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**ABSTRACT**

**Background:** The novel SARS-CoV-2 has caused the COVID-19 pandemic. Currently, with insufficient worldwide vaccination rates, the identification of treatment solutions to reduce the impact of the virus is urgently needed. **Method:** An adaptive, multicentric, open-label, and randomized controlled phase I/II clinical trial entitled the “SENTAD-COVID Study” was conducted by the Abu Dhabi Stem Cells Center under conditional exceptional approval by the Emirates Institutional Review Board (IRB) for COVID-19 Research Committee from April 4 to July 31, 2020, using an autologous peripheral blood nonhematopoietic enriched stem cell cocktail (PB-NHESC-C) administered by compressor (jet) nebulization as a complement to standard care therapy. The primary endpoints include safety and efficacy assessments, adverse events, the mortality rate within 28 days, and the time to clinical improvement as measured by a 2-point reduction on a seven-category ordinal scale or discharge from the hospital, whichever occurred first. **Results:** The study included a total of 139 randomized COVID-19 patients with 69 in the experimental group and 70 in the control group (standard care). Overall survival was 94.20% for the cocktail-treated group vs. 90.27% for the control group. Adverse events occurred in 43 (62.32%) patients receiving PB-NHESC-C vs. 44 (62.86%) in the control group, and most adverse events were related to the disease. After the first nine days of the intervention, 67.3% of cocktail-treated patients recovered and were released from hospitals compared to 53.1% (RR=0.84; 95% CI, 0.56-1.28) in the control group. Improvement, i.e., at least a 2-point reduction in the severity scale, was more frequently observed in cocktail-treated patients (42.0%) than in controls (17.0%) (RR=0.69; 95% CI, 0.56-0.88). **Conclusions:** Cocktail treatment improved clinical outcomes without increasing adverse events. Thus, the nebulization of PB-NHESC-C was safe and effective for treatment in most of these patients. **Trial registration:** ClinicalTrials.gov. NCT04473170. Registered 16 July 2020. Retrospectively registered.
INTRODUCTION

In December 2019, a novel member of the family of severe acute respiratory syndrome coronaviruses named SARS-COV-2 rapidly spread from China throughout the globe, causing a pandemic of the human respiratory illness recognized as COVID-19 [1]. The United Arab Emirates (UAE) has shown a sharp and chaotic increase in the number of confirmed COVID-19 patients [2] despite the country's nationwide efforts to control the disease by introducing a series of strict measures to stop the spread of infection. After a noticeable surge of cases, the UAE declared a state of emergency to fight coronavirus, emphasizing the need for a new approach for treatment to decrease disease progression and related deaths. SARS-CoV-2 infection can cause a wide spectrum of symptoms, ranging from mild to severe illness. Serious COVID-19 cases are characterized by severe pneumonia, acute respiratory distress syndrome (ARDS), excessive acute inflammatory responses, development of cytokine storms, and multiple organ failures leading to death, whereas nonsevere cases present known clinical manifestations of respiratory system infection [3, 4]. Although clinical aspects of COVID-19 patients have been widely reported and their management has increased tremendously, safe and effective medications are still lacking. Indeed, several clinical trials have been developed and are still under investigation for COVID-19 prevention, treatment, and diagnosis [5-7].

Currently, cellular therapy, a new field of medicine that uses cell-based products, is considered a pillar of regenerative and personalized medicine [8]. Different types of stem
cells, including embryonic stem cells and adult stem cells, such as hematopoietic stem cells (HSCs), mesenchymal stem cells (MSCs), induced pluripotent stem cells (iPSCs), and, more recently, very small embryonic-like stem cells (VSELs), have been considered “biocandidates” in several therapies [9, 10]. Adult stem cells are described as somatic, rare, and undifferentiated populations located in their niches within several adult solid organs and play a key role in maintaining tissue homeostasis. Additionally, several studies have reported that adult stem cells, including VSELs, are self-renewing and multipotent, exist in a dormant state, and are recruited to the peripheral blood (PB) under an alarm recall to support the role of innate immunity [11, 12]. Accordingly, adult stem cells are most likely present at high concentrations in the bloodstream of COVID-19 patients in response to exuberant inflammatory processes. However, good immune phenotyping of stem cells in the PB after SARS-CoV-2 infection is needed to develop potential cell therapeutic applications. To date, MSCs have shown a potential therapeutic effect in COVID-19, and several clinical trials have been registered for MSC-based therapies using different routes for delivery, including intravenous, intratracheal instillation, and inhalation/nebulization [13, 14]. Unfortunately, the challenge in adult stem cell therapy is the lack of well-established procedures for stem cell characterization, including cell isolation, purification, and expansion, without altering their phenotypes and functions [15]. To overcome these challenges, our group has developed a patented method for isolating an autologous peripheral blood nonhematopoietic enriched stem cell cocktail (PB-NHSC-C) [16] to be administered by the nebulization route. The objective of this study was to assess the safety and efficacy of PB-NHESC-C stem cell cocktail nebulization as an add-on therapy to standard care for SARS-CoV-2-infected patients during the COVID-19 outbreak in Abu Dhabi, UAE.
METHODS

Participants and Study Design

The safety and efficacy of an autologous PB-NHESC-C in patients with COVID-19 were evaluated by an adaptive, multicentric, open-label, and randomized controlled phase I/II clinical trial called the “SENTAD-COVID Study”. The study protocol was designed by the Abu Dhabi Stem Cells Center (ADSCC), received conditional exceptional approval by the Ministry of Health and Prevention via the Emirates Institutional Review Board (IRB) for COVID-19 Research Committee, and was performed in Abu Dhabi from April 4 to July 31, 2020, using procedures based on ethical standards of the Helsinki Declaration of 1975 (as revised in 1983), such as informed consent for all participants. Patients of at least 15 different nationalities who resided in the emirate of Abu Dhabi were hospitalized and treated in four major government hospitals with Sheikh Khalifa Medical City Hospital serving as the main primary care clinical trial unit. The sample size of the study was established to detect a clinically important difference between both groups concerning hospital stay and mortality with 80% power and a 5% alpha error. Permitted block randomization was used to allocate patients to the treatment groups. Each “block” had a specified number of randomly ordered treatment assignments. On the clinical site, selected investigators/physicians in charge of patient care communicated with the trial subjects. When a patient met the inclusion/exclusion criteria, they were recruited only after informed consent was signed by them (or their legal representative), and a doctoral supervisor approved the collection of data from clinical records and an initial clinical evaluation. A total of 139 COVID-19 patients with HCoV-19 RNA confirmed by RT-PCR assay [17] were randomly included, as shown in Figure 1.

Figure 1. Patient allocation during the SENTAD-COVID Study.

Legend: Four initially screened patients were excluded before randomization because two
had been previously diagnosed with malignant diseases and two were previously included in other clinical trials. After randomization, data from four additional patients were not analyzed. Two patients in the control group had data error identifications. Two patients were not followed in the PB-NHSC-C group: one was released from the hospital, and the other unfortunately passed away before the initial day of treatment.

Patients were excluded from the trial based on the following criteria: pediatric patients (aged <18 years), history of cancer, organ transplant in the last 3 months, pregnant or lactating women, inability to provide signed consent and to comply with test requirements, such as having anemia or bloodborne infectious diseases as well as the inability to collect peripheral blood stem cells and participate in other clinical trials during or within 3 months. Finally, patients were categorized according to their disease severity and clinical manifestations at the recruitment based on guidelines established by the interim guidance of the World Health Organization (WHO) as follows: scores of 1-2 represent patients in ambulatory conditions (1 indicates a completely asymptomatic patient, and 2 represents a patient with some limitation of activity); scores of 3-4 represent hospitalized patients with mild disease (3 indicates a hospitalized patient without oxygen therapy, and 4 indicates a patient receiving oxygen by mass or nasal prongs); scores of 5-7 represent patients hospitalized with severe disease (5 represents a patient with no invasive ventilation or high flow oxygen, 6 represents a patient with intubation and mechanical ventilation, and 7 represents a patient under ventilation plus additional support, including vasopressor, renal replacement therapy, or extracorporeal membrane oxygenation); a score of 8 indicates a deceased patient [18].

PB-NHESC-C: Preparation, and Characterization

Preparation of the Investigational Product
As an autologous stem cell investigational product, PB-NHESC-C was prepared in a closed system using a collection of 300 mL of PB. Stem cells were characterized by the presence of hematopoietic stem cell subsets expressing CD34⁺, CD133⁺, CD90⁺, and CD45⁺, and nonhematopoietic stem cells identified by their lack of CD45⁺ expression. PB-NHESC-C was suspended in 30 mL of platelet growth factors (PGFs) obtained by activating autologous platelets with sonication and stored at 4-8 °C for 24 h before clinical application within 5 days [16].

