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ARTICLE

Five year follow-up after autologous peripheral blood hematopoietic stem cell transplantation for refractory, chronic, corticosteroid-dependent systemic lupus erythematosus: effect of conditioning regimen on outcome

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Abstract

Some patients with systemic lupus erythematosus (SLE) are refractory to traditional therapies, dependent on chronic corticosteroids, have organ damage, and are at high risk of mortality. In this group of patients, we report outcome at a median of five years after autologous hematopoietic stem cell transplant (HSCT) using two different non-myeloablative regimens. Four patients received a conditioning regimen of cyclophosphamide (200 mg/kg) and alemtuzumab (60 mg), while 26 patients underwent conditioning with cyclophosphamide (200 mg/kg), rATG (Thymoglobulin) (5.5 mg/kg), and rituximab 1000 mg. Unselected peripheral blood stem cells were infused on day 0. There were no treatment related deaths. Of the four patients treated with cyclophosphamide and alemtuzumab, none entered remission. For the 26 patients treated with cyclophosphamide, rATG, and rituximab, disease remission defined as no immune suppressive drugs except hydroxychloroquine and/or 10 mg or less of prednisone a day was 92% at 6 months, 92% at one year, 81% at 2 years, 71% at 3 years, and 62% at 4 and 5 years post-HSCT. Autologous HSCT outcome is dependent on the conditioning regimen but prior organ damage may cause lingering symptoms.

Introduction

SLE is a chronic autoimmune multi-system disease with diverse manifestations [1]. The English physicians Willan and Bateman were credited with the name Lupus (Latin for wolf) which emphasizes its nature to devour tissues with greater blood supply, while the French physician Pierre Cazenave was credited with the name Lupus Erythematosus consistent with its cutaneous visual presentation [2]. The incidence and prevalence of SLE in the United States has been reported to be 7.3 [3] and 124 [4] per 100,000, respectively. SLE is more prevalent in females [3].

Treatment is not curative and the current 10-year survival rates range from 85% to 92% [5]. Fifteen-year survival drops significantly and has been reported to be 73% [6]. Patients at high risk for mortality are those with persistent disease activity resulting in infectious complications and accumulated chronic organ damage [7].

Autologous hematopoietic stem cell transplantation (HSCT) is a treatment modality designed for patients with SLE refractory to standard therapy. After our initial publications of HSCT using CD34 selected stem cells [8, 9], we evaluated the effect of HSCT using unselected peripheral blood stem cells (PBSC), and herein report the outcome of HSCT using two different regimens and unselected PBSC.

Methods

Patients

Patients enrolled in this open-label, single group assignment study were treated at Northwestern Memorial Hospital

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(Chicago, Illinois, USA) on an FDA approved protocol (www.clinicaltrials.gov NCT00278629). Eligible patients were 16–60 years old, met at least 4 of 11 American College of Rheumatology criteria for SLE [10], had active disease (SLEDAI > 10) [11] despite at least a minimum of 6 months of either cyclophosphamide or mycophenolate mofetil, were unable to taper prednisone below 10 mg/day due to active disease, and had any of the following internal organ involvement: renal (World Health Organization class III or IV glomerulonephritis), pulmonary (vasculitis, pneumonitis, alveolar hemorrhage), central nervous system (cerebritis, cognitive dysfunction, psychosis, confusional state, seizures, or transverse myelitis), vasculitis (confirmed by biopsy or angiogram), myositis, autoimmune cytopenias, severe serositis (symptomatic pericardial or pleural effusions causing shortness of breath, hemodynamic compromise, or chronic and disabling pain despite narcotic use, or shrinking lung syndrome), persistent non-infectious fevers, or antiphospholipid syndrome (APS) secondary to lupus as defined by the Sapporo criteria [12] with recurrent coagulopathy despite therapeutic anticoagulation. The SLICC/ACR Damage Index was determined before HSCT but not on post-HSCT evaluations [13].

Stem cell collection and transplant regimen

PBSC were mobilized with intravenous cyclophosphamide (2 g/m^2) and 5–10 mcg/kg subcutaneous filgrastim daily beginning five days after cyclophosphamide. Apheresis was performed on day 10 after cyclophosphamide, and PBSC were cryopreserved without manipulation. The conditioning regimen was either cyclophosphamide (200 mg/kg IV) given in four equal fractions (50 mg/kg) on day −5 through day −2 and alemtuzumab 60 mg equally divided on days −3 and −2, or intravenous cyclophosphamide (200 mg/kg) given in four equal fractions (50 mg/kg) on day −5 through day −2, rATG (Thymoglobulin) IV dosed at 0.5 mg/kg on day −5, 1 mg/kg on days −4, and −3, and 1.5 mg/kg on days −2, and −1 (total dose 5.5 mg/kg), and rituximab 500 mg IV on days −6 and +1. Eight patients received plasmapheresis on the day prior to admission.

