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Mesenchymal Stromal Cells

Impact of mesenchymal stromal/stem cell infusions on circulating inflammatory biomarkers in COVID-19 patients: analysis of a phase I-IIa trial

Roberto Tonelli[a,b,c], Francesca Pischiutta[v], Francesca Elice[e], Elisa R Zanier[v], Giulia Grisendi[c,f], Giuseppe Astori[e], Anna Valeria Samarelli[b,c], Giulia Bruzzi[a,b], Linda Manicardi[g], Carlotta Spano[c,f], Giovanni Nattino[h], Fabiola Signorini[h], Martina Bernardi[e], Daniela Catanzaro[e], Anna Merlo[e], Ilaria Lisi[v], Laura Pasetto[d], Valentina Bonetto[d], Laura Fiammenghi[i], Laura Boschi[i], Simona Guidi[i], Olivia Candini[j], Tommaso Zoerle[k,l], Erica Dander[m], Giovanna D'Amico[m], Ferruccio De Pierri[f], Michela Maur[n], Elisa Pettorelli[f], Valentina Ruggieri[b], Stefania Cerri[o], Giorgio Mari[p], Giorgia De Berardis[q], Pasquale Mighali[r], Maria Cristina Baschieri[f], Lorenza Lazzari[s], Franco Bambi[t], Rachele Ciccocioppo[u], Enrico Clini[a,b,c,†,*], Massimo Dominici[c,f,†], for the RESCAT study group

[a] Respiratory Intermediate Care Unit, University Hospital of Modena, Modena, Italy
[b] Department of Medical and Surgical Sciences, Experimental Pneumology Laboratory, University of Modena and Reggio Emilia, Modena, Italy
[c] Health Extended Alliance (HEAL) ITALIA for innovative therapies, Italy
[d] Department of Neuroscience, Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Milan, Italy
[e] Haematology Unit, Laboratory of Advanced Cellular Therapies, San Bortolo Hospital, Vicenza, Italy
[f] Laboratory of Cellular Therapy, Department of Medical and Surgical Sciences and Department of Oncology and Hematology, University Hospital of Modena, Modena, Italy
[g] Lung Disease Unit, Arcispedale IRCCS Santa Maria Nuova, Reggio Emilia, Italy
[h] Department of Medical Epidemiology, Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Milan, Italy
[i] ProPharma llc, Leiden, The Netherlands
[j] Evotec Modena ltd, Medolla, Modena, Italy
[k] Department of Anesthesia and Critical Care, Neuroscience Intensive Care Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy
[l] Department of Pathophysiology and Transplantation, University of Milan, Milan, Italy
[m] Tettamanti Center, Fondazione IRCCS San Gerardo dei Tintori, Via Pergolesi, Monza, Italy
[n] Department of Oncology, Azienda Sanitaria Universitaria Friuli Centrale (ASUFC), Udine, Italy
[o] Rare Lung Disease Unit, University Hospital of Modena, Modena, Italy
[p] EIR Biotherapies Ltd, Mirandola, Modena, Italy
[q] Center for Outcomes Research and Clinical Epidemiology (CORESEARCH) S.r.l., Pescara, Italy
[r] Innovation and Research Office, University Hospital of Modena, Modena, Italy
[s] Unit of Cell and Gene Therapies, Cell Factory, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy
[t] Unità Operativa Complessa di Immunoematologia, Medicina Trasfusionale e Laboratorio Azienda Ospedaliera Universitaria Meyer, Firenze, Italy
[u] Department of Medicine, Gastroenterology Unit, A.O.U.I. Policlinico G.B. Rossi & University of Verona, Verona, Italy
[v] Department of Acute Brain and Cardiovascular Injury, Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Milan, Italy
*Correspondence: Enrico Clini, MD, PhD, FERS Director, Lung and Respiratory Intensive Care Unit, Laboratory of Experimental Pneumology, University Hospital of Modena, Policlinico Via del Pozzo, 41 - 41100 Modena, Italy. *E-mail address:* enrico.clini@unimore.it (E. Clini).
[†]These authors share senior authorship.

Abbreviations: SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus 2; COVID-19, Coronavirus Disease 2019, MSCs, mesenchymal stromal/stem cells; UC-MSCs, umbilical cord derived MSCs AT-MSCs, adipose derived MSCs; CTRL, control group; mCTRL, matched control group; BMI, body mass index; MEWS, Modified Early Warning Score; SOFA, Sequential Organ Failure Assessment; HACOR, Heart rate, Acidosis, Consciousness, Oxygenation and Respiratory rate; ROX, ratio of SpO_2/FiO_2 to respiratory rate; GCS, Glasgow Coma Scale; MAP, mean arterial pressure; PaO_2/FiO_2, arterial blood partial pressure of oxygen to fraction of oxygen inspired ratio; RR, respiratory rate; NIV, noninvasive mechanical ventilation; HFNO, High Flow Nasal Oxygen; COT, conventional oxygen therapy; OSAS, obstructive sleep apnea syndrome; AMI, acute myocardial infarction; CAD, coronary artery disease; AE, adverse event; SAE, severe adverse event; NA, data not available; NFL, neurofilament light chain; GFAP, glial fibrillary acidic protein

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A B S T R A C T

Background aims: SARS-CoV-2 infection triggers respiratory inflammation with potentially fatal systemic effects. Mesenchymal stromal/stem cells (MSCs) are promising for treating severe COVID-19 due to their anti-inflammatory and regenerative capacities. This study investigates the effects of allogeneic MSCs in severe COVID-19 pneumonia.

Methods: In the phase I/IIa RESCAT trial (May 2021–Feb 2022), patients with severe COVID-19 pneumonia received two intravenous MSC infusions and were compared to a control group (CTRL). To assess cytokine and biomarker responses, the MSC group was matched 1:2 with standard care patients (mCTRL) by age, gender, BMI, and PaO2/FiO2 (Nov 2020–Feb 2021). Random-effects linear regression evaluated cytokine and biomarker trends over time between MSC and control groups.

Results: Seventeen patients (MSC = 5, CTRL = 2, mCTRL = 10) were analyzed. Two MSC infusions were feasible and safe, with all patients discharged on average 15 ± 3.7 days postsecond infusion. While IL1RA and IL18 levels significantly increased in CTRL-mCTRL patients ($P = 0.044$ and $P = 0.032$), MSC treatment averted these rises, showing a distinct trajectory, particularly for IL1RA. MSC treatment also reduced IL6 levels compared to CTRL-mCTRL, while both groups showed similar reductions in Long pentraxin. Furthermore, MSC infusions prevented the neurofilament light chain surge observed in CTRL patients.

Conclusions: MSC in COVID-19 patients resulted safe and feasible, effectively modulating inflammatory cytokines, in particular mitigating brain damage related biomarker, suggesting both reduced inflammation and a potential neurological protection.

