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Umbilical cord mesenchymal stem cell transplantation in active and refractory systemic lupus erythematosus: a multicenter clinical study

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Abstract

Introduction

Umbilical cord (UC)-derived mesenchymal stem cells (MSCs) have shown a good safety profile and therapeutic effect in severe and refractory systemic lupus erythematosus (SLE) in our single-center pilot study. The present multicenter clinical trial was undertaken to assess the safety and efficacy of allogenic UC MSC transplantation (MSCT) in active and refractory SLE patients.

Methods

Forty patients with active SLE were enrolled from 4 clinical centers in China. Allogenic UC MSCs were infused intravenously on days 0 and 7. Primary endpoints were safety profiles. Second endpoints included major clinical response (MCR), partial clinical response (PCR) and relapse. Clinical index including SLEDAI score, BILAG score, renal functional indices were also determined.

Results

The overall survival rate was 92.5% (37/40). UC-MSCT was well tolerated, and no transplantation-related adverse event was observed. Thirteen and eleven patients achieved MCR (13/40, 32.5%) and PCR (11/40, 27.5%) during 12 months follow-up, respectively. Then three and four patients experienced disease relapse at 9 (12.5%) and 12 (16.7%) months follow-up, after a prior clinical response. SLEDAI score significantly decreased at 3, 6, 9 and 12 months follow-up. Total BILAG score markedly decreased at 3 months and continued to decrease at subsequent follow-up visits. BILAG scores for renal, hematopoietic and cutaneous systems significantly improved. For those with lupus nephritis, 24-hour proteinuria declined after transplantation, with statistical differences at 9 and 12 months. Serum creatinine and urea nitrogen decreased to the lowest level at 6 months, while these values slightly increased at 9 and 12 months as a result of 7 relapsed cases. In addition, serum levels of albumin and complement 3 increased after MSCT, peaked at 6 months and then slightly declined at 9 and 12 months follow-up. Serum antinuclear antibody and anti-double-strand DNA antibody decreased after MSCT, with statistical differences at 3 months follow-up.

Conclusion

UC-MSCT results in satisfactory clinical response in SLE patients. However, several cases experienced disease relapse after 6 months, indicating the necessity to repeat MSCT after 6 months.

Registration number: NCT01741857

Introduction

Systemic lupus erythematosus (SLE) is a common and potentially fatal autoimmune disease characterized by autoantibodies associated with multiorgan injury, including the renal, cardiovascular, neural, musculoskeletal, and cutaneous systems [1]. Although disease severity and organ involvement vary significantly among SLE patients, abnormalities of T and B lymphocytes are universal [2-4]. A deeper understanding of the underlying pathology is crucial to develop optimal therapies for the restoration of immune homeostasis [5].

In addition to conventional immunosuppressive therapies, such as cyclophosphamide and mycophenolate mofetil, several new strategies have been developed to target specific activation pathways relevant to SLE pathogenesis [6]. For instance, B-cell-depleting therapies, using the monoclonal antibodies rituximab and the B-lymphocyte stimulator (BLyS) inhibitor belimumab have been beneficial to a specific subpopulation of lupus patients [7,8]. Recently, hematopoietic stem cell transplantation (HSCT) has been reported to improve disease activity in treatment-refractory SLE [9] and reverse organ dysfunction in several animal models [10], but the rates of relapse and treatment-related toxicity are high, as are the rates for the development of a secondary autoimmune disorder [11].

Mesenchymal stem cells (MSCs) are widely studied as an alternative cell source for their ability to differentiate into multiple mesenchymal lineages, including bone, fat, and cartilage [12]. Recent studies have indicated that these pluripotent cells can also differentiate into endoderm and neuroectoderm lineages, including neurons, hepatocytes, and cardiocytes [13-15]. MSCs have been found to possess immunomodulatory effect on various activated immune cells, such as T cells, B cells, natural killer cells, and dendritic cells [16-18]. Additionally, MSCs are able to escape alloantigen recognition because of their low immunogenicity and accompanying lack of expression of costimuatory molecules. These properties make MSCs promising candidate cells for preventing rejection in organ transplantation and treatment of autoimmune disease.

In recent years, we have published pilot single-center clinical studies reporting on the safety and efficacy of allogeneic bone marrow or umbilical cord derived MSCs in treating drug-resistant SLE patients, and the clinical results are encouraging [19,20]. However, we found some relapsed cases during long-term follow-ups [21]; thus, we found it is necessary to conduct a multicenter clinical study to further confirm the efficacy and to explore the best effective time of MSC-based treatment in lupus patients. In this multicenter clinical study, we found that UC MSC transplantation intravenously was safe and no transplantation related adverse event was observed. UC MSC treatment resulted in disease clinical remission and systemic amelioration in refractory lupus patients. However, some patients underwent disease relapse after 6 months, therefore, a repeated MSC infusion is feasible and necessary after 6 months to avoid disease relapse.

Methods

Patients

From December 2009 to August 2011, 40 SLE patients ranging in age from 17 to 54 years old were enrolled in this trial. Informed consent was obtained from each patient and donor. All enrolled patients met at least 4 of the 11 American College of Rheumatology criteria for

SLE. The eligibility criteria included treatment-refractory and active disease, with a SLE Disease Activity Index (SLEDAI) score of more than 8, or at least 1 British Isles Lupus Assessment Group (BILAG) grade A or at least 2 BILAG grade B manifestations, and refractory treatment meant lack of response to treatment with monthly intravenous pulse CYC (500–750 mg/m^2) for ≥ 6 months [22,23] or lack of response to treatment with oral MMF (≥1,000 mg/day) [24] or leflunomide (20 mg/day) for ≥ 3 months, or continued daily doses of ≥20 mg of prednisone or its equivalent. Patients were excluded from the study if they had uncontrolled infection, heart functional class 3 or 4, failure of one of the vital organs, or were pregnant or lactating. Active lupus nephritis was defined by at least one of the following: (1) laboratory tests documented active lupus nephritis three consecutive times: decrease in renal function (serum creatinine > 1.2 mg/dL), increase in proteinuria (defined as more than 1.0 g of protein excretion in a 24-hour urine specimen), deterioration in microscopic hematuria (defined as >10 red cells per high power-field) or the presence of cellular casts; (2) renal biopsy documenting lupus nephritis according to the International Society of Nephrology/Renal Pathology Society classification of active or active/chronic lupus nephritis in renal biopsy class III, class IV-S or IV-G, class V, class III + V, or class IV + V [25]. The study was conducted in compliance with current Good Clinical Practice standards and in accordance with the principle set forth under the 1989 Declaration of Helsinki. The protocol was approved by the Ethics Committee at The Drum Tower Hospital of Nanjing University Medical School, The Affiliated Hospital of Jiangsu University, Jiangsu Provincial People's Hospital and Subei People's Hospital of Jiangsu Province.

Study design

UC MSCs were prepared by the Stem Cell Center of Jiangsu Province, which is the National Stem Cell Institute in China and a member of the International Society for Cellular Therapy (ISCT). The Stem Cell Center was also certified by American Association of Blood Banks (AABB). Fresh umbilical cords were obtained from informed healthy mothers in a local maternity hospital after normal deliveries. The cords were rinsed twice in phosphate buffered saline in penicillin and streptomycin, and the cord blood was removed during this process. The washed cords were cut into 1-mm^2 pieces and floated in low-glucose Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum. The pieces of cord were subsequently incubated at 37°C in a humidified atmosphere consisting of 5% CO_2. Nonadherent cells were removed by washing. The medium was replaced every 3 days after the initial plating. When well-developed colonies of fibroblast-like cells appeared after about 10 days, the cells were trypsinized and passaged into a new flask for further expansion.

Cell viability was determined by trypan blue testing. Culture supernatant was analyzed for pathogenic microorganisms by direct cultivation analysis. Supernatant levels of alanine aminotransferase and endotoxin for each cell preparation were determined by automatic biochemistry analyzer and tachypleus amebocyte lysate (TAL) analysis, respectively. In addition, supernatant virus indexes were determined by enzyme-linked immunosorbent assay (ELISA). Cell surface labeling markers, including CD29, CD73, CD90, CD105, CD45, CD34, CD14, CD79 and HLA-DR, as well as their isotype controls, were all purchased from eBioscience (USA) and cell phenotypes were analyzed by flow cytometric analysis (FCM). We used GMP (Good Manufacturing Practice) conditions and clinical grade reagents to prepare the cells and the protocol was conducted in compliance with Good Clinical Practice (GCP) standards.

One million cells per kilogram of body weight were administered by intravenous infusion on days 0 and 7.

Endpoints

Each patient returned for follow-up at 1, 3, 6, 9 and 12 months after MSC transplantation (MSCT). Evaluation performed at these follow-up visits included a physical examination, determination of SLEDAI score, BILAG analysis, serologic studies, and evaluation of organ functions. Adverse events and their severity were assessed and recorded throughout the study. Primary efficacy endpoints were major clinical response (MCR) and partial clinical response (PCR) assessed during the 12-months study. A major clinical response was defined as achieving BILAG C scores or better in all organs at 6 months without experiencing a severe flare, defined, in turn, as one new domain with a BILAG A score or 2 new domains with BILAG B scores from MSC infusion and maintaining this response throughout the 12-month study period. A partial clinical response was defined as 1) achieving BILAG C scores or better and maintaining this response without a new BILAG A or B score within 3 months, 2) achieving no more than one organ with a BILAG B score at 6 months without achieving ≥1 new BILAG A or B score throughout the 12-month study period [26]. No clinical response was defined as failure to meet the definition of a major or partial clinical response. Clinical relapse was defined as experiencing ≥1 new domain with a BILAG A or B score after a previous major or partial clinical response. Secondary efficacy endpoints included SLEDAI score, lupus serologic changes, systemic evaluations, such as renal functional indexes and hematological involvements. Transplantation-related mortality included all deaths associated with UC MSC transplantation, except those related to recurrence of underlying disease. The investigators assessed and recorded adverse events and their severity throughout the study.

