AI-assisted quantitative lung CT evaluation following umbilical cord mesenchymal stem cell treatment in severe COVID-19 patients

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Abstract

Background

The COVID-19 has high transmission and mortality. Previous studies support the efficacy and safety of mesenchymal stem cells (MSCs) in the treatment of lung injury. In this study, We aimed to evaluate the CT changes of lung lesions in severe COVID-19 patients treated with umbilical cord mesenchymal stem cells (UC-MSCs) by using AI-assisted quantification method.

Methods

46 patients with severe COVID-19 from March 5 to April 1, 2020 were selected by single-blind, non-randomized controlled clinical study and divided into three groups: 11 cases in UC-MSCs treatment group 1 (MSC-1, with cells infusion once), 26 cases in UC-MSCs treatment group 2 (MSC-2, with cells infusion twice or three times), and 9 cases in control group with routine treatment. Repeated measure ANOVA was used to compare the effects of treatment factors on chest CT parameters of COVID-19 patients between control and experimental groups, and pairwise comparison using LSD test.

Findings

The differences between the percentage of GGO in total lung or the percentage of total lung infection volume on day 0 and that in day 60 as well as in day 90 were statistically significant among the three groups. The P values were 0.034 and 0.018 respectively. Pairwise comparison results showed that the percentage difference of the whole lung GGO and total lung infection volume in MSC-1 group was smaller than that in control group and MSC-2 group respectively, but there was no statistical difference between control group and MSC-2 group. The distribution characteristics and other CT parameters post-proceeded by AI software were not significantly different among the three groups. There were no serious adverse events related to stem cell infusion in all treated patients.

Interpretation

UC-MSC infusion is safe for the treatment of severe COVID-19 patients. The absorption of lung lesions at 60 days and 90 days after UC-MSC infusion once was more obvious than that in the control group. AI quantification of lung lesions is more suitable for comparative studies before and after treatment.

Introduction

In the December 2019, several cases of unexplained pneumonia emerged in Wuhan, China. By deep sequencing and virus strain isolation, it has been identified as a new type of acute respiratory infectious disease caused by a novel coronavirus (SARS-CoV-2)[1, 2]. Of all infected patients, 4–6% developed severe pneumonia, about 5% of patients end up in ICU with acute respiratory distress syndrome, septic shock and/or multiple organ failure, and more than 75% of inpatients need oxygen[3–6]. According to the current diagnosis and treatment protocol in “Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia (Trial Version 8)”, the treatment of mild to moderate patients is generally based on basic treatment, with good recovery and low mortality. However, severe patients need to be admitted to the ICU for supportive treatment such as breathing and circulation. Several studies have shown that most patients with severe infectious pneumonia will continue to develop pulmonary fibrosis due to poor inflammatory lesion absorption[7–10]. With the development of fibrotic ‘scars’, the lung tissue structure is gradually destroyed, especially in the alveolar area, which eventually leads to reduced gas exchange and chronic respiratory failure[11]. Although current studies have reported that dexamethasone can reduce the 28-day mortality of patients who require oxygen, and remdesivir can shorten the recovery time[12, 13], no specific treatment has been found to reduce mortality and improve the lesion absorption in convalescence.

Umbilical cord mesenchymal stem cells (UC-MSCs) is one kind of multipotent stem cells with high differentiation potential that exist in neonatal umbilical cord tissue and can be differentiated in multiple directions. Previous studies have shown that, UC-MSCs has an important role in immune regulation and damage repair. It can regulate the activation and proliferation of T lymphocytes as well as the activation and maturation of antigen-presenting cells, moreover, it can regulate the repair of airway epithelial cells after injury, alleviate the pulmonary inflammation and reduce the pulmonary fibrosis[14–19]. Hence, intravenous infusion of UC-MSCs is attractive therapy against severe COVID-19. Our study was aimed to use UC-MSCs to treat severe COVID-19 and evaluate the absorption of lung lesions on CT imaging by AI.

Materials And Methods

Study design.

This prospective, single-blind and non-randomized controlled clinical study was screened for COVID-19 patients in The Sixth Hospital of Wuhan (Jianghan University affiliated Hospital) and Taikang Tongji (Wuhan) Hospital between March 5 and April 1, 2020. This project passed the academic review and ethical review in Shanghai East Hospital (East Hosipital affiliated to Tongji University) on January 26, 2020 and January 28, 2020 respectively, and get the projects approval of the Ministry of Science and Technology of the People's Republic of China for 'Emergency project on prevention and control of the epidemic caused by COVID-19' on March 03, 2020. The Sixth Hospital of Wuhan and Taikang Tongji (Wuhan) Hospital had also passed the ethical review and participated in this study.

Participants.

Inclusion criteria: All patients with severe pneumonia (18-75 years old) caused by SARS-Cov-2 infection were selected according to the criteria of 'Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia (Trial Version 8)'. Confirmed patients at 18-75 years old were included if one meet any of the following criteria: an increased respiratory rate (≥30 times/min), an oxygen saturation≤93% when inhaled, or a PaO2 : FiO2 ≤ 300 mmHg. All patients were fully informed, agreed and signed informed consent.

Exclusion criteria:

1. Long term use of immunosuppressive drugs or organ transplantation; 2. T lymphocyte abnormality (the use of allogeneic may be considered, according to the clinical opinion), HIV positive; 3. High allergic constitution or severe allergic history, especially IL-2 allergic history; 4. Pregnant and lactating women; 5. Patients with a history of serious autoimmune diseases; those who are allergic to all biological agents in the treatment, such as IL-2; 6. Patients with serious complications: Patients with chronic cardiac insufficiency (NYHA cardiac function grade IV), chronic renal insufficiency (CKD stage 4 or above), chronic liver insufficiency (child Pugh score > 12), or malignant tumors. 7. There are other situations that the researcher thinks are not suitable for participating in this clinical study.

Randomization and masking.

Selected patients were divided into 3 groups. Principle investigators knew the group assignment for safety reasons, while participants, staff at the test site, computed tomography (CT) radiologists, and laboratory staff were unaware of the group assignment.

Procedures.

The day on which the patient was treated with UC-MSCs or placebo for the first time was defined as D0, and 30, 60 or 90 days after that were defined as D30, D60 or D90 respectively. Patients in control group (n=9) received intravenous infusion of placebo (100 ml normal saline containing 5% HAS) on the basis of the routine treatment that according to the treatment principle of severe and critical cases in "Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia (Trial

Version 8)". Patients in MSC-1 group (n=11) received intravenous infusion of UC-MSCs (5×10^7 cells in 100 ml normal saline containing 5% HSA) for once on the basis of the routine treatment, and patients in MSC-2 group received intravenous infusion of UC-MSCs for 2 (n=10) or 3 (n=16) times, each with an interval of 5 days. The UC-MSC treatments was basically according to the clinical program in "Expert guidance on clinical research and application of stem cell treatment of COVID-19" which was joint published by the Stem Cell Biology Branch of Chinese Society for Cell Biology and Infectious Disease Branch of Chinese Medical Association. The vital signs after treatment were observed and recorded daily by the clinician. Each participant underwent a chest CT examination at D0、D30、D60 and D90 and recorded clinical scores and laboratory-related indicators.