Supportive care guidelines

Hydration (125 to 150 ml/h of normal saline) and diuretics with each dose of cyclophosphamide continued for 24 h. Blood products were irradiated, cytomegalovirus (CMV) safe, and leukocyte depleted. Filgrastim (5–10 mcg/kg/day) was started on day +4 and continued until engraftment. Intravenous cefepime or piperacillin-tazobactam was started on day zero. Oral acyclovir or valacyclovir was started upon admission and continued for 12 months after transplant. Patients received either oral daily fluconazole or voriconazole and either oral trimethoprim-sulfamethoxazole three times a week or monthly aerosolized pentamidine for three to six months post-HSCT. Cytomegalovirus viral load was monitored post-transplant for 90 days and pre emptively treated by switching from acyclovir to oral valganciclovir (900 mg twice a day) until negative.

Study endpoints

The primary outcome was remission defined as no immune suppressive medications except 10 mg or less of daily prednisone with or without hydroxychloroquine [14]. Patient follow-ups were scheduled for six months, 12 months, and then at yearly intervals for five years following transplantation. Secondary outcomes were SLEDAI, lupus serology (antinuclear antibody [ANA], anti–double stranded DNA antibody (anit-dsDNA), anticardiolipin antibody (ACLA)), complement C3 and C4, quality of life questionnaire by SF-36, and end-organ function monitored by creatinine clearance, total lung capacity (TLC) and diffusing capacity of the lungs for carbon monoxide (DLCO) corrected for hemoglobin.

Statistical analysis

Statistical analysis was performed by the two-tailed paired Student's *t*-test. The effects of time on the binary outcomes for IgG and IgM ACLA were evaluated using a generalized estimating equation model in SAS v9.4.

Results

Demographics

Thirty-two patients were enrolled and 30 underwent transplant (Tables 1–3). Two were declined HSCT: one for end-stage fibrotic renal disease on biopsy and one for severe lupus-related left ventricular dysfunction. Of the patients who underwent HSCT, 28 were female, 2 male, 19 Caucasian, 6 Hispanic, 4 African American, and 1 Asian. The mean age and disease duration was 31 and 10.5 years, respectively. Patients were corticosteroid-dependent for a mean of 9.9 years and prior mean cumulative exposures were 90.7 grams of prednisone and 7.21 grams of methylprednisolone. Co-existing complications and number of afflicted patients included: dialysis [3], insulin dependence [3], assistance to ambulate [6], cardiac dysfunction [6], immune-mediated cytopenias [10], narcotic dependence [13], hypertension [17], thrombotic events [10], oxygen dependent [5], prior intubation [4], chest tubes [2], vertebral fractures [2], and one patient each of avascular necrosis, total parenteral nutrition,

Table 1 Demographic, clinical, and serologic features of SLE patients

Demographics	Number of patients or Mean/Median/SD (range)
Male / female	2 / 28
Caucasian / Hispanic / African American / Asian	19 / 6 / 4 / 1
Age (years)	31 / 28.5 / 10.9 (15–54)
Disease duration (years)	10.5 / 6.5 / 9.1 (1–33)
Disease activity	
SLEDAI	23.6 / 20 / 10 (8-48)
Disease chronicity / complications	
Years on corticosteroids	9.87 / 6 / 8.6 (1–33)
Approximate cumulative oral prednisone dose (grams)	90.7 / 65.7 / 85.6 (7.3–328)
Approximate cumulative IV methylprednisolone (mg)	7210 / 4000 / 8093 (0–36,000)
Dialysis / diabetes on insulin	3 / 3
Assistance to ambulate	6 -Wheelchair (4), walker (1), coma (1)
Oxygen dependent/ prior intubation	5 / 3
Cardiac dysfunction	6 - AMI (2), low LVEF (3), PAH (2)
Chest tubes	2
ITP, AIHA, immune neutropenia	10 - ITP (7), AIHA (2), immune neutropenia (1)
Narcotic dependent	13
Vertebral fractures / AVN	2 / 1
Hypertension	17 - one drug (4), two drugs (6), three drugs (3), four or more drugs (4)
PE / DVT / TIA / CAPS	10 - PE (5), DVT (7), TIA (2), CAPS (1), subarachnoid hemorrhage (1)
Other	TPN (1), growth retardation (1), leg amputation (1) foot amputation (1)
SLICC / ACR Damage Index	6.1 / 5.5 / 2.87 (1–13)
Serology abnormalities	
ANA	28 / 30
Anti-dsDNA	20 / 30
Anti-SSA and or anti-SSB	10
Anti-Sm	6
Anti-cardiolipin IgG and or anti-beta 2 GP1	8
Low C3 / C4	10 / 7

