Safety and Long-term Improvement of Mesenchymal Stromal Cell Infusion in Critically COVID-19 Patients: A Randomized Clinical Trial.

Carmen Kuniyoshi Rebelatto (carmen.rebelatto@pucpr.br)
Pontifical Catholic University of Parana: Pontificia Universidade Catolica do Parana https://orcid.org/0000-0003-1407-0416
Alexandra Cristina Senegaglia
Pontifícia Universidade Católica do Paraná: Pontificia Universidade Catolica do Parana
Claudio Luciano Franck
Hospital de Clinicas
Debora Regina Daga
Pontifícia Universidade Católica do Paraná: Pontificia Universidade Catolica do Parana
Patrícia Shigunov
Fundacao Oswaldo Cruz
Marco Augusto Stimamiglio
Fundacao Oswaldo Cruz
Daniela Boscaro Marsaro
Pontifícia Universidade Católica do Paraná: Pontificia Universidade Catolica do Parana
Bruna Schaidt
Pontifícia Universidade Católica do Paraná: Pontificia Universidade Catolica do Parana
Andressa Micosky
Pontifícia Universidade Católica do Paraná: Pontificia Universidade Catolica do Parana
Ana Paula de Azambuja
Hospital de Clinicas
Cleverson Alex Leitão
Hospital de Clinicas
Ricardo Rasmussen Petterle
Universidade Federal do Paraná: Universidade Federal do Parana
Valderez Ravaglio Jamur
Pontifícia Universidade Católica do Paraná: Pontificia Universidade Catolica do Parana
Isadora May Vaz
Pontifícia Universidade Católica do Paraná: Pontificia Universidade Catolica do Parana
Antônio Paulo Mallmann
Hospital Maternidade Brígida
Hipólito Carraro Junior
Hospital de Clinicas
Eduardo Ditzel
Hospital Pilar
Paulo Roberto Slud Brofman
Pontifícia Universidade Católica do Paraná: Pontificia Universidade Catolica do Parana
Alejandro Correa
Pontifícia Universidade Católica do Paraná: Pontificia Universidade Catolica do Parana

Research Article

Keywords: COVID-19, Mesenchymal Stromal Cells, PostAcute Sequelae, Cell Therapy

Posted Date: December 30th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-1206247/v1

Version of Record: A version of this preprint was published at Stem Cell Research & Therapy on March 21st, 2022. See the published version at https://doi.org/10.1186/s13287-022-02796-1.

Abstract

Background: COVID-19 is a multisystem disease that presents acute and persistent symptoms, the PostAcute Sequelae (PASC). Long-term symptoms may be due to consequences from organ or tissue injury caused by SARS-CoV-2, associated clotting or inflammatory processes during acute COVID-19. Various strategies are being chosen for by clinicians to prevent severe cases of COVID-19; however, a single treatment would not be efficient in treating such a complex disease. Mesenchymal stromal cells (MSCs) are known for their immunomodulatory properties and regeneration ability; therefore, they are a promising tool for treating disorders involving immune dysregulation and extensive tissue damage, as is the case with COVID-19. T*his study aimed* to assess the safety and explore the long-term efficacy of three intravenous doses of UC-MSCs (umbilical cord-MSCs) as an adjunctive therapy in the recovery and postacute sequelae reduction caused by COVID-19. To our knowledge, this is the first report that presents the longest follow-up after MSC treatment in COVID-19 patients.

Methods: This was a phase I/II, prospective, single-center, randomized, double-blind, placebo-controlled clinical trial. Seventeen patients diagnosed with COVID-19 who require intensive care surveillance and invasive mechanical ventilation – critically ill patients – were included. The patient infusion was three doses of $5x10^5$ cells/kg UC-MSCs, with a dosing *interval* of 48 hours (n=11) or placebo (n=6). These evaluations consisted of a clinical assessment, viral load, laboratory testing, including blood count, serologic, biochemical, cell subpopulation, cytokines and CT scan.

Results: The results revealed that in the UC-MSC group, there was a reduction in the levels of ferritin, IL-6 and MCP1-CCL2 on the fourteen day. In the second month, a decrease in the levels of reactive C-protein, D-dimer, and neutrophils and an increase in the numbers of TCD3, TCD4 and NK lymphocytes were observed. A decrease in lung extension was observed at the fourth month. The improvement in all these parameters was maintained until the end of patient follow-up.

Conclusions: UC-MSCs infusion is safe and can play an important role as an adjunctive therapy, both in the early stages, preventing severe complications and in the chronic phase with postacute sequelae reduction in critically ill COVID-19 patients.

Trial registration: Brazilian Registry of Clinical Trials (ReBEC), UTN code **-** U1111-1254-9819. Registered 31 October 2020 - Retrospectively registered, https://ensaiosclinicos.gov.br/rg/RBR-3fz9yr

Introduction

COVID-19 has rapidly spread[1-4] and was officially declared a pandemic by the World Health Organization (WHO) in March 2020[4,5]. Initially, infection dissemination was related to the Wuhan seafood wholesale market, and by February 2020, the disease was already reported in the Western Pacific, Southeast Asia, Eastern Mediterranean, Europe and the Americas[1,4]. By July 2020, approximately 1.4 million cases were reported worldwide, while over 1 million were reported only in the Americas[4]. Today, the world has reported more than 190 million cases with over 4 million deaths[4].

Brazil has been intensively affected by the COVID-19 pandemic. A total of over 22 million cases(almost 10% of the total cases in the world) have already been reported, with daily new cases reaching 87,000[4]. The highest daily death report was on April 18th, 2021, with 4,195 deaths, and Brazil has arduously accumulated over 614,000 deaths until November 2021[4].

General mortality due to COVID-19 was initially reported at approximately 3.8%[1] but varies according to many factors, such as case definition[6], population immunization rate, access to health care, age, severity of disease and comorbidities[7], and according to different regions of the world[4]. From July to August 2020, period in which this study was carried out, the general mortality in the Americas varied between 2.66% and 3.09% among all reported cases[4], while in Paraná (Brazil), the municipality where the investigation has taken place, general mortality was reported to be 2.5%[8,9].

A systematic review and meta-analysis suggested that mortality among patients inan intensive care unit (ICU)was 41.6% anddecreased over time from above 50% in March to approximately 40% at the end of May 2020[10], suggesting thatimprovement of COVID-19 knowledge and better intensive care have a direct impact on survival. On the other hand admission to the ICU has been related to a lower case fatality rate, suggesting that mortality may be higher in the absence of intensive treatment[11]. Among patients admitted to the ICU, noninvasive mechanical ventilation and invasive mechanical ventilation (IMV) were both associated with higher mortalities, with statistically significant hazard ratios of 2.36 and 3.77, respectively[7].In Brazil, COVID-19 mortality among patients admitted to hospital care was 24.4%, and approximately 55,7% of the hospitalized patients needed intensive care[12]. Patients admitted to the ICU and on MVin Brazil were shown to have higher mortality rates, as 28-day mortality has been reported to range from 56.3% to 61.5%[13]. Age, cardiovascular disease, neurological disorders and pneumopathies were related to a higher probability of death[14].

COVID-19 is a multisystromal disease, and the pathological features at the beginning are fever, cough, and headache. The second phase exhibits high-grade fever, difficulty breathing, and pneumonia-like symptoms[15]. The progression to the third stage is mediated by inflammatory cytokines and chemokines and massive infiltration of inflammatory cells, causing cytokine release syndrome (CRS). This syndrome induces pulmonary edema, dysfunction of air exchange, severe acute respiratory distress syndrome (SARS), vascular dysfunction through microvascular thrombosis[16], tissue injury in various organs, secondary infections, and multiple organ failure (MOD), which ultimately lead to death[17,18].

Currently, one of the major concerns isthe persistent symptoms after apparent resolution from COVID-19. Patients who develop chronic symptoms after acute COVID-19 are being given the diagnosis Long COVID or Postacute sequelae of COVID-19 (PASC)[19]. Long-term symptoms may be due to consequences from organ or tissue injury caused by SARS-CoV-2 or associated clotting or inflammatory processes during acute COVID-19[20]. PASC prevalence is high, although it diverges in different studies and represents very significant public health and economic consequences[19,21-24]. The most common symptoms were fatigue, shortness of breath, brain fog, stress/anxiety and neurological complications[23,25].

The dynamic equilibrium maintained by innate and adaptive immunity is essential to avoid the progression of COVID-19[26]. The formation of an appropriate innate immune response in the early stages of the disease, followed by an effective adaptive immune response, limits the virus's progression and prevents tissue damage. In patients infected with SARS-CoV-2, the plasma levels of inflammatory cytokines increase[27]. In contrast, there is a significant decrease in the total number of T cells, T helper (CD4) and cytotoxic suppressor (CD8) T cells, NK cells and regulatory T cells, compromising the immune systromal[28]. Reduced expression of memory T cells may be a plausible explanation for the increased reinfection rates by SARS-CoV-2[29].

Various treatment strategies are being chosenfor by clinicians to combat this disease; however, so far, no definite therapy has been proven to completely control the cytokine storm and to restore the organ damage caused by COVID-19 infection. PASC patients who develop chronic symptoms after hospitalization for acute COVID-19 may be more likely to suffer from injury to one or more body sites[30]. Hence, different therapeutic approaches for COVID-19 should not only eliminate the virus and treat CRS but also accelerate recovery and minimize chronic symptoms.

Mesenchymal stromal cells (MSCs) are known for their immunomodulatory properties that occur directly via interaction with host immune cells or indirectly through paracrine secretion of various cytokines[31,32]. Additionally, these cells can produce an antimicrobial effect[33,34], antiapoptotic effect and regeneration ability. MSCs can be more beneficial than other anti-inflammatory agents because they can provide immunomodulatory effects based on host cells. In addition, MSCs can reduce inflammation, prevent fibrosis of tissues, enable reversal of lung dysfunction, protect and repair alveolar cells, and aid in the regeneration of damaged tissue, which can be significantly beneficial for COVID-19-associated organ[35,36]. MSCs do not express angiotensin-*converting*enzyme 2 (ACE2) and transmembrane serine protease 2 (TMPRSS2), which specifically recognize and bind with the spike (S) protein of SARS-CoV-2. Since the S protein plays an essential role in virus infection and transmission[37,38], MSCs can not be infected with SARS-CoV-2[39].

Umbilical cord MSCs (UC-MSCs) can be obtained using a noninvasive method; they are easily isolated and have great potential for cell expansion, and cells from young donors are less susceptible to oxidative damage[40,41]. Intravenously infused UC-MSCs enable metabolism's first-pass effect, where MSCs are entrapped in the lung vasculature[42,43]. Therefore, they may be effective in treating lung diseases.

MSCs are a promising tool for treating disorders involving immune dysregulation and extensive tissue damage, as is the case with COVID-19. Studies have shown that intravenous MSC infusion in patients with COVID-19 is safe and welltolerated. It prevents or reduces ARDS and other serious complications, decreases inflammatory cytokines and mortality, and in some cases improves pulmonary[39,44-56]. Therapeutic interventions must decrease excess inflammation, thus preventing end-organ damage and long-term functional disability in moderate or severe COVID-19 patients. MSCs, due to their immunomodulatory, anti-inflammatory and tissue regeneration potential, could be a new therapeutic strategy to treat critically ill COVID-19 patients and minimize sequelae symptoms.

T*hepresentstudy aimed* to assess the safety and explore the long-term efficacy of UC-MSCs infusion asan adjunctive therapy in the recovery and PASC reduction caused by COVID-19. The primary outcome of this study was the safety of allogenic UC-MSC infusion after the observation of infusional reactions and adverse events (AEs). The second outcome included patient recovery demonstrated through viral load, blood tests and plasma levels of inflammatory cytokines, peripheral blood*mononuclear*cell (PBMC) assessment of T cell populations and PASC reduction evaluated by biochemical markers and CT scan.

Material And Methods

Study Design and Patient Population

This study was a phase I/II, prospective, single-center, randomized, double-blind, placebo-controlled clinical trial. The subjects were recruited from the Complexo Hospital de Clínicas, Universidade Federal do Paraná, a referral public hospital for the treatment of patients with COVID-19, and UC-MSCs were processed at Cell Core Technology (CCT) from the Pontificia UniversidadeCatólica do Paraná (PUCPR). UCs were obtained from full-term neonates, and the mother of the donor signed an informed consent form approved by the institutional review board. Written informed consent was also obtained from the patients' parents for the collection and publication of clinical data (Figure 1). The experimental design was conducted in accordance with the Helsinki Declaration for human studies and was approved by the Ethics Committee (CAAE: 30833820.8.0000.0020). This study was registered inthe Brazilian Registry of Clinical Trials (ReBEC), UTN code - U1111-1254-9819.Registered 31 October 2020 - Retrospectivelyregistered, https://ensaiosclinicos.gov.br/rg/RBR-3fz9yr.

Patients over 18 years old, diagnosed with COVID-19 (as evaluated by reverse-transcription polymerase chain reaction (RT-PCR) test confirming infection with SARS-CoV-2), SARS associated-coronavirus, who require intensive care surveillance and IMV – critically ill patients (WHO ordinal scale score 6 and 7), arterial oxygen partial pressure (PaO_2)/oxygen absorption concentration (FiO_2) ≤ 300 mmHg, were eligible for inclusion. Exclusion criteria were: use of any investigational products, previous or current history of malignancy under treatment; pre-existing thromboembolic disease; concomitant infection of human immunodeficiency virus (HIV) or tuberculosis infection; pregnancy; pre-existing transplant or use of immunosuppressive therapy; inability to provide informed consent and greater than 72 hours of ICU admission.

The primary safety endpoints encompassed the occurrence of prespecified infusion-associated AEs within 24 hours after intravenous administration of UC-MSCs or placebo. According to the Common Terminology Criteria for Adverse Events version 5.0, it was assessed by recording all AEs based on duration, intensity, and possible association with the treatment under study. Investigators conducted assessments for the presence of any AEs from enrollment throughout the study.The secondary endpoint was exploratory efficacy defined by clinical outcomes, changes in viral load, inflammatory, immunological and biochemical biomarkers and acute lung injury (ALI) score.

Seventeen eligible patients were enrolled in an approximate 1:2 randomization to IV of three doses of $5x10^5$ cells/kg UC-MSCs,adosing*interval*of 48 hours (n=11), or placebo (n=6). Concomitant corticosteroids and anticoagulants were allowed, and conventional treatment was performed together with the infusion of cells during the study period. All patients were assessed at baseline and the pre-established follow-up points on days 2, 4, 6 and 14, as well as at 2 and 4months post-infusion. These evaluations consisted of a clinical assessment, viral load, laboratory testing, including blood count, serologic, biochemical, cell subpopulation, cytokine and CT scan evaluations.

Advanced therapy product

The study was conducted following the Good Clinical Practice Guidelines for Advanced Therapy Products (ATP) (RDC 508/2021). One week before UC collection, healthy donors provided written informed consent, and serology for infectious diseases and RT–PCR for COVID-19 were performed.

