Impact of Placenta-Derived Mesenchymal Stem Cells Treatment on Patients with Severe Lung Injury Caused by COVID-19 Pneumonia: Clinical and Immunological Aspect

Mei-Chuan Chen
Taipei Medical University Hospital
Kevin Shu-Leung Lai
Taipei Medical University Hospital
Ko-Ling Chien
Taipei Medical University Hospital
Sing-Teck Teng
Taipei Medical University Hospital
Yuh-Rong Lin
Taipei Medical University
Wei Chao
Taipei Medical University
Meng-Jung Lee
Taipei Medical University
Po-Li Wei
Taipei Medical University Hospital
Yen-Hua Huang
Taipei Medical University
Han-Pin Kuo
Taipei Medical University
Chih-Ming Weng
Taipei Medical College: Taipei Medical University
Chun-Liang Chou (drchou1024@gmail.com)
Taipei Medical University Hospital https://orcid.org/0000-0002-8061-703X

Research Article

Keywords: COVID-19, Mesenchymal stem cell, severe lung injury, immune response

Posted Date: November 30th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-1013382/v1

Abstract

Background:

The novel coronavirus disease 2019 (COVID-19) has been a global pandemic health issue since 30, January, 2020. Mortality rate was as high as more than 50% in critically ill patients. The Stem cell treatment is effective in refractory severe critically ill COVID-19 patients, but immune regulation mechanisms have not been reported well. Therefore, we evaluate the clinical efficacy and immune modulation of placenta-derived mesenchymal stem cells (pcMSCs) (MatriPlax) in severe critically ill COVID-19 infection who are refractory to current standard therapies.

Methods:

Intravenous infusion of 1×10^7 MatriPlax was given to five severe COVID-19 patients at Day 0 and day 4. Serum inflammatory markers and immune profiles were studied at Day 0, 4 and 8. Clinical parameters and 28-days mortality were compared between treated group and control group.

Results:

The treatment group had no 28-days mortality and Murray's lung injury score was significantly improved compared with control group. After treatment, Ferritin, C-reactive protein (CRP) and Lactate dehydrogenases (LDH) were significantly reduced and lymphopenia was improved. IL-6, IL-1β, IFN-γ and IL-2 were significantly decreased together with decrease in IL-10 reflecting decreasing intensity of inflammation. Immune cell profiles showed increase in $CD4^+$ T cells (CD4+ naïve T cells, CD4+ memory T cells subtypes), Treg cells, $CD19^+$ B cells (and $CD19^+$ naive B cells, $CD27^+$ switched B cells subtypes) and dendritic cells, and a significant decrease in $CD14^+$ monocytes (and $CD16^-$ classical, $CD16^+$ non-classical subtypes) monocytes as well as plasma/plasmablast cells. pc-MSCs treatment suppressed hyper-inflammatory states of innate immune responses to COVID-19 infection by increasing Treg cells, decreasing monocytes and plasma/plasmablast cells, and promoted $CD4^+$ T cells and $CD19^+$ B cells towards adaptive immune responses.

Conclusion:

The intravenous transplantation of Matriplax was safe and effective for severe critically ill COVID-19 patients, especially those who were refractory to current standard care and immunosuppressive therapies

Introduction

The novel coronavirus disease 2019 (COVID-19) has been a global pandemic health issue since 30 January, 2020 [1]. In Taiwan, the pandemic occurs in the middle of May, 2021. Among those infected in the world, around 80% of the cases were mild disease. Nearly 20% of the infected patients were in severe clinical courses and 5% were in critical condition [2]. In critically ill patients, mortality rates were found

>50% [3, 4]. The severity of COVID-19 infection varies among individuals [5], and is attributed to the different magnitude of dysregulation of cytokine responses to viral infection [6]. Therefore, immunomodulatory therapies including Dexamethasone [7], Tocilizumab [8] and Barcitinib [9] have been applied to restore the immune dysregulation, and have been shown effective in reducing mortality in severe COVID patients. However, despite of intensive care accompanied with those immunotherapies, there is a persistent high mortality rate in severe COVID-19, especially those with respiratory failure [10]. To this end, there are lots of emerging inhibitors of specific inflammatory pathways proposed [1]. However, most treatments are pending to be validated in large-scale trials.

Mesenchymal stem cells (MSCs) are multipotent adult stem cells derived from various tissues and organs, including bone marrow, adipose, umbilical cord and placenta with variable magnitudes of proliferation and behaviors [11, 12]. The beneficial effect of MSCs in alleviating the diseased state is attributed to their cytokine secretion, migration ability and the immunomodulatory function. MSCs exert anti-inflammatory effects by secreting cytokines such as prostaglandin E2 (PGE2), Interleukin 10 (IL-10), Interleukin 6 (IL-6), transforming growth factor β (TGFβ), hepatocyte growth factor (HGF) etc. on various cells of immune system such as dendritic cells, NK cells, monocyte, macrophage and T cells [13–16]. The immunosuppressive properties of MSCs are often associated with a concomitant increase in the regulatory T cell (Treg) fraction [13, 17].

MSCs have been demonstrated to show a tropism for lung tissue due to hemodynamic matter within 5 min, and retained in the lungs ranging from hours to days [13]. Thus, MSCs have notable strengths for the treatment of lung diseases. Therapeutic benefit of MSC-based therapies in ARDS is found in experimental and clinical studies [18]. A MSCs clinical trial in severe COVID-19 patients conducted in the United States demonstrated therapeutic benefit in attenuating clinical severity and associated cytokine changes [19]. The therapeutic impact on immune responses of MSCs was recently investigated in China in a small cases series which included only one severe COVID-19 patient [20].

Among various tissue origins, placenta choriodecidual-membranes derived mesenchymal stem cells (pcMSCs) are a rare form of MSCs. pcMSCs treatment has been shown to suppress airway inflammation in asthma rats by increasing the number of Treg cells, followed by a reduced presence of lung infiltrated Th17 cells, macrophages, neutrophils, and eosinophils [21, 22]. MatriPlax consists of ex vivo culture-expanded human MSCs isolated from the placenta choriodecidual membrane of healthy adult mothers[11]. MatriPlax has higher lung tissue penetration [11], and has been reported to improve survival rate and promote recovery in the LPS-induced acute lung injury (ALI) animal model via immuno-modulatory properties [21, 23]. Thus, MatriPlax is anticipated to be a feasible therapy for severe or critically ill Covid-19 patients. In this study, we explored the therapeutic efficacy and safety of pcMSCs (MatriPlax) transfusion in severe COVID-19 patients with respiratory failure or impending failure, and also compared the immune profiles changes before and after treatment with pcMSCs.

Subjects And Methods

This was an academic, investigator-initiated compassionate trial performed at Taipei Medical University Hospital, Taipei, Taiwan. This trial was designed to evaluate safety and explore efficacy endpoints of placenta-derived-MSCs (MatriPlax) in COVID-19 infection with severe lung injury from May 14, 2021 to June 18, 2021.

Regulatory, ethical, and institutional review board approvals were obtained by the Taipei Medical University Institutional Review Board in accordance with local institutional requirements. Informed consent was obtained for each patient. The trial was conducted in accordance with the principles of the Declaration of Helsinki and consistent with the Good Clinical Practice guidelines of the International Conference on Harmonization.

Patients' enrollment

Patients who met the following criteria were recruited: 1) age 18-90 years old; 2) laboratory confirmed COVID-19 infection by using a reverse transcriptase polymerase chain reaction (RT-PCR) assay from nasopharyngeal specimen; 3) severe COVID-19 pneumonia who required supplemental high concentration oxygen therapy with respiratory failure or impending failure (Murray's lung injury score >1.7) [24] and/or shock requiring inotropes; 4) persistent or deteriorating lung injury with hyper-inflammatory states despite use of dexamethasone and anti-IL-6 antibody, tocilizumab. Patients were treated according to local clinical practice protocol including oral Dexamethasone 6 mg/day for severe patients, and additional Tocilizumab 8 mg/kg for steroid refractory patients. Patients who were known allergy to dimethyl sulfoxide (DMSO; a component of pcMSCs), prior stem cell therapy, pre-existing terminal illness, need of extracorporeal membrane oxygenation (ECMO), dialysis dependent or multi-organ failure were excluded. Five subjects received compassionate treatment with pc-MSC. Nineteen compatible subjects hospitalized in the ICU wards for COVID 19 and severe lung injury were assigned to the control group.

Cell preparation and treatment protocol

An average of MatriPlax pc-MSCs were administered per infusion. The viability of pc-MSCs (MatriPlax) at the time of product release for administration was found to be 88±2% by trypan blue and 90 %±2% by flow cytometry using fixable viability stain. No differences in cell dose, cell viability, or degree of apoptosis were observed between pc-MSCs prepared for the first or second infusion. Stability studies demonstrated stability of the pc-MSC investigational product for up to 8 hours after thawing and preparation, as assessed by cell count, viability by trypan blue and flow cytometry, and apoptosis assessed by flow cytometry. Cell surface marker analysis demonstrated a typical surface marker profile characteristic of MSCs: CD90 of 99% ±0.2%, CD105 of 95%±0.2%, and CD34/CD45 of 0%±0.1%.