AIHA autoimmune hemolytic anemia, *AMI* acute myocardial infarct, *ANA* antinuclear antibody, AVN = avascular necrosis, *CAPS* catastrophic anti-phospholipid syndrome, *DVT* deep venous thrombosis, *ITP* idiopathic thrombocytopenic purpura, *LVEF* left ventricular ejection fraction, *PAH* pulmonary artery hypertension, *PE* pulmonary embolism, *SLEDAI* systemic lupus erythematosus disease activity index, *TIA* transient ischemic attack, *TPN* total parenteral nutrition

growth retardation, and leg and foot amputation due to catastrophic antiphospholipid syndrome (CAPS) (Table 1).

While patients had multiple organ systems involved (Table 2) and while some patients had more than one predominant indication, the main reason(s) for HSCT were: nephritis [11], cerebritis [6], secondary anti-phospholipid syndrome [6], lung [5] (pneumonitis [3], alveolar hemorrhage [1], shrinking lung [1]), persistent SLE fevers [3], chronic idiopathic thrombocytopenic purpura (ITP) [2], and one each for pseudo-obstruction with transverse myelitis, and growth retardation with vertebral collapse (Table 2). Other than corticosteroids, patients had a mean of 6.5 different immune suppressive drugs, and had a mean SLICC / ACR Damage Index of 6.1 (Table 1). Infections that required intravenous antibiotics and/or surgery before being evaluated for HSCT were sepsis [7], bacterial pneumonia [3], cytomegalovirus (CMV) hepatitis [2], urosepsis [2], varicella zoster [2], sinusitis [2], and one patient each with prior CMV pneumonia, liver abscess, brain abscess, streptococcus pharyngitis, cellulitis, meningitis, and esophageal candidiasis (Table 3).

Table 2 Pre-transplantation disease manifestations

Condition	No of patients	No of patients for whom manifestation was primary indication for HSCT
Pleuritis / pleural effusion	20	
Arthralgia	20	
Rash	20	
Nephritis	18	11
Persistent headache	13	
Secondary anti-phospholipid syndrome / CAPS	12 / 1	5 / 1
Neuropsychiatric / cerebritis	10	6
Pericarditis / pericardial effusion	10	
Oral ulcers	10	
SLE fever	8	3
Raynaud phenomenon	8	
Pneumonitis	7	3
ITP (chronic)	7	2
Alopecia-scarring alopecia	7 / 2	
Seizures	6	
Neuropathy	6	
Pancytopenia	6	
Hepatitis	5	
Myopathy / myositis / myalgia	8	
Cutaneous vasculitis	5	
Photosensitivity	4	
Psychosis	2	
Chorea	2	
Autoimmune hemolytic anemia	2	
Myocarditis	2	
Peritonitis	2	
PAH	2	
Colitis	2	
Alveolar hemorrhage	2	1
Adenopathy	2	
Ataxia, meningitis, transverse myelitis, optic neuritis, shrinking lung, cardiomyopathy, pancreatitis, macrophage activation syndrome, TIA, pseudo-obstruction, gangrenous colitis, shrinking lung, growth retardation and vertebral collapse	1 case of each	1 transverse myelitis with pseudo-obstruction 1 shrinking lung 1 growth retardation with vertebral collapse

CAPS catastrophic antiphospholipid syndrome, *HSCT* hematopoietic stem cell transplantation, *ITP* idiopathic thrombocytopenic purpura, *PAH* pulmonary artery hypertension, *TIA* transient ischemic attack

Table 3 Prior immune suppressive medications and documented pre-transplant infections

Medications failed before HSCT	Number of patients (percentage)
Corticosteroids	30 (100%)
Mycophenylate mofetil	30 (100%)
Cyclophosphamide	28 (93%)
Chloroquine	23 (77%)
Rituximab	20 (67%)
Other biologics	10 (33%) belimumab 5, abatacept 2, infliximab 1, adalimumab 1, etanercept 1
Azathioprine / methotrexate	18 (60%), 13 (43%)
IVIG, plasmapheresis, splenectomy	8 (27%), 4 (13%), 3 (10%)
Calcineurin inhibitor	3 (10%) cyclosporine 2, tacrolimus 1
Prior HSCT	2 (8%)
Other immune drugs	6 (20%) - 6-MP 1, vincristine 1, thalidomide 1, gold 1, arava 1
Anticoagulation	10 (and 1 IVC filter)
Number of prior immune medications	Mean 6.5, Median 6, SD 1.5, range (4-10)
Serious infections before evaluation for HSCT	
CMV hepatitis	2
CMV pneumonia	1
Bacterial pneumonia	3
Urosepsis	2
Sepsis (no source, blood culture positive): achromobacter, MSSA, bacteroides fragilas, clostridium septicum, staphylococcus aureus, enterobacter, MRSA	7 (one each)
Streptococcus pharyngitis	1
Meningitis	1
Liver abscess	1
Brain abscess (MSSA)	1
Recurrent VZV	2
Sinusitis	2
Recurrent cutaneous staphylococcus cellulitis	1
Esophageal candidiasis	1