After UC MSCT, the doses of steroids as well as immunosuppressive drugs were tapered according to the amelioration of disease conditions. The dose of prednisone was tapered by 5–10 mg every 2 weeks during the first month following transplantation for responders. If the clinical index was not improved or disease activity was not declined, which defined as non-response, the dose of drugs was not tapered, or even new drugs would be chosen. When “relapse” occurred, the dose of prednisone or immunosuppressive drug would be added, or new drugs would be given. This is the uniform protocol that each center adhered to, and the trial was monitored by the third party (The Stem Cell Center of Jiangsu Province).

Statistical analysis

Data were analyzed as of last data collection in August 2011. Patients were censored at the time of death or last follow-up. We used Fisher’s exact test to compare distribution of categorical variables. Pairwise comparisons of pre- and post-MSCT variables were analyzed by paired *t* test analysis using statistical software (SPSS 13.0). The comparisons of clinical response between patients with or without CYC were analyzed by Chi-Square test. The BILAG index for different organ systems was used to assess response, and scores were converted to numeric values (A = 9, B = 3, C = 1, D = 0, E = 0) to enable evaluation [27,28] All *P* values were two-sided, and $P < 0.05$ was considered statistically significant.

Results

Participant characteristics

Forty patients, including thirty-eight females and two males, were enrolled in this trial. Twenty-six patients were enrolled from The Department of Rheumatology, the Affiliated Drum Tower Hospital of Nanjing University Medical School, Nanjing, China, 6, 5 and 3 patients were enrolled from The Department of Rheumatology, the Affiliated Hospital of Jiangsu University, Zhenjiang, China, the Department of Rheumatology, Subei People's Hospital of Jiangsu Province, Yangzhou, China, and the Department of Rheumatology, Jiangsu Provincial People's Hospital, Nanjing, China, respectively. The mean disease duration was 90.9 months, ranging from 15 to 264 months. Baseline demographics and clinical manifestations for each patient are shown in Table 1. Thirty-nine patients (39/40, 97.5%) underwent two times of UC MSC infusions with an interval of one week, and one patient (1/40, 2.5%) was exempted from the second MSC infusion because of uncontrolled disease progression.

Table 1 Clinical manifestation for each patient at baseline (n = 40)*

Patient/age	Disease duration, months	Baseline SLEDAI	Baseline BILAG	Total cumulative dose of IS	Clinical outcome after MSCT	Clinical manifestations
01/46	40	17	12	CYC 0.8gm/mo × 28mo	PCR	LN, A, C, V, H, ANA+, anti-dsDNA+
02/37	41	12	12	CYC 0.8gm/mo × 35mo	PCR	A, LN, V, ANA+, anti-dsDNA+, H
03/21	50	11	9	MMF 1.5gm/d × 31mo	NR	V, LN, C, anti-SM+
04/28	98	9	9	CYC 0.8gm/mo × 10mo, CYC 0.8gm/mo combined with MMF 1.0gm/d × 28mo (discontinue), LEF 20 mg/d × 31mo	MCR	V, A, alopecia, LN, C, ANA+, anti-dsDNA+
05/26	120	12	8	MMF 2.0gm/d × 50mo (discontinue), CYC 0.8gm/mo × 20mo	NR	V, A, LN, ANA+, anti-dsDNA+
06/23	15	14	19	CYC 0.8gm/mo × 15mo, LEF 20 mg/d × 10mo	NR	V, A, F, LN, P, ANA+, anti-dsDNA+
07/20	62	12	18	MMF 1.5gm/d × 34mo (discontinued), CYC 0.8gm/mo × 24mo	PCR	A, F, LN, C, P, ANA+
08/43	26	34	20	CYC 0.8gm/mo × 10mo (discontinued), LEF 20 mg/d × 10mo	PCR → R	C, V, LN, A, seizures, ANA+
09/36	97	10	26	CYC 0.8gm/mo × 29mo	MCR → R	C, V, A, LN, P, ANA+
10/39	60	10	7	CYC 0.8gm/mo × 25mo (discontinued), LEF 20 mg/d × 30mo	PCR	LN, A, V, ANA+, anti-SM+
11/22	40	8	16	CYC 0.8gm/mo × 25mo	NR	LN, C, P, ANA+, anti-dsDNA+
12/20	50	14	13	CYC 0.8gm/mo × 15mo (discontinued), VCR 1 mg/week × 4times (discontinued)	NR	A, severe thrombocytopenia, V, F, ANA+, anti-dsDNA+, anti-SM+
13/17	75	7	6	MMF 1.5gm/d × 13mo (discontinued), LEF 20 mg/d × 30mo	NR	Severe thrombocytopenia, LN, A, ANA+, anti-dsDNA+
14/21	39	12	11	CYC 0.8gm/mo × 17mo	NR	LN, F, P, A, anti-dsDNA+
15/36	60	10	7	LEF 20 mg/d × 20mo (discontinued), CYC 0.8gm/mo × 37mo	MCR	LN, V, P, A, ANA+, anti-SM+
16/16	49	11	15	CYC 0.8gm/mo × 17mo (discontinued), LEF 20 mg/d × 20mo	NR	LN, A, V, ANA+
17/44	145	4	8	CYC 0.8gm/mo × 64mo	NR	LN, A, V, C, ANA+
18/44	85	8	9	CYC 0.8gm/mo × 40mo	PCR	A, LN, F, ANA+, anti-dsDNA+
19/29	86	10	5	CYC 0.8gm/mo × 24mo	PCR → R	LN, A, P, F, ANA+, anti-dsDNA+
20/54	264	8	4	CYC 0.8gm/mo × 36mo (discontinued), MMF 1.5gm/d × 12mo, then MMF 1.0gm/d × 28mo	MCR	LN, A, V, C, ANA+

21/36	121	13	13	CYC 0.8gm/mo × 25mo, LEF 20 mg/d × 40mo	PCR → R	LN, A, V, C
22/40	24	12	8	CYC 0.8gm/mo × 18mo	NR	F, V, LN, C, ANA+
23/35	25	14	24	CYC 0.8gm/mo × 21mo	NR	F, A, V, LN, P
24/27	48	12	7	LEF 20 mg/d × 4mo (discontinued), CYC 0.8gm/mo × 40mo	MCR	LN, F, A, P, ANA+, anti-SM+
25/30	102	10	7	MMF 2.0gm/d × 6mo,then tapered to 1.5gm/d × 36mo, LEF 20 mg/d × 12mo, then tapered to 10 mg/d × 59mo	PCR	V, A, LN, ANA+, anti-dsDNA+
26/31	62	8	3	MMF 1.5gm/d × 8mo,then tapered to 1.0gm/d × 50mo, LEF 20 mg/d × 19mo	MCR → R	LN, V, P, ANA+, anti-dsDNA+
27/51	108	13	29	CYC0.8gm/mo × 41mo,CsA150mg/d × 30mo	NR	LN, V, A, C, seizures
28/50	110	10	11	LEF 20 mg/d × 39mo (discontinued), CYC 0.8gm/mo × 36mo	MCR	A, V, LN, ANA+
29/45	102	10	9	CYC1.2gm/mo × 22mo	NR	A, V, LN, ANA+, anti-dsDNA+
30/33	62	10	9	CYC0.8gm/mo × 21mo, LEF 20 mg/d × 12mo	MCR	LN, A, P, C
31/32	156	14	12	CYC0.8gm/mo × 36mo(discontinued), MMF 1.5gm/d × 6mo, then tapered to 1.0gm/d × 56mo	MCR → R	LN, A, C, P
32/53	146	12	10	CYC0.8gm/mo × 24mo, then tapered to 0.6gm/mo × 42mo, LEF 20 mg/d × 12mo, then tapered to 10 mg/d × 40mo	NR	LN, A, C, anti-dsDNA+
33/30	157	8	7	CYC0.8gm/mo × 16mo(discontinued)	NR	V, LN, A, C, H
34/35	123	10	9	CYC0.8gm/mo × 18mo, LEF 20 mg/d × 22mo	NR	LN, A, H, C, ANA+
35/33	216	10	3	CYC0.8gm/mo × 26mo	PCR	F, LN, V, C, A
36/39	99	5	7	CYC0.8gm/mo × 14mo	MCR	LN, C, V, anti-dsDNA+
37/35	109	6	6	LEF 20 mg/d × 34mo	PCR → R	LN, C, H, ANA+
38/31	160	9	7	LEF 20 mg/d × 13mo (discontinued), CYC0.8gm/mo × 35mo	MCR	LN, C, V, A
39/50	108	10	12	CYC0.8gm/mo × 14mo	MCR	F, LN, A, V, C
40/35	96	8	8	CYC0.8gm/mo × 28mo (discontinued), LEF 20 mg/d × 7mo	MCR	LN, V, P, C, ANA+

A arthralgia, *F* febrile, *H* hypocomplementemia, *LN* lupus nephritis, *V* vasculitis, *P* polyserositis, *C* cytopenia, *ANA* anti nuclear antibody, *anti-dsDNA* anti double strand DNA antibody, *VCR* vincristine, *MCR* major clinical response, *PCR* partial clinical response, *NR* non-response, *R* relapse, *IS* immunosuppressive drugs.

