells (4). Diffuse cutaneous disease subtype, with involvement of skin proximal to the elbows and knees, predicts internal organ involvement in SSc and thus portends a poor prognosis, with mortality rates between 5 to 10% per year (5).

To date, no therapy has been shown to reverse the natural course of the disease (6). Immune-suppressive drugs are commonly utilised to treat patients, but randomised trials have generally failed to demonstrate any long-term benefit (6).

Autologous stem cell transplantation (ASCT) has emerged as a potential treatment option for refractory autoimmune diseases, including SSc (4, 7, 8). The mechanism of action of ASCT in SSc is unknown, although proposed mechanisms include ablation, or reduction of the aberrant immune cells followed by re-constitution of a new immune system that is self-tolerant (9).

The process of ASCT is outlined in Figure 1 and involves mobilisation of CD34 haematopoietic stem cells (HSC) from peripheral blood followed by collection and cryopreservation of the stem cells by leukapheresis. Patients then undergo a conditioning regimen that causes partial or complete bone marrow ablation (10). Finally, HSC are re-infused. HSCs have the capacity for self-renewal and the ability to differentiate into mature blood cell lines, caus-

ing a re-formation of the recipient's blood or immune system (4).

The two types of conditioning regimens include myeloablative conditioning, which results in irreversible bone marrow failure (*e.g.* total body irradiation [TBI], busulfan) (10, 11), and non-myeloablative conditioning (*e.g.* cyclophosphamide, fludarabine and anti-thymocyte globulin [ATG]) resulting in only partial bone marrow suppression (10, 11).

Initial phase I/II studies showed high transplant related mortality (around 10-20%) in SSc patients undergoing ASCT (9). This has improved with time secondary to pre-transplant evaluation and by treating patients earlier in the SSc disease course (9).

The aim of this study was to systematically review the current literature and identify factors influencing outcome in ASCT in adult patients with SSc.

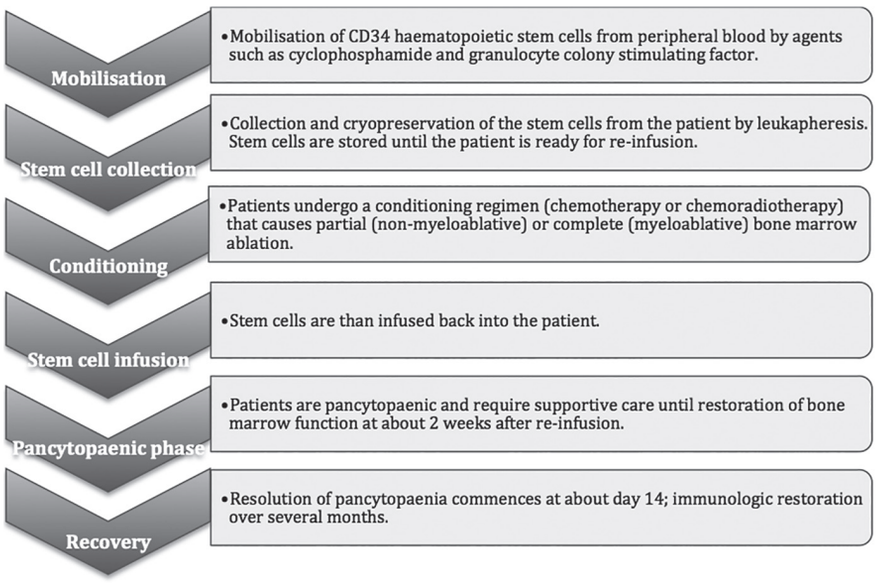

Fig. 1. The process of autologous stem cell transplantation.