HUC-MSCs were donations from healthy women who have undergone caesarean section, and have been approved by the ethics committee of Shanghai East Hospital, Shanghai, China. Full informed consents were obtained before delivery and donation. The manufacture and quality testing of the UC-MSC preparations used in this study were all carried out in the GMP lab of Shanghai East Hospital. Briefly, the Wharton's Jelly was aseptically separated from the umbilical cord tissue and shredded in to small pieces with sterile scissors. After shredding, the tissue was cultured in α-MEM medium (Thermo) containing 5% serum substitute (UltraGRo-Advanced). After 7-10 days, the monolayer cells crawling around the tissue block were harvested and recorded as passage 0 (P0). Cells at P5 were used for clinical treatment and comprehensive quality testing. Qualified requirements for UC-MSC: 1) No gross agglomeration; 2) Cell survival rate ≥ 90%, showing logarithmic expansion; 3) No pathogenic microorganisms (bacteria, mycoplasma, syphilis, hepatitis B virus, hepatitis C virus, HIV, cytomegalovirus and fungus); 4) Endotoxin ≤0.5EU/mL; 5) characterization and purity: with CD73, CD90 and CD105 positive (≥95%), CD45, CD34, CD14 and HLA-DR negative (≤2%); 6) with the potential of adipogenesis, chondrogenesis and osteogenesis; 7) No tumorigenicity.

Fresh UC-MSCs were prepared once the informed consent was obtained. Qualified UC-MSCs were suspended in 100 ml normal saline containing 5% human serum albumin (HSA) and delivered to the bedside under 2-10℃ within 8 hours. In another case, cryopreserved UC-MSC preparations which were stored in 10 mL clinical grade cryopreservation solution under -196℃ were thawed, suspended in 100 ml normal saline containing 5% human serum albumin (HSA) and delivered to the bedside under 2-10℃ within 6 hours. UC-MSCs were infused into the patients by intravenous within around 90 min. Concerned that UC-MSC infusion may cause temporary obstruction of the pulmonary microcirculation or respiratory damage, blood pressure, heart rate, respiratory parameters and percutaneous oxygen saturation were close monitoring in real-time at the bedside before, during and 6 hours after UC-MSCs infusion.

CT image acquisition and image feature analysis.

Chest CT images were obtained from two scanners: SIEMENS SOMATOM go. Top&ALL and GE MEDICAL SYSTEMS Revolution CT with tube voltage at 130 kV or 120 kV and automatic tube current modulation at 100-400 mA. The reconstructed layer thickness is 0.8 mm or 0.6 mm, respectively. The interlayer spacing is 1.0 mm or 1.25 mm, respectively. All imaging features were reviewed and evaluated through Picture Archiving and Communication Systems (PACS, Tianjian, China) by three experienced radiologists (with 7, 8, and 28 years of chest CT diagnostic experience, respectively) with no knowledge of the group information.

CT manifestations include reticular pattern, fibrotic strips (irregular fibrotic streak shadow), halo sign, reversed halo sign, traction bronchiolectasis and structural distortion. All terms were described according to the Fleischner society glossary and the peer-reviewed literature on viral pneumonia. The distribution of lesions included peripheral, central and diffuse types. Lesions on the extra 1/3 of the lung was defined as peripheral type, lesions on the internal 2/3 was defined as central type, and lesions suffused through the lungs on both sides was defined as diffuse type. Plural effusion, mediastinal and hilar lymph nodes were also evaluated.

Imaging quantification.

UAI Discover-2019 nCoV (Shanghai United Imaging Intelligent Medical Co., Ltd) was used to quantitatively analyze the lung volume of patients with COVID-19. The software provided the function of segmenting lung lobes, lung segments or infection regions and quantitative analysis. After the DICOM format images of the chest CT were imported into the software, the software can quickly and automatically segment the normal areas and infection areas. Prior to this, the software has received a lot of training for COVID-19 cases in which each case was manually sketched by a senior radiologist. Based on these labeled data, the multiscale neural network model was trained to do the segmentation. In the application stage, the machine automatically extracts the lung lobe, lung segment and infected lesion area through the model after learning. The threshold range of -750 ~ -300 Hu or -300 ~ 50 Hu were defined as GGO or Consolidation[20, 21] respectively according to the segmentation results. Finally, the infection volume and percentage of the total lung, as well as the volume and percentage of the whole lung GGO, consolidation were automatically calculated by the machine (Figure 2).

Statistical analysis.

Numerical variables were expressed as median and interquartile spacing, and intergroup comparisons were performed using one-way ANOVA and Kruskal-Wallis H tests. Categorical variables calculate frequency, and chi-square test was used for comparison. Repeated measure ANOVA was used to compare the effects of treatment factors on chest CT parameters of COVID-19 patients between control and experimental groups, and pairwise comparison using LSD test. All the statistical analysis were completed in the SPSS.25 software. The generalized linear mixed effect model analysis of ordered multiple classification repeated measurement data was carried out using GLIMMIX program in SAS 9.4 software. Significant level $P < 0.05$.

Results

A total of 50 patients were screened with 4 patients exits, and were randomly divided into three groups. Among them, 37 cases were treated with UC-MSCs and were divided into MSC-1 group (with cells infusion once, n=11. 8 cases received fresh prepared UC-MSCs) and MSC-2 group (with cells infusion twice or three times, n=26. 8 cases received fresh prepared UC-MSCs). 9 cases with routine treatment were included in the control group (Figure 1). The baseline characteristics of all patients, including age, sex, course of disease, clinical symptoms, comorbid diseases, clinical scores and laboratory test indicators were recorded (Table 1).

Adverse events during UC-MSCs treatment

During the entire study period, no serious adverse events related to the infusion of UC-MSCs occurred. Two patients treated with UC-MSCs developed skin itching 2 hours after infusion of UC-MSCs, and the symptoms subside the next morning. The results indicate that UC-MSCs are safe and tolerable in the treatment of COVID-19.

Clinical and Laboratory Analyses

The clinical and laboratory results of all 46 patients are presented in Table 1. There was a significant difference in gender among the control group, MSC-1 group and MSC-2 group (P =0.015). 2 patients in control group had symptoms of nausea, while no cases in the other two groups (P = 0.035). The sleep score and fatigue score among three groups were significantly different (P = 0.006, P = 0.027). However, there was no significant difference among three groups in terms of age, course of disease, presence of comorbidities or the other main initial symptoms. Laboratory examination showed that, there were no significant differences in the white-cell count, lymohocyte count, monocyte count, neutrophil count, C-reaction protein, D-Dimer, PaO_2 or $PaCO_2$ among three groups.

