



Safety and immune-supportive potential of the food supplement 5-aminolevulinic acid phosphate for patients with COVID-19: An open-label, non-randomized pilot study

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Submission Date: November 11th, 2023; **Acceptance Date:** January 15th, 2024; **Publication Date:** January 19th, 2024

Please cite this article as: Darwish A., Almadani A., Als Salman J., Atkin S., Murad M., Nakajima M., Rehani R., Ebeling A., Stocker M., Stummer W., Berenzen N. Safety and immune-supportive potential of the food supplement 5-aminolevulinic acid phosphate for patients with COVID-19: An open-label, non-randomized pilot study. *Bioactive Compounds in Health and Disease* 2024; 7(1): 17-35. DOI: <https://doi.org/10.31989/bchd.v7i1.1290>

ABSTRACT

Background: One mode of action of 5-ALA with SFC is the induction of Heme Oxygenase-1, a key regulator in anti-oxidative and immunological processes, leading to the hypothesis that the product supports faster recovery from infections such as COVID-19.

Objective: The main objective was to assess the safety and immune-supportive potential of the food supplement 5-aminolevulinic acid phosphate (5-ALA) with sodium ferrous citrate (SFC) in patients with COVID-19 when co-administered with standard of care medications (SoC).

Methods: A patient group with moderate COVID-19 symptoms (four males and three females, mean age 43 years) and one group with severe symptoms (12 males, mean age 48 years) received daily oral doses of 500 mg/750 mg 5-ALA with

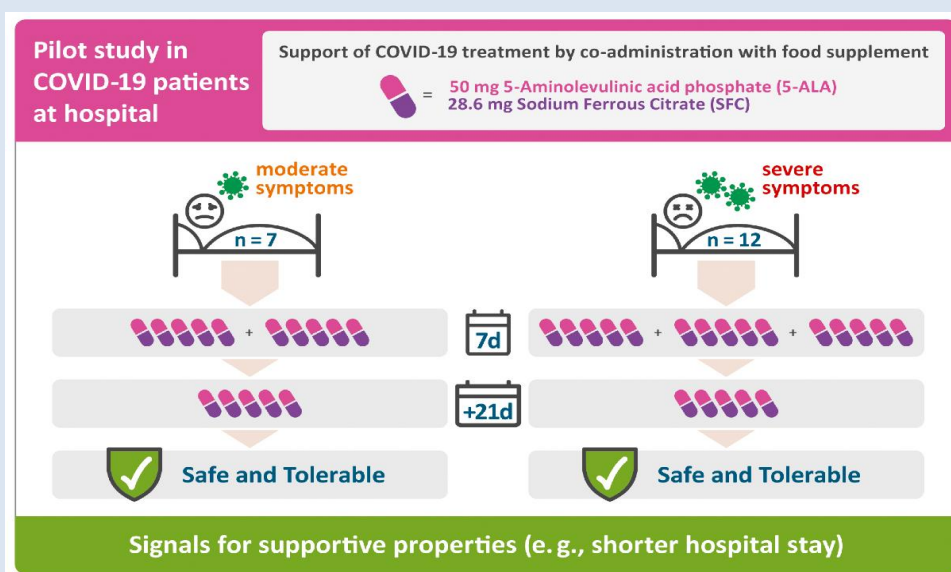
286 mg/430 mg SFC for the first 7 days (≤ 7.5 times higher than the recommended dose for the marketed product). For the subsequent 21 d, the daily dose was reduced to 250 mg 5-ALA with 143 mg SFC in both groups. Adverse events and several immunological laboratory parameters were collected. Moreover, the mean hospital stay was compared with historical data of patients solely treated with SoC.

Results: Two patients in the moderate group showed elevated liver enzymes; however, these seemed to be related to SoC. In the severe group, one patient experienced constipation. No serious adverse events were observed. In the severe group, a significant decrease of C-reactive protein (109.42 to 5.41 mg/L; $p < 0.005$), procalcitonin (0.87 to 0.07 ng/mL; $p < 0.005$), and interleukin 6 (20.07 to 5.06 pg/mL; $p < 0.05$) and an increase of the cluster of differentiation 4 (250 to 880 cells/ μ L; $p < 0.05$) and the cluster of differentiation 8 (190 to 623 cells/ μ L; $p < 0.05$) were detected. The hospital stay of the severe group was markedly shorter (8 d only) than that of the control group (16 d).

Conclusions: 5-ALA with SFC was evaluated as safe for administration in COVID-19 patients. Moreover, there were signals detecting its immune-supportive potential. The small number of included patients limits interpretation of the significance of the results; however, the study is useful in deciding future development strategies.

