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Umbilical cord mesenchymal stem cells in ulcerative colitis treatment: efficacy and possible mechanisms

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Abstract

Background Mesenchymal stem cells (MSCs) possess powerful immunomodulatory ability. This study aimed to assess the efficacy and safety of human umbilical cord-derived mesenchymal stem cells (UMSCs) in patients with ulcerative colitis (UC) and to explore the potential mechanisms.

Methods This prospective, self-controlled clinical study was conducted at Henan Provincial People's Hospital. Patients with moderate-to-severe active UC, unresponsive to traditional drugs were continuously enrolled from September 2018 to March 2023. UMSCs were administered intravenously monthly for two months at a cell dosage of 1×10^6 per kg. The primary outcome was a clinical response at 2 months. The levels of cytokines and progerin in the plasma of the patients were analyzed using enzyme-linked immunosorbent assay kits, and longitudinal data was analyzed using generalized estimation equation.

Results Forty-one patients were enrolled and received UMSC therapy. At 2 months, 73.2% (30/41) of patients achieved a clinical response, and 41.5% (17/41) achieved a clinical remission. At 6 months, 2 patients were lost to follow-up; the corresponding figures were 70.0% (25/41) and 34.2% (14/41), respectively. After UMSC therapy, the Mayo score, Mayo endoscopy score, mean and maximum values of Ulcerative Colitis Endoscopic Index of Severity and Nancy index were significantly reduced compared with baseline values. Additionally, the levels of progerin and inflammatory markers, such as interleukin (IL)-1β, IL-6, IL-8, IL-12, and IL-17 A decreased, while hemoglobin, albumin, and IL-10/IL-17 A ratio increased, particularly in the response group. Multiple stepwise logistic regression analysis showed age was an independent risk factor affecting efficacy (odds ratio, 0.875 (95% confidence interval (0.787,

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0.972)); the area under the receiver operating characteristic curve for age was 0.79. No serious adverse events were observed during or after UMSC therapy.

Conclusion UMSCs are safe and effective for patients with UC, with age being an independent risk factor affecting efficacy. Mechanistically, UMSC treatment may ameliorate cell senescence and suppress the secretion of pro-inflammatory cytokines.

Trial registration The study was retrospectively registered at www.chictr.org.cn/ (ChiCTR1900026035) on September 18, 2019.

Keywords Umbilical cord mesenchymal stem cells, Ulcerative colitis, Inflammation, Aging

Introduction

Ulcerative colitis (UC) is an inflammatory disease that primarily affects the intestine. UC is characterized by chronic and recurrent episodes and is caused by a combination of environmental, genetic, microbial, and immune-mediated factors [1]. The latest epidemiological data has shown a steady increase in both the incidence and prevalence of this disease, leading to a significant rise in disease burden. As the disease progresses, there is an elevated cumulative risk of colectomy and colorectal carcinoma [2, 3]. Treatment for inflammatory bowel disease (IBD) includes untargeted approaches, like 5-aminosalicylic acid (5-ASA) and glucocorticoids, and targeted approaches, such as anti-tumor necrosis factor (TNF) or anti-α4β7 integrin antibodies. However, not all patients respond adequately to the aforementioned treatments, and some may face intolerance [4]. Furthermore, colectomy, especially with the production of an ileoanal pouch, has the risk of pouchitis, pouch failure, and the decline of female fertility [5]. Consequently, new approaches are urgently needed.

Mesenchymal stem cells (MSCs) possess self-renewal, multidirectional differentiation, immune system regulation abilities, and low immunogenicity. They can inhibit inflammation and apoptosis, regulate immunity, and promote angiogenesis. Because of the aforementioned properties, stem cell therapy has become an attractive therapeutic strategy for intestinal injury in IBD [6]. MSCs have powerful immunomodulatory effects, which can affect both the innate immune response and the adaptive immune response [7, 8]. Numerous studies have verified the excellent therapeutic potential of MSCs in intestinal inflammation in colitis mice. Previous studies have shown that umbilical cord mesenchymal stem cells (UMSCs) significantly reduced the disease activity index (DAI) of the mice through the rebalancing of Th1/Th17/

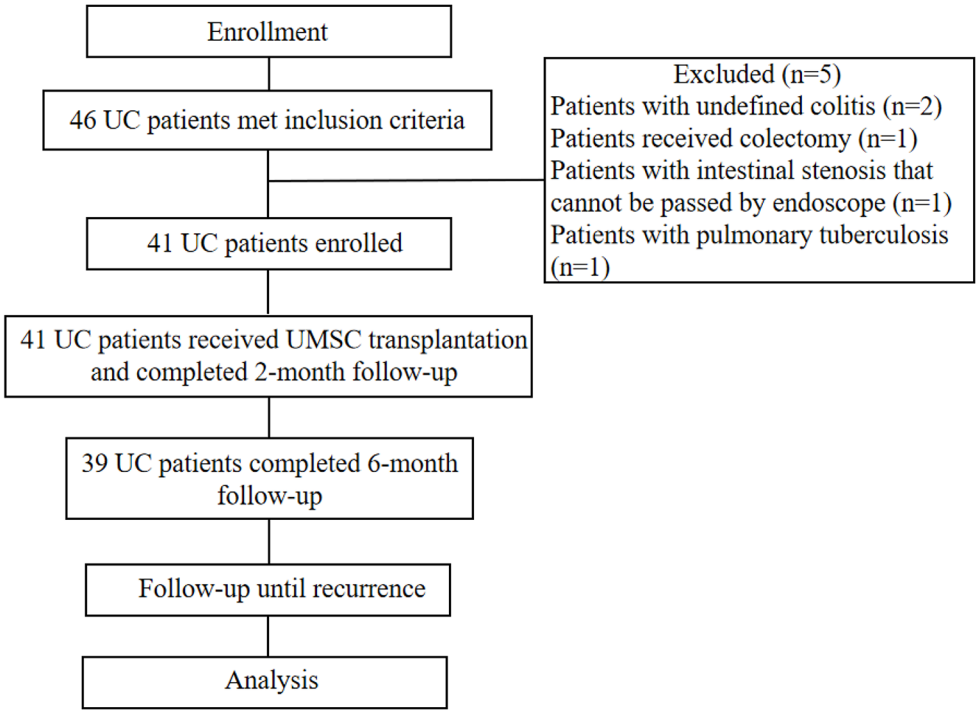

Fig. 1 Trial procedure. UC: ulcerative colitis; UMSCs: umbilical cord mesenchymal stem cells

Treg cells, reducing the secretion of pro-inflammatory cytokines and regulating intestinal flora [6, 9]. Regarding the optimization of stem cell therapy, a 2023 review has highlighted recent advances, including culturing MSCs in 3D vectors, changing culture medium of MSCs to promote cell proliferation and differentiation, genetic modification of MSCs to increase the function and efficacy of MSCs, and introducing cellular markers to MSCs to improve the homing rate of MSCs to injured tissues. Furthermore, extracellular vesicles (EVs), especially exosomes, released by MSCs have been proven to possess powerful therapeutic potential in preclinical and clinical studies in inflammatory diseases [6]. However, there are some controversies in the field, such as the heterogeneity of stem cell therapy and the potential tumorigenicity of MSCs. Adipose-derived stem cells (Cx-601) have been reported to effectively induce and maintain fistula closure in patients with Crohn's disease [10], but few clinical trials have used MSCs in the treatment of active UC.