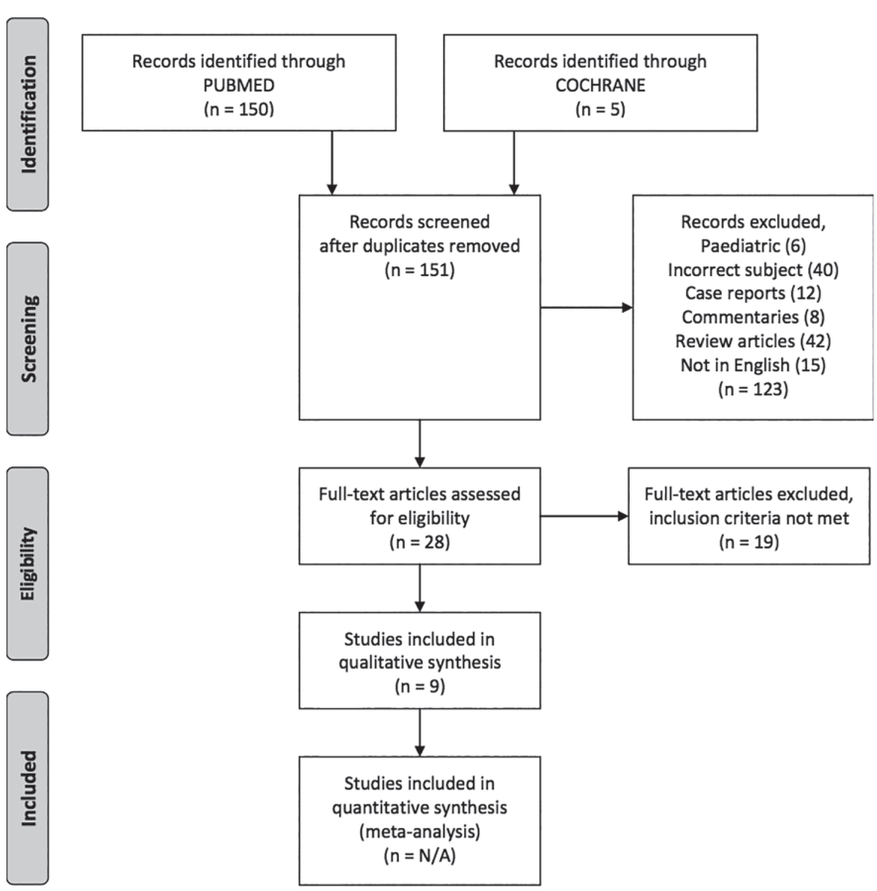

Fig. 2. Flow diagram summarising the literature search.

Methods

A literature search was performed using the PubMed database and the Cochrane library. Search terms included "autologous stem cell transplant in systemic sclerosis." The MESH terms were: "autologous" [All Fields] AND ("stem cell transplantation"[MeSH Terms] OR ("stem"[All Fields] AND "cell"[All Fields] AND "transplantation"[All Fields]) OR "stem cell transplantation"[All Fields]) AND ("scleroderma, systemic"[MeSH Terms] OR ("scleroderma"[All Fields] AND "systemic"[All Fields]) OR "systemic scleroderma"[All Fields] OR ("systemic"[All Fields] AND "sclerosis"[All Fields]) OR "systemic sclerosis"[All Fields]). The defined search period from January 2005 to March 2016 was selected in order to compare studies from the same epoch given the changes in stem cell transplantation protocols over time. Given the nature of the review no ethics approval was required.

The search was performed by two investigators (LH and JR). A total of 155 studies were identified (PubMed: 150, Cochrane: 5). Two investigators then reviewed these articles, initially by title and abstract and then in detail, using a customised data abstraction form. Studies were excluded if they were in paediatric patients (6), had incorrect subject matter (40), were duplications (4), were case reports (12), commentaries or editorials (8) or review articles (42). Only studies in English were included. Twenty-eight studies were identified for full text review as they contained original data.

Primary outcomes evaluated were transplant related mortality (TRM) and total mortality. Secondary outcomes

Table I. Study characteristics.

First Author Year [ref]	Study design	Study centres (no. locations)	Study enrolment date range	ASCT Regimen	No. SSc patients (ASCT *vs*. control)	Study follow-up (years)
Van Laar 2014 [12]	RCT (ASTIS)	Multi-centre* (28 Europe, 1 Canada)	March 2001 to October 2009	Non-myeloablative	156 (79 v 77)	7
Burt 2011 [13]	RCT (ASSIST)	Single centre (USA)	January 2006 to November 2009	Non-myeloablative	19 (10 v 9)	5
Henes 2014 [14]	Retrospective	Single centre* (Germany)	December 2008 to May 2012	Non-myeloablative	6	1
Burt 2013 [15]	Retrospective	Multi-centre§ (1 USA, 1 Brazil)	November 2002 to July 2011	Non-myeloablative	90	5
Henes 2012 [16]	Retrospective	Single centre* (Germany)	November 1997 to October 2009	Non-myeloablative	26	3
Farge 2010 [17]	Retrospective	Multi-centre* (172 Europe)	1996 to December 2007	Myeloablative / Non-myeloablative	175¶	5
Vonk 2008 [18]	Retrospective	Multi-centre* (1 Dutch, 1 French)	March 1998 to May 2004	Non-myeloablative	26	7
Nash 2007 [19]	Prospective	Multi-centre (5 USA)	July 1997 to March 2005	Myeloablative	34	8
Oyama 2007 [20]	Prospective	Single centre§ (USA)	Not stated	Non-myeloablative	10	3

ASCT: autologous stem cell transplantation; ASSIST: American Scleroderma Stem Cell versus Immune Suppression Trial; ASTIS: the Autologous Stem Cell Transplantation International Scleroderma trial; No.: number of; RCT: randomised controlled trial; SSc: systemic sclerosis.
*Includes European Group for Blood and Marrow Transplantation registered facilities.§Includes the ASSIST study centre.¶900 patients with autoimmune disease were included overall.

included change in modified Rodnan Skin Score (mRSS), progression/event free survival (P/EFS), forced vital capacity (FVC) and quality of life measures. Progression/event free survival was defined as survival without mortality, relapse or progression of SSc.
Specific attention was paid to the mobilisation and conditioning regimens, the associated TRM and overall mortality rates. Studies were excluded if they did not clearly identify the stem cell transplant regimen used or if they contained ≤5 patients. Of the 28 studies identified for full text review, only 9 studies met the inclusion criteria and were analysed (Fig. 2).

Results

Analysed studies in this systematic review included 2 placebo-controlled trials: autologous haematopoetic stem cell transplantation *versus* intravenous pulse cyclophosphamide in diffuse cutaneous systemic sclerosis: a randomised clinical trial (ASTIS) (12); and autologous non-myeloablative haematopoetic stem-cell transplantation compared with pulse cyclophosphamide once per month for systemic sclerosis (ASSIST) (13); and 7 observational and cohort studies (14-20). Table I outlines the characteristics of the studies included.