Key Words: acute respiratory failure, ARDS, COVID-19, cytokine storm, lung inflammation, mesenchymal stem cell.

Introduction

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) causes acute, postacute, and long-term consequences through a dysregulated inflammatory response primarily targeting but not limited to the lungs [1–3]. This exaggerated immune response is linked with a cytokine storm leading to severe tissue damages [4]. A hallmark of severe Coronavirus Disease 2019 (COVID-19) is the increase of IL-6 and TNF-α, with severe cases also exhibiting increasedlevels of IL-2, IL-7, IL-10, CXCL10 (IP-10), CCL2 (MCP-1), CCL3 (MIP-1α), and G-CSF compared to milder infection [5]. The resulting cytokine storm spreads inflammation throughout multiple organs (liver, bone marrow, heart, kidneys, brain) [6]. In particular, neurological complications—ranging from mild to severe, and potentially leading to long-COVID–have received increased attention, with recent studies showing that COVID-19 can cause brain damage detectable through biomarkers like serum neurofilament light chain (NFL) and glial fibrillary acidic protein (sGFAP) [7].

Over the last years, relevant efforts have been made to evaluate the efficacy of early systemic anti-inflammatory treatments to limit SARS-CoV-2 induced tissue damage [8]. Among the others, several trials were underway globally to evaluate the safety and efficacy of mesenchymal stromal/stem cells (MSCs) infusion, which are known for their anti-inflammatory and immunomodulatory properties, also in COVID-19 patients [9]. Preliminary results suggested the potential of MSC-based therapies [10], while a more recent meta-analysis demonstrated that MSC infusion in patients with COVID-19 pneumonia is safe and reduces the occurrence of adverse events (AEs) and mortality rate [10]. Thus, it is possible that MSCs regulate the immune system and potentially prevent severe systemic consequences and clinical symptoms even in the long term [11,12]. In addition, MSCs can release extracellular vesicles containing bioactive molecules for immune modulation and tissue repair [13,14]. This study is an exploratory analysis of the phase I-IIa randomized controlled trial (RCT) RESCAT, conducted in a cohort of patients hospitalized with respiratory failure due to COVID-19 pneumonia. Specifically, we aimed at exploring and comparing the cytokine profile of patients receiving allogeneic MSCs to treat severe pneumonia related to SARS-CoV-2 with a control population. A sensitivity analysis on circulating biomarkers of multiorgan damage has been implemented and feasibility, safety together with the impact on respiratory function are additionally reported.

Materials and Methods

Study design and setting

This explorative investigation encompasses an immunological analysis on biological samples of patients enrolled within a prospective, multicenter, phase I/IIa RCT (https://www.aifa.gov.it/web/guest/ricerca-aifa?searchKeywords=RESCAT, RESCAT, EUDRACT code 2020-001577-70) evaluating the feasibility, safety, and efficacy of two intravenous infusions of allogeneic mesenchymal stromal/stem cells (MSCs) for severe SARS-CoV-2 pneumonia. Patients were recruited from Azienda Ospedaliero-Universitaria di Modena and Ospedale San Bartolo in Vicenza (Italy). (Italy). Written informed consent to participate in the study and to analyze and disseminate clinical data was obtained from all admitted patients.

Study aims

The primary aim was to explore and compare the circulating cytokine profile of patients receiving two allogeneic MSCs infusions to treat severe SARS-CoV-2 pneumonia compared to those treated with standard therapy. A sensitivity analysis on circulating biomarkers of multiorgan damage was also implemented. Furthermore, the feasibility, safety profile, and respiratory impact of MSCs treatment in the cohort of patients enrolled in the RESCAT trial were reported and described.

Patients

Adult patients with from SARS-CoV-2 and admitted to semi-intensive or intensive COVID Units between May 17th, 2021, and February 10th, 2022, for the Azienda Ospedaliero-Universitaria in Modena and between December 11th, 2021 to February 28th, 2022 for the Ospedale San Bortolo in Vicenza were considered eligible for enrollment. The inclusion criteria required patients to be aged 18–80 years, have a confirmed virological diagnosis of SARS-CoV-2 infection via real-time Polymerase Chain Reaction, be hospitalized due to clinical and radiological diagnosis of pneumonia, and have a PaO_2/FiO_2 value between 150 and 300 mmHg in the anticipated requirement for escalation to noninvasive respiratory support. Additionally, patients needed to have a systolic artery pressure greater than 90 mmHg without amine support and a Modified Early Warning Score (MEWS) of less than 3. Exclusion criteria included a history of previous embolism, known active malignancy, systemic viral, bacterial or fungal infections, the presence of significant comorbidities such as uncontrolled hypertension, invalidating psychiatric or neurological disorders as well as the presence of chronic advanced cardio-pulmonary diseases or organ failure. Additional exclusion criteria were pregnancy or nursing, major surgery (either open or laparoscopic) within the 3 months prior to screening, previous hematopoietic stem cell or organ transplantation, active immunosuppressive therapy, and other investigational drug within 2 months prior to enrollment. Patients who denied informed consent were also excluded. For detailed exclusion criteria see https://www.aifa.gov.it/web/guest/ricerca-aifa?searchKeywords=RESCAT.

All patients who met the inclusion and exclusion criteria were enrolled without the application of blinding. A nonblinded, randomized 2:1 design was adopted. Participants were assigned to either the experimental (MSC) or comparator (CTRL) arm using a central randomization service implemented in the electronic case report form The choice of the MSC product was determined by the center of enrollment, as each center adhered to its established protocols and utilized either adipose-derived or umbilical cord-derived MSCs accordingly. No placebo was administered to CTRL patients.

To compare the cytokine profile of patients receiving MSC to that of patients treated with standard therapy, the MSC cohort was further matched 1:2 by age, gender, BMI, and PaO_2/FIO_2 ratio (measured at the time of admission) to a group of COVID-19 patients not exposed to MSC (matched controls—mCTRL) extracted from a dataset of COVID-19 patients admitted to intensive care unit, Rianimazione 1 Fiera Milano COVID-19 (Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy) between November 2020 and February 2021 and previously characterized [15]. Matching procedure is showed in eFigure 1, Supplement.

Biomarkers analysis

Blood samples were collected at randomization (day 0) and at days 3, 6, 14, and 28, processed at the contributing centers, aliquoted, and cryopreserved at −80°C. Cytokine levels in human plasma were assessed using the Human 17-Plex Discovery Assay (BDNF, beta-NGF, CCL2, CCL3, CX3CL1, ICAM-1, IL-10, IL-18, IL-1B, IL-1ra, IL-2, IL-33, IL-4, IL-6, IL-8, PDGF-AB, TNFa) or the Human IL-17 alpha 1-Plex High Sensitivity Cytokine A Performance Assay, both from R&D Systems (Minneapolis, MN, USA) following the manufacturer's instructions (more details in the Supplement).