Compared with MSCs derived from adipose and bone marrow, UMSCs are more attractive due to several specific advantages. For instance, UMSCs are easier to obtain, involve less ethical controversy, exhibit lower immunogenicity, and do not pose age-related senescence challenges [11]. Notably, a large number of UMSCs can be produced in vitro after several passages. Moreover, UMSCs exhibited more pronounced anti-inflammatory and immunoregulatory abilities than adipose- and bone marrow-derived MSCs [12–14]. Therefore, in this study, we investigated (Trial Registration No. ChiCTR1900026035) the safety and efficiency of UMSCs in the treatment of UC and the changes of cytokines and progerin in sera of the patients with UC pre- and post-UMSC transplantation to explore the possible mechanisms of UMSCs on UC.

Methods

Trial design and patient recruitment

This was a prospective self-controlled before and after clinical study conducted at Henan Provincial People's Hospital. Patients were continuously enrolled from September 2018 to March 2023. The study was approved by the Committee on the Ethics of Henan Provincial People's Hospital (approval number: (2018) NO.03−01), and all patients provided written informed consent.

Inclusion criteria comprised (1) patients aged 18–65; (2) patients with moderate-to-severe active UC; and (3) those who failed to respond to conventional drugs, including 5-ASA or glucocorticoids. The diagnosis of UC in patients was based on the comprehensive judgment of experienced physicians. They exhibited active UC, with a Mayo score [15] ranging from 6 to 12 points, and moderate-to-severe active disease according to the "modified Truelove and Witts Disease Severity Scale" [16]. The range of colonic lesions was determined according to the "Montreal classification" [17]. None of the patients responded well to conventional treatments, including those inadequately responding to 5-ASA for at least 2–4 weeks and those resistant to hydrocortisone, steroid-dependent, or immunosuppressant resistance [18].

Patients with chronic colitis resulting from other causes (e.g., radiation- or drug-induced colitis, unexplained colitis), intestinal stenosis not passable by an endoscope, positive tuberculin skin tests, and a history of colectomy or malignant tumors were excluded. Moreover, patients with allergic constitution or allergies to UMSC treatment were also excluded. Patients exhibiting steroid dependence, steroid resistance, immunosuppressive resistance, or non-response to biologics constituted the refractory group, while others were categorized into the non-refractory group.

Screening and baseline studies

Assessments conducted before the first MSC infusion included demographic information, symptom questionnaires, physical examinations, blood tests, stool analysis, tuberculin tests, and chest radiography. Subsequently, an endoscopy was conducted, and the baseline Mayo score and Ulcerative Colitis Endoscopic Index of Severity (UCEIS) value [19] were determined. All blood samples were processed and tested at Henan Provincial People's Hospital.

Isolation, culture, and identification of UMSCs

Umbilical cord samples were taken from full-term healthy pregnant women with written informed consent obtained from all donors. Then, MSCs derived from the umbilical cords were isolated and identified according to our previous methods [20]. UMSCs were cultured in serum-free medium (#NC0106, Yocon, Beijing, China) in a cell incubator at 5% CO_2 and 37℃ to eliminate potential interference from serum exosomes. Consistent with previous studies, UMSCs were identified based on the morphology and phenotype of the third generation and were used in subsequent experiments (Supplementary Fig. 1) [21]. Cell surface antigens of the expanded cells, including CD73, CD90, CD105, CD44, CD34, CD11b, CD19, CD45 and human leukocyte antigen-antigen D related (HLA-DR), were detected using the BD Stemflow Human MSCs Analysis kit (BD Biosciences, New Jersey, USA) and the data were analyzed using FlowJo software (version VX) [22].

UMSC therapy and procedures

UMSCs suspended in 100 mL of normal saline were administered intravenously each month for two consecutive months with a cell dosage of 1×10^6 per kg each time, based on a previous study in patients with Crohn's