Patient selection

Table II outlines the inclusion and exclusion criteria of the studies in this review. Patients selected for ASCT were less than 65 years old. Most studies required patients to have diffuse SSc with mRSS ≥14 and disease duration of <4 years. Internal organ involvement was a further inclusion criteria in some studies and the definition of this varied amongst studies. The baseline characteristics of study patients are outlined in Table III. It is likely that there is some duplication of patients between the studies identified in this review conducted at the same study centres (see Table I), although this is often not clearly defined by the authors themselves.

Outcomes analysed

Primary outcomes varied among the studies and therefore the results could not be meta-analysed. The majority of studies included outcomes relating to mortality, disease progression, organ dysfunction and quality of life. Table IV identifies the common outcomes evaluated by each study, including primary outcomes and definitions for how these were met.

Modified Rodnan Skin Score

The maximum mRSS is 51, where higher scores indicate more severe and extensive skin fibrosis (21, 22). All of the studies that evaluated improvement in mRSS as a primary outcome met this endpoint.
In the ASSIST study, 12 months after randomisation, the mean mRSS increased in controls (19 to 22) and decreased (28 to 15, p=0.0004) in pa-

Table II. Inclusion and exclusion criteria for studies.

First Author Year [ref]	Inclusion criteria	Exclusion criteria
van Laar 2014 [12]	18-65yo, diffuse SSc, mRSS ≥15, disease duration ≤4 years, and evidence of heart, lung or kidney disease*	End stage organ failure, severe PAH (mean PASP >50 mmHg), serious co-morbidities, extensive pre-treatment with CYC (>5g IV or up to 2mg/kg body weight PO for 3 months)
Burt 2011 [13]	<60yo, diffuse SSc, mRSS >14, and internal organ involvement** Patients with little cutaneous involvement (mRSS < 14) were eligible if they had coexistent pulmonary involvement	>6 previous IV doses of CYC, TLC <45% of predicted volume, LVEF <40%, symptomatic cardiac disease, duration of SSc >4yrs, HIV positive, hepatitis B surface antigen positive, renal insufficiency (creatinine >177µmol/L), pregnancy, PASP >40mmHg, or mPAP >25mmHg
Henes 2014 [14]	Consecutive SSc patients with pre-existing cardiac involvement	None stated
Burt 2013 [15]	Diffuse SSc, mRSS ≥14 and internal organ involvement*** Patients with little cutaneous involvement (mRSS <14) were eligible if they had coexistent pulmonary involvement	>55 years or had a disease duration >4 years§ TLC <45%, LVEF <40% or PASP >42mmHg, or positive serology for HIV or hepatitis B surface antigen
Henes 2012 [16]	Inefficacy of CYC or rapidly progressive diffuse SSc with strong indicators for a bad prognosis¶	Karnofsky index <70%¥, PAH with PASP >50mmHg and DLCO <40%
Farge 2010 [17]	All consecutive patients with autoimmune disease reported to the EBMT registry for first ASCT	None stated
Vonk 2008 [18]	<66yo, rapidly progressive disease (2 years duration, mRSS >20, ESR >25mm/h and/or Hb <11g/dL, not explained by other causes than active SSc) or a disease duration >2 years plus a progression of the mRSS (>20%) plus major organ involvement related to SSc§§	Uncontrolled arrhythmia, LVEF <50% or mPAP >50mmHg, DLCO <45% of predicted, creatinine clearance <20ml/min, platelets <80000/mm^3, haemorrhagic cystitis, HIV or HTLV1 seropositivity, malignancy, pregnancy, a cardiac or vascular prosthesis, and no vascular access
Nash 2007 [19]	<65yo, ≤4 year disease duration, diffuse SSc, mRSS ≥16, significant visceral organ involvement. Other inclusions; progressive pulmonary involvement (FVC or DLCO reduction of >15% in the previous 6 months with any skin involvement)	None stated
Oyama 2007 [20]	Diffuse SSc, mRSS ≥14 and evidence of internal organ involvement¶¶	TLC <45%, LVEF <40%, or PASP >45mmHg

ASCT: autologous haematopoietic stem cell transplant; CYC: cyclophosphamide; DLCO: diffusion capacity of the lungs for carbon monoxide; EBMT: European Group for Blood and Marrow Transplantation; ECG: electrocardiogram; ESR: erythrocyte sedimentation rate; FVC: forced vital capacity; GIT: gastro-intestinal tract; Hb: haemoglobin; HRCT: high resolution computed tomography; IV: intravenous; LVEF: left ventricular ejection fraction; mPAP: mean pulmonary artery pressure; mRSS: modified Rodnan Skin Score; PAH: pulmonary arterial hypertension; PASP: pulmonary artery systolic pressure; PO: per oral; SSc: systemic sclerosis; TLC: total lung capacity; VC: vital capacity; yo: years old.

*Inclusion criteria modified in 2004 to allow inclusion of patients with disease duration ≤2 years AND major organ dysfunction as long as they had mRSS ≥20 and ESR >25mm/h and/or Hb <11g/L.

**Internal organ involvement defined as at least one of the following: DLCO <80% or decline in FVC by ≥10% in the previous 12 months, pulmonary fibrosis or ground-glass appearance on HRCT, abnormal ECG or GIT involvement.

