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RESEARCH ARTICLE

# Pilot Study of Application of Combined Transbronchial and Intravenous Ultraviolet C (UVC) and Laser Beam Application for the Treatment of Critical COVID-19 Infection

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## ABSTRACT

**Objective and background:** Light-based antimicrobials, mainly ultraviolet C (UVC) and laser light irradiation, have a potential to inactivate severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The aim of our study was to evaluate the effect of transbronchial and intravenous application of UVC and laser light irradiation on treatment of patients with severe COVID-19.

**Methods:** The clinical outcome of six patients (age 42-69 years) with severe COVID-19 infection who were directly applied UVC (254 nm) transbronchially, and UVC plus green (630 nm) and red laser (535 nm) lights to the blood circulation in addition to standard pharmacotherapy (UVC group) were prospectively evaluated in comparison to six patients (age 50-69 years) treated only with pharmacotherapy (standard treatment group).

**Results:** The patients in UVC group had shorter stay in intensive care unit (median length of stay 1 vs. 8.5 days; p=0.015), more negative PCR results after treatment (5/6 vs. 0/6 patients; p=0.003), higher discharge rate (5/6 vs. 3/6 patients), and lower mortality (1/6 vs. 3/6 patients), as compared to patients in standard treatment group. Serum D-dimer level, which reached up to 2500 ng/mL (six times of baseline value) seven days after treatment in standard treatment group, was much lower in UVC group (1000 ng/mL). Serum ferritin level was 1.5 to 1.9-fold higher and CRP level was up to 1.7-fold higher in standard treatment group during ten days after treatment as compared to UVC group. No adverse effects have been observed.

**Conclusions:** Combined transbronchial and intravenous UVC and laser irradiation may improve outcome of severe COVID-19 cases.

**Keywords:** COVID-19, SARS-CoV-2, ultraviolet C irradiation, light therapy

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## INTRODUCTION

Coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a respiratory disease with high transmission and mortality rate responsible for ongoing global pandemic. While lungs are the most commonly involved organs, extrapulmonary systems, including the cardiac, gastrointestinal, hepatic, renal etc., are also affected during COVID-19 disease [1]. Analysis of published databases reporting angiotensin-converting enzyme-2 (ACE2) expression indicated that, ACE2 expression is quite highly positive in various types of human tissues, mostly in respiratory tract, heart, kidney and gastrointestinal tract, making these tissues susceptible to COVID infection [2-4].

The cardiac manifestations accompanying COVID-19 include cardiac arrhythmias, myocarditis, pericarditis, acute coronary syndrome and heart failure [1]. In a meta-analysis of six studies involving more than 1,500 patients, prevalence of cardiovascular diseases in COVID-19 was reported as 16.4% [5]. In another meta-analysis based on 16 studies with more than

2,000 patients, it was shown that 24.4% of hospitalized COVID-19 patients developed cardiac injury [6].

Li Y et al. reported that, five percent of the COVID-19 patients had experienced cerebrovascular disease during COVID-19 disease [7]. In a systematic review of 41 studies involving 4700 patients, focusing on neurological disorders accompanying COVID-19, Wang et al reported that frequency of olfactory symptoms were reported to be between 36% and 86%, and gustatory symptoms between 33% and 89%. Emergence of Guillain–Barré syndrome and acute inflammation of the brain, spinal cord, and meninges were also reported during COVID-19 disease [8].

Gastrointestinal symptoms such as nausea, vomiting, diarrhea and abdominal pain are observed quite frequently in COVID-19 patients [1,9]. In a systematic review of 57 studies, including almost 11,000 patients, Sultan et al showed that the prevalence rates of nausea and/or vomiting, diarrhea and abdominal pain were 7.8%, 7.7% and 3.6%, respectively [10].

Despite intensive preclinical and clinical studies, there is currently no effective treatment against COVID-19 [11,12]. Light-based antimicrobials, such as laser light and ultraviolet C (UVC) irradiation, has a potential to inactivate SARS-CoV-2 [13-15]. It has been known for the last 100 years that UVC light is highly germicidal, thus proposed to be used to combat infectious microorganisms [13]. With its short wavelength (200–280 nm) and high energy, UVC irradiation can inactivate various microorganisms including bacteria, fungi, and viruses by damaging nucleic acids in their DNA and RNA through inhibition of cyclobutane pyrimidine dimers [13,16-18]. UVC at 254 nm wavelength has the highest potential to be absorbed by the nucleic acids of microbial cells [13].

In contrary to long wavelength UVB (290–340 nm) and UVA (340–400 nm) which may have damaging effects on healthy tissues on chronic exposure, UVC causes minimum DNA damage in mammalian cells at its effective wavelength range of 250–270 nm that can be quickly repaired by DNA repair enzymes [19,20]. Lachert even suggested that 254 nm wavelength irradiation is not absorbed by proteins, therefore conventional toxicity tests are not required [21].

Recent studies also showed promising activity of UVC irradiation against human coronavirus [14,15,18]. The inactivating effects of UVC on coronaviruses on platelet concentrates [18], personal protective equipment [22,23] and other in vitro conditions [24-26] have been reported recently. The viruses studied in these experiments included SARS-CoV-2 [23,26] and also other coronaviruses including human betacoronavirus HCoV-OC43 [24], MERS (EM/2012 strain) [22], SARS-CoV (200300592 strain) [22] and SARS-CoV (Urbani strain) [25]. As all human coronaviruses have similar genomic sizes, the results of studies performed on human coronaviruses would be expected to be extrapolated to SARS-CoV-2.

Some authors have suggested that photobiomodulation or photodynamic therapies may be promising in the management of COVID-19 [27,28]. Fekrazad proposed that non-invasive or minimally invasive photobiomodulation or photodynamic therapy administered by intratracheal or intravenous routes may have a potential as an adjuvant to pharmacotherapy or even alternative therapy for COVID-19 [27]. Having minimum side effects and drug interactions, light-based therapies may be beneficial to patients with COVID-19 infection. Laser lights with different wavelengths may be effective against COVID-19 infection by increasing oxygenation of red cells and improving immune system [27]. Domínguez discussed that regular transdermal application of laser therapy 30 minutes per day for 3-5 days, can also control the cytokine storm in patients with COVID-19 [28]. Ferreira [29] and Camacho [30] emphasized that clinical studies are clearly needed to evaluate whether it is possible to use minimally invasive photobiomodulation therapy into the tissues to produce a systemic antimicrobial effect in the treatment of COVID-19. Because of the lack of effective and safe UVC-based technologies to deliver UVC into the tissues *in vivo*, these claims made in previous studies have not been tested in clinical studies yet. UVC has been used exclusively for superficial infections, transplant organs, or blood products [13-15].

In this study, we aimed to evaluate the effect of UVC and laser light, applied by an innovative UVC-generator device, directly into bronchial system and blood circulation.

## MATERIALS AND METHODS

### Study Design and Patients

This study is a prospective, two-arm, open-label, randomized, controlled, multicenter trial conducted in Turkey. Study population included patients who have applied to the emergency service with symptoms of fever, weakness, cough, and shortness of breath; whose COVID-19 PCR test positive (Abbott Biorad CFX 96 RT-PCR) or those having findings of atypical pneumonia on computed tomography; and treated in the intensive care unit. Pregnant and lactating women and those with suspected pregnancy, and patients previously diagnosed with mental disorders were excluded from the study. Twelve patients complying with selection criteria among 16 subjects were randomly assigned into two groups (see **Figure 1** for the study flow diagram). Patients in control group (standard treatment group) were administered standard treatment for COVID-19, which is a combination of antiviral, antimalarial and antibacterial drugs (i.e., favipiravir, hydroxychloroquine, azithromycin) according to the Guidelines of Turkish Ministry of Health [31]. Patients in the UVC group were applied transbronchial and intravenous UVC light and laser therapy with a newly developed UVC-generator device in addition to standard COVID-19 therapy based on the Guidelines of Turkish Ministry of Health.

  