Subjects in the pc-MSC treatment group received two intravenous infusions of $100 \pm 20 \times 10^6$pc-MSCs each, in 50 mL normal saline, infused over 10 ± 5 minutes, at days 0 and 4. The patients were premedicated with antihistamine before each cell treatment. The patients were monitored closely after

cell treatment and their clinical conditions were recorded in details. Best standard of care was provided in both groups following the current institutional COVID-19 guidelines.

Analysis of viral load by SARS-CoV-2 RT-PCR

The RealStar SARS-COV-2 RT-PCR kit (Altona Diagnostics GmbH, Hamburg, Germany) was used to detect the SARS-CoV-2-specific E gene and quantify the number of copies per mL of nasopharyngeal swab. The assay was performed following the manufacturer's instruction, using nasopharyngeal swab samples collected from the enrolled subjects on day 0 and every 6 days till cycle threshold (ct) >30 or negative.

Analysis of inflammatory cytokines, chemokines, and immune profiles in peripheral blood

Multiplex cytokine assay (Aimplex Biosciences, CA) was performed according to the manufacturer's instruction to determine plasma levels of a set of inflammatory cytokines interferon [IFN]γ, interleukin [IL]-1β, IL-2, IL-4, IL-5, IL-13, IL-6, IL-18, IL-10, IL-22, IL-17A, tumor necrosis factor [TNF]α). The assay was performed using serum samples collected from the pc-MSC treated subjects on day 0, day 4 and day 8. Immune profiles were performed with multiple parameter cytometry (Sony ID7000) to determine the proportion of T cells, B cells, NK cells, Monocytes, Dendritic cells and their subsets in the peripheral blood of the pc-MSC treated subjects on day 0, day 4 and day 8 (Figure S3).

Outcomes

Clinical outcomes included the followings: (a) Murray's lung injury score at day 7 after treatment; (b) 28-day survival after treatment; (c) adverse events and serious adverse events (SAEs). Laboratory testing and mechanistic analyses included the following: (a) viral load by SARS-CoV-2 real-time polymerase chain reaction (RT-PCR) in nasopharyngeal swab samples, (b) change of inflammatory markers and (c) changes of immune profiles before and after the treatment

Statistical methods

Statistical analysis was conducted with Prism 9.0 software. Comparisons of AEs, demographics, clinical characteristics, comorbidities, and concomitant treatments between the two groups were performed using Fisher's exact test and Wilcoxon two-sample tests for categorical and continuous variables, respectively. Survival and survival in absence of SAE (SAE-free survival) were estimated in each group with Kaplan-Meier survival estimates. Log-rank tests were used to compare hazards between groups. Nonparametric Wilcoxon rank-sum test or Mann-Whitney t test was used for paired or unpaired data which were non-normally distributed.

Results

From May 14, 2021 to June 18, 2021, a total of 5 subjects with deteriorating COVID-19 pneumonia despite use of dexamethasone and tocilizumab, consented to receive compassionate pc-MSC treatment. Twenty-four subjects were hospitalized in ICU for COVID-19 pneumonia during this time period. Among

them, nineteen subjects with severe lung injury with Murray's lung injury score >1.7 were assigned as the control group. At enrollment, both control and treatment group met the Berlin Criteria of ARDS with an average PaO2/FiO2 of 148.5+/-15.3 and 108.7+/-13.6 respectively. 3 subjects (60%) in the pc-MSC treatment group were receiving invasive mechanical ventilation, and 2 (40%) were on high flow oxygen therapy (FiO2>60%) via high flow nasal cannula prior to initiation of treatment. Eleven subjects (57.9%) in the control group were receiving invasive mechanical ventilation, and eight (42.1%) were on high flow oxygen therapy via high flow nasal cannula.

Demographics and baseline characteristics for two groups of subjects, along with concomitant treatment information were presented in Table 1. All patients were of medium age with male gender predominant. The pc-MSC treatment group was younger than the controlled group with a mean age of 55.5+/-7.09 (N=5) and 70.47+/-2.15 (N=19) years, respectively (p =0.012). Though statistically insignificant, the PaO2/FiO2 ratio of the pc-MSC treatment group was lower than the controlled group (P=0.16). Mean BMI in the pc-MSC treatment group (32.01+/-2.50) was significantly higher than the controlled group (25.75+/-1.40) (P=0.04). There were no significant differences in concomitant treatments between the groups (Table 1). At the time of initial enrollment, white blood cell count, lymphocyte count, and inflammatory markers were compatible in these two groups (Table 1).

Outcomes and estimations

A total of 9 deaths were documented by day 28 after the enrollment of this study. All the deaths occurred in the control group. Thus, patients in the control group were further divided into survival (Survival control, N=10) and mortality subgroups (Mortality control, N=9). The patients in the pc-MSC treatment group were younger than the Mortality control group (Figure S1a). There was no significant difference among Mortality control, Survival control and those in the pc-MSC treatment group (MSC) in terms of number of comorbidities (Figure S1b), baseline hypoxemia (Figure S1c), Murray lung injury scores which evaluates pneumonia extent, PaO2/FiO2, lung compliance and tidal volume (Figure S1d) or length of ICU stay (Figure S1e).

Treatment with 2 doses of pc-MSC significantly improved patients' clinical courses by dramatically improving lung injury assessed with Murray's Lung Injury Score compared with the control group (Figure 1a) and its effect was more significant while compared with Mortality control group (Figure 1b). The extents of lung injury were significantly improved in the pc-MSC group and those in the Survived control group before discharge from ICU (Figure 1b). In contrast, most Mortality control group patients failed to improve the extent of lung injury with the best standard of care treatment (Figure 1b). Treatment with pc-MSC also enhanced viral clearance (Figure 1d), and sustained survival compared with the control group (Figure 1c).

The improvement in COVID-19 pneumonia induced lung injury by pc-MSC treatment (Figure 1e) was associated with decreases in systemic hyper-inflammatory states, in terms of plasma levels of ferritin, CRP, LDH (Figure 1f). Serum D-dimer was not showed any significant changes with pc-MSCs treatment. (Figure 1f).

Analysis of immune profiles and inflammatory cytokines and chemokines levels in peripheral blood plasma

The blood plasma levels of 18 inflammation-related proteins were assessed by multiplex cytokine assay in the pc-MSC group on days 0, 4 and 8. We observed a significant decrease in both IL-6 and IL-2 at 4^{th}day after pc-MSC treatment initiation, and decrease in IL-6, IL-2, IL-1βand IFN-γ at the 8^{th} day of treatment (Figure 2). There was no significant change in plasma levels of IL-17A (not shown), TNFα, IL-18 (not shown) or IL-22 (not shown). There was also a significant increase in type 2 cytokines, IL-5 and IL-13 after pc-MSC treatment (Figure 2). IL-10, a known inhibitory cytokine, was found to have a trend to decrease after pc-MSC treatment.

Peripheral blood of the patients of pc-MSC treatment group was analyzed by high dimensional flow cytometry and the immune cell cluster was visualized through t-Distributed Stochastic Neighbor Embedding (t-SNE) (Figure S2). Its distribution was also checked after treatment and showing significant increase in Treg cells and B cells with reduction in plasma/plasmablast cell and monocyte (Figure S2b).

Patients in the pc-MSC treatment group had lower absolute lymphocyte counts in the peripheral blood (667.1$\pm$137.1/μl, N=5) when compared with healthy controls (1798.6$\pm$145.2/μl, N=5, $P<0.01$). The absolute lymphocyte counts increased after pc-MSC treatment (1161$\pm$180.7/μl, $P<0.05$, N=5) (Figure 3a). Analysis of sub-population of each cell cluster was performed with proportion of each cell type and absolute cell counts of each cell type in peripheral blood mononuclear cells (PBMC). The proportions of $CD4^+$ T and $CD8^+$ T cells of total lymphocytes were not significantly different from healthy controls (Figure 3b). The changes of T cell subpopulation proportion after treatment were shown as heat map (Figure 3c). Patients had lower $CD4^+$ T and $CD8^+$ T cell counts (321.7$\pm$80.1/μl, and 160.4$\pm$44.5/μl, respectively, N=5) compared with healthy controls (813.5$\pm$76.5/μl and 296.5$\pm$53.1/μl, respectively N=5, $P<0.05$) (Figure 3b). Treatment with pc-MSC significantly increased $CD4^+$ T cell counts (655.5$\pm$163.6/μl, N=5, $P<0.05$) (Figure 3d), but not $CD8^+$ T cells (269.2$\pm$121.7/μl, N=5) (Figure 3e). Among the $CD4^+$ T cells, pc-MSC treatment increased the proportion and absolute cell number of $CD4^+$ naïve T cells and $CD4^+$ memory T cells (Figure 3d). In the $CD8^+$ T cell subtypes, pc-MSC treatment had limited effects on its proportion or the absolute count (Figure 3e). Treg cells were significantly increased in both the proportion (Figure 3b, Figure 3c) and absolute count (Figure 3f). NKT cells were not significantly changed with pc-MSC treatment (Figure 3f).