***Internal organ involvement defined as pulmonary fibrosis or ground glass changes on CT chest, abnormal ECG, or GIT involvement.

§Exclusion not applicable to Brazil study centre.

¶Bad prognostic indicators are positivity for Scl-70 antibodies, rapid progression of skin/organ manifestations, diffuse cutaneous form, male sex, alveolitis, early PAH.

¥The Karnofsky Performance Scale Index is an assessment tool for functional impairment.

§§Major organ involvement as defined by either: (1) lung involvement with a VC or DLCO below 70% predicted, or a mPAP >40mmHg (2) GIT involvement with serum albumin <25g/L or weight loss exceeding 10% body weight in the preceding year; (3) kidney involvement with 24hr urinary protein above 0.5g or serum creatinine above 120mmol/L.

¶¶A revision of entry criteria was made after the study began to accommodate a single patient with disabling lung disease, who did not meet one of the original entry criteria of a skin score greater than 14.

tients receiving a transplant (13). Although not a primary outcome, the ASTIS study showed an improvement in mRSS with a mean change from baseline to 2 years of -19.9 in the treatment arm compared with the control group of -8.8 (difference 11.1 [95% CI: 7.3–15.0]; p<0.001) (12).

Henes *et al*. (2014), reported >25% improvement in mRSS in 4 of 6 patients (14). In the study by Henes *et al*. (2012), 18 of the 23 patients (78.3%) that survived reached the primary target of >25% improvement in mRSS at 6 months (16). Nash *et al*. (2007), showed a significant improvement in mRSS with a mean decrease at final evaluation of 22.08 (baseline 30.12, improvement of 70.3%, p<0.001) (19). Similarly in the study by Vonk *et al*. (2008), there was a significant decrease in mRSS in 73% (n=19/26) of the patients who had ASCT after 1 year and in 94% (15/16) after 5 years (18).

Table III. Baseline characteristics of study patients.

First Author Year [ref.]	van Laar 2014 [12] (ASCT *vs*. control)	Burt 2011 [13] (ASCT *vs*. control)	Henes 2014 [14]	Burt 2013 [15]	Henes 2012 [16]	Farge 2010 [17]	Vonk 2008 [18]	Nash 2007 [19]	Oyama 2007 [20]
Mean age (years)	44.2 *vs*. 43.3	45 *vs*. 44	42	42*	39*	41	42*	41*	44
Women (%)	54.4 *vs*. 63.6	90.0 *vs*. 88.9	33.3	81.0	69.2	71.0	73.1	76.5	90.0
Disease duration from diagnosis (mean years)	1.2 *vs*. 1.4	1.13 *vs*. 1.5	2.0	2.1	2.25*	2.5	2.0*	1.75	3.03
Diffuse SSc (%)	100 *vs*. 100	80.0 *vs*. 77.7	–	80.0	92.3	–	100	100	100
Mean mRSS	24.8 *vs*. 25.8	28.0 *vs*. 19.0	25.8	24.0*	18.2	–	32.0*	30.0*	28.5
Scl 70 positive (%)	66.7 *vs*. 80.5	50.0 *vs*. 77.8	83.3	48.0	73.0	–	46.0	32.4	30.0

*Data presented as median. – Data unavailable.

Pulmonary function

Pulmonary function was evaluated differently across the studies, but 6 studies reported FVC as an outcome. At 2 years follow-up the ASTIS trial, showed a mean change in FVC of 6.3% predicted *vs*. –2.8% in the control group (95% CI: –14.7 to –2.5; p=0.004) (12). In the ASSIST study, the 11 patients who had ASCT who were available for follow-up showed an improvement in FVC 15% compared with –9% in controls at 12 months (p=0.006) (13). The other four studies also showed improved FVC, although not sustained over five years in one study (15), and not statistically significant in another study (14).

Progression/event free survival

The ASTIS trial showed an improved event-free survival in the group that underwent ASCT compared to cyclophosphamide, with a hazards ratio of 0.34 at 4 years (95% CI: 0.16–0.74) (12). A striking benefit in long-term survival was noted in non-smokers.

In the smaller ASSIST trial no patient who received ASCT progressed at 12 months (odds ratio 110, 95% CI: 14·04–∞; p=0.00001), compared to 8 of 9 patients in the control group (13). Of the 8 patients who progressed on cyclophosphamide, 7 were re-allocated to receive ASCT after a mean of 14 months of enrolment and all improved. The Oyama *et al*. (2007) study, formed the basis for the ASSIST trial and showed a 90% survival at median follow-up of 2.13 years (20).

An overall survival of 78% at 5 years with a P/EFS of 70% in patients who received ASCT was reported by Burt *et al*. (2013) (15). Henes *et al*. (2012), reported a P/EFS at 3 years of 74% with an overall mortality of 27% (16).

Of the 900 patients reported by Farge *et al*. (2010), 175 of them had ASCT for SSc (17). Although the study states that the five year overall survival rate is 76%, it is not clear how this figure was arrived at, as the quoted overall mortality rate at 5 years was 27.5% (48/175), suggesting a five year survival rate of 72.6% (95% CI: 69-83%). The 5 year P/EFS in this study was 55% (95% CI: 46-64%).

Vonk *et al*. (2008), reported a P/EFS of 64.3% (95% CI: 47.9-86.3%) at 5 years and 57.1% (95% CI: 39.3-83.1%) at 7 years (18). The study by Nash *et al*. (2007), reported a P/EFS of 64% at 5 years (19).