UCs were obtained from full-term newborns by cesarean section and they were aseptically stored in sterile Iscove'smodified Dulbecco's medium (IMDM) (Gibco BRL, Grand Island, NY) supplemented with 100 U/mL penicillin and 100 µg/mL streptomycin (Gibco BRL, Grand Island, NY). The umbilical cord was washed three times with phosphate buffered saline (PBS) (Gibco BRL, Grand Island, NY) and antibiotics, sectioned into small fragments (1–2 mm^2pieces), and centrifuged at 280 *g*for 10 minutes. After removing the supernatant fraction, the precipitate was washed with *IMDM*and centrifuged at 280 *g* for 10 minutes. The tissue was treated with 0.1% collagenase type II (Sigma, St Louis, MO, USA) at 37°C for 16 hours, washed, and further digested with 0.25% trypsin-EDTA (Gibco, Grand Island, NY, USA) at 37°C for 15 minutes. Fetal bovine serum (FBS) (HyClone™, South Logan, USA) was added to the MSCs to neutralize the excess trypsin[57].

Cells were plated in 75 cm^2 culture flasks (Greiner Bio-One, Kremsmünster, AT) with IMDM supplemented with 20% FBS, 100 U/mL penicillin, and 100 mg/mL streptomycin and *incubated*at 37°C and 5% CO_2. At 96 hours, nonadherent cells were removed and washed with PBS, and the culture medium was replaced with fresh medium every three days. When the cell culture reached 70% to 80% confluence, cells were detached by treatment with 0,25% trypsin-EDTA and replated at a density of 8,000 cells/cm^2into 150 cm^2culture flasks. At passage 3 (P3), cytogenetic analysis was performed. UC-MSCs were harvested and cryopreserved using a rate-controlled freezer at a final concentration of 10% d*imethyl sulfoxide* (Origen, Texas, USA) and 90% FBS. Four days before infusion, cells were thawed and replated at a density of 8,000 cells/cm^2into 150 cm^2culture flasks. When the number of cells was sufficient for administration, confluent UC-MSCs were detached with 0,25% trypsin-EDTA and washed twice with saline, and samples were collected for quality control. This control includes viability and cell surface markers by flow cytometry[58], cytogenetics analysis by GTG-banding method[59], microbiological tests (Bact/Alert 3D, Biomerieux, Durham, USA), endotoxin (Endosafe ™ PTS, Charles River, Charleston, USA) and Mycoplasma (KIT MycoAlert ™ PLUS Mycoplasma Detection, Lonza, Rockland, USA), accordingto the manufacturer's instructions. These quality control tests were performed before each batch of cells was released. For infusion, $5×10^5$UC-MSCs/kg of body weight were resuspended in a final volume of 30 mL of vehicle solution composed of saline (JP, Ribeirão Preto, Brazil), 5% *Anticoagulant*Citrate Dextrose (ACD) (JP, Ribeirão Preto, Brazil) and 20% albumin (BlauFarmacêutica, São Paulo, Brazil). The placebo group received a vehicle solution. Cells were infused between P3 and P5.

The release criteria for the clinical use of UC-MSCs included the absence of contamination with pathogenic microorganisms (bacteria, Mycoplasma and fungi) or endotoxin (≤0.5 EU/mL), cell viability ≥70%, identity and purity pattern characterized by positive (≥95%) of CD73, CD90, CD105 and CD29 and negative expression (≤2%) of CD45, CD34, CD14, CD19, and HLA-DR.

RNA extraction and RT–qPCR

RNA was extracted from 140 µl pulmonary aspirate samples from COVID-19 patients by using aQIAamp Viral RNA Kit (Qiagen, Hilden, Germany). RT–qPCR was performed using the GoTaq Probe 1-Step RT–qPCRSystromal (Promega, Madison, EUA)accordingto the manufacturer's instructions. A 10 µL reaction contained 2.5µL of total RNA, 5 µL of GoTaq® Probe qPCR Master Mix with dUTP (2X), 0,2 µl GoScript™ RT Mix for 1-Step RT–qPCR, 600 nMRdRp primer Forward, 800 nMRdRP primer Reverse, 100 nMRdRP probe and 50 nMRNApol probe and primer Forward and Reverse. The primer and probe sequences were as follows: (1) RdRP (NC_045512.2):RdRp_SARSr-F GTGARATGGTCATGTGTGGCGG; RdRp_SARSr-R CARATGTTAAASACACTATTAGCATA and RdRp_SARSr-P2 FAM-CAGGTGGAACCTCATCAGGAGATGC-BBQ[60]; (2) Homo sapiens RNA polymerase II subunit A (POLR2A) (NM_000937.5): RNA Pol-F TGGACAGGCAAGCAAATCTTC; RNA Pol-R AAGGGCCACTGTCTTCATCATC and RNA Pol-Probe Cy5-TACCCACAGCACCCATCCCGATG-BBQ. *R is G/A; S is G/C. FAM: 6-carboxyfluorescein; Cy-5: Cyanine-5; BBQ: blackberry quencher.The reactions were performed in triplicate using the LightCyclersystromal (Roche, Basiléia, Suíça), and thermal cycling was performed at 45°C for 15 minutes for reverse transcription, followed by 95°C for 3 minutes and then 45 cycles of 95 °C for 15 s and 58 °C for 45 s. For viral quantification analysis, the Cq results for the RdRP gene were normalized based on POLR2A quantity. The relative quantification of RdRP was calculated in relation to the preinfusion time of MSCs or placebo using ΔΔCq methods. The angular coefficient and R^2were established after linearizing the data (ln (x)) and obtaining the linear equation of each patient.

Multiparameter Flow Cytometry

Multiparametric flow cytometry was performed on all patients at baseline and on specific days after infusion (days 2, 4, 6 and 14, as well as at 2 and 4 months). Absolute leukocyte counts were performed using a Sysmex XN-3000 counter at the time of MFC analysis.

Comercial antibodies were used to analyze the expression of the cell surface markers CD3, CD4, CD8, CD19, CD38, CD127, CD25, and HLA-DR (Becton Dickinson, San Diego, USA). Immunophenotypic characterization of peripheral blood (PB) lymphocytes was performed with conventional staining sample preparation techniques according to Kalina et al.[61]. A total of 1,000,000 cells/events per tube were acquired using a FACSCanto II® flow cytometer (Becton Dickinson, Franklin Lakes, USA) and Infinicyt™ software (Cytognos, Salamanca, Spain—version 2.0) for flow cytometry analyses. The analysis protocol included removal of threshold debris, and lymphocytes were initially identified based on low frontal (FSC) and side scatter (SSC) and strong CD45 staining. The frequency and cell number of total, CD4+ and CD8+ T cells, as well as B (CD19+) and plasmablasts (CD19+CD38++) in patients, was determined using a Boolean strategy[62]. CD19-positive staining identified B cells, and strong CD38 positivity in B cells was used to identify the plasmablast population (CD19++CD38++). To identify T cell regulatory CD4 lymphocytes (Tregs),it was used CD25 positivity and CD127 negativity in the CD4 lymphocyte gate.

Analysis of inflammatory cytokines, chemokines, and growth factors in peripheral blood plasma

Blood samples were collected in EDTA Vacutainer® tubes (BD Biosciences, Curitiba, PR) and immediately transported to the laboratory for processing. Plasma was obtained by centrifugation (1,600 *g* for 15 minutes at 4°C), divided into aliquots and stored at −80°C until analysis.

The BD™ Cytometric Bead Array Systromal(CBA Flex Set Systromal,BD Biosciences, San Diego, EUA) was used todetermine plasma levels of a set of inflammatory cytokines, chemokines, and growth factors, including granulocyte-macrophage colony-stimulating factor(GM-CSF), interleukin(IL) -2, IL-6, IL-7, IL-8, tumor necrosis factor(TNF)α,monocyte chemoattractant protein-1 (MCP1/CCL2) and macrophage inflammatory protein 1-alpha (MIP1a/CCL3),according to the manufacturer's recommendations.The assay was performed at preinfusion and on days 2, 4, 6 and 14, as well as 2 and 4monthspost-infusion. All samples were measured in duplicate. Standard curves for each cytokine were generated using the premixed lyophilized standards provided in the kits. The cytokine concentrations in samples were determined by measuringtheir fluorescent intensities and referencingfrom the appropriate standard curve.Data were analyzed using the FlowJo™ v.10 software.

Image evaluation

All scans were obtained using a 64-row multidetector scanner (Toshiba Aquilion 64 TSX 101A, Tokyo, Japan). Chest CT evaluation was blinded and the following characteristics were assessed: ground glass opacities, linear opacities, consolidation, interlobular septal thickening, crazy-paving pattern, subpleural lines, bronchial wall thickening, lymph node enlargement and pleural effusion. Lesions were quantified by assigning a score to all abnormal areas involved[11]. Each lobe was assigned a score of 0 (0% involvement), 1 (1-25% involvement), 2 (26-50% involvement), 3 (51-75% involvement) or 4 (76%-100% involvement). The total score was the sum of all lobes, ranging from 0 to 25.

Statistics

In the descriptive analysis, it was used absolute and relative frequencies for categorical variables, and for quantitative variables,it was calculated the average values with theirrespective standard deviations. To investigate the change in response variable over time, it was used the framework of Gaussian copula marginal regression models[63]for longitudinal data analysis. First, it was select the probability distribution for the response variable from either Gaussian or gamma. Thereafter, it was select the available correlation structure: (i) independent, (ii) exchangeable, (ii) autoregressive of order 1 (AR1) and (iv) moving average of order 1 (MA1). Next, it was investigated the interaction effect between the group (placebo or UC-MSCs) and the evaluation times (time) that were treated as a factor with seven levels (baseline, 2, 4, 6 and 14 days, 2 and 4 months). When the interaction was not significant (p>0.05), it was used the additive effect on the linear predictor. On the other hand, when the interaction effect was significant, it was conducted a multiple comparison test, where the $p$values were obtained through Bonferroni correction[64]. It was adopted the Akaike (AIC) and Bayesian (BIC) information criteria and the maximized value of the log-likelihood function (logLik) to select the probability distribution for each response variable and the structure for the correlation matrix.The statistical data analysis was performed in R software version 4.0.3[65]using the R package GCMR[66].

Results

Advanced Therapy Product

In this study, samples from four UC donors were used, and all of them were negative for RT-*PCRtests*for*COVID*-19and serology for infectious diseases. In this clinical trial, fresh infused cells were used, and the interval between product release and patient IV infusion was up to three hours.

The ATP infused into the patients was negative for microbiological tests, and no clonal chromosomal abnormalities were observed. For each infusion, the average cell viability was 96.6±0.01, 95.4±0,03, and 95.5±0,02 for each infusion. Cell characterization was performed following the criteria defined byInternational Society for Cellular Therapy (ISCT) Guidelines (An additional movie file shows this in more detail [see Additional Table 1). UC-MSCs show the potential to differentiate into osteoblasts, adipocytes, and chondroblasts, and the immunomodulation potential is higher than 50% (Figure 2). The results from all evaluations were in conformation with those established by CCT/PUCPR.

Patient baseline characteristics and study population

A total of 17 patients were included in this study from 12 June to 13 July 2020. After randomization, 11 patients were included in the UC-MSC group, and 6 patients in the placebo group. In the UC-MSC group, one patient was excluded on the twelfth day because she did not undergo follow-up after cell infusion. At enrollment, all patients had ARDS and were in IMV in critical condition rated 6-7 on the WHO scale.

The average age of the UC-MSC group was 53±15.3 years, while the average age ofthe placebo group was 61,7±9.7 years. The baseline symptoms were fever, cough, nausea or vomiting, diarrhea, loss of taste or smell, shortness of breath, disorientation and confusion. No differences were observed when comparing the interval between symptom onset and hospital admission or the interval between symptom onset and first cell injection. Two patients in the UC-MSC group had no basic chronic diseases, and all other patients had comorbidities when they were admitted to thehospital, such asdiabetes, hypertension, kidney disease, chronic obstructive pulmonary disease, schizophrenia and obesity. The patients received standard treatment with anticoagulants,steroids, and antibiotics if there was evidence of bacteriological infection. Two patients from the UC-MSC group were treated with antiviral drugs as a concomitant treatment.Patient baseline characteristics are demonstrated in Table 1.

Table 1. Baseline Characteristics			
Characteristics		**UC-MSC (n=11)**	**Placebo (n=6)**
Age (years)	Mean ± SD	53±15.3	61.7±9.7
Gender, n (%)	Female	3 (27.2)	2 (33.3)
	Male	8 (72.7)	4 (66.6)
Symptoms, n (%)	Fever	8 (72.7)	5 (83.3)
	Cough	9 (81.8)	4 (66.6)
	Nauseaorvomiting	3 (27.3)	2 (33.3)
	Diarrhea	4 (36.4)	0 (0)
	Lossoftasteorsmell	4 (36.4)	3 (50)
	Shortnessofbreath	9 (81.8)	3 (50)
	Disorientationandconfusion	2 (18.2)	0 (0)
***Oxigenation index (PaO_2/FiO_2) atenrollment, n (%)**	Mild	4 (36.4)	5 (83.3)
	(200≥PaO_2/FiO_2≤300 mmHg)		
	Moderate	6 (54.5)	0 (0)
	(100≥PaO_2/FiO_2≤200 mmHg)		
	Severe	1 (9.1)	1 (16.6)
	(PaO_2/FiO_2≤100 mmHg)		
Intervalbetweensymptomsonsetand hospital admission (days)	Mean ± SD	6.8±3.2	8±1.6
Intervalbetweensymptomsonsetandfirstcellinjection (days)	Mean ± SD	10.7±3.9	12.1±2.2
Comorbidities, n (%)	Diabetes	4 (36.4)	3 (50)
	Hypertension	6 (54.5)	3 (50)
	Kidneydisease	1 (9.1)	0 (0)
	Chronicobstructivepulmonary	0 (0)	1 (16.7)
	disease		
	Schizophrenia	1 (9.1)	0 (0)
	Obesity (BMI>30)	6 (54.5)	3 (50)
Concomitattreatment, n (%)	Anticoagulant	11 (100)	6 (100)
	Steroids	11 (100)	6 (100)
	Antibiotics	2 (18.2)	1 (16.7)
	Antiviral drugs	2 (18.2)	0 (0)

Abbreviations: n, number; PaO_2, Arterial OxygenPartialPressure; FiO_2 ,OxygenAbsorptionConcentration; UC-MSC, Umbilical Cord MesenchymalStromalCell.

Laboratory tests, such as blood count and serologic, biochemical and cell subpopulations, were evaluated at baseline (Table 2).All these parameters were considered response variables in the statistical analysis(An additional movie file shows this in more detail [see Additional Tables 2 and 3).

Table 2. Patientlaboratoryfindings.				
Laboratorytests		**UC-MSC (n=11)**	**Placebo (n=6)**	***p*value**
BloodCount	Total lymphocyte (µL)	737±299.55	1652.33±2032.80	0.01
	Leukocytes (µL)	10959.73±4591.77	9605±3998.52	n.s.
	Neutrophils (µL)	9899.73±4354.47	8266±3671.02	n.s.
	Hemoglobin (µL)	13.35±1.69	12.68±2.56	n.s.
	Hematocrit (%)	41±5	38±7	n.s.
	Platelets (µL)	271690.91±75889.73	296500±54013.89	n.s.
Serologic	D-Dimer (mg/L)	7.75±10.81	6.45±4.83	n.s.
	C-Reactiveprotein (mg/dL)	38±81	9±5	n.s.
	Ferritin (ng/mL)	2760.53±3167.67	1600.38±1258.86	n.s.
	Troponin I (pg/mL)	218±655	132±209	n.s.
Biochemical	Creatinin (mg/dL)	2.07±1.71	1.92±1.84	n.s.
	ALT (µ/L)	49±40	29±14	n.s.
	AST (µ/L)	60±33	45±19	n.s.
	Total Bilirubin (mg/dL)	0.84±1.41	0.33±0.12	n.s.
	Direct bilirubin (mg/dL)	0.59±1.17	0.18±0.06	n.s.
	Indirectbilirubin (mg/dL)	0.24±0.28	0.15±0.10	n.s.
Mainlymphocytessubgroups	TCD3	242.17±176.44	471.90±205.33	0.02
	TCD4	195.120±94.84	346.06±194.50	0.01
	TCD8	105.83±85.91	107.06±41.42	n.s.
	B	90.47±53.31	143.56±146.16	n.s.
	NK	56.54±35.54	41.92±27.21	n.s.
	Treg	11.26±6.20	21.57±6.66	n.s.
	Plasmablasts	17.04±17.01	14.94±7.88	n.s.