Chest CT Analysis on Admission

The chest CT findings of patients were presented in Table 2. There were no significant differences in the lesion distribution and processing data based on artificial intelligence among three groups.

Effects of stem cells on CT parameters during follow-up

Repeated measures ANOVA was used to compare the imaging features of control group and MSC groups in 30-90 days follow-up. The results were shown in Table 3. Within 30-90 days of follow-up, the differences between the percentage of GGO in total lung or the percentage of total lung infection volume on day 0 and that on the three follow-up time points showed a decreasing trend with time. However, the differences between the percentage of consolidation on day 0 and that on the three follow-up time points shows no significant downward trend and there was no significant difference between the control group and the two MSC treated groups. (Figure 3).

Repeated measures ANOVA was used to compare the imaging features of control group and MSC groups in 60-90 days follow-up. The results were shown in Table 4. The differences between the percentage of GGO in total lung or the percentage of total lung infection volume on day 0 and that on day 60 as well as on day 90 showed a decreasing trend with time. The results of pairwise comparison showed that the differences between the percentage of GGO in total lung or the percentage of total lung infection volume on day 0 and that on day 60 as well as on day 90 in MSC-1 group were smaller than those of control group and MSC-2 group, but there was no statistical significance between control group and MSC-2 group.

Effects of stem cells on CT structural characteristics during follow-up

A generalized linear model was used to analyze the dynamic changes of CT structural characteristics between the control group and MSC groups within 90 days follow-up. The results are shown in Table 5. There were no statistically significant differences in the terms of traction bronchiolectasis, architectural distortion and reticular pattern among three groups. It can be concluded that UC-MSC treatment have no effect on the dynamic changes of these three CT structures. The three structures all changed over time ($P < 0.001$).

Discussion

The results of our non-randomized controlled single-blind clinical trial showed that, severe COVID-19 patients who received UC-MSC infusion once had a statistical significant difference in the absorption of lung CT lesions at 60 days and 90 days compared with patients in control group. However, UC-MSC infusion 2 or 3 times not only did not improve the efficacy, but appeared to worsen the prophetic efficacy. This might be mainly due to the usage of freshly thawed cryopreserved cells which will be discussed in details below. By the way, the AI-assisted evaluation method of lung CT lesions is more efficient and comparable.

For severe COVID-19 patients, after a comprehensive treatment in the acute phase, pulmonary fibrosis will also cause long-term functional damage in the convalescence. Currently, there was no specific treatment in the world. Many researchers have studied the pathogenesis of severe cases and believe that inflammatory storm may be one of the key causes of serious lesions in patients[22–24], so immunomodulatory therapy was considered and MSCs was chosen to treat severe COVID-19 due to its excellent immunomodulatory ability. Zhang et al. performed MSC treatment for one COVID-19 patient. The lung function improved 2 days after MSC transplantation and was discharged 7 days later, emphasizing the potential of stem cells in the treatment of COVID-19[25]. Zhao Chunhua's group used UC-MSCs to treat 7 patients with COVID-19. Their results confirmed that UC-MSCs were safe for COVID-19 patients and can promote the absorption of local lung lesions and reduce multiple inflammation indicators[26]. Recently, Wang Fusheng's group published a phase I clinical study of stem cell treatment of COVID-19. A total of 18 patients were enrolled, 9 of which were treated with UC-MSCs. Their results showed that the three-times infusion of UC-MSCs was safe and well tolerated, except for two patients experienced

transient facial flushing and fever and one patient experienced transient hypoxia 12 hours post UC-MSCs infusion. No serious adverse events related to UC-MSCs infusion were observed[27].

Compared with the above three studies, our study expanded the number of patients enrolled. A total of 37 severe COVID-19 patients were treated with UC-MSCs, and no serious adverse events were observed. The baseline imaging signs, total lung infection percentage, lung GGO and consolidation volume of the control group and UC-MSC treatment groups (MSC-1 and MSC-2) were similar with no statistical difference. Compared with the control group, the absorption of CT lung lesions at 60 days and 90 days was more obvious in MSC-1 group, and the difference was statistically significant. However, there were no significant difference in the absorption of lung lesions at 60 days and 90 days between the MSC-2 group and the control group. In addition, the MSC-1 group had more obvious lung lesion absorption than the MSC-2 group. The possible reasons analyzed were as follows:

Firstly, in our study, 8 of the 11 patients in the MSC-1 group were treated with fresh prepared UC-MSCs, however, 8 of the 26 patients in the MSC-2 group received fresh prepared UC-MSCs only for the first infusion and others all received cryopreserved MSCs for twice or three times, which suggested that cryopreserved MSCs which were freshly thawed may have certain defects in terms of efficacy. In 2009, a multi-center phase III clinical trial used industrial bone marrow mesenchymal stem cell (BM-MSC) products to treat GvHD, but did not reach its main clinical endpoint. Compared with placebo control, there was no significant improvement of the patients in the BM-MSC treatment group and the 180-day mortality rate of the two groups was equivalent[28]. When analyzing the causes[29], the investigator pointed out that the infusion of cryopreserved MSC immediately after thawing maybe an important reason to cause clinical ineffectiveness. Studies found that cryopreserved human MSCs started a heat shock program within 4 hours post-thaw, and significantly reduced the immunosuppressive properties that depend on indoleamine2,3-dioxygenase (IDO)[30], and its metabolic activity was also significant decrease[31]. Compared with fresh prepared MSCs, the cytoskeleton protein F-actin was reduced by 60% in cryopreserved MSCs, and its ability to bind to human endothelial cells was reduced by 80%, and its survival time after infused into mice was significantly shortened[32]. However, this decline in immunosuppressive ability post-thaw can be recovered by culturing in vitro for 24 hours[30]. But it was still difficult to predict whether this function recovery can be carried out in vivo, because even “live” MSCs may be eliminated due to abnormal metabolic function[33]. Therefore, we speculate that patients in the MSC-2 group did not show efficacy, which may be caused by the infusion of cryopreserved MSCs with decreased activity. This also suggests that in future clinical applications, cryopreserved stem cells need to be cultured for 24–48 hours pre-transplantation to restore their optimal immune regulation and biological activity.

Furthermore, as Killer et al. reported[31], the immunosuppressive ability of the same batch of MSCs to T cells from different donors was quite different. Therefore, the individual differences in severe COVID-19 patients may also be the cause for the difference in efficacy between the MSC-1 and MSC-2 groups. The influence of such factors can be reduced by expanding the sample size.

AI-assisted evaluation of pneumonia lesions has been reported in a large number of literatures[34–37]. The currently accepted conclusion is that the accuracy of AI used to evaluate COVID-19 is no worse than manual evaluation, and the influence of artificial subjective factors is excluded[38]. These previous studies, especially our research aimed to make a comparison of the absorption of lung lesions before and after treatment, indicate that AI-assisted evaluation was more comparable. We believe that the results of AI evaluation were more reliable than manual evaluation, so we did not compare the differences between AI and manual evaluation.