CMV cytomegalovirus, *IVC* inferior vena cava, *IVIG* intravenous immunoglobulin, *6-MP* 6 mercaptopurine, *MRSA* methicillin resistant staphylococcus aureus, *MSSA* methicillin sensitive staphylococcus aureus, *VZV* varicella zoster virus

Toxicity during transplant hospitalization

There were no treatment related deaths. Grade 3 and 4 toxicities during transplant are listed in Table 4. Grade 4 electrolyte toxicities due to hyper-hydration and furosemide were subsequently mitigated by addition of prophylactic oral electrolyte supplements and twice a day electrolyte monitoring. The grade 4 troponin elevation was not

Table 4 Hematopoietic stem cell transplant toxicity during transplant hospitalization

Transplant toxicity	Number of patients
Treatment related mortality	0
Grade 4	
Hypokalemia	3
Elevated Troponin	1
Intracranial Hemorrhage	1
Grade 3	
Neutropenic Fever	10
Hypokalemia	8
Hypertension	7
Hyperglycemia	6
Hypophosphatemia	5
Dyspnea	5
Hypomagnesemia	3
Hypocalcemia	3
Elevated Transaminase	2
Chest Pain	2
Rituximab allergy, hypotension, bronchospasm, headache, epistaxis, POTS, bradycardia, altered mental status, rash due to sweets syndrome	1 each

POTS postural orthostatic tachycardia syndrome

treatment related but from ongoing disease related lupus pericarditis. No infections occurred during neutropenia or transplant hospitalization.

Outcome of cyclophosphamide / alemtuzumab regimen (4 patients)

Of four patients treated with cyclophosphamide and alemtuzumab, none entered a clinical remission following HSCT (Table 5). One died 30 days after transplant from a pulmonary embolism following a half-day motor vehicle trip, and one died four years after HSCT of active disease and urosepsis. Of the two surviving patients, one entered remission after another three years of ongoing immune suppression and one remained with active disease for five years. Post-transplant discharge infectious events were septic bursitis, central line infection, pneumonia, influenza, sinusitis, candida esophagitis, recurrent urinary tract infections, urosepsis, staphylococcus sepsis, and aspergillosis. Late cytopenias included autoimmune hemolytic anemia and autoimmune neutropenia. Late sequelae of chronic disease-related damage and its treatment were rib fracture, hip avascular necrosis, shoulder surgery, recurrent deep venous thrombosis, cardiac catheterization, idiopathic seizures, chronic adrenal insufficiency, and new insulin dependent diabetes.

Table 5 Post-transplant hospital discharge events during 5 years of follow-up

Alemtuzumab/cyclophosphamide regimen (4 patients)
Disease remission - 0 (0%)
Treatment related mortality - 0 (0%)
Non-treatment related mortality - 2 (50%) Pulmonary embolus- 1 month post HSCT, active SLE urosepsis (renal stones) 4 years post HSCT
Infections - sinusitis, pneumonia, septic bursitis, PICC infection, influenza, candida esophagitis, urosepsis, sepsis (MSSA), aspergillosis
Musculoskeletal - AVN, rib fracture, shoulder surgery, hip labral tear
Vascular - DVT, cardiac catheterization (normal)
Endocrine - diabetes, chronic adrenal insufficiency
Hematologic - AIHA, autoimmune neutropenia
Neuropyschiatric - seizure
Other - appendectomy (2)
Rituximab / rATG / cyclophosphamide regimen (26 patients)
Disease remission - 24 / 26 (92%)
Treatment related mortality - 0 (0%)
Non-treatment related mortality - 1 (4%) Cardiac arrhythmia on dialysis 2 months post-HSCT
Infection - sinusitis (5), UTI (6), VZV (3), URTI (7), clostridium difficile (3), influenza (3), cellulitis (2), PTLD, otitis media, meningitis (MSSA), skin abscess I&D, empyema, strep throat, walking pneumonia, urosepsis, wisdom tooth abscess skin wart
Musculoskeletal - AVN (5), herniated vertebral disc (2), bone infarct (2) knee surgery (3), vertebral fractures, cataracts
Vascular - POTS, DVT (relapse), Budd Chiari (relapse), new HTN
Endocrine - Adrenal insufficiency, hyperthyroidism, diabetes
Hematologic - acute ITP (no relapse) (3)
Neuropsychiatric- chorea (transient no treatment no relapse), seizure (relapse)
Other- adenopathy (biopsy & culture negative) (3), myringotomy, hysterectomy, hypogammaglobulinemia, pregnancy (healthy baby), drug facial rash, cholecystectomy, drug induce suicidal ideation