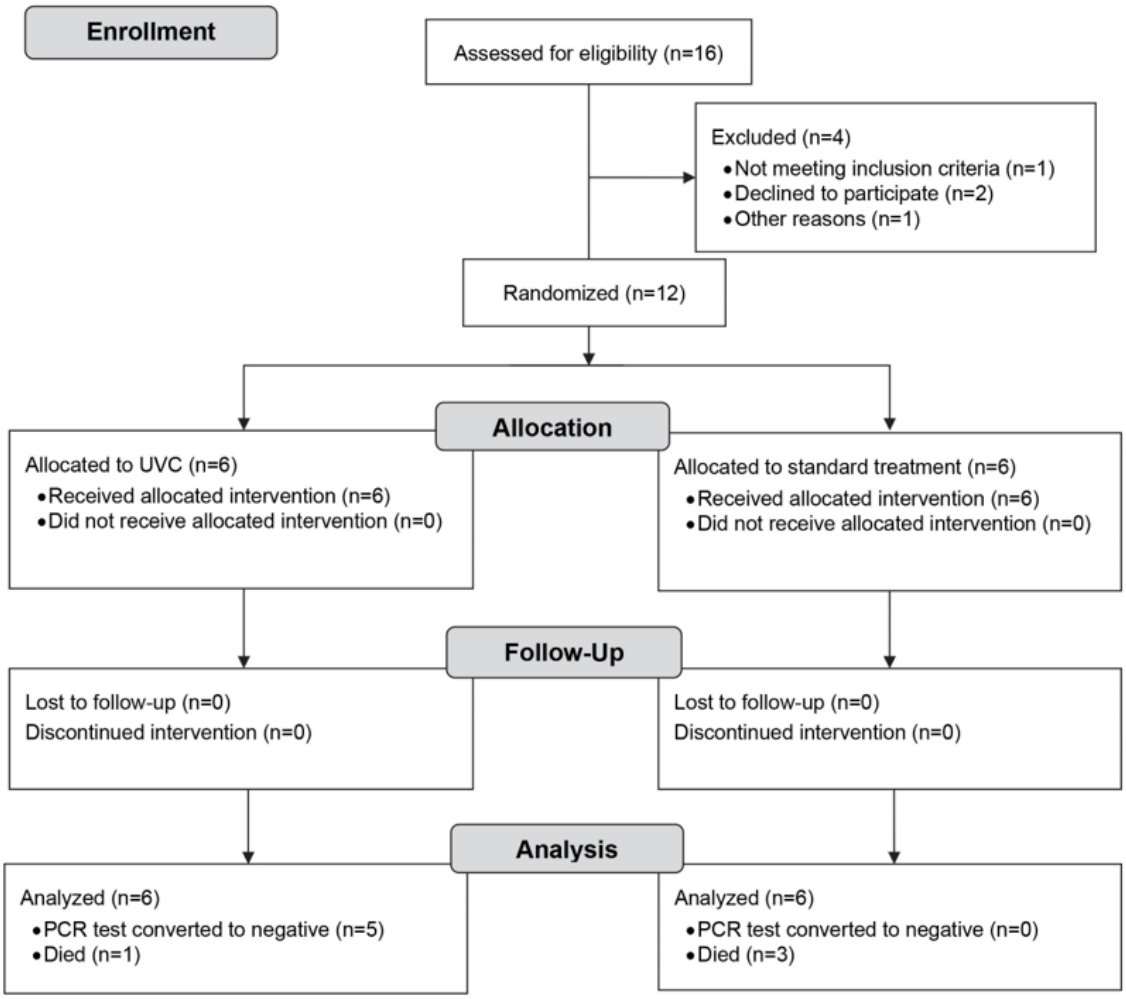


**Figure 1.** Study flow diagram

The study was approved by the Institutional Ethics Committee for Clinical Studies of Gazi University Faculty of Medicine (date 13th Apr 2020, no 253) and The Turkish Medicines and Medical Devices Agency COVID-19 Scientific Council (date 4th May 2020, no 108225), and conducted in accordance with latest version of Helsinki Declaration. All of the study patients or legal representatives were informed about the study and gave written consent before any study-related procedures.

**UVC and Laser Light Generator Device**

An innovative UVC and laser light generator (Voltran®, RD Global® Inc., Florida, USA) which is a light treatment application device designed specifically for tracheobronchial tree and veins was used in this study. In addition to UVC light (254 nm), the device allows application of green laser (630 nm) and red laser (535 nm) into the body. It consists of a custom-made UVC (100±5 W) and laser light (3±0.2 W) generators and a semi-flexible fiberoptic disposable camera catheter. The technical parameters of the UVC and laser light generator are summarized in **Table 1**.

The UVC and laser light generator is a medical device that has passed the *in vitro* and *in vivo* biocompatibility and safety tests (acute and subchronic systemic toxicity, cytotoxicity, genotoxicity, mutagenicity, hemolytic impact, pyrogenicity, skin sensitization, intradermal irritation) which were performed in an independent laboratory based at Gazi University Medical Faculty, Ankara, Turkey, in accordance with ISO 10993-1, and current EU and national regulations (**Supplementary Appendices**).

**Application of UVC and Laser Therapy**

All patients in the study group were applied both transbronchial UVC, and intravenous UVC and laser therapy by the UVC-generator (Voltran®, RD Global® Inc., Florida, USA).

For transbronchial UVC therapy, the fiberoptic catheter system was slowly pushed through the trachea down into the lower bronchia, and 20 mW/cm$^2$ power beam energy was applied for 30 sec every 5 cm segment (**Figure 2**). The procedure was performed on the closed-circuit laryngeal mask airway under general anesthesia.

For intravenous UVC and laser therapy, the fiberoptic catheter system was slowly pushed through the antecubital vein by using an intravenous catheter, and approximately 5 cm of the tip of the catheter was adjusted to remain in the

**Table 1.** Technical parameters of UVC and laser light generator device

| | | |
|---|---|---|
| **Manufacturer** | RD Global® Inc., Florida, USA | |
| **Model identifier** | Voltran® | |
| **Production year** | 2019 | |
| **Number and type of emitters** | UVC light emitter: | 100±5 W, 200-280 nm (×4) |
| | Green laser light emitter: | 3±0.2 W, 535±10 nm (×1) |
| | Red laser light emitter: | 3±0.2 W, 660±10 nm (×1) |
| **Specific wavelength** | UVC: | 200-280 nm |
| | Green laser: | 525-545 nm |
| | Red laser: | 650-670 nm |
| **Power density** | UVC — Respiratory route: | 2 W/m$^2$ |
| | UVC — Intravenous route: | 20 W/m$^2$ |
| | Green and red laser: | 5-25 W/m$^2$ |
| **Area irradiated** | Respiratory system and whole blood | |
| **Application technique** | Intratracheal and intravenous | |
| **Duration of each treatment session** | Respiratory route: | 5±1 min (30 seconds for each 5 cm bronchial segment) |
| | Intravenous route: | 30±5 min |
| **Frequency of treatment** | Usually once, but may be extended up to five applications every other day | |
| **Cumulative dose (total radiant energy over entire treatment course)** | Respiratory route: | UVC 0.06 J/cm$^2$ |
| | Intravenous route: | UVC 3.6 J/cm$^2$ |

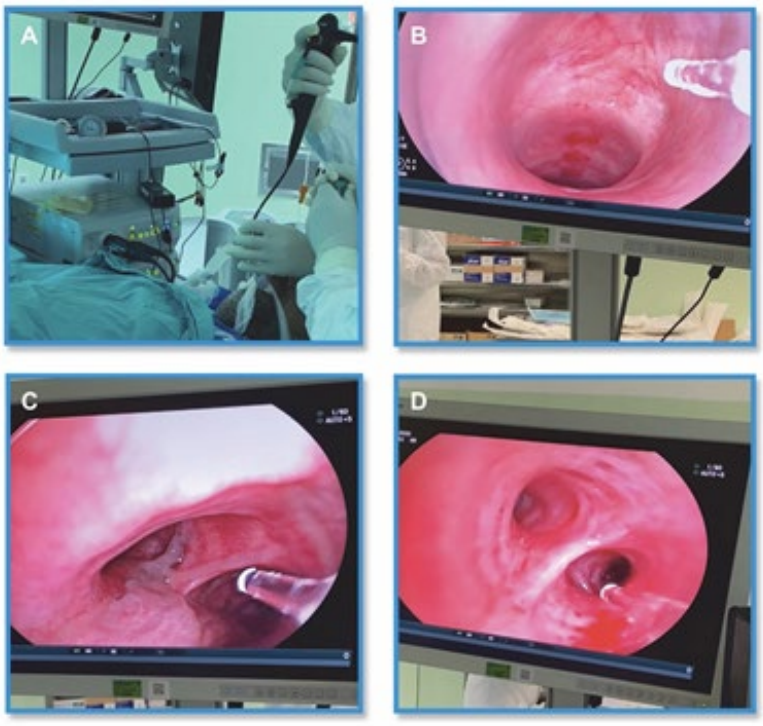


**Figure 2.** Respiratory UVC beam application by using UVC-generator device (A). The images as fiberoptic catheter system passes through the trachea (B) and bifurcation (C, D)

intravascular area. UVC and laser therapy was applied to the blood for 30±5 minutes with a 200 mW/cm$^2$ power beam energy for virus inactivation, and the fiberoptic catheter was then slowly withdrawn.

The dose and the duration of exposure were kept as minimum as possible during the study. The patients had been monitored for possible lung toxicity very closely during and after the procedure for 48 hours, with complete equipment and experienced medical staff for noninvasive and invasive ventilation.

Considering the clinical condition of the patient, the maximum time for application of the beam was 30±5

  

minutes for intravenous route, 5±1 minutes for transbronchial route.

**Study Outcome Endpoints**

Primary endpoint to be analyzed was defined as conversion of positive PCR test to negative in bronchoalveolar lavage fluid (BAL) within 24 hours following the procedure.

Secondary end-points we intended to analyze were shortening of length of stay in intensive care unit, in medical ward, and total length of stay after treatment, lowering the elevated levels of serum D-dimer, ferritin and CRP values, not worsening of serum biochemistry values (blood urea nitrogen, creatinine, electrolytes, transaminases, bilirubin), hemostatic parameters (prothrombin time and activated partial thromboplastin time) and hematologic parameters (hemoglobin, hematocrit, red blood cell count, white blood cell count, neutrophil percentage, platelet count).

Patients' clinical status were defined using World Health Organization (WHO) R&D Blueprint Ordinal Clinical Scale scores at baseline and daily during the 10-days' follow-up [32]. Faster change in this clinical status was another secondary study endpoint.

**Safety Issues**

The patients had been monitored for possible lung toxicity (vital signs and clinical symptoms, physical examination, chest X-ray, chest computed tomography as needed, arterial oxygen, carbon dioxide and pH monitoring) very closely during and after the procedure for 48 hours, with complete equipment and experienced medical staff for noninvasive and invasive ventilation. Ophthalmologic examinations of the patients were performed daily for ten days following the procedure, in order to diagnose any eye toxicity finding.

**Statistical Analysis**

D-dimer, ferritin, C-reactive protein (CRP), serum biochemistry tests, and hematologic tests were performed every 1-3 days, depending on the clinical status of the patients. Last-observation-carried-forward method was implemented in order to impute the missing data between two consequent measurements, i.e., in case of any missing data the valid measurement at the nearest previous time point was copied to the day with missing data.