Patients in the pc-MSC treatment group had a higher portion of IgM^+ memory B cells and $CD27^+CD38^+$ plasma/plasmablast cells in their peripheral blood, compared to healthy controls (Figure 4a). pc-MSC treatment significantly increased the absolute number of $CD19^+$ B cells, $CD19^+$ naïve B cells, and increased the proportion as well as absolute number of $CD27^+$ switched activated B cells (Figure 4b). pc-MSC treatment did not change the proportion of IgM^+ memory B cells, but significantly suppressed the proportion and absolute number of $CD27^+CD38^+$ plasma/plasmablast cells (Figure 4a,4b).

The proportions of $CD14^+$ monocyte in PBMC, $CD14^+CD16^+$ or $CD14^+CD16^-$ subpopulations were not significantly different between patients in the pc-MSC group and healthy controls (Figure 5a). Treatment with pc-MSC significantly decreased the proportion of $CD14^+$ and the $CD14^+CD16^-$ subpopulation, and a significantly lower proportion of $CD14^+CD16^+$ subpopulation compared with healthy control (Figure 5a). The absolute cell numbers of $CD14^+$ monocytes and their subpopulations, $CD14^+CD16^-$ and $CD14^+CD16^+$ were significantly decreased after pc-MSC treatment (Figure 5b).

There was no significant change in the proportion of stage 4, stage 5-6 NK cells or dendritic cells (Figure 5c). pc-MSC treatment only significantly increased the absolute number of dendritic cells (Figure 5d).

Adverse events

No serious adverse events (SAEs) were observed in the pc-MSC group or in the control group after follow up for 8 weeks.

Discussion

In this study, we have shown that pc-MSCs treatment was safe and effective in improving critically ill COVID19 patients with severe pneumonia. Nine out of 19 patients in the control group were documented in day-30 mortality marking 47% of deaths in our institution which was consistent with the reported mortality rate of ~50% of critically ill COVID patients [10]. There is no day-30 mortality in the pc-MSC treatment group and 2 out of 5 patients were able to prevent intubation, despite those patients having risk factors such as higher BMI and poor PaO2/FiO2 ratio.

Patients in both groups had high inflammation markers like C-reactive protein (CRP), D-dimer, Lactate dehydrogenase (LDH), ferritin as reported in COVID-19 cohorts [25, 26], even though patients had received anti-inflammatory agents, including dexamethasone and tocilizumab. Treatment with pc-MSC significantly inhibited hyper-inflammatory states by decreasing serum levels of ferritin, LDH and CRP.

It has been shown that SARS-CoV-2 RNAs, acting as pathogen-associated molecular patterns and sensing toll-like receptors, trigger downstream cascades in innate immune cells, resulting in the production of pro-inflammatory cytokines, such as (IL)-1β, IL-6 and interferon (IFN)-γ that induce synthesis of several defense proteins [27] from liver, including CRP, LDH and ferritin. These multi-functional peptides, especially LDH and ferritin levels are independent factors for predicting disease severity and mortality [28, 29]. Ferritin may activate nuclear factor-κB (NF-κB) [30], and create a vicious loop by further increase pro-inflammatory cytokines production, including IL-1β, IL-6 and IFN-γ, and contributing to the development of a cytokine storm syndrome. The decrease in serum levels of ferritin after pc-MSC treatment was associated with a concomitant decrease in IL-1β, IL-6 and IFN-γ serum levels, suggesting pc-MSC effectively broke the vicious cycle and attenuated cytokine storm syndrome. COVID-19 pneumonia severity was also significantly lessened in terms of Murray's lung injury scores.

MSC therapy offers a promising treatment option for severe lung diseases caused by autoimmune, sepsis, and in COVID-19 infection [18-20]. The underlying cellular and molecular mechanisms of MSC-mediated immunomodulation, though still elusive, is proposed through a synergy of cell contact-dependent mechanisms and soluble factors [13, 31], via cytokine-dependent and cytokine-independent functional changes of monocytes/macrophages, dendritic cells, T cells, B cells, and natural killer cells [14, 15, 17, 32]. Peripheral blood immune profiles of the patients in the pc-MSC group were studied on days 0, 4 and 8 by using multi-parameter flow-cytometry analysis. TSNE cell clusters revealed a significant change in monocytes, Th cells, B cells, Treg cells and plasma/plasmablast cells subpopulations after pc-MSC treatment.

Monocytes and macrophages are the most important innate immune cells against viral infections. They mainly respond to SARS-CoV-2 infections by producing pro-inflammatory mediators through ACE2-independent and ACE2-dependent pathways to remove pathogens and repair tissue injury . However, dysregulation of their function such as through inappropriate activities to induce cytokine storm can result in the acute respiratory distress syndrome and other vital organs damage of the body including the heart [33]. During SARS-CoV-2 infection, circulating monocytes subpopulation, $CD14^{+}CD16^{+}$ monocytes, exhibits notable potency in contributing cytokine storms through producing a high level of TNF-α, IL-10, and IL-6 that are related to the deterioration of patients and increasing their admission to the ICU [2, 34]. The decrease in the absolute number of $CD14^{+}CD16^{+}$ subpopulation may be contributory to the attenuation of hyper-inflammation responses in the pc-MSC treatment group.

In severe COVID-19, lymphocytes are significantly reduced, and lymphopenia (absolute counts<1000 cells/μL) is considered an indicator for disease severity [35], therapeutic response [36], and disease outcome [37]. High levels of pro-inflammatory cytokines in cytokine storm such as TNF-α and IL-6 could induce lymphocyte deficiency [37]. All the patients in the pc-MSC treatment group had absolute lymphocyte counts <1000/ μL before treatment. The lymphocyte numbers in the peripheral blood significantly increased after treatment, probably due to the inhibitory effect of pc-MSC on pro-inflammatory cytokines, such as IL-6.

Lymphocytes in the peripheral blood including T cells, B cells and natural killer cells (NK cells) are critical to generate early control, viral clearance and disease resolution after SARS-CoV-2 infection [38, 39]. Regulatory T cells (Treg) play an important role in the prevention of excessive immune responses to SARS-CoV-2 infection [40]. Analysis of T lymphocyte subpopulations, patients of the pc-MSC treatment group had lower $CD4^{+}$ and $CD8^{+}$ T cell counts, butnot in the proportion of $CD4^{+}$, $CD8^{+}$ or Treg cells, compared with age-matched healthy controls. Treatment with pc-MSC significantly increased the proportion and absolute cell number of $CD4^{+}$ T cells and Treg cells. Among $CD4^{+}$ T cell subpopulations, memory T cells are crucial in viral clearance during the re-infection. Although the memory T cells were not identified as SARS-CoV2-specific, an increased number of memory T cell may suggest an adaptive immunity was being developed. MSCs have been shown to facilitate the formation of Treg cells [17, 41], via induction of Treg from conventional T cells [42, 43]. The increase in Treg cells is considered responsible for pc-MSC-mediated suppression of hyper-inflammatory responses in our patients.

Additionally, MSC treatment has been shown to cause a shift from proinflammatory Th1 to anti-inflammatory Th2 cells [44]. Increased serum levels of IL-5, IL-4 and IL-13 after pc-MSC treatment may support this notion.

Effective immune response to SARS-CoV infection depends on the activation of $CD8^+$ cytotoxic T cells through the killing of virus-infected cells [45]. However, although there was a trend for $CD8^+$ T cells to increase with pc-MSC treatment, statistical significance was not reached. Our data about the role of NK cells in the pathogenesis of COVID-19 were limited. In one study there were no differences in the levels of NK cells among responders and non-responders before and following the treatment [46]. In another study, this trend was increasing among survivors and decreasing among non-survivors [47]. In our study, there was no significant change in NK cells after pc-MSC treatment.

B cells mediated antibody responses via coordination of other adaptive immune responses, including $CD4^+$ T cells, $CD8^+$ T cells are essential for effective control of COVID-19 infection [48]. B cells participate in the antiviral immune response by first rapidly releasing germline or near-germline antibodies from plasmablasts, via an extrafollicular pathway. Increased portions of IgM^+ memory B cells and $CD27^+CD38^+$ plasma/plasmablast cells in the peripheral blood, compared to healthy controls, suggesting a protective memory response occurred in those patients with COVID-19 infection [5]. Upon appropriate cytokine stimulation, B cells undergo class switching and/or enter germinal centers within secondary lymphoid organs to undergo affinity maturation. This maturation process produces both long-lived plasma cells and memory B cells capable of responding to secondary challenge with homotypic or heterotypic antigenic challenge. Pc-MSC treatment significantly increased the proportion and absolute number of $CD27^+$ switched activated B cells and concomitantly suppressed the proportion and absolute number of $CD27^+CD38^+$ plasma/plasmablast cells. These results suggest pc-MSC treatment shifted B cells from a protective memory response to COVID-19 infection to enhancing generation of virus-specific immunoglobulin switched B cells or long-lived plasma cells [49].