Parenthesis are number of patients or percent of patients. Unless otherwise numbered, the event involves one patient

AIHA autoimmune hemolytic anemia, *AVN* avascular necrosis, *CAD* Coronary artery disease, *DVT* deep vein thrombosis, *HTN* hypertension, *I&D* incision and drainage, *ITP* idiopathic thrombocytopenic purpura, *MSSA* methicillin sensitive staphylococcus aureus, *PICC* peripherally inserted central catheter, *POTS* postural orthostatic tachycardia syndrome, *PTLD* post-transplant lymphoproliferative disease, *rATG* rabbit anti-thymocyte globulin, *URTI* upper respiratory tract infection, *UTI* urinary tract infection, *VZV* varicella zoster virus

Outcome of cyclophosphamide / rituximab / rATG (26 patients)

Survival

Of twenty-six patients treated with cyclophosphamide, rATG, and rituximab, one died two months after HSCT from an arrhythmia while tapering off dialysis (Table 5).

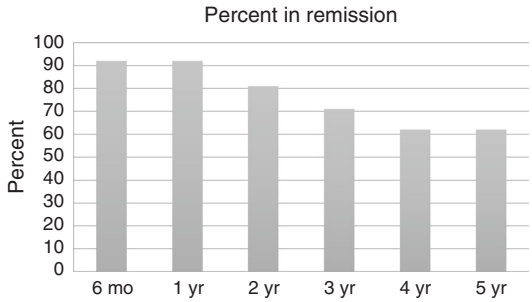

Fig. 1 SLE Remission post-HSCT with cyclophosphamide, rATG, rituximab

Beyond the five year study follow-up, one patient died six years after transplant from relapsed disease and pulmonary aspergillosis while on a TNF inhibitor and despite a second transplant. Disease remission was 92% at 6 months and 1 year, 81% at 2 years, 71% at 3 years, and 62% at 4 and 5 years post-HSCT, respectively (Fig. 1).

Infections

No infections occurred during transplant hospitalization. Infections over a median of five years of follow-up and number of patients affected were sinusitis [5], upper respiratory tract infection [7], urinary tract infection [6], cutaneous varicella zoster [3], influenza [3], *clostridium difficile* diarrhea [3], cellulitis [2], and one case each of periodontal abscess, subcutaneous abscess incision and drainage, cutaneous verrucae (skin wart), otitis media, suspected meningitis, streptococcus pharyngitis, urosepsis, pneumonia, and post-transplant lymphoproliferative disease (Epstein Barr virus positive) that was treated with 2 doses of rituximab. After hospital discharge, there were a total of 40 infections (1.5 infections per patient). The majority of infections, 88% (35 of 40), were treated with oral antibiotics

Non-infectious events

Non-infectious events over the five years post-transplant and number of patients affected were: hip avascular necrosis [5], ITP (not associated with relapse) [3], herniated lumbar discs [2], bone infarcts [2], ruptured cruciate ligament [2], knee surgery [3], and one patient each with vertebral fractures, coronary artery disease, adrenal insufficiency, cataracts, new insulin-dependent diabetes, new hypertension, hyperthyroidism, hypogammaglobulinemia, chorea (transient), seizure (at relapse), deep venous thrombosis (at relapse), Budd Chiari (at relapse), cholecystectomy, hysterectomy, myringotomy, pregnancy (healthy baby), resection of chronic small bowel adhesions, syncope (no etiology), postural orthostatic tychycardia syndrome (POTS), drug-induced suicidal ideation (transient), and drug-induced facial rash (transient).

Outcome parameters

The SLEDAI mean (standard deviation (SD)) improved from 23.5 (10.2) pre-HSCT to 4.6 (4.6) at six months and 1.5 (1.9) at 1 year, 4.5 (6.5) at 2 years, 4.9 (4.1) at 3 years, 6.8 (6.5) at 4 years, and 2.9 (5) at 5 years post-HSCT (all p values < 0.01) (Fig. 2A). The mean (SD) C3 complement (mg/dl) (normal > 75 mg/dl) improved from 98 (44) pre-HSCT to 137 (43) at six months, 135 (38) at 1 year, 121 (30) at 2 years, 145 (44) at 3 years, 129 (19) at 4 years, and 119 (28) at 5 years (all $p < 0.01$) (Fig. 2B). Mean (SD) C4 complement (mg/dl) (normal > 20 mg/dl) improved from 17 (10) pre-HSCT to 38 (28) at 6 months, 28 (9) at 1 year, 25 (15) at 2 years, 32 (13) at 3 years, 28 (9) at 4 years, and 24 (11) at 5 years (all $p < 0.01$) (Fig. 2D).