The pathological changes of pulmonary fibrosis and the absorption of fibrotic lesions require a long-term process. Whether UC-MSCs treatment has a positive effect on slowing down the development of pulmonary fibrosis in patients still needs long-term observation. Therefore, we are continuing to follow up these patients.

Limitations of this study: 1, the research design did not strictly follow the 1:1 allocation of the UC-MSC group and the control group, resulting in fewer cases in the MSC-1 group and the control group. 2, only the imaging results of the follow-up patients were analyzed, and the clinical laboratory and other indicators were not integrated. 3, there were only 3 cases of once infusion with cryopreserved UC-MSCs, therefore, it was impossible to make further statistical analysis on the difference between freshly prepared UC-MSCs and cryopreserved UC-MSCs in the absorption of lung lesions. Our future research will expand the sample size, implement a strict randomized controlled design, and combine multiple indicators to evaluate the safety and efficacy of UC-MSCs in the treatment of COVID-19.

Conclusion

For severe COVID-19 patients, the infusion of UC-MSCs can improve the absorption of lung lesions with no serious adverse events. AI is more suitable for the comparative evaluation of CT lung lesions before and after treatment.

Abbreviations

COVID-19: Coronavirus disease 2019; UC-MSCs: umbilical cord mesenchymal stem cells; CT: Computed tomography; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; ICU: Intensive care units; AI: artificial intelligence; PaO2: arterial oxygen partial pressure; FiO2fraction of inspiration O2; GGO: ground-glass opacity.

Declarations

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Authors' contributions

H., J.L., X.W., Z.L., S.G., L.H., Y.S. and L.Q., collection and/or assembly of data, data analysis and interpretation. H.Z. and J.L., manuscript writing. D.H. and L.Z., data analysis and interpretation. Z.L., L.Q., S.C., E.J. and W.J., conducting experiments, conception and design, data analysis and interpretation, administrative and financial support. The authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Ethics approval and consent to participate

We obtained written informed consent from the conscious patients or first of kin of the unconscious patients. All procedures were conducted in accordance with the relevant approved regulations, guidelines, and the Declaration of Helsinki. The Ethical Committee of Shanghai East Hospital (EC.D(BG).020.02.0, and EC.D(BG).016.01.1) approved this study.

Consent for publication

Not applicable.

Competing interests

The other authors have no conflict of interest.

References

1. Zu ZY, Jiang MD, Xu PP, Chen W, Ni QQ, Lu GM, Zhang LJ: **Coronavirus Disease 2019 (COVID-19): A Perspective from China**. *Radiology* 2020, **296**(2):E15-E25.
2. Sun P, Lu X, Xu C, Sun W, Pan B: **Understanding of COVID-19 based on current evidence**. *J Med Virol* 2020, **92**(6):548-551.
3. Wiersinga WJ, Rhodes A, Cheng AC, Peacock SJ, Prescott HC: **Pathophysiology, Transmission, Diagnosis, and Treatment of Coronavirus Disease 2019 (COVID-19): A Review**. *JAMA* 2020, **324**(8):782-793.
4. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, Wang B, Xiang H, Cheng Z, Xiong Y *et al*: **Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China**. *JAMA* 2020, **323**(11):1061-1069.
5. Du Y, Tu L, Zhu P, Mu M, Wang R, Yang P, Wang X, Hu C, Ping R, Hu P *et al*: **Clinical Features of 85 Fatal Cases of COVID-19 from Wuhan. A Retrospective Observational Study**. *Am J Respir Crit Care Med* 2020, **201**(11):1372-1379.
6. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, Qiu Y, Wang J, Liu Y, Wei Y *et al*: **Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study**. *Lancet* 2020, **395**(10223):507-513.
7. George PM, Wells AU, Jenkins RG: **Pulmonary fibrosis and COVID-19: the potential role for antifibrotic therapy**. *Lancet Respir Med* 2020, **8**(8):807-815.
8. Ojo AS, Balogun SA, Williams OT, Ojo OS: **Pulmonary Fibrosis in COVID-19 Survivors: Predictive Factors and Risk Reduction Strategies**. *Pulm Med* 2020, **2020**:6175964.
9. Schwensen HF, Borreschmidt LK, Storgaard M, Redsted S, Christensen S, Madsen LB: **Fatal pulmonary fibrosis: a post-COVID-19 autopsy case**. *J Clin Pathol* 2020.
10. Vasarmidi E, Tsitoura E, Spandidos DA, Tzanakis N, Antoniou KM: **Pulmonary fibrosis in the aftermath of the COVID-19 era (Review)**. *Exp Ther Med* 2020, **20**(3):2557-2560.
11. Wynn TA: **Integrating mechanisms of pulmonary fibrosis**. *J Exp Med* 2011, **208**(7):1339-1350.
12. Beigel JH, Tomashek KM, Dodd LE: **Remdesivir for the Treatment of Covid-19 - Preliminary Report. Reply**. *N Engl J Med* 2020, **383**(10):994.
13. Group RC, Horby P, Lim WS, Emberson JR, Mafham M, Bell JL, Linsell L, Staplin N, Brightling C, Ustianowski A *et al*: **Dexamethasone in Hospitalized Patients with Covid-19**. *N Engl J Med* 2021, **384**(8):693-704.
14. Djouad F, Charbonnier LM, Bouffi C, Louis-Plence P, Bony C, Apparailly F, Cantos C, Jorgensen C, Noel D: **Mesenchymal stem cells inhibit the differentiation of dendritic cells through an interleukin-6-dependent mechanism**. *Stem Cells* 2007, **25**(8):2025-2032.
15. Du YY, Zhou SH, Zhou T, Su H, Pan HW, Du WH, Liu B, Liu QM: **Immuno-inflammatory regulation effect of mesenchymal stem cell transplantation in a rat model of myocardial infarction**. *Cytotherapy* 2008, **10**(5):469-478.
16. Li T, Yan Y, Wang B, Qian H, Zhang X, Shen L, Wang M, Zhou Y, Zhu W, Li W *et al*: **Exosomes derived from human umbilical cord mesenchymal stem cells alleviate liver fibrosis**. *Stem Cells Dev* 2013, **22**(6):845-854.