Limitation of this study:

The study was a small sample-sized study without true, randomized control group to provide strong evidence of treatment benefit. According to local government law and legislation, compassionate study for stem cell is limited to 3 patients. Due to pandemic issue, our study was performed up to 5 patients. At the time of treatment, all the patients were already treated with anti-viral and anti-inflammatory agents like Dexamethasone and Tocilizumab. The anti-inflammatory effect of pure Matriplax was hard to identify. Nevertheless, low mortality rate was noted at treatment group. In the control group, low anti-viral usage was due to local central disease control policy and drug regulation. Even so our interested patients were in severe condition and anti-viral has limited benefit on severe patients. Despite these limitations, we showed the desirable good prognosis in treatment group and biochemical response. Matriplax also balance dysregulation of immune response and it accelerated its homeostasis.

Conclusion

This compassionate study demonstrates safety and well tolerability of Matriplax in treatment of COVID-19 pneumonia-induced severe lung injury. The clinical efficacy of pc-MSC results from modulatory effects on the immune responses in COVID-19 infection by balancing host defense immunity and anti-inflammation.

Declarations

Acknowledgement

We thank BioSeed International Co., Ltd for providing pc-MSCs (Matriplax).

Funding

This study was supported by Taiwan Ministry of Science and Technology grant, 109-2314-B-038-097 and 108-2314-B-038-134-MY2.

Authors' contributions:

*M-C. Chen and Kevin SL Lai contributed equally to this work

† Authors to whom correspondence should be addressed

MC Chen and Kevin SL Lai wrote the manuscript. H.P Kuo, YH Huang, CM Weng and CL Chou contributed to the design and implementation of the research. KL Chien, ST Teng, PL Wei involved in clinical care and collecting clinical specimen. YJ Lin, W Chao and M-J Lee carried out laboratory analysis. H.P Kuo, CL Chou and CM Weng revised the manuscript and provided additional comments. All authors read and approved the final manuscript.

Availability of data and materials: The data that support the findings of this study are available from the corresponding author upon reasonable request

Ethics approval and consent to participate:

Regulatory, ethical, and institutional review board approvals were obtained by the Taipei Medical University Institutional Review Board in accordance with local institutional requirements. Informed consent was obtained for each patient.

Consent for publication:

Informed consent of each patient was obtained for analysis and publication.

Conflict of interest

HP Kuo**,** has also been renumerated for speaking engagements for GSK, Novartis, Elli Lily, Pfizer, Roche, Boehringer Ingelheim, Astra-Zeneca, Sanofi-Aventis and MSD. The other authors have no conflict of

interest to declare.

References

1. Hu B, Guo H, Zhou P, Shi ZL, (2021) Characteristics of SARS-CoV-2 and COVID-19. Nature reviews Microbiology 19: 141-154
2. Wu Z, McGoogan JM, (2020) Characteristics of and Important Lessons From the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72 314 Cases From the Chinese Center for Disease Control and Prevention. Jama 323: 1239-1242
3. Bhatraju PK, Ghassemieh BJ, Nichols M, Kim R, Jerome KR, Nalla AK, Greninger AL, Pipavath S, Wurfel MM, Evans L, Kritek PA, West TE, Luks A, Gerbino A, Dale CR, Goldman JD, O'Mahony S, Mikacenic C, (2020) Covid-19 in Critically Ill Patients in the Seattle Region - Case Series. The New England journal of medicine 382: 2012-2022
4. Yang X, Yu Y, Xu J, Shu H, Xia J, Liu H, Wu Y, Zhang L, Yu Z, Fang M, Yu T, Wang Y, Pan S, Zou X, Yuan S, Shang Y, (2020) Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. The Lancet Respiratory medicine 8: 475-481
5. Mathew D, Giles JR, Baxter AE, Oldridge DA, Greenplate AR, Wu JE, Alanio C, Kuri-Cervantes L, Pampena MB, D'Andrea K, Manne S, Chen Z, Huang YJ, Reilly JP, Weisman AR, Ittner CAG, Kuthuru O, Dougherty J, Nzingha K, Han N, Kim J, Pattekar A, Goodwin EC, Anderson EM, Weirick ME, Gouma S, Arevalo CP, Bolton MJ, Chen F, Lacey SF, Ramage H, Cherry S, Hensley SE, Apostolidis SA, Huang AC, Vella LA, Betts MR, Meyer NJ, Wherry EJ, (2020) Deep immune profiling of COVID-19 patients reveals distinct immunotypes with therapeutic implications. Science (New York, NY) 369
6. Yang L, Xie X, Tu Z, Fu J, Xu D, Zhou Y, (2021) The signal pathways and treatment of cytokine storm in COVID-19. Signal transduction and targeted therapy 6: 255
7. Horby P, Lim WS, Emberson JR, Mafham M, Bell JL, Linsell L, Staplin N, Brightling C, Ustianowski A, Elmahi E, Prudon B, Green C, Felton T, Chadwick D, Rege K, Fegan C, Chappell LC, Faust SN, Jaki T, Jeffery K, Montgomery A, Rowan K, Juszczak E, Baillie JK, Haynes R, Landray MJ, (2021) Dexamethasone in Hospitalized Patients with Covid-19. The New England journal of medicine 384: 693-704
8. (2021) Tocilizumab in patients admitted to hospital with COVID-19 (RECOVERY): a randomised, controlled, open-label, platform trial. Lancet (London, England) 397: 1637-1645
9. Kalil AC, Patterson TF, Mehta AK, Tomashek KM, Wolfe CR, Ghazaryan V, Marconi VC, Ruiz-Palacios GM, Hsieh L, Kline S, Tapson V, Iovine NM, Jain MK, Sweeney DA, El Sahly HM, Branche AR, Regalado Pineda J, Lye DC, Sandkovsky U, Luetkemeyer AF, Cohen SH, Finberg RW, Jackson PEH, Taiwo B, Paules CI, Arguinchona H, Erdmann N, Ahuja N, Frank M, Oh MD, Kim ES, Tan SY, Mularski RA, Nielsen H, Ponce PO, Taylor BS, Larson L, Rouphael NG, Saklawi Y, Cantos VD, Ko ER, Engemann JJ, Amin AN, Watanabe M, Billings J, Elie MC, Davey RT, Burgess TH, Ferreira J, Green M, Makowski M, Cardoso A, de Bono S, Bonnett T, Proschan M, Deye GA, Dempsey W, Nayak SU, Dodd LE, Beigel JH,

(2021) Baricitinib plus Remdesivir for Hospitalized Adults with Covid-19. The New England journal of medicine 384: 795-807

10. Arentz M, Yim E, Klaff L, Lokhandwala S, Riedo FX, Chong M, Lee M, (2020) Characteristics and Outcomes of 21 Critically Ill Patients With COVID-19 in Washington State. Jama 323: 1612-1614
11. Zhuang WZ, Lin YH, Su LJ, Wu MS, Jeng HY, Chang HC, Huang YH, Ling TY, (2021) Mesenchymal stem/stromal cell-based therapy: mechanism, systemic safety and biodistribution for precision clinical applications. Journal of biomedical science 28: 28
12. Thompson M, Mei SHJ, Wolfe D, Champagne J, Fergusson D, Stewart DJ, Sullivan KJ, Doxtator E, Lalu M, English SW, Granton J, Hutton B, Marshall J, Maybee A, Walley KR, Santos CD, Winston B, McIntyre L, (2020) Cell therapy with intravascular administration of mesenchymal stromal cells continues to appear safe: An updated systematic review and meta-analysis. EClinicalMedicine 19: 100249
13. de Witte SFH, Luk F, Sierra Parraga JM, Gargesha M, Merino A, Korevaar SS, Shankar AS, O'Flynn L, Elliman SJ, Roy D, Betjes MGH, Newsome PN, Baan CC, Hoogduijn MJ, (2018) Immunomodulation By Therapeutic Mesenchymal Stromal Cells (MSC) Is Triggered Through Phagocytosis of MSC By Monocytic Cells. Stem cells (Dayton, Ohio) 36: 602-615
14. Luz-Crawford P, Djouad F, Toupet K, Bony C, Franquesa M, Hoogduijn MJ, Jorgensen C, Noël D, (2016) Mesenchymal Stem Cell-Derived Interleukin 1 Receptor Antagonist Promotes Macrophage Polarization and Inhibits B Cell Differentiation. Stem cells (Dayton, Ohio) 34: 483-492
15. Németh K, Leelahavanichkul A, Yuen PS, Mayer B, Parmelee A, Doi K, Robey PG, Leelahavanichkul K, Koller BH, Brown JM, Hu X, Jelinek I, Star RA, Mezey E, (2009) Bone marrow stromal cells attenuate sepsis via prostaglandin E(2)-dependent reprogramming of host macrophages to increase their interleukin-10 production. Nature medicine 15: 42-49
16. Vignali DA, Collison LW, Workman CJ, (2008) How regulatory T cells work. Nature reviews Immunology 8: 523-532
17. Ge W, Jiang J, Arp J, Liu W, Garcia B, Wang H, (2010) Regulatory T-cell generation and kidney allograft tolerance induced by mesenchymal stem cells associated with indoleamine 2,3-dioxygenase expression. Transplantation 90: 1312-1320
18. Harrell CR, Sadikot R, Pascual J, Fellabaum C, Jankovic MG, Jovicic N, Djonov V, Arsenijevic N, Volarevic V, (2019) Mesenchymal Stem Cell-Based Therapy of Inflammatory Lung Diseases: Current Understanding and Future Perspectives. Stem cells international 2019: 4236973
19. Singh S, Chakravarty T, Chen P, Akhmerov A, Falk J, Friedman O, Zaman T, Ebinger JE, Gheorghiu M, Marbán L, Marbán E, Makkar RR, (2020) Allogeneic cardiosphere-derived cells (CAP-1002) in critically ill COVID-19 patients: compassionate-use case series. Basic research in cardiology 115: 36
20. Leng Z, Zhu R, Hou W, Feng Y, Yang Y, Han Q, Shan G, Meng F, Du D, Wang S, Fan J, Wang W, Deng L, Shi H, Li H, Hu Z, Zhang F, Gao J, Liu H, Li X, Zhao Y, Yin K, He X, Gao Z, Wang Y, Yang B, Jin R, Stambler I, Lim LW, Su H, Moskalev A, Cano A, Chakrabarti S, Min KJ, Ellison-Hughes G, Caruso C, Jin