Long pentraxin 3 (PTX3) concentration was evaluated by a commercially available solid-phase enzyme-linked immunosorbent assay based on the sandwich principle (HycultBiotech, Uden, The Netherlands). In brief, patients' plasma was diluted at least ten folds with the supplied dilution buffer and tested according to the manufacturer's instructions. 450nm absorbance was evaluated by means of a multimodal microplate reader (Spark, Tecan Life Science, Switzerland) and PTX3 concentration was determined from the standard curve by means of Magellan software (Tecan). Circulating biomarkers of neuronal damage (NFL, TAU, UCHL-1) and astrocytic/blood-brain-barrier (BBB) damage (GFAP) were quantified using a commercially available single molecule array assay multiplex kit (Neuro 4-Plex B (#103345)) on an SR-X Analyzer, as per the manufacturer's instructions (Quanterix, Billerica, MA). Each analyte was assessed using a single batch of reagents. The BBB damage marker MMP-9 was measured using an AlphaLISA kit specific for the human protein (#AL3138, PerkinElmer). AlphaLISA signals were detected and quantified using an Ensight Multimode Plate Reader (PerkinElmer).

All analyses were centralized. PTX3 was measured at Fondazione IRCCS San Gerardo dei Tintori of Monza (Italy), while all the other inflammatory markers and acute brain injury markers were measured at Istituto di Ricerche Farmacologiche Mario Negri IRCCS of Milan, (Italy).

MSCs manufacturing and feasibility

Umbilical cord derived MSCs (UC-MSC) and adipose derived MSCs (AT-MSCs) were produced at the Laboratory for Advanced Cellular Therapies (authorization code: aM-49/2019, Vicenza, Italy) and the Cell Factory Rigenerand s.r.l. now EVOTEC Modena s.r.l. (authorization code: aM-25/2020, Medolla, Modena, Italy) respectively according to Good Manufacturing Practice [16,17]. For details regarding AT-MSC and UC-MSC manufacturing and cell identity see Supplementary Materials (eFigure 2–8 and eTable 1–5). Feasibility was assessed by evaluating the production capacity of the participating Cell Factories to produce an adequate quantity of cellular product lots sufficient to treat 100% of the recruited patients. The process of transferring the cellular product to the Clinical Units was validated to ensure that all quality, safety, and efficacy requirements were met, with a particular focus on maintaining the potency and cell viability of the cellular product.

Clinical assessment

Safety was assessed by recording and reporting all adverse events, coded according to the Common Terminology Criteria for Adverse Events version 5.0, based on their duration, intensity, and possible association with the treatment under study. Patients underwent short-term follow-up at 4 and 48 hours after each MSC infusion to monitor for early reactions related to the infusion. Additionally, medium-term follow-up was conducted at 2 and 4 weeks after the end of MSC treatment, and long-term follow-up on safety was conducted at 6 months. The assessment of adverse events and their potential relation to MSC administration was conducted by a dedicated data monitor who was not involved in the review or interpretation of the study results. The clinical effect of MSCs infusion was assessed by comparing several parameters between patients and controls. These included the trend of the daily PaO_2/FiO_2 ratio at protocolized time points, and the mortality rate at 2 and 4 weeks after the end of treatment. Respiratory parameters were also evaluated, such as the need to escalate to mechanical ventilation, time to independence from noninvasive respiratory support and oxygen therapy and time to hospital discharge. The decision to upgrade to invasive mechanical ventilation was made by the attending physician based on clinical judgment. This decision considered factors such as a significantly worsened PaO_2/FiO_2 ratio, the need to protect airways due to neurological deterioration or massive secretions, hemodynamic instability or major electrocardiographic abnormalities, unchanged or worsened dyspnea, and the persistence of respiratory distress (respiratory rate $>$ 35 breath per minute [bpm], gasping for air, psychomotor agitation requiring sedation, or abdominal paradox). Similarly, the decision to wean from noninvasive respiratory support was based on the attending physician's clinical judgment, considering a respiratory rate $<$ 25 bpm, absence of signs of respiratory distress, and $SpO_2 > 94\%$ after a 1-hour trial of O_2 support via a Venturi mask with FiO_2 set $< 40\%$. Weaning from oxygen support was decided based on the presence of arterial $pO_2 > 60$ mmHg in room air during blood gas analysis.

Statistics

Patient demographics and clinical characteristics were reported individually. Descriptive statistics were used to summarize the data, presenting categorical variables as frequencies and percentages, and continuous variables as medians and interquartile ranges, as appropriate. Given the exploratory and descriptive nature of this investigation, we have confined the use of inferential statistics to the analysis of biomarker data and have excluded them from the clinical evaluations to prevent overstating the findings. To assess whether the change in the concentration of the circulating biomarkers differed over time and between treated and control patients, we used random-effect (RE) linear regression [18], whose details are reported in the Supplement. Analyses were performed with R, version 4.3.2, using the lme4 package.

Results

Study population

During the study period, 7 patients (1 from Vicenza and 6 from Modena) admitted to semi-intensive or intensive COVID units were enrolled in the RESCAT® protocol and randomized into the MSC

group (n = 5) and the CTRL group (n = 2). Among MSC-patients, 4 received AT-MSCs, while one received UC-MSCs. Following the matching procedure, 10 patients formed the mCTRL group. The demographics features and clinical characteristics of the study populations, are presented in Table 1, *part A* for MSC and CTRL groups and *part B* for the mCTRL. Patients in the MSC cohort were prevalently male (n = 4/5, 80.0%) with a median age of 58.3 years IQR (56.5–60.2). At the time of randomization, all patients presented with mild to moderate respiratory failure, with none having a $PaO_2/FiO_2 < 100$ mmHg. The SOFA score was 2 for all patients enrolled in the RESCAT®, while the ROX index ranged from 13.4 to 9.3. All patients received steroid treatment before or at the time of randomization. One patient in the MSC group underwent Tocilizumab infusion 7 days before MSC infusion, while one in the CTRL group received antiviral therapy. Three out of 5 patients treated with MSC and all patients in the mCTRL cohort received HFNO and/or noninvasive mechanical ventilation (NIV) as first-line therapy, whereas none in the CTRL cohort did.