Stem cell transplant regimen and associated survival measures

The mobilisation regimen for ASCT in the studies included intravenous cyclophosphamide (CYC), at doses from 1-4g/m^2, in combination with Granulocyte-colony stimulating factor (G-CSF) or G-CSF alone. The conditioning regimens showed further variability in agents and dosage used. Table V outlines the conditioning regimen in each study and associated progression/event free survival trends, transplant related mortality (TRM) and overall mortality rates (defined as per Table IV).

TRM in the ASCT group was significant in the ASTIS study accounting for 8 of the 11 deaths that occurred in the first year, producing a TRM of 10.1% (8/79). This compares to none of the 7 deaths that occurred in the first year in the control group being related to treatment (12). There were 42 deaths total in the ASTIS study during the median follow-up of 5.8 years, with a higher mortality in the ASCT group in the first year, but better long-term survival compared to those treated with CYC. In the ASCT group there were 19 deaths with the causes being transplant related (n=8), disease progression (n=9), cerebrovascular accident (n=1) and one patient dying of malignancy. In the control group there were 23 deaths, with the causes being disease progression (n=12), cardiovascular disease (n=4), malignancy (n=5) and 'other' causes (n=2). A further 7 patients in the control group died of disease progression outside the median follow-up (12).

In the ASSIST trial no deaths occurred in either group after 12 months of follow-up (13). Similarly in the Oyama *et al*. (2007) study, there was no TRM reported (20). The ASTIS study had a higher TRM and overall mortality compared to the ASSIST study (10.1% and 24.0% *vs*. 0% and 0% respectively). Patients who underwent ASCT in the ASTIS study had stem cells mobilised with a higher dose of intravenous (IV) CYC (4g/m^2 on 2 consecutive days). The conditioning regime included IV CYC 200mg/kg with methylprednisolone 1mg/kg over 4 consecutive days and IV rabbit anti-thymocyte globulin (rATG) 7.5mg/kg administered over three consecutive days. In the ASSIST study, stem cells were mobilised using IV CYC 2g/m^2. The conditioning regimen included a lower dose of IV rATG (total 6.5mg/kg) and CYC 200mg/kg was given in 4 equal fractions on day -5 to day -2. Intravenous methylprednisolone 1,000mg was infused before each dose of rATG.

In the Henes *et al*. (2014) study, of 6 patients there was no TRM (14). Over-

Table IV. Summary of outcomes of included studies.

First Author Year [ref.]	mRSS	PFT	P/EFS	TRM	QoL Measures	Additional measures
van Laar 2014 [12] (ASTIS)	Changes within 24 months (mid: 3.2-5.3)	DLCO, FVC, residual volume, TLC	*Time in days from randomisation until "Event": all cause mortality or persistent major organ failure§	Death adjudicated as not attributable to disease progression	EQ-5D, SF-36, HAQ-DI (mid: 0.10-0.14)	Toxicity, body weight, need for additional immunosuppressive therapy between 12 to 24 months
Burt 2011 [13] (ASSIST)	*>25% improvement at 12 months	DLCO, HRCT, TLC *FVC >10% change	Disease progression defined as increase >25% mRSS or decrease >10% FVC	Not further defined	SF-36	N/A
Henes 2014 [14]	*>25% improvement at 6 and 12 months	HRCT, FVC, DLCO	*Progression or relapse defined as increase >25% mRSS or new ground glass pattern on HRCT. Cardiac event defined as death or hospitalisation due to heart failure or ICD discharge.	Any death within 100 days of treatment	N/A	NT-proBNP, troponin, TTE
Burt 2013 [15]	>25% improvement	DLCO, FVC, TLC	Relapse defined as increase >25% mRSS from best improvement, decreased >10% FVC, renal crisis, initiation of parenteral nutrition or restarting immune suppressive or modulating medication.	*Deaths not attributable to relapse of SSc	SF-36	N/A
Henes 2012 [16]	*>25% improvement at 6 months	DLCO, HRCT, VC	*Progression or relapse defined as increased mRSS or decreased DLCO with new ground glass pattern on HRCT.	Death within 100 days of transplant without relapse or progression of autoimmune disease	N/A	N/A
Farge 2010 [17]	N/A	N/A	*Survival without any evidence of increase of disease activity index as compared to baseline	*Death within 100 days of transplant without relapse or progression of autoimmune disease	N/A	*Overall survival time to death irrespective of cause
Vonk 2008 [18]	Decrease >25%	Increase in FEV_1 >15% DLCO, FVC, TLC	Relapse defined by onset of progression after prior response to therapy. Progression defined as increase >25% mRSS or decrease >15% FVC or DLCO and/or major organ dysfunction§	Death within 6 months not attributable to disease progression	N/A	*Overall survival and disease response including improved mRSS, FEV_1, WHO PS ≤1, LVEF >45% and serum creatinine <180µmol/L
Nash 2007 [19]	*mRSS change (>5 for baseline ≤20; >25% for baseline >20)	*DLCO >15% change or FVC >10% change	Progression could be disease or treatment-related deaths or major organ failure§	Within 6 months of transplant other than for myelodysplastic syndrome	*mHAQ change more than 0.4	Dermal fibrosis grading, LVEF
Oyama 2007 [20]	Change at 6,12 and 24 months post-transplant	DLCO, TLC, VC	Not further defined	*Not further defined	N/A	LVEF, renal function