*: Patients clinical manifestations were recorded within one week before UC MSC transplantation.

No. 1 to 26 enrolled from The Department of Rheumatology, the Affiliated Drum Tower Hospital of Nanjing University Medical School, Nanjing, China. No. 27 to 32 enrolled from The Department of Rheumatology, the Affiliated Hospital of Jiangsu University, Zhenjiang, China. No. 33 to 37 enrolled from the Department of Rheumatology, Subei People's Hospital of Jiangsu Province, Yangzhou, China. No. 38 to 40 enrolled from the Department of Rheumatology, Jiangsu Provincial People's Hospital, Nanjing, China.

UC MSCs characteristics

All the infused UC MSCs were derived from passages 2 to 4, with rigorous purification and quality control. Cell viability of purified MSCs was >92%, Culture supernatant was negative for pathogenic microorganisms, including aerobic and anaerobic bacteria, as well as negative for hepatitis B surface antigen, hepatitis B core antibody, hepatitis C virus antibody, human immunodeficiency virus antibodies I and II, cytomegalovirus IgM, and syphilis antibody. Flow cytometric analysis showed CD29, CD73, CD90 and CD105 expression of >95%, in

parallel with CD45, CD34, CD14, CD79 and HLA-DR expression of <2%. In addition, levels of alanine aminotransferase and endotoxin in the supernatants of each cell preparation were strictly controlled within 40 IU/liter and 5EU, respectively. The capacity of MSCs to differentiate into adipogenic and osteogenic lineages was also assayed.

Safety

After 12 months, the overall survival rate was 92.5% (37/40). Three patients died of uncontrolled disease activity and organ failures. One patient had active lupus with malar rash, arthralgia, uncontrolled hypertension, and rapid deterioration of renal function, hypoproteinemia and severe proteinuria. She died seven days after the first MSC infusion from uncontrolled progressive disease and acute heart failure. Another patient had lupus relapse 8 months after MSC infusion, with pulmonary hypertension and died from right-sided heart failure 256 days after MSCT. The third patient also had disease relapse 6 months after MSC transplantation, with steroid-resistant thrombocytopenia and uncontrolled septicemia, finally dying of respiratory failure 192 days post MSC infusion. Two patients underwent moderate herpesvirus infection 291 and 135 days after MSC treatment, respectively and one patient experienced tuberculosis infection at 326 days. All the infection events were treated by conventional therapies. Adverse events were not considered to be possibly related to UC MSC transplantations and all the adverse events were listed in Table 2.

Table 2 Adverse events by UC MSC treatment within 12 months

Patient no.	Adverse event	Severity	Time (day)	Related to MSCT
02	Herpesvirus infection	AE	291	No relation
03	Herpesvirus infection	AE	135	No relation
03	Herpesvirus infection	AE	187	No relation
09	Death	SAE	7	No relation
12	Tuberculosis infection	AE	326	No relation
14	Death	SAE	256	No relation
27	Death	SAE	192	No relation

AE adverse event, *SAE* severe adverse event.

Clinical outcomes

Clinical responses

Thirteen and eleven patients achieved MCR (13/40, 32.5%) and PCR (11/40, 27.5%) during 12 months follow-up, respectively. In total, 16 patients had no clinical response (16/40, 40%). Three and four patients experienced disease relapse at 9 (12.5%) and 12 (16.7%) months follow-up, respectively, after a prior major or partial clinical response. Twenty-six patients had CYC for a basal treatment and the other 14 patients did not. However, we did not observe any difference in the rate of clinical remission between the two groups ($P > 0.05$ by Chi-Square test).

Disease activity assessment

Lupus disease activity, as shown by SLEDAI, significantly decreased after MSC transplantation (mean ± SD, 10.83 ± 4.63 at baseline, 8.55 ± 3.99 at 1 month, 7.43 ± 3.93 at 3 months, 6.30 ± 3.63 at 6 months, 6.40 ± 3.84 at 9 months, 6.48 ± 3.52 at 12 months; all $P < 0.01$ versus baseline levels, Figure 1a). Total BILAG score markedly ameliorated after UC

MSC infusions (mean ± SD, 10.78 ± 6.09 at baseline, 5.35 ± 4.48 at 1 month, 5.28 ± 4.71 at 3 months, 4.23 ± 4.43 at 6 months, 3.85 ± 4.73 at 9 months, 3.55 ± 4.33 at 12 months; all $P < 0.001$ versus baseline levels, Figure 1b).

Figure 1 Assessments of changes in Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) score (a) and total British Isles Lupus Assessment Group (BILAG) score (b) before and after umbilical cord mesenchymal stem cells (MSCs) transplantation. **: $P < 0.01$ versus before MSCT, ***: $P < 0.001$ versus before MSCT.

Serology changes

Serum albumin levels improved shortly after UC MSC infusions, achieved normal levels at the 1-month follow-up visit, and maintained normal levels in the succeeding 9 months until the 12-month follow-up visit, when they declined (mean ± SD, 3.17 ± 0.75 g/dl at baseline, 3.70 ± 0.58 g/dl at 1 month, 3.80 ± 0.67 g/dl at 3 months, 3.84 ± 0.63 g/dl at 6 months, 3.89 ± 0.64 g/dl at 9 months, 3.67 ± 0.78 g/dl at 12 months; all $P < 0.05$ versus baseline levels, Figure 2a). Serum complement 3 improved, with statistical significance at 6 months (Figure 2b). Serum complement 4 levels showed no obvious changes after MSC treatment in those patients. We observed that serum anti-double-strand DNA (dsDNA) antibody levels decreased after MSCT, with statistical difference at 6 and 12 months follow-up visits (mean ± SD, 710.83 ± 814.05 U/ml at baseline, 526.78 ± 666.7 U/ml at 1 month, 590.41 ± 702.99 U/ml at 3 months, 492.67 ± 615.15 U/ml at 6 months, 513.58 ± 378.6 U/ml at 9 months, 212.62 ± 244.77 U/ml at 12 months, n = 16), along with decreased serum antinuclear antibody (ANA) (mean ± SD, 5.77 ± 2.32 at baseline, 5.40 ± 2.08 at 1 month, 5.24 ± 2.66 at 3 months, 4.85 ± 2.83 at 6 months, 4.46 ± 2.21 at 9 months, 4.73 ± 2.36 at 12 months, Figure 2c, d).

Figure 2 Allogenic umbilical cord mesenchymal stem cells (MSCs) transplantation improved serum albumin (a) and complement 3 levels (b) in refractory lupus patients. Serum anti-double-strand DNA (ds-DNA) antibody **(c)** and anti nuclear antibody (ANA) **(d)** levels decreased after MSC infusions. *: $P < 0.05$ versus before MSCT.

Organ functional improvement

Thirty-eight patients (38/40, 95%) had active lupus nephritis (LN) (renal BILAG A or B score) at baseline, but their renal BILAG score significantly decreased after two times of UC MSC infusions (Figure 3a). Twenty-four-hour proteinuria levels significantly decreased after UC MSC treatment (mean ± SD, 2.24 ± 1.43 g at baseline, 2.13 ± 1.35 g at 1 month, 1.91 ± 1.20 g at 3 months, 1.65 ± 1.11 g at 6 months, 1.24 ± 1.09 g at 9 months, 1.41 ± 1.33 g at 12 months, $P < 0.05$ at 9 and 12 months follow-up visits, Figure 3b). Renal function index, as assessed by serum creatinine and blood nitrogen, also decreased, and both showed statistical differences at the 6 months follow-up visit (Figure 3c, d), while, however, increasing at the 12 months follow-up visit. Twenty-five (25/40, 62.5%) and twenty-eight (28/40, 70%) patients had hematopoietic and cutaneous system involvements at baseline, respectively. The BILAG score for the two systems also ameliorated after MSC treatment (Figure 3e, f).

Figure 3 Renal system BILAG score markedly ameliorated after umbilical cord mesenchymal stem cells (UC MSCs) transplantation (a). Twenty-four hour proteinuria **(b)**, serum creatinine **(c)** and blood nitrogen **(d)** declined after systemic UC MSC administration. BILAG score for hematopoietic **(e)** and cutaneous **(f)** systems ameliorated after UC MSC treatment.

Therapy schedule after UC MSC infusion

The dose of prednisone was tapered from 5 to 10 milligrams every 2 weeks in the first month following transplantation, according to clinical status and laboratory indicators of disease amelioration. During the 12 months follow-up visit, 30 out of 37 (81.08%) patients underwent steroid tapering, and while 19 out of 35 (54.29%) patients had immunosuppressant tapering after MSC transplantation, two patients were excluded, as they had not been taking immunosuppressive drugs at baseline (Table 3).