The measurements during follow-up period were summarized with geometric mean and 95% confidence interval (CI) for D-dimer, ferritin and CRP levels, and median and 95%CI or minimum-maximum values for other tests. Increase in D-dimer, ferritin and CRP levels at follow-up days as the ratio over baseline values were also calculated (in order to avoid daily random fluctuations, moving three-day averages were used). These variables showed dispersion within very wide ranges. Therefore, geometric means were calculated, in order to avoid the disproportionate influence of extreme values on the average values.

Since the number of subjects was quite low, bootstrapping technique was applied to calculate CIs. Bias-corrected accelerated 95%CIs were calculated.

Chi-square test or Fisher's exact test, when needed, was used to compare the proportions of categorical variables (conversion of positive PCR test to negative and death) between study groups. Nonparametric approach for the analysis of numerical variables, since the number of cases is low and/or the distribution of the values are non-normal. Mann-Whitney U test used to compare the geometric means or medians of numerical variables, and also median WHO R&D Blueprint Ordinal Clinical Scale scores between study groups.

SPSS v22 was used to perform the statistical analysis.

## RESULTS

**Baseline Patient Characteristics**

Individual patient data including demographic, hospital stay and clinical outcomes are presented in **Table 2**. Patients in the UVC group (median age: 50.5 years (42 to 69),

**Table 2.** Clinical follow-up of study patients in UVC group and standard treatment group

| Study group | Initials | Age | Sex | Length of stay before UVC therapy (days) | PCR test results after UVC therapy | | | Length of stay in intensive care unit after UVC therapy (days) | Length of stay in medical ward (days) | Total length of stay (days) | Final outcome | Last follow-up since admission (days) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | *BAL* | *Trachea* | *Blood* | | | | | |
| UVC | MO | 46 | M | 8 | (–) | (–) | (–) | 1 | 6 | 15 | Alive / PCR negative | 28 |
| UVC | CÇ | 47 | F | 2 | (–) | (–) | (–) | 1 | 3 | 6 | Alive / PCR negative | 19 |
| UVC | NA | 63 | M | 8 | (–) | (–) | (–) | 0 | 5 | 13 | Alive / PCR negative | 22 |

[a] PCR was negative on day 19, but positive on day 25.

Bal: Bronchoalveolar Lavage Fluid

Std Rx: Standard treatment

**Table 2 (continued).** Clinical follow-up of study patients in UVC group and standard treatment group

| Study group | Initials | Age | Sex | Length of stay before UVC therapy (days) | PCR test results after UVC therapy | | | Length of stay in intensive care unit after UVC therapy (days) | Length of stay in medical ward (days) | Total length of stay (days) | Final outcome | Last follow-up since admission (days) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | *BAL* | *Trachea* | *Blood* | | | | | |
| UVC | AG | 42 | M | 10 | (–) | (–) | (–) | 1 | 3 | 14 | Alive / PCR negative | 27 |
| UVC | RG | 54 | M | 9 | (–)[a] | (–) | (–) | 0 | 3 | 12 | Alive / PCR negative | 25 |
| UVC | BD | 69 | F | 7 | (+) | (–) | (–) | 8 | 0 | 15 | Died / PCR positive | 15 |
| Std Rx | BA | 55 | M | 8 | (+) | — | — | 7 | 0 | 15 | Alive / PCR positive | 15 |
| Std Rx | KE | 69 | F | 4 | (+) | — | — | 6 | 0 | 10 | Alive / PCR positive | 16 |
| Std Rx | SO | 68 | M | 3 | (+) | — | — | 19 | 0 | 22 | Alive / PCR positive | 25 |
| Std Rx | VA | 50 | M | 2 | (+) | — | — | 4 | 0 | 6 | Died / PCR positive | 6 |
| Std Rx | EM | 63 | M | 9 | (+) | — | — | 24 | 0 | 33 | Died / PCR positive | 33 |
| Std Rx | Nİ | 56 | M | 5 | (+) | — | — | 10 | 0 | 15 | Died / PCR positive | 15 |

[a] PCR was negative on day 19, but positive on day 25.

Bal: Bronchoalveolar Lavage Fluid

Std Rx: Standard treatment

male/female (M/F): 4/2) and the standard group (median age: 59.5 years (50 to 69); M/F: 5/1) were included in the study (for age p=0.24, Mann-Whitney U test; for gender p=1.00, Fisher's exact test). Patients in the UVC group and standard treatment group had been in hospital for 2 to 10 days when they were enrolled. Median length of stay before enrollment were 8 and 4.5 days, in the study groups, respectively (p=0.31; Mann-Whitney U test) (**Table 3**).

**Clinical Outcome and PCR Test Results**

While no PCR test result for COVID-19 converted to negative in any patient in standard treatment group, five out of six patients in the UVC group had negative PCR test results after UVC treatment in all samples of BAL fluid, trachea and blood (p=0.003; chi-square test) (**Table 3**).

Five of six patients in UVC group could be transferred to medical ward (two of them on the same day and other three one day after the UVC treatment). These patients stayed in medical ward for 3-6 more days and were discharged from the hospital with negative PCR test. The sixth patient in the UVC group died with positive PCR test result after staying eight days in the intensive care unit following the UVC treatment. Her peripheral oxygen saturation ($SpO_2$) value increased from 56% to 90% just after the study procedure. Although PCR in BAL fluid did not convert to negative, the viral load decreased as evidenced with improvement in quantitative PCR from 16 to 30 cycle threshold.

On the other hand, all patients in the standard treatment group continued to stay in the intensive care unit for 4-22 days. None of them could reach to a clinical status good enough to be transferred to medical ward. PCR test of all patients in the standard treatment group continued to be positive—three were discharged and three were dead (**Table 3**).

Median values for length of stay in intensive care unit after treatment were 1 day (95%CI: 0 to 1 days) and 8.5 days (95%CI: 4 to 24 days) (p=0.015; Mann-Whitney U test). Median values for total length of stay after treatment were 4.5 days (95%CI: 3.5 to 7.5 days) and 8.5 days (95%CI: 4 to 24 days) (p=0.093; Mann-Whitney U test) (**Table 3**).

Median WHO R&D Blueprint Ordinal Clinical Scale scores at baseline were 4 in both study groups (3 to 5 in UVC group and 3 to 6 in standard treatment group; p=0.82, Mann-Whitney U test). The median score declined gradually during the ten days following the study procedure and happened to be 2 on Day-10, in UVC group. On the other hand, the median score in standard treatment group did not show any downward improvement, and on Day-10 median score was 4.5. The p values corresponding to differences of

  

**Table 3.** Clinical outcome parameters in UVC group and standard treatment group

| | UVC group | | Standard treatment group | | p value |
|---|---|---|---|---|---|
| | n/N | | n/N | | |
| Negative PCR test after treatment (in BAL fluid) | 5/6 | | 0/6 | | 0.003* |
| Death | 1/6 | | 3/6 | | 0.54** |
| | **Average** | **95%CI*** ** | **Average** | **95%CI** | |
| **Length of stay before enrollment (days)** | | | | | |
| Median | 8 | (8-8) | 4.5 | (2-8.5) | 0.31 |
| Mean | 7.3 | (4.2-9.4) | 5.2 | (3.2-7.4) | |
| **Length of stay after treatment (days)** | | | | | |
| in intensive care unit | | | | | |
| Median | 1 | (0-1) | 8.5 | (4-24) | 0.015 |
| Mean | 1.8 | (0.3-4.3) | 11.7 | (6.7-16.9) | |
| in medical ward | | | | | |
| Median | 3 | (3-4) | 0 | - | 0.015 |
| Mean | 3.3 | (1.5-5.0) | 0 | - | |
| total stay | | | | | |
| Median | 4.5 | (3.5-7.5) | 8.5 | (4-24) | 0.093 |
| Mean | 5.2 | (4.0-6.6) | 11.7 | (6.7-16.9) | |
| **WHO R&D Blueprint Ordinal Clinical Scale score** | **Median** | **Range** | **Median** | **Range** | |
| Day-0 | 4 | (3-5) | 4 | (3-6) | 0.82 |
| Day-1 | 3 | (3-6) | 4 | (4-8) | 0.093 |
| Day-2 | 3 | (3-6) | 4 | (4-8) | 0.041 |
| Day-3 | 3 | (2-6) | 4 | (3-8) | 0.093 |
| Day-7 | 2 | (2-6) | 4.5 | (2-8) | 0.18 |
| Day-10 | 2 | (2-8) | 4.5 | (2-8) | 0.24 |

*UVC: Ultraviolet-C; CI: Confidence interval; PCR: Polimerase chain reaction; BAL: Bronchoalveolar lavage*

** Chi-square test*

*** Fisher's exact test*

*Other p values are calculated by Mann-Whitney U test*

**** 95%CIs (bias-corrected accelerated: BCa) are calculated with bootstrapping technique*

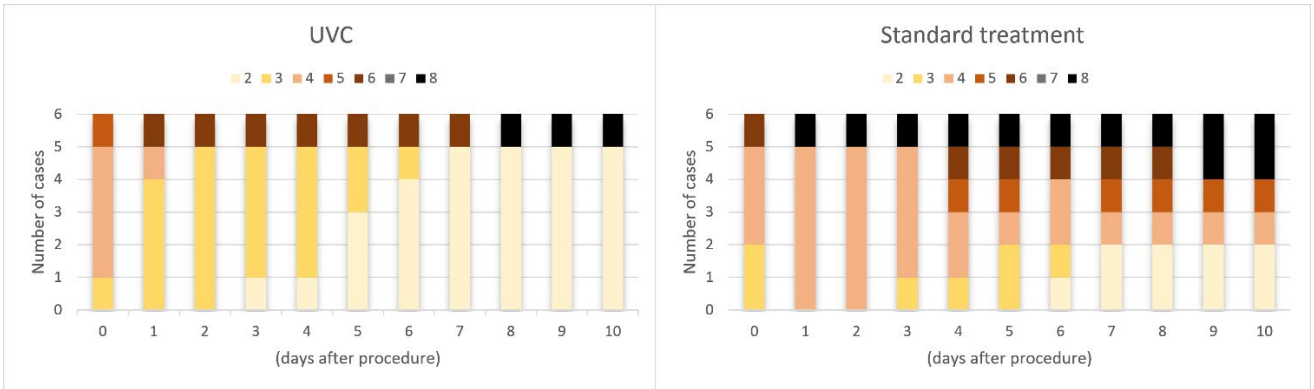


**Figure 3.** Distribution of the patients among WHO R&D Blueprint Ordinal Clinical Scale scores at baseline and during the ten days following the procedure in UVC group and standard treatment group

median scores between the groups was lower than 0.10 during the five days following the procedure, even statistically significant on Day-2 (p=0.041; Mann-Whitney U test) (**Table 3**, **Figure 3**).