17. Ortiz LA, Gambelli F, McBride C, Gaupp D, Baddoo M, Kaminski N, Phinney DG: **Mesenchymal stem cell engraftment in lung is enhanced in response to bleomycin exposure and ameliorates its fibrotic effects**. *Proc Natl Acad Sci U S A* 2003, **100**(14):8407-8411.
18. Yagi H, Soto-Gutierrez A, Parekkadan B, Kitagawa Y, Tompkins RG, Kobayashi N, Yarmush ML: **Mesenchymal stem cells: Mechanisms of immunomodulation and homing**. *Cell Transplant* 2010, **19**(6):667-679.
19. Yang SH, Park MJ, Yoon IH, Kim SY, Hong SH, Shin JY, Nam HY, Kim YH, Kim B, Park CG: **Soluble mediators from mesenchymal stem cells suppress T cell proliferation by inducing IL-10**. *Exp Mol Med* 2009, **41**(5):315-324.
20. Jacobs C, van Rikxoort EM, Twellmann T, Scholten ET, de Jong PA, Kuhnigk JM, Oudkerk M, de Koning HJ, Prokop M, Schaefer-Prokop C *et al*: **Automatic detection of subsolid pulmonary nodules in thoracic computed tomography images**. *Med Image Anal* 2014, **18**(2):374-384.
21. Kauczor HU, Heitmann K, Heussel CP, Marwede D, Uthmann T, Thelen M: **Automatic detection and quantification of ground-glass opacities on high-resolution CT using multiple neural networks: comparison with a density mask**. *AJR Am J Roentgenol* 2000, **175**(5):1329-1334.
22. Giamarellos-Bourboulis EJ, Netea MG, Rovina N, Akinosoglou K, Antoniadou A, Antonakos N, Damoraki G, Gkavogianni T, Adami ME, Katsaounou P *et al*: **Complex Immune Dysregulation in COVID-19 Patients with Severe Respiratory Failure**. *Cell Host Microbe* 2020, **27**(6):992-1000 e1003.
23. Harrison C: **Focus shifts to antibody cocktails for COVID-19 cytokine storm**. *Nat Biotechnol* 2020, **38**(8):905-908.
24. Teuwen LA, Geldhof V, Pasut A, Carmeliet P: **COVID-19: the vasculature unleashed**. *Nat Rev Immunol* 2020, **20**(7):389-391.
25. Zhang Y, Ding J, Ren S, Wang W, Yang Y, Li S, Meng M, Wu T, Liu D, Tian S *et al*: **Intravenous infusion of human umbilical cord Wharton's jelly-derived mesenchymal stem cells as a potential treatment for patients with COVID-19 pneumonia**. *Stem Cell Res Ther* 2020, **11**(1):207.
26. Leng Z, Zhu R, Hou W, Feng Y, Yang Y, Han Q, Shan G, Meng F, Du D, Wang S *et al*: **Transplantation of ACE2(-) Mesenchymal Stem Cells Improves the Outcome of Patients with COVID-19 Pneumonia**. *Aging Dis* 2020, **11**(2):216-228.
27. Meng F, Xu R, Wang S, Xu Z, Zhang C, Li Y, Yang T, Shi L, Fu J, Jiang T *et al*: **Human umbilical cord-derived mesenchymal stem cell therapy in patients with COVID-19: a phase 1 clinical trial**. *Signal Transduct Target Ther* 2020, **5**(1):172.
28. Kebriaei P, Hayes J, Daly A, Uberti J, Marks DI, Soiffer R, Waller EK, Burke E, Skerrett D, Shpall E *et al*: **A Phase 3 Randomized Study of Remestemcel-L versus Placebo Added to Second-Line Therapy in Patients with Steroid-Refractory Acute Graft-versus-Host Disease**. *Biol Blood Marrow Transplant* 2020, **26**(5):835-844.
29. Galipeau J: **The mesenchymal stromal cells dilemma–does a negative phase III trial of random donor mesenchymal stromal cells in steroid-resistant graft-versus-host disease represent a death knell or a bump in the road?** *Cytotherapy* 2013, **15**(1):2-8.
30. Francois M, Copland IB, Yuan S, Romieu-Mourez R, Waller EK, Galipeau J: **Cryopreserved mesenchymal stromal cells display impaired immunosuppressive properties as a result of heat-shock response and impaired interferon-gamma licensing**. *Cytotherapy* 2012, **14**(2):147-152.
31. Killer MC, Nold P, Henkenius K, Fritz L, Riedlinger T, Barckhausen C, Frech M, Hackstein H, Neubauer A, Brendel C: **Immunosuppressive capacity of mesenchymal stem cells correlates with metabolic activity and can be enhanced by valproic acid**. *Stem Cell Res Ther* 2017, **8**(1):100.
32. Chinnadurai R, Garcia MA, Sakurai Y, Lam WA, Kirk AD, Galipeau J, Copland IB: **Actin cytoskeletal disruption following cryopreservation alters the biodistribution of human mesenchymal stromal cells in vivo**. *Stem Cell Reports* 2014, **3**(1):60-72.

33. Copland IB, Galipeau J: **Death and inflammation following somatic cell transplantation**. *Semin Immunopathol* 2011, **33**(6):535-550.
34. Li Z, Zhong Z, Li Y, Zhang T, Gao L, Jin D, Sun Y, Ye X, Yu L, Hu Z *et al*: **From community-acquired pneumonia to COVID-19: a deep learning-based method for quantitative analysis of COVID-19 on thick-section CT scans**. *Eur Radiol* 2020, **30**(12):6828-6837.
35. Mansoor A, Bagci U, Foster B, Xu Z, Papadakis GZ, Folio LR, Udupa JK, Mollura DJ: **Segmentation and Image Analysis of Abnormal Lungs at CT: Current Approaches, Challenges, and Future Trends**. *Radiographics* 2015, **35**(4):1056-1076.
36. Wang YH, Cao ZY, Li QH, Lin GQ, Zhou J, Tian P: **Activating Pronucleophiles with High pKa Values: Chiral Organo-Superbases**. *Angew Chem Int Ed Engl* 2020, **59**(21):8004-8014.
37. Xu Y, Ma L, Yang F, Chen Y, Ma K, Yang J, Yang X, Chen Y, Shu C, Fan Z *et al*: **A collaborative online AI engine for CT-based COVID-19 diagnosis**. *medRxiv* 2020.
38. Harmon SA, Sanford TH, Xu S, Turkbey EB, Roth H, Xu Z, Yang D, Myronenko A, Anderson V, Amalou A *et al*: **Artificial intelligence for the detection of COVID-19 pneumonia on chest CT using multinational datasets**. *Nat Commun* 2020, **11**(1):4080.