ASCT: autologous haematopoietic stem cell transplant; ASSIST: American Scleroderma Stem Cell versus Immune Suppression Trial; ASTIS: the Autologous Stem Cell Transplantation International Scleroderma trial; DLCO: diffusion capacity of the lungs for carbon monoxide; EQ-5D: European five dimensional quality of life questionnaire; FEV_1: forced expiratory volume in first second; FVC: forced vital capacity; HAQ-DI: Health Assessment Questionnaire Disability Index; HRCT: high resolution computed tomography; ICD: implantable cardioverter defibrillator; LVEF: left ventricular ejection fraction; mHAQ: modified Health Assessment Questionnaire; mid: minimally important difference; mRSS: modified Rodnan Skin Score; N/A: not applicable; P/EFS: progression/event free survival without mortality, relapse or progression of disease; PFT: pulmonary function tests; SF-36: 36-item Short Form General Health Survey; sHAQ: Scleroderma Health Assessment Questionnaire; SSc: systemic sclerosis; TLC: total lung capacity; TRM: transplant related mortality; TTE: transthoracic echocardiogram; VC: vital capacity; WHO PS: World Health Organisation Performance Status.
*Designated primary outcome of the study.
§Major organs defined as heart, lung and kidney.

Table V. Stem cell transplant regimen vs progression free survival and mortality (transplant related and overall).

Study	Control	ASCT REGIMEN			P/EFS	MORTALITY		
		Type	Mobilisation	Conditioning		TRM	Overall mortality	Years follow-up*
van Laar 2014 [12] (ASTIS)	CYC IV 750mg/m² monthly for 12 months	Non-myeloablative	CYC IV 4g/m² G-CSF 10µg/kg	CYC IV 200mg/kg day -5 to -2 rATG 7.5mg/kg for 3 days	81% (4yrs)	10.1% (8/79)	24.0% (19/79)	5.8 (IQR 4.1-7.8)
Burt 2011 [13] (ASSIST)	CYC IV 1g/m² monthly for 6 months	Non-myeloablative	CYC IV 2g/m² G-CSF 10µg/kg	CYC IV 200mg/kg day -5 to -2 rATG IV 0.5mg/kg day -5, then 1.5mg/kg for 4 days, Methylprednisolone IV 100mg (pre rATG doses)	80% (2.6yrs)	0% (0/17)	0% (0/17)	Mean 2.6 (1-5)
Henes 2014 [14]	N/A	Non-myeloablative	CYC IV 1g/m² rG-CSF 105mg	Thiotepa 2x5mg/kg day -5, CYC IV 2x50mg/kg day -2 to -1, rATG 4x10mg/kg day -4 to -1.	66.6% (1.6yrs)	0% (0/6)	16.6% (1/6)	1.6 (1-3.8)
Burt 2013 [15]	N/A	Non-myeloablative	CYC IV 2g/m² G-CSF 5–10µL/kg daily	CYC IV 200mg/kg day –5 to –2 rATG IV 0.5mg/kg day –5 and then either 1.0mg/kg or 1.5mg/kg every day –4 to day –1. Methylprednisolone IV (250–1000mg) before rATG or as part of each rATG infusion	70% (5yrs)	5.5% (5/90)	22.7% (20/88)	Target 5
Henes 2012 [16]	N/A	Non-myeloablative	CYC IV 2g/m² G-CSF 10µg/kg/day	CYC IV 4×50mg/kg day –5 to –2, rATG 4×10mg/kg day –4 to –1.	74% (3yrs)	4.0% (1/26)	27.0% (7/26)	4.4 (1.1-12.2)
Farge 2010 [17]	N/A	Myeloablative/ non-myeloablative	CYC IV 1.5–4g/m² + G-CSF or with G-CSF alone according to local protocols	TBI (7%) or various combinations of chemotherapy alone (93%). CYC at 150 or 200 mg/kg total dose (52%), busulfan (4%), and BEAM (carmustine, cytarabine, melphalan, and etoposide) (34%). Antithymocyte globulin (55%)	55% (5yrs)	6.8% (12/175)	27.4% (48/175)	2.83 (0.04-9.17)
Vonk 2008 [18]	N/A	Non-myeloablative	CYC IV 4g/m² rG-CSF 5-10µg/kg/day	CYC IV 50mg/kg/day day -5 to -2 ALG (no dose given)	64.3% (5yrs) 57.1% (7yrs)	3.8% (1/26)	19.2% (5/26)	5.2 (1-7.5)
Nash 2007 [19]	N/A	Myeloablative	G-CSF (16g/kg/d) subcutaneously	Fractionated TBI 800cGy (first 8 patients, subsequently lung shielding to 200cGy), CYC IV 120mg/kg, Equine ATG 90mg/kg; Methylprednisolone 1mg/kg IV with each dose of ATG.	64% (5yrs)	23.0% (8/34)	35.3% (12/34)	4.0 (1-8)
Oyama 2007 [20]	N/A	Non-myeloablative	CYC IV 2g/m² G-CSF 10 µg/kg/day	CYC IV 50mg/kg/day day -5 to-2 rATG 1.5mg/kg/day day -5 to -1 Methylprednisolone 1.0g/day (prior to rATG)	70% (2.13yrs)	0% (0/10)	10% (1/10)	2.13 (0.83-3.33)