Abbreviations: ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; NK, Natural killer; n.s, notsignificant; Treg, T cellregulatory CD4 lymphocytes; UC-MSC, umbilical cordmesenchymalstromalcell.

The primary safety outcome

Safety was evaluated through AEs observed within 24 hours after each infusion, including clinical examinations and measurement of vital signs. No serious complications associated with UC-MSC infusion were observed. One patient from the UC-MSC group had transient hypotension after the first infusion. In the placebo group, one patient had tachycardia immediately after the first infusion; however, the alveolar recruitment maneuver was performed by physiotherapy, and it is not possible to be sure that this would not be the cause of the observed tachycardia. There were no clinical repercussions for the patient and no need for intervention. No AEs were observed in the second and third cell infusions. Critically ill patients with severe COVID-19 showed no immediate deaths or acute anaphilatic shock after UC-MSC infusion. The other patients showed stable vital signs after the treatment. Investigators conducted assessments for the presence of any AEs from enrollment throughout the study.

In this study, five patients from the UC-MSC group and one patient from the placebo group (35% of the patients) passed away, although no significant difference was observed between the groups. Five patients were male and one female, and their ages ranged from 41 to 78 years. None of the deaths seemed to be related to UC-MSC infusion. The cause of death of five patients was secondary to bacterial septic shock, and one patient died secondary to ARDS and multiorgan dysfunction syndrome. There was no *association of mortality*and *elderly patients*.Five of the six patients had comorbidities such asobesity, diabetes, hypertension and schizophrenia, whichwas not associated with mortality. However, patients who presented dialysis kidney dysfunction during the course of the disease had higher associated mortality (p=0,029). The table 3 shows the details of death.

Table 3. Survival status of COVID-19 patients.

Patient	Sex	Age	Intervalbetweenfirstcellinfusionand death (days)	StudyGroup	Oxygenation PaO_2/FiO_2	Comorbidities	Cause of Deaths
1	F	50	8	UC-MSC	173 mmHg - moderate	Hypertension, obesity	Multi-organdysfunctionsyndrome
6	M	78	8	UC-MSC	175 mmHg - moderate	Diabetes, hypertension	Bacterialsepticshock
8	M	71	20	UC-MSC	250 mmHg - mild	Hypertension	Bacterialsepticshock
10	M	57	17	UC-MSC	180 mmHg- moderate	None	Bacterialsepticshock
15	M	53	38	Placebo	99 mmHg - severe	Diabetis	Bacterialsepticshock
17	M	41	23	UC-MSC	96 mmHg- severe	Diabetis, Schizophrenia	Bacterialsepticshock

Abbreviations: PaO2, arterial oxygenpartialpressure; FiO2 ,oxygenabsorptionconcentration; UC-MSC, umbilical cordmesenchymalstromalcell.

The efficacy outcome after four month follow-up

HCoV-19 nucleic acid detection

Viral load was performed at baseline and after cell therapy on days 2, 4, 6, and 14, and blood count, serologic tests, biochemistry, cell subpopulation analysis and inflammatory cytokines were performed at all times already mentioned, including 2 and 4 months.

Quantification of viral load was assessed in patient samples at baseline and 2, 4, 6, and 14 days after infusion with UC-MSCs or placebo (Figure 3). The angular coefficient (slope) and R^2 value were established for each patient. Most patients had an angular coefficient below zero, which means that the viral load decreased over time (Figure 3 C-E). Only two patients (1 UC-MSC and 1 placebo) did not obtain strong R^2 values (<0.4, Figure 3 E). The data showed no significant difference in viral load, as determined by the angular coefficient, between the UC-MSC and placebo groups (Figure 3 F). In this study, in both groups, there was a reduction in viral load over time, without significant differences.

Laboratory Assessments

Analysis of inflammatory markers

To determine the patients' inflammatory status, ferritin, C-reactive proteinand cytokine levels were analyzed. Ferritin values in the UC-MSC group were higher at baseline than at day 14 (p=0.03), 2 months (p=0.01) and 4 months (p=0.01). In the fourth month, there was a marked and statistically significant decrease in ferritin values and a return to normal levels. In the placebo group, the levels were always higher than the reference ranges. In the fourth month, there was an increase in the placebo group, opposite to the UC-MSC group (p=0.01). C-reactive protein, which is the main inflammatory marker in COVID-19 patients, showed a decrease in the comparison between baseline at 2 months (p=0.01) and baseline at 4 months (p=0.01) in the UC-MSC group. In the second month, the values were within normal levels, while in the placebo group, although the values were lower than those in the UC-MSC group, there were no differences over time, always maintaining levels above the reference (Figure 4).

Regardingthe cytokines IL-2, MIP1a-CCL3, G-CSF and TNFα assays, there were no differences between and within thetime points of each group(Figure 5).

Conversely, IL-6 levels in the UC-MSC group showed differences between baseline and thefourteen day (p=0.02), second month (p=0.01) and fourth month (p=0.01). From the fourteen day onwards, IL-6 levels decreased significantly (fourteen day x second month, p=0.01; fourteen day x fourth month, p=0.01). During this same period, in the placebo group, the levels remained high, with no differences between evaluation time points. In the comparison between groups, at baseline (p= 0.01), day 2 (p=0.01) and day 4 (p=0.04), IL-6 values were higher in the UC-MSC group than in the placebo group. However, at month 4, there was a significant decrease in the UC-MSC group and an increase in the placebo group (p=0.01). The levels of IL-8 in the UC-MSC group until the fourteen day were always higher than those in the placebo group. In both groups, there was a large reduction in values at 2 and 4 months (UC-MSC group, baseline x 2 months, p=0.01; baseline x 4 months, p=0.01; placebo group, baseline x 2 months, p=0.01, baseline x 4 months, p=0.01). The UC-MSC group showed a decrease in MCP1 levels, with differences between baseline and thefourteen day (p=0.01), 2 months (p=0.01) and 4 months (p=0.01). Comparing groups, this cytokine level was higher in the UC-MSC group than in the placebo group at baseline (p=0.01), 2 days (p=0.01) and 4 days (p=0.01). However, from the sixth day to thefourth

month, there was a decrease, with no differences in relation to the placebo group. Regarding the cytokines IL-6, IL-8 and MCP1-CCL2, all had higher levels in the UC-MSC group than in the placebo group until the fourth day. After this period, the levels decreased and were lower than those in the placebo group in the fourth month, suggesting that MSCs were effective in decreasing inflammation. The level of IL-7, which is a pleiotropic cytokine essential for lymphocyte survival and expansion, showed a decrease in the fourth month in the placebo group (baseline x 4 months, p=0.02), indicating a worsening for patients in this group (Figure 6).

On the other hand, there was an increasing trend of anti-inflammatory cytokine IL-10 levels in the UC-MSC group at all evaluation times, although with no significant difference. A study with a larger number of patients will be necessary to confirm the possible stimulation of IL-10 production exerted by UC-MSCs, which reduces the inflammatory process. The results of inflammatory markers strongly suggest that UC-MSCs had an important anti-inflammatory action from the fourteen day of evaluation in most cases and that MSC immunomodulatory function contributed to the main efficacy outcome (Figure 6).

Analysis of *coagulationparameters*

Coagulation markers were also evaluated, including D-dimer, platelets and neutrophils. With respect toD-dimer, both groups presented values above the reference in all evaluations. However, in the UC-MSC group, a decrease in D-dimer values was observed in the second month compared to baseline (p=0.01). At 2 months, values in the UC-MSC group were very close to the reference and significantly lower than the value in the placebo (UC-MSC x placebo, p=0.01). In the UC-MSC group, there was a decrease in the number of platelets in the comparison between baseline and 2 months (p=0.01) and 4 months (p=0.01).In the second and fourth months, platelets in the UC-MSC group were within the reference range, whereas in the placebo group, they were out of the reference range In the fourth month, there was a significant difference, with higher levels in the placebo group (p=0.01). A difference in the number of neutrophilsin both groups was also observed. The UC-MSC group had a lower number of neutrophils than the placebo group in the second month (p=0.03) and fourth month (p=0.01) after treatment. These results demonstrate that two months after cell infusion, there was a decrease in coagulation markers that could reduce the risk of thrombus formation compared to the placebo group (Figure 4).

Analysis of cell subpopulation

In this trial, the main cell subpopulations were evaluated by flow cytometry. The total lymphocyte count at baseline was below the reference range in the UC-MSC group and significantly lower than that in the placebo group (p=0.01). From the sixth day, there was an increase in the lymphocyte count at UC-MSC group, with a return to the normal range and a difference between baseline and 2 months (p=0.04). In the placebo group, at all evaluation times, values were within reference. The numbers of TCD3 and TCD4 lymphocytes were also lower in the UC-MSC group than in the placebo group at baseline (p=0.02 and p=0.01, respectively) and on the secondday after infusion (p=0.04 and p=0.01, respectively), with no significant differences in the other evaluations. These results indicate that the placebo group was in better condition than the UC-MSC group at the beginning of the study. From the second month, an increase in the absolute TCD3 lymphocyte values was observed, with differences between baseline and 2 months (p=0.01) and 4 months (p=0.01) in the UC-MSC group. The same was observed in relation to TCD4 lymphocytes (baseline x 2 months, p=0.01; baseline x 4 months, p=0.01). In the placebo group, there were no differences over time. Values of NK cellsincreased significantly when comparing baseline and 2 months (p=0.01) in the UC-MSC group. These data indicate thatlymphopenia, which has important prognostic potential and is present in patients who need intensive treatment, was more common in patients in the UC-MSC group, and after cell infusion, there was an improvement in immune systromal function (Figure 4).

Even with a small number of individuals per group, the results presented thus far strongly suggest that treatments with MSCs significantly improve several of the patients' functional and inflammatory parameters.

The efficacy outcome – Hepatic, Cardiac, Kidney and Pulmonary Sequelae

Months after COVID-19 infection, patients could still present some persistent symptoms that need to be monitored. Some biochemical tests to evaluate liver function, such as bilirubin, alanine aminotransferase (AST) and aspartate aminotransferase (ALT), showed no differences between groups. Regarding the function of the kidneys and heart, the levels of troponin I and creatinine were analyzed. Troponin I showed decreased levels in both groups. Values were within thenormal range at the second and fourthmonths after treatment (placebo group, baseline x 2 months, p=0.01; baseline x 4 months, p=0.01/UC-MSC group, 6 days x 2 months, p=0.04; 6 days x 4 months, p=0.01). Creatinine values indicative of renal function in the UC-MSC group were increased compared to those in the placebo group on days 4 (p=0.03), 6 (p= 0.01) and 14 (p= 0.02). On day 14, after three cell infusions, values were within the reference range, showing an improvement in renal function. In the second and fourthmonths, the UC-MSC group remained within the reference range, while there was an increase in levels in the placebo group above normal values, but there was no significant difference between groups.

In this trial, chest CT was used to detect lung damage in COVID-19 patients. All patients suffered from serious pulmonary damage and needed oxygen inhalation support during the course of disease. CT imaging results revealed bilateral, multilobular involvement as well as segmental consolidation and characteristics of pneumonia. Concerning chest CT abnormalities, there was no significant difference between groups (An

additional movie file shows this in more detail [see Additional Table 4). However, there was a reduction in the extension of opacities related to COVID-19 in chest CT scans for both groups(An additional movie file shows this in more detail [see Additional Table 2). Visually, there was a higher degree of clearence in patients from the UC-MSC group than in the placebo group, with statistical analysis showing a significant difference in the degree of opacification in those patients when comparing baseline and 4 months (p=0.01) and 14 days and 4 months (p=0.01). Patients with pulmonary fibrosis were not observed(Figure 7).

Discussion

Severe SARS-CoV-2 infection induces a cytokine storm, leading to ARDS and MOD, which are very serious health conditions.Those characteristics of COVID-19 make disease controlchallenging if using a single strategy.MSC properties allow the systemic distribution of positive immunomodulatoryand regenerative effects throughout the body, thereby ensuring a systemic effect in addition to local modulation[67]. To our knowledge, this is the first report that presents the longest follow-up after MSC treatment in COVID-19 patients. This trial was conducted during the early stages of the COVID-19 outbreakand proposed UC-MSC infusion as an adjunctive therapy in the recovery and postacute sequelae reduction caused by COVID-19. The results indicate tolerability and safety, and suggest the efficacy of UC-MSC infusion in critically ill patients.

For MSC therapy to be feasible in patients with critical illnesses such as COVID-19, cells must be obtainable within a very short time and in adequate numbers from a reproducible production process[68]. In this context, the use of allogeneic UC cells, available in a Biobank, *allows postthaw, culture recovery*and infusion within 72 hours after the patient's inclusion in the study. Some studies[69,70] show that MSCs need to restart their metabolism and other biochemical processes before infusion, recovered by a 24–72hours subculture, because thawed MSCs seem to be unresponsive directly after thawing. If MSCs are allowed to recover in culture, they restore their functionality. For this reason, in this trial, fresh cells were used in all infusions.

Sample randomization was conducted in this study to protect against imbalance in biasing caused by enrollment. However, with small numbers of patients, there is still potential for imbalance. Some differences between groups were observed at baseline, demonstrating that the UC-MSC group seemed to be more compromised than the placebo group.

All patients received standard treatment with steroids because it is recommended to use steroids in crically ill patients to inhibit the inflammatory response, especially for those requiring respiratory support[71]. Glucocorticoids, including dexamethasone, also reduce neutrophil extracellular trap (NET) formation, most likely by suppressing the expression of inflammatory mediators that activate neutrophils[72]. Heparin was also used as a standard treatment because it reduces NET formation[73], as it has been shownto have therapeutic value in COVID-19 treatment[74].

In this research, two patients in the UC-MSC group had no previous chronic diseases, while all other patients had comorbidities. This is in line with studies that show that the presence of underlying conditions such as cardiovascular disease, chronic pulmonary disease, and diabetes are risk factors that will require critical care[75].

The interval between the first infusion and hospital discharge was similar between groups (average of 16.3 days for the UC-MSC group and 14.4 days for the placebo group), showing that MSCs do not accelerate patient recovery. Similar results were observed by Adas et al.[50], when compared the control and experimental groups of criticaly ill patients. The average length of stay was 45 days in the control group and 47 days for the placebo group, with no significant differences.

After three IV UC-MSC infusions, none of the patients developed a thromboembolic event, and only mild AEs resolved naturally in a small number of cases. These AEs are unrelated to cell infusion, showing safety with no life-theatening complications. Safety was shown in other studies that also performed MSC-based interventions. They did not noticed any acute infusion-related issues, alergic reaction, delayed hypersensitivity or secondary infections in the patients either[39,45,47,48,54,76,77].