Table 3 Treatments used before and after UC MSC transplantation in each patient

No.	Baseline	1mo	3mo	6mo	12mo
01	Pred5mg/d, CYC0.8gm/mo, LEF20mg/d, HCQ200mg/d	Pred5mg/d, CYC0.8gm/mo, LEF20mg/d, HCQ200mg/d	Pred5mg/d, CYC0.8gm/mo, LEF20mg/d, HCQ200mg/d	Pred5mg/d, CYC0.8gm/mo, LEF20mg/d, HCQ200mg/d	Pred5mg/d, CYC0.8gm/mo, LEF20mg/d, HCQ200mg/d
02	Pred30mg/d, CYC0.8gm/mo	Pred15mg/d, CYC0.8gm/mo	Pred10mg/d, CYC0.8gm/mo	Pred10mg/d, CYC0.8gm/mo	Pred10mg/d, CYC0.8gm/mo
03	Pred15mg/d MMF1.5gm/d HCQ200mg/d	Pred15mg/d MMF1.5gm/d HCQ200mg/d	Pred10mg/d MMF1.5gm/d HCQ200mg/d	Pred15mg/d MMF1.5gm/d HCQ200mg/d	Pred15mg/d MMF1.0gm/d HCQ200mg/d
04	Pred10mg/d LEF20mg/d HCQ400mg/d	Pred5mg/d LEF20mg/d HCQ400mg/d	Pred5mg/d LEF20mg/d HCQ400mg/d	Pred5mg/d LEF10mg/d HCQ400mg/d	Pred5mg/d LEF10mg/d HCQ200mg/d
05	Pred10mg/d CYC0.8gm/mo HCQ400mg/d	Pred10mg/d CYC0.8gm/mo HCQ400mg/d	Pred5mg/d CYC0.8gm/mo HCQ400mg/d	Pred5mg/d CYC0.8gm/mo HCQ400mg/d	Pred5mg/d CYC0.6gm/mo HCQ400mg/d
06	Pred20mg/d CYC0.8gm/mo LEF20mg/d HCQ400mg/d	Pred20mg/d CYC0.8gm/mo LEF20mg/d HCQ400mg/d	Pred15mg/d CYC0.8gm/mo LEF20mg/d HCQ400mg/d	Pred15mg/d CYC0.8gm/mo LEF20mg/d HCQ400mg/d Triptolide 60 mg/d	Pred15mg/d CYC0.8gm/mo LEF20mg/d HCQ400mg/d Triptolide 60 mg/d
07	Pred15mg/d CYC0.8gm/mo HCQ300mg/d	Pred10mg/d CYC0.8gm/mo HCQ300mg/d	Pred10mg/d CYC0.8gm/mo HCQ300mg/d	Pred10mg/d CYC0.8gm/mo HCQ300mg/d	Pred10mg/d CYC0.6gm/mo HCQ300mg/d
08	Pred20mg/d LEF20mg/d HCQ400mg/d	Pred15mg/d LEF20mg/d HCQ400mg/d	Pred15mg/d LEF20mg/d HCQ400mg/d	Pred30mg/d LEF20mg/d HCQ400mg/d	Pred10mg/d LEF20mg/d HCQ300mg/d
09	Pred40mg/d CYC0.8gm/mo HCQ400mg/d	Pred25mg/d CYC0.8gm/mo HCQ400mg/d	Pred15mg/d CYC0.8gm/mo HCQ400mg/d	Pred10mg/d CYC0.8gm/mo HCQ400mg/d	/
10	Pred10mg/d LEF20mg/d HCQ400mg/d	Pred5mg/d LEF20mg/d HCQ400mg/d	Pred5mg/d LEF10mg/d HCQ400mg/d	Pred5mg/d LEF10mg/d HCQ400mg/d	Pred5mg/d LEF10mg/d HCQ400mg/d
11	Pred20mg/d CYC0.8gm/mo	Pred20mg/d CYC0.8gm/mo	Pred15mg/d CYC0.8gm/mo	Pred10mg/d CYC0.8gm/mo	Pred10mg/d CYC0.4gm/mo

		HCQ400mg/d	HCQ400mg/d	HCQ400mg/d	HCQ400mg/d
12	Pred20mg/d	Pred15mg/d	Pred15mg/d	Pred10mg/d	Pred10mg/d
	HCQ400mg/d	HCQ400mg/d	HCQ400mg/d	HCQ400mg/d	HCQ400mg/d
13	Pred20mg/d	Pred15mg/d	Pred15mg/d	Pred10mg/d	Pred10mg/d
	LEF20mg/d	LEF20mg/d	LEF20mg/d	LEF20mg/d	LEF20mg/d
	HCQ400mg/d	HCQ400mg/d	HCQ400mg/d	HCQ400mg/d	HCQ400mg/d
14	Pred15mg/d	/	/	/	/
	CYC0.8gm/mo				
15	Pred15mg/d	Pred15mg/d	Pred15mg/d	Pred10mg/d	Pred10mg/d
	CYC0.8gm/mo,	CYC0.8gm/mo,	CYC0.8gm/mo,	CYC0.8gm/mo,	CYC0.4gm/mo
	HCQ400mg/d	HCQ400mg/d	HCQ300mg/d	HCQ300mg/d	HCQ300mg/d
16	Pred15mg/d	Pred15mg/d	Pred10mg/d	Pred10mg/d	Pred20mg/d
	LEF20mg/d	LEF20mg/d	LEF20mg/d	LEF20mg/d	LEF20mg/d
	HCQ200mg/d	HCQ200mg/d	HCQ200mg/d	HCQ200mg/d	HCQ200mg/d
17	Pred15mg/d	Pred15mg/d	Pred15mg/d	Pred10mg/d	Pred10mg/d
	CYC0.8gm/mo	CYC0.8gm/mo	CYC0.8gm/45d	CYC0.8gm/45d	CYC0.4gm/mo
	HCQ200mg/d	HCQ200mg/d	HCQ200mg/d	HCQ200mg/d	HCQ200mg/d
18	Pred20mg/d	Pred15mg/d	Pred15mg/d	Pred15mg/d	Pred10mg/d
	CYC0.8gm/mo	CYC0.8gm/mo	CYC0.8gm/mo	CYC0.8gm/mo	CYC0.8gm/mo
	HCQ400mg/d	HCQ400mg/d	HCQ400mg/d	HCQ400mg/d	HCQ400mg/d
19	Pred15mg/d	Pred15mg/d	Pred10mg/d	Pred10mg/d	Pred15mg/d
	CYC0.8gm/mo	CYC0.8gm/mo	CYC0.8gm/mo	CYC0.6gm/mo	CYC0.8gm/mo
	HCQ400mg/d	HCQ400mg/d	HCQ400mg/d	HCQ400mg/d	HCQ400mg/d
20	Pred5mg/d	Pred5mg/d	Pred5mg/d	Pred5mg/d	Pred5mg/d
	MMF1.0gm/d	MMF1.0gm/d	MMF1.0gm/d	MMF1.0gm/d	MMF0.5gm/d
	HCQ200mg/d	HCQ200mg/d	HCQ200mg/d	HCQ200mg/d	HCQ200mg/d
21	Pred7.5 mg/d	Pred7.5 mg/d	Pred5mg/d	Pred15mg/d	Pred15mg/d
	CYC0.8gm/mo	CYC0.8gm/mo	CYC0.8gm/mo	CYC0.8gm/mo	CYC0.8gm/mo
	LEF10mg/d	LEF10mg/d	LEF10mg/d	LEF10mg/d	LEF10mg/d
	HCQ400mg/d	HCQ400mg/d	HCQ400mg/d	HCQ400mg/d	HCQ200mg/d
22	Pred20mg/d	Pred15mg/d	Pred15mg/d	Pred10mg/d	Pred10mg/d
	CYC0.8gm/mo	CYC0.8gm/mo	CYC0.8gm/mo	CYC0.8gm/mo	CYC0.6gm/mo
	HCQ400mg/d	HCQ400mg/d	HCQ400mg/d	HCQ400mg/d	HCQ400mg/d
23	Pred45mg/d	Pred15mg/d	Pred10mg/d	Pred10mg/d	Pred10mg/d
	CYC0.8gm/mo	CYC0.8gm/mo	CYC0.8gm/mo	CYC0.8gm/mo	CYC0.6gm/mo
	HCQ400mg/d	HCQ400mg/d	HCQ400mg/d	HCQ400mg/d	HCQ200mg/d
24	Pred15mg/d	Pred15mg/d	Pred10mg/d	Pred5mg/d	Pred5mg/d
	CYC0.8gm/mo	CYC0.8gm/mo	CYC0.8gm/mo	CYC0.8gm/mo	CYC0.6gm/mo
25	Pred15mg/d	Pred10mg/d	Pred10mg/d	Pred10mg/d	Pred10mg/d
	MMF1.5gm/d	MMF1.5gm/d	MMF1.5gm/d	MMF1.0gm/d	MMF1.0gm/d
	LEF10mg/d	LEF10mg/d	HCQ400mg/d	HCQ400mg/d	HCQ400mg/d
	HCQ400mg/d	HCQ400mg/d			
26	Pred20mg/d	Pred15mg/d	Pred10mg/d	Pred10mg/d	Pred20mg/d
	MMF1.0gm/d	MMF1.0gm/d	MMF1.0gm/d	MMF1.0gm/d	MMF1.0gm/d
	LEF20mg/d	LEF20mg/d	LEF20mg/d	LEF20mg/d	LEF20mg/d
	HCQ200mg/d	HCQ200mg/d	HCQ200mg/d	HCQ200mg/d	HCQ200mg/d
27	Pred50mg/d	Pred30mg/d	Pred15mg/d	MP80mg/d	/
	CYC0.8gm/mo	CYC0.8gm/mo	CYC0.8gm/mo	CYC0.6gm/d × 3d	
	CsA150mg/d	CsA150mg/d	CsA150mg/d	Gamma globulin20g × 3d	
28	Pred20mg/d	Pred15mg/d	Pred15mg/d	Pred5mg/d	Pred5mg/d
	CYC0.8gm/mo	CYC0.8gm/mo	CYC0.8gm/mo	CYC0.8gm/mo	CYC0.8gm/mo
	HCQ200mg/d	HCQ200mg/d	HCQ200mg/d	HCQ200mg/d	HCQ200mg/d