The median ANA improved from a titer of 1:680 pre-HSCT to 1:80 at six months, 1:80 at one year, 1:80 at two years, 1:60 at 3 years, 1:40 at 4 years, and 1:80 at 5 years (all p values < 0.01) (Fig. 2C). The median anti-double stranded DNA (anti-dsDNA) titer improved from 1:160 pre-HSCT to 0 at 6 months ($p = 0.05$), 0 at 1 year ($p < 0.01$), 0 at 2 years ($p = 0.12$), 0 at 3 years ($p = 0.14$), 0 at 4 years ($p = 0.25$), 0 at 5 years ($p = 0.24$).

Elevated IgG ACLA were present in seven of 26 patients before HSCT, two of 26 at 6 months, two of 23 at 1 year, two of 15 at 2 years, one of 16 at 3 years, and one of 10 at 4 and 5 years ($p = 0.6$) (Fig. 2E). Elevated IgM ACLA was present in nine of 26 patients before HSCT, 2 of 26 at 6 months, one of 23 at 1 year, one of 16 at 2 years, zero of 16 at 3 years, zero of 10 at 4 years and zero of 12 at 5 years ($p = 0.002$) (Fig. 2F). Of ten patients on anticoagulation before transplant, seven became and remained free of anticoagulation post-HSCT. Post-hepatitis B vaccination, one patient relapsed and developed Budd Chiari and DVT.

For patients with renal insufficiency before HSCT, the creatinine clearance (CrCl) did not change significantly. Pre-HSCT and one year post-transplant CrCl was 65 ml/min and 71 ml/min, respectively ($p = 0.06$). Three patients were on dialysis for a duration of two weeks, two months, and six months, respectively, before HSCT. The two week and two month dialysis-dependent patients became dialysis-independent for three years (restarting dialysis after delivery of a healthy baby), and for five years (the length of study), respectively. The patient dependent on dialysis for six months died two months after HSCT from an arrhythmia. Six patients, not on dialysis pre-HSCT, with biopsy documented renal pathology (one with mild chronicity, four with grade 3 chronicity, and one with acute APS micro-vascular thrombosis) all remained dialysis free for the five years of follow-up.

Median DLCO corrected for hemoglobin was 60% pre-transplant and 60% one year post-transplantation. Pre-HSCT total lung capacity improved significantly from