The mortality rate in this study was 35%. Out of six patients, five had at least one comorbidity, and the most common cause of death was secondary bacterial infection. A similar study carried out in Brazil showed that in the same period of time, the mortality rate in ICU patients in theSouth region was 53%[78]. Hashemian et al.[52] studied critically ill patients with severe hypoxemia who requiredMV, and observed a 45% mortality rate- most of which had signs of multiorgan failure or sepsis, and died 5-19 days after the first infusion. Also Dilogo et al.[51] showed that the mortality rate was 65% in a group of intubated critically ill patients with COVID-19 in the ICU, and higher mortality was associated with patients who had two or more comorbidities. Another trial with critically ill patients was conducted by Adas et al.[50], and they observed 33% mortality in patients who received MSC therapy. The most common cause of death was secondary infections due to bacteria, followed by myocardial infarction and thromboembolism. Data fromsuch studies reinforce that critically ill COVID-19 patients had small chances of survival.

Gender was also shown to be related to mortality. The ACE2 gene localizes on the Xchromosome, and ACE2 levels in the blood are higher in males than in females as well as in patients with diabetes or cardiovascular disease[79-89]. In this study, it was observed that six patients (83.3%) were male. Therefore, male patients might be more likely to die from COVID-19 because of the high expression of ACE2.

A high mortalityrate of patients who presented kidney dialysis dysfunction was also observed[83]. The incidence of acute kidney injury (AKI) secondary to COVID-19 is high[84]. Kidney failure appears to occur late in the course of disease and is strongly associated with high mortality among hospitalized COVID-19[85,86].Ghonimi et al.[87] reported a strong association between death related to COVID-19 infection in dialysis patients, which was also observed in the study of Costa et al.[88], showing that COVID-19 patients with AKI who need dialysis had worse outcomes. Lino et al.[89] also showed that a worse prognosis is frequently associated with a more rapid evolution to intensive and respiratory care or even dialysis[88].

According to Saleh et al.[53], the optimal time for cell infusion is the second week of the disease, namely, the second phase, where there is hyperinflammation beginning on days 7 to 15. Zhu et al.[56]also suggested that MSCs can improve the outcome of patients with severe/critical symptoms more significantly than common/mild patients. In this trial, only critically ill patients were included, and the average interval between symptom onset and first cell injection was 10.7 days for the UC-MSC group and 12.1 days for the placebo group. Possibly due to a combination of suitable patient and the ideal time points for patient treatment, an overall patient benefit after IV UC-MSC infusion was noticed.

Verifying the presence of genomic material of the virus in serum or plasma represents a useful approach to evaluate the impact of the extrapulmonary dissemination of viral material on disease severity and on the host response to the infection[90,91]. The systromalic dissemination of the virus or viral components is associated with the severity of COVID-19 and with a number of parameters indicating the presence of a dysregulated response to the infection[92]. In this study, in both groups, 14 days after infusion, there was a reduction in viral load over time, without significant differences. This is in line with Lanzoni et al.[46], who showed no differences between the UC-MSC treatment and the control group. According totheauthors, UC-MSC treatment seems to be more closely associated with a decrease in inflammatory cytokines rather than a change in viral load.Leng et al.[39] also observed that critically severe patients became negative for hCoV-19 nucleic acid 13 days after transplantation.

In the acute phase reaction of an inflammatory process, there is a variation in the concentrations of various plasma proteins, including C-reactive protein and ferritin. They are important biomarkers of inflammation in the context of COVID-19 progression because they are predictive of in-hospital[93,94]. Patients with the highest ferritin levels also presented significantly higher levels of C-reactive protein and serum creatinine[89]. In this study, analysis of inflammatory markers showed that C-reactive protein and ferritin values, in the UC-MSC group, decreased in the second and fourthmonths compared with baseline. In the placebo group, the levels were always higher than the reference ranges. Those results are in accordance with studies that show that the inflammatory biomarkers were increased in COVID-19 patients[95,96].

Critically ill COVID-19 patients have a CRS storm that involves elevated levels of circulating cytokines and immune-cell hyperactivation[97]. It occurs due to the combination of a defective (or delayed) first line of defense, followed by persistent hypercytokinemia and a dysfunctional Tcell response. That results in impaired clearance of apoptotic cells or infected/activated macrophages, followed by multiple cytokine release, hemophagocytosis, coagulopathy, and ARDS[98-100]. The clinical manifestation is the sharp rise of a large number of cytokines within a short time frame. Liu et al.[101]identified that serum levels of IL-6 (>32.1 pg/mL), one of the mediators of hyperinflammation, have a significant correlation with the severity of COVID-19, and can be used to predict disease risk. As part of this study, analysis of plasma cytokine levels was performed, andincreased IL-6 levels were observed in both groups. However, in the UC-MSC group, there was a significant reduction from day 14 (mean 9.59 pg/mL in the second month and 3.70 pg/mL in the fourth month), while in the placebo group, the levels remained high (mean 45.97 pg/mL at the second month and 100.97 pg/mL at thefourth month). A gradual decline in IL-6 levels, as shown in the present study, might be a biologically relevant marker of the efficacy of UC-MSC treatment in patients with COVID-19.

Levels of IL-8 in the UC-MSC group until the fourteen day were always higher than those in the placebo group. In both groups, there was a large reduction in values at 2 and 4 months. IL-8 is known as a neutrophil chemotactic factor and plays a major role in the recruitment of neutrophils to the site of infection. Ma et al.[102] did not observe any association of IL-8 concentrations with the severity of COVID-19, butdid observe an association between IL-8 serum levels and the duration of illness in patients with severe COVID-19. Thus, IL-8 will be a signaling pathway in the evolution of COVID-19. This same result was obtained by Li et al.[103], who showed that serum levels of IL-8 correlated to the overall clinical disease scores at different stages of the same COVID-19 patients. Hence, IL-8 may act as a biomarker for COVID-19 disease prognosis. The higher levels in the UC-MSC group enforced that patients in this group had a worse prognosis than those in the placebo group. When there was patient recovery, in the second month, there was a reduction in IL-8 levels in both groups.

The MCP-1-CCL2 chemokinehas a critical role in the process of inflammation, where it attracts or enhances the expression of other inflammatory factors/cells. It is a biomarker associated with the severity of COVID-19 disease and can be related to the risk of death in COVID-

19 patients[104].The MCP1-CCL2 chemokine level in the UC-MSC group, until the fourth day, was higher than that in the placebo group, decreasing from the fourteen day to the fourth month, reaching levels with no differences in relation to the placebo group. Resultsreveal that there was decreased inflammation and clinical improvement in these patients after cell treatment.

There were no differences in IL-7 levels in the UC-MSC group at any evaluation time; on the other hand, in the placebo group, the IL-7 level was decreased in the fourth month, with a significant difference compared to baseline. IL-7 is a pleiotropic cytokine essential for lymphocyte survival and expansion. Most likely, for this reason, a recovery in the TCD3, TCD4 and NK lymphocyte numbers was not observed in the placebo group at different evaluation times. IL-7 promotes lymphocyte expansion and possibly reverses T-cell exhaustion and may be useful in restoring immune systemic homeostasis[105]. Studies show that IL-7 exerts antiapoptotic properties and induces potent proliferation of naive and memory T-cells, leading to replenishment of circulating TCD4+ and TCD8+ [106,107].

At the inflammatory stage, there is a discharge of cytokines, chemokines and growth factors triggering neutrophil and monocyte recruitment[108]. Neutrophils and the imbalance between NET formation and degradation play a central role in the pathophysiology of inflammation, coagulopathy, organ damage, and immunothrombosis, which characterize severe cases of COVID-19[109]. Therefore, some clinical studies have found that the number of neutrophils in the bronchoalveolar lavage fluid of ARDS patients is correlated with the severity of COVID-19 and the cytokine storm[110]. Activatedplatelets form aggregates with leukocytes, particularly in patients with severe disease[111,112]. Circulating platelets bind neutrophils and may result in NET formation in the pulmonary and renal microcirculation[109], thereby contributing to immunothrombosisin patients with COVID-19[90]. Zhu et al.[56] found that MSC treatment can reduce plasma NET-DNA levels in COVID-19 patients. In this research, in the second month after treatment, there was a reduction in the number of neutrophils to the reference range in the UC-MSC group, while in the placebo group, the values were always above the normal range. The number of platelets was at the reference range in both groups in all evaluations. However, in the second and fourth months, in the placebo group, there was an increase in values above the reference. The decreased number of neutrophils and normal platelet values indicate a lower risk of thrombosis in the UC-MSC group.

D-dimer, a fibrin degradation product, is also used as a biomarker for thrombotic disorders and has been identified as a potential indicator for prognosis in COVID-19 patients[113,114,115]. According to this study, both groups presented D-dimer values above the reference in all evaluations. However, in the UC-MSC group, a decrease in D-dimer values was observed in the second month. This indicates that cell infusion was effective in reducing D-dimer levels in the UC-MSC group, decreasing the risk of thrombosis formation in these patients.

Lymphopenia is a typical profile in patients with COVID-19[116,117]and might be related to disease severity and mortality[118,119]; therefore, it is very important to determine these parameters when evaluating critically ill patients. In this trial, the number of TCD3 and TCD4 lymphocytes was lower in the UC-MSC group than in the placebo group at baseline and day 2. The number increased at thesecond and fourth months compared to baseline. The number of NK cells in the UC-MSC group was higher in the fourth month than at baseline. These results are in line with studies that have shown that all subsets of lymphocytes were decreased in COVID-19 patients[116,118,119,120] and that T-cells exhibit elevated exhaustion levels and reduced functional diversity[116,121,122]. Patients with a severe form of COVID-19 have fewer multifunctional and nonfunctional CD4+ Tcells and fewer nonexhausted CD8+ Tcells than patients with mild COVID-19[122]. Several studies observed that lymphocyte count returned to the normal range in the experimental group, and the time was significantly faster after stromal cell infusion compared with the control treatment[48,123,124].

The persistent follow-up of discharged patients with COVID-19 is essential to find ways to improve quality of life and reduce morbidity and mortality by efficient prevention. In this study, some markers of cardiac and kidney function were evaluated,and a CTscan was performed for pulmonary evaluation. In this trial, no differences were observed in relation to troponinI levels, corroborating the results observed by Johnsen et al.[23], who analyzed patients with long COVID-19 sequelae three months posthospitalization and observed no signs of cardiac dysfunction.

Huang et al.[25]showed that 13% of patients without acute kidney injury at the acute phase had a decreased glomerular filtration rate at follow-up, exhibiting an underestimation of patients with kidney dysfunction. Persistent impairment in renal function can occur following an episode of acute kidney injury, with the potential to progress to end-stage kidney disease with dialysis[125], which highlights the importance of long-term follow-up. Creatinine values in the UC-MSC group were above the reference until thesecond month, followed by a reduction at the fourth month. In the placebo group, there was an increase above the reference at the second and fourth months. It is probable that kidney lesions acquired during the disease's activity remain sequelae that may result in a slow and asymptomatic progression toward advanced stages and *chronic kidney*failure (CKD). Thus, patients who have recovered from COVID-19 who present proteinuria, hematuria, elevated creatinine and AKI should be monitored for CKD[126]. Increased creatinine values may also be associated with the patient's nutritional status; however, these patients underwent nutritional assessment at 2 and 4 months, and it was observed that all were in good nutritional status.

The benefits of corticosteroid treatment for accelerating the recovery of lung injury,according to pulmonary function assessment and chest imaging in patients with COVID-19, are controversial[71,125,127]. Therefore, new strategies to avoid pulmonary sequelae need to be developed.

Once injected intravenously, a significant amount of MSCs accumulate in the lungs, and they secrete numerous factors that play an important role in immunomodulation, protect alveolar epithelial cells, restore thepulmonary alveolar niche, prevent fibrosis, and improve overall pulmonary function, which is a great benefit for treating severe pulmonary disease in COVID-19[48,128].In addition, lung function and chest CT changes may be impaired months after the infection[129]. Huang et al.[25] observed that a considerable proportion (22–56%) of patients had a pulmonary diffusion abnormality 6 months after symptom onset. In this trial,there was a decrease in lung lesion extension in the UC-MSC group after 4 months of follow-up. The improvement of pulmonary lesions directly affects the recovery of lung function and the remission of clinical symptoms[48]; therefore, the results observed in this study could reflect reduced lung inflammation in the UC-MSC group mediated by immune regulation.

Throughout this trial, there were some limitations such as the patient assessment time, between the fourteen day and 2 month, was very long. Many parameters may have improved before the 2 months, but the exact moment could not be observed. Sample randomization was conducted in this study; however, based on some inflammation markers andlymphocyte subpopulations, the UC-MSC group seemed to be more compromised than the placebo group at baseline. Although the sample size was not large enough to stratify subgroups, it was difficult to exclude bias. The emergency condition in ICUs did not allow us to carry out CT evaluations in all patients at different times.

The results of this study revealed that in the UC-MSC group, there was a reduction in the levels of ferritin, IL-6 and MCP1-CCL2 on the fourteen day. In the second month, a decrease in the levels of reactive C-protein was observed, as well as D-dimer and neutrophils and an increase in the numbers of TCD3, TCD4 and NK lymphocyteswere observed. A decrease in lung extension was observedinthe fourth month. The improvement in all the parameters was maintained until the end of patient follow-up. Those data show that UC-MSCs can play an important role both in the early stages, by preventing *more*severe complicationsand in the chronic phase, with a reduction in sequelae.

COVID-19 is a complex multifactorial disease that makes treatment difficult using a single strategy. The promising long-term safety and efficacy results shown in this trial indicate that UC-MSCs could be used as adjunctive therapy for critically ill COVID-19 patients. UC-MSCs showed beneficial effects for patient recovery in the shortterm through a decrease inCRS by secreting anti-inflammatory factors, reducing risk of thrombosis and, in the longterm, viareduction in kidney and pulmonary sequelae based on tissue repair. The combination of immunomodulatory therapy based on UC-MSCs and antiviral drugs could help accelerate patient recovery, attenuating disease progression.

Abbreviations

Acute Respiratory Distress Syndrome (ARDS), Acute Kidney Injury (AKI), Acute Lung Injury (ALI), Advanced Therapy Products (ATP), Adverse Events (Aes),Alanine Aminotransferase (ALT), Angiotensin-Converting Enzyme 2 (ACE2), Anticoagulant Citrate Dextrose (ACD), Arterial Oxygen Partial Pressure (PaO_2), Aspartate Aminotransferase (AST), Body Mass Index (BMI), Cell Core Technology (CCT), Chronic Kidney Failure (CKD), Colony-Stimulating Factor (GM-CSF), Coronavirus Disease 2019 (COVID-19), Cytokine Release Syndrome (CRS),Computed Tomography (CT); Fetal Bovine Serum (FBS), Immunodeficiency Virus (HIV), Intensive Care Unit (ICU),International Society For Cellular Therapy (ISCT), Interleukin (IL), Iscove'sModified Dulbecco's Medium (IMDM), Intravenous Infusion (IV), Invasive Mechanical Ventilation (IMV),Macrophage Inflammatory Protein 1-Alpha (MIP1a), Mechanical Ventilation (MV), Mesenchymal Stromal Cells (MSCs), Monocyte Chemoattractant Protein-1 (MCP1),Multiple Organ Failure (MOD), Natural Killer (NK), Neutrophil Extracellular Trap (NET), Oxygen Absorption Concentration (FiO_2), Passage (P), Phosphate Buffered Saline (PBS),Pontificia UniversidadeCatólica do Paraná (PUCPR), Polymerase Chain Reaction (PCR), Polymerase II Subunit A (POLR2A), Postacute Sequelae (PASC), Reverse-Transcription Polymerase Chain Reaction (RT–PCR), Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-Cov-2),Spyke Protein (S), T Cell Regulatory CD4 Lymphocytes (Tregs), Transmembrane Serine Protease 2 (TMPRSS2), Tumor Necrosis Factor (TNF), Umbilical Cord (UC), Umbilical Cord Mesenchymal Stromal Cells (UC-MSCs), World Health Organization (WHO).