ALG: anti-lymphocyte globulin; ASSIST: American Scleroderma Stem Cell *versus* Immune Suppression Trial; ASTIS: the Autologous Stem Cell Transplantation International Scleroderma trial; cGY: centigray; CYC: cyclophosphamide; G-CSF: granulocyte-colony stimulating factor; IQR: interquartile range; IV: intravenous; N/A: not applicable; P/EFS: progression/event free survival as defined in Table IV; rATG: rabbit anti-thymocyte globulin; rG-CSF: recombinant G-CSF; TBI: total body irradiation; TRM, transplant related mortality as defined in Table IV.
Data are median (range) years unless otherwise stated.

all mortality was 16.6% (1/6) with a median follow-up of 1.6 years. The one death occurred 1.6 years post-ASCT and was secondary to disease progression (skin and pulmonary arterial hypertension). This study used CYC at a lower dose of 1g/m² with recombinant G-CSF for mobilisation. The conditioning regimen involved thiotepa 2x5mg/kg on day -5 and low-dose CYC (2x50mg/kg body weight on days -2 and -1) in conjunction with

rATG 4x10mg/kg on day -4 to -1. All 6 patients partaking in this study had cardiac manifestations of their SSc prior to ASCT (14). Pre-ASCT patient screening included a 24hr holter monitor (4 of 6 were abnormal), transthoracic echocardiogram (2 of 6 were abnormal), right heart catheterisation, myocardial biopsy (all 6 had biopsy proven myocardial fibrosis) and 4 patients had a cardiac MRI (2 of 4 were abnormal). All patients received an implantable cardiac defibrillator (ICD) before ASCT (14). Half of patients had ICD events post-ASCT. No reduction in left ventricular function was found at median follow-up. As a consequence, this study recommended ICD implantation in this subgroup of patients prior to ASCT.

In the Burt *et al*. (2013) cohort, TRM was 5.5% (5/90), with an overall mortality at 5 years being 22.7% (20/88), with 2 patients being lost to follow-up (15). This study therefore gave a survival rate of 77.3% at 5 years. Eight of 13 patients who had relapsed disease had died (6 from cardiac causes, 2 from renal crisis). Burt *et al*. (2013) mobilised their patients' stem cells using CYC at a dose of 2g/m^2 together with G-CSF (15). The conditioning regime involved CYC IV 200mg/kg day -5 to -2 and rATG IV 0·5mg/kg day -5 and then either 1·0mg/kg or 1·5mg/kg IV every day -4 to day -1. The rATG was combined with methylprednisolone.

In Burt *et al*. (2013), 4 of 5 TRM deaths were from cardiovascular complications and the fifth death was from sepsis (15). Given the high early cardiac mortality rate, a standard set of investigations to evaluate cardiac risk was applied to the last 12 patients of the study, including; an echocardiogram, right heart catheterisation and cardiac MRI. The study concluded that cardiac screening guidelines for SSc should incorporate echocardiogram, as well as confrontational right heart catheterisation, including a fluid challenge test and cardiac MRI to appropriately assess cardiac risk in the setting of ASCT (15).

The study by Henes *et al*. (2012), differentiated between 100-day transplant-related mortality, (defined as death without relapse or progression of autoimmune disease) rate 4% (1/26) whereby this single death was attributed to an adverse reaction to the G-CSF/CYC regimen, compared to treatment-related mortality (any death within 100 days after treatment) rate 11.5% (3/26), and included 2 additional deaths secondary to disease progression during or shortly after mobilisation. The total mortality was 27% with a further four deaths due to disease progression over the study period (16). The transplant regimen in Henes *et al*. (2012), included mobilisation with CYC at 2g/m^2 and G-CSF at 10 μg/kg/day. Conditioning included CYC IV 4×50mg/kg days -5 to -2 and rATG 4×10 mg/kg days -4 to -1 (16).

Farge *et al*. (2010), reviewed 175 patients and found a 6.8% 100-day TRM (12/175). The total mortality was 27.4 % (48/175) with deaths due to disease progression being 13.3% (23/175). The other 13 deaths were attributed to infection (n=4), haemorrhage (n=2), malignancy (n=2, oesophageal, bronchial), interstitial pneumonitis (n=1), cardiac toxicity (n=1) and 3 patients died from other or unknown causes (17). The mobilisation and conditioning regimens in the Farge *et al*. (2010) study varied depending on the centre and local protocols (17). Mobilisation regimens included CYC IV 1.5-4g/m^2 and G-CSF, or G-CSF alone. The conditioning regime included total body irradiation (TBI) (7%) or various combinations of chemotherapy alone (93%).

Farge *et al*. (2010) found that in multivariate analysis, the 100-day TRM was lower in experienced centres (p=0.003). The centres with greater experience also showed better overall survival (p<0.0001) (17). An age <35 years at time of transplant was associated with increased P/EFS (p=0.004) and overall survival (p=0.01). It is important to note that these analyses were of the combined autoimmune cohort, not just the SSc patients. In contrast to this finding in the ASTIS study no centre effect was found, with 5 of 8 cases of TRM observed in 3 of the 4 most active autoimmune disease transplant centres in Europe (12).

