Autologous Hematopoietic Stem Cell Transplantation for Systemic Sclerosis

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Abstract: Systemic sclerosis is a rare disorder manifesting as skin and internal organ fibrosis, a diffuse vasculopathy, inflammation, and features of autoimmunity. Patients with diffuse cutaneous disease or internal organ involvement have a poor prognosis with high mortality. To date no therapy has been shown to reverse the natural course of the disease. Immune suppressive drugs are commonly utilized to treat patients, but randomized trials have generally failed to demonstrate any long-term benefit. In phase I/II trials, autologous hematopoietic stem cell transplantation (HSCT) has demonstrated impressive reversal of skin fibrosis, improved functionality and quality of life, and stabilization of internal organ function, but initial studies were complicated by significant treatment-related mortality. Treatment-related mortality was reduced by better pre-transplant evaluation to exclude patients with compromised cardiac function and by treating patients earlier in disease, allowing selected patients the option of autologous HSCT treatment. There are currently three ongoing randomized trials of autologous HSCT for systemic sclerosis: ASSIST (American Systemic Sclerosis Immune Suppression versus Transplant), SCOT (scleroderma cyclophosphamide versus Transplant), and ASTIS (Autologous Stem cell Transplantation International Scleroderma). The results from these trials should clarify the role of autologous HSCT in the currently limited therapeutic arsenal of severe systemic sclerosis.

Keywords: Scleroderma, systemic sclerosis, hematopoietic stem cell, transplantation.

INTRODUCTION

Systemic sclerosis (SSc) is a chronic systemic autoimmune disease of unknown etiology. Its prevalence is estimated between 38 and 76/1,000,000, having geographical variations, with higher rates reported in USA and Australia. SSc exhibits a female predominance with a sex ratio ranging between 4 and 14:1 and manifesting more often in the fourth and fifth decades of life [1-5].

The terms systemic sclerosis and scleroderma are often and inaccurately used interchangeably. Scleroderma is fibrosis or sclerosis of the skin and subcutaneous tissue, and by definition includes both localized scleroderma and systemic sclerosis. Localized scleroderma is a type of scleroderma that dermatologist refer to as morphea. It is localized to the skin and is almost always without Raynaud's phenomena, acrosclerosis, or internal organ involvement. Systemic sclerosis is fibrosis of the skin and subcutaneous tissue and is associated with internal organ involvement and Raynaud's phenomena. It is a systemic process that includes, to varying degrees, fibrosis, systemic inflammation, autoimmunity, and a vasculopathy.

In 1980, the American College of Rheumatology (ACR) defined scleroderma as possessing either one major criterion or 2 of 3 minor criteria. The one major criterion is sclerosis of the skin proximal to the metacarpal-phalangeal joints. The 3 minor criteria are sclerodactyly (sclerosis of the fingers), digital pitting, or bilateral basal pulmonary fibrosis [6]. This definition incorporates a large and heterogeneous spectrum of scleroderma disorders. For example, a patient with digital pitting and bilateral lung fibrosis is defined as having scleroderma even without any cutaneous sclerosis. In 1988, the ACR went further in characterizing scleroderma by defining subsets of scleroderma. These subsets were based on clinical characteristics and not distinct criteria and were categorized as: 1) diffuse cutaneous systemic slcerosis (dcSSc), 2) limited cutaneous systemic sclerosis (lcSSc), 3) localized scleroderma and 4) overlap syndromes in which features of systemic sclerosis (usually dcSSc) occurred along with characteristic features of other systemic inflammatory autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus, polymyositis, Sjogren's syndrome, or mixed connective tissue disease [7].

The general clinical characteristics of dcSSc are: onset of Raynauds within 1 year of skin sclerosis (puffy swelling or hidebound), involvement of truncal and acral skin, early internal organ involvement (lung, kidney, gastrointestinal tract, heart), presence of tendon friction rubs, usually absence of anti-centromere antibodies, nailfold capillary dilatation and destruction and, in approximately 30% of patients, presence of anti-topoisomerase antibodies. In contrast, the general clinical characteristics of lcSSc are depicted as: Raynauds for years or decades preceding skin involvement that is either limited to hands, face, feet, and forearms (i.e. acral) or with no skin involvement at all; late and delayed incidence of pulmonary hypertension with or without interstitial lung disease; skin calcifications or telangiectasias; a high inci-

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dence (70-80%) of anti-centromere antibodies; and dilated nailfold capillaries without destruction or dropout.

The 1988 ACR criteria are useful for identification of subsets of scleroderma spectrum disorders and have prognostic value for disease-associated mortality. That is, dcSSc is associated with high mortality; lcSSc with low mortality; and localized scleroderma (morphea), that has skin hardening without the vasculopathy or internal organ involvement associated with systemic sclerosis, has an excellent prognosis.

This scheme, based on distinguishing diffuse SSc from limited SSc, has become accepted as the gold standard for the classification of patients with SSc, and has been largely validated in clinical trials. However, other classifications and subsets of scleroderma spectrum disorders such as "intermediate" SSc and "systemic sclerosis sine scleroderma" (no skin involvement) are continually being proposed in order to better define mortality risk and predisposition to specific disease manifestations (Table **1**). It is, therefore, important that physicians and investigators clarify the terminology used to define scleroderma subsets, especially the extent of skin involvement used to categorize diffuse versus limited systemic sclerosis.

