Treatment of Severe COVID-19 with human Umbilical Cord Mesenchymal Stem Cells

Lei Shu
Sir Run Run Hospital
Changming Niu
Nanjing Medical University Second Affiliated Hospital
Ruyou Li
Huangshi Center Hospital
Tingrong Huang
Huangshi Hospital of Traditional Chinese Medicine
Yan Wang
Jiangsu Cell Tech Medical Research Institute
Ningfei Ji
the first affiliated hospital of nanjing medical university
You Zheng
Huangshi Hospital of Traditional Chinese Medicine
Xiaolin Chen
Sir Run Run Hospital
Lei Shi
Sir Run Run Hospital
Mingjing Wu
Sir Run Run Hospital
Kaili Deng
Sir Run Run Hospital
Jing Wei
Sir Run Run Hospital
Xueli Wang
Sir Run Run Hospital
Yang Cao
Sir Run Run Hospital
Jiaxin Yan
Sir Run Run Hospital
ganzhu feng (✉ fenggz@njmu.edu.cn)
Nanjing Medical University Second Affiliated Hospital

Research

Keywords: Coronavirus disease-19 (COVID-19), clinical characteristics, coronavirus pneumonia, human Umbilical Cord Mesenchymal Stem Cells

Posted Date: April 29th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-23696/v1

Version of Record: A version of this preprint was published on August 18th, 2020. See the published version at https://doi.org/10.1186/s13287-020-01875-5.

Abstract

Background

COVID-19 is a highly infectious respiratory disease. No effective therapeutics have yet been proved for treating of severe COVID-19.

Objectives

To determine whether human Umbilical Cord Mesenchymal Stem Cells infusion may be effectiveness and safety in the treatment of severe COVID-19.

Methods

The severe COVID-19 randomly divided into 2 groups, standard treatment group and standard treatment plus hUC-MSCs infusion group. The incidence of severe patients aggravated to critically illness, 28-day mortality, clinical symptoms improvement, time to clinical symptoms improvement, hematologic indicators including C-reaction protein, lymphocyte number, interleukin 6 and imaging changes were observed and compared between two groups.

Measurements and Main Results

The incidence of severe patients aggravated to critically illness and 28-day mortality were 0 in hUC-MSCs treatment group, while 4 patients in control group were deteriorated to critical illness and been used invasive ventilation, 3 of them died, and 28-day mortality was 10.34%. In hUC-MSCs treatment group, the time to clinical improvement was shorter, clinical symptoms of weakness and fatigue, shortness of breath, and low oxygen saturation had improved obviously began the third day of stem cells infusion, and reached the significant difference on the day 7, CRP and IL-6 were significantly decreased from day 3 of infusion, the time for lymphocyte count returned to normal range was significant faster, and lung inflammation absorption was significantly shorter from CT imaging.

Conclusions

Intravenous transplantation of hUC-MSCs is a safe and effective way that can be considered as salvage and priority choice in the treatment of severe COVID-19.

Introduction

In December of 2019, a series of pneumonia broke out in Wuhan City, Hubei Province and other parts of China. Recently it has been identified as a novel coronavirus, designated SARS-CoV–2[1, 2], and named this type pneumonia as Coronavirus Pneumonia (COVID–19). Since then, the number of COVID–19 patients have sharply increased not only in China, but also most of the World. Up to April 5, 2020, surpassed 1,000,000 confirmed COVID–19 patients and more than 57,000 deaths in 207 countries, areas or territories all over the world[3], according to the data from WHO, the mortality of COVID–19 is 5.17%, in some places, however, the mortality rate was more higher, reaching at 16.7%[4], which depending on the sample size included and the severity of the outbreak. So, controlling the mortality rate of critically ill patients is of paramount importance. Considering the highly prevalence and infection, The World Health Organization (WHO) has declared COVID–19 as a pandemic[5], and has grown to be a public health emergency of international concern and poses a huge threat to global health. However, thus far, there are no specific drugs or vaccines could be available to treat COVID–19 patients.

Recently studies have found that SARS-COV–2 virus interacts with human mucosal epithelial cells ACE2 depends on binding of the viral spike (S) proteins to cellular receptors and on S protein priming by host cell proteases[6, 7], which unravelling cellular factors may be used as therapeutic targets for controlling SARS-CoV–2 transmission.

Mesenchymal stem cells (MSCs) have been widely used for solution of clinical issues, not only just in autoimmune diseases[8, 9], but also in infectious diseases[10–12], and the safety and effectiveness have been well elucidated. Umbilical cord mesenchymal stem cells (hUC-MSCs), as one kind of MSCs, can be easily obtained and expansion in vitro.

Numerous studies have shown hUC-MSCs play significant immune-modulation and tissue repair functions for its low immunogenicity[13–15]. As an ideal candidate for allogenic adoptive transfer therapy, hUC-MSCs have been shown to play a protect role in A/H5N1-associated acute lung injury[16], Although one article have been published recently on the therapeutic effects of stem cells on COVID–19, it is about bone marrow mesenchymal stem cells[17],and did not focus on the treatment of severe COVID–19. So far, the safety and therapeutic effect of hUC-MSCs on severe COVID–19 have not been reported.

In order to evaluate the efficacy of hUC-MSCs on severe COVID–19, we conducted this hUC-MSCs transplantation pilot study intend to elucidate the potential therapeutic role in treatment of severe COVID–19.

Methods

Study design and participants

This study was a single-center open-label, individually randomized, standard treatment-controlled trial conducted in Huangshi Hospital of Traditional Chinese Medicine of Hubei Province from Feb 12 to March 25, 2020, and it was performed in according to the Declaration of Helsinki and approved by the Ethics Committee of the Huangshi Hospital of Traditional Chinese Medicine (No. HSZYPJ–2020–009–01).Written informed consent was obtained from all patients or their representatives when data were collected retrospectively.