Tables

Table 1. Clinical Characteristics of 46 Patients with COVID-19 on Admission

	Control（n=9）	MSC-1（n=11）	MSC-2 (n=26)	*P* value
Age (years), Median (IQR)	66.9±7.3	65.9±6.7	66.5±10.1	0.969
Male gender, no.(%)	7(77.8)	3(27.3)	20(76.9)	0.250
Course of disease (days), Median (IQR)	40.0(33.0-45.0)	35.0(33.0-45.0)	35.0(30.0-42.0)	0.474
Comorbidity, no.(%)				
Diabetes	2(22.2)	1(11.1)	3(13.0)	0.627
Hypertension	3(33.3)	3(33.3)	9(39.1)	0.722
Initial symptom, no.(%)				
Cough	5(55.6)	5(55.6)	16(69.6)	0.388
Fatigue	5(55.6)	1(11.1)	8(34.8)	0.651
Fever (>37.3°)	7(77.8)	4(44.4)	18(78.3)	0.489
Laboratory findings, Median (IQR)				
White-cell count(×10^9/L)	5.24(4.1-6.2)	6.00(5.4-7.3)	6.20(5.3-7.2)	0.36
Lymohocyte count(×10^9/L)	1.26(0.9-1.8)	1.80(1.39-2.31)	1.35(0.9-2.0)	0.054
Monocyte count(×10^9/L)	0.54(0.5-0.6)	0.43(0.3-0.6)	0.61(0.4-0.7)	0.292
Neutrophil count(×10^9/L)	2.90(2.2-5.8)	3.54(3.3-4.2)	3.76(2.7-4.7)	0.982
C-reactionprotein(mg/L)	3.14(1.8-13.2)	2.16(1.4-4.2)	3.96(1.7-12.5)	0.455
D-dimer quantification(mg/L)	0.27(0.02-0.60)	0.42(0.2-0.9)	0.44(0.2-0.6)	0.580
PaO2(mmHg)	106.00(83.0-152.0)	89.00(85.8-130.8)	103.50(70.5-129.0)	0.977
PaCo2(mmHg)	41.60(38.9-47.7)	49.00(42.1-52.0)	42.50(36.8-48.6)	0.295
mMRC	3.10(3.0-3.73)	3.00(2.9-3.2)	3.35(3.1-3.5)	0.079
SleepScore	13.00(12.0-14.5)*	15.00(15.0-18.0)*Δ	14.00(13.0-16.0) Δ	0.006
FatigueScore	24.00(20.0-26.0)#	23.00(19.0-26.0) Δ	19.00(15.0-22.0)# Δ	0.027

Pairwise comparison between different groups LSD test：*P＜0.05 control vs MSC-1, #P＜0.05 control vs MSC-2，ΔP＜0.05 MSC-1 vs MSC-2

Table 2. CT Characteristics of 46 Patients with COVID-19 on Admission

	Control (n=9)	MSC-1 (n=11)	MSC-2 (n=26)	*P* value
The lesion distribution				
Centrilobular type	4(44.4)	2(22.2)	8(34.8)	0.857
Subpleural type	0(0.0)	0(0.0)	1(4.3)	0.403
Diffuse type	5(55.6)	7(77.8)	14(60.9)	0.929
CT findings				
Architectural distortion, no.(%)	8(26.7)	6(20.0)	16(53.3)	0.400
Halosign, no.(%)	4(44.4)	1(11.1)	9(39.13)	0.245
Reversed halosign, no.(%)	2(22.2)	2(22.2)	4(17.4)	0.654
Plural effusion, no.(%)	2(22.2)	1(6.3)	0(0)	0.120
Traction bronchiolectasis,no.(%)	9(100.0)	8(88.9)	15(65.2)	0.069
Reticular pattern,no.(%)	6(66.7)	4(44.4)	13(56.1)	0.636
Processing data based on artificial intelligence				
Total lung volume (cm), Median (IQR)	3132.0(2695.0-3875.5)	2707.0(2422.0-3377.0)	3352.0(2720.3-4083.5)	0.190
Percentage of total lung infection(%), Median (IQR)	24.5(19.5-35.6)	29.9(21.7-54.8)	29.9(11.0-45.2)	0.637
Percentage of Consolidation(%), Median (IQR)	4.6(2.6-8.5)	2.8(0.8-6.0)	2.8(1.1-7.4)	0.361
Percentage of GGO (%), Median (IQR)	14.6(10.2-21.3)	20.0(9.0-34.7)	14.8(6.2-30.3)	0.654

Table 3. Comparison of CT image changes of COVID-19 patients within 30-90 days of follow-up

		Follow up			
		30 days	60 days	90 days	Total
GGO Percentage Difference	Control	-1.0(-7.4~0.1)*	-4.1(-10.3~-1.3)	-8.0(-11.8~-5.0)*	-12.0(-36.4~-8.6)
	MSC-1	-20.4(-25.7~-5.1)*#	-10.6(-28.3~-6.4)	-17.7(-35.9~-8.8)*#	-45.4(-79.8~-12.9)#
	MSC-2	-3.1(-4.7~0.5)#	-5.4(-13.7~-2.6)	-5.3(-13.75~-3.1)#	-11.0(-33.0~-6.6)#
	Total	-7.3(-10.7~-3.9)●	-9.5(-13.3~-5.6)□	-11.0(-15.2~-6.8)●□	
	HF coefficient		0.001		
	Intergroup F, *P*		3.286, 0.058		
	Time F, *P*		4.284, 0.047		
	Interaction F, *P*		0.180, 0.855		
Consolidation Percentage Difference	Control	-2.9(-4.5~-0.9)Δ	-3.6(-7.4~-1.3)	-3.8(-7.8~-1.6)	-8.6(-13.3~-2.5)
	MSC-1	-1.9(-2.5~-0.5)	-2.4(-5.5~-2.4)	-2.5(-5.0~-1.0)	-3.9(-6.3~-1.6)
	MSC-2	-0.9(-1.6~-0.5)Δ	-1.2(-3.8~-0.8)	-1.0(-2.4~-0.6)	-2.7(-4.75~-1.7)
	Total	-1.7(-2.5~-0.9)	-1.9(-2.9~-0.9)	-2.0(-3.0~-0.9)	
	HF coefficient		0.001		
	Intergroup F, *P*		1.048, 0.369		
	Time F, *P*		1.059, 0.329		
	Interaction F, *P*		0.136, 0.909		
Total infection lung Percentage Difference	Control	-1.8(-8.2~5.9)*	-3.2(-14.0~1.3)*	-7.6(-15.2~-4.7)*	-7.3(-51.9~0.5)*
	MSC-1	-20.5(-37.1~-11.1)*#	-21.1(-45.4~-9.6)*	-25.6(-52.4~-19.4)*#	-69.7(-122.9~-27.5)*#
	MSC-2	-4.1(-9.9~-1.0)#	-8.1(-25.7~-3.0)	-11.5(-27.1~-5.2)#	-20.3(-43.1~-12.3)#
	Total	-10.3(-15.9~-4.8)●□	-14.7(-21.0~-8.4)□□	-17.6(-23.9~-11.3)●□□	
	HF coefficient		0.001		
	Intergroup F, *P*		2.746, 0.087		
	Time F, *P*		9.808, 0.002		
	Interaction F, *P*		0.444, 0.696		

Pairwise comparison between different groups at the same time LSD test□*P□0.05 MSC-1 vs control, #P□0.05 MSC-1 vs MSC-2□ΔP□0.05 MSC-2 vs control. Pairwise comparison between different time LSD test □●P□0.05 D30 vs D90□□P□0.05 D60 vs D90□□P□0.05 D30 vs D60