29	Pred30mg/d CYC1.2gm/mo	Pred25mg/d CYC1.2gm/mo	Pred20mg/d CYC1.2gm/mo	Pred10mg/d CYC1.2gm/mo Triptolide 60 mg/d	Pred5mg/d CYC0.8gm/mo Triptolide 60 mg/d
30	Pred25mg/d CYC0.8gm/mo LEF20mg/d	Pred20mg/d CYC0.8gm/mo LEF20mg/d	Pred15mg/d CYC0.8gm/mo LEF20mg/d	Pred10mg/d CYC0.8gm/mo LEF20mg/d	Pred10mg/d CYC0.6gm/mo LEF10mg/d
31	Pred30mg/d MMF1.0gm/d	Pred25mg/d MMF1.0gm/d	Pred25mg/d MMF1.0gm/d	Pred30mg/d MMF1.0gm/d	Pred15mg/d MMF1.0gm/d
32	Pred25mg/d CYC0.6gm/mo LEF10mg/d HCQ400mg/d	Pred20mg/d CYC0.6gm/mo LEF10mg/d HCQ400mg/d	Pred15mg/d CYC0.6gm/mo LEF10mg/d HCQ400mg/d	Pred15mg/d CYC0.6gm/mo LEF10mg/d HCQ400mg/d	Pred15mg/d CYC0.6gm/mo LEF10mg/d HCQ200mg/d
33	Pred25mg/d	Pred15mg/d	Pred15mg/d	Pred35mg/d	Pred35mg/d
34	Pred15mg/d CYC0.8gm/mo LEF20mg/d	Pred15mg/d CYC0.8gm/mo LEF20mg/d	Pred15mg/d CYC0.8gm/mo LEF10mg/d	Pred15mg/d CYC0.8gm/mo LEF10mg/d	Pred10mg/d CYC0.8gm/mo LEF10mg/d
35	Pred20mg/d, CYC0.8gm/mo	Pred15mg/d, CYC0.8gm/mo	Pred15mg/d, CYC0.8gm/mo	Pred10mg/d, CYC0.8gm/mo	Pred10mg/d CYC0.6gm/mo
36	Pred25mg/d CYC0.8gm/mo HCQ400mg/d	Pred15mg/d CYC0.8gm/mo HCQ400mg/d	Pred15mg/d CYC0.8gm/mo HCQ400mg/d	Pred10mg/d CYC0.6gm/mo HCQ300mg/d	Pred10mg/d CYC0.6gm/mo HCQ300mg/d
37	Pred20mg/d LEF20mg/d	Pred15mg/d LEF20mg/d	Pred15mg/d LEF20mg/d	Pred15mg/d LEF20mg/d	Pred20mg/d, LEF20mg/d
38	Pred10mg/d CYC0.8gm/mo HCQ200mg/d	Pred10mg/d CYC0.8gm/mo HCQ200mg/d	Pred10mg/d CYC0.8gm/mo HCQ200mg/d	Pred7.5 mg/d CYC0.8gm/mo HCQ200mg/d	Pred7.5 mg/d CYC0.6gm/mo HCQ200mg/d
39	Pred20mg/d CYC0.8gm/mo HCQ200mg/d	Pred15mg/d CYC0.8gm/mo HCQ200mg/d	Pred10mg/d CYC0.8gm/mo HCQ200mg/d	Pred10mg/d CYC0.8gm/mo HCQ200mg/d	Pred10mg/d CYC0.8gm/mo HCQ200mg/d
40	Pred10mg/d LEF20mg/d	Pred10mg/d LEF20mg/d	Pred10mg/d LEF20mg/d	Pred10mg/d LEF20mg/d	Pred5mg/d LEF20mg/d

UC MSCs umbilical cord derived mesenchymal stem cells, *MSCT* MSC transplantation, *Pred* prednisone, *CYC* cyclophosphamide, *HCQ* hydroxychloroquine, *MMF* mycophenolate mofetil, *LEF* leflunomide, *CsA* Ciclosporin A, *mo* month, *d* day.

No. 1 to 26 enrolled from The Department of Rheumatology, the Affiliated Drum Tower Hospital of Nanjing University Medical School, Nanjing, China. No. 27 to 32 enrolled from The Department of Rheumatology, the Affiliated Hospital of Jiangsu University, Zhenjiang, China. No. 33 to 37 enrolled from the Department of Rheumatology, Subei People's Hospital of Jiangsu Province, Yangzhou, China. No. 38 to 40 enrolled from the Department of Rheumatology, Jiangsu Provincial People's Hospital, Nanjing, China.

Discussion

MSCs are multipotent, non-haematopoietic progenitor cells that are being explored as a promising new treatment for tissue regeneration. Although their immunomodulatory properties are not yet completely understood, their low immunogenic potential, together with their effects on immune responses, make them a promising therapeutic tool for severe and refractory autoimmune diseases. MSCs have already been applied in clinical treatment for acute graft-versus-host disease (GVHD) following allogeneic HSCT [29,30], ischemic cardiomyopathy [31,32], and for autoimmune diseases like systemic sclerosis [33],

inflammatory bowel disease [34,35], dermatomyositis/polymyositis [36], rheumatoid arthritis [37], Sjogren's syndrome [38] and type 1 or type 2 diabetes mellitus [39,40].

To date, only limited clinical investigations have been performed with MSC treatment in lupus patients. We previously conducted a small-scale and short-term study of intravenous delivery of umbilical cord MSCs [19]. Recently a larger-scale study with 87 lupus cases enrolled and longer-term follow-up time of 4 years further suggested the clinical responses of allogenic MSC transplantation [21]. However, we did not have the evidence of a multicenter study to further confirm the results. The present multicenter study has substantiated clinical safety and efficacy by UC MSC transplantation in lupus patients, as determined in single-center studies. Sixty percent of patients achieved major or partial clinical response after 12 months of follow-up visits, while another 40% of patients had no clinical response. Intravenous infusion of UC MSCs is a safe practice, demonstrating treatment efficacy in ameliorating renal function and serologic index. In addition to significant decline of disease activity assessed by SLEDAI and BILAG, UC MSC infusion ameliorated systemic manifestations in hematopoietic and cutaneous systems.

We have previously compared the clinical efficacy between single and double MSC infusions in lupus patients, and the results showed that the treatment efficacy was comparable between the two groups [41]. In this multicenter study, 39 out of 40 of the enrolled patients received double UC MSC infusions, with a one-week interval. After 12 months follow-up, we found a comparable rate of clinical response and safety profile. The results further indicate that single infusion is enough in clinical treatment.

However, the role of MSCs *in vivo* is not permanent. In the present study, 12.5% and 16.7% patients had disease relapse at 9 and 12 months follow-up, respectively, after a prior major or partial clinical response. Serologic index, such as serum albumin and complement 3 levels, recurred slightly, concomitant with relapsed renal function index, as assessed by serum creatinine and blood nitrogen levels. Based on the safety profile of MSC infusion in clinical application, our data further suggest the necessity of repeating MSC infusions after 6 months in refractory lupus patients.

MSCs can be isolated from many tissues, including bone marrow, umbilical cord, umbilical cord blood, placenta or adipose tissues. Bone marrow-derived MSCs are widely used in clinical applications, whether autologous or allogenic. However, more and more studies have recently shown that MSCs from bone marrow are difficult to obtain, have ethics issues and are easily contaminated. Moreover, autologous bone marrow-derived MSCs are functionally abnormal in some disorders like lupus [42,43], rheumatoid arthritis [44] and systemic sclerosis [45], which may limit their clinical applications. Umbilical cords are off-fall after delivery, but rich in MSCs. UC MSCs have many advantages over bone marrow MSCs, including easy access, less possibility of contamination and no ethics problems. Furthermore, UC MSCs do not express tumor-associated fibroblasts phenotype and therefore have no opportunity to grow solid tumors, compared to bone marrow MSCs [46]. Moreover, UC MSCs have a higher rate of gene expression related to cell adhesion, morphogenesis, angiogenesis and neurogenesis compared to umbilical cord blood derived MSCs [47], and can accumulate more mineralized matrix than placenta-derived MSCs [48], indicating that UC MSCs may be used as an optimal option for cell therapy.