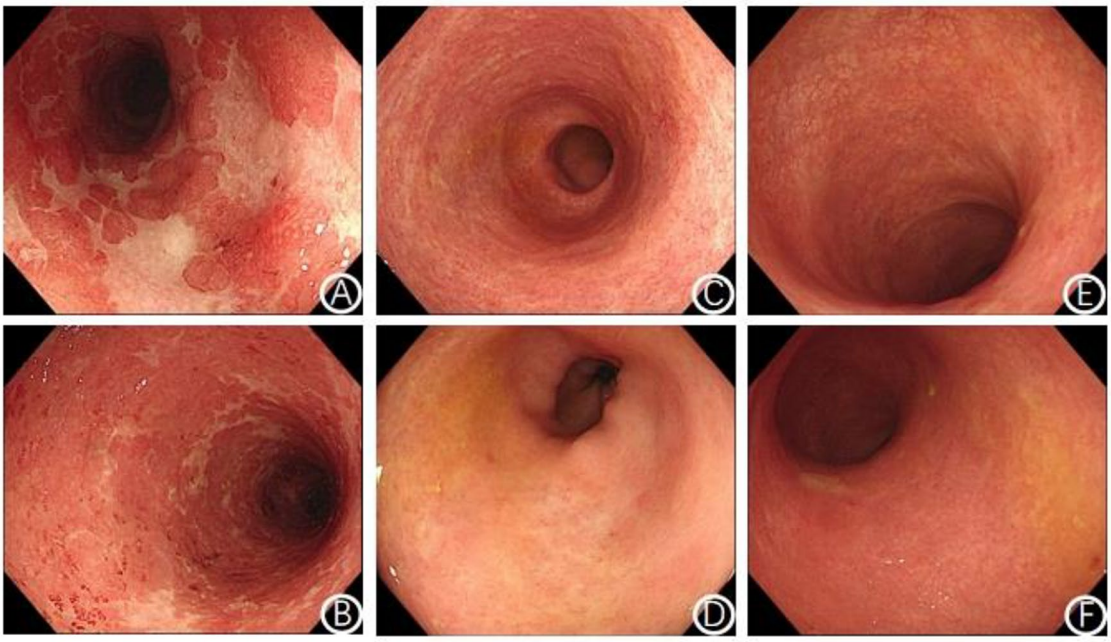

Fig. 2 Representative endoscopic images of patients with UC before and after UMSC therapy. **A–B**: Endoscopic presentation within the sigmoid colon and rectum before UMSC treatment. **C–D**: Endoscopic presentation within the sigmoid colon and rectum 2 months after UMSC treatment. **E–F**: Endoscopic presentation within the sigmoid colon and rectum 6 months after UMSC treatment. UC: ulcerative colitis; UMSC: umbilical cord mesenchymal stem cell

disease [10]. After UMSC transplantation, the dose of prednisone was gradually reduced, initially by one tablet per week to a dose equivalent to 20 mg of prednisone per day, then by half a tablet per week until discontinuation. The doses of other related medications such as 5-ASA remained constant. Corticosteroids and biologics via intravenous or rectal administration were not permitted during stem cell therapy. The therapy was deemed unsuccessful if any of these events occurred.

Cytokines and progerin detection

Fasting blood samples of patients with UC and healthy controls (HC) were collected. The plasma Interleukin-1β (IL-1β), IL-6, IL-8, IL-10, IL-12, IL-17 A, and plasma progerin levels were detected using enzyme-linked immunosorbent assay (ELISA) kits (Quanzhou Ruixin Biological Technology Co., Ltd., Quanzhou, China) following the manufacturer's instructions.

Follow-up

The final evaluation before the first UMSC infusion served as the baseline for all analyses. During the first 6 months, the patients were evaluated every month. Blood and stool samples were collected at 0, 2, and 6 months, and colonoscopies were performed simultaneously. The Mayo score (0 ~ 12) and UCEIS score (UCEIS average and maximum value) were calculated accordingly. The Nancy index (0 ~ 4; a higher grade indicates a more active disease) was employed to evaluate the histological disease activity of the biopsy samples obtained during colonoscopies [23]. Subsequently, patients were followed up by phone every 2 months until disease recurrence or a change in the treatment. Adverse events and concomitant treatments were recorded at each follow-up.

Outcomes

The primary outcome of our study was clinical response at 2 months, referred to a decline of ≥ 3 points and 30% in the total Mayo score from the scores before MSC therapy, and simultaneously a decline of ≥ 1 point or an absolute score of ≤ 1 point in rectal bleeding subscale. Secondary outcomes included clinical response at 6 months, clinical remission (referred to the total Mayo score of ≤ 2 points, accompanied by no subscore of ≥ 1 point) and mucosal healing (referred to an absolute endoscopy subscore of ≤ 1 point) at 2 and 6 months, sustained clinical response and remission (response/remission at both 2 and 6 months), and glucocorticoid-free remission at 6 months in patients treated with corticosteroids before UMSC therapy.

Patients who took prohibited medication, discontinued the study treatment due to poor efficacy, underwent intestinal surgery, or were lost to follow-up were all considered treatment failures in stem cell therapy, regardless of their Mayo scores. Relapse or aggravation refers to the recurrence or aggravation of the disease after complete symptom control with previous therapy [24].

Table 1 Demographics of patients with UC treated with UMSCs

Variable	Case ($n=41$)
Male n (%)	25 (61.0)
Age (in years, Mean ± SD)	45.0 ± 12.5
Duration of UC (years, Mean ± SD)	5.5 ± 4.7
Smoking n (%)	
Never smokers	30 (73.2)
Former smokers	10 (24.4)
Current smokers	1 (2.4)
Clinical severity n (%)	
Moderate	24 (58.5)
Severe	17 (41.5)
The colonic area involved n (%)	
Left side of colon	26 (63.4)
Extensive colon	15 (36.6)
Previous treatment n (%)	
5-ASA alone	17 (41.5)
5-ASA + Glucocorticoids	16 (39.0)
5-ASA + Glucocorticoids + Immunosuppressant	5 (12.2)
5-ASA + Glucocorticoids + Biological agents	1 (2.4)
5-ASA + Biological agents	2 (7.3)

Abbreviations UC: ulcerative colitis; UMSCs: umbilical cord mesenchymal stem cells; SD: standard deviation; 5-ASA: 5-Aminosalicylic acid

Table 2 Outcome indicators of UMSCs on patients with UC ($n=41$)

Variable	T2	T6
Clinical response		
Total, n (%)	30 (73.2)	25 (61.0)
Non-refractory group[†], n/total n (%)	14/19 (73.7)	13/19 (68.4)
Refractory group[‡], n/total n (%)	16/22 (72.7)	12/22 (54.6)
Clinical remission		
Total, n (%)	17 (41.5)	14 (34.2)
Non-refractory group, n/total n (%)	10/19 (52.6)	7/19 (36.8)
Refractory group, n/total n (%)	7/22 (31.8)	7/22 (31.8)
Mucosal healing, n (%)	18(43.9)	17 (41.5)
Sustained clinical response[§], n (%) n (%) {n (%)}		24 (58.5)
Sustained clinical remission[¶], n (%)		11 (26.8)
Glucocorticoid-free remission, n/total n (%) {no./total no. (%) }		3/10 (30.0)

T2: 2 months after UMSC therapy; T6: 6 months after UMSC therapy

[†] Patients not belonging to refractory group constituted the non-refractory group;

[‡] Patients exhibiting steroid dependence, steroid resistance, immunosuppressive resistance, non-response to biologics constituted the refractory group;

[§] A sustained clinical response means that patients obtained clinical response at both 2 and 6 months;

[¶] A sustained clinical remission means that patients obtained clinical remission at both 2 and 6 months

Abbreviations UC: ulcerative colitis; UMSCs: umbilical cord mesenchymal stem cells

Additionally, if the patient changed treatments or was readmitted to the hospital due to aggravating symptoms during follow-up, it was considered a relapse.