Declarations

Acknowledgments

We are grateful to all ICU staff at the Complexo Hospital de Clínicas - Universidade Federal do Paraná and the Clinical Analysis Laboratory Unit (ULAC) of the Complexo Hospital de Clínicas - Universidade Federal do Paraná for carrying out the laboratory tests. We also thank Yara Schlunga to support *cytometry analysis and GrazielaBoccasanta*for **umbilical cord selecting donors.**

Authors' Contribution:

CLKR, conceived and designed experiments, performed the experiments, administrative support, wrote the paper and edited the paper; **ACS, c**onceived and designed experiments, performed the experiments, collect and analyze the data, administrative support, co-wrote the manuscript; **CLF,** performed patient enrolment, intervention and follow-up; **DRD,** cell culture; quality control management; P**S, viral load assay**; **MAS,** evaluation of inflammatory *cytokines*; **DBM, cell culture** and assembly of data; **BS, cell culture** and assembly of data and umbilical cord

collection; **AM, p**erformed the experiments and assembly of data; **APA, flow cytometry**; **CAL,** CT scan **analysis; RRP, statistical analysis; VRJ, cytogenetic analysis**; **IMV, cytogenetic analysis**; **APM, umbilical cord selecting donors; HCJ, p**erformed patient enrolment and intervention; **ED, p**erformed patient enrolment; **PRSB** conceived and designed experiments and analyze the data; **AC, c**onceived and designed experiments, analyze the data and co-wrote the manuscript. All the authors reviewed and approved the final manuscript. PRSB and AC: these authors contributed equally to this work.

Funding

This work was supported by grants from the ProjetosIndividuais no Combateà*Covid-19* - Universidade Federal do*Paraná*(N°3748), PontifíciaUniversidadeCatólica do Paraná with resources from *Banco Regional de Desenvolvimento do Extremo Sul* (BRDE) (N° 01/2020, 03/2020) and Brazilian Ministry of Health - Brazilian National Program of Genomic and Precision Health/CNPq (N° 403624/2020-7).

Avaiability of data and materials

The majority of the data generated or analyzed during this study are included in this article.

Ethical Approval and consent to participate

All procedures in this study were conducted in accordance with the Ethics Committee in Human Research of the PontifíciaUniversidadeCatólica do Paraná (PUCPR) (CAAE: 30833820.8.0000.0020) and Comissão Nacional de ÉticaemPesquisa (CONEP).Written informed consent was obtained from the patient for their anonymized information to be published in this article.

Competing interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Consent for publication

Not applicable.

References

1. Ahn DG, Shin HJ, Kim MH, Lee S, Kim HS, Myoung J, et al. Current status of epidemiology, diagnosis, therapeutics, and vaccines for novel coronavirus disease 2019 (COVID-19). J Microbiol Biotechnol. 2020; 30: 313-324.
2. Jin Y, Yang H, Ji W, Wu W, Chen S, Zhang W, et al. Virology, Epidemiology, Pathogenesis, and Control of COVID-19. Viruses. 2020; 12:372.
3. Khan M, Adil SF, Alkhathlan HZ, Tahir MN, Saif S, Khan M, et al. COVID-19: A Global Challenge with Old History, Epidemiology and Progress So Far. Molecules. 2020; 26:39.
4. World Health Organization. World Health Organization COVID-19 Dashboard. Genebra: WHO, 2021. http://covid19.who.int. Accessed 30 Nov 2021.
5. Pollard CA, Morran MP, Nestor-Kalinoski AL. The COVID-19 pandemic: a global health crisis. Physiol Genomics. 2020; 52:549-557.
6. Preskorn SH. COVID-19: Why has the mortality rate declined? J Psychiatr Pract. 2020; 26:394-399.
7. Grasselli G, Greco M, Zanella A, Albano G, Antonelli M, Bellani G, et al. Risk factors associated with mortality among patients with COVID-19 in Intensive Care Units in Lombardy, Italy. JAMA Intern Med. 2020; 180: 1345-1355.
8. Secretaria da Saúde, Governo do Estado do Paraná. Boletim Epidemiológico Coronavírus 31-07-2021 (COVID-19). Curitiba, PR: SESA, 2021. http://saude.pr.gov.br/Pagina/Coronavirus-COVID-19. Acessed 14 Aug2021.
9. Secretaria da Saúde, Governo do Estado do Paraná. Boletim Epidemiológico Coronavírus 30-08-2021 (COVID-19). Curitiba, PR: SESA, 2021. http://saude.pr.gov.br/Pagina/Coronavirus-COVID-19. Acessed14Aug 2021.
10. Armstrong RA, Kane AD, Cook TM. Outcomes from intensive care in patients with COVID-19: a systematic review and meta-analysis of observational studies. Anesthesia. 2020; 75: 1340-1349.
11. Immovilli P, Morelli N, Antonucci E, Radaelli G, Barbera M, Guidetti D. COVID-19 mortality and ICU admission: the Italian experience. Crit Care. 2020; 24:228.
12. de Andrade CLT, Pereira CCA, Martins M, Lima SML, Portela MC. COVID-19 hospitalizations in Brazil's Unified Health System (SUS). PLoS One. 2020; 15: e0243126.
13. Tomazini BM, Maia IS, Cavalcanti AB, Berwanger O, Rosa RG, Veiga VC, et al. Effect of dexamethasone on days alive and ventilator-free in patients with moderate or severe acute respiratory distress syndrome and COVID-19: The CoDEX Randomized Clinical Trial. JAMA. 2020; 324:1307-1316.

14. Sousa GJB, Garces TS, Cestari VRF, Florêncio RS, Moreira TMM, Pereira MLD. Mortality and survival of COVID-19. Epidemiol Infect. 2020; 148:e123.
15. Huertas A, Montani D, Savale L, Pichon J, Tu L, Parent F, et al. Endothelial cell dysfunction: a major player in SARS-CoV-2 infection (COVID-19)? Eur Respir J. 2020; 56:2001634.
16. Pons S, Fodil S, Azoulay E, Zafrani L. The vascular endothelium: the cornerstone of organ dysfunction in severe SARS-CoV-2 infection. Crit Care. 2020; 24:353.
17. Chen G, Wu D, Guo W, Cao Y, Huang D, Wang H, et al. Clinical and immunological features of severe and moderate coronavirus disease 2019. J. Clin Invest. 2020; 130: 2620-2629.
18. 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.
19. Proal AD, VanElzakker MB. Long COVID or post-acute sequelae of COVID-19 (PASC): An overview of biological factors that may contribute to persistent symptoms. Front Microbiol. 2021; 12:698169.
20. Del Rio C, Collins LF, Malani P. Long-term health consequences of COVID-19. JAMA. 2020; 324: 1723-1724.
21. Adeloye D, Elneima O, Daines L, Poinasamy K, Quint JK, Walker S, et al. The long-term sequelae of COVID-19: an international consensus on research priorities for patients with pre existing and new-onset airways disease. Lancet Respir Med. 2021; 9: 1467-1478.
22. Bell ML, Catalfamo CJ, Farland LV, Ernst KC, Jacobs ET, Klimentidis YC, et al. Postacute sequelae of COVID-19 in a nonhospitalized cohort: Results from the Arizona CoVHORT. Plos One. 2021; 16.
23. Johnsen S, Sattler SM, Miskowiak KW, Kunalan K, Victor A, Pedersen L, et al. Descriptive analysis of long COVID sequelae identified in a multidisciplinary clinic serving hospitalised and non-hospitalised patients. ERJ Open Res. 2021; 7:00205-2021..
24. Logue JK, Franko NM, McCulloch DJ, McDonald D, Magedson A, Wolf CR, et al. Sequelae in Adults at 6 Months After COVID-19 Infection. JAMA Netw Open. 2021; 4:e210830.
25. Huang Y, Pinto MD, Borelli JL, Mehrabadi MA, Abrihim H, Dutt N, et al. COVID Symptoms, Symptom Clusters, and Predictors for Becoming a Long-Hauler: Looking for Clarity in the Haze of the Pandemic. medRxiv. 2021; 5:2021.03.03.21252086.
26. Li C, Zhao H, Wang B. Challenges for Mesenchymal Stem Cell-Based Therapy for COVID-19. Drug Des DevelTher. 2020; 14:3995-4001.
27. Zhang JY, Wang XM, Xing X, Xu Z, Zhang C, Song JW, et al. Single-cell landscape of immunological responses in patients with COVID-19. Nat Immunol. 2020; 21:1107-1118.
28. Tufan A, AvanoğluGüler A, Matucci-Cerinic M. COVID-19, immune system response, hyperinflammation and repurposing antirheumatic drugs. Turk J Med Sci. 2020; 50:620-632.
29. Bamba C, Singh SP, Choudhury S. Can mesenchymal stem cell therapy be the interim management of COVID-19? Drug DiscovTher. 2020; 14: 139-142.
30. Huang C, Huang L, Wang Y, Li X, Ren L, Gu X, et al. 6-month consequences of COVID-19 in patients discharged from hospital: a cohort study. Lancet. 2021; 397:220-232.
31. Prockop DJ, Oh JY. Mesenchymal stem/stromal cells (MSCs): role as guardians of inflammation. Mol Ther. 2012; 20:14-20.
32. Prockop DJ. The exciting prospects of new therapies with mesenchymal stromal cells. Cytotherapy. 2017; 19:1-8. https://dx.doi.org/10.1016/j.jcyt.2016.09.008.
33. Sutton MT, Fletcher D, Ghosh SK, Weinberg A, van Heeckeren R, Kaur S, et al. Antimicrobial Properties of Mesenchymal Stem Cells: Therapeutic Potential for Cystic Fibrosis Infection, and Treatment. Stem Cells Int. 2016;2016:5303048. https://dx.doi.org/10.1155/2016/5303048.
34. Alcayaga-miranda F, Cuenca J, Khoury M. Antimicrobial activity of mesenchymal stem cells: current status and new perspectives of antimicrobial peptide-based therapies. Frontier in Immunolog. 2017; 8.
35. Akkoc T. COVID-19, and mesenchymal stem cell tretament: mystery or not. Adv Exp Med Biol. 2020; 10: 167-176.
36. Pinky, Gupta S, Krishnakumar V, Sharma Y, Dinda AK, Mohanty S. Mesenchymal stem cell derived exosomes: a nano platform for therapeutics and drug delivery in combating COVID-19. Stem Cell Rev Rep. 2021; 17:33-43.
37. Kuba K, Imai Y, Rao S, Gao H, Guo F, Guan B, et al. A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury. Nat Med. 2005; 11:875-9.
38. Ge XY, Li JL, Yang XL, Chmura AA, Zhu G, Epstein JH, et al. Isolation and chracterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature. 2013; 503: 535-8.
39. Leng Z, Zhu R, Hou W, Feng Y, Yang Y, Han Q, et al. Transplantation of ACE2- Mesenchymal Stem Cells Improves the Outcome of Patients with COVID-19 Pneumonia. Aging Dis. 2020; 11:216-228.

40. Stolzing A, Jones E, McGonagle D, Scutt A. Age-related changes in human bone marrow-derived mesenchymal stem cells: consequences for cell therapies. Mech Ageing Dev. 2008; 129:163-73.
41. Wagner W, Bork S, Horn P, Krunic D, Walenda T, Diehlmann A, et al. Aging and replicative senescence have related effects on human stem and progenitor cells. PLoS One. 2009; 4:e5846.
42. Fischer UM, Harting MT, Jimenez F, Monzon-Posadas WO, Xue H, Savitz SI, et al. Pulmonary passage is a major obstacle for intravenous stem cell delivery: the pulmonary. Stem Cells Dev. 2009; 18: 683-691..
43. Li X, An G, Wang Y, Liang D, Zhu Z, Tian L. Targeted migration of bone marrow mesenchymal stem cells inhibits silica-induced pulmonary fibrosis in rats. Stem Cell Res Ther. 2018; 9:335.
44. Atluri S, Manocha V, Boddu N, Bhati S, Syed Z, Diwan S, et al. Safety and effectiveness of intravascular mesenchymal stem cells to treat organ failure and possible application in COVID-19 complications. Pain Physician. 2020; 23: 391-420.
45. Feng Y, Huang J, Wu J, Xu Y, Chen B, Jiang L, t al. Safety and feasibility of umbilical cord mesenchymal stem cells in patients with COVID-19 pneumonia: A pilot study. Cell Prolif. 2020; 53.
46. Lanzoni G, Linetsky E, Correa D, Messinger Cayetano S, Alvarez RA, Kouroupis D, Umbilical cord mesenchymal stem cells for COVID-19 acute respiratory distress syndrome: A double-blind, phase 1/2a, randomized controlled trial. Stem Cells Transl Med. 2021;10:660-673.
47. Meng F, Xu R, Wang S, Xu Z, Zhang C, Li Y, 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; 5:172.
48. Shu L, Niu C, Li R, Huang T, Wang Y, Huang M, et al. Treatment of severe COVID-19 with human umbilical cord mesenchymal stem cells. Stem Cell Res Ther. 2020; 11:361.
49. Tang L, Jiang Y, Zhu M, Chen L, Zhou X, Zhou C, et al. Clinical study using mesenchymal stem cells for the treatment of patients with severe COVID-19. Front Med. 2020; 14:664-673.
50. Adas G, Cukurova Z, Yasar, KK, Yilmaz R, Isiksacan N, Kasapoglu P, et al. The systematic effect of mesenchymal stem cell therapy in critical COVID-19 patients: A prospective double controlled trial. Cell Transplant. 2021; 30: 1-41.
51. Dilogo IH, Aditianingsih D, Sugiarto A, Burhan E, Damayanti T, Sitompul PA, et al. Umbilical cord mesenchymal stromal cells as critical COVID-19 adjuvant therapy: A randomized controlled trial. Stem Cells Transl Med. 2021; 10: 1279-1287.
52. Hashemian SR, Aliannejad R, Zarrabi M, Soleimani M, Vosough M, Hosseini SE, et al. Mesenchymal stem cells derived from perinatal tissues for treatment of critically ill COVID-19-induced ARDS patients: a case series. Stem Cell Res Ther. 2021; 12:91.
53. Saleh M, Vaezi AA, Aliannejad R, Sohrabpour AA, Kiaei SZF, Shadnoush M, et al. Cell therapy in patients with COVID-19 using Wharton's jelly mesenchymal stem cells: a phase 1 clinical trial. Stem Cell Res Ther. 2021; 12:410.
54. Shi L, Huang H, Lu X, Yan X, Jiang X, Xu R, et al. Effect of human umbilical cord-derived mesenchymal stem cells on lung damage in severe COVID-19 patients: a randomized, double-blind, placebo-controlled phase 2 trial. Signal Transduct Target Ther. 2021; 6:58.
55. Xu Z, Shi L, Wang Y, Zhang J, Huang L, Zhang C, et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. Lancet Respir Med. 2020; 8:420-422.
56. Zhu R, Yan T, Feng Y, Liu Y, Cao H, Peng G, et al. Mesenchymal stem cell treatment improves outcome of COVID-19 patients via multiple immunomodulatory mechanisms. Cell Res. 2021; 31:1244-1262.
57. Rebelatto CK, Aguiar AM, Moretão MP, Senegaglia AC, Hansen P, Barchiki F, et al. Dissimilar differentiation of mesenchymal stem cells from bone marrow, umbilical cord blood, and adipose tissue. Exp Biol Med (Maywood). 2008; 233:901-13.
58. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy. 2006; 8: 315-7.
59. Borgonovo T, Vaz IM, Senegaglia AC, Rebelatto CLK, Brofman PRS. Genetic evaluation of mesenchymal stem cells by G-banded karyotyping in a Cell Technology Center. Rev Bras HematolHemoter. 2014; 36: 202-207. https://dx.doi.org/10.1016/j.bjhh.2014.03.006 .
60. Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DK, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT–PCR. Euro Surveill. 2020; 25: 23-30.
61. Kalina T, Flores-Montero J, van der Velden VH, Martin-Ayuso M, Böttcher S, Ritgen M, et al. EuroFlow standardization of flow cytometer instrument settings and immunophenotyping protocols. Leukemia. 2012; 26:1986-2010.
62. Flores-Montero J, Grigore G, Fluxá R, Hernández J, Fernandez P, Almeida J, et al. EuroFlow Lymphoid Screening Tube (LST) data base for automated identification of blood lymphocyte subsets. J Immunol Methods. 2019;475:112662.
63. Masarotto G, Varin C. Gaussian copula marginal regression. Electron. J. Statist. 2012; 6:1517–1549.
64. Bretz F, Hothorn T and Westfall P. Multiple Comparisons Using R. 1st ed. New York: Chapman and Hall/CRC, 2010.
65. Dessau RB, Pipper CB. R–enprogrampakketilstatistiskdatabehandlingoggrafik ["R"–project for statistical computing]. UgeskrLaeger. 2008; 170:328-30.