The diagnosis of COVID–19 is based on the WHO interim guidance[18] and New coronavirus pneumonia diagnosis and treatment program (6th ed.) (in Chinese)[19], the severe patients should be positive of SARS-CoV–2 nucleic acid test by the real-time reverse transcription polymerase chain reaction (RT-PCR) assay of HCoV–19 RNA in Chinese Center for Disease Control and Prevention followed the protocol described previously[10, 20], and at the same time coincide with any of the following criteria: (a) respiratory distress, respiration rate (RR) ≥ 30 times / min; (b) the oxygen saturation ≤ 93% in the resting state; (c) PaO_2 / FiO_2 ≤ 300 mmHg (1mmHg = 0.133 kPa). In general, severe COVID–19 would be recommended to participate in this pilot study with the clinical symptoms were not significantly alleviated under standard treatment for 7 to 10 days, and randomly divided into 2 groups, standard treatment group (Control group) and standard treatment plus umbilical cord mesenchymal stem cells infusion group (hUC-MSCs group). Exclusion criteria included the following: any kind of cancer, severe liver disease, known allergy or hypersensitivity to hUC-MSCs, and other conditions that the clinician deems inappropriate to participate.

Cell preparation and transplantation

The clinical grade hUC-MSCs were supplied, for free,by The Jiangsu Cell Tech Medical Research Institute and The Jiangsu Cell Tech Biotechnology Co.Ltd. The product has been registered and reviewed by China Clinical Trial Center

(Registration No. ChiCTR2000031494). It has passed the demonstration by the Ethics Committee of Huangshi Hospital of Traditional Chinese Medicine (Approval Letter No. HSZYPJ–2020–009–01). The preparation is completed in GMP workshop. The intravenous administration is used. Before the intravenous drip, the MSCs were suspended in 100 ml of normal saline, and the total number of transplanted cells was calculated by 2 x 10^6 cells/kg of weight. The infusion was lasted about 1 hour.

Clinical outcome assessment

The patients were observed through the 2 weeks after hUC-MSCs infusion, the clinical symptoms, laboratory tests and radiological results were recorded and confirmed by experienced physicians. The primary clinical outcomes included the incidence of severe patients aggravate to critically illness, time to clinical improvement of two points on a seven-category ordinal scale which used widely in clinical symptoms assessment previously or discharge from hospital[21]. In our study, the NEWS2 score and seven-category ordinal scale were used to assess the clinical symptoms and conditions of enrolled patients[22]. The secondary clinical outcomes included statuses at day 7 and 14 assessed with seven-category ordinal scale, hospital staying, changes in oxygenation index, hematology inflammatory factors and imaging.

Statistical analysis

Continuous variables with normal distribution were expressed as mean±standard deviation (SD); non-normal continuous variables were reported as median (interquartile range, IQR). For P value, the Mann-Whitney U test were used for analyzing the normally distributed continuous variables, and Kruskal-Wallis test for non-normally distributed data. The categorical variables were presented as percentage and analyzed by Chi-square test or Wilcoxon rank-sum test. All statistical analyses were performed with Stata version 14.2 for Mac (StataCorp, College Station, TX), and P value less than 0.05 was considered statistically significant.

Results

hUC-MSC treatment procedure and general patient information

This study was conducted from Feb 12, 2020, to March 25, 2020. Total 12 patients were enrolled in hUC-MSC treatment group, and 29 patients were enrolled in control group (Figure 1). The median age of total patients was 61 years old (interquartile range [IQR], 50 to 70.5 years, 65 years in hUC-MSCs group vs. 58 years in control group, P = 0.576), and 58.54% of total patients were men (66.67% in hUC-MSCs group vs. 55.17% in control group, P = 0.794, table 1). The median time from onset of symptoms to enrollment was 13 days (11.5 days in hUC-MSCs group vs. 14 days in control group, P = 0.135, Table 2). There were no significant differences between hUC-MSCs treatment and control groups in demographic characteristics, laboratory test results, distribution of sequential scale scores, and NEWS2 scores at enrollment. In the trial, all patients used antiviral drugs for 7 to 10 days, and systemic glucocorticoids also been included in use, the median days was 5 (7.5 day in hUC-MSCs group vs. 5 days in control group, P = 0.195, Table 2)

Primary Outcome

In hUC-MSCs treatment group, all patients improved and discharged, no invasive ventilation occurred in 12 patients, the incidence of severe patients aggravated to critically illness and 28-day mortality were 0, while 4 patients in control group were deteriorated to critical illness and been used invasive ventilation, 3 of them died, and 28-day mortality was 10.34%. The time to clinical improvement in hUC-MSCs treatment group was shorter than that in control group (median, 9.0 days in hUC-MSCs group vs. 14.0 days in control group, $P = 0.006$). In age≤65 years' subgroups, the time improvement in hUC-MSCs treatment group was 6.0 days (3.00, 7.00) vs. 12 days (7.25, 15.50) in control group, in Age>65 years' subgroups, the time to clinical improvement were significantly prolonged in both groups, 13 days (11.75, 14.00) in hUC-MSCs treatment group vs. 23 days (18.50, 29.00) in control group. Symptoms of weakness and fatigue, shortness of breath, and low oxygen saturation had improved obviously in hUC-MSCs group than in control group, at Day 3 of infusion, 2 patients (16.67%) in hUC-MSCs group had reduction of above symptoms, however, only 1 patient (3.45%) appeared symptoms relief in control group, at Day 7, more than half of the patients (58.33%) in hUC-MSCs group had symptoms relief, and 66.67% of patients were not requiring supplemental oxygen (Table 3), but only 5 patients (17.24%) in control group felt relief and 3 patients (10.34%) were not oxygen supplementation. At day 14, 11 patients (91.67%) felt obviously clinical symptoms improvement in hUC-MSCs group, usually manifested as significant remission of dyspnea, obvious absorption on imaging, however, only 15 patients (51.72%) felt symptoms relief in control group. All the hUC-MSCs treatment patients had no adverse reactions. (such as rash, allergy, and febrile reaction after infusion).