Trial Registration: The study was registered on ClinicalTrials.gov (<https://clinicaltrials.gov/>) on September 06, 2020 (registration no. NCT04542850).

Keywords: 5-aminolevulinic acid; COVID-19; SARS-CoV-2; food supplement



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INTRODUCTION

In 2020, coronavirus disease 19 (COVID-19) evolved from a single case in Wuhan, China, to a global pandemic, with over 622 million cases and 6.56 million deaths reported [1-2]. Over 2,700 clinical trials were registered with the

aim of investigating medications for the treatment of COVID-19. For several drugs, rapid approvals, at least as emergency use authorizations for COVID-19 therapy, were given (e.g., remdesivir, tocilizumab, baricitinib). However, these drugs have multiple side effects;

therefore, products with good safety profiles are required, such as food supplements which can augment established COVID-19 therapies and may lead to a reduction in the treatment duration. One overarching goal of the study presented here is to provide research that can lead to the development of functional food products: in our case in the indication of COVID-19. The definition for functional foods, as provided by the Functional Food Center (FFC) located in Texas, United States, is as follows: "Natural or processed foods that contain biologically-active compounds; which, in defined, effective, non-toxic amounts, provide a clinically proven and documented health benefit utilizing specific biomarkers, to promote optimal health and reduce the risk of chronic/viral diseases and manage their symptoms." A promising candidate is 5-aminolevulinic acid with sodium ferrous citrate (5-ALA with SFC), which has been marketed as a food or food supplement in several countries as e.g., in Japan for many years. The bioactive component 5-ALA is a natural ingredient of many foods or fermented products like Banana (0.4

mg/kg), Shitake mushrooms (0.6 mg/kg) and Baker's yeast (140 mg/kg) [3], However, it can also be produced by fermentation processes and added to foods or used as a food supplement in capsule form.

5-ALA is metabolized into protoporphyrin IX (PpIX) in the mitochondria via a cascade of metabolites. This photoactive agent accumulates in the cells of different cancers, including brain tumors. This mechanism forms the foundation for the use of 5-ALA, in its hydrochloric acid salt form, as a pharmaceutical agent, such as in Gliolan, for the diagnosis and photodynamic therapy of cancers [4-6]. However, as a food supplement, 5-ALA is combined with iron (Fe) as an SFC, which causes PpIX to not accumulate and instead directly convert to heme via a ferrochelatase-catalyzed reaction. Increased heme concentration indirectly and strongly induces Heme Oxygenase-1 (HO-1). Fujino et al. [7] described its metabolic pathways in detail and highlighted the key role of 5-ALA as an inducer of HO-1, which regulates antiviral, anti-inflammatory, antioxidant, and immunological processes within the body (Figure 1).