Statistical analysis

Measurement data is presented as mean (standard deviation (SD)) or median (interquartile range (IQR)), and enumeration data with numbers (percentages). The two independent sample *t*-test was applied to normally distributed measurement data, and the Wilcoxon (Mann–Whitney) test was applied to non-normally distributed measurement data to compare baseline characteristics between UC patients and HC or responders and non-responders. The generalized estimation equation was employed to compare baseline and results at 2 and 6 months of the longitudinal data. Single-factor binary logistic regression analysis was employed to identify factors affecting the treatment efficacy of UMSCs on UC patients, and the independent variable with $p<0.05$ was included in the multivariate binary logistic regression model. In order to assess the accuracy of risk factors in predicting efficacy, the area under the receiver operating characteristic (ROC) curve (AUC) was calculated. A p-value <0.05 was considered statistically significant.

Results

Demographics of the patients

Patients with moderate-to-severe active UC who were admitted to our hospital between September 2018 and March 2023 were accessed for eligibility. Among the evaluated patients, 46 met the inclusion criteria and expressed willingness to undergo UMSC treatment. However, five patients were excluded based on specific criteria. Finally, 41 patients were enrolled in the study (Fig. 1). Of these, 25 patients were male, and 16 patients were female; the longest duration of UC was 21 years. All patients had received a full dose of 5-ASA. Additionally, 53.7% (22/41) of patients had previously received glucocorticoids. Within this sub-group, five patients underwent a combined therapy of immunosuppressive agents (i.e., azathioprine), and one underwent anti-TNF antibody (i.e., infliximab) treatment. Among the 22 patients who had received glucocorticoids, 6 (27.3%) were steroid-dependent, 14 (63.7%) were steroid-resistant, and 2 (9.1%) were steroid-sensitive (Table 1).

Clinical outcomes and safety

Efficacy

At 2 months, 73.2% (30/41) of patients undergoing UMSCs transplantation achieved a clinical response, 41.5% (17/41) exhibited a clinical remission, and 43.9% (18/41) experienced mucosal healing. At 6 months, 2 patients were lost to follow-up, 61.0% (25/41) achieved a clinical response, 34.2% (14/41) exhibited a clinical remission, 41.5% (17/41) experienced mucosal healing, and 30.0% (3/10) attained a glucocorticoid-free remission. Furthermore, 58.5% (24/41) and 26.8% (11/41) of

patients attained sustained clinical response and remission at 6 months, respectively (Table 2).

Following UMSC treatment, patients experienced a gradual improvement in clinical symptoms, including reduced abdominal pain, decreased defecation frequency, and less bloody purulent stool. Endoscopy revealed a significant reduction in colonic inflammation and ulcer disappearance, and mucosal remission was gradually achieved. Histopathology showed decreased inflammatory cell infiltration and gradual normalization of glandular structure. Representative endoscopic images are shown in Fig. 2.

Compared with baseline, Mayo score, Mayo endoscopic score, UCEIS average and maximum values, and Nancy index decreased, while albumin (ALB) and hemoglobin (HB) increased in patients with UC following UMSC transplantation. These differences were statistically significant at 2 and 6 months (Table 3).

In order to explore the risk factors affecting the efficacy of UMSC transplantation, patients achieving clinical response at 2 months were divided into a response group ($n=30$), while the other patients constituted a no-response group ($n=11$). The baseline indicators of the response and no-response groups were not significantly different (Table S1). Compared with baseline, the response group displayed statistically significant decreases in Mayo score, Mayo endoscopic score, UCEIS average and maximum values, and Nancy index at 2 and 6 months, while ALB and HB levels increased significantly. In contrast to the no-response group, Mayo score, Mayo endoscopic score, UCEIS average and maximum values, and Nancy index were lower and ALB level was higher in response group at 2 and 6 months, and the difference was statistically significant (Fig. 3).

Table 3 The changes in clinical indicators of patients with UC treated with UMSCs

Variables	T0 ($n=41$)	T2 ($n=41$)	T6 ($n=39$)	$P^{\dagger}$
Mayo score (Mean ± SD)	10.6 ± 1.6	4.9 ± 3.9**	5.1 ± 4.0*	< 0.001
Mayo endoscopic score, Median (IQR)	3.0 (3.0–3.0)	2.0 (1.0–2.5)**	2.0 (1.0–3.0)*	< 0.001
UCEIS average value (Mean ± SD)	2.7 ± 1.0	1.8 ± 0.9**	1.6 ± 0.7*	< 0.001
UCEIS maximum value (Mean ± SD)	4.9 ± 1.0	3.2 ± 1.3**	2.9 ± 1.4*	< 0.001
Nancy index, Median (IQR)	4.0 (3.0–4.0)	2.0 (1.0–3.0)**	2.0 (1.0–3.0)*	< 0.001
WBC (10^9/L, Mean ± SD)	6.9 ± 2.4	6.5 ± 2.3	6.6 ± 3.7	0.520
HB (g/L, Mean ± SD)	111.5 ± 22.6	117.5 ± 25.1**	118.2 ± 25.2*	0.014
ESR, Median (IQR)	21.0 (10.0–40.0)	23.0 (12.0–44.0)	22.0 (11.8–46.5)	0.458
CRP, (mg/L, Median (IQR))	5.7 (1.9–18.7)	1.5 (0.0–7.2)	1.3 (0.0–7.7)	0.105
ALB (g/L, Mean ± SD)	36.1 ± 5.7	40.4 ± 7.1**	39.6 ± 6.6*	< 0.001

T0: before UMSC therapy; T2: 2 months after UMSC therapy; T6: 6 months after UMSC therapy

† The generalized estimation equation was employed to compare baseline and results at 2 and 6 months of the longitudinal data. **$p<0.01$ T2 versus T0; *$p<0.05$ T6 versus T0

Abbreviations UC: ulcerative colitis; UMSCs: umbilical cord mesenchymal stem cells; UCEIS: Ulcerative Colitis Endoscopic Index of Severity; SD: standard deviation; IQR: interquartile range; WBC: white blood cell; HB: hemoglobin; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; ALB: albumin

Recurrence

A total of 30 patients achieved a clinical response at 2 months, and 2 patients were lost to follow-up by 6 months. Furthermore, 14 patients experienced no recurrence during the follow-up, with maintenance time ranging from 8 months to 5 years. Among them, 13 patients had a maintenance duration of over 1 year, 6 patients over 3 years (Table 4).

Safety

The most common adverse reactions associated with the UMSC infusion were low fever and fatigue, resolved with symptomatic treatment and rest. One patient developed dizziness, nausea, and vomiting after the first transplantation, while the symptoms disappeared after symptomatic treatment, and no significant discomfort occurred during the second infusion. After the second infusion, the other patient developed headache and hand numbness. A magnetic resonance imaging examination of the head was performed, and no significant abnormalities were found. The patient's discomfort resolved after symptomatic treatment. No serious adverse events, infections, or cancers were found during MSC treatment or subsequent follow-up (Table 5).