The efficacy outcome

The efficacy of hUC-MSCs in treatment was reflected in changes of indicators. Compared with control group, C-reaction protein and IL−6 were significantly decreased from day 3 of stem cells infusion in hUC-MSCs group. Arterial blood gas analysis showed that the time for the oxygenation index to return to the normal range was faster in hUC-MSCs treatment group than that in control group, the difference was obviously significant started from day 7 of hUC-MSCs infusion, it was consistent with the time window for the patients' clinical symptom relief. The time for lymphocyte count to return to normal range was significant faster after stem cells infusion (Figure 2). Chest CT scanning indicated that the absorption of lung inflammation in the stem cell treatment group was significantly faster than that in the control group (Figure 3).

Discussion

As the epidemic continues to spread and escalate, more and more patients are diagnosed with new coronary pneumonia globally. However, to date, especially in the treatment of severe and critically ill patients, there is still no effective medical drugs or methods for treating.

At present, the mortality rate of COVID−19 is different due to the different sample sizes populations included in different regions, different severity of the epidemic is different.[23, 24]

In our study, we found that there was no invasive ventilation occurred in 12 hUC-MSCs treated patients, the proportion of severe patients converted to critically ill patients and 28-day mortality were 0, while 4 patients in control group were converted to critical illness and been used invasive ventilation, 3 of them died, and 28-day mortality was 10.34%. Although the statistics were not significant, the improve trend is clear, and there is every reason to believe that it will be significant differences if the sample is large enough.

We also found that in the hUC-MSCs treatment group, patients' clinical symptoms, including chest tightness, shortness of breath, fatigue, etc., were significantly relieved and alleviated in a relatively short time compared with the control group. Inflammatory factors, including IL–6, CRP could be rapidly reduced, and the lymphocyte count could return to normal levels in less time. As the patient's chest tightness and shortness of breath quickly relieved, arterial blood gas suggested that the oxygenation index could improve in a shorter time than the control group. With the improvement of clinical symptoms, the changes in imaging absorption were also obvious. The positive effect of hUC-MSCs on severe COVID–19 is clearly and obviously, whereas, the specific molecular mechanism of hUC-MSCs was not clear and still needs to be further illustrated.

MSCs therapy can suppress immune system excessive activation and promote endogenous repair by improving the microenvironment. Studies have found that MSCs can enter the human body by intravenous infusion, then some mesenchymal stem cells accumulate in the lungs, which can improve the lung microenvironment, protect alveolar epithelial cells, prevent pulmonary fibrosis, and improve lung function[25–27]. Based on previous studies, and combined with our results, we speculate that hUC-MSCs can reduce the inflammatory response in the lungs through reducing the release of inflammatory factors mediated by immune regulation.

In our study, in addition to the above results, we found another interesting phenomenon: patients with diabetes complication had significantly less exogenous insulin use after hUC-MSCs infusion than usual. The effects of hUC-MSCs on diabetes have been reported in many studies previously[28–30]. It has been reported that diabetes is a death risk factor of COVID–19[31–33], so for patients of severe COVID–19 with diabetes, hUC-MSCs therapy may be the most ideal treatment. Previously studies indicated older age is a potential risk factor for the mortality of COVID–19[34, 35], in our study, patients younger than 65 years old had well reaction to hUC-MSCs therapy, which indicated the therapeutic effect of stem cells in severe patients indirectly, the specific mechanism needs to be further clarified.

It is obviously that our study found the therapeutic effect of hUC-MSCs on severe COVID–19, this is a single-center, small-sample controlled cohort study and has certain limitations. First, the sample size isnot large enough to stratify subgroups, and some bias are difficult to be excluded.Second, it is a preliminary comparative clinical study, the relevant mechanism needs to be further elucidated.

Conclusions

As a non-invasive treatment, hUC-MSCs therapy is a very effective and worthy method for clinical application and promotion at the current critical moment for lacking effective ways in treating of severe COVID–19.

Abbreviations

COVID–19: Corona Virus Disease 2019; hUC-MSCs: human Umbilical Cord Mesenchymal Stem Cells; CRP: C-reaction protein; IL–6: interleukin 6; IQR: interquartilerange; WBC: white blood cells; NEU: neutrophils; LYM: lymphocytes; Mon: monocytes; PLT: platelets; Hb: hemoglobin; PT: prothrombin time; APTT: activated partial thromboplastin time; D-D: DD dimers; CK: creatine kinase; LDH: lactate dehydrogenase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; Cr: creatinine; BUN: urea nitrogen; ECMO denotes extracorporeal membrane oxygenation, HFNC high-flow nasal cannula for oxygen therapy, and NEWS2 National Early Warning Score 2.

Declarations

Acknowledgements

We thank the authors of the primary studies for their timely and helpful responses to our information requests.

Authors' contributions

L.Shu, C.N., R.L., T.H., N.J., and Y.Z. conceived and designed the study, and take responsibility for the integrity of the data and the accuracy of the data analysis. L.Shu, X.C., and Y. W. contributed to analysis the infection situation and write the paper. L.Shi, M.W., K.D., J.W., X.W., Y.C., and J. Y. assisted in data collection, extraction and evaluation the eligibility of the original data. L.Shu and C.N. analyzed the data. L.Shu and G.F. interpreted the data and contributed to the writing of the final version of the manuscript.

All authors reviewed and approved the final manuscript.

Funding: This work was supported by grant of National Natural Science Foundation of China (81670013) and Key Research and Development Project of Jiangsu Province (BE2020616)

Availability of data and materials

The majority of the data generated or analyzed during this study are included in this article. Unpublished data are available from the corresponding author upon reasonable request

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Huangshi Hospital of Traditional Chinese Medicine (No. HSZYPJ-2020-009-01). Written informed consent was obtained from all patients or their representatives when data were collected retrospectively.

Consent for publication

Not applicable.

Competing interests

Lei Shu, Changming Niu, Ruyou Li, Tingrong Huang, Yan Wang, Ningfei Ji, You Zheng, Xiaolin Chen, Lei Shi, Mingjing Wu, Kaili Deng, Jing Wei, Xueli Wang, Yang Cao, Jiaxin Yan, Ganzhu Feng declared that there were no competing interests.