### D-dimer, Ferritin and CRP Levels

The geometric mean of serum D-dimer level was initially almost similar in UVC and standard treatment groups on treatment day (Day-0), but the course was quite different in

**Table 4.** Mean serum D-dimer, ferritin and CRP levels at treatment day (Day-0) and at Days 1, 3, 7 and 10, in UVC group and standard treatment group. Ratios of mean level at specified day over mean level at Day-0 are also presented in the lower part of the table

| | UVC group | | Standard treatment group | | |
|---|---|---|---|---|---|
| | Geometric mean | 95%CI* | Geometric mean | 95%CI | p value** |
| **D-dimer (ng/mL)** | | | | | |
| Day-0 | 323 | (142-681) | 414 | (254-709) | 0.70 |
| Day-1 | 406 | (187-867) | 686 | (238-3,206) | 0.82 |
| Day-3 | 728 | (242-1,690) | 1’290 | (430-4,130) | 0.70 |
| Day-7 | 786 | (273-2,608) | 2’535 | (3010-28,099) | 0.24 |
| Day-10 | 630 | (228-2,179) | 1’191 | (293-6,860) | 0.39 |
| **Ferritin (ug/L)** | | | | | |
| Day-0 | 575 | (268-1,325) | 841 | (444-1,894) | 0.39 |
| Day-1 | 547 | (271-1,141) | 1’108 | (658-1,885) | 0.18 |
| Day-3 | 519 | (250-1,142) | 971 | (468-1,898) | 0.18 |
| Day-7 | 512 | (238-1,173) | 961 | (497-1,750) | 0.18 |
| Day-10 | 512 | (238-1,173) | 911 | (460-1,648) | 0.25 |
| **CRP (mg/L)** | | | | | |
| Day-0 | 50.8 | (22.2-100.9) | 58.9 | (28.4-118.5) | 0.94 |
| Day-1 | 42.1 | (184-80.6) | 64.0 | (34.8-108.3) | 0.48 |
| Day-3 | 31.4 | (18.1-63.9) | 32.2 | (10.5-80.9) | 1.00 |
| Day-7 | 9.8 | (4.9-21.1) | 18.4 | (2.9-111.8) | 0.59 |
| Day-10 | 8.0 | (3.0-21.3) | 12.2 | (2.7-60.2) | 0.70 |
| **D-dimer increase ratio (compared to Day-0)** | | | | | |
| Day-1 | 1.26 | (1.13-1.39) | 1.66 | (0.77-5.28) | 0.48 |
| Day-3 | 2.26 | (1.52-4.00) | 3.12 | (1.18-6.70) | 0.24 |
| Day-7 | 2.44 | (0.93-9.19) | 6.11 | (0.75-75.25) | 0.48 |
| Day-10 | 1.95 | (0.81-7.70) | 2.87 | (0.63-17.83) | 0.82 |
| **Ferritin increase ratio (compared to Day-0)** | | | | | |
| Day-1 | 0.95 | (0.84-1.06) | 1.32 | (1.07-1.65) | 0.093 |
| Day-3 | 0.90 | (0.79-1.00) | 1.15 | (0.73-1.80) | 0.31 |
| Day-7 | 0.89 | (0.72-1.08) | 1.14 | (0.66-2.00) | 0.54 |
| Day-10 | 0.89 | (0.72-1.08) | 1.08 | (0.55-2.02) | 0.54 |
| **CRP increase ratio (compared to Day-0)** | | | | | |
| Day-1 | 0.83 | (0.65-1.11) | 1.09 | (0.57-2.08) | 0.82 |
| Day-3 | 0.62 | (0.29-1.36) | 0.55 | (0.14-2.24) | 0.82 |
| Day-7 | 0.19 | (0.10-0.38) | 0.32 | (0.03-3.47) | 0.94 |
| Day-10 | 0.16 | (0.06-0.37) | 0.21 | (0.03-1.11) | 0.82 |

*UVC: Ultraviolet-C; CI: Confidence interval*

** 95%CIs (bias-corrected accelerated: BCa) are calculated with bootstrapping technique*

*** Mann-Whitney U test*

the following ten days. Mean D-dimer level showed a remarkable increase from 414 ng/mL at baseline to higher than 2500 ng/mL during the first post-treatment week, in standard treatment group (**Table 4**, **Figure 4**). The ratio of D-dimer level at Day-7 over Day-0 was higher than six times. This course was then followed by a slow decline, but mean D-dimer level was still higher than 1000 ng/mL at Day-10 (almost three times higher than Day-0 value). On the other hand, this incline was quite suppressed in UVC group. Mean D-dimer level showed increase from 323 ng/mL at baseline to about 1100 ng/mL maximum during the first post-treatment week. This corresponded to almost three times increase as compared to Day-0. This course was then followed by a decline. At Day-10, mean D-dimer level was almost twice the Day-0 value. D-dimer level was 1.3 to 2.6-

  

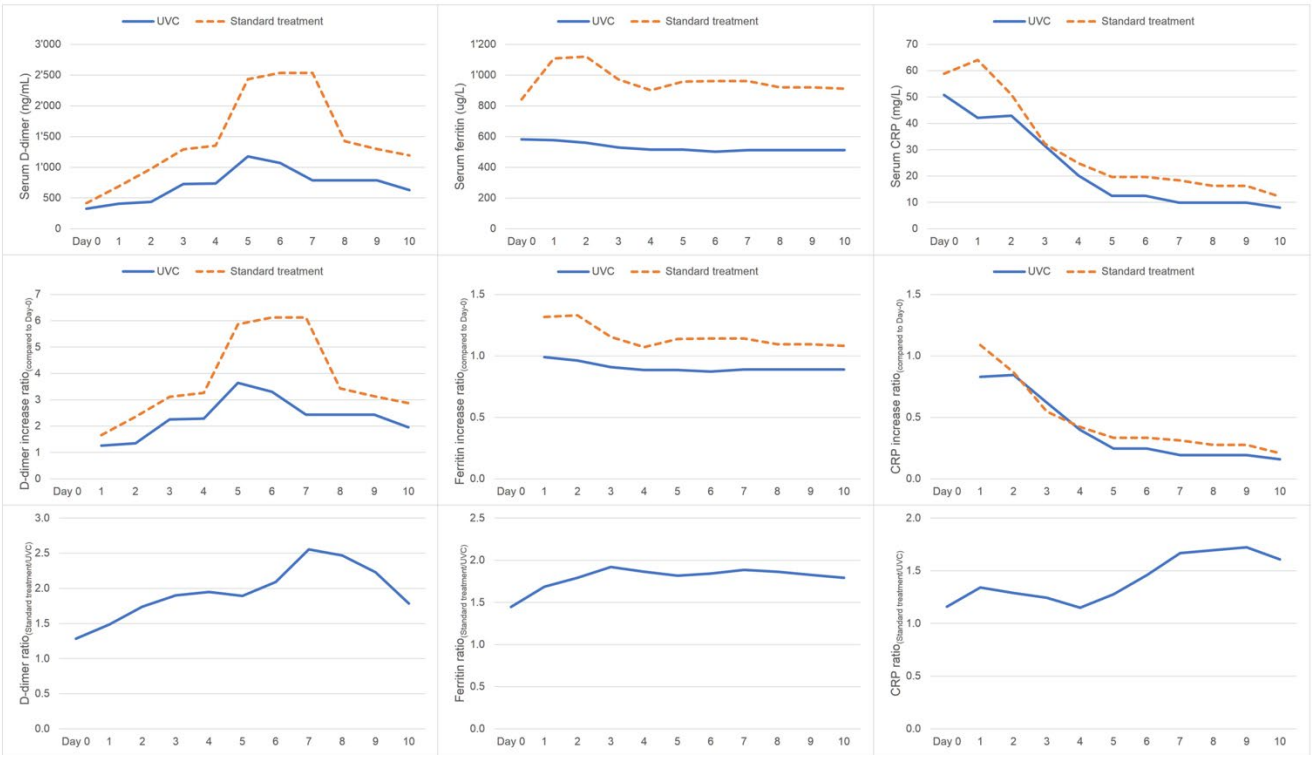


**Figure 4.** The courses of serum D-dimer, ferritin and CRP levels of patients in UVC group and standard treatment groups during follow-up after the treatment. Geometric means are on the rows, increase ratios (mean level at specified day divided by mean level at Day-0) are on the middle rows and ratios of standard treatment to UVC group (mean level in standard group divided by mean level in UVC group) are on the lower rows

fold higher during to ten days after Day-0, in standard treatment group as compared to UVC group.