66. Masarotto G, Varin C. Gaussian Copula Regression in R. Journal of Statistical Software. 2017; 77:1–26.
67. Öztürk S, Elçin AE, Elçin YM. Mesenchymal stem cells for coronavirus (COVID-19)-Induced pneumonia: Revisiting the paracrine hypothesis with new hopes? Aging Dis. 2020; 11:477-479.
68. Tufan AC. Mesenchymal stem cells as a treatment strategy for coronavirus disease 2019 (COVID-19): Need for authority regulations and clinical guidelines. Curr Stem Cell Res Ther. 2021; 16:465-480.
69. Galipeau J, Krampera M, Barrett J, Dazzi F, Deans RJ, DeBruijn J, et al. International Society for Cellular Therapy perspective on immune functional assays for mesenchymal stromal cells as potency release criterion for advanced phase clinical trials. Cytotherapy. 2016; 18: 151–159.
70. Moll G, Geißler S, Catar R, Ignatowicz L, Hoogduijn MJ, Strunk D, et al. Cryopreserved or Fresh Mesenchymal Stromal Cells: Only a Matter of Taste or Key to Unleash the Full Clinical Potential of MSC Therapy? Adv Exp Med Biol. 2016; 951:77-98.
71. Horby P, Lim WS, Emberson JR, Mafham M, Bell JL, Linsell L, et al. Dexamethasone in Hospitalized Patients with Covid-19. N Engl J Med. 2021; 384:693-704.
72. Vargas A, Boivin R, Cano P, Murcia Y, Bazin I, Lavoie JP. Neutrophil extracellular traps are downregulated by glucocorticosteroids in lungs in an equine model of asthma. Respir Res. 2017; 18:207.
73. Sun Y, Chen C, Zhang X, Wang S, Zhu R, Zhou A, et al. Heparin improves alveolarization and vascular development in hyperoxia-induced bronchopulmonary dysplasia by inhibiting neutrophil extracellular traps. BiochemBiophys Res Commun. 2020; 522:33-39.
74. Wang T, Chen R, Liu C, Liang W, Guan W, Tang R, et al. Attention should be paid to venous thromboembolism prophylaxis in the management of COVID-19. Lancet Haematol. 2020; 7:e362-e363.
75. Sharma D, Zhao F. Updates on clinical trials evaluating the regenerative potential of allogenic mesenchymal stem cells in COVID-19. NPJ Regen Med. 2021; 6:1-11.
76. Guo Z, Chen Y, Luo X, He X, Zhang Y, Wang J. Administration of umbilical cord mesenchymal stem cells in patients with severe COVID-19 pneumonia. Crit Care. 2020; 24: 420.
77. Sánchez-Guijo F, García-Arranz M, López-Parra M, Monedero P, Mata-Martínez C, Santos A, et al. Adipose-derived mesenchymal stromal cells for the treatment of patients with severe SARS-CoV-2 pneumonia requiring mechanical ventilation. A proof of concept study. EClinicalMedicine. 2020; 25:100454.
78. Ranzani OT, Bastos LSL, Gelli JGM, Marchesi JF, Baião F, Hamacher S, et al. Characterisation of the first 250,000 hospital admissions for COVID-19 in Brazil: a retrospective analysis of nationwide data. Lancet Respir Med. 2021; 9:407-418.
79. Patel SK, Velkoska E, Burrell LM. Emerging markers in cardiovascular disease: where does angiotensin-converting enzyme 2 fit in? Clin Exp Pharmacol Physiol. 2013; 40:551-9. https://dx.doi.org/10.1111/1440-1681.12069.
80. Bunders M, Altfeld M. Implications of Sex Differences in Immunity for SARS-CoV-2 Pathogenesis and Design of Therapeutic Interventions. Immunity. 2020; 53: 487-495.
81. Li Q, Guan X, Wu P, Wang X, Zhou L, Tong Y, et al. Early Transmission Dynamics in Wuhan, China, of Novel Coronavirus–Infected Pneumonia. N Engl J Med. 2020; 382:1199-1207.
82. Zhang JJ, Dong X, Cao YY, Yuan YD, Yang YB, Yan YQ, et al. Clinical characteristics of 140 patients infected with SARS-CoV-2 in Wuhan, China. Allergy. 2020; 75:1730-1741.
83. Robbins-Juarez SY, Qian L, King KL, Stevens JS, Husain SA, Radhakrishnan J, et al. Outcomes for patients with COVID-19 and acute kidney injury: A Systematic review and meta-analysis. Kidney Int Rep. 2020; 5:1149-1160.
84. Chan L, Chaudhary K, Saha A, Chauhan K, Vaid A, Baweja M, et al. Acute kidney injury in hospitalized patients with COVID-19. J Am Soc Nephrol. 2020; 32: 151-160.
85. Kellum JA, van Till JWO, Mulligan G. Targeting acute kidney injury in COVID-19. Nephrol Dial Transplant. 2020; 35:1652-1662.
86. Arikan H, Ozturk S, Tokgoz B, Dursun B, Seyahi N, Trabulus S, et al. Characteristics and outcomes of acute kidney injury in hospitalized COVID-19 patients: A multicenter study by the Turkish society of nephrology. PLos One. 2021; 16: e0256023.
87. Ghonimi TAL, Alkad MM, Abuhelaiqa EA, Othman MM, Elgaali MA, Ibrahim RAM, et al. Mortality and associated risk factors for COVID-19 infection in dialysis patients in Qatar: A nationwide cohort study. Plos One. 2021; 16:
88. Costa RLD, Sória TC, Salles, Gerecht AV, Corvisier MF, Menezes MAM, et al. Acute kidney injury in patients with Covid-19 in a Brazilian ICU: incidence, predictors and in-hospital mortality. J Bras Nefrol. 2020; 43: 349-358.
89. Lino K, Guimarães GMC, Alves LS, Oliveira AC, Faustino R, Fernandes CS, et al. Serum ferritin at admission in hospitalized COVID-19 patients as a predictor of mortality. Braz J Infect Dis. 2021; 25:101569.
90. Middleton EA, He XY, Denorme F, Campbell RA, Ng D, Salvatore SP, et al. Neutrophil extracellular traps contribute to immunothrombosis in COVID-19 acute respiratory distress syndrome. Blood. 2020; 136:1169-1179.

91. Moskalev A, Stambler I, Caruso C. Innate and Adaptive Immunity in Aging and Longevity: The Foundation of Resilience. Aging Dis. 2020; 11:1363-1373.
92. Bermejo-Martin JF, González-Rivera M, Almansa R, Micheloud D, Tedim AP, Domínguez-Gil M, et al. Viral RNA load in plasma is associated with critical illness and a dysregulated host response in COVID-19. Crit Care. 2020; 24: 691.
93. Henry BM, de Oliveira MHS, Benoit S, Plebani M, Lippi G. Hematologic, biochemical and immune biomarker abnormalities associated with severe illness and mortality in coronavirus disease 2019 (COVID-19): a meta-analysis. Clin Chem Lab Med. 2020; 58:1021-1028.
94. Sharifpour M, Rangaraju S, Liu M, Alabyad D, Nahab FB, Creel-Bulos CM, et al. C-Reactive protein as a prognostic indicator in hospitalized patients with COVID-19. PLoS One. 2020; 15:e0242400.
95. Payán-Pernía S, Gómez Pérez L, Remacha Sevilla ÁF, Sierra Gil J, Novelli Canales S. Absolute lymphocytes, ferritin, C-Reactive protein, and lactate dehydrogenase predict early invasive ventilation in patients with COVID-19. Lab Med. 2021; 52:141-145.
96. Kaftan AN, Hussain MK, Algenabi AA, Naser FH, Enaya MA. Predictive Value of C-reactive Protein, Lactate Dehydrogenase, Ferritin and D-dimer Levels in Diagnosing COVID-19 Patients: a Retrospective Study. Acta Inform Med. 2021; 29:45-50.
97. Fajgenbaum DC, June CH. Cytokine Storm. N Engl J Med. 2020; 383: 2255-2273.
98. Schulert GS, Grom AA. Pathogenesis of macrophage activation syndrome and potential for cytokine- directed therapies. Annu Rev Med. 2015; 66:145-59.
99. Brisse E, Wouters, CH, Matthys P. Advances in the pathogenesis of primary and secondary hemophagocytic lymphohistiocytosis: differences and similarities. Br J Hematol. 2016; 174: 203-2017.
100. Crayne CB, Albeituni S, Nichols KE, Cron RQ. The Immunology of Macrophage Activation Syndrome. Front Immunol. 2019; 10.
101. Liu F, Li L, Xu M, Wu J, Luo D, Zhu Y, et al. Prognostic value of interleukin-6, C-reactive protein, and procalcitonin in patients with COVID-19. J Clin Virol. 2020; 127:104370.
102. Ma A, Zhang L, Ye X, Chen J, Yu J, Zhuang L, et al. High Levels of Circulating IL-8 and Soluble IL-2R Are Associated With Prolonged Illness in Patients With Severe COVID-19. Front Immunol. 2021; 12:626235.
103. Li L, Li J, Gao M, Fan H, Wang Y, Xu X, et al. Interleukin-8 as a Biomarker for Disease Prognosis of Coronavirus Disease-2019 Patients. Front Immunol. 2021; 11:602395.
104. Chen Y, Wang J, Liu C, Su L, Zhang D, Fan J, et al. IP-10 and MCP-1 as biomarkers associated with disease severity of COVID-19. Mol Med. 2020; 26: 97.
105. Jamilloux Y, Henry T, Belot A, Viel S, Fauter M, El Jammal T, Walzer T, François B, Sève P. Should we stimulate or suppress immune responses in COVID-19? Cytokine and anti-cytokine interventions. Autoimmun Rev. 2020; 19:102567.
106. Nanjappa SG, Kim EH, Suresh M. Immunotherapeutic effects of IL-7 during a chronic viral infection in mice. Blood. 2011; 117:5123-32.
107. Perales MA, Goldberg JD, Yuan J, Koehne G, Lechner L, Papadopoulos EB, et al. Recombinant human interleukin-7 (CYT107) promotes T-cell recovery after allogeneic stem cell transplantation. Blood. 2012; 120:4882-91.
108. Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, Locati M. The chemokine system in diverse forms of macrophage activation and polarization. Trends Immunol. 2004; 25:677-86.
109. Ackermann M, Anders HJ, Bilyy R, Bowlin G, Daniel C, Lorenza R, et al. Patients with COVID-19: in the dark-NETs of neutrophilis. Cell Death and Differ. 2021; 28: 3125-3139.
110. Perrone LA, Plowden JK, García-Sastre A, Katz JM, Tumpey TM. H5N1 and 1918 pandemic influenza virus infection results in early and excessive infiltration of macrophages and neutrophils in the lungs of mice. PLoSPathog. 2008; 4:e1000115.
111. Hottz ED, Azevedo-Quintanilha IG, Palhinha L, Teixeira L, Barreto EA, Pão CRR, et al. Platelet activation and platelet-monocyte aggregate formation trigger tissue factor expression in patients with severe COVID-19. Blood. 2020; 136:1330-1341.
112. Leppkes M, Knopf J, Naschberger E, Lindemann A, Singh J, Herrmann I, et al. Vascular occlusion by neutrophil extracellular traps in COVID-19. EBioMedicine. 2020; 58:102925.
113. Soni M, Gopalakrishnan R, Vaishya R, Prabu P. D-dimer level is a useful predictor for mortality in patients with COVID-19: Analysis of 483 cases. Diabetes MetabSyndr. 2020; 14:2245-2249.
114. 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; 395:1054-1062.
115. Hayıroğlu Mİ, Çiçek V, Kılıç Ş, Çınar T. Mean sérum D-dimer level to predict in-hospital mortality in COVID-19. Rev Assoc Med Bras (1992). 2021; 67:437-442.
116. Diao B, Wang C, Tan Y, Chen X, Liu Y, Ning L, et al. Reduction and functional exhaustion of T cells in patients with coronavirus disease 2019 (COVID-19). Front Immunol. 2020; 11:

117. Wang F, Hou H, Luo Y, Tang G, Wu S, Huang M, et al. The laboratory tests and host immunity of COVID-19 patients with different severity of illness. JCI Insight. 2020; 5:e137799.
118. Qin C, Zhou L, Hu Z, Zhang S, Yang S, Tao Y, et al. Dysregulation of immune response in patients with coronavirus 2019 (COVID-19) in Wuhan, China. Clin Infect Dis. 2020; 71:762-768.
119. Wang F, Nie J, Wang H, Zhao Q, Xiong Y, Deng L, et al. Characteristics of peripheral lymphocyte subset alteration in COVID-19 pneumonia. J Infect Dis. 2020; 221:1762-1769.
120. Hadjadj J, Yatim N, Barnabei L, Corneau A, Boussier J, Smith N, et al. Impaired type I interferon activity and inflammatory responses in severe COVID-19 patients. Science. 2020; 369: 718-724.
121. Zheng M, Gao Y, Wang G, Song G, Liu S, Sun D, et al. Functional exhaustion of antiviral lymphocytes in COVID-19 patients. Cell Mol Immunol. 2020; 17:533-535.
122. Zheng HY, Zhang M, Yang CX, Zhang N, Wang XC, Yang XP, et al. Elevated exhaustion levels and reduced functional diversity of T cells in peripheral blood may predict severe progression in COVID-19 patients. Cell Mol Immunol. 2020; 17:541-543.
123. Chen J, Hu C, Chen L, Tang L, Zhu Y, Xu X, et al. Clinical study of mesenchymal stem cell treatment for acute respiratory distress syndrome induced by epidemic influenza A (H7N9) infection: A Hint for COVID-19 treatment. Engineering. 2020; 6: 1153-1161.
124. Liang B, Chen J, Li T, Wu H, Yang W, Li Y, et al. Clinical remission of a critically ill COVID-19 patient treated by human umbilical cord mesenchymal stem cells: A case report. Medicine (Baltimore). 2020; 99:e21429.
125. Forni LG, Darmon M, Ostermann M, Oudemans-van Straaten HM, Pettilä V, Prowle JR, et al. Renal recovery after acute kidney injury. Intensive Care Med. 2017; 43: 855-866.
126. Herrera-Valdés R, Almaguer-López M, López-Marín L, Bacallao-Méndez R, F J, Guerra-Bustillo G. COVID-19 and the Kidneys: Risk, Damage and Sequelae. MEDICC Rev. 2020; 22:87-88.
127. Sterne JAC, Murthy S, Diaz JV, Slutsky AS, Villar J, Angus DC, et al. Association between administration of systemic corticosteroids and mortality among critically Ill patients with COVID-19: A Meta-analysis. JAMA. 2020; 324:1330-1341.
128. 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; 34:2548-2558.
129. Lerum TV, Aaløkken TM, Bronstad E, Aarli B, Ikdahl E, Lund KMA, et al. Dyspnoea, lung function and CT findings 3 months after hospital admission for COVID-19. Eur Respir J. 2021; 57:2003448.