The present study has some limitations. First, 95% of patients had active lupus nephritis at the entry of the study, but we cannot provide the pathologic data of the present enrolled

patients; thus, we do not know whether MSCs can, indeed, ameliorate renal pathology, aside from the improvements in renal function. Second, this is not a controlled and randomized study. We still lack of a group of patients with conventional therapies, but not combined with allogeneic MSC infusion. Therefore, the current data only provide evidence that allogeneic MSCT could induce renal remission on the basis of other drugs taken by patients enrolled in this study. Third, because of the discrepancy of each patient at enrollment, we cannot assure the uniformity and standards for quality control among different centers or different patients. We will consider performing a randomized and multicenter controlled study in China to assess the safety and efficacy of MSCT in lupus nephritis patients, to compare the clinical safety and efficacy between steroid combined MSC treatment and steroid combined traditional immunosuppressive drug therapy, like CYC. In the incoming trial, repeated renal biopsy will be designed to further determine whether MSC transplantation could alleviate renal pathology in LN patients. In addition, we will try to make uniformity for the enrolled patients, to keep quality control of the study.

Conclusions

Our multicenter clinical study illustrated the safety and efficacy of systemic administration of UC MSCs in SLE patients. Moreover, a repeated MSC infusion is feasible and necessary after 6 months to avoid disease relapse.

Abbreviations

ANA, Anti nuclear antibody; anti-dsDNA, Anti double strand DNA antibody; BILAG, British Isles Lupus Assessment Group; BlyS, B-lymphocyte stimulator; CsA, Ciclosporin A; CYC, Cyclophosphamide; DMEM, Dulbecco's modified Eagle's medium; GMP, Good manufacturing practice; GVHD, Graft-versus-host disease; HCQ, Hydroxychloroquine; HSCT, Graft-versus-host disease; LEF, Leflunomide; LN, Lupus nephritis; MCR, Major clinical response; MMF, Mycophenolate mofetil; MSCs, Mesenchymal stem cells; MSCT, Mesenchymal stem cell transplantation; PCR, Partial clinical response; Pred, Prednisone; SLE, Systemic lupus erythematosus; SLEDAI, Systemic lupus erythematosus disease activity index; TAL, Tachypleus amebocyte lysate.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

DW: conception and design, data collection and analysis, manuscript writing and final approval of the manuscript. JL: study design, data collection and analysis, manuscript revision and final approval of the manuscript. YZ: study design, data collection and analysis, manuscript revision and final approval of the manuscript. MZ: study design, data collection and analysis, manuscript revision and final approval of the manuscript. JC: data analysis, manuscript writing and final approval of the manuscript. XL: conception and design, data collection, manuscript drafting and final approval of the manuscript. XH: conception and design, critical revision and final approval of manuscript. SJ: conception and design, data collection, manuscript drafting and final approval of the manuscript. SS: conception and

design, critical revision and final approval of the manuscript. LS: conception and design, data collection and analysis, manuscript writing and finical approval of the manuscript. All authors read and approved the final manuscript.

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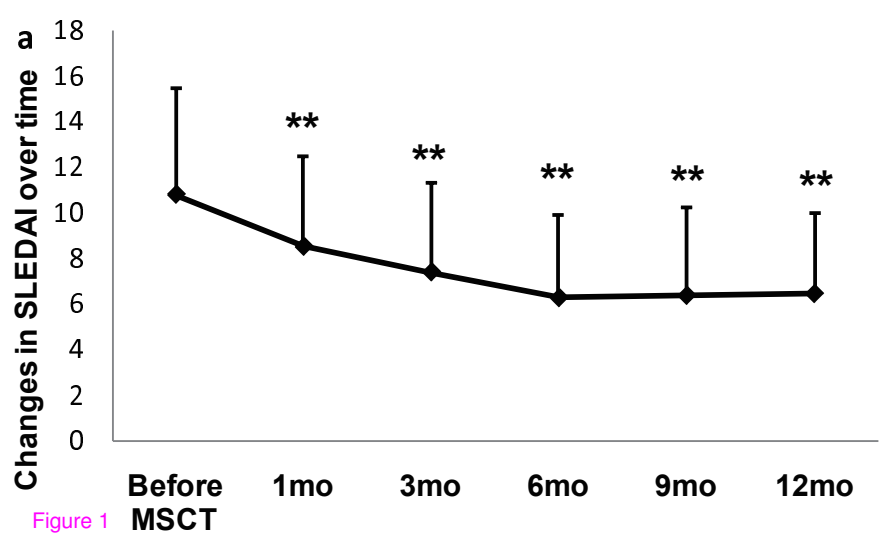

a
Changes in SLEDAI over time
18
16
14
12
10
8
6
4
2
0
**
**
**
**
**
Before MSCT
1mo
3mo
6mo
9mo
12mo

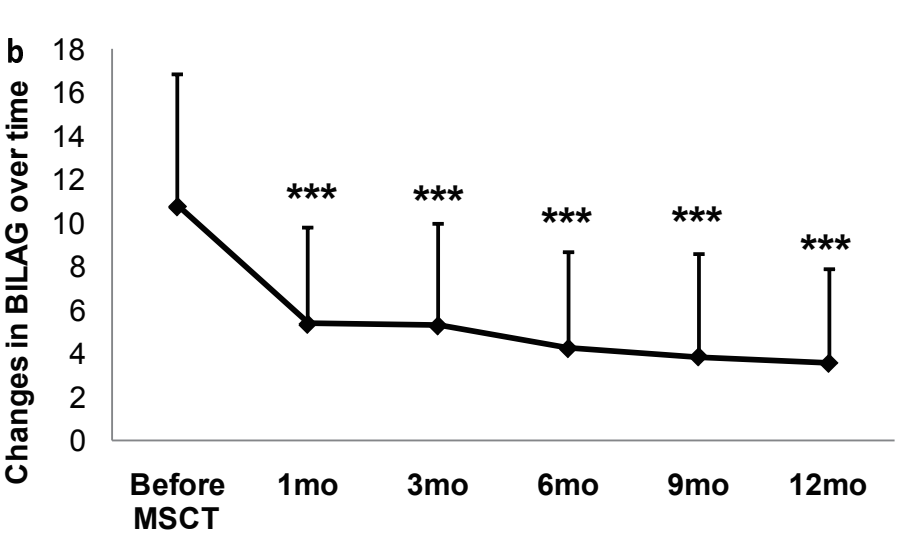
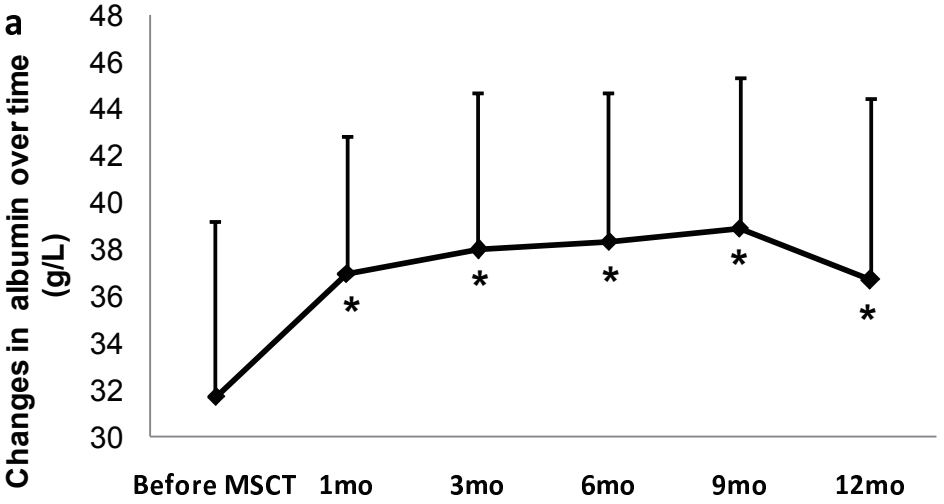
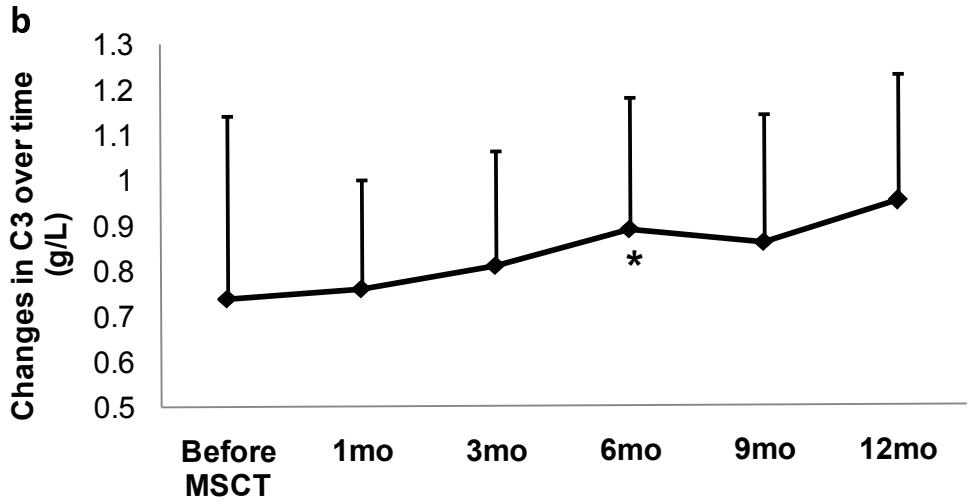
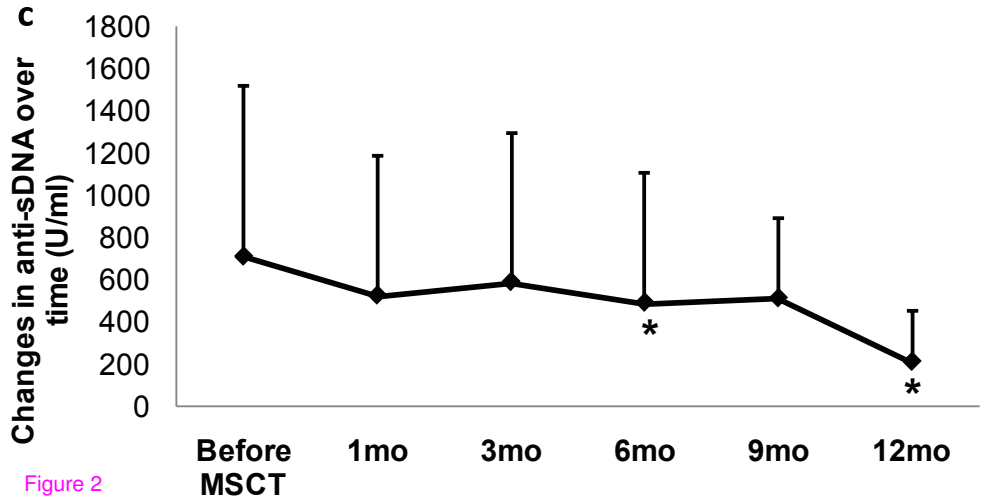