The course of serum ferritin level was quite different between UVC and standard treatment groups. In standard treatment group, mean serum ferritin level showed up to 33% increase in the first three days following Day-0, followed by a slow decline (**Table 4**, **Figure 4**). Then it stayed in between 900 and 1000 ug/L from Day-3 to Day-10. On the other hand, mean ferritin level did not show any increase after Day-0. It even declined slowly during ten days after Day-0. Mean ferritin level was 1.5 to 1.9-fold higher during ten days after Day-0, in standard treatment group as compared to UVC group.

Serum CRP level declined in both UVC and standard treatment groups (except the very first day in standard treatment group). In Day-7 and Day-10, mean serum CRP level was declined down to 32% and 21% of the CRP level at Day-0, respectively, in standard treatment group (**Table 4**, **Figure 4**). Corresponding figures were 19% and 16% in UVC group. CRP level was up to 1.7-fold higher during ten days after Day-0, in standard treatment group as compared to UVC group.

**Safety**

Patients in study groups were comparable with regards to baseline serum biochemistry values, hemostatic parameters and hematologic parameters (**Supplementary Table**). The courses of white blood cell count and platelet count were similar throughout the ten days following the study procedure. Neutrophil percentage, which was similar in study groups at baseline, show a continuous decline starting the next day after the procedure and lasting until Day-10. However, neutrophil percentage did not show any change during the ten days.

Patients did not experience any deterioration in their clinical symptoms, physical examinations, laboratory findings, or any ophthalmologic diagnosis, which could not be explained by the natural course of COVID-19. No other undefined adverse effects related with UVC application have been observed.

## DISCUSSION

In this report, we presented for the first time transbronchial and intravenous UVC and laser light by an innovative UVC and laser generator device in six patients with severe COVID-19, and proposed that UVC and laser light therapy added to available pharmacotherapy has a potential to improve the clinical outcome of patients.

UVC light has long been known to have antimicrobial properties, thus suggested to be used for treatment of superficial and catheter-related infections, and sterilization of surfaces, indoor environments, blood products and donor organs [13,33-37]. In addition to its antibacterial and antiprotozoal effects, previous studies reported promising findings on the inhibitory effect of UVC light on viruses including human coronavirus. UVC light was shown to

**Table 5.** Literature summary on virus inactivation studies and opinion/comment/proposals on the use of UVC on coronaviruses

| Author, year (ref. no) | Condition | Virus | Findings/Topic |
|---|---|---|---|
| **Virus inactivation studies** | | | |
| Banerjee, 2020 (13) | PPE | SARS-CoV-2 | 3-, 4- and 5-log reduction in virus in 19, 30 and 80 minutes of UVC exposure, respectively |
| Inagaki, 2020 (16) | in vitro | SARS-CoV-2 | Rapidly inactivation of a strain of SARS-CoV-2 (isolated from a patient who developed COVID-19 in the cruise ship Diamond Princess) with UV irradiation |
| Eickmann, 2020 (8) | Platelet/plasma samples | SARS-CoV | UVC at dose of 0.1 J/cm$^2$ and 30 J/cm$^2$ reduced the infectivity of SARS-CoV with virus reduction factor of ≥3.4 and ≥3.1 for SARS-CoV in platelet concentrates and plasma samples |
| Buonanno, 2020 (12) | Aerosolized media contaminated with virus | Human alpha coronavirus HCoV-229E and betacoronavirus HCoV-OC43 | Proposal for continuous far-UVC exposure at dose of 3 mJ/cm$^2$/hour to result in 90%, 99% and 99.9% viral inactivation in 8, 16 and 25 minutes |
| Heimbuch, 2019 (14) | PPE | MERS (EM/2012 strain) and SARS (200300592 strain) | Log reduction rates reached by UV dose of 1 J/cm$^2$, ≥4.50 and ≥4.81 for MERS and SARS, respectively |
| Darnell, 2004 (15) | *in vitro* | SARS-CoV Urbani strain | Complete viral inactivation in 15 minutes at a distance of 3 cm of UV exposure |
| **Opinion/Comment/Recommendation on use of light therapy on SARS-CoV-2** | | | |
| Nogueira, 2020 (4) | | SARS-CoV-2 | Clinical use of light therapy |
| Fernandes, 2020 (34) | | SARS-CoV-2 | Clinical use of light therapy |
| Domínguez, 2020 (18) | | SARS-CoV-2 | Transdermal application |
| Camacho, 2020 (10) | | SARS-CoV-2 | Transdermal application |
| Fekrazad, 2020 (17) | | SARS-CoV-2 | Intravenous or intratracheal application |
| Ferreira, 2020 (19) | | SARS-CoV-2 | Intravascular application |
| Narla, 2020 (31) | | SARS-CoV-2 | Using on PPE |
| Yang, 2020 (32) | | SARS-CoV-2 | Using on PPE |
| Sagripanti, 2020 (33) | | SARS-CoV-2 | Model |

** PPE: Personal protective equipment*

inactivate enveloped or nonenveloped, single-stranded RNA or DNA viruses in platelet concentrates [38], hepatitis C virus ex vivo in donor lungs during preservation [33-37], and aerosolized H1N1 influenza virus [39]. SARS-CoV-2 is also an enveloped, positive-sense single-stranded RNA coronavirus [40].

There are some studies showing evidence of viral inactivation induced by UVC on coronaviruses. Also there have been a couple of papers, in which scientists experienced on light therapy, have noted their opinions, comments and recommendations A summary of these publications are presented in **Table 5**.

In their model, Banerjee et al tested the effectiveness of UVC 254 nm at a dose of ≥1 J/cm$^2$, on disinfecting respirators and personal protective equipment contaminated with SARS-CoV-2. They reported that 3-, 4- and 5-log reduction targets were reached in 19, 30 and 80 minutes, respectively. The average exposure of UV-C applied in 20 minutes was 3.5 kJ (11.5 J/cm$^2$), which corresponded to 3.1 log reduction of virus. Total exposure needed to reach 5-log reduction was 46.6 J/cm$^2$ in 80 minutes [23].

In an *in vitro* study, UVC (280 nm) has been shown to inactivate SARS-CoV-2 obtained from a COVID-19 patient [26].

There are also several studies on the effects of UVC on coronaviruses other than SARS-CoV-2 published in recent years. As all human coronaviruses have similar genomic sizes, UVC would be expected to show similar inactivation efficiency against other human coronaviruses including SARS-CoV-2. It has been reported that UVC at half of the full UVC dose (0.1 J/cm$^2$) reduced the infectivity of SARS-CoV and virus reduction factor ≥3.4 for SARS-CoV was achieved with the UVC-based pathogen inactivation system applied on platelet concentrates [18]. Buonanno et al. showed that low doses of UVC (222 nm, 1.2 mJ/cm$^2$) inactivated aerosolized human betacoronavirus HCoV-OC43, which is a member of beta coronaviruses, as SARS-CoV-2 is, too [24]. Based on the results of UVC on betacoronavirus HCoV-OC43, which is in the same genus as SARS-CoV-2, it has been proposed that continuous far-UVC exposure at dose of 3 mJ/cm$^2$/hour is expected to result in 90%, 99% and 99.9% viral inactivation in 8, 16 and 25 minutes. Thus, while staying within current regulatory dose

  

limits, low-dose-rate far-UVC exposure can potentially safely provide a major reduction in the level of coronaviruses [24].

In a report released by Heimbuch and Harnish for Applied Research Associates in 2019, the efficiency of UV on MERS (EM/2012 strain) and SARS (200300592 strain) on personal protective equipment was reported. Log reduction rates reached by UV dose of 1 J/cm$^2$, were ≥4.50 and ≥4.81 (equivalent to no detectable viable virus) for MERS and SARS, respectively. These values were still in the range between ≥3.87 and ≥4.28, even in the presence of artificial skin oil (sebum) and artificial saliva (mucin buffer), resembling real tissue conditions [22].

In their experiment, when Darnell et al. applied UVC to the SARS-CoV Urbani strain containing wells at a distance of 3 cm, partial inactivation started in 1 minute of UVC exposure with increasing efficiency up to 6 minutes and complete inactivation in 15 minutes [25].

UVC has also been suggested to be used for sterilization of N-95 masks commonly used for protection from COVID-19 and decontamination of respirators [41,42]. In their model, Sagripanti and Lytle claimed that coronaviruses are estimated to be least twice as sensitive to UVC (254 nm) as influenza viruses [43].

In a very recent review, Fernandes et al. reported that, other than UVC, photobiomodulation by application of laser light with various wavelength might be considered to be applied in COVID-19, expecting the effect on promoting immune system by increasing the production of adenosine triphosphate and oxygenation of red blood cells, and eventually increasing defense mechanisms of body against intense inflammatory processes induced by COVID-19 [44]. However, application of UVC irradiation and laser light in COVID-19 patients has not been reported yet.

UVC and laser light generator device used in the present study was first developed in 2017 for the treatment of infections within body with a semiflexible catheter and a custom-made light source. Upon rapid spread of COVID-19 cases, the device was adapted to be applied by transbronchial and intravenous route for severe COVID-19 cases. The germicidal spectrum range of UVC is 200-280 nm [19-38]. Therefore, UVC-generator that we used in our study generates UVC at a wavelength of 200-280 nm. An effective dose of 2-3.7 mJ/cm2 UVC is required to destroy the SARS-CoV virus population in infected blood products [18,45]. We determined the duration and dose of UVC irradiation for the lungs and blood, based on this dose. The time it takes to sterilize the blood from the virus depends on the duration of circulation of the whole blood. Therefore, duration of the circulation of blood in the body as well as the volume of blood in the body are the main factors in determining the energy level of UVC and laser therapy. In intravenous application, the dose was calculated by converting the blood volume to the surface area. For the transbronchial route, the dose was calculated by the surface area of respiratory tract and alveoli.