Flow Cytometry Product Analysis

A Beckman Coulter Navios EX cytometer (Beckman Coulter, USA) was used; 100 μL of PBNHE-SC was dispensed for staining with fluorochrome-conjugated monoclonal antibodies from the same manufacturer: anti-CD45 (Kro; clone J33); anti-CD90 (PB; clone Thy-1/310); anti-CD 133 (APC; clone W6B3C1); anti-CD34 (ECD; clone 581), and 7-amino actinomycin D (7-AAD) for cell viability staining, to exclude dead cells from the analysis. After incubation, cells were treated for 10 minutes with 500 µL of OptiLyse C, and 100 µL flow-count fluorospheres were added for absolute cell counts.

Angiotensin-Converting Enzyme 2 (ACE2) Surface Expression Determination

One hundred microliters of PBNHE-SC were incubated for 20 minutes in the dark with 20 µL of Beckman-Coulter anti-CD45 (Kro; clone J33), 7-amino actinomycin D (7-AAD), and 5 µL of anti-CD143 (APC; clone 5-369, Biolegend). Acquisition data were processed using Kaluza C Software V1.1 with a minimum of 150,000 acquired events. The gating strategy is shown in Figure 2.

[Insert figure 2 here]
Legend: Immunophenotype characterization of peripheral blood nonhematopoietic enriched stem cell cocktail. Figure 2a) Logic and manual gating strategy for cell characterization using five monoclonal antibody-conjugated CD markers simultaneously, including 7-amino-actinomycin D (7-AAD). Figure 2b) Expression of angiotensin-converting enzyme 2 (ACE2) on the surface of cells.

**Immunofluorescence Analysis**

Samples of PB-NHESC-Cs were fixed with 3.5% paraformaldehyde for 20 min, pre-blocked with 2% bovine serum albumin (BSA) for 10 min at room temperature (RT), and subsequently stained with fluorescein isothiocyanate (FITC)-conjugated CD45 (1:100, mouse monoclonal IgG; Beckman Coulter) for 30 min at RT in the dark. Hoechst 33342 nucleic acid stain (Sigma Aldrich) was added at 10 µg/mL to the cell suspensions for 20 min at RT in the dark. After washing, PB-NHESC-Cs was acquired using a laser scanning microscope (Leica SP8 confocal microscope, Leica) with FITC (emission 496-598 nm) and Hoechst (emission 415-470 nm) channels using a 60x objective.

**SARS-CoV-2 Antibody Detection**

SARS-COV-2 antibody levels were assessed in 63 PGF samples of treated patients (13 severe cases; 50 moderate cases) using semiquantitative Ortho VITROS® Anti-SARS-CoV-2 Total (CoV2T) and Anti-SARS-CoV2-IgG (CoV2G) antibodies (Ortho Clinical Diagnostics, Raritan, New Jersey) and analyzed using the VITROS ECi/ECiQ 3600 Immunodiagnostic System following the manufacturer’s instructions. The results were reported as either reactive (S/CO≥1.0) or nonreactive (S/CO<1.0), and the S/CO was obtained using the manufacturer system [19].
**PGF Metabolite Measurement**

Human PGF concentrations of angiopoietin-2 (Ang-2), epidermal growth factor (EGF), erythropoietin (EPO), fibroblast growth factor (FGF-basic), granulocyte-colony stimulation factor (G-CSF), granulocyte/macrophage-colony stimulation factor (GM-CSF), hepatocyte growth factor (HGF), macrophage-colonystimulation factor (M-CSF), platelet-derived growth factor AA (PDGF-AA), platelet-derived growth factor BB (PDGF-BB), stem cell factor (SCF), T-cell growth factor alpha (TGF-α), and vascular endothelial growth factor (VEGF) were determined in 20 PGF samples of COVID-19 patients (10 moderate and 10 severe) using a 13-plex bead-based multiplex assay kit (LEGENDplex™ Human Growth Factor Panel; Cat. No. 740180) as described by the manufacturer. PGF metabolite concentrations were determined by fluorescence intensity using a Navios EX cytometer and analyzed with LEGENDplex V.8.0 software as recommended by the manufacturer.

**Treatment Procedures and Follow-up**

PB-NHESC-C was delivered to the patients of Group A following compressor (jet) nebulization for a total of two doses 24 hrs apart. Both doses were administered through a sterile humidifier and a regular concentrated oxygen supply at a flow rate of 5-6 L/min. For clinical and laboratory evaluations, stem cell treatments were performed on days 0 and 1, and daily follow-up was performed for 28 days. Group B (control) was treated exclusively with the standard care provided to both groups.

All clinical, laboratory, and radiological data were recorded during patient follow-up. From a clinical point of view, a detailed record included primary safety data (nebulization or standard treatment-induced allergic reactions, secondary infection, and severe and non-severe adverse events), and efficacy data were reported for the measurement of endpoints. Laboratory tests included a complete blood count determined using a Hematology Analyzer.
DHX900 (Beckman Coulter, USA) and acute phase reactants, such as C-reactive protein (CRP), which was assessed using a Chemistry Analyzer AU480 (Beckman Coulter, USA), and fibrinogen and D-dimer, which were assessed using a Cobas t 511 (Roche, Switzerland), according to the manufacturer's instructions and in compliance with ADSCC standard operating procedures (SOP). SARS-CoV-2 RNA was assessed by real-time reverse transcription PCR before recruitment and after treatment [17]. Both groups were followed up using different imaging approaches until discharge, and the control radiological image reports and computed tomography CT were evaluated as previously described [20].

Outcomes Evaluation

Primary Safety Data: Adverse Effects and Disease Assessment

Adverse events were assessed using the “Common Terminology Criteria for Adverse Events (CTCAE) v5.0” [21]. Nebulized patients were closely followed up for possible acute adverse events of treatment (AET) during the first 3 days after receiving stem cell nebulization, and this close follow-up continued for 28 days. AET in patients not treated with stem cells was also compared during the same time period.

Primary Endpoints

a) Hospital discharge. Assessed after the first 9 days of randomization: (1st. tertile of the follow-up).

b) Mortality. Death by any cause in the 28 days of follow-up.

Secondary Endpoints

a) Accelerated clinical improvement after 9 days (1st. tertile of follow-up): Defined as a net decrease of at least 2 points on the scale (excluding patients who experienced
increases in points in the period analyzed).

b) Clinical improvement after 9 days (1st tertile of follow-up). Defined as a net decrease of at least one point on the scale (excluding patients who experienced increases in points in the period analyzed).

Exploratory Endpoints

a) The persistence of lymphopenia.
b) The appearance of lymphopenia.
c) The persistence of an elevated neutrophil/lymphocyte ratio (NLR).
d) The persistence of high C-reactive protein (CRP) levels.
e) The persistence of elevated D-dimer levels (in the stem cell-treated group).

All assessments were performed on the day of randomization and 5 days (1st quintile) after the intervention. Control group: evaluated on the day of randomization and 6 days later.

Statistical Analysis

Efficacy was assessed in the intention-to-treat and safety-as-treated groups in the study population. All statistical tests were performed to demonstrate the superiority of the assessed experimental therapy against the standard of care established for COVID-19. The Shapiro-Wilk test was performed to assess the normality of data distribution. The Mann-Whitney U nonparametric test was used for the analysis of two independent groups. Spearman’s rho correlation was applied in the comparison of downward changes in scores. The Wilcoxon test for nonparametric paired samples in laboratory variables before and after interventions as well as Fisher’s exact probability test for a small number of samples were also applied in the adverse events comparison, whereas the chi-square test was used for categorical data, such as in the comparison of patient demographics and clinical proportions, adverse events and
some laboratory variable analyses. To evaluate the clinical impact of interventions, the relative risk (RR) was calculated with a 95% confidence interval (CI), and the RR and number needed to treat (NNT) were reduced. Most of the statistical analyses were performed with GraphPad Prism v.8 (La Jolla, America) [22] and MedCalc software [23]. All p-values represented were two-sided and considered statistically significant when p<0.05.

RESULTS

Patient Groups

Figure 1 shows the number of screened, excluded, and finally enrolled patients as well as the randomized groups before treatment, the numbers of patients lost after randomization and before any interventions (together with reasons for loss), and the final outcomes of all participants. The main demographic data and clinical status of the COVID-19 patients are shown in Table 1. [Insert Table 1 here]

Finally, a total of 139 SARS Cov-2 confirmed by PCR patients were enrolled. The clinical score on the day of randomization (before initial treatment) was taken as a reference. The recruited patients were composed of 44 critical disease patients: 31 critically severe type (score 7), 13 severe patients (scores 5 and 6), and 95 moderate COVID-19 types (score 3: n=77, and score 4: n=18). No significant differences were noted between the two groups based on the Fisher's exact probability test. For treatment randomization, COVID-19 patients were divided into two groups. Group A included 69 patient donors (65 males, 4 females) aged 45.93+/−9.75 years old (mean +/- SD; minimum 27, maximum 71) who were recruited to assess the investigational product whole receiving the standard COVID-19 care treatment established by the Ministry of Health and Prevention of the United Arab Emirates (MOHAP) [24]. Group B served as the control group with a total of 70 patients (64 males, 6 females)
aged 44.31 +/- 11.22 years old (mean +/-SD; minimum 26, maximum 73). These patients were from the same hospitals. These patients exclusively received the same standard care for COVID-19 and did not receive cell therapy products.