PATHOGENESIS

The pathogenesis of systemic sclerosis involves: 1) fibrosis of skin as well as of internal organs (interstitial lung disease, pulmonary fibrosis, biliary cirrhosis, retroperitoneal fibrosis, myocardial fibrosis, gastrointestinal fibrosis with

Table 1. Classification of Systemic Sclerosis Subsets

Study, Year of Publication	Classification System
Goetz, 1945 [8]	2 subsets based on skin thickening: acrosclerosis (limited to extremities) and diffuse (includes trunk)
Tuffanelli, 1962 [9]	2 subsets: acrosclerosis: RP, acral skin involvement; diffuse SSc: no RP, skin involvement beginning centrally
Winterbauer, 1964 [10]	CRST syndrome: calcinosis, RP, sclerodactyly, teleangiectasia
Barnett, 1969 [11]	3 subsets based on skin involvement: limited (fingers only), moderate (limbs and face), extensive (trunk)
Rodnan, 1979 [12]	3 subsets: classical disease involving skin of the trunk, face and proximal extremities and early involvement of esophagus, intestine, heart, lung and kidney; CREST syndrome; Overlap syndromes including sclerodermatomyositis and mixed connective tissue disease
Giordano, 1986 [13]	3 subsets based on skin involvement: limited (fingers, face, neck, axillae; intermediate (proximal to fngers); diffuse (trunk)
Holzmann, 1987 [14]	5 subsets (type I-IV) based on presence/absence of RP, sclerosis, extracutaneous manifestations, ANA
LeRoy, 1988 [7]	2 subsets: dcSSc: onset of Raynaud within 1 year, truncal and acral skin involvement, tendon friction rubs, early incidence of ILD, renal failure, diffuse GI disease, myocardial involvement, absence of ACA, abnormal NC; lcSSc: RP for years, skin involvement limited to hands, face feet, forearms or absent, late incidence of PAH, trigeminal neuralgia, calcinosis, teleangiectasia, high incidence of ACA, abnormal NC.
Masi, 1988 [15]	3 subsets based on skin involvement: digital (fingers or toes); proximal extremity (proximal extremities or face); truncal (thorax or abdomen)
LeRoy, 2001 [16]	4 subsets: limited SSc (LSSc) consists of (1) objective RP plus any one of NC changes or SSc selective antibodies OR (2) subjective RP plus both NC changes and SSc selective autoantibodies; lcSSc: criteria for LSSc plus distal cutaneous changes; dcSSc: criteria for lcSSc plus proximal cutaneous changes; diffuse fasciitis with eosinophilia: proximal cutaneous changes without criteria for LSSc or lcSSc
Ferri, 2002 [17]	4 subsets: sine scleroderma SSc: absence of cutaneous involvement with visceral involvement, NC changes and autoantibodies; limited cutaneous: skin involvement of fingers with or without involvement of neck, face, and axillae; intermediate cutaneous: skin involvement of upper and lower limbs, neck and face without truncal involvement; diffuse cutaneous: distal and truncal skin involvement
Scussel-Lonzetti, 2002 [18]	4 subsets: normal skin; limited: skin involvement restricted to fingers, with RP, calcinosis, esophageal involvement and teleangiectasia; intermediate: skin involvement of arms proximal to metacarpophalangeal but not trunk; diffuse: skin involvement of the trunk
Maricq, 2004 [19]	6 subsets: diffuse, intermediate, digital, scleroderma sine scleroderma, UCTD with scleroderma, CREST syndrome
Nishimagi, 2004 [20]	3 subsets: lcSSc; RPSSc: dcSSC with mRSS higher than 15 points within a year from the first symptoms; non-RPSSc : all the other dcSSc patients
Hunzelmann, 2008 [21]	5 subsets: lcSSc, dcSSc, SSc sine scleroderma, overlap-syndrome and UCTD with scleroderma features.

Adapted from Johnson *et al*. [22]

ACA: anicentromere antibodies; CREST: calcinosis, RP, esophageal involvement, sclerodactyly, teleangiectasia; dcSSc: diffuse cutaneous systemic sclerosis; GI: gastrointestinal; ILD: interstitial lung disease; lcSSc: limited cutaneous systemic sclerosis; mRSS: modified Rodnan skin score; NC: nailfold capillary; PAH: pulmonary artery hypertension; RP: Raynaud`s phenomenon; RPSSc: rapid progressive systemic sclerosis; UCTD: undifferentiated connective tissue disease

poor peristalsis, pseudo-obstruction, hide bound intestine, and patulous esophagus), 2) a diffuse vasculopathy (Raynaud's phenomena, pulmonary artery hypertension, telangiectasia, renal crises, digital ulcerations, and gastric antral vascular ectasis or GAVE), and 3) evidence of systemic immune activation/inflammation (pleuritis, pericarditis, myositis, synovitis, alveolitis, and numerous autoantibodies).

The extent and severity of skin involvement has been recognized as a surrogate marker for internal organ involvement. It is assessed by repeated measurements of the modified Rodnan skin score (mRSS) [23].

Skin thickness is assessed by clinical palpation of 17 body areas on a scale of 0 to 3 (normal, mild, moderate, severe). However, a lack of significant skin sclerosis does not exclude organ manifestations and the possibility of severe complications [24, 25].

Nailfold capillary microscopy (NBM) is a method to visualize the vasculopathy of SSc by examining finger nail bed capillaries under a microscope. NBM scores capillaries according to: 1) presence or absence of capillary telangiectasia, 2) degree of capillary dilatation (0=normal, 1 is < 2 x normal, 2 = 2 to 4 times normal, 3 is greater than 4 times normal diameter), and extent of avascular areas (A = no capillary loss, B= rare avascular areas, C = moderate capillary loss, D = extensive capillary loss). In general, localized scleroderma (morphea) has no capillary abnormalities, limited SSc has capillary dilatation without capillary loss, and diffuse SSc has dilated capillaries with capillary loss [26].

Systemic sclerosis has also been associated with diminished number of circulating endothelial progenitor cells (EPC) in the peripheral blood. EPCs (CD133+Lin-) are marrow-derived hematopoietic cells that differentiate into endothelial cells and participate in angiogenesis. Circulating number of EPCs indirectly correlates with disease severity, in that patients with digital ulcerations, and patients with higher severity scores (Medsger score) have the lowest circulating EPC number [27]. Patients with SSc also have diminished *ex vivo* function of EPCs demonstrated by lower hypoxia-induced expression of vascular endothelial growth factor-1 receptor (VEGF-1R) compared to healthy patients [28].

Anti-nuclear antibodies (ANA) are detected in more than 90% of SSc sera. SSc-associated ANA include anticentromere (anti-CENP-B), ani-Th/To, anti-topoisomerase I (anti-topo 1, also known as Scl-70) and anti-RNA polymerase III (anti-RNAPIII). CENP-B is a centromere-located protein located in the DNA region on the chromosome where the two sister chromatids (one from each parent) come into contact. Anti-Th/To antibodies recognize RNAse MRP / RNase P ribonucleoproteins (nuclear proteins that contain RNA). Topoisomerases are proteins that wind and unwind DNA to allow transcription and/or replication; RNA polymerase III transcribes DNA into small RNAs such as transfer RNA (tRNA) and ribosomal RNA.