Positive PCR tests converted to negative just after the procedure in five patients who were applied UVC. We think that the single patient whose PCR in BAL fluid did not convert to negative, might benefit from repeated applications of UVC irradiation, if we had done, considering the decrease in viral load in this patient. UVC patients also had shorter stay in intensive care unit, higher discharge rate and lower mortality compared to six patients who received only pharmacotherapy. On the basis of these critical findings, we suggest that UVC therapy may improve the patients' outcome in severe cases of COVID-19 without causing any side effects.

It is also remarkable that serum D-dimer levels declined faster and stayed at lower level in UVC group as compared to standard treatment group. There was no increase in ferritin levels in UVC group in contrary to significant increase in standard treatment group. High levels of D-dimer and ferritin have been suggested to be predictors of severe clinical course and poor prognosis in patients with COVID-19 [46-49]. On the other hand, CRP level showed a parallel decline in both UVC and standard treatment groups, suggesting that UVC and laser light applied directly into the circulation had no obvious additional anti-inflammatory effect, contrary to what previous authors proposed [44]. However, it should be noted that UVC did not cause any extra inflammation in patients with COVID-19. Therefore, the biochemical findings of our case series may support the effectiveness and safety of UVC in COVID-19.

## CONCLUSIONS

In conclusion, transbronchial and intravenous application of UVC (254 nm) and laser light (green laser 630 nm; red laser 535 nm) may improve the outcome of severe COVID-19 cases. Further studies involving more patients are needed to confirm the promising effect of UVC and laser light and to consider the use of light-based therapies in combination with pharmacotherapy, particularly for critical cases of COVID-19.

We suggest that our treatment protocol might be implemented on other organs/tissues, including wound or nasal mucosa, skin ulcers, teeth, toenail, stomach, intestines, i.e. tissues where light can be delivered safely and effectively [13].

### Limitation

The major limitation of our study is the low number of patients. Actually, we had the opportunity to increase the number of patients easily before the publication, but since we observed that the preliminary results of our study in the UVC group were significantly better than the control group, we have decided to share the interim results of the first cases as a rapid contribution to the literature, because we are going through an extraordinary pandemic course and dealing with severe patient groups who need urgent treatment options.

Before moving to clinical studies with larger sample size, we wanted our findings to be discussed openly within the scientific literature frame.

**Author contributions:** All authors have sufficiently contributed to the study, and agreed with the results and conclusions.

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**Declaration of interest:** The authors declare no conflict of interest.

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## SUPPLEMENTARY APPENDICES – BIOCOMPATIBILITY TEST RESULTS

**Appendix A: Acute systemic toxicity**

**Test date:** 05.07.2019

**Report date:** 30.07.2019

**Report no:** 20190122-03

**Laboratory:** Gazi University Faculty of Medicine Laboratory, Ankara, Turkey

***Summary***

Acute Systemic Toxicity Test, was performed according to "ISO 10993-11: 2006 Biological evaluation of medical device: Systemic toxicology" "ISO 10993-2: 2006 Biological evaluation of medical devices- Part 2: Animal welfare requirements" and "ISO 10993-12: 2012 Biological evaluation of medical devices".

***Methods***

The sample was tested using 8-12 weeks old 5 female and 5 male mice / BALB-C. 5 female and 5 male BALB-C rats of 8-12 weeks old were used for negative control.

Mice were dosed 50 mL/kg intraperitoneally. Incubation was performed at 37°C for 72 hours and the extraction preparation rate was accepted as 6 cm/mL. As stated in ISO 10993-11: 2006 and ISO 10993-12: 2012 during the 72-hour test, the systemic effects of the product were followed according to the clinical observation criteria. Food and water consumption of all groups is normal. Out of weight loss was not observed in any of the control and test group, female and male mice. Liver weight index values obtained for all test and control mice are also within normal limits (4-6%). No anomaly was observed in the gross pathology examination performed for all test and control mice as a result of autopsy. At the end of the 72 hours test period, tissues of lung, liver, kidney and spleen taken from the control and test groups have been subjected to fixation with 4% paraformaldehyde. Tissue cuts of 5 pm thickness taken from the paraffin blocks, hematoxylin-eosin painting was made.

***Results***

Repeated dose subacute systemic toxicity test was performed with the extract obtained from the test material, and the test was terminated after a 72-hour observation period. As a result of evaluation and analysis studies including clinical observation, gross pathology, histopathology examinations, it was concluded that the product does not have an acute systemic toxic effect according to ISO 10993-11:2006.

**Appendix B: Subchronic systemic toxicity**

**Test date:** 03.07.2019

**Report date:** 25.07.2019

**Report no:** 20190122-04

**Laboratory:** Gazi University Faculty of Medicine Laboratory, Ankara, Turkey

***Summary***

Subchronic Systemic Toxicity Test was performed according to "ISO 10993-11 Biological evaluation of medical device: Systemic toxicology", "ISO 10983-2 Biological evaluation of medical devices: Animal welfare Requirements" and "ISO 10993-12 Biological evaluation of medical devices Sample Preparation" protocols.

***Methods***

The test was carried out using 5 females and 5 male mice / BAB-C of 8-12 weeks old sample. As recommended in the ISO 10993-11 test protocol, 5 female and 5 male mice / BAB-C were used for the control group for negative control.

The method of preparing the extract was chosen because the product cannot be applied directly. Incubation was performed at 37°C for 72 hours, and the extraction preparation rate was accepted as 3 $cm^2$/mL.

The systemic effects of the product were followed during the 90-day test according to the clinical observation criteria stated in in ISO10993-11 and ISO10993-12, and no clinical findings were found. Food and water consumption of all groups is normal. No mice weight change was recorded in any mouse within the start and end time of the experiment. The liver weights of the experimental animals were found within normal limits (4-6%).

The product was applied for 90 days according to the ISO 10993-11 test protocol. In the gross pathology examination, no anomaly was detected.

***Results***

Repeated dose subchronic systemic toxicity test was performed with the extract obtained from the test material, and the test was terminated after 90 days of observation. It has been observed that as a result of clinical observation and gross pathology examinations according to ISO 10993-11, the product does not have a subchronic systemic toxic effect.

  

**Appendix C: Cytotoxicity**

**Test date:** 03.07.2019

**Report date:** 28.07.2019

**Report no:** 20190122-01

**Laboratory:** Gazi University Faculty of Medicine Laboratory, Ankara, Turkey

***Summary***

Cytotoxicity Tests have been performed according to "Biological Assessment of Medical Products: ISO 10993-5 Tests for In-vitro Cytotoxicity" Standards.

***Methods***

The cytotoxicity of UVC/laser application for 1, 3 and 5 minutes were investigated by MTT ((3-4,5-dimethyl thiazol 2-yl) 2,5-diphenyl tetrazolium bromide) colorimetric assay.

The L929 healthy mouse fibroblast cells (ATCC, USA) were cultured in DMEM (Sgima ® D6429, Germany), supplemented with 10% FBS (Sigma® f7524, Germany) and 1% penicillin/streptomycin (GibcoTM, Germany) and at 37°C in 5% CO2. L929 cells at 2x104 cell/well were plated in to 96 well black plate (Costar™, NY, USA) and incubated for 24 hours. After incubation, cells were exposed to UVC/laser application for 1, 3 and 5 minutes. After exposure, cell medium was discarded. MTT solution (0.5 mg/mL) was added to wells and cells were incubated for an additional 2 h at 37°C. After incubation, cell culture medium was discarded and 100 µL of isopropanol was added to wells to dissolve formazan. The absorbance was measured at 570 nm wavelength by ELISA microplate reader (Thermo Multiskan Spectrum, Finland). The percentage of cell viability compared to the negative control was calculated by using the following equation:

$$\text{Viability\%} = (\text{Absorbance}_{\text{treatment group}}) / (\text{Absorbance}_{\text{control}}) \times 100\%.$$

***Results***

The proportion of viable cells did not change significantly with increasing exposure time. This figure was 95.4%±4.7% after first minute, as compared to 100%±5.2%. Corresponding figures were 93.2%±7.3% and 86.7%±6.7%, at third and fifth minutes, respectively.

There was no significant decrease in cell viability within the exposure duration.

**Appendix D: Genotoxicity**

**Test date:** 02.07.2019

**Report date:** 16.08.2019

**Report no:** 20190122-05

**Laboratory:** Gazi University Faculty of Medicine Laboratory, Ankara, Turkey

***Summary***

Bacterial Reverse Mutation Test AMES test was performed according to "OECD Guideline for Testing of Chemicals: Bacterial Reverse Mutation Test (No: 471, Adopted: 21 st July 1997)" using test materials and Salmonella strains from Molecular Toxicology (Moltox).

The Ames test is based on bringing the mutated cell into another state by applying another mutation or reverse mutation. Salmonella typhimurium is used as microorganism in the test.

***Methods***

The cultures of S. typhimurium used in the test were prepared as follows:

a) S. typhimurium TA 1535 disc to 20 mL Oxoid # 2 Nutrient Broth,

b) S. typhimurium TA97a, TA98 and TA100 discs each containing 20 mL of Oxoid#2 Nutrient Broth containing ampicillin antibiotic with a final concentration of 25 μg/mL,

c) S. typhimurium TA102 disc was thrown into 20 mL Oxoid # 2 Nutrient Broth containing ampicillin and tetracycline antibiotics with final concentrations of 25 μg/mL and 2 μg/mL, respectively.