Table 4. Comparison of CT image changes of COVID-19 patients within 60-90 days of follow-up

		Follow up		
		60 days	90 days	Total
GGO Percentage Difference	Control	-4.1(-10.3~-1.3)	-8.0(-11.8~-5.0)*	-12.4(-21.9~-6.3)*
	MSC -1	-10.6(-28.3~-6.4)	-17.7(-35.9~-8.8)*#	-24.9(-60~-17.3)*#
	MSC-2	-5.4(-13.7~-2.6)	-5.3(-13.75~-3.1)#	-10.3(-24.3~-5.0)#
	Total	-9.5(-13.3~-5.6)□	-11.0(-15.2~-6.8)□	
	HF coefficient		0.001	
	Intergroup F, *P*		3.842, 0.034	
	Time F, *P*		12.872, 0.001	
	Interaction F, *P*		1.782, 0.187	
Consolidation Percentage Difference	Control	-3.6(-7.4~-1.3)	-3.8(-7.8~-1.6)	-7.4(-15.2~-2.9)
	MSC-1	-2.4(-5.5~-2.4)	-2.5(-5.0~-1.0)	-4.3(-10.9~-1.2)
	MSC-2	-1.2(-3.8~-0.8)	-1.0(-2.4~-0.6)	-3.8(-7.8~-1.6)
	Total	-1.9(-2.9~-0.9)	-2.0(-3.0~-0.9)	
	HF coefficient		0.001	
	Intergroup F, *P*		1.992, 0.156	
	Time F, *P*		2.494, 0.126	
	Interaction F, *P*		0.728, 0.197	
Total infection lung Percentage Difference	Control	-3.2(-14.0~1.3)*	-7.6(-15.2~-4.7)*	-8.8(-29.1~-5.3)*
	MSC-1	-21.1(-45.4~-9.6)*	-25.6(-52.4~-19.4)*#	-50.4(-103.5~-36.3)*#
	MSC-2	-8.1(-25.7~-3.0)	-11.5(-27.1~-5.2)#	-21.6(-49.0~-6.8)#
	Total	-14.7(-21.0~-8.4)□	-17.6(-23.9~-11.3)□	
	HF coefficient		0.001	
	Intergroup F, *P*		4.699, 0.018	
	Time F, *P*		14.749, 0.001	
	Interaction F, *P*		0.836, 0.444	

Pairwise comparison between different groups at the same time LSD test: *P□0.05 MSC-1 vs control, #P□0.05 MSC-1 vs MSC-2, ΔP□0.05 MSC-2 vs control. Pairwise comparison between different time LSD test: □P□0.05 D60 vs D90.

Table 5. Comparison of CT structure changes of COVID-19 patients during follow-up 0-90 days

		Estimate	*P* value
Tractionbronchiolectasis	MSC Groups	1.37	0.06
	Time	0.89	0.000
Architectural distortion	MSC Groups	-0.96	0.16
	Time	-1.04	0.000
Reticular pattern	MSC Groups	1.09	0.14
	Time	0.53	0.000