In general, morphea is associated with anti-Th/To autoantibodies; limited SSc is associated with both anti- CENP-B and anti-Th/To antibodies; and diffuse SSc is associated with anti-Topo I and anti-RNA polymerase III antibodies. None of these antibodies are pathognomonic for morphea or systemic sclerosis and whether any of these antibodies are pathogenic or an epiphenomena remains unclear [29].

Recently, several additional autoantibodies directed against non nuclear antigens have been detected in scleroderma sera and experimental evidence is accumulating supporting their role in tissue damage. They include anti-endothelial cell antibodies (AECAs), antifibrillin-1 (anti-FBN1) antibodies, antibodies against matrix metalloproteinases (MMP), and anti-platelet-derived growth factor receptor l (PDGFR) antibodies. AECAs may be pathogenetic in the vascular damage of SSc. AECAs induce apoptosis of endothelial cells *in vitro* which could lead *in vivo* to the widespread loss of capillaries and obliterative intimal proliferation in small arteries which is characteristic of SSc [30, 31]. FBN1 is a 350 kDa glycoprotein which is the major constituent of microfibrils in the extracellular matrix (ECM). Circulating autoantibodies to FBN1 have been detected in most SSc patients and are specific for the disease [32]. Recent in-vitro studies have shown that they activate normal fibroblasts, resulting in increased production of collagens and other ECM components [33].

Antibodies to MMP1 and MMP3, whose functions are to break down collagens and other matricellular components, also appear to be specific for SSc sera. Such antibodies have been proposed to cause a failure of degradation of those ECM components that accumulate as fibrotic material in the ECM of patients with scleroderma [34, 35]. Anti-PDGFR autoantibodies were shown to activate PDGFR, thus inducing the Ha- Ras-ERK1/2 pathways leading to increased synthesis of reactive oxygen species which stimulated type I collagen gene expression and promoted the conversion of normal human fibroblasts into myofibroblasts [36, 37].

Anticardiolipin antibodies (ACLA) have been reported to occur in 0 to 25% of patients with SSc but reports of clinical associations are limited [38]. The association of thrombosis and antiphospholipid antibodies, detected as lupus anticoagulant (LA) and/or anticardiolipin antibodies (ACLA), although rare, has been described in SSc, supporting SSC as a cause for "secondary" *antiphospholipid syndrome* [39]. While APS-related anti-beta-2 glycoprotein 1 (anti-β2-GP1) is less well studied, one study has shown their correlation with pulmonary hypertension and raised mean pulmonary artery pressure [40].

SURVIVAL

Important to any classification system is the ability to predict survival. Localized scleroderma (i.e. morphea) may occur in limited patches, be generalized, or in rare and severe cases, be pansclerotic. While the prognosis of morphea is generally very favorable, does not shorten life-expectancy, and generally does not cause joint contractures, the pansclerotic variant of morphea is associated with disabling joint contractures and development of squamous cell cancer in areas of chronic skin inflammation. In terms of systemic sclerosis, the prognosis for limited SSc is significantly better than diffuse SSc. As a rough rule of thumb, mortality for limited versus diffuse SSc sclerosis is 1-4% versus 5-10% per year, respectively [41].

Since systemic sclerosis is a heterogeneous clinical and pathologic entity, several studies have evaluated extent of skin and organ involvement, nail bed capillary involvement, autoantibodies, and signs of inflammation or immune activation for prognostic significance. In univariate analysis numerous poor prognostic factors have been reported including: diffuse skin involvement [17, 18, 42-48, 58, 59], male sex [17, 45, 47, 50], older age at the onset of the SSc [17, 18, 42-44, 46, 51-53, 59], internal organ involvement including the heart [17, 18, 42-46, 49-52, 54-56, 58], kidney [17, 18, 43-45, 52-54, 56, 57, 59], gastrointestinal tract [43, 54, 57], lung [17, 18, 42-46, 49, 50, 53, 54, 57] presence of pericardial effusion or pericarditis [44, 45, 53], clinical signs of right heart failure [46, 50], anti-topoisomerase antibody positivity [18, 42, 46], anaemia [42-44, 52, 53, 57], increased erythrocyte sedimentation rate (ESR) [18, 42-44, 49, 52, 59], increased C reactive protein [45], abnormal urine sediment [42, 55], proteinuria [42, 52], pigmentation disturbances [43, 59], and elevated peripheral blood soluble tumor necrosis factor (sTNF) alpha-receptor, or soluble interleukin-2 receptor (sIL-2R) [60]. Conversely, anticentromere antibody positivity [17, 59], limited skin involvement [13], or improvement in skin thickening (decrease in modified Rodman skin score by 25% or more) conveys a favourable prognosis [25, 61].

Since there exists considerable overlap in skin, organ, and laboratory abnormalities for any one patient with systemic sclerosis, a few studies have attempted multivariate analysis to identify independent predictors of mortality (Table **2**). Independent predictors for survival from the multivariate analysis are shown in Table **2** and include: pulmonary involvement (DLCO or FVC < 70%), renal involvement (proteinuria, elevated creatinine, low creatinine clearance, diastolic hypertension, prior renal crisis, or elevated BUN), cardiac involvement (arrhythmia, heart failure, persistent moderate to large pericardial effusion, pulmonary artery hypertension), elevated sedimentation rate, low hemoglobin, dcSSc, or skin involvement of the trunk. In the future, presence or absence of these independent prognostic values may allow stratification of mortality and/or enrollment and randomization in clinical trials independent of just the currently accepted limited versus diffuse SSc classification.

STANDARD THERAPY

Better medical supportive care for prevention or control of systemic sclerosis-related vasculopathy has likely contributed to a gradual improvement in survival in more recent studies for patients with SSc compared to prior studies. Angiotension enzyme inhibitors treat and prevent renal crises, calcium channel blockers such as nifedipine or amlodipine treat Raynaud's phenomena, and pulmonary artery vasodilators such as bosentan (endothelin 1 inhibitor) or sildenafil (phosphodiesterase V inhibitor) decrease pulmonary artery pressures in patients with pulmonary artery hypertension.