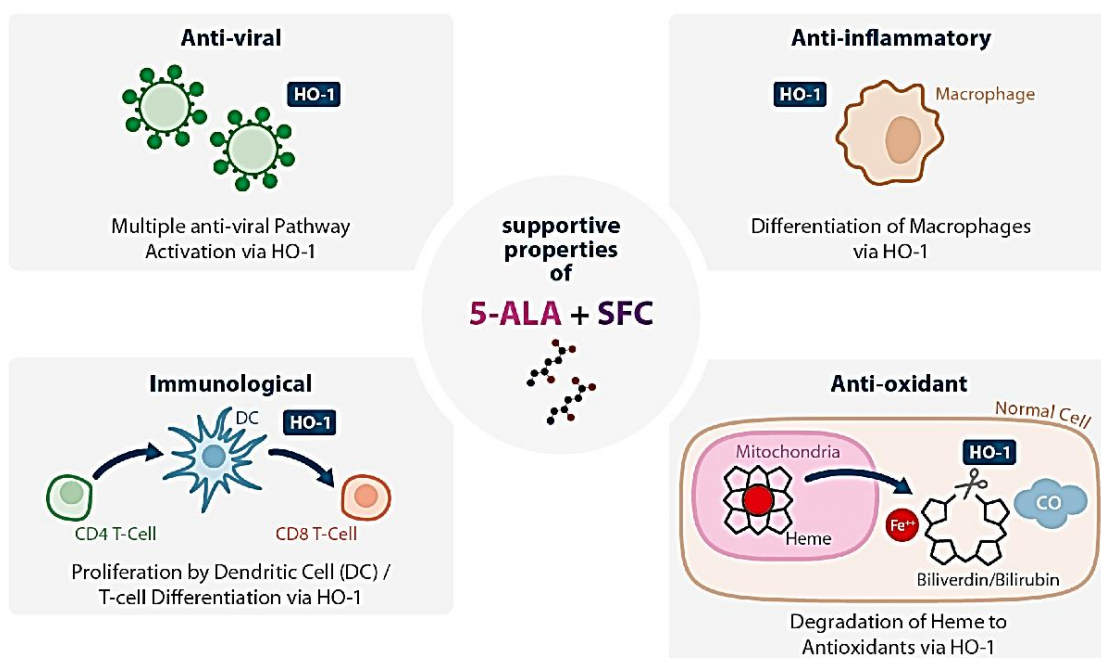


Figure 1. Supportive properties of 5-aminolevulinic acid phosphate with sodium ferrous citrate (5-ALA with SFC). The role of 5-ALA with SFC as a key inducer of Heme Oxygenase-1 (HO1), which triggers and activates anti-viral, anti-inflammatory, anti-oxidant, and immunological processes.

Anti-viral properties of 5-ALA with SFC have been demonstrated for several severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants in an *in vitro* study published in 2022 [8]. Moreover, an antiviral effect based on HO-1 has been observed in several other viruses such as human immunodeficiency virus (HIV) [9], Zika [10], herpes [11], influenza [12], dengue [13], hepatitis B [14], and Ebola [15].

Anti-inflammatory supportive properties can also be attributed to the combination of 5-ALA with SFC, and indirectly via HO-1, which can activate M2 macrophages [7, 16]. Anti-oxidant activity of the heme degradation products, biliverdin, carbon monoxide (CO), and Fe, is catalyzed by HO-1 [7]. Supplementation of 5-ALA with SFC may lead to increased heme levels and initiation of HO-1 activity; thus, the degradation products of heme should also be increased. *In vitro* and *in vivo* testing has shown that these products protect against apoptosis, inflammation, and oxidative stress [17-18]. Wegiel and Otterbein reported that Biliverdin “emerged as a cytoprotective and important anti-inflammatory molecule [19].”

The immunologically supportive properties of 5-ALA with SFC are attributed to the fact that it strengthens the mitochondrial function of T cells [20]. Patients with COVID-19 have reduced T cell concentrations [21]. T cells are crucial for defense against acute viral lung infections [22]. The presence of memory CD8 T cells with antibodies protects the body from further viral infections [23]. Furthermore, chronic inflammation due to mitochondrial malfunction can cause a rapid increase in cytokine levels, which can lead to critical symptoms of COVID-19 [24]. However, 5-ALA with SFC specifically supports mitochondrial function by increasing heme synthesis, which upregulates the respiratory cascade [25]; therefore, it is necessary to provide virus-infected

patients with products that specifically strengthen the immune system in areas weakened by the disease.

The primary hypothesis and stimulus for conducting our study was that co-administration of 5-ALA with SFC complements the current therapy for COVID-19 and that patient administration is safe and tolerable. This was investigated in a case study of six patients with severe COVID-19 symptoms at the Hanzoman Clinic in Tokyo, Japan [26]. Co-administration of 5-ALA with SFC reduced the recovery time compared to that of patients who received standard of care (SoC) against COVID-19. 5-ALA with SFC was administered at daily doses ≤ 2250 mg, however, with no fixed-dose regimen.

To simplify the administration recommendations for physicians and self-administration by patients, our pilot clinical study was designed and conducted to use standardized dosing regimens. This is the first study to perform a group comparison between patients with moderate and severe symptoms to provide the first estimate for the most appropriate target population. However, the groups were very small, with only seven patients assigned to the moderate group and 12 patients to the severe group. This was due to massive recruitment problems, as 994 of the 1017 patients contacted refused to participate in the study and four patients did not meet the screening requirements.

MATERIALS AND METHODS

This open-label (not blinded for patients as well as for investigators), non-randomized (patients were assigned to the moderate or severe group according to their severity of COVID-19 symptoms) pilot study was approved by the ethical committees of the Bahrain Defense Force (BDF) hospital and Salmaniya Medical Complex. This study was conducted following good clinical practice and in accordance with the guidelines of the Declaration of Helsinki. All patients provided written

informed consent. Data integrity and quality were regularly checked by a clinical monitor during on-site monitoring visits.

Study duration, number of patients, and inclusion and exclusion criteria/group assignment: The first patient was recruited in November 2020, and the last patient's visit was in September 2021. This study was conducted in two Bahraini hospitals (BDF hospital and Salmaniya Medical Complex hospital). Patients with moderate-to-severe symptoms of COVID-19 were asked to participate directly after hospital admission. Patient eligibility was assessed at the screening visit. Patients were only administered 5-ALA with SFC when they met all inclusion and exclusion criteria.