PB-NHESC-C Characterization

The PB-NHESC-C was characterized by flow cytometry (figure 2a) and two main cell fractions with greater than 95% viability were noted: hematopoietic stem cells (CD45$^{\text{dim}}$) in the range of 25-49% (median 36%) and nonhematopoietic stem cells (CD45$^{-}$) in the range of 53-70% (median 64%) with one or more of the following markers: CD133$^{+}$, CD90$^{+}$ and CD34$^{+}$. The PB-NHESC-C also contains anti-SARS-CoV-2 antibodies and PGF.

The median total number of nebulized cells was 2.2x10$^6$ for the whole group of treated patients, 1.8x10$^6$ among severe COVID-19 patients, and 2.5x10$^6$ in moderate cases. PB-NHESC-C was predominated comprised of CD45 CD133$^{+}$ and CD34$^{+}$ cells. CD45$^{\text{dim}}$-positive cell counts were significantly higher in moderate cases (p<0.0001), and the CD45$^{-}$ cell count was higher but not statistically significant in severe cases (p=0.6). A positive correlation was observed between the patient’s absolute lymphocyte count and the number of CD45 cells in PB-NHESC-C (p=0.006) and its absence with respect to CD45$^{-}$ cells (p=0.79). The CD45$^{-}$ CD90$^{+}$ marker predominated in the PB-NHESC-C of severe patients compared with moderate patients (p=0.04), as shown in Table 2.

ACE2 receptor expression was demonstrated in all CD45$^{-}$ and CD45$^{\text{dim}}$ cells (figure 2b). On the other hand, the presence of anti-SARS-CoV-2 total antibodies (COV2T) was detected in all cocktail samples of severe patients and 94% of moderate cases (p=0.36), and IgG anti-SARS-CoV-2 antibodies (COV2G) were more frequently observed (84.3%) in the autologous preparations of severe compared with moderate patients (48%) (p=0.01). The semiquantitative values of total anti-CoV-2 (102.0 vs. 16.4,
p=0.007) and anti-anti-CoV-2 IgG (6.9 vs. 1.6, p=0.005) were higher in severe cases compared with moderate cases.

**Table 2.** Cellular and humoral components of the peripheral blood non-hematopoietic enriched stem cell cocktail.

<table>
<thead>
<tr>
<th>Cellular elements, median (range)</th>
<th>Severe cases (n = 19)</th>
<th>Moderate cases (n = 50)</th>
<th>Total (n = 69)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total stem cell dose (× 10⁶ cells)</td>
<td>1.8 (0.2-12)</td>
<td>2.5 (0.4-23)</td>
<td>2.2 (0.4-23)</td>
<td>0.34</td>
</tr>
<tr>
<td>Total CD markers (× 10⁵ cells)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD45⁺</td>
<td>6.4 (0.1-49.5)</td>
<td>3.5 (0.1-7.1)</td>
<td>4.6 (0.1-7.1)</td>
<td>0.6081</td>
</tr>
<tr>
<td>CD45dim⁺</td>
<td>0 (0-10.5)</td>
<td>5.2 (0.1-45.7)</td>
<td>3.7 (0-45.7)</td>
<td>&lt;0.0001**</td>
</tr>
<tr>
<td>CD133⁺</td>
<td>5.2 (1.1-31.2)</td>
<td>7.1 (0.2-69)</td>
<td>6.8 (0.2-69)</td>
<td>0.3056</td>
</tr>
<tr>
<td>CD34⁺</td>
<td>4.2 (0.2-37.1)</td>
<td>7.7 (0.1-52.7)</td>
<td>6.1 (0.1-52.7)</td>
<td>0.0920</td>
</tr>
<tr>
<td>CD90⁺</td>
<td>3.9 (0-13)</td>
<td>0.6 (0-12)</td>
<td>0.1 (0-13)</td>
<td>0.0485*</td>
</tr>
</tbody>
</table>

Proportion of patients and semiquantitative values of anti-SARS-CoV-2 antibodies related to severity (S1 subunit of SARS-CoV-2 spike protein)

<table>
<thead>
<tr>
<th>Humoral factor</th>
<th>Severe cases</th>
<th>Moderate cases</th>
<th>Total evaluated</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients’ anti-SARS-CoV2 antibodies, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total class antibodies</td>
<td>13 (100)</td>
<td>47 (94.0)</td>
<td>60 (95.2)</td>
<td>0.36</td>
</tr>
<tr>
<td>IgG class</td>
<td>11 (84.3)</td>
<td>24 (48.0)</td>
<td>35 (55.5)</td>
<td>0.018*</td>
</tr>
<tr>
<td>Optical density (OD) median value</td>
<td>(n = 13)</td>
<td>(n = 50)</td>
<td>(n = 63)</td>
<td></td>
</tr>
<tr>
<td>Total class antibodies</td>
<td>102.0</td>
<td>16.45</td>
<td>28.4</td>
<td>0.007**</td>
</tr>
<tr>
<td>IgG class</td>
<td>6.920</td>
<td>1.60</td>
<td>1.27</td>
<td>0.005**</td>
</tr>
</tbody>
</table>

Concentration of platelet growth factor in 20 selected COVID-19 patient samples of PB-NHESC- cocktail

<table>
<thead>
<tr>
<th>Platelet growth factor name (abbreviation)</th>
<th>Total evaluated (n = 20), pg/mL [median (range)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stem Cell Factor (SCF)</td>
<td>4.26 (4.26-21.51)</td>
</tr>
<tr>
<td>Platelet Derived Growth Factor-AA (PDGF-AA)</td>
<td>382.3 (148.8-2542)</td>
</tr>
<tr>
<td>Granulocyte-Colony Stimulation Factor (G-CSF)</td>
<td>18.28 (18.28-97.25)</td>
</tr>
<tr>
<td>Hepatocyte Growth Factor (HGF)</td>
<td>25.91 (19.53-2885)</td>
</tr>
<tr>
<td>Epidermal Growth Factor (EGF)</td>
<td>44.02 (4.40-125.2)</td>
</tr>
<tr>
<td>Granulocyte Macrophage-Colony Stimulation Factor (GM-CSF)</td>
<td>8.99 (8.99-17.97)</td>
</tr>
<tr>
<td>T-Cell Growth Factor alpha (TGF-α)</td>
<td>1.61 (9.53-188.68)</td>
</tr>
<tr>
<td>Platelet Derived Growth Factor-BB (PDGF-BB)</td>
<td>51.09 (16.04-187.2)</td>
</tr>
<tr>
<td>Macrophage-Colony Stimulation Factor (M-CSF)</td>
<td>152 (73.69-268.8)</td>
</tr>
<tr>
<td>Angiopoietin-2 (Ang-2)</td>
<td>35.51 (2.56-327.4)</td>
</tr>
<tr>
<td>Fibroblast Growth Factor (FGF-basic)</td>
<td>1.6 (1.66-39.29)</td>
</tr>
<tr>
<td>Vascular Endothelial Growth Factor (VEGF)</td>
<td>1.7 (8.72-91.96)</td>
</tr>
<tr>
<td>Erythropoietin (EPO)</td>
<td>7.63 (14.46-65.55)</td>
</tr>
</tbody>
</table>

χ²-test; bMann-Whitney test for nonparametric samples; *: significant difference; **: highly significant difference; pg: picograms.
Therefore, PB-NHESC-C was also characterized by the presence of human PGF derived from autologous platelet-enriched plasma, and PDGF-AA and M-SCF exhibited the highest concentrations followed by PDGF-BB, EGF, Ang-2, and HGF. In addition, after the isolation of stem cells from the PB of treated patients, a representative image of PB-NHESC-C shows very small cells negative for CD45 with a diameter of approximately 5-7 µm and a fraction of cells dimly stained with CD45 and slightly higher in number compared with the negatively stained cells (Figure 3).

Figure 3. Representative immunofluorescence images of PB-NHESC-C.

Legend: PB-NHESCs were stained with FITC-conjugated monoclonal surface antibody CD45 (1:100) and Hoechst nucleic acid dye 33342 (10 µg/ml). Images were acquired using a Leica SP8 confocal microscope using a 63x objective. Two main populations were found: nonhematopoietic (*) and hematopoietic stem cells.

Outcomes

Primary Safety Data: Assessment of Adverse Events and Safety.

Patients with adverse events were reported from both groups with a total of 101 (72.66% of enrolled patients) affected. There were two groups. In total, 50 (72.46%) patients received stem cell treatment compared to 51 (72.85%) patients in the control group (p=0.9419). A total of 240 adverse events were reported during the 28-day follow-up for all enrolled patients (Table 3). Serious events, including one patient death, were assessed. In total, 35 serious events occurred in the PB-NHESC-C-treated group, and 57 were noted in the control group.
Table 3. Adverse events reported during the follow-up: SENTAD-COVID Study.