A definitive answer to the question of whether immune suppressive medications benefit patients with systemic sclerosis is not available, despite their widespread and decades long history of clinical usage of immune modulating and suppressive medications such as prednisone, methotrexate, azathioprine, cyclophosphamide, mycophenolate mofetil, cyclosporine, and chloroquine are commonly used for patients with diffuse SSc and overlap syndrome. However, randomized trials that support statistically significant improvement are generally non-existent. In two small randomized trials of 29 and 70 patients, respectively, methotrexate was not significantly better than placebo in improving skin score or pulmonary function [63, 64]. D-penicillamine demonstrated no improvement in skin score in a randomized double-blinded trial [65]. Randomized trials of azathioprine, chloroquine, mycophenolate mofetil or cyclosporine versus placebo have not been reported.

Besides increasing risk of infection, two immune modulating medications, corticosteroids and especially cyclosporine, have been reported to precipitate renal crisis [66, 67]. Radiation, also an immune-suppressive agent, is not used as a treatment for SSc. In fact, only one trial using radiation (total nodal irradiation) to treat SSc has been reported. The trial was stopped following randomization of only 6 patients after 3 patients on the radiation treatment arm developed either gastrointestinal or lung related SSc exacerbation [68]. The assessment of risk benefit for immune suppressive medications is especially relevant for design of hematopoietic stem cell transplant trials since autologous hematopoietic stem cell transplantation is a method to deliver intense short-term immune suppression.

The immune suppressive drug most commonly used for diffuse SSc and / or for SSc-related interstitial lung disease (ILD) is cyclophosphamide, administered either orally or intravenously. It is the only immune suppressive drug with reports of short-term efficacy in randomized trials and in systemic sclerosis-related ILD. As shown in Table **3**, cyclophosphamide as been given either orally at daily intervals for up to 1 year or intravenously at doses between 500 mg to 1000 mg /m2 at monthly intervals for 6 to 18 months. Three randomized trials and one meta-analysis have been performed [69-72].

One study by Nadashkevich *et al*. compared intravenous cyclophosphamide to azathioprine, a drug commonly used but not proven effective in a randomized trial. Patients were randomized to 18 months of oral cyclophosphamide (1-2 mg/kg/day) versus oral azathioprine (2-2.5 mg/kg/day). Skin tightness (modified Rodnan skin score) improved significantly in the cyclophosphamide treated group. DLCO and FVC did not decline in the cyclophosphamide group but significantly worsened for patients receiving azathrioprine. In comparison, two studies compared cyclophosphamide to placebo [72]. Hoyles *et al*. reported that six monthly infusions of cyclophosphamide (600mg/m^2) followed by maintenance daily oral azathioprine was not significantly different than placebo in slowing pulmonary function decline (DLCO and TLC), although there was a non-significant trend towards improvement in FVC in the treatment arm [69]. Taskin *et al*. performed a randomized trial of oral cyclophosphamide (< 2 mg/kg/day) versus placebo for 1 year and then followed patients off therapy for an additional year. Skin score improved significantly at 1 year in the treated group. FVC and DLCO declined in both treated and placebo groups but the decline in FVC was significantly less at 1 year in patients receiving cycloposphamide [70]. With longer follow-up, there was no significance difference in FVC and

Table 2. Independent Predictors of Mortality in Systemic Sclerosis by Multivariate Analysis

Study, Year of Publication	No. of Pts	Statistical Method / How Results Reported	Independent Predictors of Mortality	Results*
Wynn, 1985 [50]	64	Cox regression analysis/Risk ratio	S3 gallop Age at onset (70 ys vs 20 ys old)	5.44 (p= 0.0037) 20 (p= 0.0017)
Zarafonetis, 1988 [51]	390	Cox proportional hazards model/Coefficient	Heart involvement (abnormal EKG, pericardial effusion or CHF by CXR)	2 (p= 0.01)
Bulpitt, 1993 [55]	48	Stepwise Cox proportional hazards analysis/Hazards ratio (95% CI)	Abnormal urine sediment	4.57 (1.48-14.1)
Nagy, 1997 [44]	171	Cox proportional hazards regression analysis with time-dependent data/Risk ratio (Pr > Chi-Square)	SRC Pigmentation disturbances, CHF Anaemia (Ht < 33%) Respiratory failure	9.38 (0.01) 6.15 (0.01) 10.71 (0.03) 7.49 (0.01) 37.8 (0.01)
Bryan, 1999 [42]	280	Logistic regression model /Odds Ratio (95% CI)	Proteinuria ESR ≥ 25 DLCO ≤ 70%	23.6 (1.9–298.6) 7.4 (2.6–21.2) 8.8 (1.8–44.5)
Clements, 2000 [49]	134	Stepwise multivariate logistic regression/Odds ratio(95% CI)	Lung involvement mRSS > 20	6.1 (1.6–23.1) 3.7 (1.2–11.7)
Jacobsen, 2001 [46]	174	Cox regression analysis/Relative risk (95% CI)	Right heart failure dcSSc SRC DLCO < 40%	12.4 (2.5-60) 7.8 (1.8-35) 6.1 (1.8-21) 4.8 (1.1-20)
Ferri, 2002 [17]	1012	Cox proportional hazards model/Hazards ratio (95% CI)	icSSc dcSSc SRC	2.88 (1.43–2.40) 4.89 (1.43–2.40) 3.76 (2.61–5.43)
Scussel-Lonzett, 2002 [18]	309	Stepwise Cox proportional hazards analysis/Hazards ratio (95% CI)	Trunk involvement DLCO ≤ 70% Increased ESR ≥ 25 mm/h Hemoglobin ≤ 12.5 g/dL	3.6 (1.57–8.3) 2.88 (1.43–5.8) 3.89 (1.68–8.95) 2.37 (1.15–4.87)
Simeon, 2003 [53]	79	Cox proportional hazards model/incidence density ratio (95% CI)	Age at diagnosis over 60 yr FVC < 70% SRC	24.7 (2.9–205.1) 22.2 (4.4–111.7) 45.9 (6.4–331.6)
Nagy, 2005 [45]	80	Cox proportional hazards regression analysis with baseline data/Risk ratio (95% CI)	SRC Pericarditis PIIINP dcSSc	2.27 (0.51-10.11) 2.31 (0.42-12.7) 5.65 (1.39-23.04) 3.53 (0.84-14.84)
Czirjak 2008 [59]	366	Stepwise Cox proportional hazards model/Risk ratio (95% CI)	Increased ESR SRC dcSSc Early malignancy	3.00 (1.83–4.93) 3.38 (1.87–6.10) 2.37 (1.49–3.78) 3.20 (1.62–6.32)
Hachulla, 2009 [62]	546	Stepwise Cox proportional hazards analysis/Hazards ratio (95% CI)	PAH (mean PA >25mmHg at rest or >30mmHg during exercise) mRSS (per 1 point)	7.246 (4-13.2) 1.05 (1.02-1.06)