References

1. Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020.
2. Xu X, Chen P, Wang J, Feng J, Zhou H, Li X, et al. Evolution of the novel coronavirus from the ongoing Wuhan outbreak and modeling of its spike protein for risk of human transmission. Sci China Life Sci. 2020;63:457-60.
3. WHO, Coronavirus disease (COVID-19) Situation Dashboard. https://experience.arcgis.com/experience/685d0ace521648f8a5beeeee1b9125cd(2020).Accessed.
4. Cao J, Tu WJ, Cheng W, Yu L, Liu YK, Hu X, et al. Clinical Features and Short-term Outcomes of 102 Patients with Corona Virus Disease 2019 in Wuhan, China. Clin Infect Dis. 2020.

5. WHO, WHO characterizes COVID-19 as a pandemic. https://www.who.int/emergencies/diseases/novel-coronavirus-2019/events-as-they-happen(2020).Accessed 2020-03-12.
6. Hoffmann M, Kleine-Weber H, Schroeder S, Kruger N, Herrler T, Erichsen S, et al. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. Cell. 2020.
7. Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet. 2020;395:565-74.
8. Le Blanc K, Rasmusson I, Sundberg B, Gotherstrom C, Hassan M, Uzunel M, et al. Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. Lancet. 2004;363:1439-41.
9. Geng L, Tang X, Wang S, Sun Y, Wang D, Tsao BP, et al. Reduced Let-7f in Bone Marrow-Derived Mesenchymal Stem Cells Triggers Treg/Th17 Imbalance in Patients With Systemic Lupus Erythematosus. Front Immunol. 2020;11:233.
10. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. 2020 2020-02-07.
11. McIntyre LA, Stewart DJ, Mei S, Courtman D, Watpool I, Granton J, et al. Cellular Immunotherapy for Septic Shock. A Phase I Clinical Trial. Am J Respir Crit Care Med. 2018;197:337-47.
12. Zheng G, Huang L, Tong H, Shu Q, Hu Y, Ge M, et al. Treatment of acute respiratory distress syndrome with allogeneic adipose-derived mesenchymal stem cells: a randomized, placebo-controlled pilot study. Respir Res. 2014;15:39.
13. Li T, Xia M, Gao Y, Chen Y, Xu Y. Human umbilical cord mesenchymal stem cells: an overview of their potential in cell-based therapy. Expert Opin Biol Ther. 2015;15:1293-306.
14. Chen P, Zhang KH, Na T, Wang L, Yin WD, Yuan BZ, et al. The hUC-MSCs cell line CCRC-1 represents a novel, safe and high-yielding HDCs for the production of human viral vaccines. Sci Rep. 2017;7:12484.
15. Jagiello J, Sekula-Stryjewska M, Noga S, Adamczyk E, Dzwigonska M, Kurcz M, et al. Impact of Graphene-Based Surfaces on the Basic Biological Properties of Human Umbilical Cord Mesenchymal Stem Cells: Implications for Ex Vivo Cell Expansion Aimed at Tissue Repair. Int J Mol Sci. 2019;20.
16. Loy H, Kuok D, Hui K, Choi M, Yuen W, Nicholls JM, et al. Therapeutic Implications of Human Umbilical Cord Mesenchymal Stromal Cells in Attenuating Influenza A(H5N1) Virus-Associated Acute Lung Injury. J Infect Dis. 2019;219:186-96.
17. Leng Z ZRHW. Transplantation of ACE2 Mesenchymal stem cells improves the outcomes of patients with COVID-19 pneumonia. Aging Dis. 2020;216:228.
18. WHO, Clinical management of severe acute respiratory infection when Novel coronavirus (nCoV) infection is suspected: interim guidance. https://www.who.int/internalpublications-detail/clinical-management-of-severe-acuterespiratoryinfection-when-novel-coronavirus-(ncov)-infection-is-suspected (2020).Accessed Jan 11, 2020.
19. New coronavirus pneumonia diagnosis and treatment program (6th ed.) (in Chinese). http://www.nhc.gov.cn/xcs/zhengcwj/202002/3b09b894ac9b4204a79db5b8912d4440.shtml(2020).Accessed.
20. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020;395:497-506.
21. Wang Y, Fan G, Salam A, Horby P, Hayden FG, Chen C, et al. Comparative effectiveness of combined favipiravir and oseltamivir therapy versus oseltamivir monotherapy in critically ill patients with influenza virus infection. J Infect Dis. 2019.