In Vonk *et al*. (2008), the TRM was 3.8% (1/26), with a total mortality of 19.2% (5/26) at median follow-up of 5.3 years. Three patients died of disease progression and 1 patient, a heavy smoker, succumbed to small cell lung cancer 64 months after ASCT (18). Mobilisation in Vonk *et al*. (2008) was with CYC 4g/m^2 and G-CSF 5-10μg/kg/day. Conditioning included CYC IV 50mg/kg/day day -5 to -2 and anti-lymphocyte globulin, the dose of which was not defined in the study (18).

The TRM for Nash *et al*. (2007), was 23% (8/34). The overall mortality rate was 35.3% (12/34). Causes of death included respiratory failure (n=6, 2 treatment related and 4 disease related), renal crisis/failure (n=2), Epstein Barr virus-post-transplant lymphoproliferative disorders (n=1), cardiac arrhythmia with cardiac involvement of SSc (n=1), multi-organ failure with pulmonary haemorrhage (n=1), and myelodysplastic syndrome (n=1). In this study the ASCT regimen included fractionated TBI 800cGy, CYC IV 120mg/kg, equine ATG 90mg/kg and Methylprednisolone 1mg/kg IV with each dose of ATG (19).

Discussion

The ASTIS (12) and ASSIST (13) studies are the only randomised control trials available in this review and their findings therefore, are more robust than the other studies described. Both of these placebo-controlled studies suggest an improved long-term patient outcome with ASCT compared to pulse cyclophosphamide in the poorer prognosis patients with diffuse cutaneous SSc. Therefore, it is not surprising that these patients are keen to be considered for ASCT.

Reviewing the literature would suggest that the type of conditioning regimen used could influence transplant related mortality. Of the two RCTs, the ASTIS study utilised a higher total dose of CYC in the mobilisation phase, a higher dose of rATG and a lower dose of methylprednisolone than the ASSIST study. The ASTIS study had a much higher TRM and overall mortality compared to the ASSIST study (10.1% and 24.0% *vs*. 0% and 0% respectively). It must be noted however that the ASTIS study included larger numbers

of patients, which could also influence the TRM. ASTIS was a multicentre study while ASSIST was a single centre study, which may have played a role in the observed differences.

The highest mortality rates were in the Nash *et al*. (2007) study with a 23% TRM and 35.2% overall mortality. The definition for time frame of TRM in this study was not clear, with one death at 20 months being attributed to a TRM (19). One other possible explanation for the higher TRM rates is that a myeloablative regimen was used in this study including fractionated TBI. This study adjusted its conditioning to include shielding of the kidney and lungs after pulmonary and renal toxicities occurred in the first 8 patients.

Henes *et al*. (2014) adopted a less toxic, thiotepa-based conditioning regimen for their patients who already had cardiac involvement from their SSc, with no TRM reported in this study (0/6).

In Farge *et al*. (2010), the conditioning regime included TBI (7%) or various combinations of chemotherapy (93%). The chemotherapeutic options included CYC at 150 or 200 mg/kg total dose (52%), busulfan (4%), and BEAM (carmustine, cytarabine, melphalan and etoposide) (34%). These more myeloablative regimes could contribute to the slightly higher mortality rates observed (TRM 6.8%, overall mortality 27.4%) (17).

A thorough cardiac work-up of patients prior to selection for transplantation was an important issue identified in this review. Deaths from cardiac disease appear prominent in SSc patient's receiving ASCT suggesting the need for a full and complete pre-transplantation cardiac assessment, beyond that usually performed in patients receiving ASCT for other conditions (12, 13).

The results of this systematic review suggest that patient selection and conditioning regimens are critical in the prognosis and mortality of SSc patients post ASCT. A non-myeloablative conditioning regime, with a lower dose of CYC and rATG may impact positively on survival by reducing treatment related mortality (Table V). The experience of the centre performing the ASCT may influence outcome, however there has been some conflicting data in relation to this (12, 17).

It is likely that disease duration at time of ASCT may influence the P/EFS. Several studies have chosen to include disease duration as part of their inclusion and exclusion criteria, with many giving a cut-off of <4 years (12, 13, 15, 19). From the data presented in this review, no clear conclusions can be drawn in relation to the association between disease duration at the time of ASCT and survival. However, the data suggest that there may be a trend towards improved P/EFS in patients with shorter disease durations prior to ASCT (12, 13), although this is not borne out by all studies (19).

Whilst this review suggests an improved outcome in selected scleroderma patients treated with ASCT, it does not suggest a more intense conditioning regime correlates with improved outcome.

A recent review by Rebeiro and Moore (2016) proposed some factors to consider for optimal outcome in SSc ASCT patients. Patient factors of age <60 years, non-smokers, mean pulmonary artery pressure (mPAP) <25mmHg, total lung capacity (TLC) >50% and a DLCO >50% were identified as protective. Diffuse scleroderma, duration of disease <5 years and pulmonary fibrosis were disease characteristics offered on which to base patient selection for ASCT (23).

Conclusion

Several studies, including 2 placebo controlled trials, show a survival benefit in a select group of patients with scleroderma undergoing ASCT compared to standard care. However, available evidence does not provide clear guidance on where ASCT should be incorporated in the treatment algorithm, the ideal pre-treatment work up and the best conditioning regimen. Further studies are required to improve our understanding of where and how ASCT should best be used in this rare disease.

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