* only listed if > 2, BP: blood pressure; CHF: congestive heart failure; CXR: chest x ray; dcSSc: diffuse cutaneous systemic sclerosis; DLCO: carbon monoxide lung diffusion; ESR: erythrocyte sedimentation rate; Ht: hematocrit; FVC: forced vital capacity; icSSC: intermediate cutaneous systemic sclerosis; mRSS: modified Rodnan skin score; PAH: pulmonary artery hypertension; PIIINP: amino terminal propeptide of type III procollagen; RF: renal failure; SRC: scleroderma renal crisis

skin score between cyclophosphamide and placebo controls after 2 years [71].

A recent meta-analysis systematically reviewed the effect of cyclophosphamide treatment on pulmonary function in patients with systemic sclerosis and interstitial lung disease. The above three randomized clinical trials and six prospective observational studies with at least 12 months follow up were included for analysis. Based on improvement defined

Table 3. Clinical Studies Performed with Cyclophosphamide in Systemic Sclerosis*

First Author Study Design	No. of Pts	Regimen	Follow-up (mo)	Outcome (ILD)	Outcome (mRSS)
Hoyles, 2006 Randomized, Double-Blind, Placebo-Controlled [69]	45	IV Cy + oral pred x 6mo / Maintenance: AZA x 6 mo vs. placebo	12	FVC n.s. DLCO n.s.	na
Tashkin, 2006 Randomized, Double-Blind, Placebo-Controlled [70, 71]	158	Oral Cy (2 mg/kg/d) vs. placebo x 12 mo.	24	At 12 mo:* Cy better FVC: (P=0.03) TLC: (P = 0.026) At 24 mo n.s.	At 12 mo:* Cy better Change -3.06 (P = 0.008). At 24 mo n.s.
Nadashkevich, 2006 Randomized unblinded trial [72]	60	oral Cy x 18 mo vs oral AZA x 18 mo	18	FVC: worsening in AZA group (P <0.01) DLCO: worsening in AZA group (P <0.01)	Improved after 12 (P <0.001) and 18 mo (P <0.01) in Cy group
White, 2000 Retrospective uncontrolled [76]	103	Oral Cy 2 x 12 mo. or IV Cy x 6–9 mo vs no treatment	13	Cy pts: FVC (72%) and DLCO (49%) stable or better Pts no Cy: FVC (–7.1%) and DLCO (-9.6%) decrease	na
Giacomelli, 2002 Prospective uncontrolled [77]	23	IV Cy + oral pred x 6 mo	6	FVC ns DLCO ns	na
Pakas, 2002 Prospective uncontrolled [78]	28	IV Cy x 12 mo + low-dose pred or high-dose pred	12	In high-dose steroid group FVC ($P \leq 0.001$) and DLCO ($P = 0.029$) improve at 12 mo.	skin involvement -5.4%; (P = 0.01) at 12 mo.
Beretta, 2007 Prospective uncontrolled [79]	33	Oral Cy + oral pred x 12 mo.	12	DLCO: increased at 12 mo. ($P < 0.001$) FVC: n.s.	na
Berezne, 2008 Retrospective uncontrolled [80]	27	IV Cy x 6mo Maintenance: AZA x 18 mo	24	FVC, TLC, DLCO n.s.	na

* only listed studies including > 20 patients, AZA: azathioprine; Cy: cyclophosphamide; dcSSc: diffuse cutaneous systemic sclerosis; DLCO: carbon monoxide lung diffusion; ESR: erythrocyte sedimentation rate; FVC: forced vital capacity; ILD: interstitial lung disease; IV: intravenous infusion; mo: months; mRSS: modified Rodnan skin score; n.a: not available; n.s.: not significant; pred: prednisone; RCT: randomized clinical trial; TLC: total lung capacity

as at least 10% increase in DLCO or FVC, cyclophsophamide treatment in patients with SSc-related ILD did not result in clinically significant improvement of pulmonary function. In summary, even cyclophosphamide, the one immune suppressive medication thought most effective for systemic sclerosis, lacks convincing data from randomized trials to unequivocally demonstrate its efficacy [73].

Despite lack of convincing evidence-based literature, immune suppressive medications are commonly employed to treat SSc. Data on duration and dosage of corticosteroids and immunosuppressive agents were analyzed from 1,729 patients who were registered in the German Network for Systemic Scleroderma. A total of 41.3% and 35.8 % of all registered SSc patients had been treated respectively with corticosteroids and immunosuppressants. The most commonly prescribed drugs were methotrexate (30.5%), cyclophosphamide (22.2%), azathioprine (21.8%), and (hydroxy) chloroquine (7.2%) [74]. These findings are consistent with those of Pope and colleagues reporting data for Canada and North America [75].

RATIONALE AND METHODS OF HSCT FOR SYSTEMIC SCLEROSIS

The hematopoietic stem cell is also the "immune" stem cell. Hematopoietic/immune stem cells are CD34+/ CD133+/lin- (lineage negative) and have no immune function themselves, but like all stem cells are capable of self-renewal and differentiation into committed progenitors. Hematopoietic stem cells differentiate into mature T-lymphocytes, B-lymphocytes, macrophages, and dendritic cells. The rationale for an autologous hematopoietic (immune) stem cell transplant is to cytoreduce deleterious autoreactive immune cells and then reinfuse hematopoietic stem cells to regenerate new immune cells, resulting in an immune reset [81].