Critical role of senescence in the treatment of umscs in UC patients

Senescence includes biological aging and stress-induced premature senescence. In order to explore risk factors affecting the effect of UMSC treatment, factors such as age, sex, body mass index, course of disease, severity of disease, and previous treatments were included in the logistic regression analysis model. The results showed that age was an independent risk factor affecting the efficacy of UMSCs in patients with UC, with an odds ratio (OR) value of 0.875 (95% confidence interval [CI]: 0.787–0.972). Furthermore, a ROC curve was employed to analyze the prediction accuracy of age for efficacy. The results showed an AUC of 0.791, a maximum Youden index of 0.518, and a corresponding cut-off point value of 54. This suggests that age ≤ 54 could be used as a reference value for forecasting efficacy. The sensitivity and specificity were 70% and 81.82%, respectively (Fig. 4).

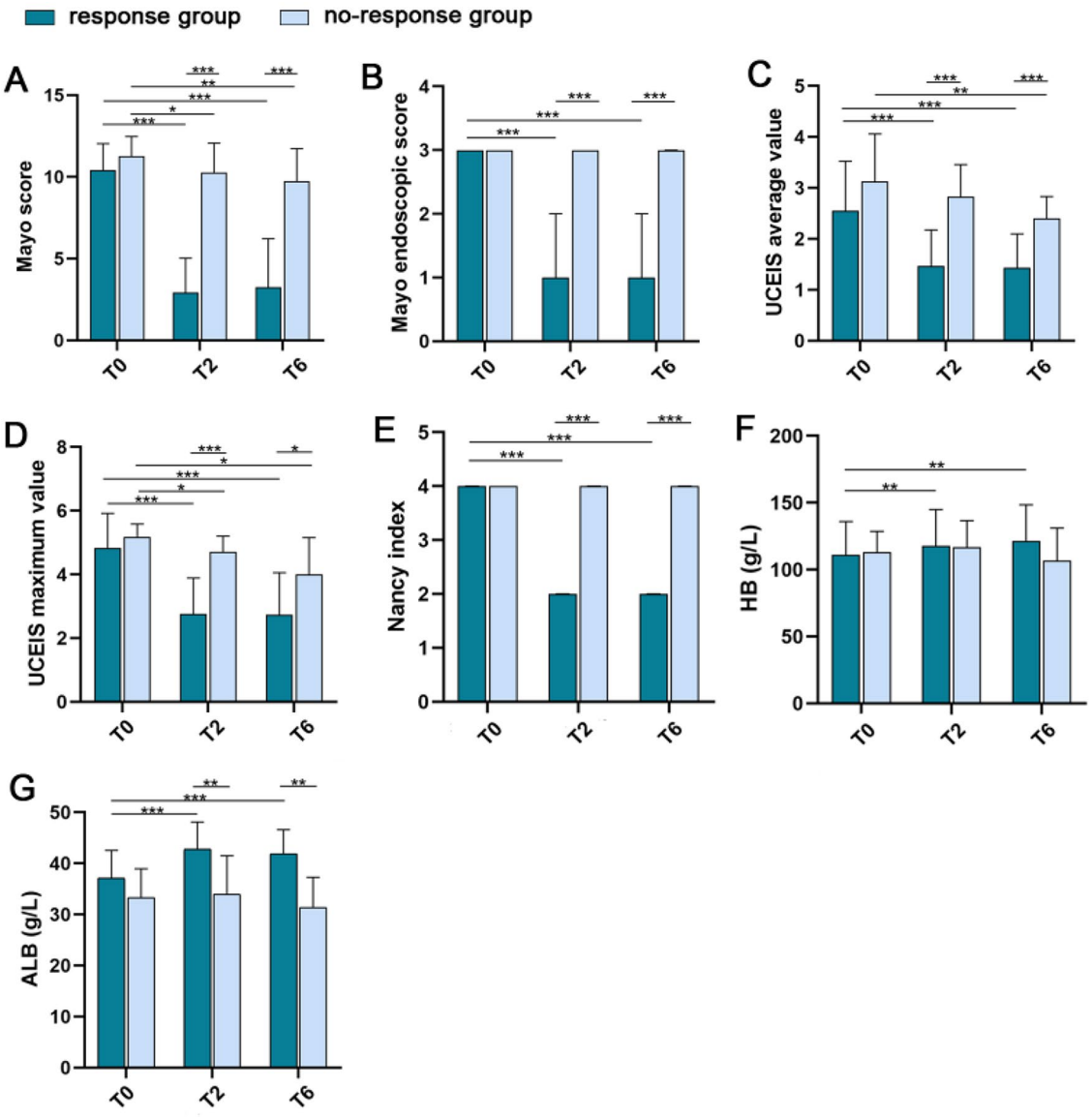

Fig. 3 The changes in the clinical indicators of the response and no-response groups after UMSC treatment. (**A**) Mayo score. (**B**) Mayo endoscopic score. (**C**) Ulcerative Colitis Endoscopic Index of Severity (UCEIS) average value. (**D**) UCEIS maximum value. (**E**) Nancy Index. (**F**) Hemoglobin (HB) concentration. (**G**) Albumin (ALB) concentration. (Response group: $n = 30$, no-response group: $n = 11$; T0: before UMSC therapy, T2: 2 months after UMSC therapy, T6: 6 months after UMSC therapy; ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$). UMSC: umbilical cord mesenchymal stem cell

Table 4 Recurrence in patients with UC treated with UMSCs

	2–6 months	6–12 months	1 year or longer	No recurrence
Total	4	6	4	14
Non-refractory group[†]	1	5	1	6
Refractory group[‡]	3	1	3	8

[†] Patients not belonging to refractory group constituted the non-refractory group

[‡] Patients exhibiting steroid dependence, steroid resistance, immunosuppressive resistance, non-response to biologics constituted the refractory group

Abbreviations UC: ulcerative colitis; UMSCs: umbilical cord mesenchymal stem cells

Table 5 Adverse events in UC patients treated with UMSCs

Event *n* (%)	*n* = 41
Fever	4 (9.8)
Dizziness	1 (2.4)
Headache	1 (2.4)
Nausea	1 (2.4)
Emesis	1 (2.4)
Abdominal pain	1 (2.4)
Hand numbness	1 (2.4)
General numbness	1 (2.4)
Upper respiratory tract infection	3 (7.3)
Fatigue	5 (12.2)