K, Zhao RC, (2020) Transplantation of ACE2(-) Mesenchymal Stem Cells Improves the Outcome of Patients with COVID-19 Pneumonia. Aging and disease 11: 216-228

21. Li Y, Li H, Cao Y, Wu F, Ma W, Wang Y, Sun S, (2017) Placenta-derived mesenchymal stem cells improve airway hyperresponsiveness and inflammation in asthmatic rats by modulating the Th17/Treg balance. Molecular medicine reports 16: 8137-8145
22. Lin SC, Liou YM, Ling TY, Chuang YH, Chiang BL, (2019) Placenta-Derived Mesenchymal Stem Cells Reduce the Interleukin-5 Level Experimentally in Children with Asthma. International journal of medical sciences 16: 1430-1438
23. Li Y, Qu T, Tian L, Han T, Jin Y, Wang Y, (2018) Human placenta mesenchymal stem cells suppress airway inflammation in asthmatic rats by modulating Notch signaling. Molecular medicine reports 17: 5336-5343
24. Murray JF, Matthay MA, Luce JM, Flick MR, (1988) An expanded definition of the adult respiratory distress syndrome. The American review of respiratory disease 138: 720-723
25. Richardson S, Hirsch JS, Narasimhan M, Crawford JM, McGinn T, Davidson KW, Barnaby DP, Becker LB, Chelico JD, Cohen SL, Cookingham J, Coppa K, Diefenbach MA, Dominello AJ, Duer-Hefele J, Falzon L, Gitlin J, Hajizadeh N, Harvin TG, Hirschwerk DA, Kim EJ, Kozel ZM, Marrast LM, Mogavero JN, Osorio GA, Qiu M, Zanos TP, (2020) Presenting Characteristics, Comorbidities, and Outcomes Among 5700 Patients Hospitalized With COVID-19 in the New York City Area. Jama 323: 2052-2059
26. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, Wang B, Xiang H, Cheng Z, Xiong Y, Zhao Y, Li Y, Wang X, Peng Z, (2020) Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. Jama 323: 1061-1069
27. Guo YR, Cao QD, Hong ZS, Tan YY, Chen SD, Jin HJ, Tan KS, Wang DY, Yan Y, (2020) The origin, transmission and clinical therapies on coronavirus disease 2019 (COVID-19) outbreak - an update on the status. Military Medical Research 7: 11
28. Henry BM, Aggarwal G, Wong J, Benoit S, Vikse J, Plebani M, Lippi G, (2020) Lactate dehydrogenase levels predict coronavirus disease 2019 (COVID-19) severity and mortality: A pooled analysis. The American journal of emergency medicine 38: 1722-1726
29. Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ, (2020) COVID-19: consider cytokine storm syndromes and immunosuppression. Lancet (London, England) 395: 1033-1034
30. Ruddell RG, Hoang-Le D, Barwood JM, Rutherford PS, Piva TJ, Watters DJ, Santambrogio P, Arosio P, Ramm GA, (2009) Ferritin functions as a proinflammatory cytokine via iron-independent protein kinase C zeta/nuclear factor kappaB-regulated signaling in rat hepatic stellate cells. Hepatology (Baltimore, Md) 49: 887-900
31. Wu Y, Hoogduijn MJ, Baan CC, Korevaar SS, de Kuiper R, Yan L, Wang L, van Besouw NM, (2017) Adipose Tissue-Derived Mesenchymal Stem Cells Have a Heterogenic Cytokine Secretion Profile. Stem cells international 2017: 4960831
32. Spaggiari GM, Capobianco A, Abdelrazik H, Becchetti F, Mingari MC, Moretta L, (2008) Mesenchymal stem cells inhibit natural killer-cell proliferation, cytotoxicity, and cytokine production: role of

indoleamine 2,3-dioxygenase and prostaglandin E2. Blood 111: 1327-1333

33. McKechnie JL, Blish CA, (2020) The Innate Immune System: Fighting on the Front Lines or Fanning the Flames of COVID-19? Cell host & microbe 27: 863-869
34. Zhou Y, Fu B, Zheng X, Wang D, Zhao C, qi Y, Sun R, Tian Z, Xu X, Wei H, (2020) Aberrant pathogenic GM-CSF⁺ T cells and inflammatory CD14⁺CD16⁺ monocytes in severe pulmonary syndrome patients of a new coronavirus. bioRxiv: 2020.2002.2012.945576
35. Zhao Q, Meng M, Kumar R, Wu Y, Huang J, Deng Y, Weng Z, Yang L, (2020) Lymphopenia is associated with severe coronavirus disease 2019 (COVID-19) infections: A systemic review and meta-analysis. International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases 96: 131-135
36. Lu C, Liu Y, Chen B, Yang H, Hu H, Liu Y, Zhao Y, (2021) Prognostic value of lymphocyte count in severe COVID-19 patients with corticosteroid treatment. Signal transduction and targeted therapy 6: 106
37. Huang G, Kovalic AJ, Graber CJ, (2020) Prognostic Value of Leukocytosis and Lymphopenia for Coronavirus Disease Severity. Emerging infectious diseases 26: 1839-1841
38. Jagadeesh A, Prathyusha A, Sheela GM, Bramhachari PV, T Cells in Viral Infections: The Myriad Flavours of Antiviral Immunity
39. Sun J, Zhuang Z, Zheng J, Li K, Wong RL, Liu D, Huang J, He J, Zhu A, Zhao J, Li X, Xi Y, Chen R, Alshukairi AN, Chen Z, Zhang Z, Chen C, Huang X, Li F, Lai X, Chen D, Wen L, Zhuo J, Zhang Y, Wang Y, Huang S, Dai J, Shi Y, Zheng K, Leidinger MR, Chen J, Li Y, Zhong N, Meyerholz DK, McCray PB, Jr., Perlman S, Zhao J, (2020) Generation of a Broadly Useful Model for COVID-19 Pathogenesis, Vaccination, and Treatment. Cell 182: 734-743.e735
40. Mahmoudi S, Rezaei M, Mansouri N, Marjani M, Mansouri D, (2020) Immunologic Features in Coronavirus Disease 2019: Functional Exhaustion of T Cells and Cytokine Storm. Journal of clinical immunology 40: 974-976
41. Aggarwal S, Pittenger MF, (2005) Human mesenchymal stem cells modulate allogeneic immune cell responses. Blood 105: 1815-1822
42. Engela AU, Hoogduijn MJ, Boer K, Litjens NH, Betjes MG, Weimar W, Baan CC, (2013) Human adipose-tissue derived mesenchymal stem cells induce functional de-novo regulatory T cells with methylated FOXP3 gene DNA. Clinical and experimental immunology 173: 343-354
43. Khosravi M, Karimi MH, Hossein Aghdaie M, Kalani M, Naserian S, Bidmeshkipour A, (2017) Mesenchymal stem cells can induce regulatory T cells via modulating miR-126a but not miR-10a. Gene 627: 327-336
44. Bai L, Lennon DP, Eaton V, Maier K, Caplan AI, Miller SD, Miller RH, (2009) Human bone marrow-derived mesenchymal stem cells induce Th2-polarized immune response and promote endogenous repair in animal models of multiple sclerosis. Glia 57: 1192-1203
45. McMahan K, Yu J, Mercado NB, Loos C, Tostanoski LH, Chandrashekar A, Liu J, Peter L, Atyeo C, Zhu A, Bondzie EA, Dagotto G, Gebre MS, Jacob-Dolan C, Li Z, Nampanya F, Patel S, Pessaint L, Van Ry A,