Allogeneic MSC intravenous infusions are feasible

Allogeneic MSCs were successfully isolated from both adipose tissue and umbilical cord. From 2.5 grams of adipose tissue, 1.22×10^{10} AT-MSCs were obtained in 16 days. Regarding the umbilical cord, a 12-cm fragment yielded 1.12×10^{9} cells after 27 days. The release criteria for AT-MSCs and UT-MSCs have been validated (Supplementary eTable 1 and eTable 2). AT-MSCs were frozen in liquid nitrogen using Miltenyi Biotec CryoMACS Freezing Bag 50. The final medicinal product consists of 50×10^{6} allogeneic AT-MSCs, formulated in 25 mL of freezing medium composed of 20 mL saline solution (80%), supplemented with 2.5 mL DMSO (10%) and 2.5 mL Albumin 200g/l (10%), as outlined in Supplementary eTable 3. UC-MSCs were similarly frozen in liquid nitrogen using the Miltenyi Biotec CryoMACS Freezing Bag 50. The final product consists of either 25 or 50×10^{6} allogeneic UC-MSCs, also formulated in 25 mL of freezing medium, which includes 20 mL saline solution (80%), 2.5 mL DMSO (10%), and 2.5 mL Albumin 200g/l (10%), as reported in Supplementary eTable 3. Shipment from the cell factories to the clinical site pharmacy was carried out for AT-MSCs using temperature-controlled nitrogen tanks by certified courier Criolab (SOLGROUP, Pavia, Italy). For UC-MSCs, shipment took place on dry ice, following a validated procedure from a hospital service located a 5-minute walk from the cell factory to the ward.

Infusion procedures were performed after thawing the bags in a water bath (37°C) under a class A hood, followed by infusion within 30 minutes. In one case, AT-MSCs viability from a minimal volume bag wash (5 mL of saline) was tested using 7-AAD at 45 minutes post-thawing, and FACS analysis showed 86.6% viable cells, consistent with the results obtained in the stability study at three months postfreezing (85.35% viability at 45 minutes). For UC-MSCs, cells were thawed at 37°C after a 1:1 dilution in thawing solution (saline, 10% v/v human albumin, 12% ACD-A). All treatments were successfully completed.

Allogeneic MSC infusions retain an acceptable safety profile with clinical impact

Among MSC patients, incidence of AEs and SAEs was 3 and 3 respectively while there were no AEs and SAEs for CTRL. All SAE occurred in the same patient and were not related to MSC infusion. Of the reported AEs, only one was deemed likely to be related to the treatment (maculo-papular rush), while the others were considered unrelated. There were no deaths in both groups at 2 and 4 weeks after the end of treatment. One former smoker patient of the MSCs group died 5 months after the end of treatment due complications related to a non-small cell lung cancer diagnosed 2 months after discharge. Complete safety profile is shown in Table 2.

The time trend of PaO_2/FiO_2 ratio between the cohorts at each time point since randomization is shown in Figure 1A. None of the patients enrolled in each cohort proceed to invasive mechanical ventilation support. MSC group reached oxygen weaning after 8 (4–13.5) days as compared to 5 (4–6) days of CTRL. Length of hospital stay was 15 (11.5–18.5) days for MSC and 11 (10–12) days for CTR. Figure 1B summarizes the clinical course of enrolled patients, highlighting the utilization of respiratory support.

Infused MSC impact on cytokine profile and circulating biomarkers

We employed a random effects linear regression to evaluate the average treatment effect on cytokines and biomarkers' changes over time, considering the individual trajectory (i.e., slope) for each patient. Figure 2A provides a graphical overview of the temporal changes in biomarkers' slopes for each group, with green indicating a decrease, white no changes and purple an increase of the plasma levels over time. Supplementary eTable 6 reports the slope estimates with standard errors and 95% confidence intervals. Slopes statistically different from zero are reported with an asterisk in Figure 2A.

In CTRL-mCTRL, IL1RA and IL18 significantly increased over time ($P = 0.044$ and $P = 0.032$ respectively) (Figure 2A and eTable 6). Treatment with MSC prevented the increase of these cytokines resulting in a significantly different trajectory of IL1RA (slope −0.007 [−0.019–0.004] versus 0.013 CI [0–0.025], $P = 0.025$), and a trend toward significance for IL18 (slope −0.002 [−0.008–0.004] versus 0.007 CI [0.001–0.013], $P = 0.068$) which remained stable (Figure 2A and B and eTable 6) as compared to CTRL-mCTRL. Notably, MSC treatment significantly reduced IL6 levels ($P = 0.033$) (Figure 2A and Supplementary eTable 6). Moreover, the trajectory of IL6 differed significantly between MSC-treated and CTRL-mCTRL COVID-19 patients ($P = 0.047$) (Figure 2A and B; eTable 6). A similar trend was observed for CRP, which also exhibited a significantly different trajectory in MSC-treated patients (Supplementary eFigure 9A and eTable 6). We also observed a significant decrease of PTX3 both in CTRL-mCTRL and MSC COVID-19 patients ($P = 0.001$ and $P < 0.001$) with no different slopes between groups ($P = 0.189$) (Figure 2A and Supplementary eFigure 9A and eTable 6).

All other inflammatory markers exhibited either no significant changes over time or comparable trends in both CTRL-mCTRL or MSC treated patients (Figure 2A and Supplementary eFigure 9A and eTable 6). Concentrations of IL-1β, IL-2, IL-17, and IL-33 were below the detection range in both groups.

By analyzing plasma levels of brain and astrocytic/BBB damage biomarkers, we observed a steep increase overtime of NFL in CTRL patients ($P < 0.001$). Of note, MSC treatment abolished the NFL temporal rise, leading to a significantly different slope compared to CTRL patients (slope 0.002 [−0.012–0.015] versus 0.029 CI [0.016–0.041], $P = 0.012$) (Figure 2A–B). Trajectories of the neuronal biomarkers TAU, UCHL-1, the astrocytic/BBB biomarkers GFAP, and MMP9 and their associated pro-survival growth factors (BDNF, NGF-beta and PDGF-AB) did not show significant differences between groups (Figure 2A, Supplementary eFigure 9B and C and eTable 6)

Discussion

The emergence of the COVID-19 pandemic, coupled with the early shortage of validated treatments, drove researchers and clinicians to urgently seek new therapeutic options. In this context, MSCs have shown promise as a treatment for severe pneumonia and ARDS related to COVID-19, with clinical trials yielding encouraging results [9]. However, MSC role in managing systemic effects and extrapulmonary complications remains unclear. This study, part of the phase I (focused on safety and feasibility)-II (exploratory efficacy analyses)

Table 1
Baseline demographic and clinical features of the study population presented according to treatment cohorts.