Abbreviations UC: ulcerative colitis; UMSCs: umbilical cord mesenchymal stem cells

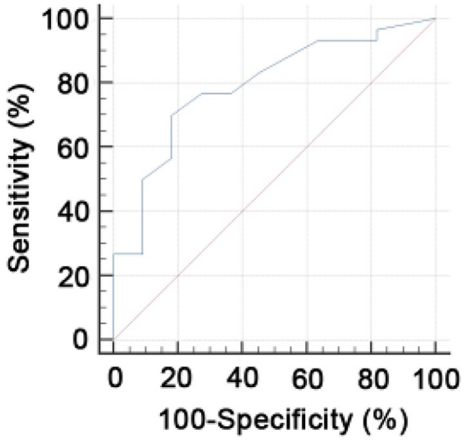

Fig. 4 ROC curve of age for predicting the efficacy of UMSC treatment in patients with UC. UC: ulcerative colitis; UMSC: umbilical cord mesenchymal stem cell

Progerin is a permanently mutated lamin A, which is an important biomarker of cell premature senescence [25]. Furthermore, we explored the change of progerin in the plasma of patients with UC pre- and post-UMSC therapy. The plasma progerin level of patients with UC was markedly high compared with that in HC; whereas, the level of progerin declined noticeably after UMSC treatment at 2 and 6 months, and the differences were statistically significant (Figs. 5A and 6A).

UMSC Treatment inhibits pro-inflammatory cytokine secretion

we explored the change of inflammation-associated factors in the plasma of patients with UC before and after UMSC transplantation.

Initially, we examined the plasma levels of cytokines of patients with UC and HC. Results revealed significantly elevated plasma levels of IL-1β, IL-6, IL-8, IL-12, IL-17 A, accompanied by a decreased ratio of IL-10/IL-17 A in patients with UC compared to HCs. However, no prominent difference was found in IL-10 levels between the two. Subsequently, we investigated the changes in cytokines in patients with UC pre- and post-UMSC transplantation. The data indicated remarkably declined levels of IL-1β, IL-6, IL-8, IL-12, IL-17 A, with a noteworthy rise in the IL-10/IL-17 A ratio after UMSC transplantation at 2 and 6 months. However, no significant change in IL-10 level was observed (Fig. 5B-H).

Furthermore, we analyzed the change of the aforementioned cytokines in the response and no-response groups

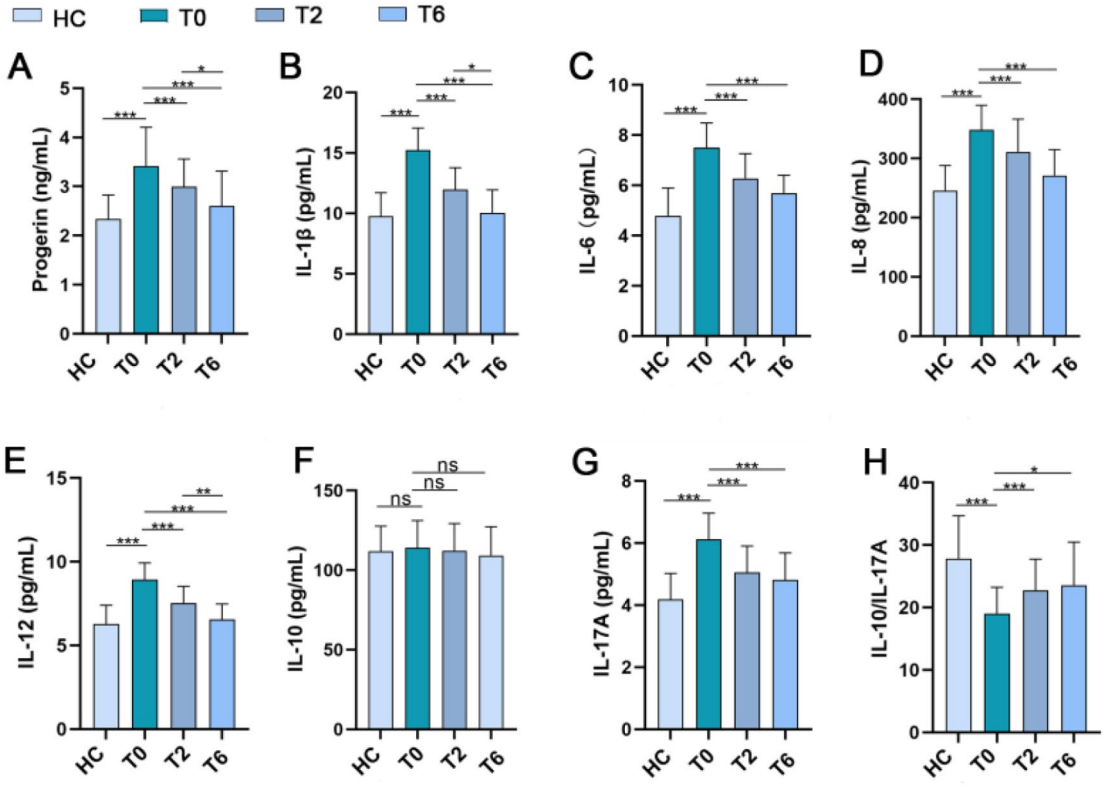

Fig. 5 The level of progerin and cytokines of HC and UC patients pre and post-UMSC treatment. (**A**) Plasma progerin concentration. (**B**) Plasma interleukin (IL)-1β. (**C**) Plasma IL-6 concentration. (**D**) Plasma IL-8 concentration. (**E**) Plasma IL-12 concentration. (**F**) Plasma IL-10 concentration. (**G**) Plasma IL-17 A concentration. (**H**) Ratio of IL-10/IL-17 A. (HC: $n = 50$, patients: $n = 41$; T0: before UMSC therapy, T2: 2 months after UMSC therapy, T6: 6 months after UMSC therapy; ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$). HC: healthy controls; UC: ulcerative colitis; UMSC: umbilical cord mesenchymal stem cell