22. National Early Warning Score (NEWS) 2: standardising the assessment of acuteillness severity in the NHS. London: Royal College of Physicians. (https://www.rcplondon.ac.uk/projects/outputs/national-early-warning-score-news-2). (2017).Accessed.
23. Lai CC, Wang CY, Wang YH, Hsueh SC, Ko WC, Hsueh PR. Global epidemiology of coronavirus disease 2019: disease incidence, daily cumulative index, mortality, and their association with country healthcare resources and economic status. Int J Antimicrob Agents. 2020:105946.
24. Weiss P, Murdoch DR. Clinical course and mortality risk of severe COVID-19. Lancet. 2020;395:1014-5.
25. Harrell CR, Sadikot R, Pascual J, Fellabaum C, Jankovic MG, Jovicic N, et al. Mesenchymal Stem Cell-Based Therapy of Inflammatory Lung Diseases: Current Understanding and Future Perspectives. Stem Cells Int. 2019;2019:4236973.
26. Yan X, Fu X, Jia Y, Ma X, Tao J, Yang T, et al. Nrf2/Keap1/ARE Signaling Mediated an Antioxidative Protection of Human Placental Mesenchymal Stem Cells of Fetal Origin in Alveolar Epithelial Cells. Oxid Med Cell Longev. 2019;2019:2654910.
27. Sinclair KA, Yerkovich ST, Chen T, McQualter JL, Hopkins PM, Wells CA, et al. Mesenchymal Stromal Cells are Readily Recoverable from Lung Tissue, but not the Alveolar Space, in Healthy Humans. Stem Cells. 2016;34:2548-58.
28. Cai J, Wu Z, Xu X, Liao L, Chen J, Huang L, et al. Umbilical Cord Mesenchymal Stromal Cell With Autologous Bone Marrow Cell Transplantation in Established Type 1 Diabetes: A Pilot Randomized Controlled Open-Label Clinical Study to Assess Safety and Impact on Insulin Secretion. Diabetes Care. 2016;39:149-57.
29. Liu X, Zheng P, Wang X, Dai G, Cheng H, Zhang Z, et al. A preliminary evaluation of efficacy and safety of Wharton's jelly mesenchymal stem cell transplantation in patients with type 2 diabetes mellitus. Stem Cell Res Ther. 2014;5:57.
30. Carlsson PO, Schwarcz E, Korsgren O, Le Blanc K. Preserved beta-cell function in type 1 diabetes by mesenchymal stromal cells. Diabetes. 2015;64:587-92.
31. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet. 2020.
32. Wu C, Chen X, Cai Y, Xia J, Zhou X, Xu S, et al. Risk Factors Associated With Acute Respiratory Distress Syndrome and Death in Patients With Coronavirus Disease 2019 Pneumonia in Wuhan, China. Jama Intern Med. 2020.
33. Guo W, Li M, Dong Y, Zhou H, Zhang Z, Tian C, et al. Diabetes is a risk factor for the progression and prognosis of COVID-19. Diabetes Metab Res Rev. 2020:e3319.
34. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet. 2020;395:1054-62.
35. Yang X, Yu Y, Xu J, Shu H, Xia J, Liu H, et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. Lancet Respir Med. 2020.

Tables

Table 1 Demographics and baseline characteristics in all patients

Variables	Total Patients (n=41)	hUC-MSCs（N=12）	Control（N=29）	P Value
Age（Mean±SD）	58.78±16.26	61.00±17.87	57.86±15.79	0.576
Male sex, n(%)	24 (58.54%)	8 (66.67%)	16 (55.17%)	0.794
Comorbidities, n(%)				
Diabetes	8 (19.51%)	3 (25%)	5 (17.24%)	0.672
Hypertension	9 (21.95%)	3 (33.33%)	6 (20.69%)	1.000
Signs and symptoms on admission, n(%)				
Fever≥37.3°C	36 (87.80%)	10 (83.33%)	26 (89.66%)	0.470
Cough	27 (65.85%)	8 (66.67%)	19 (65.52%)	1.000
Respiratory rate >24/min	31 (75.61%)	11 (91.67%)	20 (68.97%)	0.231
Blood routine				
WBC count ($x10^9$/L) Mean (IQR)	6.88 (5.06, 9.20)	7.37 (5.06, 11.16)	6.88 (5.06, 8.71)	0.449
3.5~9.5($x10^9$/L), n(%)	29 (70.73%)	7 (58.34%)	22 (75.86%)	0.068
<3.5($x10^9$/L), n(%)	3 (7.31%)	1 (8.33%)	2 (6.89%)	1.000
>9.5($x10^9$/L), n(%)	7 (17.07%)	4 (33.33%)	3 (10.34%)	0.165
LYM count($x10^9$/L) median (IQR)	0.82 (0.59, 1.19)	0.77 (0.43, 1.72)	0.82 (0.59, 1.11)	0.783
<1($x10^9$/L) , n(%)	18 (43.90%)	5 (41.67%)	13 (44.83%)	1.000
≥1($x10^9$/L) , n(%)	23 (56.10%)	7 (58.33%)	16 (55.17%)	1.000
MON count($x10^9$/L) median (IQR)	0.50 (0.32, 0.75)	0.41 (0.26, 0.65)	0.62 (0.33, 0.0.91)	0.187
Hb(g/L) Mean (IQR)	120.0 (112, 128.5)	119.5 (100.3, 127.8)	120.0 (112.0, 133.0)	0.322
PLT count($x10^9$/L) median (IQR)	205.0 (145.0, 242.0)	207.0 (170.5, 327.8)	205.0 (141.0, 236.0)	0.338
<100($x10^9$/L), n(%)	3 (7.32%)	1 (8.33%)	2 (6.90%)	1.000
≥100($x10^9$/L), n(%)	38 (92.68%)	11 (91.67%)	27 (93.10%)	1.000
PT(s) median (IQR)	12.30 (11.60, 13.20)	11.85 (11.33, 13.10)	12.40 (11.80, 13.20)	0.296
APTT(s) median (IQR)	37.10 (32.53, 41.80)	34.45 (29.60, 43.45)	38.80 (34.00, 41.80)	0.317
D-D(μg/L) median (IQR)	0.54 (0.02, 0.54)	0.89 (0.24, 2.78)	0.34 (0.20, 1.34)	0.224
Myocardial enzymes				
CK(U/L) median (IQR)	109.0 (40.5, 215.0)	162.5 (75.0, 360.8)	106.0 (36.0, 201.0)	0.132
<310(U/L), n(%)	36 (87.80%)	9 (75.00%)	27 (93.10%)	0.139
≥310(U/L), n(%)	5 (12.20%)	3 (25.00%)	2 (6.90%)	0.139
LDH(U/L) median (IQR)	331.0 (229.0, 410.5)	285.5 (220.0, 392.0)	331.0 (237.5, 441.0)	0.338
<250(U/L), n(%)	14 (34.15%)	4 (33.33%)	10 (34.48%)	1.000
≥250(U/L), n(%)	27 (65.85%)	8 (66.67%)	19 (65.52%)	1.000
Biochemical indicators				
ALT(U/L) median (IQR)	56.00 (42.50, 74.50)	67.00 (47.50, 104.00)	42.50 (42.00,74.50)	0.065
<50 (U/L), n(%)	9 (21.95%)	2 (16.67%)	7 (24.14%)	0.702
≥50 (U/L), n(%)	32 (78.05%)	10 (83.33%)	22 (75.86%)	0.702
AST(U/L) median (IQR)	31.00 (24.50, 38.00)	34.00 (25.25, 45.00)	31.00 (24.00, 37.50)	0.576
<40(U/L), n(%)	32 (78.05%)	8 (66.67%)	24 (82.76%)	0.407
≥40 (U/L), n(%)	9 (21.95%)	4 (33.33%)	5 (17.24%)	0.407
Cr(μmol/L) Mean (SD)	60.27 (49.28, 69.46)	53.84 (44.97, 65.57)	65.85 (49.41, 71.59)	0.360
BUN(mmol/L) median (IQR)	5.05 (3.43, 6.15)	5.43 (3.14, 6.59)	4.83 (3.43, 5.90)	0.827