Inclusion criteria:

- Willing and able to provide written informed consent, or with a legal representative who can provide informed consent.
- Aged ≥ 21 to 70 years
- Acute Respiratory Syndrome Coronavirus (SARS-CoV)-2 infection confirmed by polymerase chain reaction (PCR) test before beginning dose regimen.
- qSOFA ≥ 1 or imaging
- Currently hospitalized
- Radiographic evidence (chest X-ray or chest CT scan) of pulmonary infiltrates
- Able to swallow 5 capsules of study product at dosing time points.

Exclusion criteria:

- Subject has critical symptoms of COVID19 infection as defined as: require high-flow oxygen therapy (>15 l/min delivered by nasal cannula or mask) or invasive mechanical ventilation signifying respiratory failure, septic shock, and/or multiple organ dysfunction.

- Subject is nourished by a nasogastric tube.
- Subject has acute or chronic type(s) of porphyria or a family history of porphyria.
- Subject has demonstrated previous intolerance of 5-ALA-Phosphate and/or SFC by topical or oral administration (except for photosensitivity)
- Pregnant or nursing women
- Males and females of reproductive potential who have not agreed to use an adequate method of contraception during the study.
- Subjects who are unable or unwilling to comply with requirements of the clinical trial.
- Participation in any other clinical trial of an experimental treatment for COVID-19
- Evidence of multiorgan failure
- Alanine Aminotransferase (ALT) or aspartate aminotransferase (AST) > 5 X upper limit of normal (ULN)
- Creatinine clearance < 50 mL/min using the Cockcroft-Gault formula for participants ≥ 18 years of age
- Subjects who may be excluded at the Investigator's discretion.

This study had a recruitment issue. Of the 1017 patients asked to participate, 994 refused. The main reason reported was the unwillingness to participate in a study in these uncertain times with this new virus infection. Moreover, some patients, already taking over 10 different medications, declined to add 10 or 15 capsules of 5-ALA with SFC per day during the initial 7 d of the study. Initially, we planned to include 40 patients; however, owing to recruitment issues, we stopped after 23 patients were included in the study (20 males, 3 females). Of these, four were screening failures (two consent withdrawals, one protocol violation, and one owing to the investigators' discretion) and were not assigned to any of the study groups before the first dose.

Nineteen patients fulfilled the criteria for the modified intention-to-treat (mITT) analysis, of which seven fulfilled

the criteria for the moderate group and 12 for the severe group (Figure 2).