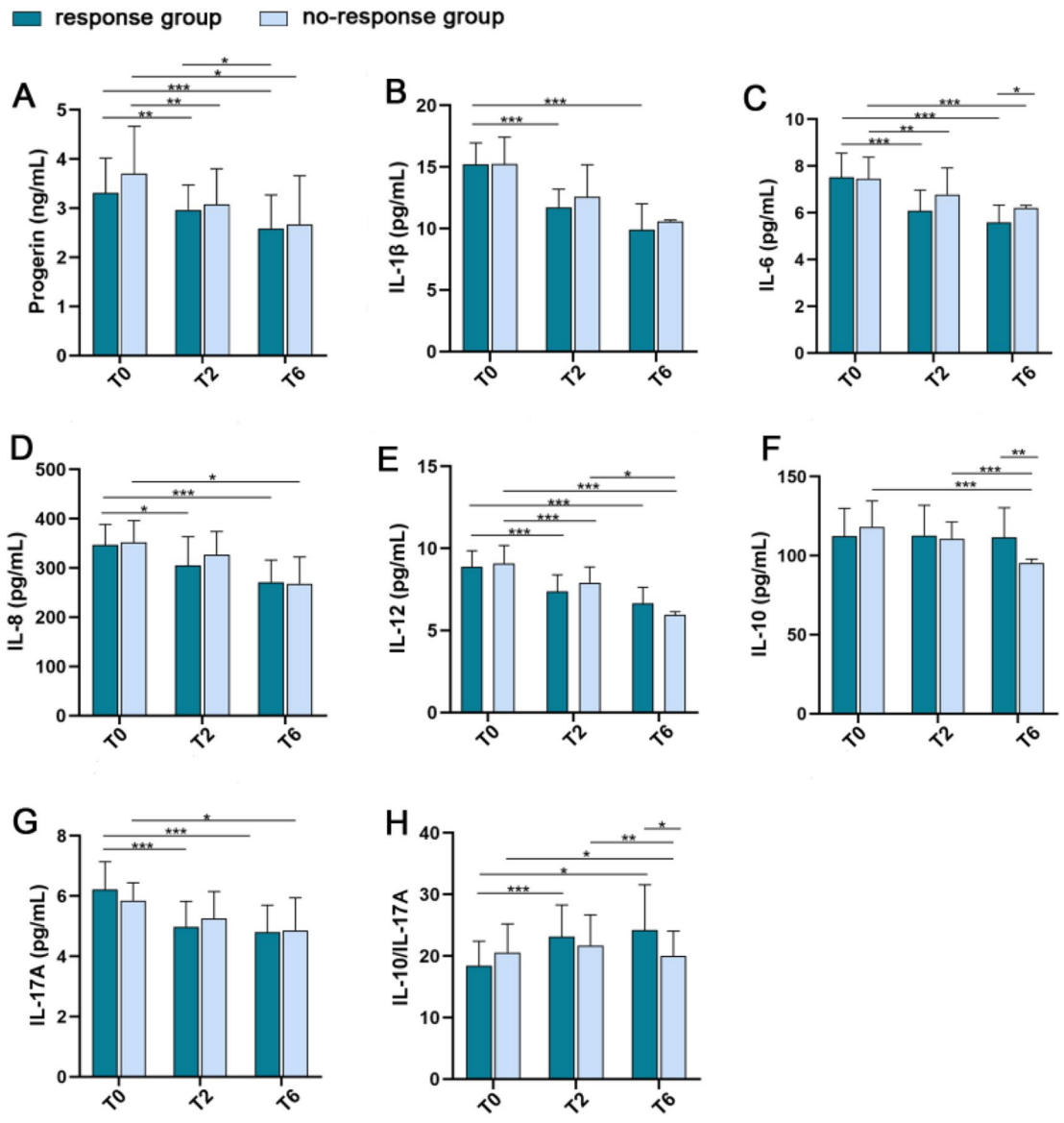

Fig. 6 The progerin and cytokine changes of the response and no-response groups after UMSC treatment. (**A**) Plasma progerin concentration. (**B**) Plasma interleukin (IL)-1β. (**C**) Plasma IL-6 concentration. (**D**) Plasma IL-8 concentration. (**E**) Plasma IL-12 concentration. (**F**) Plasma IL-10 concentration. (**G**) Plasma IL-17 A concentration. (**H**) Ratio of IL-10/IL-17 A. (Response group: $n=30$, no-response group: $n=11$; T0: before UMSC therapy, T2: 2 months after UMSC therapy, T6: 6 months after UMSC therapy; ***$p<0.001$, **$p<0.01$, *$p<0.05$). UMSC: umbilical cord mesenchymal stem cell

pre- and post-UMSC transplantation. At baseline, no prominent differences were observed in the levels of the aforementioned cytokines between the two. The results revealed that the IL-1β, IL-6, IL-8, IL-12, IL-17 A levels all prominently decreased, while the IL-10/IL-17 A ratio markedly increased after UMSC transplantation at 2 and 6 months in the response group. However, in the no-response group, only the IL-6, IL-12 levels decreased at 2 and 6 months after the UMSC transplantation. At 6 months, compared with the no-response group, the IL-6 level in the response group was lower, and the IL-10/IL-17 A ratio was higher, with the difference being statistically significant. Additionally, compared with the no-response group, the IL-17 A, IL-1β levels in the response group at 2 and 6 months were lower, though with no statistically significant differences (Fig. 6B-H).

Discussion

Our study's findings indicate that UMSC transplantation is effective and safe for patients with moderate-to-severe UC. Premature senescence is observed in UC patients, and UMSC treatment may mitigate cell senescence in UC patients. Age is identified as an independent risk factor impacting the efficacy of UMSC treatment. Furthermore,

UC patients exhibit significant pro-inflammatory secretion, and UMSC treatment may inhibit the secretion of such cytokines.

MSC-based therapy has become a hopeful treatment strategy for chronic inflammatory diseases in recent years, including IBD [26, 27]. A mounting animal experiments have confirmed the remarkable role of MSC in colitis mice [28], but clinical trials of MSCs in IBD, especially in UC were few, and most of the previous studies were small sample case reports with low quality [6]. The therapeutic effects of MSC in UC deserves further study. Our results indicate that UMSC therapy is effective in patients who are unresponsive to traditional therapeutic approaches. At 2 months, 73.2% of patients achieved a clinical response, and 41.5% obtained clinical remission. In comparison, the clinical response and remission proportion for infliximab were 61.5–69.4% and 27.5–38.8%, respectively, and for vedolizumab, the corresponding figures were 47.1% and 16.9%, respectively, according to previous studies [15, 29]. Furthermore, among the 30 patients obtaining a clinical response at 2 months, 13 remained recurrence-free for over 1 year, and 6 remained recurrence-free for over 3 years. Importantly, these patients only underwent stem cell transplantation twice and did not receive maintenance therapy. MSCs possess powerful self-renewal, multilineage differentiation and immunomodulatory and tissue repair properties, possibly accounting for the remarkable effects of UMSCs in patients with UC. MSCs can modulate various immune cells and secrete numerous immune mediators to create an environment with immune tolerance properties [30, 31]. MSC-derived EVs were demonstrated have the abilities of anti-inflammation, anti-apoptosis, promotion of angiogenesis [26].