Abbreviation: IQR: interquartilerange; WBC: white blood cells; NEU: neutrophils; LYM: lymphocytes; Mon: monocytes; PLT: platelets; Hb: hemoglobin; PT: prothrombin time; APTT: activated partial thromboplastin time; D-D: DD dimers; CK: creatine kinase; LDH: lactate dehydrogenase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; Cr: creatinine; BUN: urea nitrogen.

Table 2 Patients' Status and Treatments Received at or after Enrollment

Variables	Total Patients (n=41)	hUC-MSCs (N=12)	Control (N=29)	P Value
NEWS2 score at day 1-median (IQR)	8.00 (7.00, 10.00)	9.0 (8.00, 10.75)	8.00 (7.00, 10.00)	0.098
Seven-category scale at day 1				
3:Hospitalization, not requiring supplemental oxygen no. (%)	3 (7.31%)	1 (8.33%)	2 (6.90%)	1.000
4:Hospitalization, requiring supplemental oxygen no. (%)	28 (68.30%)	7 (58.33%)	21(72.41%)	0.469
5:Hospitalization, requiring HFNC or noninvasive mechanical ventilation no. (%)	10 (24.39%)	4 (33.33%)	6 (20.69%)	0.441
6:Hospitalization, requiring ECMO, invasive mechanical ventilation, or both no. (%)	0	0	0	1.000
Days from illness onset to randomization median (IQR)	13.00 (9.00, 17.50)	11.50 (6.00, 20.00)	14.00 (10.00, 18.00)	0.135
Earlier (≤12 days of symptom onset) no. (%	17 (41.46%)	7 (58.33%)	10 (34.48%)	0.184
Later (>12 days of symptom onset) no. (%)	24 (58.54%)	5 (41.67%)	19 (65.52%)	0.184
Treatments during study period no. (%)				
Noninvasive mechanical ventilation	5 (12.20%)	3 (25%)	2 (6.70%)	0.139
Invasive mechanical ventilation	4 (9.76%)	0	4 (13.79%)	0.302
Antibiotic agent	36 (87.80%)	10 (83.33%)	26 (89.66%)	0.620
Antiviral treatment	41 (100%)	12 (100%)	29 (100%)	1.000
Vasopressors	7 (17.07%)	0	7 (24.14%)	0.085
Renal-replacement therapy	0	0	0	1.000
ECMO	0	0	0	1.000
Glucocorticoid therapy	41 (100%)	12 (100%)	29 (100%)	1.000
Days of glucocorticoid therapy-median (IQR)	5.00 (3.00, 8.50)	7.50 (5.00, 9.75)	5.00 (3.50, 9.00)	0.195

Notes: Plus–minus values are means ±SD. ECMO denotes extracorporeal membrane oxygenation, HFNC high-flow nasal cannula for oxygen therapy, and NEWS2 National Early Warning Score 2.

Table 3 Clinical outcomes of the patients

Variables	Total Patients (n=41)	hUC-MSCs (N=12)	Control (N=29)	P Value
Time to clinical improvement-median no. of days (IQR)	13.00 (7.00, 18.50)	9.00 (6.00, 13.00)	14.00 (9.50, 21.00)	0.006
Age≤65 years- median no. of days (IQR)	9.50 (6.75, 14.00)	6.00 (3.00, 7.00)	12.00 (7.25, 15.50)	0.0014
Age>65 years -median no. of days (IQR)	17.00 (13.00, 23.00)	13.00 (11.75, 14.00)	23.00 (18.50, 29.00)	<0.001
Day 28 mortality-no. (%)	3 (7.31%)	0	3 (7.31%)	0.543
Earlier (≤12 days after onset of symptoms)	0	0	0	
Later (>12 days after onset of symptoms)	3 (7.31%)	0	3 (7.31%)	0.543
severe converted to critically ill patients-no. (%)	4 (9.76%)	0	4 (13.79%)	0.667
Clinical improvement - no. (%)				
Day 3	3 (7.32%)	2 (16.67%)	1 (3.45%)	0.200
Day 7	12 (29.27%)	7 (58.33%)	5 (17.24%)	0.020
Day 14	26 (63.41%)	11 (91.67%)	15 (51.72%)	0.03
Day 28	37 (90.24%)	12 (100%)	25 (86.21%)	0.302
Hospital stay -median no. of days (IQR)	22.00 (19.50, 25.00)	20.00 (16.00, 24.00)	24.00 (20.00, 26.50)	0.054
Score on seven-category scale at day 7 of enrollment -no. of patients (%)				
2: Not hospitalized, but unable to resume normal activities	1 (2.44%)	1 (8.33%)	0	0.293
3: Hospitalization, not requiring supplemental oxygen	10 (24.39%)	7 (58.33%)	3 (10.34%)	0.0028
4: Hospitalization, requiring supplemental oxygen	22 (53.66%)	3 (25.00%)	19 (65.52%)	0.037
5: Hospitalization, requiring HFNC or noninvasive mechanical ventilation	6 (14.63%)	1 (8.33%)	5 (17.24%)	0.651
6: Hospitalization, requiring ECMO, invasive mechanical ventilation, or both	2 (4.88%)	0	2 (4.88%)	1.000
7: Death	0	0	0	1.000
Score on seven-category scale at day 14 of enrollment- no. of patients (%)				
2: Not hospitalized, but unable to resume normal activities	10 (24.39%)	5 (41.67%)	5 (17.24%)	0.124
3: Hospitalization, not requiring supplemental oxygen	23 (56.10%)	6 (50.00%)	17 (58.62%)	0.734
4: Hospitalization, requiring supplemental oxygen	2 (4.88%)	1 (8.33%)	1 (3.45%)	0.505
5: Hospitalization, requiring HFNC or noninvasive mechanical ventilation	2 (4.88%)	0	2 (6.70%)	1.000
6: Hospitalization, requiring ECMO, invasive mechanical ventilation, or both	1 (2.44%)	0	1 (3.45%)	1.000
7: Death	3 (7.32%)	0	3 (10.34%)	0.543