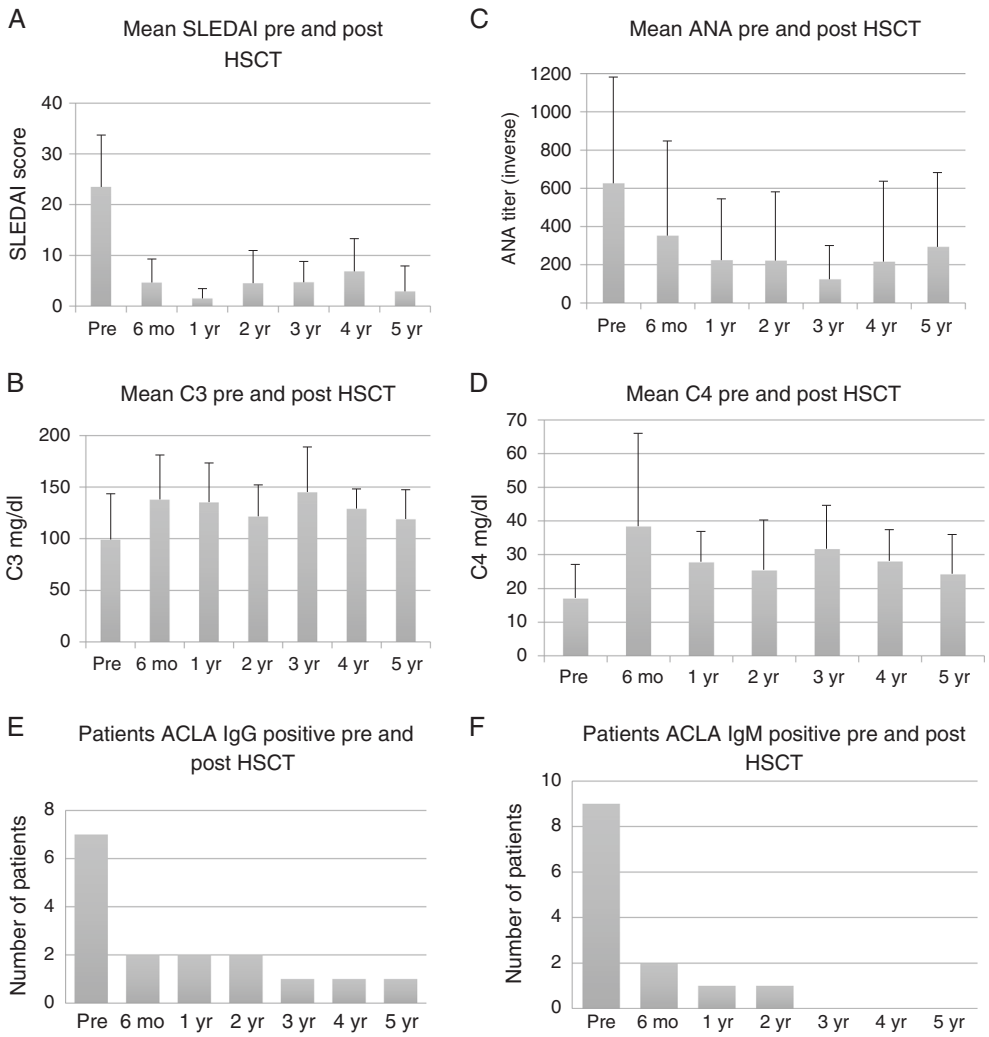

Fig. 2 SLE disease activity, serology, and complement pre and post HSCT with cyclophosphamide, anti-thymocyte globulin, and rituximab A) SLEDAI, B) C3, C) ANA, D) C4, E) ACLAG, Ig6, F) ACLA IgM

79% to 87% at one-year post transplant ($p < 0.01$). Before HSCT, four patients had been intubated: two for alveolar hemorrhage, one for catastrophic anti-phospholipid syndrome, and one for heart failure while no patients have been intubated during or after HSCT. Before HSCT, four patients required nasal cannula oxygen, three of which became oxygen independent after HSCT.

Quality of life and ambulation

Post-transplant quality of life by SF-36 improved ($p < 0.05$) in all scales and dimensions including physical health, mental health and total SF-36 score for six months, 1, 2, 3, and 5 years after HSCT (Table 6). Before HSCT, two patients were in a wheelchair due to vertebral compression fractures. One improved to ambulation without assistance. Another patient was bed-confined with acute quadriplegia and pseudo-obstruction due to transverse myelitis and subsequently improved post-HSCT to ambulation without assistance. Of eleven patients on narcotics pre-transplant, six became and remained narcotic-free after transplantation. Seventeen patients were on anti-hypertensive medications pre-HSCT. Nine were able to discontinue all anti-hypertensive medications after transplantation.

Discussion

The phrase "autologous hematopoietic stem cell transplantation" is a misnomer. The rationale of the conditioning regimen is to lymphodeplete and stop inflammation with a non-myeloablative and immune-specific regimen. Since the ability of the marrow stem cell compartment to recover is not compromised, spontaneous hematopoietic recovery occurs even without stem cell reinfusion. In reality, there is no transplant. The stem cells are autologous, not foreign,

Table 6 SF-36 quality of life before and after HSCT with cyclophosphamide, ATG, rituximab

	Pre-HSCT	6 month	1 year	2 year	3 year	4 year	5 year
Physical score							
Median (IQR)	22.00 (15.20–35.50)	45.50 (33.25–76.00)	53.00 (36.00–87.00)	70.00 (41.25–87.55)	56.00 (36.00–73.00)	49.50 (30.15–70.50)	74.00 (64.00–87.00)
Mean (SD)	27.42 (18.95)	53.59 (25.84)	58.18 (28.34)	63.46 (29.19)	54.80 (25.96)	51.57 (30.53)	70.00 (25.32)
95% CI	19.23-35.61	41.50-65.68	43.61-72.75	46.61-80.31	41.45-68.15	19.53-83.61	50.54-89.46
P-value@		< 0.001	0.001	0.004	0.005	0.066	0.002
Mental score							
Median (IQR)	48.00 (31.80–53.50)	63.00 (50.25–79.00)	61.80 (50.00–87.40)	80.50 (62.25-83.75)	71.00 (53.30-79.00)	63.45 (49.23-65.00)	78.00 (66.00-86.00)
Mean (SD)	45.30 (15.33)	61.49 (20.89)	64.05 (24.97)	74.11 (15.10)	65.42 (20.22)	59.07 (21.71)	72.67 (24.49)
95% CI	38.67-51.93	51.71-71.27	51.21-76.89	65.39-82.83	55.02-75.82	36.29-81.85	53.85-91.49
P-value@		0.003	0.005	0.001	0.048	0.083	0.004
Total Score							
Median (IQR)	42.00 (25.50-47.00)	57.50 (40.50-79.50)	59.00 (47.00-89.00)	79.50 (57.00-87.75)	71.00 (53.00-82.00)	59.22 (42.36-72.75)	83.00 (73.00-91.00)
Mean (SD)	39.53	60.10 (23.17)	63.09 (26.25)	71.88 (21.63)	63.81 (23.01)	58.33 (25.52)	75.56 (24.61)
95% CI	32.45-46.61	49.26-70.94	49.59-76.58	59.39-84.37	51.98-75.64	31.55-85.11	56.64-94.48
P-value@		< 0.001	0.002	0.002	0.011	0.068	0.001