Part A

	MSC					*CTRL*	
Patient ID	1	3	4	5	7	2	6
Type of MSC	AT	AT	AT	UC	AT	NA	NA
Age, years	58	60	56	52	78	57	57
Ethnicity	White	White	White	White	White	White	White
Male sex, y/n	y	y	y	n	y	y	y
BMI, kg/m^2	25.2	26.5	28.7	26.2	40	29.9	26.9
Days from diagnosis to randomization, days	8	6	14	8	2	8	8
MEWS, score	2	2	2	1	1	1	2
Comorbidities	None	Diverticulosis, dyslipidemia	Prostatitis, previous intestinal occlusion	None	Diabetes, CAD, hypertension, OSAS	Hypertension	Hypertension, spondylarthritis
Smoking habits	No	Former	No	No	Former	No	Former
SOFA, score	2	2	2	2	2	2	2
HACOR, score	2	0	2	2	3	3	0
ROX, index	12.3	13.4	9.3	12.5	11.9	11.8	12.9
GCS, score	15	15	15	15	15	15	15
MAP, mmHg	85	110	97	80	92	98	85
RR, bpm	26	24	24	20	20	20	26
PaO_2/FiO_2 at enrollment, mmHg	193	209	183	200	171	175	238
HFNO, *y/n*	y	n	y	y	n	n	n
NIV, *y/n*	n	n	n	y	n	n	n
COT only, y/n	n	y	n	n	y	y	y
Pharmacological therapy	Steroids	Steroids	Steroids, Tocilizumab	Steroids	Steroids	Steroids	Steroids, casirivimab-imdevimab
Days with HFNO after enrollment, days	3	0	10	10	0	0	0
Days with NIV after enrollment, days	0	0	0	10	0	0	0
Days with COT after enrollment, days	3	2	2	5	8	6	4
Length of stay, days	13	18	15	19	10	10	12

Part B

	mCTRL									
Patient ID	8	9	10	11	12	13	14	15	16	17
Age, years	59	59	67	66	58	58	50	49	75	74
Ethnicity	Caucasian	Hispanic	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian
Male sex, y/n	y	y	y	y	y	y	n	n	y	y
BMI, kg/m^2	26.1	31.2	29.3	29.4	27.8	26.1	27.3	38.1	24.3	26.2
MEWS, score	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Comorbidities	Hypertension, vasculopathy	None	Hypertension, diabetes	Hypertension	Asthma, Hyperthyroidism	Hypertension	Hyper-tension	None	Previous AMI	Hypertension
Smoking habits	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
SOFA, score	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
HACOR, score	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
ROX, index	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
GCS, score	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
MAP, mmHg	109	107	87	75	81	75	80	97	81	93
RR, bpm	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
PaO_2/FiO_2 at admission, mmHg	142	156	198	187	180	172	171	250	134	210
HFNO, *y/n*	y	y	y	y	y	y	y	y	y	y
Pharmacological therapy	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Days with HFNO after admission, days	16	51	14	30	43	44	30	7	15	19
Days with COT after admission, days	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Length of stay, days	17	81	14	33	48	47	35	12	17	18

Part A refers to data from the RESCAT® trial while *Part B* reports the characteristics of matched controls from the cohort described in Bonetto *et al.* [15].
BMI, body mass index; MEWS, modified early warning score; SOFA, sequential organ failure assessment; HACOR, heart rate, acidosis, consciousness, oxygenation and respiratory rate; ROX, ratio of SpO_2/FiO_2 to respiratory rate; GCS, Glasgow Coma Scale; MAP, mean arterial pressure; PaO_2/FiO_2; arterial blood partial pressure of oxygen to fraction of oxygen inspired ratio; RR, respiratory rate; HFNO, high flow nasal oxygen; COT, conventional oxygen therapy; MSC, mesenchymal stromal/stem cell treatment group; CTRL, control group; mCTRL, matched controls; OSAS, obstructive sleep apnea syndrome; AMI, acute myocardial infarction; CAD, coronary artery disease.

Table 2
Adverse events reported for MSC patients.

AE category	AEs			SAEs		
Type of event	Dyspnea	Rash maculo-papular	Hemorrhoids	Respiratory failure	Rectal hemorrhage	Cardiac arrest
Patient ID	4	5	5	7	7	7
Days since randomization	15	35	NA	33	33	161
Severity	Mild	Moderate	Mild	Moderate	Moderate	Severe
Relation to study drug	Unlikely	Probably	Unlikely	Unlikely	Unlikely	Unlikely
Concomitant medication	N	y	n	y	n	n
Outcome	Resolved	Resolved	Resolved	Resolved	Resolved	Unresolved

AE, adverse event; SAE, severe adverse event; NA, data not available.

RESCAT RCT, investigated the circulating cytokine profiles of patients hospitalized with COVID-19 pneumonia, comparing those treated with allogeneic MSCs to control subjects. Additionally, we reported data on the safety profile and clinical course of the enrolled patients. Our findings showed that two MSCs infusions led to a distinct cytokine profile, characterized by reduced levels of IL-1RA and IL-6 in treated patients. Further and for the first time, we report that MSCs treatment mitigated the increase in the brain damage biomarker NFL. In terms of safety, MSCs treatment resulted in only a single transient and self-limited adverse event, while the clinical improvement observed in MSC-treated patients was comparable to that of the control group.