<table>
<thead>
<tr>
<th>Adverse events</th>
<th>PB-NHESC-C + standard care (n = 69)</th>
<th>Standard care (n = 70)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total patients affected by adverse events, n (%)</td>
<td>50 (72.46)</td>
<td>51 (72.85)</td>
<td>0.9590a</td>
</tr>
<tr>
<td>Total adverse events, n (%)</td>
<td>n = 240</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adverse events by group, n (%)</td>
<td>107 (44.58)</td>
<td>133 (55.41)</td>
<td>0.8206a</td>
</tr>
<tr>
<td>Number of serious adverse events by group (including deaths)</td>
<td>35 (32.71)</td>
<td>57 (42.85)</td>
<td>0.1040a</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Adverse event type</th>
<th>Description</th>
<th>PB-NHESC-C</th>
<th>Standard care</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total deaths, n (%)</td>
<td>Death</td>
<td>4 (5.79)</td>
<td>7 (10)</td>
<td>0.5319c</td>
</tr>
<tr>
<td>Other serious adverse events, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe anemia</td>
<td>Hemoglobin reduction &lt; 100 g/dL</td>
<td>10 (14.49)</td>
<td>8 (11.42)</td>
<td>0.5912a</td>
</tr>
<tr>
<td>Disease progression</td>
<td>Any score increased</td>
<td>5 (7.24)</td>
<td>9 (12.85)</td>
<td>0.3989c</td>
</tr>
<tr>
<td>Sepsis</td>
<td>Isolation of pathological fungal or bacterial from blood cultures indicating disseminated infections</td>
<td>5 (7.24)</td>
<td>15 (21.42)</td>
<td>0.0278c</td>
</tr>
<tr>
<td>Acute renal failure</td>
<td>Hemodialysis of at least 2 days</td>
<td>4 (5.79)</td>
<td>6 (8.57)</td>
<td>0.7447c</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>Oxygen saturation (SpO₂) &lt;88%</td>
<td>3 (4.34)</td>
<td>4 (5.71)</td>
<td>0.7184c</td>
</tr>
<tr>
<td>Acute respiratory distress syndrome</td>
<td>Respiratory rate &gt;20 or &lt;12 + SpO₂ ≤88% + score ≥6</td>
<td>3 (4.34)</td>
<td>5 (7.14)</td>
<td>0.7184c</td>
</tr>
<tr>
<td>Multiorgan failure</td>
<td>Score changed to 7</td>
<td>1 (1.44)</td>
<td>3 (4.28)</td>
<td>0.6195c</td>
</tr>
<tr>
<td>Other nonserious adverse events</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increased respiratory breath rate</td>
<td>O₂ saturation levels ≥100</td>
<td>30 (43.47)</td>
<td>34 (48.57)</td>
<td>0.7348a</td>
</tr>
<tr>
<td>Hypertension</td>
<td>Blood pressure ≥120/80 mmHg</td>
<td>17 (24.63)</td>
<td>12 (17.14)</td>
<td>0.2789a</td>
</tr>
<tr>
<td>Fever</td>
<td>Temperature ≥38 °C</td>
<td>13 (18.84)</td>
<td>14 (20)</td>
<td>0.8633a</td>
</tr>
<tr>
<td>Severe decreased absolute lymphocyte count</td>
<td>Lymphocytes &lt;0.8 × 10⁹/L</td>
<td>8 (11.59)</td>
<td>9 (12.85)</td>
<td>1.0000a</td>
</tr>
<tr>
<td>Sinus tachycardia</td>
<td>Heart rate (beats/min) ≥100</td>
<td>2 (2.89)</td>
<td>3 (4.28)</td>
<td>1.0000c</td>
</tr>
<tr>
<td>Sinus bradycardia</td>
<td>Heart rate (beats/min) &lt;60</td>
<td>1 (1.45)</td>
<td>2 (2.85)</td>
<td>1.0000c</td>
</tr>
<tr>
<td>Hypotension</td>
<td>Blood pressure &lt;100/60 mmHg</td>
<td>1 (1.45)</td>
<td>2 (2.85)</td>
<td>1.0000c</td>
</tr>
</tbody>
</table>

PB-NHESC-C: peripheral blood non-hematopoietic enriched stem cell cocktail, a: χ² test; b: exact Poisson method; c: Fisher’s exact test; *: significant difference.

Primary Endpoints: PB-NHESC-C Treatment Partially Demonstrated Superior Effects on Hospital Discharge and Mortality Reduction

Hospital Discharge: After 9 days of follow-up (in the evaluation of the first tertile after cell therapy), 63.3% of PB-NHESC-C-treated patients recovered and were discharged from hospitals, while in the control group, this percentage was only 57.1%, but a nonsignificant
difference was found. This patient’s hospital discharge after the first 9 days was related to a higher dose of CD45\textsuperscript{dim} cells compared to the other cases (4.6x10\textsuperscript{6} vs. 0.7x10\textsuperscript{6}, p=0.004), specifically those with the CD45\textsuperscript{dim} CD34\textsuperscript{+} phenotype (1.4x10\textsuperscript{6} vs. 0, p=0.0002) and treated with a low dose of CD45\textsuperscript{-} CD90\textsuperscript{+} cells (0.6x10\textsuperscript{6} vs. 3.2x10\textsuperscript{6}, p=0.04). Nevertheless, no significant differences in the total number of nebulized cells were noted in patients discharged before and after 9 days (1.6x10\textsuperscript{6} vs. 2.3x10\textsuperscript{6}, p=0.71). Positivity for anti-SARS-CoV-2 IgG antibodies was less frequently noted in the PB-NHESC-C administered to the cases with hospital discharge during the first 9 days (43.1% vs. 84.2%, p=0.002), and similar effects were noted for the quantitative values of anti-SARS-CoV-2 total antibody classes COV2T (13.0 vs. 102.0, p=0.001) and anti-COV2G (0.5 vs. 6.6, p=0.008). \textbf{Mortality:} The death rate in the treated group was 5.8% vs. 9.73% in the controls.

\textit{Secondary Endpoints: Clinical Improvement over Time due to PB-NHESC-C Treatment}

\textit{Observed in Severe and Moderate COVID-19 Patients.}

[Insert figure 4 here]

\textbf{Figure 4. Clinical improvement. Different trend line slopes during the clinical trial follow-up.}

Legend: Group A/S: Peripheral Blood Non-Hematopoietic Enriched Stem Cell Cocktail (PB-NHESC-C) Treated classified as severe; Group B/S: Controls classified as severe; Group A/M: PB-NHESC-C Treated classified as moderate; Group B/M: Controls classified as moderate.

From a clinical point of view, as seen in Figure 4, severe COVID-19 patients treated with PB-NHESC-C resulted in more rapid clinically improvement compared with the corresponding control subgroups, as measured by the number of patients with clinical score reductions on a
daily basis. A trend line of $y=-0.1076x+6.4331$ ($R^2=0.951$) was noted in severe stem cell-treated COVID-19 patients, whereas the same evaluation in severe controls yielded a trend line of $y=-0.0343x+5.8026$ ($R^2=0.3372$) during the 28 days of monitoring. An extremely significant difference in slopes of the lines were noted between both groups ($p\leq0.0001$).

Moderate stem cell-treated COVID-19 patients also improved and showed a linear trend of $y=-0.1185x+3.4715$ ($R^2=0.6732$) compared with moderate COVID-19 controls with $y=-0.0806x+3.341$ ($R^2=0.3354$) during the first 15 days of monitoring, and a significant difference in slopes was noted, indicating better results for the stem cell-treated patients ($p=0.0074$).

**Clinical Impact: The PB-NHESC-C-Treated Group Exhibited a Greater Proportion of Patients with Clinical Improvements**

The PB-NHESC-C-treated group showed a greater proportion of patients with clinical improvement than the control group after the first 9 days of hospitalization after randomization, as shown in Figure 5.

![Figure 5](image)

**Figure 5. Clinical impact on the assessed outcomes.**

**Legend:** NLR: Neutrophil to Lymphocyte Ratio; CRP: C-Reactive Protein; EI0: Exposure Incidence in controls; EI: Exposure Incidence in peripheral blood nonhematopoietic enriched stem cell cocktail treated-patients; RR: relative risk; 95% CI: confidence interval; RRR: relative risk reduction; NNT: number needed to treat to produce the effect; a: Z-test. *: significant; **: highly significant.