Before transplant, hematopoietic stem cells are collected from the blood *via* outpatient leukapheresis. In order to mobilize sufficient stem cells into the blood to be harvested by leukapheresis, patients are treated with either cyclophosphamide and neupogen (G-CSF) or G-CSF alone. The Europeans and Chicago groups mobilize stem cells by intravenous infusion of cyclophosphamide (2-4 g/m^2) over 1-2 hours, followed by G-CSF 5-10 μg/kg beginning 5 days later, and collection of stem cells 10 days after cyclophosphamide. Mobilization with cyclophosphamide and G-CSF provides a cyclophosphamide-related treatment ameliorating affect. Cyclophosphamide-induced lymphopenia also provides a partial *in vivo* purge of lymphocytes. However, cyclophosphamide-induced neutropenia occurring 8-9 days after infusion requires judicious monitoring for infections. The Seattle group avoids neutropenia by collecting stem cells using only daily G-CSF 16 μg/kg and corticosteroids with leukapheresis on the 4^{th} or 5^{th} day of neupogen. Daily oral steroids are administered to minimize neupogen-induced disease flare, which in SSc manifests as telangiectasias and arthralgias.

The term peripheral blood stem cells (PBSC) is used to describe the mobilized leukapheresis product. PBSCs are a collection of mononuclear cells including progenitor (stem) cells and lymphocytes and may be cryopreservated without further purification or undergo CD34 selection to further reduce lymphocytes from the product before cryopreservation. The therapeutic value of CD34+ selection of PBSC remains unclear. Stem cell selection of the graft reduces the risk of re-infusing potential disease causing lymphocytes. Whether this translates into a longer clinical remission has yet to be clarified. On the adverse side, CD34+ selection increases the risk of a post-transplant infection with cytomegalovirus (CMV) during the first 3 months after transplant for patients with malignancies [82], and will likely convey the same risk for patients with scleroderma.

In order to fulfill FDA requirements for sterility cultures on the mobilized PBSC, a minimum 14 day interval is required between outpatient stem cell leukapheresis and inpatient admission for transplant. The first week of a transplant admission is for administration of the conditioning regimen, i.e. the immune suppressive drugs and subsequent infusion of the PBSCs (defined as day 0) followed in 8-12 days by peripheral blood count recovery (engraftment) and hospital discharge. In practice, there are currently two philosophical approaches to the conditioning regimen, namely non-myeloablative versus myeloablative [81].

Non-myeloablative regimens (utilized by the Europeans and Chicago) are designed to maximally suppress the immune system without destruction of the bone marrow stem cell compartment. Recovery from conditioning regimen cytopenias will occur without reinfusion of stem cell. The infused stem cells, while not necessary for recovery, hasten recovery and shorten the interval of neutropenia and duration of hospitalization. PBSCs are more properly viewed as a supportive autologous blood product. In fact, the term autologous transplant is a misnomer. Regardless of whether the autologous transplant is non-myeloablative or myeloablative, there is no transplant of foreign tissue or cells, only infusion of the patient's own (autologous) stem cells either to shorten the neutropenic interval (non-myeloablative regimen) or prevent mortality from marrow failure (myeloablative regimen). Standard treatment of autoimmune disease is the art of giving immune suppressive drugs and non-myeloablative regimens are, therefore, based on dose escalation of immune suppressive agents used in standard practice.

Myeloablative regimens (utilized by the Seattle/NIH consortium) are adapted from myeloablative regimens utilized for transplant of leukemia. In particular, the myeloablative regimen for systemic sclerosis employs total body irradiation (TBI) with lung and kidney shielding. The rationale for TBI is that, while it is not a standard agent used to treat inflammatory or autoimmune disorders, it is strongly immune suppressive. The argument against TBI is that is associated with a high incidence of MDS/leukemia within 5 years and a progressively increasing incidence of radiation-induced solid tumors beginning 10 years after treatment [83]. Autologous hematopoietic stem cell transplant for patients with low grade lymphomas using TBI containing regimens resulted in 8.5% developing myelodysplastic syndrome/leukemia and 13.5% a second malignancy [84]. In comparison, for lymphoma patients treated with regimens containing intense alkylating chemotherapy without radiation, 1.7% developed MDS/leukemia and 3.5% any second malignancy [84]. The risk of second cancer may be even higher in systemic sclerosis patients exposed to TBI since cells from SSc patients have, for unclear reasons, a high incidence of increased genetic instability with abnormal chromosome fragility and breakage compared to the general population [85-90]. As a further note of caution, MDS/leukemia has already been reported in patients treated with TBI-containing transplant regimens [91]. Current data for transplant of autoimmune diseases supports the concept that non-myeloablative regimens have lower mortality when compared to myeloablative regimens [81]. However, randomized comparisons between regimens concerning long-term disease response, survival, and late toxicities such as regimen-related second malignancies have not been performed in patients with SSc. Regardless of the regimen, data on immune reconstitution following autologous stem cell transplant for SSc [92], as well as immune studies in patients transplanted for other autoimmune diseases [93-95], suggest that transplant results in an "immune reset" and perhaps even immunologic tolerance.

Table 4. Autologous HSCT in Systemic Sclerosis: Phase 1-2 Studies

Author date	Center (# sites)	# patients	Mean Age (yrs)	Disease Subset	Mobilization	Cell Selection	Conditioning Regimen
Mixed Regimens							
Binks, 2001 [96]	Multi (18)	41	41	37 dSSc 4 lSSc	Cy + G-CSF or G-CSF	Mixed	Mixed
Farge, 2002 [97]	Multi	11	42	NA	Cy 4 g/m2 + G-CSF	Yes	Cy 200 mg/kg or melphalan 140 mg/m2
Myeloablative Regimens							
Mcsweeney, 2002 [98]	Multi (4)	19#	40	NA	G-CSF	Yes	TBI 800 cGy +/- lung shielding +120 mg/kg Cy + eATG 90 mg/kg
Nash, 2007 [91]	Multi (5)	34&	41	NA	G-CSF	Yes	TBI 800 cGy + lung shielding +120 mg/kg Cy + eATG 90 mg/kg
Non-Myeloablative Regimen							
Farge, 2004 [99]	Multi (22)	57@	40	50 dSSc 4 lSSc 3 NS	Cy + G-CSF or G-CSF	mixed (yes 87%)	Cy 150-200 mg/kg (61%) or Cy 200 + ATG (21%)
Tsukamoto, 2006 [100]	Single	6	54	NA	Cy 4 g/m2 2+ G-CSF	Yes	Cy 200 mg/kg
Oyama, 2007 [101]	Single	10	47	9 dSSc 1 lSSc	Cy2 g/m2 + G-CSF	No	Cy 200 mg/kg + 7.5 mg/kg rATG
Vonk, 2008 [102]	Multi (3)	26	42	DSSc	Cy 4 g/m2+ G-CSF	Yes	Cy 200 mg/kg