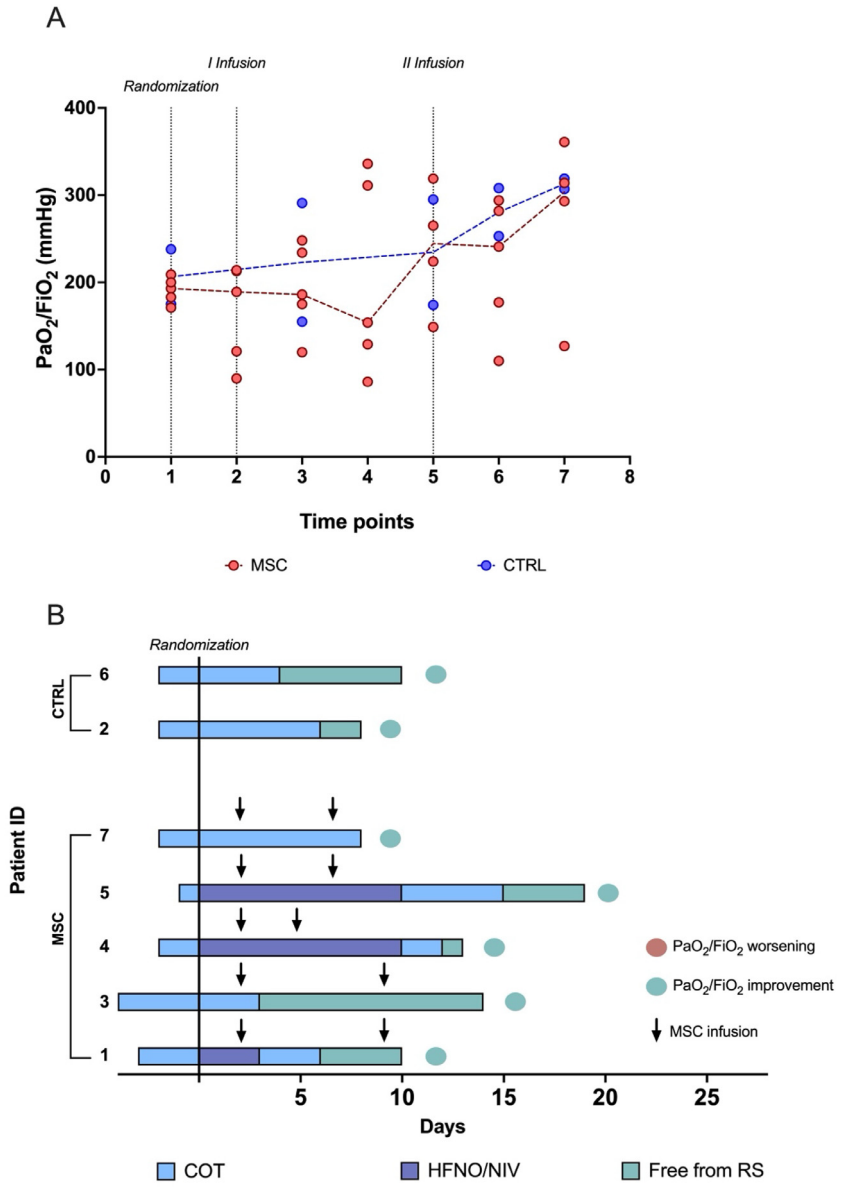

Fig. 1. Daily trend of PaO_2/FiO_2 ratio for MSC and CTRL. (A) Time trend of patients' gas exchange, expressed by the PaO_2/FiO_2 ratio at each per-protocol time point, for the MSC (n = 5) and CTRL (n = 2) groups. (B) Clinical course from admission to discharged for each patient enrolled reporting the use of respiratory support. MSC, mesenchymal stromal/stem cell treatment group; CTRL, control group, HFNO, high flow nasal oxygen; NIV, noninvasive mechanical ventilation; COT, conventional oxygen therapy; RS respiratory support. (Color version of figure is available online.)

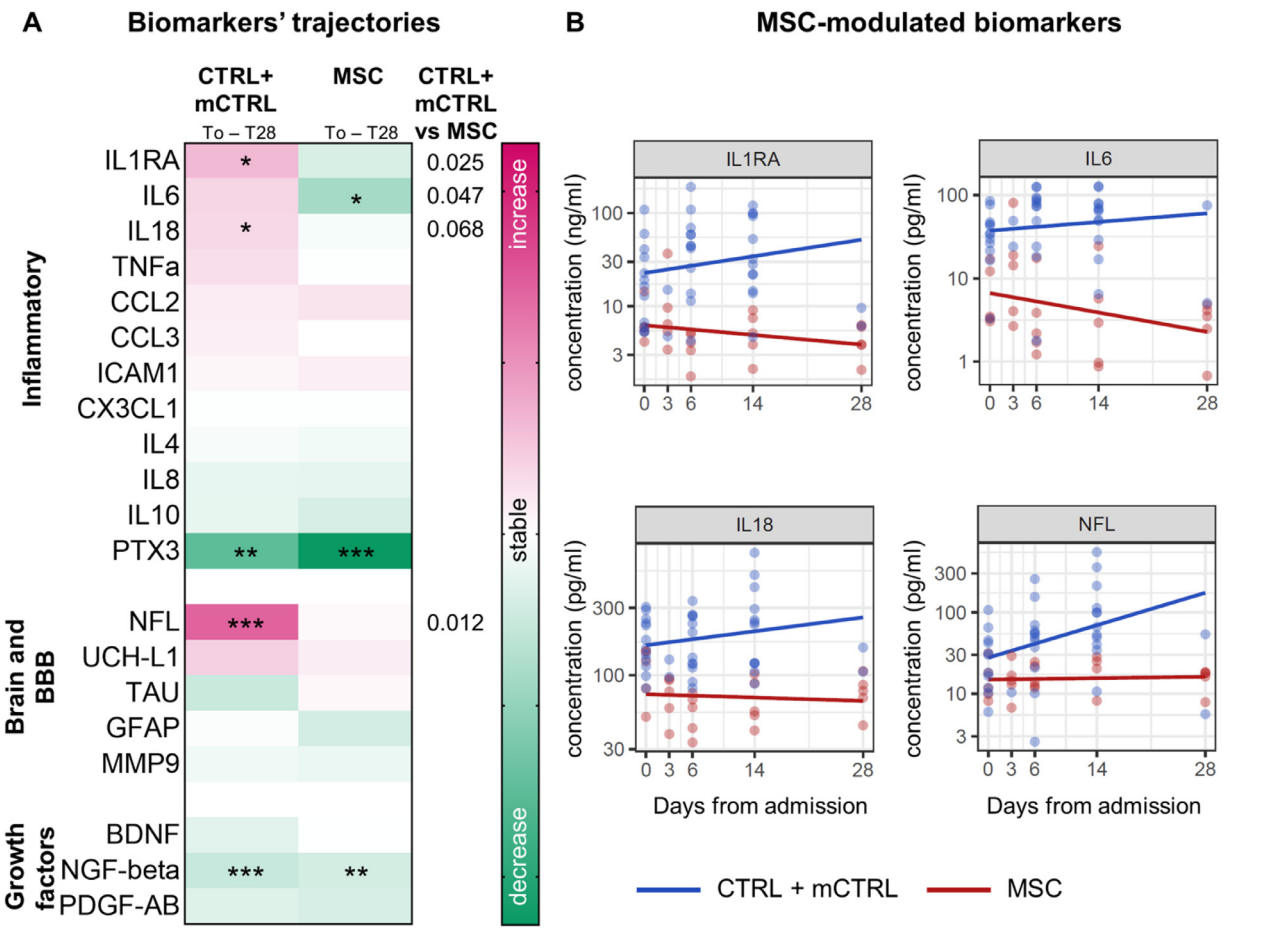

Fig. 2. Plasma biomarkers' trajectories. (A) Graphical overview of biomarkers' temporal changes based on biomarkers' slopes in COVID-19 patients either untreated or treated with mesenchymal stroma cells. Green indicates a decrease, white no changes, and purple an increase of each biomarker over time.*Asterisks refer to biomarkers' slopes significantly different from 0. *P* values are reported for significant, or trend-towards-significance, differences in the biomarkers' slopes in CTRL-mCTRL (n = 12) versus MSC (n = 5) COVID-19 patients. (B) Temporal trajectories of biomarkers resulted significantly modulated in MSC treated (red) versus untreated (blue) COVID-19 patients. Each plot represents the average "typical" lines for treated and untreated patients as estimated by random-effect linear regression. Samples were collected at the time of randomization (0) and after 3, 6, 14 and 28 days. COVID-19, coronavirus disease 2019, MSC, mesenchymal stromal/stem cell treatment group; CTRL, control group; mCTRL, matched control group; IL1RA, interleukin-1 receptor antagonist; IL6, interleukin-6; IL18, interleukin-18; TNFα, tumor necrosis factor alpha; CCL2, C-C motif chemokine ligand 2 (monocyte chemoattractant protein-1, MCP-1); CCL3, C-C motif chemokine ligand 3 (macrophage inflammatory protein-1α, MIP-1α); ICAM1, intercellular adhesion molecule 1; CX3CL1, C-X3-C motif chemokine ligand 1 (fractalkine); IL4, interleukin-4; IL8, interleukin-8; IL10, interleukin-10; PTX3, pentraxin 3; NFL, neurofilament light chain; UCH-L1, ubiquitin C-terminal hydrolase L1; TAU, tau protein; GFAP, glial fibrillary acidic protein; MMP9, matrix metalloproteinase-9; BDNF, brain-derived neurotrophic factor; NGF-beta, nerve growth factor beta; PDGF-AB, platelet-derived growth factor AB. (Color version of figure is available online.)