This finding was evidenced by an increased incidence of hospital discharges as previously stated during that period in treated patients compared to the controls (63.3% vs. 57.1%, respectively) ($RR=0.84$). The clinical improvement, which was evidenced by a greater than
two-point reduction, demonstrated the effectiveness of the treatment (RR=0.69). If this type of therapy was applied to the control group, the number of patients who did not experience this type of improvement would have been reduced by 31%. Only 4 patients were required to be treated with nebulization, and one patient improved by greater than 2 points in 9 days. More improvements of at least a one-point reduction were noted in the treated group, but the result was nonsignificant. The stem cells treatment does not result in equivalence or inferiority, it rather maintains superiority compared with controls. Based on the laboratory data, appearance of lymphopenia was noted in 26.3% vs. 0% (RR=0.03; p=0.012), persistence of high levels of C-reactive protein was 76.2% vs. 36.0% (RR=0.47; p=0.011) and persistence of high levels of D-dimer was 92.3% vs. 47.1% (RR=0.51; p=0.010) in the control group vs. the PB-NHESC-C-treated group, respectively.

**Exploratory Endpoints: Lymphocyte, Neutrophils and Acute Phase Reactant Changes in PB-NHESC-C-Treated Patients**

Table 4 shows the comparison of laboratory results before and after in each group of patients and the comparison between these groups after 28 days of follow-up. After treatment with PB-NHESC-C, all COVID-19 patients showed better results in normalizing the absolute number of lymphocytes and improving the neutrophil/lymphocyte ratio compared with the control group. In addition, fibrinogen, IL-6, and C-reactive protein levels also significantly decreased in the treated group during the follow-up. In the control group, only statistically significant changes were observed in the reduction of C-reactive protein levels. Comparing blood cell counts in both groups, differences were observed in the levels of white blood cells, absolute

<table>
<thead>
<tr>
<th>Variables</th>
<th>PB-NHESC-C treated</th>
<th>Control</th>
<th>p-value&lt;sup&gt;a&lt;/sup&gt;</th>
<th>p-value&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>Time</td>
<td>Time</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White blood cells (10&lt;sup&gt;9&lt;/sup&gt;/L)</td>
<td>1&lt;sup&gt;st&lt;/sup&gt; 51</td>
<td>6.8 (4, 20.45)</td>
<td>0.5377</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2&lt;sup&gt;nd&lt;/sup&gt; 51</td>
<td>7.6 (3.84, 18.76)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutrophils (10&lt;sup&gt;9&lt;/sup&gt;/L)</td>
<td>1&lt;sup&gt;st&lt;/sup&gt; 51</td>
<td>4.4 (1.6, 17.75)</td>
<td>0.2251</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2&lt;sup&gt;nd&lt;/sup&gt; 43</td>
<td>4.3 (1.5, 15.18)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphocytes (10&lt;sup&gt;9&lt;/sup&gt;/L)</td>
<td>1&lt;sup&gt;st&lt;/sup&gt; 51</td>
<td>1.305 (0.55, 3.2)</td>
<td>&lt;0.0001**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2&lt;sup&gt;nd&lt;/sup&gt; 2.1</td>
<td>(0.802, 3.38)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monocytes (10&lt;sup&gt;9&lt;/sup&gt;/L)</td>
<td>1&lt;sup&gt;st&lt;/sup&gt; 51</td>
<td>0.6 (0.3, 1.25)</td>
<td>0.9838</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2&lt;sup&gt;nd&lt;/sup&gt; 0.6</td>
<td>(0.3, 1.504)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eosinophils (10&lt;sup&gt;9&lt;/sup&gt;/L)</td>
<td>1&lt;sup&gt;st&lt;/sup&gt; 51</td>
<td>0.1 (0, 0.3)</td>
<td>&lt;0.0001**</td>
<td>0.075 (0.00, 0.4155)</td>
</tr>
<tr>
<td></td>
<td>2&lt;sup&gt;nd&lt;/sup&gt; 0.2</td>
<td>(0, 0.82)</td>
<td></td>
<td>0.018 (0.00, 0.81)</td>
</tr>
<tr>
<td>Basophils (10&lt;sup&gt;9&lt;/sup&gt;/L)</td>
<td>1&lt;sup&gt;st&lt;/sup&gt; 51</td>
<td>0.0 (0, 0.1)</td>
<td>&lt;0.0001**</td>
<td>0.02 (0.00, 0.0645)</td>
</tr>
<tr>
<td></td>
<td>2&lt;sup&gt;nd&lt;/sup&gt; 0.1</td>
<td>(0, 0.2)</td>
<td></td>
<td>0.03 (0.00, 0.158)</td>
</tr>
<tr>
<td>NLR (U)</td>
<td>1&lt;sup&gt;st&lt;/sup&gt; 51</td>
<td>3 (0.86, 15.66)</td>
<td>0.0011**</td>
<td>2.6 (0.84, 15.7)</td>
</tr>
<tr>
<td></td>
<td>2&lt;sup&gt;nd&lt;/sup&gt; 1.8</td>
<td>(0.56, 16.79)</td>
<td></td>
<td>4.3 (1.1, 57.66)</td>
</tr>
<tr>
<td>D-Dimer (µg FEU/mL)</td>
<td>1&lt;sup&gt;st&lt;/sup&gt; 49</td>
<td>0.4 (0.2, 8.1)</td>
<td>0.1113</td>
<td>0.5764 &lt;0.0001**</td>
</tr>
<tr>
<td></td>
<td>2&lt;sup&gt;nd&lt;/sup&gt; 0.2</td>
<td>(0, 3.6)</td>
<td></td>
<td>0.3931 &lt;0.0001**</td>
</tr>
<tr>
<td>Fibrinogen (g/dL)</td>
<td>1&lt;sup&gt;st&lt;/sup&gt; 31</td>
<td>718 (380, 1136)</td>
<td>&lt;0.0001**</td>
<td>420 (240, 1940)</td>
</tr>
<tr>
<td></td>
<td>2&lt;sup&gt;nd&lt;/sup&gt; 350</td>
<td>(220, 69)</td>
<td></td>
<td>470 (390, 980)</td>
</tr>
<tr>
<td>IL-6(pg/mL)</td>
<td>1&lt;sup&gt;st&lt;/sup&gt; 31</td>
<td>20.3 (1.5, 3361)</td>
<td>&lt;0.0001**</td>
<td>0 ND</td>
</tr>
<tr>
<td></td>
<td>2&lt;sup&gt;nd&lt;/sup&gt; 3.05</td>
<td>(1.4, 3384)</td>
<td></td>
<td>ND ND</td>
</tr>
<tr>
<td>C-reactive protein (mg/L)</td>
<td>1&lt;sup&gt;st&lt;/sup&gt; 31</td>
<td>34 (90.6, 381.8)</td>
<td>&lt;0.0001**</td>
<td>21.79 (0.4, 335.3)</td>
</tr>
<tr>
<td></td>
<td>2&lt;sup&gt;nd&lt;/sup&gt; 3.6</td>
<td>(0.42, 111)</td>
<td></td>
<td>16.45 (0.6, 350)</td>
</tr>
</tbody>
</table>

<sup>a</sup>: Wilcoxon test for nonparametric pair samples; <sup>b</sup>: Mann-Whitney U test, comparing the two groups; Time: comparing 1<sup>st</sup> and 2<sup>nd</sup> studies inside each group; n: sample size; NLR: neutrophil/lymphocyte ratio; ND: not determined.

As an example of the fast recovery of PB-NHESC-C-treated patients, the CT images of a COVID-19 patient with a score of 4 is shown in Figure 6.
Figure 6: High-resolution computer tomography images of a patient’s chest.

Legend: Patient No. 4. Group A (peripheral blood non-hematopoietic enriched stem cell cocktail + standard care): Images a) Day of recruitment (April 1st). Images b) Day 4 after the first dose of stem cell treatment (April 13th, nebulization was initiated on April 9th, and the second dose was initiated on April 10th).

On the day of recruitment, his CT chest images showed patchy consolidations in the bilateral lower lobes and right middle lobe as well as few peripheral consolidations in the left upper lobe and patchy peripheral ground-glass opacities in the upper lobes. An important change in improvement was noted after 4 days of nebulization.

DISCUSSION

The clinical data of recruited patients at the time of inclusion included all signs and symptoms that more frequently noted in COVID-19 patients, including fever, weakness, shortness of breath, secondary bacterial sepsis, and lower oxygen saturation [25].

The loss of patients during the follow-up was the main limitation for the collection of data because many of the patients in the first and second tertiles recovered and were released from hospitals, rendering some statistical limitations and bias in this study. However, a proper quantitative meta-analysis with a larger sample could be designed to validate this study and achieve a better assessment of the effect of treatment. The quantities of each allocation group changed daily, so data monitoring was performed to evaluate clinical improvements and adverse events, including deaths.

From the 139 initial randomized patients, 2 from each group were lost before the interventions. In Group A, one patient was critically ill and passed away, and another was almost
asymptomatic, completely recovered and was released from the hospital. In Group B, two
patients were excluded due to an error of identification to facilitate accurate data collection for
proper analysis. Therefore, randomization yielded 69 patients for Group A and 70 for Group
B. Age, gender, nationalities, and other demographic and clinical information obtained at the
time of recruitment were similar between both groups, and the only significant difference was
found in a portion of body mass index (BMI) categories, such as normal weight and
overweight. For both of these factors, the control group exhibited better values, and a smaller
number of patients with these disadvantages were included in the stem cell-treated group
because some reports have noted a poor prognosis for overweight patients [26]. Patients in
the trial represent 15 nationalities, which is similar to that noted in the UAE population, giving
strong meaning to the study.