Blade K, Yalley-Ogunro J, Cabus M, Brown R, Cook A, Teow E, Andersen H, Lewis MG, Lauffenburger DA, Alter G, Barouch DH, (2021) Correlates of protection against SARS-CoV-2 in rhesus macaques. Nature 590: 630-634

46. Wang F, Nie J, Wang H, Zhao Q, Xiong Y, Deng L, Song S, Ma Z, Mo P, Zhang Y, (2020) Characteristics of Peripheral Lymphocyte Subset Alteration in COVID-19 Pneumonia. The Journal of infectious diseases 221: 1762-1769
47. Deng Z, Zhang M, Zhu T, Zhili N, Liu Z, Xiang R, Zhang W, Xu Y, (2020) Dynamic changes in peripheral blood lymphocyte subsets in adult patients with COVID-19. International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases 98: 353-358
48. Rydyznski Moderbacher C, Ramirez SI, Dan JM, Grifoni A, Hastie KM, Weiskopf D, Belanger S, Abbott RK, Kim C, Choi J, Kato Y, Crotty EG, Kim C, Rawlings SA, Mateus J, Tse LPV, Frazier A, Baric R, Peters B, Greenbaum J, Ollmann Saphire E, Smith DM, Sette A, Crotty S, (2020) Antigen-Specific Adaptive Immunity to SARS-CoV-2 in Acute COVID-19 and Associations with Age and Disease Severity. Cell 183: 996-1012.e1019
49. Rodda LB, Netland J, Shehata L, Pruner KB, Morawski PA, Thouvenel CD, Takehara KK, Eggenberger J, Hemann EA, Waterman HR, Fahning ML, Chen Y, Hale M, Rathe J, Stokes C, Wrenn S, Fiala B, Carter L, Hamerman JA, King NP, Gale M, Jr., Campbell DJ, Rawlings DJ, Pepper M, (2021) Functional SARS-CoV-2-Specific Immune Memory Persists after Mild COVID-19. Cell 184: 169-183.e117

Tables

Table 1 Demographics and baseline characteristics of the treated and control group with serum inflammatory markers and clinical outcome

Characteristics and treatments		pc-MSC (n =5)	Control (n=19)	p value
Age, years, mean (SD)		55.4 +/- 7.1	70.5 +/- 2.2	0.012
Gender				0.24
	Male	3 (60%)	16 (84.2%)	
	Female	2 (40%)	3 (15.7%)	
PaO2/FiO2 ratio at enrollment, mean (IQR)		108.7+/-13.6	148.5+/-15.3	0.16
Murray's lung injury score, n(%)				0.6
	Moderate-to-sever >1 <2	0 (0%)	1 (5.2%)	
	Severe>2	5 (100%)	18 (94.73%)	
BMI, kg/m2, mean (SD)		32.0+/-2.5	25.8+/-1.4	0.04
Smoker (including former smoker), n(%)		0	4 (21.0%)	0.54
Comorbidities, n(%)		4 (80%)	13 (68.4%)	0.93
	DM	3 (60%)	5 (26.3%)	0.28
	Hypertension	4 (80%)	7 (36.8%)	0.14
	Obesity (BMI >30)	2 (40%)	5 (26.3%)	0.6
	Cancer	1 (20%)	1 (5.2%)	0.38
	Heart disease	1 (20%)	4 (21.0%)	0.99
Concomitant treatments, n(%)		5 (100%)	19(100%)	0.99
	Heparin, Therapeutic dose	5 (100%)	16 (84.2%)	0.99
	Remdesivir	5 (100%)	3 (15.8%)	0.001
	Corticosteriods	5 (100%)	19 (100%)	0.99
	Tocilizumab	5 (100%)	19 (100%)	0.99
Intubation at enrollment		3 (60%)	11 (57.9)	0.99
Mortality, n(%)		0	8 (42.1%)	0.13
At the time of enrollment				
WBC (x 1000/µl)		12.25 +/- 3.22	7.71 +/- 0.89	0.065

Lymphocytes (x 1000/µl)	8.84+/-3.46	11.92+/-2.12	0.501
CRP (mg/dl)	6.6+/- 2.74	8.17 +/- 1.39	0.613
Ferritin (ng/ml)	2345 +/- 441.9	2423+/- 449	0.933
D- Dimer (ug/mL)	5.77 +/- 4.58	3.61+/- 1.47	0.561
Procalcitonin (ng/ml)	0.39+/-0.27	0.57+/-0.26	0.746
LDH (U/L)	679.2 +/- 84.06	569.9+/- 37.41	0.209

WBC = white blood cells, LDH = Lactate Dehydrogenase, CRP = C-reactive protein

Figures

Figure 1

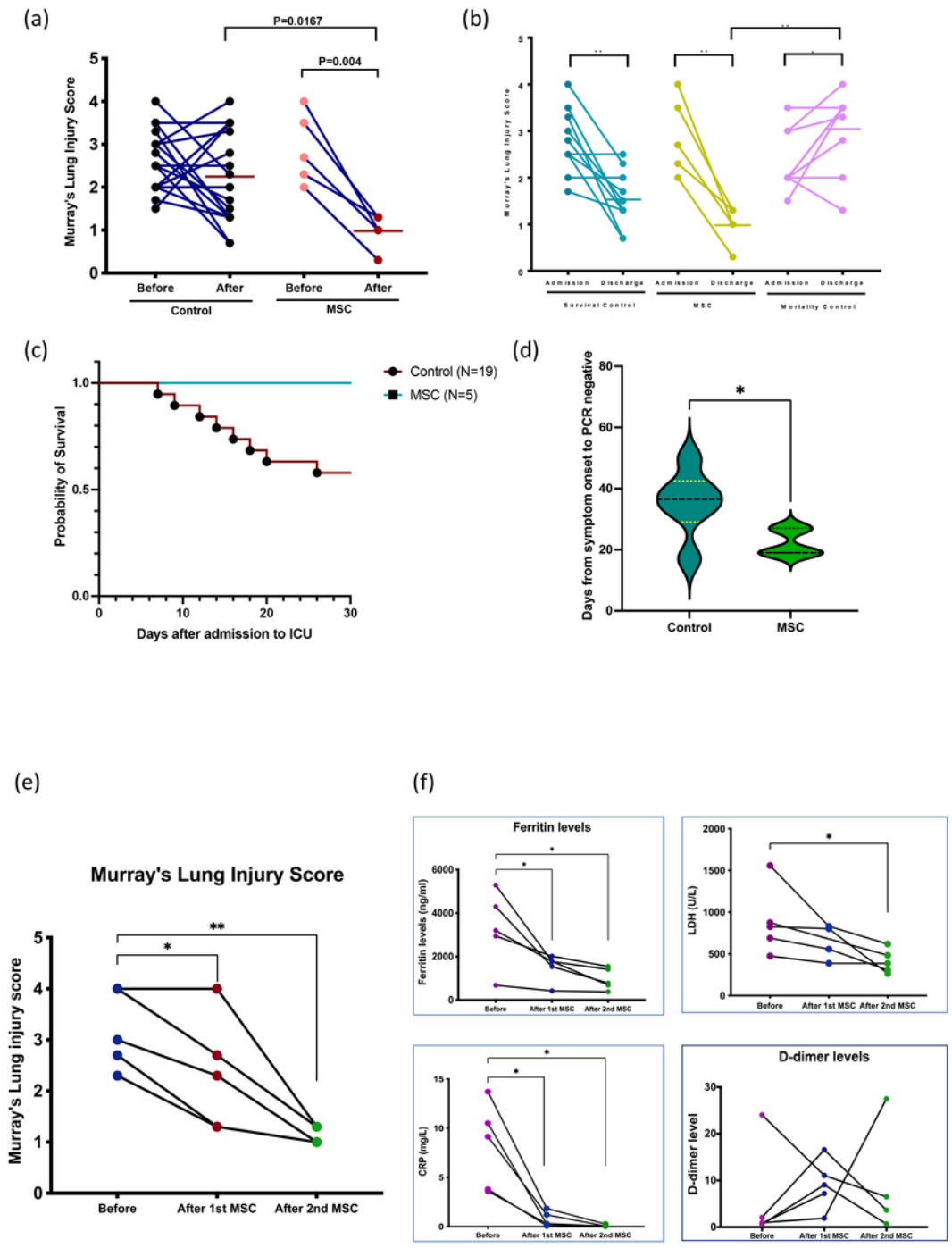

Figure 1

(a)Murray's lung injury score of control and treatment group was shown as before and after treatment; timing of after was adjusted as one week after recruitment (b) Murray's lung injury score compared at admission and discharge condition as survival control, mortality control group and MSC group; (d) survival analysis of control and MSC group; (d): duration of viral clearance by detecting viral PCR in control and treated group; Five treated patients were analyzed at day 0, 4 and 8 for Murray's lung injury(e);

serum proinflammatory markers like Ferritin, LDH, CRP and D-dimer (f) *P < 0.05 and **P < 0.01, nonparametric paired Wilcoxon t test