There are a few points worth noting. First, 53.7% of patients enrolled in the study had been treated with glucocorticoids alone or in combination with immunosuppressants. As is well known, prolonged application of glucocorticoids and immunosuppressants leads to various side effects [32, 33]. MSC therapy demonstrated promising efficacy in patients with refractory UC in our study. Second, in addition to the Mayo score, UCEIS was also used to more comprehensively and accurately evaluate the mucosal conditions in endoscopy and Nancy index was used to examine the histological disease activity of the biopsy samples in our study.

Senescence occurs in patients with IBD, as evidenced by upregulated expression of the cellular senescence markers p16 and p21, telomere shortening, and expression of the DNA damage response pathways [34]. Improving senescence has been shown to reduce colon inflammation [34–36]. Progerin is an important biomarker of premature cell senescence. Our results showed plasma progerin level in patients with UC was high compared with HC, and progerin level declined after UMSC treatment. EVs derived from MSCs were demonstrated could alleviate endothelial cells senescence in vitro and in vivo mouse wound-healing models though regulation of miR-146a/Src [37]. In addition, multiple progressive logistic regression analysis showed that age was an important risk factor affecting the efficacy of UMSCs in UC patients. The transplantation of bone marrow-derived MSCs in elderly patients with UC was less effective than in patients of young and middle age, which is consistent with our study [38]. As patients age, the mechanical barrier of the intestinal mucosa is impaired, intestinal flora and metabolites become disturbed, and the immune system and intestinal epithelial stem cells experience senescence. These factors can promote the development of IBD and influence the efficacy of MSC treatment [35, 39, 40].

Th17 cell-mediated abnormal immune response plays a crucial part in the pathophysiology of IBD and IL-17 A is the main effector cytokine of Th17 cells. IL-1β located at the upstream of the inflammatory response can aggravate the secretion of various pro-inflammatory cytokines including IL-6 and IL-8 [26, 41, 42]. High levels of IL-17 A and IL-1β were detected in colitis animal models and in the serum and colonic mucosa of patients with active UC. Neutralizing these cytokines reduces colonic inflammation in mice models [26, 27, 43–45]. IL-12 is a main Th1 cell-stimulating factor, and remarkably raised secretion of IL-12 was observed in IBD patients [46]. Our results showed there is elevation secretion of pro-inflammatory factors in UC patients. After UMSC treatment, the secretion of such cytokines declined, which was more obvious in the response group than in the no-response group. MSCs restrain the differentiation and activation of Th1 and Th17 cells directly or indirectly, thus inhibiting effector cytokine expression, such as IL-1β, interferon IFN-γ, and IL-17 A. The expression of IL-12 is downregulated by MSCs through inducing the production of prostaglandin E2 or suppressing the activation of macrophages. EVs derived from MSCs have been shown could effectively suppress expression of inflammatory markers in colitis models, such as IL-1β, IL-6, IL-8 and oxidative stress markers [26, 31].

Although MSC transplantation demonstrated a high level of efficacy in patients with UC in our study, not all patients respond well to MSC therapy. There are several possible reasons for this. Our results have indicated that age may be an important factor affecting the efficacy of stem cell treatment, a theory supported by previous research [40]. Additionally, the heterogeneity of MSC may also affect the efficacy of MSC therapy, resulting in significant individual differences [6, 26].

There are some limitations in our study. First, this was a single-center, non-randomized, single-arm clinical

study. A larger sample size and multicenter randomized controlled trial is required. Moreover, patients in this study received weight-based doses of UMSCs (1×10^6 cells /kg body weight) according to previous research in patients with Crohn's disease [10]. Clinically, we may need to establish different dose subgroups to explore the most suitable dose of UMSCs for UC patients. Finally, each patient received only two stem cell infusions and no maintenance therapy. Approximately 50% of the patients achieving a clinical response at 2 months relapsed at the subsequent follow-up, suggesting that repeated MSC therapy is of great significance. Notably, six patients maintained a 3-year relapse-free status. Therefore, patients who need maintenance therapy and the factors leading to relapse warrant further investigation.

Conclusion

In conclusion, our preliminary study demonstrated that intravenous infusion of UMSCs in patients with moderate-to-severe UC was safe and effective and age was an independent risk factor affecting the efficacy of UMSC treatment. UMSC transplantation may ameliorate cell senescence and suppress pro-inflammatory cytokine secretion.

Abbreviations

ALB	Albumin
AUC	Area Under the Curve
CI	Confidence Interval
CRP	C-reactive Protein
DAI	Disease Activity Index
ESR	Erythrocyte Sedimentation Rate
EVs	Extracellular Vesicles
HB	Hemoglobin
HC	Healthy Controls
HLA-DR	Human Leukocyte Antigen-Antigen D Related
IBD	Inflammatory Bowel Disease
IL	Interleukin
IQR	Interquartile Range
MSCs	Mesenchymal Stem Cells
OR	Odds Ratio
ROC	Receiver Operating Characteristic
SD	Standard Deviation
Th1	T Helper 1
Th17	T Helper 17
TNF	Tumor Necrosis Factor
UCEIS	Ulcerative Colitis Endoscopic Index of Severity
UC	Ulcerative Colitis
UMSCs	Umbilical Cord Mesenchymal Stem Cells
WBC	White Blood Cell

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

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Not applicable.

Author contributions

XKJ, BYZ, SYH, XLL and HY contributed to the conception and design of the study. XKJ, XYL, YQB, HD, HY, and YLL provided study materials and completed patient enrollment. YL, CP, HRH, ML, HMZ, ZYY, ZJL and XYL collected clinical samples, performed experiments, and completed patient follow-up. YJS evaluated the histological disease activity of the biopsy samples. CHC conduced data analysis. XKJ interpreted the data. XKJ and XYL drafted the manuscript, and all authors approved the final manuscript.

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Data availability

Datasets are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The study was authorized by the Ethics Committee of Henan Provincial People's Hospital (project title: Clinical study of mesenchymal stem cells in the treatment of moderately refractory ulcerative colitis; Approval number: (2018) NO.03 – 01; date of approval: 25 January 2018). All patients provided written informed consent.

Consent for publication

Not applicable.

Competing interests

All authors declare there are no conflicts of interest.

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