The strains used were controlled phenotypically in the four-chamber phenotype confirmation plate. Plates were incubated at 37°C for 24-48 hours. As a negative control, sterile phosphate buffered saline solution (Phosphate buffered saline, PBS) used in the preparation of the sample extract was used. Sodium Azide [CAS no. 26628-22-8] for strains of S. typhimurium TA 1535 and TA 100, ICR 191 Acridine [CAS no. 17070-45-0] for TA97 a juice, Daunomycin for [CAS no. 23541-50-6] TA98 strain, Mitomycin C [CAS no. 50-07-7] for TA102 strain. To perform the metabolic activation control, 2-Aminoanthracene [CAS no. 613-13-8], for strains TA98 and TA 100 Benzo (a) pyrene [CAS no. 50-32-8] was used as a positive control.

As the metabolic activation system in the test, NADPH Regensys™ A (contains 0.1 M phosphate buffer, glucose-6-phosphate, $MgCl_2$'KCI in pH 7.4) and Aroclor 1254-induced male Sprague Dawley supported by NADPH Regensys™ B (NADPH) co-factors. The postmitochondrial S9 fraction prepared from the liver of the rat was used. The analysis was carried out using a %10 (v/v) S9 mixture, and 500 μL of S9 mixture was used per plate.

Sample extraction was performed by incubation at 37°C for 72 hours by applying the v/v volume ratio PBS was used for extraction The sample extract was tested without waiting and 100 μL extract was used per plate.

The analysis was carried out in duplicate for all samples. Revertant (reverse mutant) colonies formed in each plate after incubation were counted visually. The average number of colonies for each duplicate study was determined. For each strain, the frequency of spontaneous reverse mutant (spontaneous reverse mutation frequency) was determined.

The AMES test of the sample was evaluated as negative (-) since the number of His reverse mutant colonies of the sample did not increase twice or above compared to spontaneous and negative control.

***Results***

The product does not cause mutation (not mutagenic) under the tested test conditions and used bacterial strains.

  

**Appendix E: Mutagenicity**

**Test date:** 01-15.04.2019

**Report date:** 14.07.2020

**Report no:** YÜEF-İKTAL.TOKSLAB/0167/14.07.2020

**Laboratory:** Drug, Cosmetic and Medical Device R&D Analysis Laboratories (YÜEF-İKTAL)

***Summary***

This test was conducted according to ISO 10993-3 tests for genotoxicity, carcinogenicity and reproductive toxicity: mutagenicity test (OECD 471).

***Methods***

The mutagenicity of UVC/laser application for 1, 3 and 5 minutes were also investigated in this study. The mutagenicity assay was performed as a standard plate incorporation test with Salmonella typhimurium strains TA98 and TA100. TA98 and TA100 are indicator strains for frame shift and base substitution mutations, respectively. Tested strains were supplied from Moltox molecular toxicology, Inc (North Carolina, USA). Sodium azide (SA) and 4-nitro-o-phenylenediamine (NPD) were used as direct positive mutagens for TA100 and TA98 strains, respectively. SA and NPD were supplied from Sigma Chemical Company (St Louis, Missouri, USA).

Briefly, 0.50 mL of phosphate buffer (0.2 M, pH 7.4) and 0.10 mL of the bacterial culture were added to a sterile amber glass 13 mm culture tubes. Then, UVC/laser application catheter of the device was immersed in this solution and bacteria were exposed to UVC/laser for 1, 3 and 5 minutes at room temperature. Then, 2.0 mL of 45°C top agar was added, mixed and then transferred to the surface of the minimal glucose agar plates. Plates were inverted and placed at 37°C for 72 h in dark and revertant colonies were counted after incubation period. Similarly, as a control, 2.0 mL of 45°C top agar was added to the tubes containing phosphate buffer and bacteria after 1, 3 and 5 minutes. In addition, to assess the susceptibility of tested strains to UV light, 2 plates of each strains were placed under the UV lamp (ESCO, CSF/UV-30A) inside the biosafety cabinet (ESCO, Class II BSC) and exposed to UV light for 5 minutes.

***Results***

Negative control values of numbers of revertant per plate were 30.2±5.2, 144.0±13.3 and 23.3±3.6 for strains TA98, TA100 and TA1535, respectively. For strain TA98, this figure was 35.0±2.8, 30.5±6.4 and 13.0±2.8 at 1, 3 and 5 minutes after exposure of UVC/laser application, respectively. For strain TA100, this figure was 145.0±7.1, 94.0±8.5 and 91.5±2.1 at 1, 3 and 5 minutes after exposure of UVC/laser application, respectively. For strain TA1535, this figure was 15.5±6.4, 17.5±10.6 and 18.5±2.2 at 1, 3 and 5 minutes after exposure of UVC/laser application, respectively.

The results revealed that up to 5 minutes application of UVC/laser application, the mutation frequencies for TA98, TA100 and TA1535 strains did not change significantly when compared to spontaneous mutation frequencies, indicating no mutagenicity to the tested strains.

**Appendix F: Hemolytic impact**

**Test date:** 01.07.2019

**Report date:** 18.07.2019

**Report no:** 20190122-05

**Laboratory:** Gazi University Faculty of Medicine Laboratory, Ankara, Turkey

***Summary***

The hemolytic effect test is carried out according to the standards of "TS EN ISO 10993-4: 2010 Selection of Blood Interaction Experiments" and "ASTM F756-13: 2013 Standard Practice For Assessment of Hemolytic Properties of Materials".

TS EN ISO 10993-4 Liquid test sample is used according to "Selection of Blood Interaction Tests" and "ASTM F 756-13" standards.

***Methods***

Solid Sample Extraction Method: Solid test sample extract is prepared according to the Standard Surface Areas and Extract Liquid Volumes Schedule in the "TS EN ISO 10993-12 Sample Preparation and Reference Materials" standard. According to this table, the extract is obtained by holding the test sample with saline (0.9% m/v NaCl) at 37°C for 72 hours. For negative control, Polyethylene injector extract, 0.1% $Na_2CO_3$ solution for positive control and Phosphate buffered saline (PBS) was used as blind solution.

- Blood taken from the ear veins of 3 rabbits was collected in a heparinized tube, approximately 5 mL from each rabbit.
- Plasma free hemoglobin and total blood hemoglobin concentrations were calculated according to ASTM 756-13 Standard Practice for Assessment of Hemolytic Properties of Materials protocol.
- The blood that was found appropriate for the standard was diluted with PBS to be approximately 10 mg/mL hemoglobin.
- Test sample, positive control, negative control extract; The blind solution was prepared so that three tubes from each were 7 mL in each tube. One ml of blood diluted with PBS was added to the prepared tubes and incubated for 3 hours at 37°C.
- After incubation, the tubes were centrifuged at 700-800 g for 15 minutes.
- One mL of the supernatants was treated with 1 mL of cyanomethemoglobin for 4-5 minutes, and their absorbance was measured with a spectrophotometer at 540 nm.
- Based on the measured absorbents, hemoglobin concentrations and hemolytic indices of the test sample and controls are standard ASTM F 756-13 9.8.3.2. and 9.8.3.3.

***Results***

When the results are evaluated according to TS EN ISO 10993-4: 2010 Selection of Blood Interaction Experiments" and "ASTM F756-13: 2013 Standard Practice For Assessment of Hemolytic Properties of Materials" standards, Hemolytic Degree of the product sample was determined to be "not hemolytic".

  

**Appendix G: Pyrogenicity**

**Test date:** 01.07.2019

**Report date:** 16.07.2019

**Report no:** 20190122-9

**Laboratory:** Gazi University Faculty of Medicine Laboratory, Ankara, Turkey

***Summary***

Pyrogenicity test was performed according to ISO 10993-11, 2017 "Biological Evaluation of Medical Devices - Part 11: Tests for Systemic Toxicity, ISO 10993-12, 2012, Biological Evaluation of Medical Devices - Part 12: Sample Preparation and Reference Materials".

***Methods***

Pyrogenicity test is the measurement of the temperature rise detected after the application of the appropriately prepared extract to the rabbits by IV injection. Four albino New Zealand rabbits (2 females, 2 males, weight 2.40-2.65 kg). were used as the experimental animal, and one as the control animal. Test and control samples were prepared in NaCl at the rates specified in the protocol.

The test sample was extracted with Sodium Chloride (NaCl) for 0.9% Injection at a rate of 1 unit per 10 mL. The appropriately prepared test sample was placed in an extraction bottle and NaCl solution was added. The Control Sample was prepared using the same procedures. Each extract was shaken vigorously before application. Test or control samples were injected into the ear vessels at a dose based on the body weight of each animal. The test sample extract was injected at a dose of 10 mL per kg. Each injection was completed 10 minutes after the start of the application. Sodium Chloride (NaCl) solution was used as the control sample for 0.9% injection treated in the same way as the test sample.

Disposable syringes and hypodermic needles were used to apply the test sample. The glassware used in the tests were heated from 200°±2°C for at least 3 hours and purified from pyrogen.

A probe was inserted into the rectum of the test rabbit to measure body temperature to a depth of not less than 7.5 cm. After a temperature stabilization period (about 1 minute), body temperature measurements were taken. At least 30 minutes before injection of the test sample, basal temperatures were determined. Body temperatures were recorded at zero hour followed by 1 to 3 hours after injection at 30-minute intervals. Temperature drops are considered zero increases. If no temperature increase of 0.5°C or above is observed in any of the test animals, the test sample is not pyrogen.