Figure 2

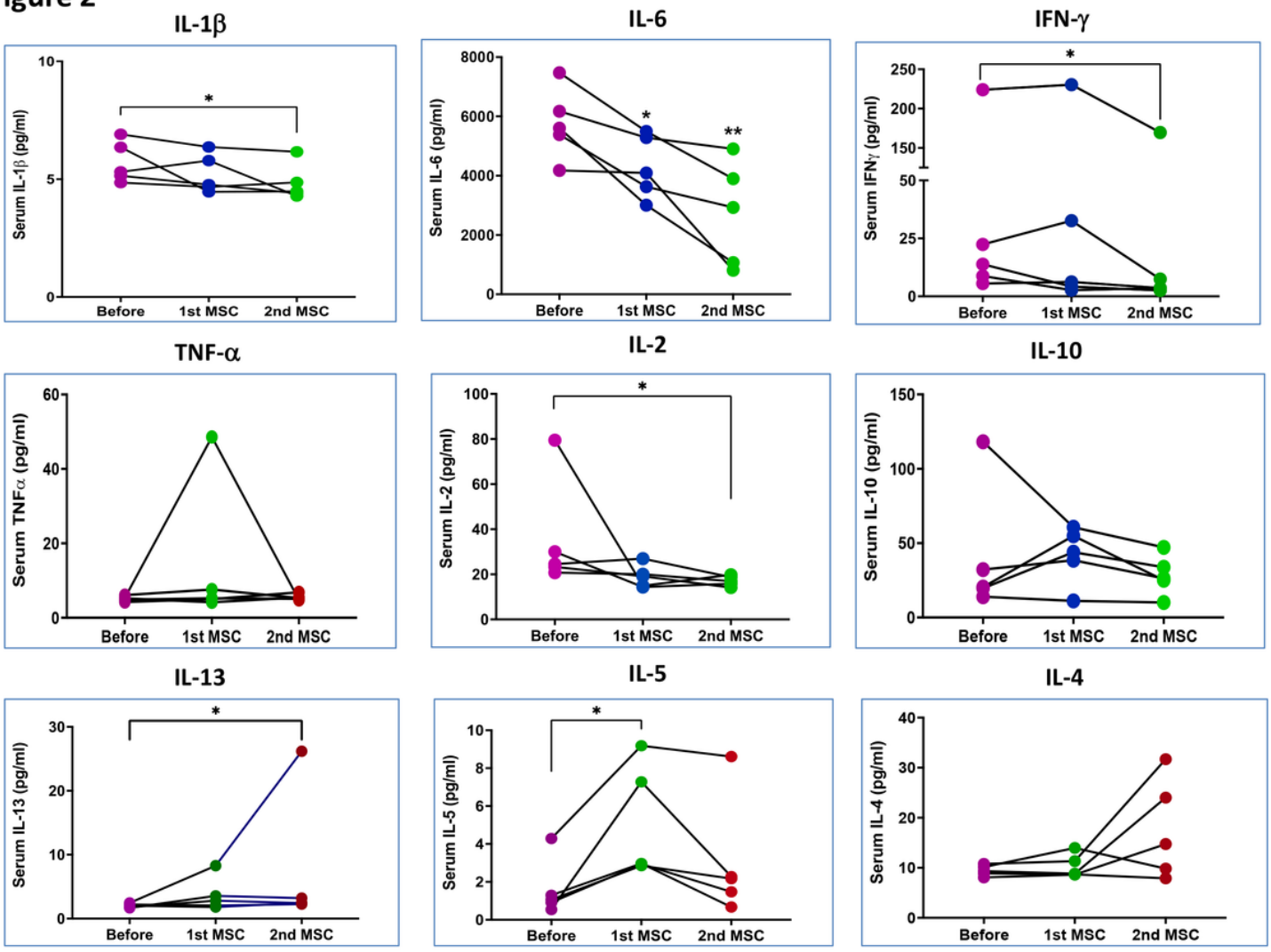

Figure 2

Proinflammatory markers, IL-10 and type 2 cytokines changes of 5 treated patients in Day 0, 4 and 8. *P < 0.05 and **P < 0.01, nonparametric paired Wilcoxon t test

Figure 3

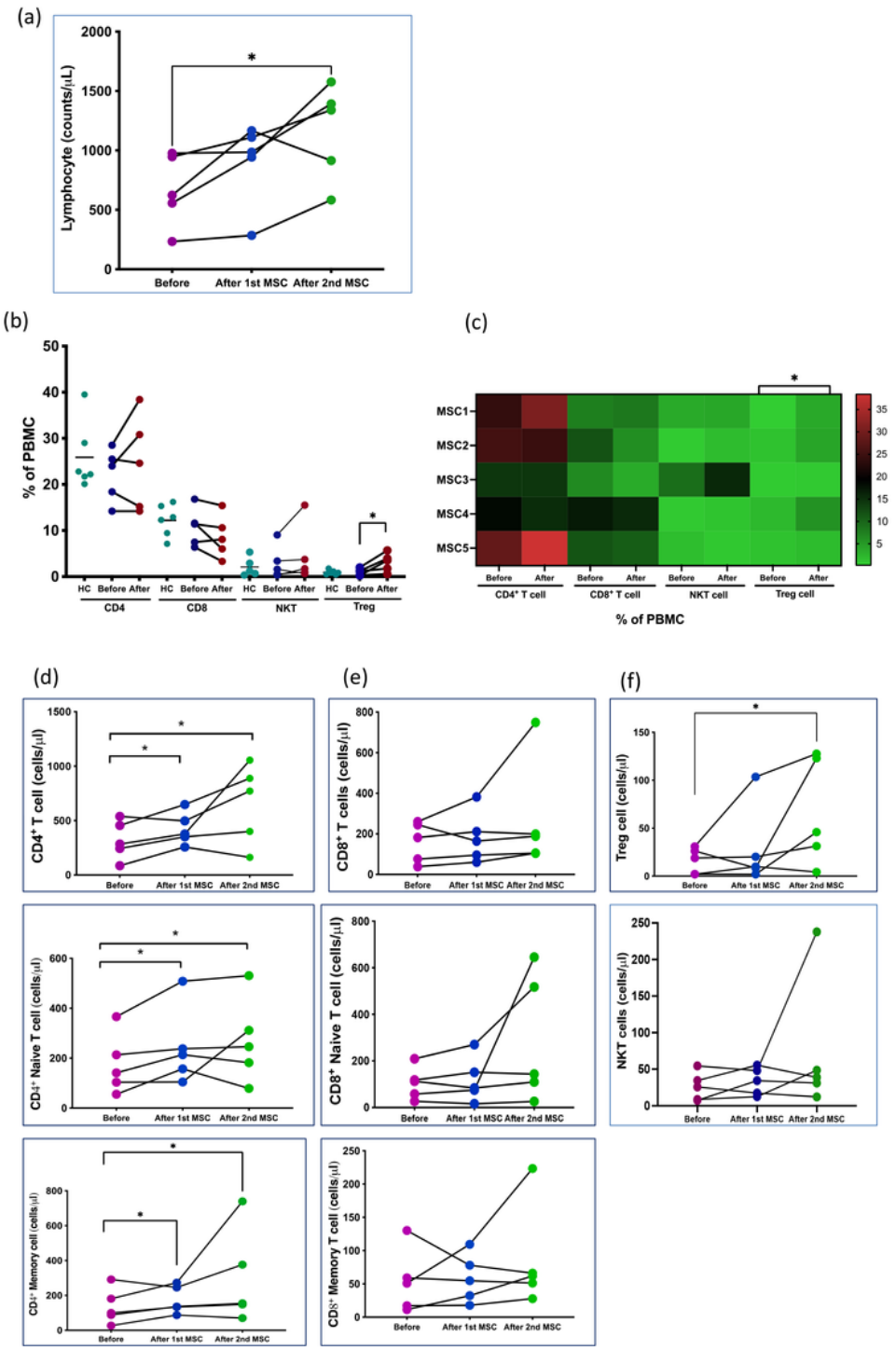

Figure 3

Increase of Treg after MSC treatment in SARS-CoV2 infected patients. (a) Total lymphocyte count changes after D0, 4 and 8 of treatment; (b) CD4, CD8, NKT and Treg proportion was compared with normal healthy individual and also after treatment showing significant increase in Treg cell proportion; (c) Distribution of proportion of T cells subtype was shown in heat map; (d) total CD4 count, naïve CD4+ T cell and CD4+ memory cell increased after treatment; (e) no change in CD8+ T cell, CD8+ Naïve T cell and

CD8+memory T cell count; (f) Treg cell count was significantly increased after D8. *P < 0.05 and **P < 0.01, nonparametric paired Wilcoxon t test