This work describes the characterization of a cocktail of autologous stem cells suspended in
PGF that also includes anti-SARS-CoV-2 antibodies. These cells express markers CD133
and CD34, which are known as pluripotent markers, and to a lesser extent CD90; the cocktail
has a similar number of circulating endothelial cells to that reported in response to endothelial
damage and dysfunction in many diseases [27]. The CD45- cell fraction is similar in size and
phenotype to that described for VSELs, but further studies are needed for better cell
characterization [28]. Given that PB-NHESC-C is a product obtained from COVID-19 patients,
the variability in the cellular number and phenotype is influenced by the immune response to
viral infection and the mobilization of progenitor cells to damaged tissue. Therefore, these
differences explain the differences found in this study between the severe and moderate
groups [29]. Indeed, the significant CD5dim cell count in the cellular preparation of moderate
cases is synergically correlated with the absolute count of lymphocytes in peripheral blood,
indicating their potential role as lymphocyte precursors. The increase in CD45- and CD90+
cell counts found in the PB-NHESC-C of severe patients could be associated with an
increase in their mobilization under stress conditions and a response to the release of inflammatory cytokines, which is known as one of the red flags of the disease [30]. The effects of stem cell therapy are dose dependent. The efficacy of a stem cell cocktail product should be evaluated based on the presence, phenotype, and function of different cellular subsets. In this work, a relationship between the total dose of stem cells administered and the efficacy of the product was not found. Our results were more correlated with specific stem cell subsets. Indeed, hospital discharge before 10 days was related to the number of CD45\(^{\text{dim}}\) cells and, more specifically, CD45\(^{\text{dim}}\)CD34\(^+\) cells. Nevertheless, due to the well-known role of CD34\(^+\) hematopoietic stem cells in hematopoietic transplantation [31], their therapeutic role in pulmonary diseases has not been widely studied, and a few supportive studies have reported their increase in peripheral blood during interstitial lung disease as a compensatory mechanism of tissue repair [32] as well as their ability to differentiate into other cell lineages, including epithelial cells [33]. CD34 is expressed in a wide range of cells in addition to hematopoietic cells and stromal, epithelial, and endothelial cells. The function of this marker has not been fully clarified, but it is associated with the inhibition or facilitation of adhesion, proliferation, and regulation of cell differentiation [34]. A significant increase in the peripheral blood of CD45\(^{\text{dim}}\)CD34\(^+\) cells in severe COVID-19 patients was reported [29] and interpreted as a response to endothelial regeneration during hypoxia. In our study, no relationship was found between the number of CD34\(^+\) cells in PB-NHESC-C and disease severity. These contradictory findings may be due to differences in study populations, methodology, and parameter analysis.

Furthermore, we found that nebulization of a high dose of CD90\(^+\) cells was associated with a longer hospital stay, and CD90\(^+\) cells were more highly expressed in PB-NHESC-C cells of severe patients who required a longer hospital stay. This finding was not expected given the immunosuppressive activity of CD90 that controls inflammation. We attributed the alteration
of CD90 activity to the high expression of proinflammatory cytokines, such as IL-1β and TNFα, which are commonly increased in COVID-19 patients [35].

On the other hand, it is well known that ACE2 is expressed in most human cells, and SARS-CoV-2 enters the host cell via binding of the S protein on the viral surface to ACE2 on the cell surface [36]. In addition to the lung, ACE2 is widely expressed in human tissues, including the heart, liver, kidney, and digestive organs [37]. Almost all endothelial cells and smooth muscle cells in organs express ACE2; therefore, we measured ACE2 expression in our investigational product. A recent study demonstrated that ACE2 and the entry-facilitating transmembrane protease TMPRSS2 are expressed on VSELs, and it is hypothesized that the interaction of its receptor activates the Nlrp3 inflammasome. Thus, hyperactivation of these cells can promote the death of infected cells by pyroptosis [38]. Nevertheless, in this trial, there was no evidence of the loss of viability in our stem cell cocktail, and the cells were not damaged by SARS-CoV-2. If the cells were damaged, the PB-NHESC-C cocktail would not demonstrate efficacy in controlling the patient’s cytokine storm.

Positivity for anti-SARS-CoV-2 IgG antibodies and the semiquantitative estimation of anti-SARS-CoV-2 T/G did not show beneficial effects on endpoints. Antibody nebulization of human plasma does not cause loss of immunoglobulin function in animal models [39] and is well tolerated for treatment in humans [40]. Nevertheless, the exclusive presence of anti-SARS-CoV-2 IgG antibodies in the nebulized product evaluated in this study also potentially influenced the effectiveness of PB-NHESC-C therapy despite not being related to antibody concentration. This notion should be clarified in other studies, and these results could be influenced because the concentration of anti-SARS-CoV-2 antibodies was significantly higher in the group of severe patients who require a longer hospital stay due to disease severity.

Additionally, we evaluated the presence of human growth factor in PGF, which was used as an excipient of the stem cell cocktail, among a small number of cases with the purpose of
better characterizing the final investigational product. Notably, it was not possible to relate these factors with efficacy. In previous communications, similar profiles of markers of angiogenesis and endothelial damage in the peripheral blood of patients were obtained in noncritical and critical phases of COVID-19 [41]. Higher concentrations of factors, such as PDGF-AA and M-SCF, play an important role in many processes related to the immune response, angiogenesis, and tissue repair [42]. A potential role of PGF and its contribution to the efficacy of the treatment was not addressed in this study and is currently under further investigation. Death, sepsis, disease progression, acute renal failure, hypoxia, acute respiratory distress syndrome, and multiorgan failure were among the less frequent adverse events found in the stem cell-treated group [41], but only the increased sepsis frequency found in the control group was significantly different between the two groups. Severe anemia, an expected adverse event after blood collection for the PB-NHESC-C preparation, was similar in both groups, indicating a correlation with SARS-CoV-2 infection and not with the treatment [43, 44]. None of the considered nonserious events assessed, such as increased respiratory breath rate, hypertension, fever, decreased absolute lymphocyte count, tachycardia, bradycardia, and hypotension, showed significant differences between the groups. In addition, administration-related or allergic reactions were not observed within two hours to 3 days after nebulization. Similarly, no delayed hypersensitivity or secondary infections were reported during the complete follow-up.

The clinical improvement observed in severe and moderate COVID-19 patients during the follow-up supported the potential application of stem cell cocktails. The trend line slope in both patients treated with stem cells showed a better adjustment to the right line and less dispersion to the center compared with patients treated with the standard of care, indicating that PB-NHESC-C facilitates accelerated clinical improvement that portend an improved recovery prognosis compared with those treated with standard care alone. However, no
relationship was found between a reduction of 2 or more points on the disease severity scale and the dose of nebulized cells, cell phenotypes, or positivity for anti-SARS-CoV2, total and IgG antibodies.

Some reports also found the appearance of lymphopenia as well as high levels of D-dimer and C-reactive protein in COVID-19 patients [45, 46]. In general, the elevation of D-dimer levels is not a specific response and is most commonly used in the diagnosis of venous thromboembolism and pulmonary embolism [47]. Recently, D-dimer levels have also been used as a diagnostic marker of acute abdomen disorders [48]. C-reactive protein is a biomarker with high sensitivity for inflammation and host response to the production of cytokines, particularly TNFα, IL-6, MCP1, and IL-8, which are secreted by several immune cells, including T cells, and increased C-reactive protein levels are also indicative of a myocardial effect [49]. Therapy with compressor-nebulized PB-NHESC-C modifies the immune response as demonstrated by statistically significant changes in acute phase serum markers and coagulation tests, such as D-dimer, after treatment in COVID-19 patients. As found in this study, the levels of some acute phase reactants in COVID-19 patients were higher than the normal range at the start of stem cell therapy, so the cytokine release syndrome caused by abnormally activated immune cells resulted in deterioration of the patient’s condition, which may alter endothelial cell function, induce capillary leakage, promote mucus blockage in the lung and induce respiratory failure. These effects could even cause an inflammatory cytokine storm leading to multiple organ failure. However, this effect was reverted in most of the patients when their levels of inflammatory reactants were normalized after treatment with PB-NHESC-C.