Note Clinical improvement was defined as a decline of two categories on the modified seven-category ordinal scale of clinical status, or hospital discharge. HFNC: high nasal flow oxygen therapy; ECMO: extracorporeal membrane oxygenation.

Figures

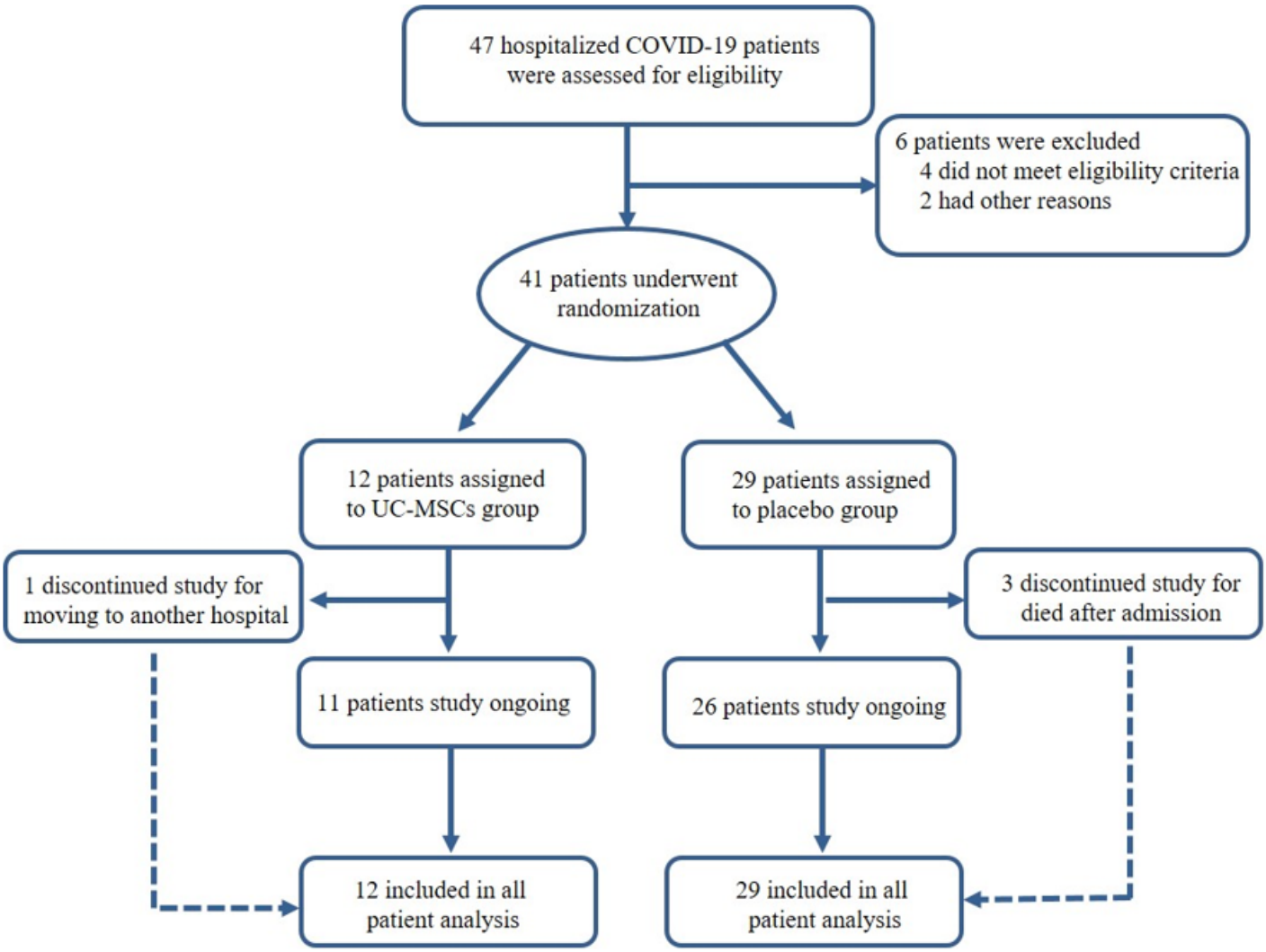


Figure 1

Flow diagram of clinical trial in the study. Abbreviation: UC-MSCs: umbilical cord matrix stem cells; COVID-19: Corona Virus Disease 2019.