Figure 4

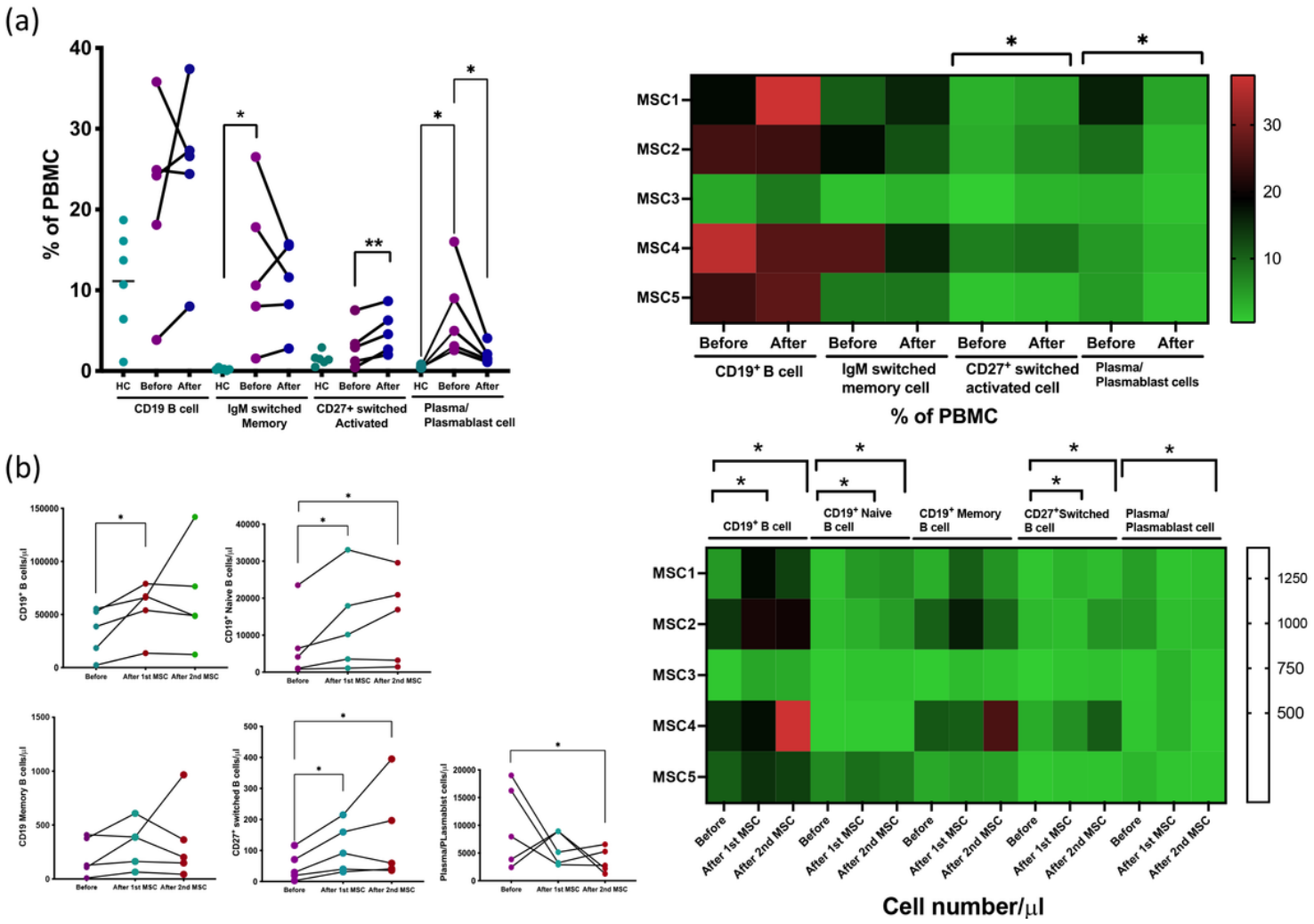

Figure 4

The shifting of B cell population after pcMSC treatment. (a) IgM+ switched memory B cell and CD27+CD38+ plasma/plasmablast cells were high before treatment compared with healthy control (left) and after treatment, increased in CD27+ switched activated B cell proportion and decreased in plasma/plasmablast cells proportion (right). (b) In subtype analysis of B cells, CD19+ total B cell count, CD19+ Naïve B cells count, CD27+ switched B cell count were significantly increased after D4 and D8 (left). Plasma cell was significantly was reduced after D8 (left). *P < 0.05 and **P < 0.01, nonparametric paired Wilcoxon t test

Figure 5

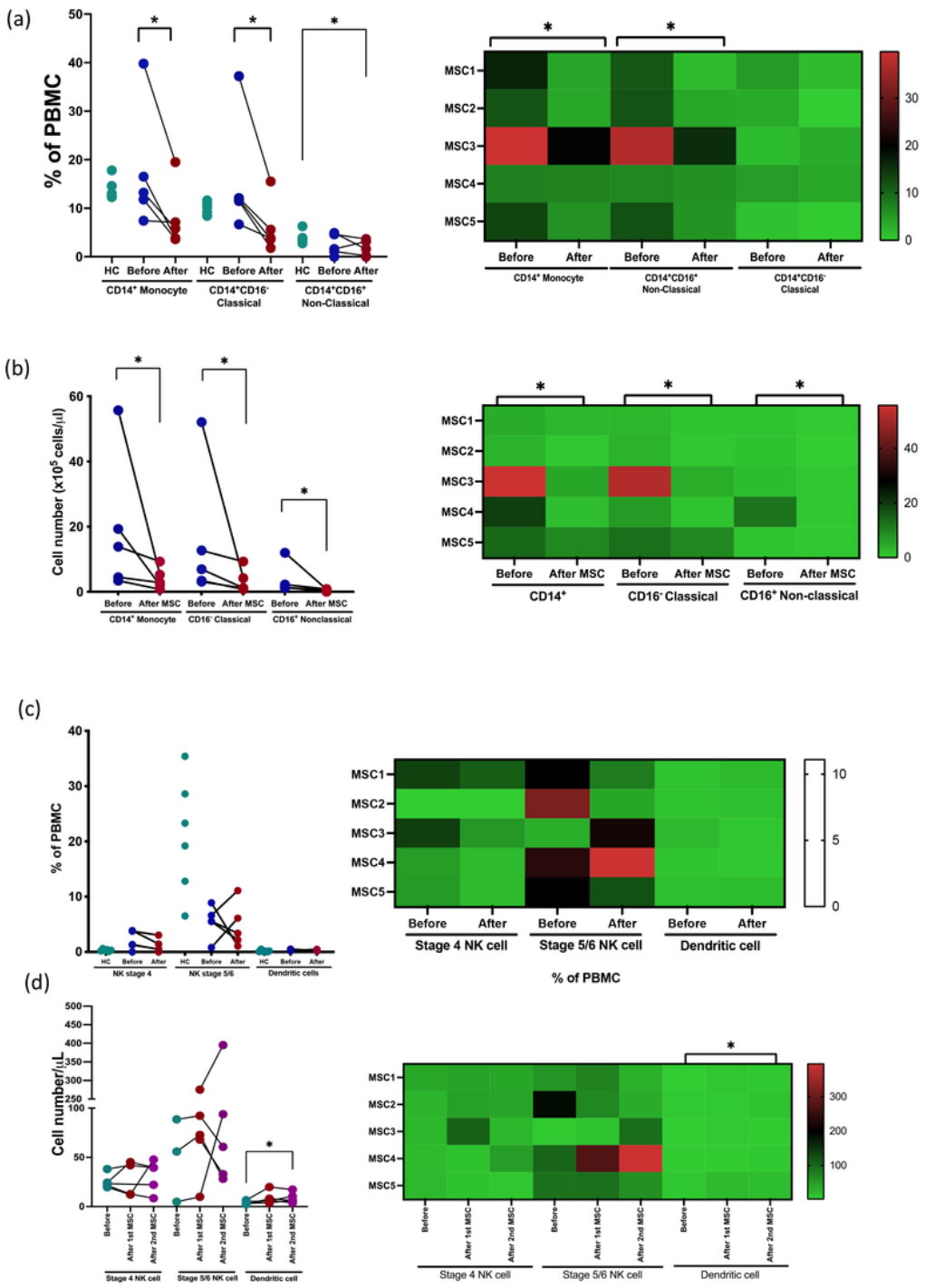

Figure 5

The shifting of monocyte and NK cell population after pcMSC treatment. (a)Proportion of total monocyte count and classical Monocyte was reduced after treatment (left) and it was also showed in heat map (right) with color gradient. Non –classical monocyte was decreased compared with healthy control after treatment (left); (b)Significant reduction of total amount of classical monocyte and non-classical monocyte though total amount reduction didn't reach significant (left) and color gradient on heat map

(right); (c)Proportion of all type of NK cell changes were not significant compared with healthy control (left) and also after treatment (right). (d)Increased in total amount of dentritic cell was seen after D8. *P < 0.05 and **P < 0.01, nonparametric paired Wilcoxon t test

Supplementary Files

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

- s1.png
- s2.png
- s3.png