A variety of chest imaging features have been reported in COVID-19, and the images are similar to those found in other types of coronavirus syndromes. In this study, the CT chest image is presented to provide an example of patients who rapidly improved after
investigational product treatment. PB-NHESC-C cocktail therapy may inhibit overactivation of the immune system and significantly improve inflammation even in severe COVID-19 patients. The majority of patients with severe COVID-19 pneumonia survive and recover. The fact that the nebulization of PB-NHESC-C improved the outcome of COVID-19 patients may be due to regulation of the inflammatory response and the promotion of tissue repair and regeneration. However, a statistically significant difference in the percentage of patients discharged from the hospital was not noted. After day 9, the value was greater in the PB-NHESC-C treated group compared with controls with a clinical impact of RR=0.84. Additionally, the proportion of patients with an improved NLR in the stem cell-treated group was greater than that in the controls, and both clinical impact factors favored nebulization, as NLR levels have been found to be a predictive marker for severity and mortality in COVID-19 [50]. More overall improvements measured by at least a 2-point reduction on the disease severity scale were noted in the treated group with a highly statistically significant difference. In contrast, more improvements measured a 1-point reduction were noted in the treated group, but these results were not significant. However, it should be noted that treatment does not yield equivalent or inferior effects. Rather, treatment maintains superiority compared with the control group. Increasing the size of the sample could lead to better statistically significant differences in results.

The cure for COVID-19 is essentially dependent on the patient's immune system. When the overactivated immune system kills the virus, it produces a large number of inflammatory factors, leading to severe cytokine storms, and older patients may be more easily affected due to immunosenescence [51]. Cells, such as mesenchymal stem cells, have been used for the treatment of respiratory viral infections using nebulization and aerosols [52, 53]. In cases of COVID-19, other cellular therapy products using different types of stem cells have also aided
in patient recovery [13, 54-56].

CONCLUSIONS

This is the first report of an autologous treatment with minimally manipulated stem cells. The main component of the cocktail is nonhematopoietic cells, which were obtained using a simplified autologous cell isolation procedure that can be implemented in blood banks or transfusion center facilities. The PB-NHESC-C was safe and improved the clinical and laboratory outcomes in the majority of treated patients with the potential to reduce hospitalization and mortality.

Abbreviations.

AAD: Amino-actinomycin D

ACE2: Angiotensin-converting enzyme 2.

ADSCC: Abu Dhabi Stem Cells Center.

Ang-2: Angiopoietin-2.

APC: Allophycocyanin.

ARDS: Acute Respiratory Distress Syndrome.

BSA: Bovine Serum Albumin.

CTCAE: Common Terminology Criteria for Adverse Events.


CoV2T: SARS-CoV2 total antibodies.

CoV2G: SARS-CoV2 IgG antibodies.

CT: Computerized tomography scans.

EGF: Epidermal growth factor.

EPO: Erythropoietin.
FGF: Fibroblast growth factor.
FICT: Fluorescein isothiocyanate.
G-CSF: Granulocyte-colony stimulation factor.
GM-CSF: Granulocyte/macrophage-colony stimulation factor.
HGF: Hepatocyte growth factor.
HSCs: Hematopoietic stem cells.
iPSCs: Induced pluripotent stem cells.
IRB: Institutional review board.
M-CSF: Macrophage-colony stimulation factor.
MSCs: Mesenchymal stem cells.
NLR: Neutrophil to lymphocyte ratio.
PB: Peripheral blood.
PB-NHESC-C: Peripheral blood-derived non-hematopoietic enriched stem cell cocktail.
PDGF-AA: Platelet-derived growth factor AA.
PDGF-BB: Platelet-derived growth factor BB.
PGF: Platelet growth factor.
REC: Research Ethics Committee.
RNA: Ribonucleic acid.
RR: Relative risk.
RT: Room temperature.
RT-PCR: Real-time Polymerase Chain Reaction.
SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus-2
SCF: Stem cell factor.
SpO₂: Oxygen Saturation.
TGF-α: T-cell growth factor alpha.
Acknowledgements.

This work was supported by different governmental entities of the United Arab Emirates, as part of the national efforts to fight the COVID-19 pandemic, and authors are grateful to Informatics and all frontline healthcare workers from ADSCC and SEHA participating hospitals, for their support and commitment to the quality of the information provided to the research team, we also would like to thank the Core Technology Platforms team at New York University Abu Dhabi for assisting with this work.

Authors’ Contributions

YVC: Leading conceptualization, funding acquisitions, project administration, also participate in resources, and writing-review and editing; FMA: Leading Resources, also participation in funding acquisition, project administration, and writing-review and editing; YMCA: Leading Methodology, also participate in conceptualization, resources, data curation, validation, visualization and writing-review and editing; CAVV: Leading Writing-review and editing, also participate in methodology, investigation, formal analysis and visualization; YMA: Resources, data curation, validation, and
visualization; **PS**: Resources and data curation, **AAA**: Resources and investigation; **AA**: Resources, investigation and validation; **GMTZ**: Resources, data curation, investigation and writing-review and editing; **MWM**: Leading data curation, also participate in formal analysis, and writing-review and editing; **DQS**: Leading formal analysis, also participate in conceptualization, methodology, data curation, and writing-review and editing; **LAH**: Leading visualization, also participate in resources, investigation, validation, and writing-review and editing. **AABH**: Leading Investigation and validation, also participate in conceptualization, methodology, formal analysis, visualization and writing-review and editing; **RARJ**: Writing original draft, conceptualization, data curation, validation, formal analysis, visualization, and writing-review and editing.

**Funding**

This whole work was supported by the Abu Dhabi Stem Cells Center management, under Mr. Hamad Al Shamsi, as CEO and employer.

**Availability of Data and Materials**

Datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**DECLARATIONS**

**Ethics Approval and Consent to Participate**

The clinical trial called “SENTAD- COVID Study” was designed by Abu Dhabi Stem Cells Center (ADSCC), according to the research project approved by the ADSCC
Research Ethic Committee on April 4th, 2020, and later by a conditional exceptional approval letter by the Ministry of Health and Prevention of the UAE, via the Emirates Institutional Review Board (IRB) for COVID-19 Research Committee, on June, 25th 2020. The Helsinki Declaration was based on patients’ informed consent and ethical conduction of all participants.

Consent for Publication

The PB-NHESC-C treated patient No. 4 gave a written consent for the publication of his CT chess images.

Competing Interests

The procedure for obtaining the PB-NHESC-C mentioned in this report was advanced through research conducted and patented for ADSCC by YVC, AABH, and FMA. We declare that YMCA, CAVV, PS, YMA, AA, LAH, GMTZ, and RARJ are also staff members of ADSCC, for the rest thereof the co-authors there are no competing interests. In May 2020, the patented procedure was approved by the Ministry of Economy of the United Arab Emirates, and also in May 2020, 5 of the authors (FMA, YVC, RARJ, AABH, and YMCA) received 3 Copyrights from the INTEROCO Copyright Office (EC-01-002809, EC-01-002810, and EC-01-002811), so this procedure including nebulization has been accredited to ADSCC. Nevertheless, YVC and the other patent authors resigned to be eligible to receive equity as a result of the licensing of this procedure.

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REFERENCES


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Tables

Table 1. Demographic data and clinical status of the enrolled patients on the recruitment day.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group A: Treated with PB-NHESC-C plus standard care (n = 69)</th>
<th>Group B: Control (standard care only) (n = 70)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographic data</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, years (mean +/- SD)</td>
<td>45.93 +/- 9.75</td>
<td>44.38 +/- 11.09</td>
<td>0.380</td>
</tr>
<tr>
<td>Gender, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>69 (48.94)</td>
<td>70 (51.06)</td>
<td>1.0000</td>
</tr>
<tr>
<td>Masculine</td>
<td>65 (46.10)</td>
<td>64 (46.81)</td>
<td>0.7447</td>
</tr>
<tr>
<td>Feminine</td>
<td>4 (2.88)</td>
<td>6 (4.32)</td>
<td>0.4964</td>
</tr>
<tr>
<td>Nationalities</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Afghanistan</td>
<td>2 (2.90)</td>
<td>1 (1.43)</td>
<td>0.6195</td>
</tr>
<tr>
<td>Bangladesh</td>
<td>11 (15.94)</td>
<td>11 (15.71)</td>
<td>1.0000</td>
</tr>
<tr>
<td>China PRP</td>
<td>0 (0)</td>
<td>1 (1.43)</td>
<td>1.0000</td>
</tr>
<tr>
<td>Egypt</td>
<td>3 (4.35)</td>
<td>3 (4.29)</td>
<td>1.0000</td>
</tr>
<tr>
<td>India</td>
<td>22 (31.88)</td>
<td>28 (40.00)</td>
<td>0.2920</td>
</tr>
<tr>
<td>Indonesia</td>
<td>1 (1.45)</td>
<td>0 (0)</td>
<td>0.4964</td>
</tr>
<tr>
<td>Jordan</td>
<td>0 (0)</td>
<td>1 (1.43)</td>
<td>1.0000</td>
</tr>
<tr>
<td>Nepal</td>
<td>5 (7.25)</td>
<td>2 (2.86)</td>
<td>0.2746</td>
</tr>
<tr>
<td>Pakistan</td>
<td>10 (14.49)</td>
<td>15 (21.43)</td>
<td>0.3777</td>
</tr>
<tr>
<td>Palestine</td>
<td>4 (5.80)</td>
<td>0 (0)</td>
<td>0.0581</td>
</tr>
<tr>
<td>Philippines</td>
<td>4 (5.80)</td>
<td>4 (5.71)</td>
<td>1.0000</td>
</tr>
<tr>
<td>Somalia</td>
<td>1 (1.45)</td>
<td>0 (0)</td>
<td>0.4964</td>
</tr>
<tr>
<td>Sudan</td>
<td>3 (4.35)</td>
<td>0 (0)</td>
<td>0.1196</td>
</tr>
<tr>
<td>Syria</td>
<td>1 (1.45)</td>
<td>2 (2.86)</td>
<td>1.0000</td>
</tr>
<tr>
<td>UAE</td>
<td>1 (1.45)</td>
<td>1 (1.43)</td>
<td>1.0000</td>
</tr>
<tr>
<td>USA</td>
<td>0 (0)</td>
<td>1 (1.43)</td>
<td>1.0000</td>
</tr>
<tr>
<td>Unknown</td>
<td>1 (1.45)</td>
<td>0 (0)</td>
<td>0.4964</td>
</tr>
</tbody>
</table>