MSC-based therapies have been widely investigated in numerous both acute and chronic conditions [19,20] and no significant risks of serious adverse events after intravascular infusion has been observed [21]. During SARS-CoV-2 pandemia, MSCs have been largely examined in severely ill patients, demonstrating the overall safety of this treatment [9,22]. Furthermore, a phase I study has validated the safety of both high and low doses of MSCs also for nonsevere COVID-19 cases [23]. Our study confirms those findings, given the absence of serious AEs related to MSCs administration in patients enrolled. We observed a self-limited maculopapular rash following MSC infusion, likely related to the treatment, but no other adverse events were linked to the therapy.

In terms of efficacy, preclinical studies in non-COVID-19 ARDS suggest that MSCs enhance alveolar fluid clearance and oxygenation [24], while clinical trials have shown encouraging trends [25,26]. However, RCTs have yet to consistently demonstrate a survival benefit. Clinical translation remains challenging due to variability in study designs, MSC sources, and administration protocols. Recent meta-analyses indicate that MSC therapy may reduce mortality with low heterogeneity, but larger, high-quality trials are needed to confirm its clinical impact [27]. In patients with severe COVID-19, MSC therapy has shown potential in modulating inflammation and improving oxygenation [9]. Shu *et al.* first observed that patients treated with umbilical cord-derived MSCs experienced rapid improvement in oxygenation [28]. Similarly, in a phase 1 trial, prenatal MSCs from umbilical cord and placental tissues increased SpO2 within 2–4 days postinfusion in 7 out of 11 patients with COVID-19-induced ARDS [29]. Notably, a multicenter, placebo-controlled trial (STROMA-CoV-2) demonstrated a significant increase in PaO2/FiO2 ratios in 21 umbilical cord MSC-treated ARDS patients, providing stronger evidence due to its controlled design [30]. Additionally, other studies have reported improvements in SpO_2/FiO_2 ratios and oxygenation levels following MSC therapy derived from menstrual blood and placenta in patients with severe and critical COVID-19 [13,31,32] However, the methodological limitations of many studies—such as open-label designs, lack of blinding, and absence of control groups—continue to limit the strength of conclusions regarding MSC therapy's efficacy in COVID-19 [33]. In our cohort, 3 out of 5 patients who received MSC treatment exhibited an early increase in PaO_2/FiO_2 following infusion, with all patients showing improved PaO_2/FiO_2 levels from baseline within 14 days (data not shown). This finding aligns with previous reports [1,34–37]. A similar trend was observed in the control group, with some patients demonstrating early improvements in oxygenation indices. Given that the average baseline PaO_2/

FiO_2 in our cohort was somewhat higher than previously reported [9], we could hypothesize that MSCs may offer more substantial benefits for patients with more severe lung impairment. Notably, none of the MSC-treated patients required intensive care, despite 3 out of 5 needing respiratory support (i.e., HNFO or NIV) at the time of infusion. All patients survived and were discharged within 3 weeks of randomization, with hospital stays comparable to those of the control group. However, the earlier improvement observed in the control group suggests that MSC treatment did not confer a clear clinical advantage in this small cohort. This could reflect the fact that our cohort consisted of patients who were not critically ill at inclusion but were candidates for escalation to respiratory support and closely monitored, distinguishing them from other MSC trials that included patients already receiving MV or NIV at study outset. These observations underscore the need for larger, controlled studies to determine the clinical benefits of MSC therapy in COVID-19 and to identify which patient populations may derive the greatest benefit.

MSCs treatment has demonstrated significant improvements in inflammatory biomarkers and cytokines associated with severe COVID-19 [9,38]. Early studies have shown reductions in C reactive protein (CRP) and IL-6 levels, as well as rapid normalization of lymphocyte counts in severe cases following umbilical cord derived-MSC infusion [28]. Further investigations reported that MSC treatment led to lower concentrations of various inflammatory markers, such as GM-CSF and TNFα, with decreased levels of cytokines like IL1RA, IL5, IL7, MCP1, interferon-inducible cytokine IP10 (IP-10), IL22, IL18, IL8, and macrophage inflammatory protein 1-alpha (MIP1), and IFNγ in patients with COVID-19 associated ARDS[1] [20,31,34,35]. Our findings align with previous studies, showing that MSC treatment prevented the significant increases in IL1RA and IL18 levels seen in the control group, thereby stabilizing these cytokines, altering their trajectories and dampening the inflammatory response. Additionally, MSC treatment significantly reduced IL6 and CRP levels compared to the control group.

As sensitivity analysis, we explored the plasma levels of brain and astrocytic/BBB damage biomarkers. In this line, NFL has been gaining recognition as a valuable blood biomarker for brain damage, being related to clinical outcomes of traumatic injured patients [39–41].

In our study, while NFL levels appeared to align between the MSC and control groups by day 28, a notable divergence was observed at day 14, suggesting a potential early effect of MSC therapy on neurological injury markers. This transient difference may reflect the short-term paracrine activity of MSCs, which warrants further investigation in larger, more detailed studies. A retrospective review of medical records at six months found no reported neurological abnormalities in surviving MSC-treated patients, while one CTRL patient experienced fatigue, mild dizziness, and lightheadedness. As these findings were not part of the protocol analysis, they should be interpreted with caution. Furthermore, given the small sample size, any observed associations between MSC treatment and NFL trajectories remain exploratory, as the study underpowered. However, if confirmed, these findings could have significant implications for long-COVID-19 syndrome, where neurological impairments are a major concern. Recent evidence from a randomized controlled phase 2a trial in mild Alzheimer's disease further supports the neuroprotective potential of MSC therapy [42]. These findings align with our hypothesis that MSCs may modulate neuroinflammatory pathways implicated in post-COVID neurological sequelae. By mitigating neuroinflammation and preserving neuronal integrity, MSC therapy could help reduce the burden of long-COVID-related cognitive dysfunction. Moreover, its ability to attenuate the rise in NFL levels suggests a potential role in preventing or limiting persistent neurological damage in this population [43]. Nevertheless, the absence of significant changes in other neurological biomarkers, such as GFAP and TAU proteins, suggests that the observed modulation of NFL levels may not fully capture the extent of neuroaxonal injury. This highlights the need for a comprehensive biomarker panel to accurately assess neurological outcomes in future studies.