@ 32 patients previously reported in Binks 2001; # 8 patients previously reported Binks 2002; & 11 patients previously reported in Mcsweeney 2002; Cy = cyclophosphamide; dSSc = diffuse cutaneous systemic sclerosis; G-CSF: neupogen; eATG: equine anti-thymocyte globulin; lSSc:limited systemic sclerosis; rATG: rabbit anti-thymocyte globulin; NA: not available; TBI: total body irradiation

In summary, the rationale for autologous HSCT is intense short-term immune suppression followed by an immune regeneration / reset. Autologous hematopoietic stem cell transplant is designed to favorably alter the inflammatory and autoimmune component of SSc but not directly alter SSc-related vasculopathy, unless SSc-related vasculopathy is also immune-mediated.

TRIALS OF HEMATOPOIETIC STEM CELL TRANSPLANT FOR SYSTEMIC SCLEROSIS.

Autologous HSCT has been safely performed for 2 patients with disabling pansclerotic morphea (1 in Chicago, USA, the other in Tubingen, Germany: Handretinger R. Personal communication) but results are not yet published. Autologous HSCT has generally been restricted to patients with diffuse SSc, although occasionally limited SSc have been included (Table **4**). In general, studies have not clarified if any of the patients had overlap syndromes accompanied by clinical or serologic findings of lupus, rheumatoid arthritis, Sjogren's, polymyositis, or other rheumatologic diseases. Non-myeloablative regimens utilize cyclophosphamide with or without anti-thymocyte globulin while myeloablative regimens are composed of cyclophosphamide, anti-thymocyte globulin, and total body irradiation (TBI) with pulmonary and renal shielding. These initial trials were complicated by high treatment-related mortality (Table **5**), although non-myeloablative regimens appear less toxic. It is also generally recognized that with experience, exclusion of high-risk candidates, and referral and treatment of patients early in disease course instead of relegating transplant to salvage therapy for end-stage disease, the procedure is safer.

TBI was initially accompanied by exacerbation of pulmonary hypoxia and pulmonary deaths as well as precipitation of renal crises leading to subsequent patients receiving both lung and kidney shielding during TBI exposure [98]. Some patients, independent of myeloablative or non-myeloablative intent, died of cardiac failure, leading to the need for careful pre-enrollment cardiac evaluation and mak-

Table 5. HSCT in Systemic Sclerosis: Outcome of Phase 1-2 Studies

Author Date	Skin Score @	Pulmonary Function Tests	Overall Mortality / TRM	Survival
Mixed Regimens				
Binks, 2001 [96]	Improved in 69%	FVC and TLC no change	27% / 17%	73% at 1 year
Farge, 2002 [97]	Improved in 66%	No change	36% / 9.1%	NA
Myeloablative Regimen				
Mcsweeney, 2002 [98]	Improved in 100%	Worse at 3 months then return to baseline	21% / 15%	79% at 2 years
Nash, 2007 [91]	Improved in 70%	Increased FVC / decreased DLCO	36% / 23%	64% at 5 years
Non-Myeloablative Regimens				
Farge, 2004 [99]	Improved in 70% at 6 month, 66% at 12 month, 78% at 24 month, 60% at 36 month	No change	23% / 8.7%	72% at 5 years
Tsukamoto, 2006 [100]	Improved in 100% at 12 months	Improved in PaO2 and HRCT	0%	100%
Oyama, 2007 [101]	Improved in 100% but 20% relapse	No change	10% / 0%	90%
Vonk, 2008 [102]	Improved in 73% at 1 year and 94% at 5 years	No change	8% / 0%	96% at 5 year

@ improvement in skin score is decline in modified rodnan skin score (mRSS) by at least 25%; FVC: forced vital capacity; HRCT: High resolution chest computed tomography; NA: not available; TLC: total lung capacity; TRM: treatment related mortality

ing patients with compromised cardiac function ineligible. Pulmonary artery hypertension (pulmonary artery systolic pressure (PASP) > 40 mmHg) should be considered an exclusion criteria for transplantation.

Nevertheless, relying solely on PASP can be misleading. For example, pulmonary vascular resistance (PVR) is defined as mean PASP minus the pulmonary capillary wedge pressure (PCWP) divided by right ventricle cardiac output (CO). A failing right ventricle will decrease cardiac output which decreases PASP but the ratio of PASP - PCWP/CO, that is PVR, will remain elevated. Therefore an elevated PVR independent of PASP should also be an exclusion criteria.

The current outcome data (Table **5**) indicates that HSCT is the single most effective therapy for improving skin flexibility, that is improving skin score (decreasing mRSS by 25% or more). Improved skin score correlates with increased mobility and functionality of hands and joints [103] as well as improved quality of life [91,102]. This improvement is usually noticeable before hospital discharge and often continues for years after the transplant (although relapse may occur). Since non-transplant studies suggest that improvement in skin score correlates with improved survival [25, 61], it is anticipated that improved skin score following autologous HSCT may translate into improved survival.