**Health status at recruitment**

| Body mass index categories, n (%) | | | |
| Unknown | 3 (4.35) | 2 (2.86) | 0.6806 |
| Normal (healthy weight) | 17 (24.64) | 29 (41.43) | 0.0472* |
| Overweight | 35 (50.74) | 19 (27.14) | 0.0054** |
| Obese Class I (moderately obese) | 11 (15.94) | 13 (18.57) | 0.8230 |
| Obese Class II (severely obese) | 0 (0) | 3 (4.29) | 0.2446 |
| Obese Class III (very severely obese) | 3 (4.35) | 4 (5.71) | 1.0000 |

Moderate COVID-19, n (%)
<table>
<thead>
<tr>
<th>Score 3</th>
<th>Score 4</th>
<th>Subtotal</th>
</tr>
</thead>
<tbody>
<tr>
<td>37 (53.62)</td>
<td>12 (17.39)</td>
<td>49 (71.01)</td>
</tr>
<tr>
<td>40 (57.14)</td>
<td>6 (8.57)</td>
<td>46 (65.71)</td>
</tr>
<tr>
<td>0.7342</td>
<td>0.1372</td>
<td>0.5852</td>
</tr>
</tbody>
</table>

Severe COVID-19, n (%)
<table>
<thead>
<tr>
<th>Score 5</th>
<th>Score 6</th>
<th>Score 7</th>
<th>Subtotal</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 (4.34)</td>
<td>2 (2.89)</td>
<td>15 (21.74)</td>
<td>20 (28.98)</td>
</tr>
<tr>
<td>7 (10.00)</td>
<td>1 (1.42)</td>
<td>16 (22.86)</td>
<td>24 (34.28)</td>
</tr>
<tr>
<td>0.3255</td>
<td>0.6195</td>
<td>1.0000</td>
<td>0.5852</td>
</tr>
</tbody>
</table>

Main comorbidities, n (%)
<table>
<thead>
<tr>
<th>Arterial hypertension</th>
<th>Diabetes mellitus</th>
<th>Cardiovascular disease and dyslipidemia</th>
<th>Chronic smoking and asthma</th>
</tr>
</thead>
<tbody>
<tr>
<td>18 (26.09)</td>
<td>18 (26.09)</td>
<td>7 (6.25)</td>
<td>11 (15.94)</td>
</tr>
<tr>
<td>19 (27.14)</td>
<td>13 (18.57)</td>
<td>6 (8.57)</td>
<td>4 (5.71)</td>
</tr>
<tr>
<td>1.0000</td>
<td>0.3142</td>
<td>0.7792</td>
<td>0.0603</td>
</tr>
</tbody>
</table>

PB-NHESC-C: peripheral blood non-hematopoietic enriched stem cell cocktail; the $p$-value for age was determined by a $\chi^2$-test; the remaining $p$-values were determined by the F-exact probability test; *: significant difference; **: highly significant difference.
Figure 1.
Figure 2.

Figure 2A)

Figure 2B)
Figure 3.
Figure 4.

A) Severe COVID-19 patients

B) Moderate COVID-19 patients
Figure 5.

<table>
<thead>
<tr>
<th></th>
<th>%Eli</th>
<th>%EI</th>
<th>RR</th>
<th>95% CI</th>
<th>RRR</th>
<th>NNT</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Discharge before 10 days</td>
<td>57.1</td>
<td>63.3</td>
<td>0.84</td>
<td>0.56-1.28</td>
<td>19%</td>
<td>15.1</td>
<td>0.425</td>
</tr>
<tr>
<td>Clinical improvement at least 2 scores</td>
<td>17.1</td>
<td>42.0</td>
<td>0.49</td>
<td>0.56-0.88</td>
<td>31%</td>
<td>4.0</td>
<td>**0.002</td>
</tr>
<tr>
<td>Clinical improvement at least 1 score</td>
<td>72.9</td>
<td>79.7</td>
<td>0.85</td>
<td>0.45-1.60</td>
<td>15%</td>
<td>39.6</td>
<td>0.345</td>
</tr>
<tr>
<td>Mortality</td>
<td>10.0</td>
<td>5.0</td>
<td>0.57</td>
<td>0.18-1.85</td>
<td>43%</td>
<td>23.8</td>
<td>0.375</td>
</tr>
<tr>
<td>Persistence of lymphopenia</td>
<td>40.0</td>
<td>33.3</td>
<td>0.53</td>
<td>0.13-2.14</td>
<td>47%</td>
<td>5.7</td>
<td>0.375</td>
</tr>
<tr>
<td>Appearance of lymphopenia</td>
<td>26.3</td>
<td>0.0</td>
<td>0.03</td>
<td>0.001-0.40</td>
<td>97%</td>
<td>4.2</td>
<td>**0.012</td>
</tr>
<tr>
<td>Persistence high level of NLR</td>
<td>70.4</td>
<td>40.0</td>
<td>0.56</td>
<td>0.29-1.11</td>
<td>44%</td>
<td>3.3</td>
<td>0.095</td>
</tr>
<tr>
<td>Persistence high level of CRP</td>
<td>76.2</td>
<td>36.0</td>
<td>0.47</td>
<td>0.27-0.84</td>
<td>53%</td>
<td>2.3</td>
<td>**0.011</td>
</tr>
<tr>
<td>Persistence high level of D-Dimer</td>
<td>92.3</td>
<td>47.1</td>
<td>0.51</td>
<td>0.30-0.85</td>
<td>49%</td>
<td>2.2</td>
<td>**0.010</td>
</tr>
</tbody>
</table>

Favor Nebulization vs Favor standard care
Figure 6.
Figure 1

Patient allocation during the SENTAD-COVID Study. Legend: Four initially screened patients were excluded before randomization because two had been previously diagnosed with malignant diseases and two were previously included in other clinical trials. After randomization, data from four additional
patients were not analyzed. Two patients in the control group had data error identifications. Two patients were not followed in the PB-NHSC-C group: one was released from the hospital, and the other unfortunately passed away before the initial day of treatment.

**Figure 2A)**

Flow cytometry gating strategy. Legend: Immunophenotype characterization of peripheral blood nonhematopoietic enriched stem cell cocktail. Figure 2a) Logic and manual gating strategy for cell characterization using five monoclonal antibody-conjugated CD markers simultaneously, including 7-
amino-actinomycin D (7-AAD). Figure 2b) Expression of angiotensin-converting enzyme 2 (ACE2) on the surface of cells.

Figure 3

Representative immunofluorescence images of PB-NHESC-C. Legend: PB-NHESCs were stained with FITC-conjugated monoclonal surface antibody CD45 (1:100) and Hoechst nucleic acid dye 33342 (10 μg/ml). Images were acquired using a Leica SP8 confocal microscope using a 63x objective. Two main populations were found: nonhematopoietic (*) and hematopoietic stem cells.
Figure 4

Clinical improvement. Different trend line slopes during the clinical trial follow-up. Legend: Group A/S: Peripheral Blood Non-Hematopoietic Enriched Stem Cell Cocktail (PB-NHESC-C) Treated classified as severe; Group B/S: Controls classified as severe; Group A/M: PB-NHESC-C Treated classified as moderate; Group B/M: Controls classified as moderate.
Figure 5

Clinical impact on the assessed outcomes. Legend: NLR: Neutrophil to Lymphocyte Ratio; CRP: C-Reactive Protein; E10: Exposure Incidence in controls; E1: Exposure Incidence in peripheral blood nonhematopoietic enriched stem cell cocktail treated-patients; RR: relative risk; 95% CI: confidence interval; RRR: relative risk reduction; NNT: number needed to treat to produce the effect; a: Z-test. *: significant; **: highly significant.
Figure 6

High-resolution computer tomography images of a patient’s chest. Legend: Patient No. 4. Group A (peripheral blood non-hematopoietic enriched stem cell cocktail + standard care): Images a) Day of recruitment (April 1st). Images b) Day 4 after the first dose of stem cell treatment (April 13th, nebulization was initiated on April 9th, and the second dose was initiated on April 10th).