Our study has several limitations. Initially, the RESCAT trial aimed to enroll a larger cohort; however, recruitment was hindered by the waning of the COVID-19 pandemic, competing trial enrollment, delays in ethical approvals across centers, and restrictive exclusion criteria. Consequently, as previously stated, the small sample size limits statistical confidence in detecting meaningful associations between MSC therapy and inflammatory biomarker trajectories, precluding definitive conclusions on clinical efficacy. Rather, this exploratory analysis provides a descriptive assessment of MSC safety and biological effects in COVID-19 pneumonia. Secondly, while our results suggest that MSC therapy modulates inflammatory responses, heterogeneity in baseline inflammatory markers adds complexity to the interpretation of cytokine trajectories. Additionally, we did not account for potential confounders, such as variations in pharmacological treatments—including prior administration of immunomodulatory agents like tocilizumab—which may have influenced cytokine profiles and inflammatory responses. To evaluate the potential impact of multiple comparisons, we computed the number of statistical tests performed in the primary biomarker analysis, specifically comparing the slopes of control and treated patients. A total of 20 tests were conducted, one for each biomarker. Under the null hypothesis of no real difference between slopes, 5% of tests performed at the 0.05 level would be expected to be significant by chance, equating to one false positive. Given that three biomarkers reached statistical significance, we are confident that the observed differences are unlikely to be solely attributable to type I error inflation from multiple testing. Nonetheless, we acknowledge the inherent limitations of multiple comparisons in small studies and have exercised caution in the interpretation of these results. Future studies should incorporate larger cohorts and appropriate statistical corrections to enhance the robustness and reproducibility of findings. Thirdly, our study design did not include long-term follow-up to assess whether the observed stabilization of NFL levels translated into clinically relevant neurological outcomes. Given the growing recognition of persistent neurological complications in post-COVID-19 syndrome, future studies should incorporate neurocognitive assessments and imaging to better characterize the potential neuroprotective effects of MSC therapy. Moreover, the study utilized two different MSC products (adipose-derived and umbilical cord-derived), which may exhibit differential biological activity. While both cell types met standard quality criteria, the small sample size did not allow for a direct comparison of their effects. Future studies should address whether tissue source influences the therapeutic properties of MSCs in respiratory and systemic inflammation. Finally, we acknowledge the temporal differences between the two periods in which mCTRL patients were treated. However, the comparability of the MSC and mCTRL groups remains supported by the consistent use of corticosteroids as the standard treatment for severe COVID-19 since mid-2020 [44], minimizing potential bias related to treatment evolution. Despite these limitations, our findings provide valuable insights into the immunomodulatory effects of MSC therapy and warrant further investigation in larger, well-powered, and controlled trials. Understanding how MSCs influence inflammatory and neurological markers may open new avenues for mitigating severe COVID-19 complications and systemic inflammation in other conditions.

Conclusion

This exploratory investigation suggests that MSC therapy may reduce inflammatory cytokine levels, particularly IL-6 and IL-1RA, which are key mediators of the cytokine storm associated with severe COVID-19. Additionally, MSC therapy appears to stabilize NFL levels, a biomarker of neuronal injury, indicating a potential neuroprotective

effect. This may reduce the risk of brain injury and neurological complications, which are increasingly recognized as significant consequences of COVID-19 [38]. By effectively dampening the hyperinflammatory response, MSCs could contribute to preventing long-COVID syndromes, including systemic and neurological complications. These findings align with the emerging recognition of persistent symptoms such as "brain fog," reported in a substantial proportion of post-COVID patients [45]. However, these preliminary observations require validation through larger, controlled studies to confirm the therapeutic potential of MSCs in this population.

Ethics Statement

The studies involving human participants were reviewed and approved by the ethics committees of the clinical centers involved. Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milano, Italy (approval number 868_2020) on October 10th, 2020), Italian Medicine Agency (AIFA), Rome (Italy) (protocol SC/P 115763) on October 20th, 2020, National Institute for Infectious Diseases Istituto Lazzaro Spallanzani, Rome (Italy) (protocol number 238) on December, 28th, 2020. EUDRACT code 2020-001577-70 The patients/participants provided their written informed consent to participate in this study.

Availability of Data and Materials

Data are available at: Respiratory Disease Unit of the University Hospital of Modena, Italy, Center for Outcomes Research and Clinical Epidemiology (CORESEARCH) S.r.l., Pescara, Italy and Department of Neuroscience, Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Milan, Italy upon request.

Declaration of Generative AI and AI-Assisted Technologies in the Writing Process

During the preparation of this work the authors used ChatGPT in order to improve language and readability. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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Declaration of Competing Interest

The authors have no commercial, proprietary, or financial interest in the products or companies described in this article.

Author Contributions

RT enrolled patients, collected clinical data, conducted clinical data analysis, wrote, and edited the manuscript, and produced the tables and the figures. FP and EZ designed the study, performed circulating biomarker analysis of patient samples, contributed to the writing and editing of the manuscript, and produced the tables and the figures. GB, LM and FE enrolled patients wrote and edited the manuscript. GG and GA made substantial contributions to cell preparation, participated in writing and editing the manuscript, and produced the tables and the figures. MB, DC, and AM were responsible for cell preparation from umbilical cord samples and assisted in drafting and editing the manuscript. GN and FS conducted the statistical analysis of circulating biomarkers for both patients and controls and contributed to the drafting and editing of the manuscript. IL, LP, VB, ED, and GDA significantly contributed to circulating biomarker analysis of patient samples and supported the manuscript's drafting and editing. LF, LB, SG and OC oversaw cell preparation from adipose tissue and regulatory activities and took part in manuscript drafting and editing. TZ enrolled patients in the mCTRL cohort and contributed to drafting and editing the manuscript. CS, SC, VR, AVS, and EP played key roles in cell preparation from adipose tissue before infusion, collected data and assisted in drafting and editing the manuscript. FDP and MM were involved in patient enrollment and cell infusion, collected data and contributed to the drafting and editing of the manuscript. GM was responsible for adipose derive cell expansion and contributed to drafting and editing the manuscript. GDB, PM, and MCB were responsible for the study governance, research documentation, ethical procedures, and contributed to drafting and editing the manuscript. LL, FB and RC supported the design of study protocol and edited the manuscript. EC and MD designed the study, were responsible for fundings, defined the endpoints, led the research group and coordinated the writing and editing the manuscript thus sharing senior authorship. All the authors read and approved the final version of the manuscript.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.jcyt.2025.04.059.

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