b
Changes in BILAG over time
18
16
14
12
10
8
6
4
2
0
***
***
***
***
***
Before MSCT
1mo
3mo
6mo
9mo
12mo

Figure 1


a
Changes in albumin over time (g/L)
48
46
44
42
40
38
36
34
32
30
*
*
*
*
*
Before MSCT
1mo
3mo
6mo
9mo
12mo


b
Changes in C3 over time (g/L)
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
*
Before MSCT
1mo
3mo
6mo
9mo
12mo


c
Changes in anti-sDNA over time (U/ml)
1800
1600
1400
1200
1000
800
600
400
200
0
*
*
Before MSCT
1mo
3mo
6mo
9mo
12mo

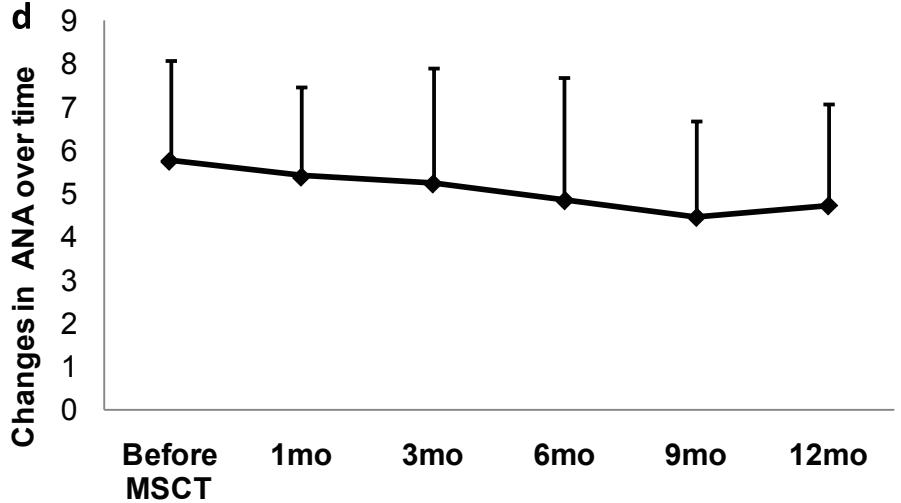
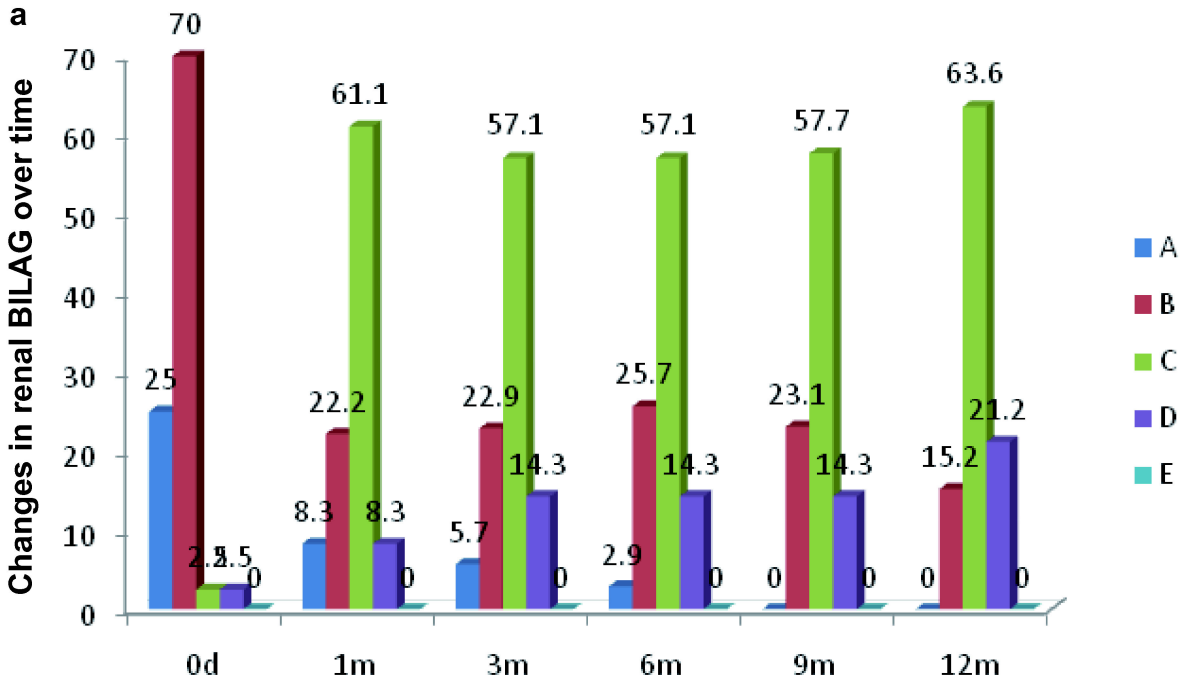
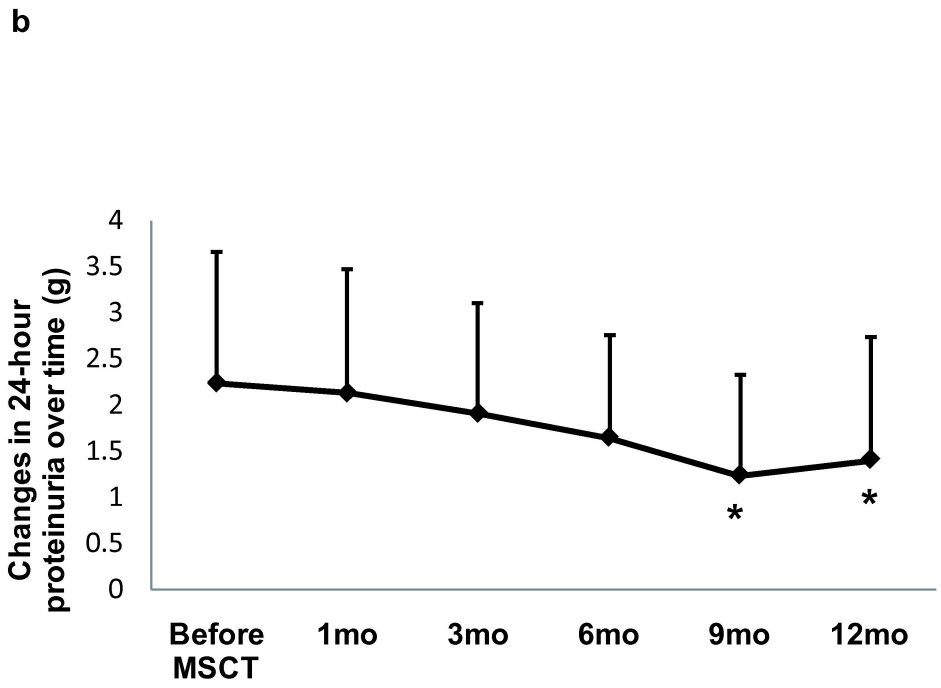
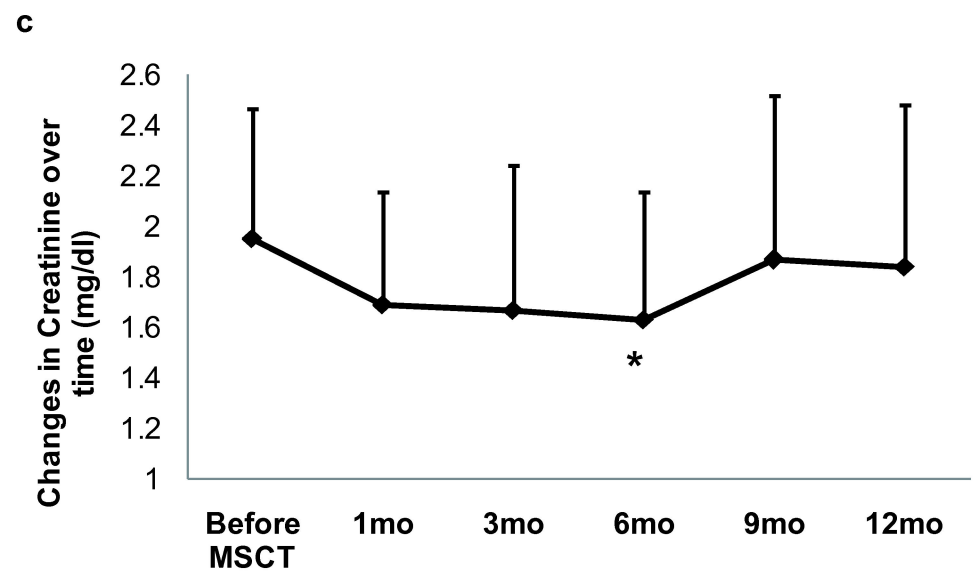
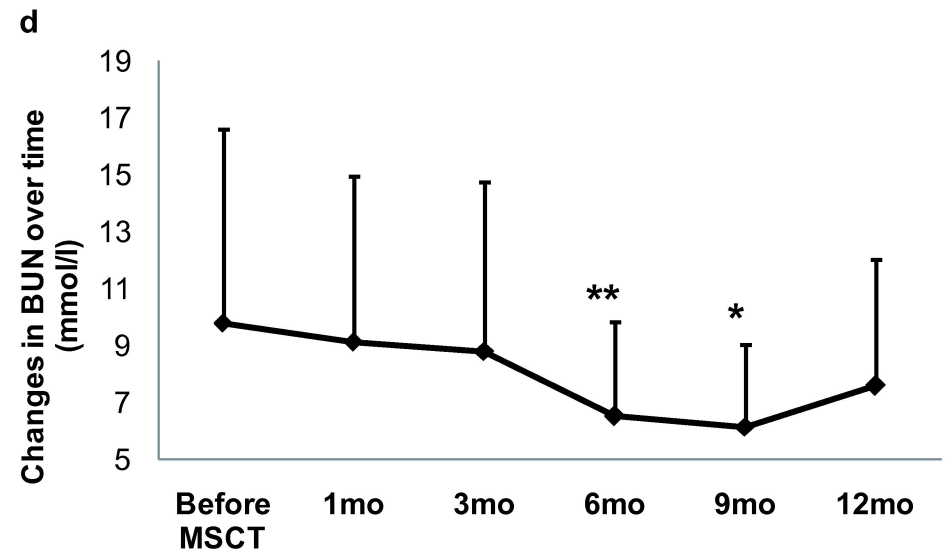