Despite some dramatic improvements in high resolution computed tomography (HRCT) of the lung in some patients, the procedure has yet to demonstrate significant improvement in pulmonary function. Improvement by at least a mean of 10% of total lung capacity (TLC), forced vital capacity (FVC) or oxygen diffusion capacity determined by DLCO has not yet been reported, and in some cases these measures have deteriorated compared to baseline. While autologous HSCT was designed to arrest inflammation/autoimmunity in patients with SSc, even with myeloablative TBI-containing regimens, serologic remission have not occurred. Titers may initially decline, but patients remain positive for anti-topoisomerase I as well as other SSc-associated antibodies. It remains unclear if antibody titer rebound will be a harbinger of clinical relapse. Since initial studies focused on skin, quality of life, and pulmonary function, there is no data on post-transplant changes in pulmonary artery hypertension (PASP), cardiac performance, or gastrointestinal function

Table 6. Prospective Randomized Clinical Trials Ongoing in Systemic Sclerosis

RCT	Inclusion Criteria	HSCT	Control Arm
ASTIS [104]	dcSSc with: a) disease duration ≤ 4 years *plus* mRSS ≥ 15 *plus* one of the following: respiratory involvement = DLCO and/or FVC < 80% *and* evidence of ILD (by CXR and/or HR-CT scan and/or BAL and/or biopsy of the lungs) renal involvement = persistent urinalysis abnormalities or microangiopathic hemolytic anemia or new renal insufficiency cardiac involvement = reversible CHF or arhythmia or pericardial effusion b) disease duration < 2 years plus mRSS ≥ 20 plus involvement of trunk plus ESR > 25 mm/1st hour and/or Haemoglobin < 11 g/dL	Mobilization: Cy 4 g/m2 plus G-CSF Conditioning: Cy 200 mg/kg plus rATG 7.5 mg/kg Graft manipulation: CD34 selection	Cy 750 mg/m2 IV pulse monthly for 12 months Cross over to HSCT: not allowed
SCOT [105]	dcSSc plus disease duration ≤ 5 years *plus* mRSS ≥ 16 *plus* one of the following: respiratory involvement = DLCO and/or FVC < 70% *and* evidence of ILD (by HR-CT scan and/or BAL) renal involvement = history of SSc related renal crisis or disease not active	Mobilization: G-CSF Conditioning: TBI 800 cGy (with bilateral lung and kidney shielding) Cy 120 mg/kg plus eATG 90 mg/kg Graft manipulation: CD34 selection	Cy 750 mg/m2 IV pulse monthly for 12 months Cross over to HSCT: not allowed
ASSIST [106]	dcSSc with: a) disease duration ≤ 4 years *plus* mRSS ≥ 15 *plus* one of the following: respiratory involvement = DLCO < 80% or decrease in lung function ≥ 10% over 12 months or Active alveolitis on BAL or evidence of ILD (by CXR and/or HR-CT scan and/or BAL) Renal involvement = two or more of the following: proteinuria, hematuria, a diastolic BP > 95 mm/hg. Cardiac involvement = abnormal EKG GI involvement = confirmed on radiological study. OR Only above pulmonary involvement	Mobilization: Cy 2 g/m2 plus G-CSF Conditioning: Cy 200 mg/kg plus rATG 6.5 mg/kg Graft manipulation: no CD34 selection	Cy 1g/m2 IV pulse monthly for 6 months Cross over to HSCT: allowed after 12 months if there is worsening > 25 % in mRSS or > 10 % deterioration in FVC or DLCO

ASSIST: American scleroderma stem cell versus immune suppression trial; ASTIS: Autologous stem cell transplantation international scleroderma; ATG: rabbit antithymocyte globulin; BAL bronchoalveolar lavage; BP: blood pressure; CHF: congestive heart failure; CXR: chest x ray; Cy: cyclophosphamide; dcSSc: diffuse cutaneous systemic sclerosis; DLCO: carbon monoxide lung diffusion; ESR: erythrocyte sedimentation rate; FVC: forced vital capacity; G-CSF: granulocyte colony-stimulating factor; HSCT: hematopoietic stem cell transplantation; ILD: interstitial lung disease; IV: intravenous infusion; mRSS: modified Rodnan skin score; RCT: randomized clinical trial; SCOT: scleroderma cyclophosphamide or transplantation

(patulous esophogous, intestinal peristalis, or gastric antral vascular ectasia) (Table **5**).

Cutaneous fibrosis has been confirmed histologically to resolve following transplant. Microcapillary regeneration also occurs following transplant using either myeloablative or non-myeloablative regimens. Nail bed capillary microscopy has demonstrated improvement in capillary microcirculation following non-myeloablative transplantation, and skin biopsies have reported capillary regeneration following myeloablative transplantation [104, 105]. Nevertheless, although generally not reported, Raynaud's phenomena persists, although occasionally with subjective improvement in frequency and severity after autologous transplantation. Therefore, at this time, autologous HSCT cannot be interpreted as a cure, but it does appear to change the natural course of SSc. Perhaps transplant is currently best viewed conservatively as converting the course of diffuse SSc from disabling, morbid, and highly lethal into a form similar to limited SSc: less disabling, less morbid and with a better prognosis.. Whether autologous transplantation will also benefit patients with limited SSc is unknown.

There are 3 ongoing randomized trials of autologous HSCT for SSc (Table **6**). Two trials are non myeoablative (ASTIS, ASSIST) while one uses a TBI-based myeloablative regimen (SCOT). Two trials CD34 select the graft (ASTIS, SCOT) and one does not (ASSIST). Two trials are survival studies and do not allow cross-over to transplant for progression on the control arm (ASTIS, SCOT). One trial is a treat-

ment failure study rather than survival study and therefore allows cross-over to transplant for disease progression (ASSIST). Two trials are multicenter (ASTIS, SCOT), while one is a single center study (ASSIST) [106-108]. These trials will be important to clarify the safety and efficacy of this procedure for patients with SSc, to compare early and late toxicity (second malignancies) from the different regimens, to determine the benefit, if any, from CD34 selection of the autograft, and to allow comparison of safety from multiple centers versus a single experienced center of excellence.

CONCLUSION

The pathogenesis of SSc involves features of fibrosis, (skin and internal organ fibrosis), autoimmunity, and micro and macro vasculopathy. There is wide heterogeneity in the classification system of SSc, although efforts have been made over time to define distinct subsets and to find early independent predictors of mortality in order to better predict clinical manifestations and survival. To date, autologous HSCT is the most effective therapy shown to reverse skin fibrosis. It is note worthy that the extent and severity of skin involvement has been recognized to correlate with internal organ involvement, and on the other hand, improvement in skin thickness correlates with improvement in survival [25, 61]. Stabilization of internal organ function has also been observed for up to 5 years post-HSCT. Non myeloablative regimens are safer than myeloablative ones that include total body irradiation, especially in risk of treatment-induced malignancies [81]. Better pretransplant evaluation and selection of patients earlier in disease has considerably decreased TRM. Results from ongoing randomized clinical trials are awaited.

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