Figures

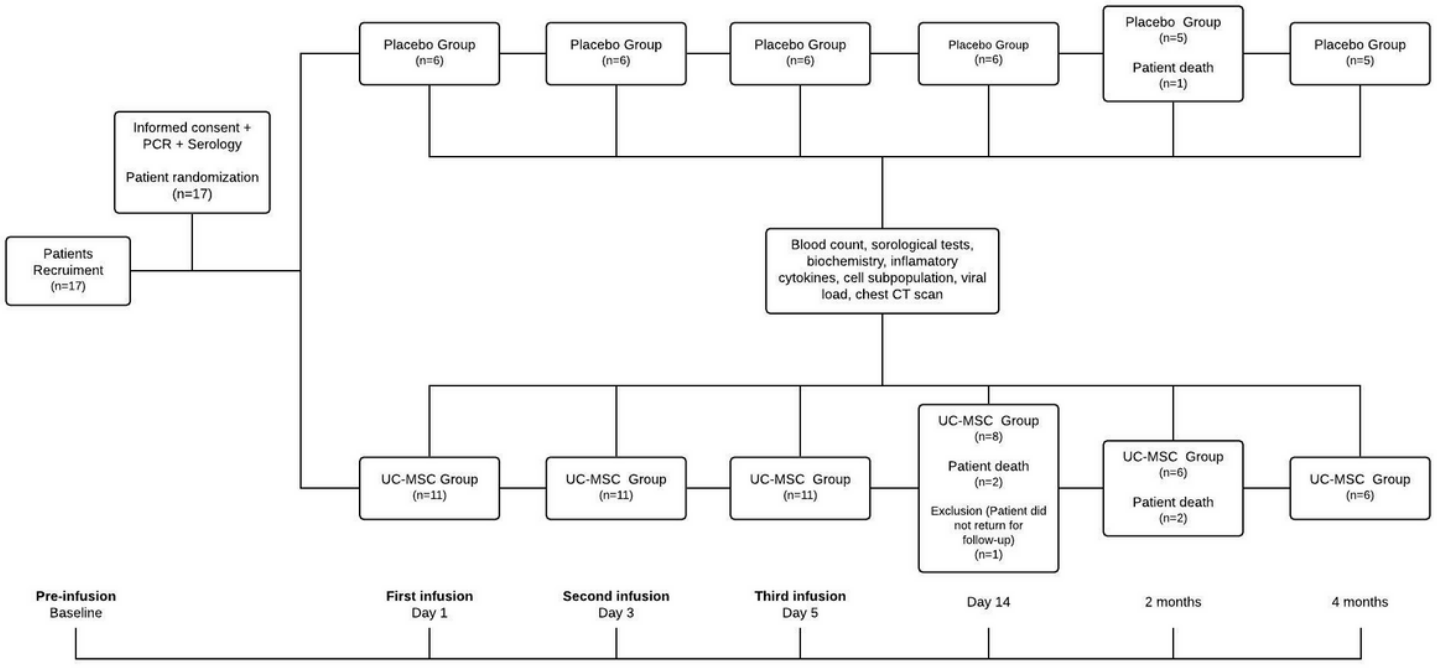

Figure 1

Flow chart for patient enrollment, intervention and follow-up.

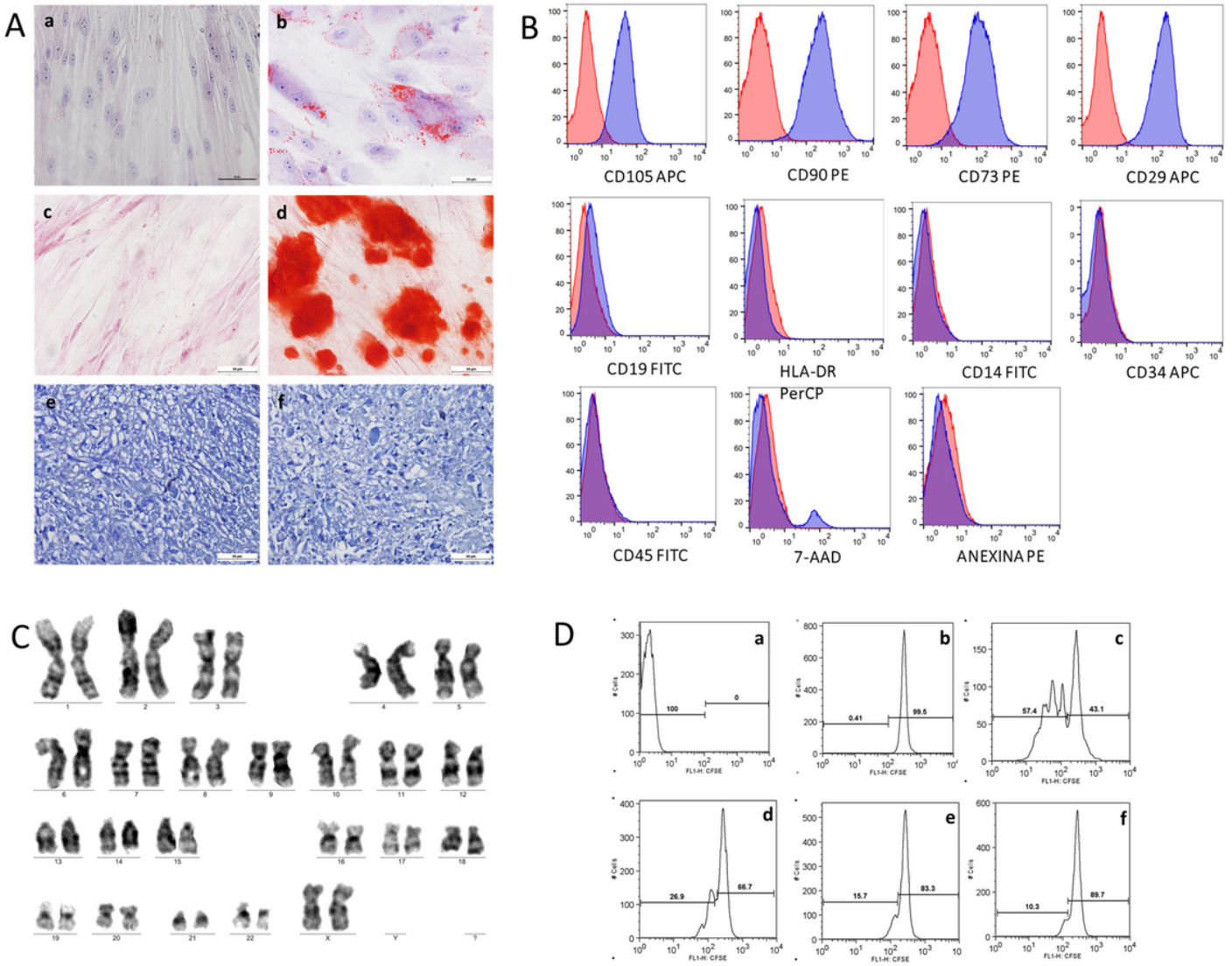

Figure 2

Characterization and quality control for UC-MSC. A. Representative image of cell differentiation.(a, c, and e) Control cells; (b) Cells differentiated into adipocytes characterized by the presence of lipidic vacuoles stained with Oil Red O; (d) Cells differentiated into osteoblasts characterized by the presence of calcium deposits stained with Alizarin Red S (red); (f) Presence of vacuoles around young chondrocytes and proteoglycan in the matrix. B.Representative histograms of UC-MSC surface markers, cell viability andapoptosis/necrosis. The isotype control is shown as a red-line histogram. C. UC-MSC karyogram after cell expansion. Normal karyotype: 46,XX. D. Representative histograms from the lymphocyte inhibition assay. MSCs were cultivated with PHA stimulated CD3+ lymphocytes labeled with CFSE. (a) CD3+ lymphocytes not labeled with CFSE; (b) CFSE-labeled CD3+ lymphocytes; (c) CD3+ lymphocytes labeled with CFSE and stimulated with PHA (1 μg/μL); (d) MSCs were cultivated with CD3+ lymphocytes labeled with CFSE 1:10; (e) MSCs were cultivated with CD3+ lymphocytes labeled with CFSE 1:5; (f) MSCs were cultivated with CD3+ lymphocytes labeled with CFSE in a 1:2 ratio.

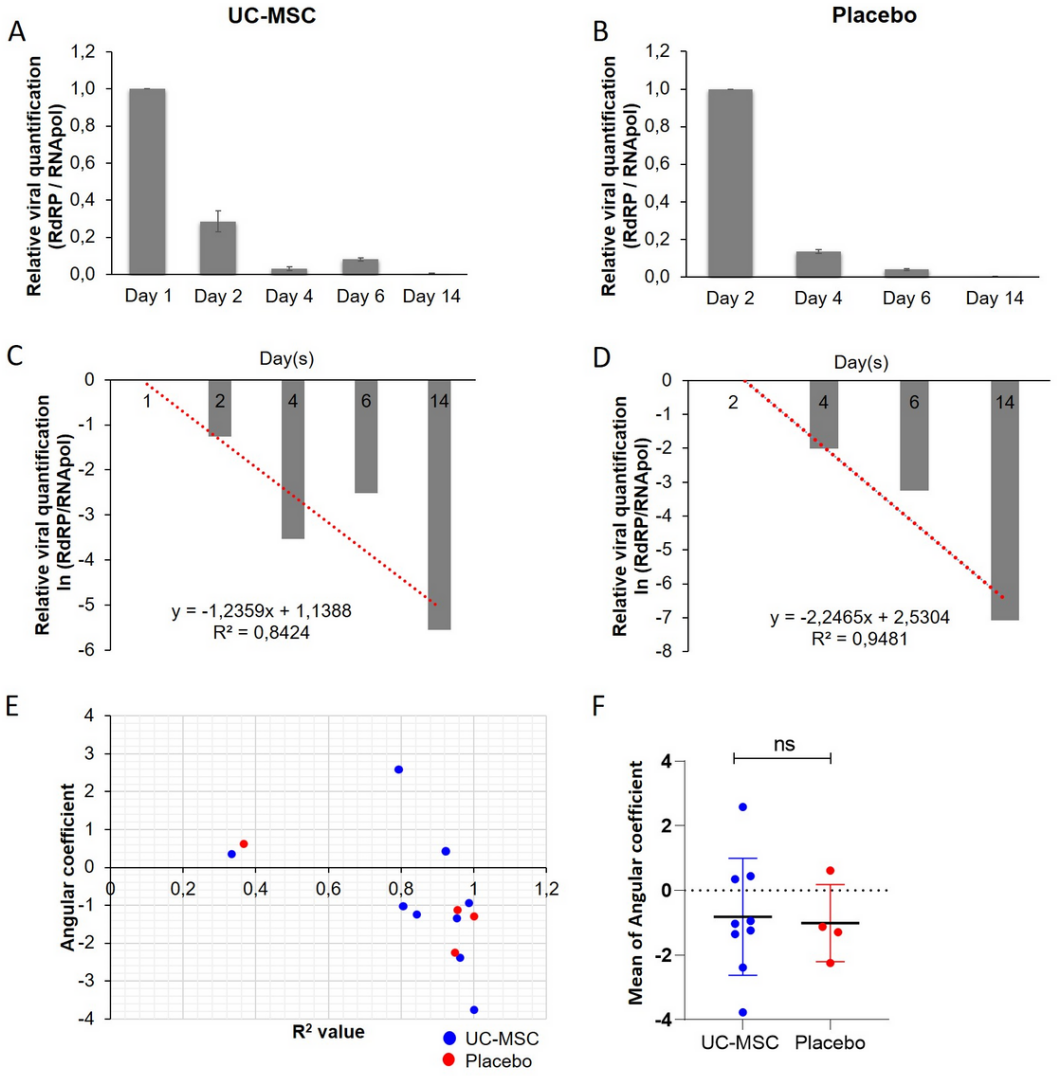

Figure 3

Viral load from day 1 (baseline) to 14 days after UC-MSC treatment. (A-D) Graphs representing relative viral quantification. Viral load was determined based on the relative expression of the viral gene RdRP in relation to the human POLR2B gene (normalizer). Viral gene expression gradually decreased in patient samples from day 1 (baseline) to day 14 (A-B). (C-D) After linearizing the data by the natural logarithm (ln (x)) and obtaining the linear equation, the angular coefficient of the viral load line (slope) and the coefficient of determination (R^2) were established. (C) UC-MSC and (D) Placebo. The angular coefficient (slope) and R^2 of each patient are plotted in (E). Average of the angular coefficient of each group (F). Mean with SEM; Student's unpaired ttest analysis. *ns, not significant.