If any rabbit shows an individual temperature increase of 0.5°C or higher, the test is repeated by including 3 more rabbits in the study. If a temperature increase of 0.5°C and above is observed in more than three of the 8 rabbits and the sum of the Eight individual maximum temperature increase does not exceed 3.3°C, the test sample is not pyrogen.

Body temperature increases for the test animals after injection were 0.1, 0.1, 0.0, 0.0 and 0.1°C. These increases did not exceed the test limit for the maximum individual temperature increase. The body temperature increase of the control animal was also determined as 0.0°C.

***Results***

Based on the criteria of the protocol, the test sample remained below the pyrogenicity limits, thus is not pyrogenic.

**Appendix H: Skin sensitization**

**Test date:** 01.07.2019

**Report date:** 20.08.2019

**Report no:** 20190122-07

**Laboratory:** Gazi University Faculty of Medicine Laboratory, Ankara, Turkey

***Summary***

Skin sensitization test was performed according to "ISO 10993-10: 2010 Biological evaluation of medical device: Tests for irritation and delayed-type hypersensitivity", "ISO 10993-2: 2006 Biological evaluation of medical devices: Animal welfare requirements" and "ISO 10993-12".

***Methods***

The purpose of this test is to evaluate whether the sample tested in the animal model causes skin hypersensitivity.

Guinea pig was used for the experiments as recommended in the standard protocol.

Each animal was injected intradermally 0.1 ml of each of the following into the injection areas (A, B and C), as shown in **Figure A1**:

Region A: Freud's full adjuvant with physiological saline is 50:50 vol.

Region B: Test sample (non-propelled extract); solvent alone is injected into control animals.

Region C: Test sample at the concentration used in the region, Freund's full adjuvant and physiological saline (50%)

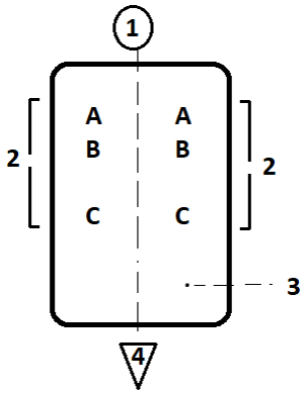


**Figure A1.** Application points. (1) Test animal head tip, (2) test region (0.1 mL intradermal injection site), (3) area between two clipped scapula, (4) caudal tip

Seven days after the completion of the intradermal induction phase, superficial application was performed to each animal with test specimens impregnated with gaseous cloth of approximately 8 $cm^2$. The skin was pretreated 48 hours prior to local application to avoid irritation with 10% sodium dodecyl sulfate. Local application was terminated after 48 hours. All competing test and test sample control animals were applied locally to control and test samples directly to the areas that could not be treated at the induction stage, using appropriate patches dipped in the test sample at the concentration in the C region 14 days after completion of the local induction phase. 24 hours later, dressings and patches were removed. Following removal of dressings in the application areas, the appearance of the control animals and competing skin areas of the test was observed between 24 and 48 hours. Skin reactions were evaluated under good lighting.

***Results***

Skin sensitization test was performed with the extraction solution obtained from the tested sample product, and the test was terminated after 27 days of observation. The test result of the product was evaluated as "0-No visible change" according to the grade scale given in Magnusson and Kligman Scale.

  

**Appendix I: Intradermal irritation**

**Test date:** 01.07.2019

**Report date:** 24.07.2019

**Report no:** 20190122-06

**Laboratory:** Gazi University Faculty of Medicine Laboratory, Ankara, Turkey

***Summary***

The intra-skin irritation test was performed as described in the "Annex B" section of the international protocol "ISO 10993-10: 2010 Tests for irritation and delayed-type hypersensitivity". Apart from this, "ISO 10993-2: 2006 Animal Welfare Requirements" and "ISO-10993-12: 2012 Sample Preparation and Reference Materials" standards are also taken into consideration.

***Methods***

Three female New Zealand albino rabbits (8-12 weeks old, less than 2 kg).

The extract was prepared as stated in the document titled ISO 10993-12: 2012, because the product cannot be applied directly. According to the 10.3.1 section of the related protocol, incubation was performed at 37°C for 72 hours. Extraction preparation rate was accepted as 6 cm/mL.

After the experimental animals were shaved to provide sufficient application area, the samples were applied by intradermal injection as shown in **Figure A2**. A total of 0.2 mL of substance was applied to 5 different points in each test region. As can be seen in **Figure A2**, extract samples obtained from the product in the region 2 and 4 were applied as polar solvent control to the region 3 and non-polar solvent positive control to the region 5. The perimeter of the injection areas was determined by marking. Following the application, each injection site was observed at 24, 48 and 72 hours.

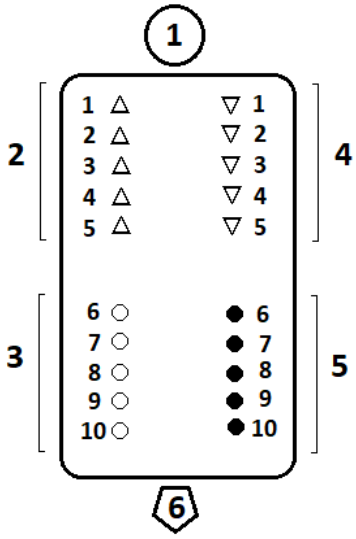


**Figure A2.** Application points. (1) experimental animal's head region, (2) test zone, (3) negative control region, (4) test zone, (5) positive control region, (6) tail side of the experimental animal

While calculating the score value for the tested samples and controls, the redness and edema values obtained for each observation time are summed up and divided into 15 (3 observation times 5 observation sites). The overall average score for tests and controls is obtained by dividing the obtained score value by the number of animals, i.e. 3. The posttest sample score is obtained by subtracting this value from the score obtained for the test sample if a value is obtained for the control.

***Results***

According to the protocol and evaluation criteria specified in ISO 10993-10: 2010 standard, it was determined that the product does not cause intradermal irritation.

## SUPPLEMENTARY TABLE

**Serum biochemistry and hematology/hemostasis parameters in UVC group and standard treatment group**

| | UVC group | | Standard treatment group | | |
|---|---|---|---|---|---|
| | **Median** | **95%CI*** | **Median** | **95%CI** | **p value**** |
| **Serum biochemistry** | | | | | |
| Blood urea nitrogen (mg/dL) | 37 | (34-51) | 33.5 | (22-83) | 0.70 |
| Serum creatinine (mg/dL) | 0.74 | (0.71-0.82) | 0.85 | (0.63-1.28) | 0.31 |
| Serum potassium (mEq/L) | 3.99 | (3.34-4.44) | 4.14 | (3.62-4.52) | 0.59 |
| Aspartate transaminase (U/L) | 27.5 | (13.0-40.0) | 33.0 | (24.0-235.0) | 0.31 |
| Alanine transaminase (U/L) | 24.5 | (15.0-36.0) | 23.5 | (16.0-123.0) | 0.59 |
| Total bilirubin (mg/dL) | 0.41 | (0.28-0.51) | 0.62 | (0.41-0.81) | 0.093 |
| **Hematology/Hemostasis** | | | | | |
| PT (sec) | 13.7 | (12.4-14.4) | 12.5 | (11.8-13.3) | 0.18 |
| aPTT (sec) | 33.4 | (30.8-47.6) | 32.8 | (28.1-36.1) | 0.82 |
| Hemoglobin (g/dL) | 13.6 | (11.2-13.9) | 13.1 | (12.3-15.1) | 0.82 |
| Hematocrit (%) | 41.4 | (38.5-43.3) | 40.4 | (37.4-49.4) | 0.82 |
| Red blood cell count ($10^3$/uL) | 4.64 | (3.95-4.91) | 4.52 | (4.26-5.31) | 0.70 |
| **White blood cell count ($10^3$/uL)** | | | | | |
| Day-0 | 8.95 | (8.17-13.25) | 7.61 | (5.29-10.17) | 0.31 |
| Day-1 | 9.23 | (7.05-10.19) | 8.30 | (7.15-9.45) | 0.59 |
| Day-3 | 7.85 | (6.73-9.42) | 9.08 | (7.21-12.03) | 0.24 |
| Day-7 | 8.68 | (6.74-14.54) | 11.40 | (7.69-16.85) | 0.48 |
| Day-10 | 9.69 | (7.99-10.48) | 10.68 | (7.09-16.85) | 0.59 |
| **Neutrophil percentage (%)** | | | | | |
| Day-0 | 82.3 | (66.0-86.4) | 87.6 | (81.2-89.9) | 0.13 |
| Day-1 | 79.3 | (63.7-90.0) | 87.0 | (83.3-89.5) | 0.48 |
| Day-3 | 73.2 | (61.3-84.0) | 89.4 | (89.2-91.6) | 0.026 |
| Day-7 | 70.6 | (57.3-91.0) | 90.8 | (89.5-92.0) | 0.065 |
| Day-10 | 64.0 | (51.3-90.9) | 88.7 | (81.6-91.6) | 0.13 |
| **Platelet count ($10^3$/uL)** | | | | | |
| Day-0 | 367 | (196-418) | 254 | (177-407) | 0.59 |
| Day-1 | 382 | (207-427) | 306 | (296-314) | 0.59 |
| Day-3 | 271 | (113-519) | 318 | (198-436) | 0.82 |
| Day-7 | 325 | (113-424) | 247 | (121-537) | 0.82 |
| Day-10 | 371 | (270-387) | 225 | (179-463) | 0.39 |

*UVC: Ultraviolet-C; CI: Confidence interval; PT: Prothrombin time; aPTT: Activated partial thromboplastin time*

** 95%CIs (bias-corrected accelerated: BCa) are calculated with bootstrapping technique*

*** Mann-Whitney U test*

  