d
Changes in ANA over time
9
8
7
6
5
4
3
2
1
0
Before MSCT
1mo
3mo
6mo
9mo
12mo

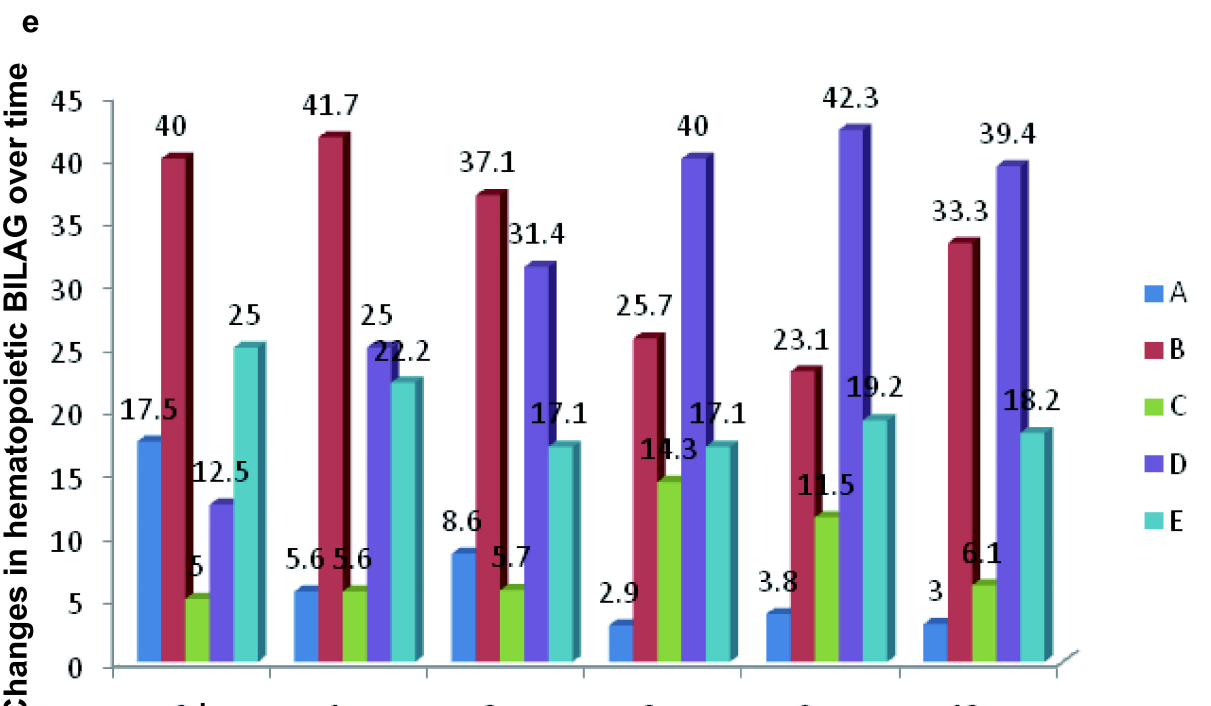
Figure 2


a
Changes in renal BILAG over time
70
61.1
57.1
57.1
57.7
63.6
25
22.2
22.9
25.7
23.1
21.2
14.3
14.3
14.3
8.3
8.3
5.7
2.9
15.2
0d
1m
3m
6m
9m
12m
A
B
C
D
E


b
Changes in 24-hour proteinuria over time (g)
Before MSCT
1mo
3mo
6mo
9mo
12mo
*
*


c
Changes in Creatinine over time (mg/dl)
*
Before MSCT
1mo
3mo
6mo
9mo
12mo


d
Changes in BUN over time (mmol/l)
**
*
Before MSCT
1mo
3mo
6mo
9mo
12mo


e
Changes in hematopoietic BILAG over time
40
41.7
37.1
40
42.3
39.4
31.4
25
25
22.2
25.7
23.1
19.2
17.1
17.1
17.5
12.5
14.3
11.5
8.6
5.7
5.6
5.6
2.9
3.8
3
6.1
0d
1m
3m
6m
9m
12m
A
B
C
D
E

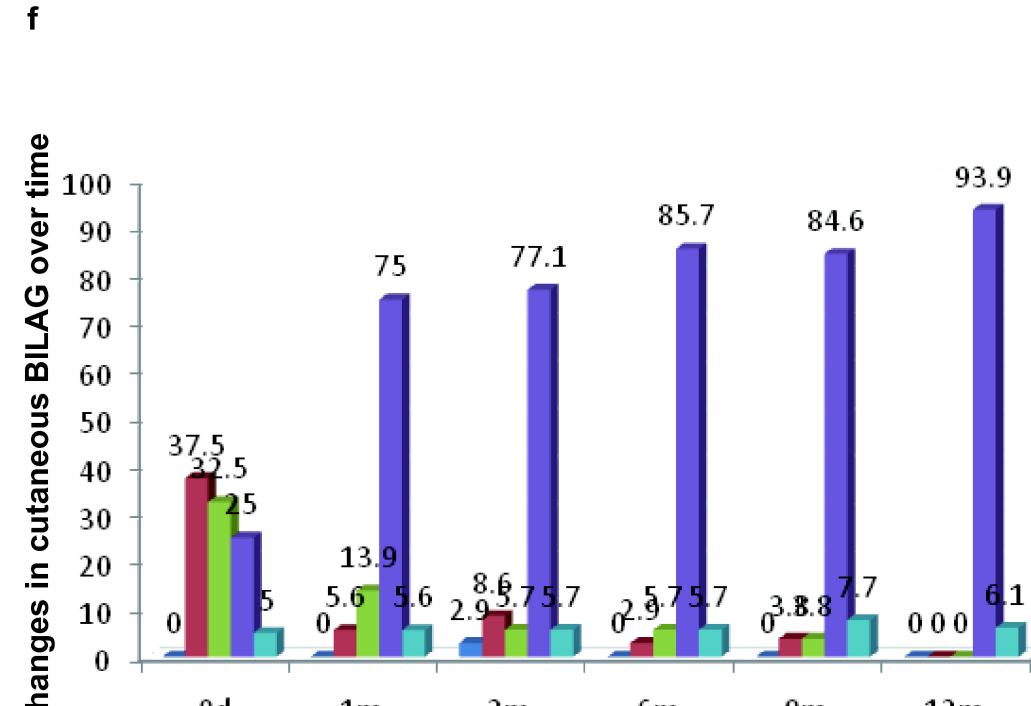

f
Changes in cutaneous BILAG over time
93.9
85.7
84.6
77.1
75
37.5
32.5
25
13.9
8.6
5.6
5.7
5.7
5.7
5.7
5.6
5
7.7
6.1
0d
1m
3m
6m
9m
12m

Figure 3