Figures

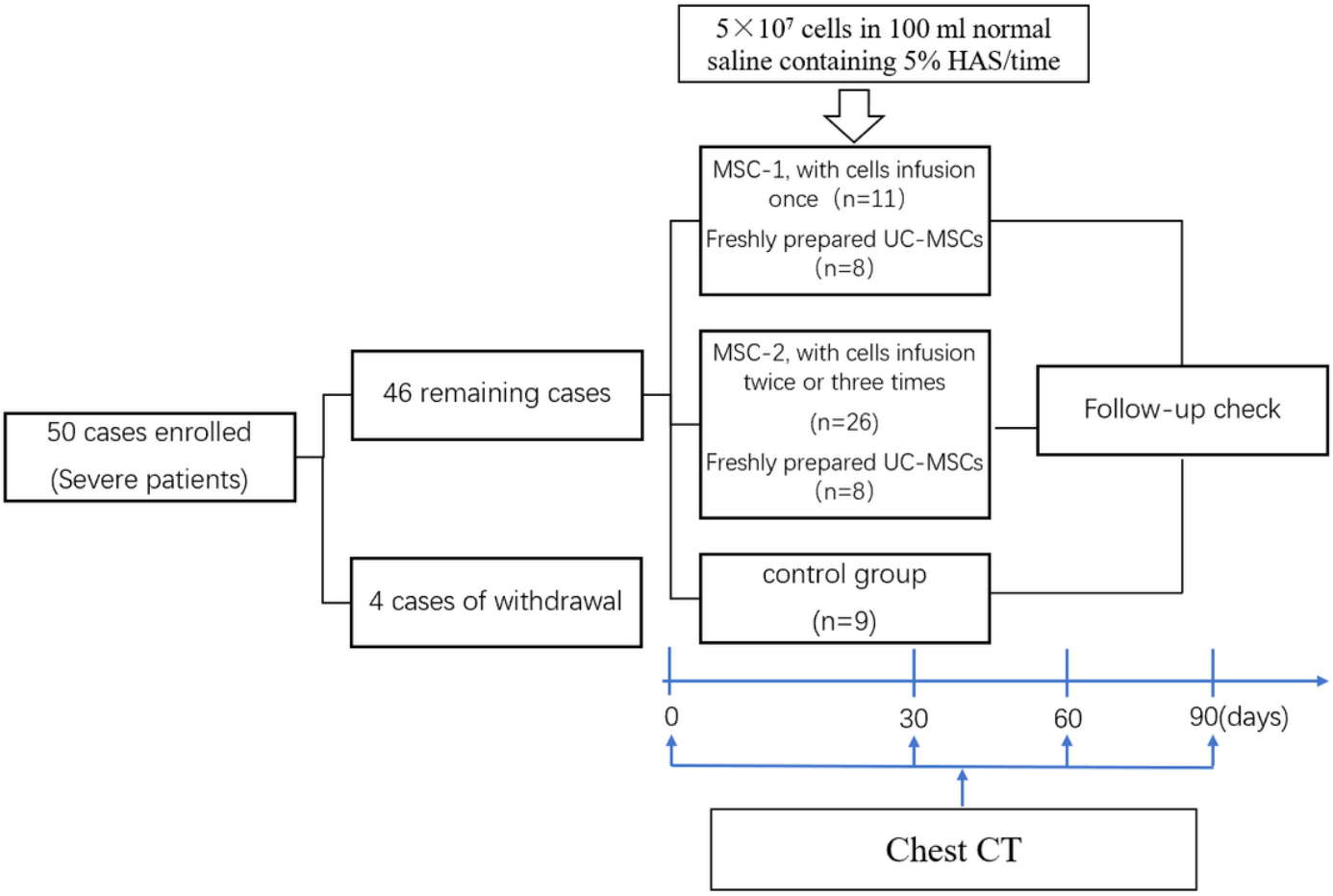

Figure 1

Structure and patient enrollment

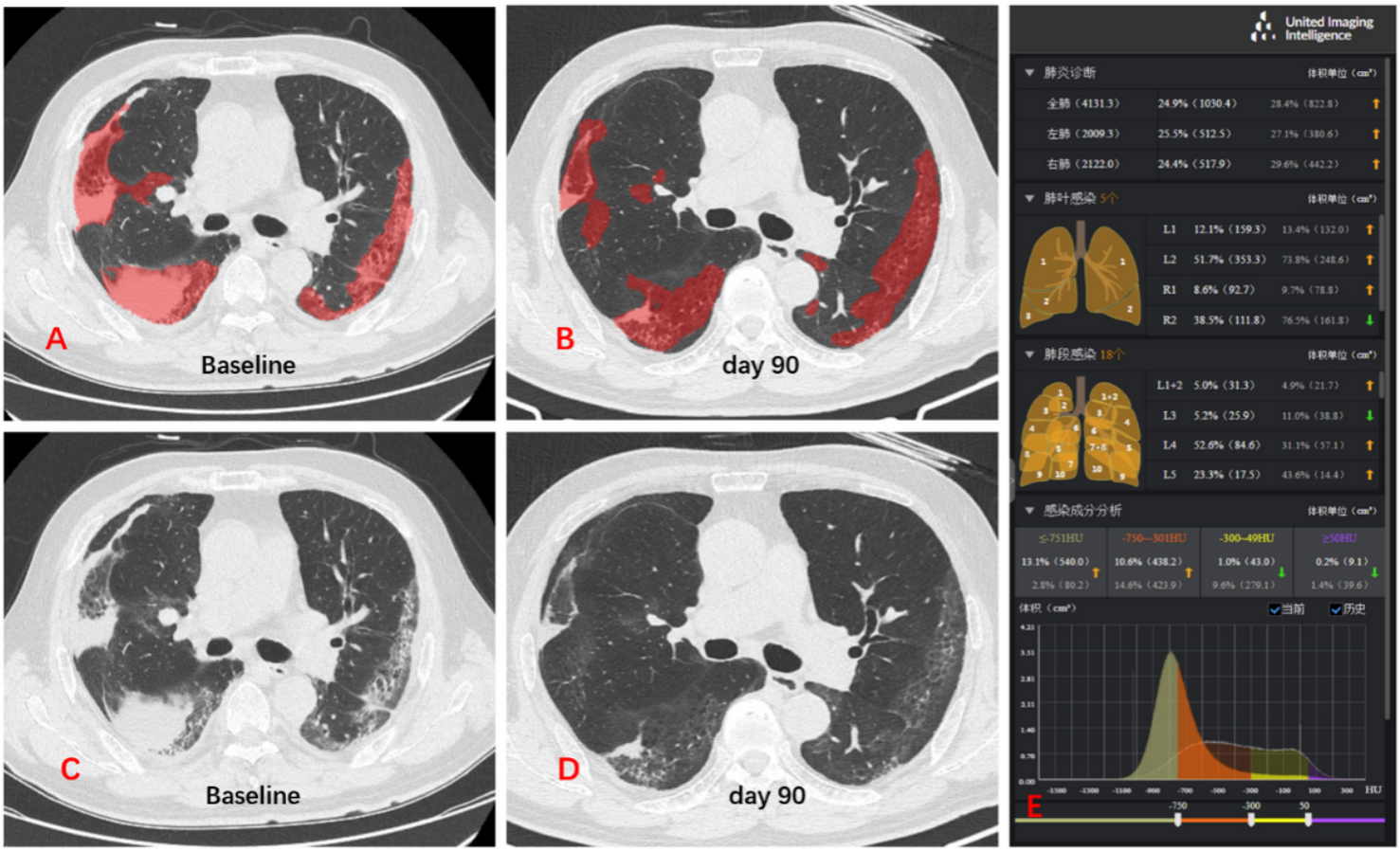

Figure 2

Chest CT scanned images of a 67-year-old men in the control group at D0 and D90. A&B, the red area was the infection area automatically identified by AI software. C&D are original images of A and B respectively indicated that the infection areas identified by AI software were quite accurate. E, the change trend of the percentage of lung infection in the two examinations at D0 and D90.

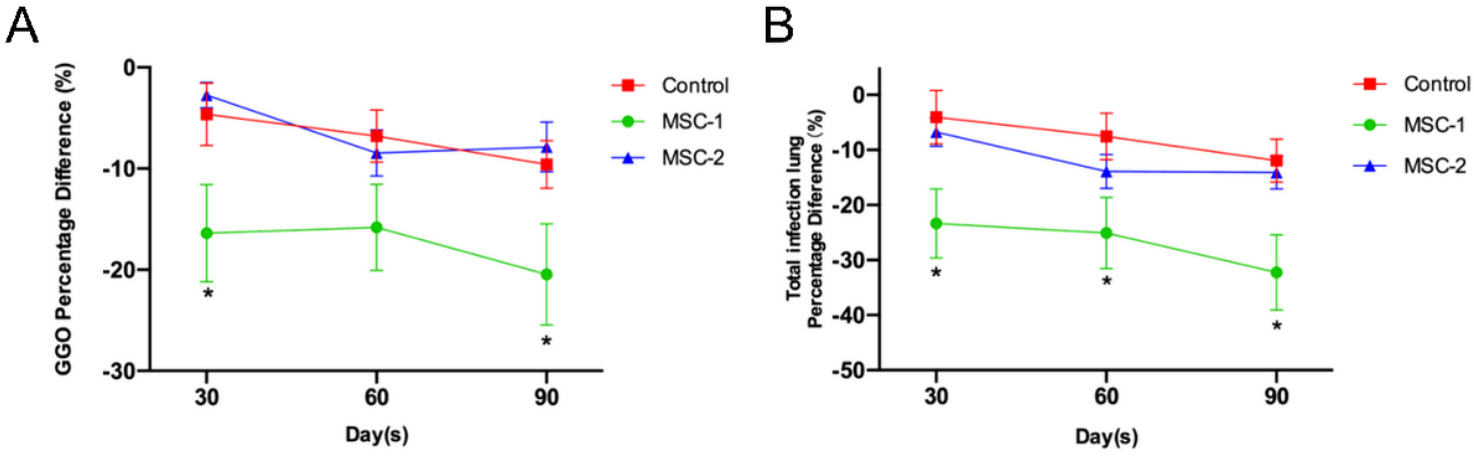

Figure 3

Line chart of the lung lesion percentage difference. A, Total lung infection percentage difference (D30, D60, and D90 respectively minus D0); B, GGO percentage difference (D30, D60, and D90 respectively minus D0). *P□0.05 vs. Control.