HSCT hematopoietic stem cell transplantation, *IQR* interquartile range, *SD* standard deviation, *CI* confidence interval

@ Comparison group is before HSCT

and when using a non-myeloablative regimen, do not need to be infused for recovery. HSC have no direct anti-lupus or immune suppressive effect and are not a drug. Autologous HSC are a homologous transfusion blood product that shortens the post-transplant interval of cytopenia.

Multiple small studies on HSCT for SLE using different regimens have been reported, but there have been no reports of long-term, i.e., five-year, follow-up or of a regimen that did not induced remission [16, 17]. We first reported on HSCT for SLE using a regimen of cyclophosphamide, rATG, and CD34 selected peripheral blood stem cells (PBSC) [8]. Subsequently, due to the inability to obtain CD34 columns from American manufacturers (Cellpro Inc, Seattle, Washington, or Baxter International, Deerfield, Illinois), we developed a regimen using alemtuzumab and the same dose of cyclophosphamide without CD34 selection. Since alemtuzumab is effective at decreasing graft versus host disease after allogeneic HSCT [15], it was hypothesized that in vivo alemtuzumab would substitute for CD34 selection by purging the reinfused unmanipulated graft. However, zero of four patients entered remission after conditioning with cyclophosphamide and alemtuzumab, and two subsequently died of complications of active disease. As a result, the regimen was changed to the original cyclophosphamide and rATG plus rituximab (a standard lupus drug) and of 26 patients, 90% entered remission with no treatment medication-related mortality and 4% non-treatment related mortality during five-years of follow-up.

It has been reported by others that HSCT depletes autoreactive SLE specific memory cells [18]. In patients who received cyclophosphamide and alemtuzumab, we did not perform a correlate immune analysis that may have elucidated why it was ineffective. However, in the set of patients who received cyclophosphamide, rATG, and rituximab, our group analyzed immune regeneration between pre- and post-transplant peripheral blood lymphocytes [19]. We previously reported, in comparison to conventional drugs, that HSCT generated a new population of TGF-beta producing LAP^{high}, $CD103^{high}$, CD8 Treg cells that repaired Treg deficiency in patients with SLE [19]. We now report the five-year clinical outcome in these patients that resulted in durable clinical remission, normalization of C3 and C4, and persistent decrease in lupus serologies.

The definition of disease remission in patients with SLE is evolving [20, 21] and durable remissions are rare [21]. Because our patients had chronic refractory disease with a high pre-transplant SLICC / ACR Damage Index, and required low dose corticosteroids for chronic adrenal insufficiency and/or chronic musculoskeletal pain that were not related to active disease per se, we allowed patients in remission to receive low dose daily prednisone (≤ 10 mg) [13]. Despite remission of active disease, prior organ damage and chronic corticosteroids usage resulted in ongoing musculoskeletal (avascular necrosis, compression fractures, damaged articular cartilage), vascular (hypertension), and endocrine complications (diabetes, hypothyroidism, adrenal insufficiency) [22–25].

Before transplant, patients suffered from a number of infectious complications (Table 3) that are likely underestimated, because the average duration of disease before

HSCT was 10 years (range 1–33 years), long-term records of outpatient infections were incomplete, and patients were unable to remember all instances of oral antibiotics. Post-transplant, there were on average 1.5 infections per patient, the majority of which were responsive to outpatient oral antibiotics. In future protocols, omitting the second (day +1) dose of rituximab would likely diminish post-HSCT upper respiratory tract infections and risk of immunoglobulin deficiency.

While still perfecting the optimal regimen and timing for intervention, HSCT using cyclophosphamide, rATG, rituximab, and unmanipulated PBSC support resulted in durable clinical remission and what appears to be a decrease in mortality compared to the natural history of refractory, chronic, corticosteroid-dependent disease. In the future, patients with high risk of organ damage might be identified earlier for HSCT by gene expression profiling [26, 27].

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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