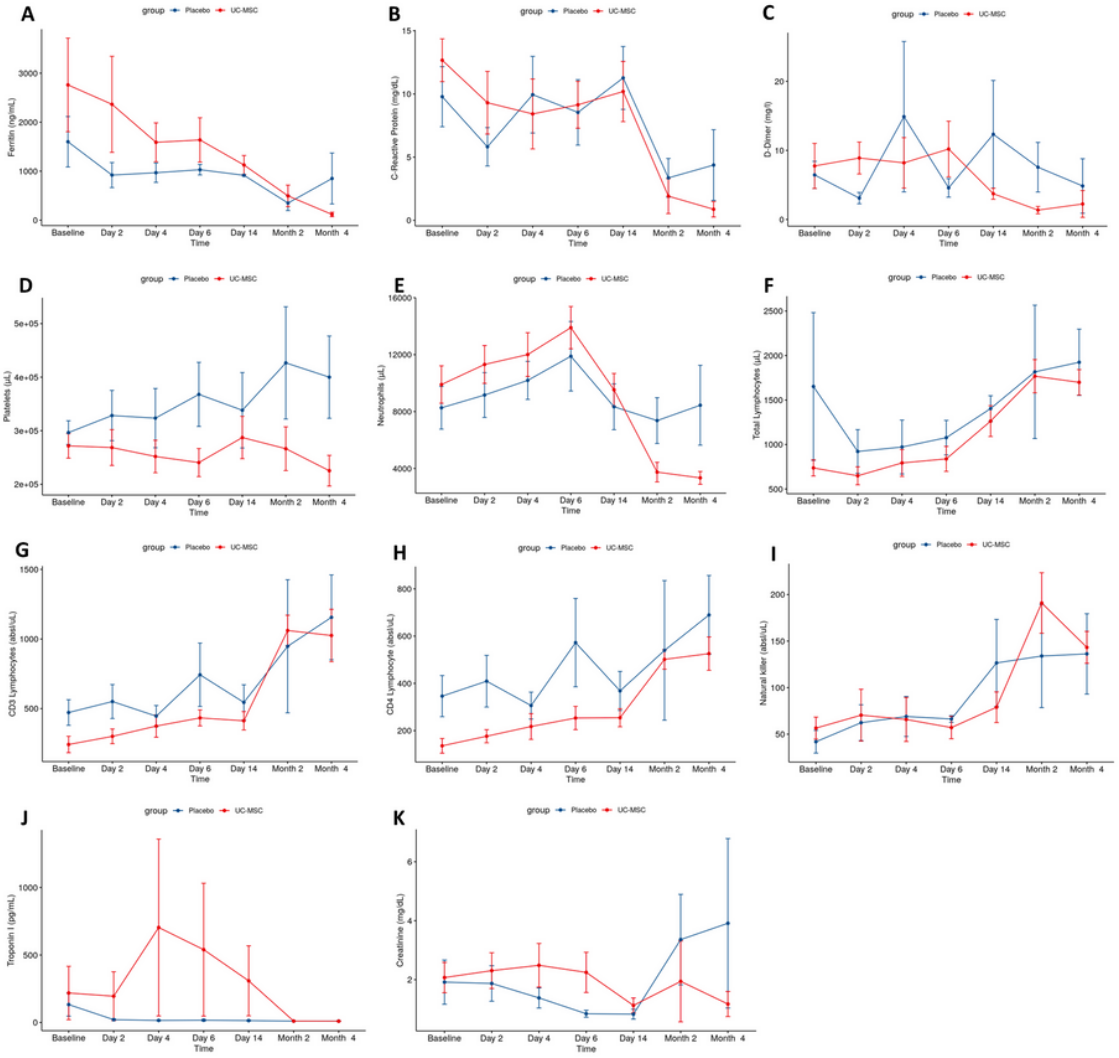

Figure 4

Change in patients' serum biomarker levels and cell subpopulations. Comparison between UC-MSC and placebo groups over time. The bars show standard deviations (SD). (A) Ferritin, (B) C-Reactive Protein, (C) D-dimer, (D) Platelets, (E) Neutrophils, (F) Total Lymphocytes, (G) TCD3 lymphocytes, (H) TCD4 lymphocytes, (I) Natural killer, (J) Troponin I and (K) Creatinin. Abbreviations: UC-MSCs, Umbilical Cord Mesenchymal Stromal Cells.

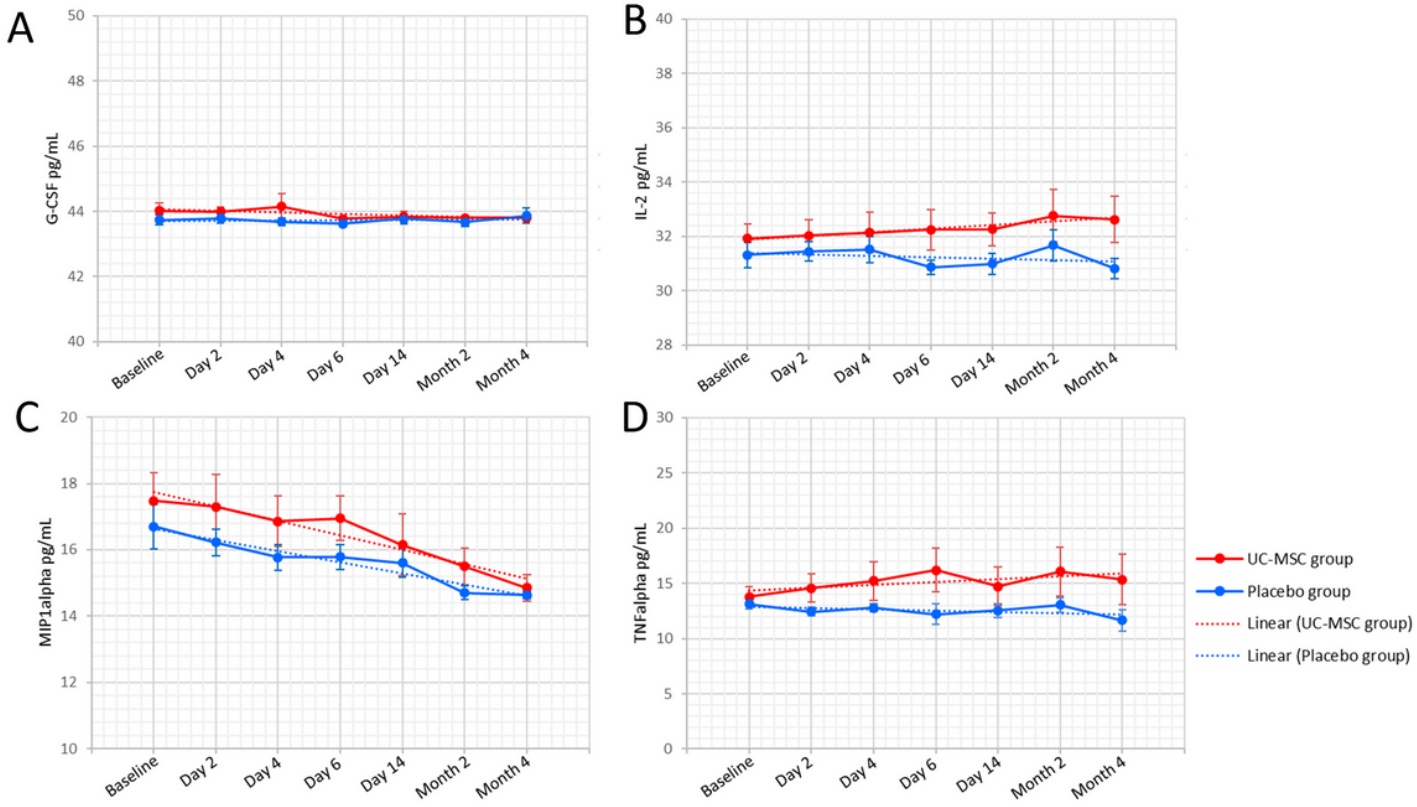

Figure 5

Profile of plasma cytokines, chemokines and growth factors in the patients during different clinical stages. Comparison between groups and times. Graphs show the results of some analytes separately, comparing the UC-MSC and placebo groups over time. The bars show standard deviations (SD), and the broken line is the trend line fitted to the data. (A) Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF), (B) Interleukin (IL) -2, (C) Macrophage Inflammatory Protein 1-Alpha (MIP1a/CCL3), (D) Tumor Necrosis Factor (TNF) A. Abbreviations: UC-MSCs, Umbilical Cord Mesenchymal Stromal Cells.

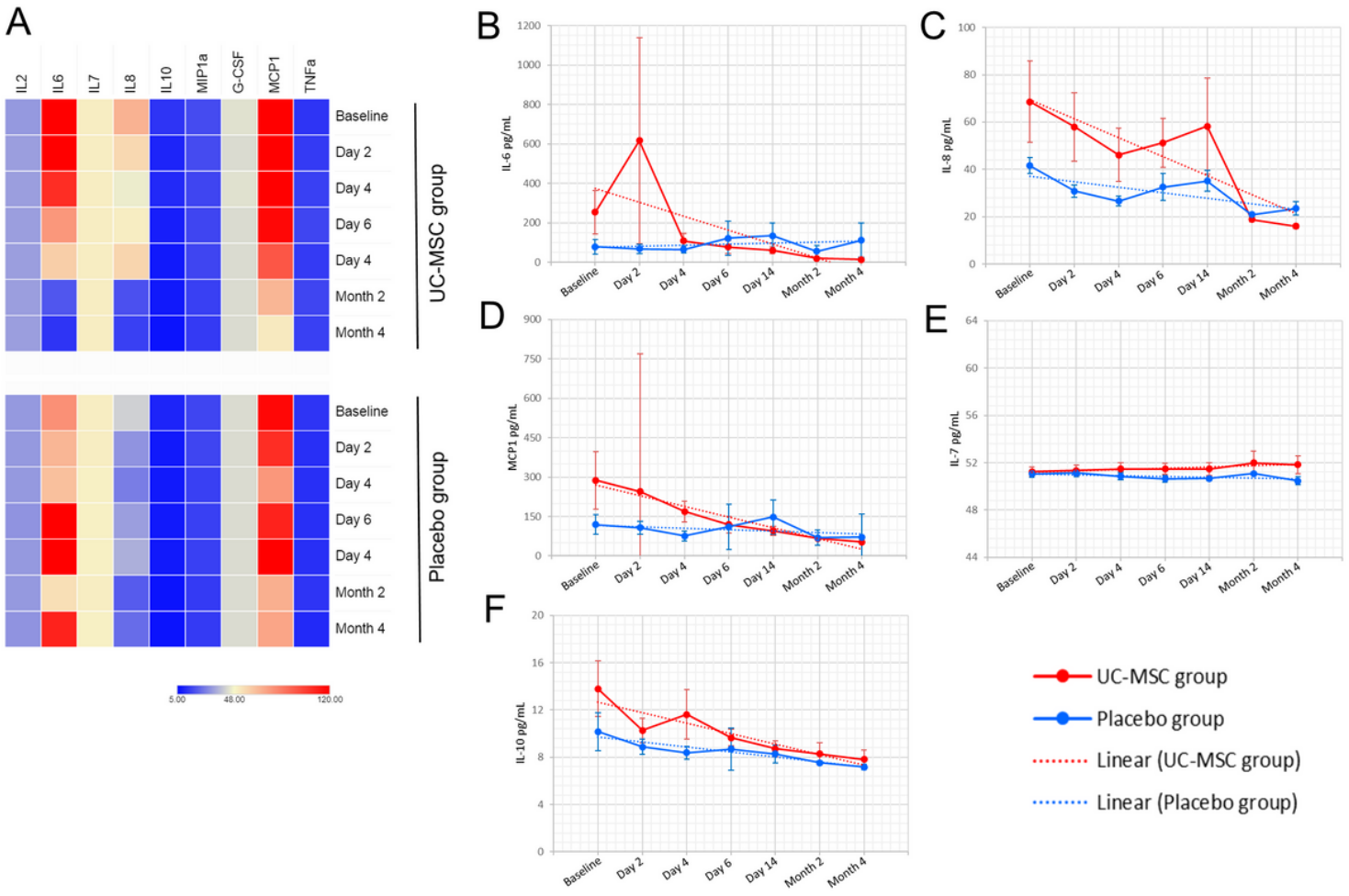

Figure 6

Profile of plasma cytokines, chemokines and growth factors in the patients during different clinical stages. Comparison between groups and times. (A) Heatmap jointly comparing all analytes evaluated over the treatment time, either with cell therapy or placebo. Graphs show the results of some analytes separately, comparing the UC-MSC and placebo groups over time. The bars show standard deviations (SD), and the broken line is the trend line fitted to the data. (B) IL-6: Interleukin 6; (C) IL-8: Interleukin 8; (D) MCP1-CCL2:Monocyte chemoattractant protein-1; (E) IL-7: Interleukin 7; (F) IL-10: Interleukin 10. *ns, not significant.

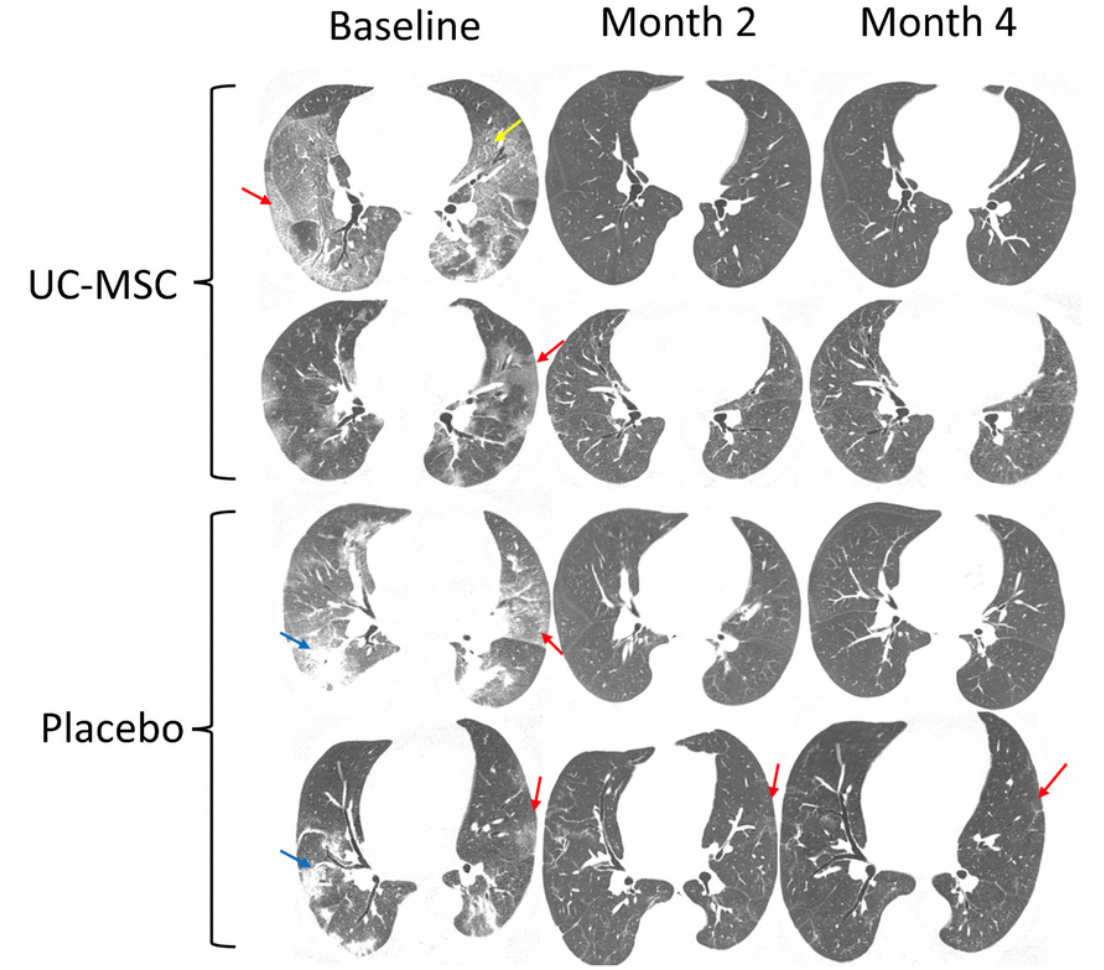

Figure 7

Representative images from chest CT at the level of the lower lobes in patients from the treatment and control groups. Red arrows point to ground-glass opacification, blue arrows to peripheral consolidations, and the yellow arrow to crazy-paving, all of which are typical features of COVID-19. Please note the higher degree of clearance in patients from the treatment group (first line) compared to the control group (last line).*ns, not significant.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- AdditionalfileTable1.xlsx
- AdditionalfileTable2.xlsx
- AdditionalfileTable3.xlsx
- AdditionalfileTable4.xlsx