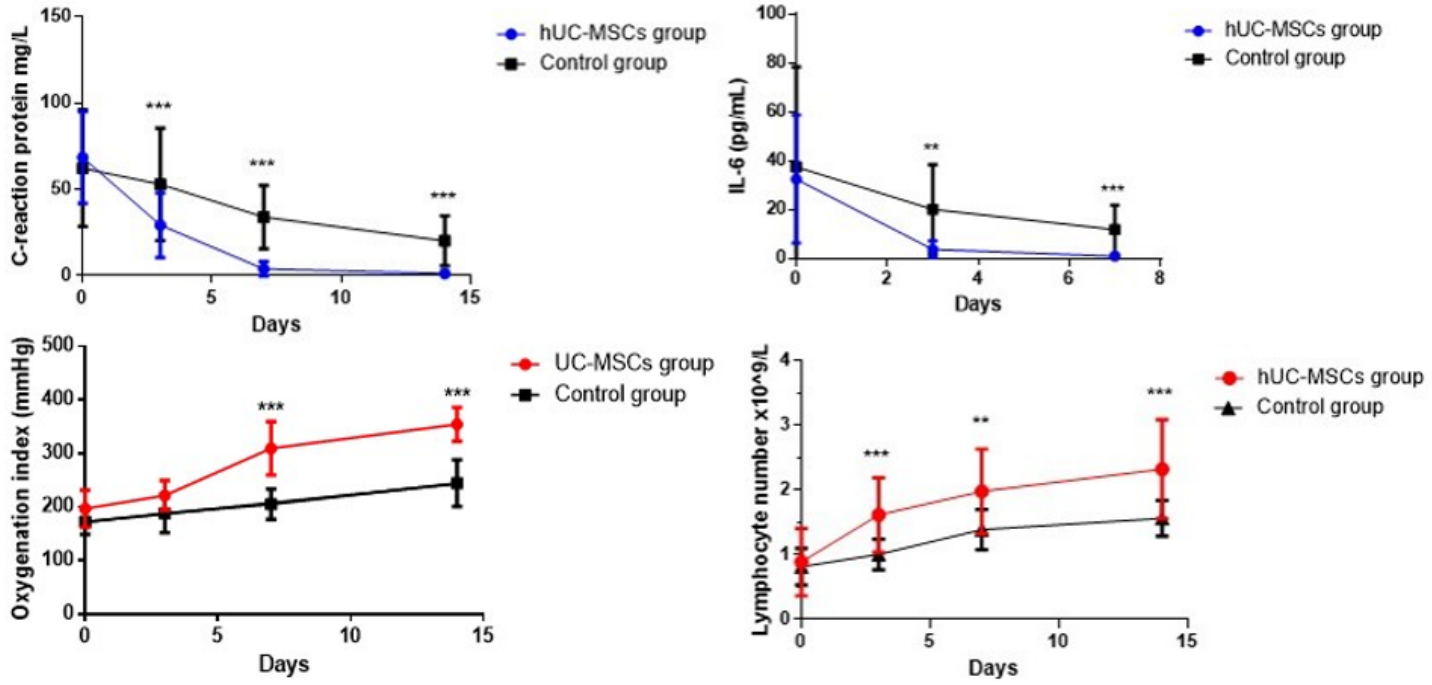
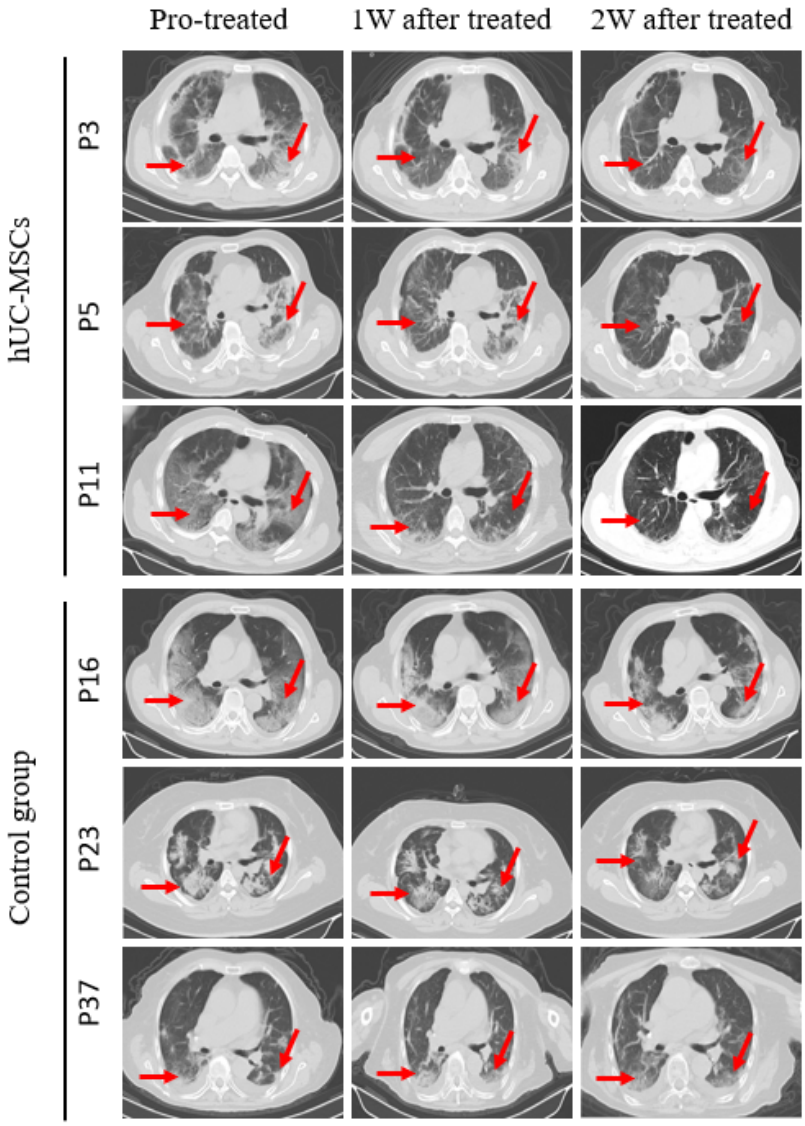


Figure 2

The dynamic changes of CRP, IL-6, Oxygenation index and lymphocyte number in patients of hUC-MSCs and control groups. (**, p<0.01, ***, p<0.001)



CT scanning showed 6 patients (P3, P5, P11, means patient no. 3, no 5, no 11, from hu-MSCs group, P16, P23, P37, means patient no. 16, no 23, no 37, from control group) imaging results at 3 time points (pro-treated, 1 week after treated, and 2 weeks after treated. The red arrows showed the sites of inflammatory exudation, consolidation, or absorption.

Figure 3

Chest computerized tomography (CT) images of the patients in hUC-MSCs and control groups