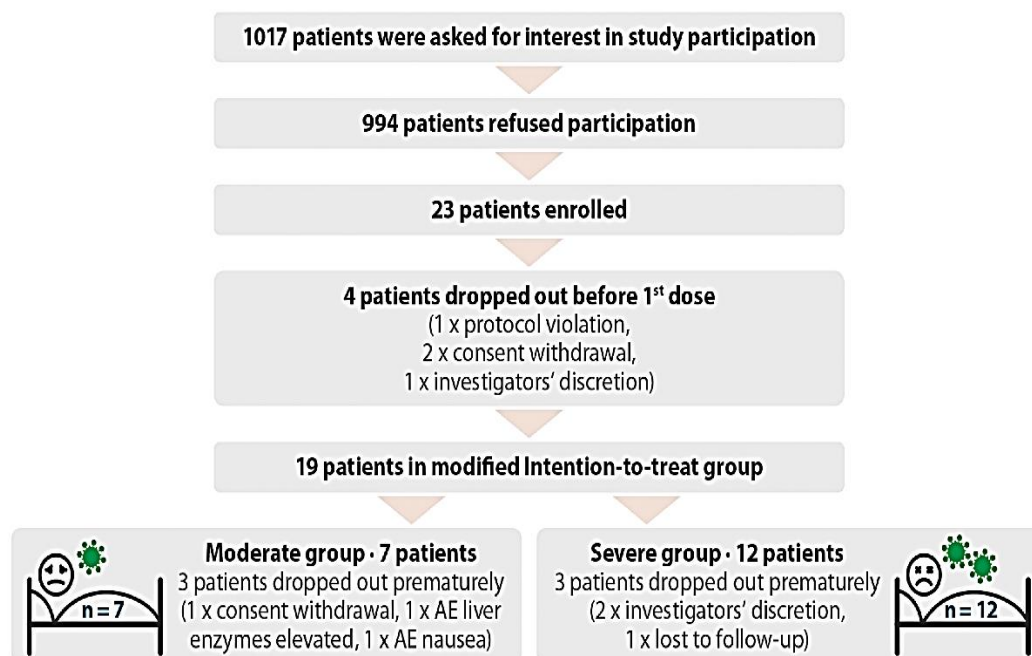


Figure 2. Flowchart of patient characteristics. AE, adverse event

Evidence of lower respiratory disease was clinically assessed using the quick Sepsis-related Organ Failure Assessment score (qSOFA ≥ 1) or imaging in patients with moderate symptoms. Patients with severe COVID-19 had a respiratory frequency of >30 breaths/min, ratio of arterial partial blood pressure of oxygen to fraction of inspired oxygen ($\text{PaO}_2/\text{FiO}_2$) <300 mmHg, and lung infiltrate of $>50\%$. In exceptional cases, the investigator decided, owing to certain signs and symptoms, to assign a moderate patient to the severe group; however, not all criteria were fulfilled. The primary objective was to assess the safety and tolerability of the administration of 5-ALA with SFC in patients with COVID-19 that underwent concomitant SoC treatment (remdesivir, dexamethasone, and other medications that mitigated symptoms used at the time of the study in Bahrain) and to identify of all treatment-emergent and serious adverse

events with grade III and IV common toxicity criteria for adverse events (CTCAE) and any causal relationship to 5-ALA with SFC.

The secondary objective of this study was to assess efficacy parameters. The secondary endpoints were the COVID-19 modified scale, biological markers for infection, immunology (C-reactive protein [CRP], D-dimer, procalcitonin, interleukin 6 [IL-6], cluster of differentiation 4 [CD4] and CD8), and the duration of hospitalization.

COVID-19 modified scale: Clinical improvement was measured using a modified version of the ordinal scale developed by the World Health Organization Committee to assess illness severity over time for patients COVID-19 (recommended by the WHO R&D Blueprint Group in 2020).

Laboratory measurements including biological markers:

Blood samples were analyzed at the Bahrain Defense Force Royal Medical Services Pathology Department, BDF Hospital, West Riffa, Kingdom of Bahrain (ISO 15189:2012 accredited in December 2017 by the Dubai Accreditation Centre, Dubai, United Arab Emirates). Alanine aminotransferase (ALT) was enzymatically analyzed using pyridoxal L-5-phosphate (Lab Assay Cobas Pro c503, Roche Diagnostics, Indianapolis, USA) with 3.48% coefficient of variation (CV). CRP and D-dimer levels were measured using immunoturbidimetric assays. CD4 and CD8 levels were measured using flow cytometry according to the manufacturer's instructions (Roche Diagnostics, Indianapolis, USA). IL-6 and procalcitonin levels were analyzed using an electrochemiluminescence immunoassay (Roche Diagnostics, Indianapolis, USA). The following laboratory assay equipment were used: Cobas Pro c503 (Roche Diagnostics, Indianapolis, USA) with 3.48% CV for ALT, 3.9% CV for CRP, STAR MAX3+ (Diagnostica Stago, USA) with <5% CV for D-dimer, Navios EX (Beckmann Coulter, Indianapolis, USA) with <5% CV for CD4 and CD8; Cobas Pro e801 (Roche Diagnostics, Indianapolis, USA) with 4.8% CV for IL-6 and 4.68% CV for procalcitonin.

Adverse Events monitoring: Adverse Events (AEs) were monitored from when the Informed Consent was signed to the end of the study for each patient. All AEs were analyzed according to the principle of treatment emergence. An adverse event was considered treatment emergent if it either did not exist before the first administration but manifested afterward, or if it was present before the first administration of the study product and subsequently worsened in severity. A subject having the same AE more than once would be

counted only once for the incidence for that AE. Well known symptoms of a COVID-19 infection were not reported as AEs.

Length of hospitalization: The duration of hospitalization was compared with the available data from 11 patients who were hospitalized at BDF Hospital, owing to severe COVID-19 symptoms, which were included in a separate audit that was not part of this study. Three patients were excluded from the analysis as they necessitated high-flow oxygen therapy either upon admission or during the hospital stay, aligning with the exclusion and discontinuation criteria outlined in our study, respectively. The audit data were suitable for a control because the patients were the same age and sex, their COVID-19 symptoms were assessed by the same investigator as severe, and they were treated at the same hospital for approximately the same time in 2020; the SoC was also comparable. Moreover, the demographic and laboratory data available at hospital admission showed no significant differences in most values (Table 1). The values only differed for neutrophils (mean, 5.94×10^9 cells/L and 3.05×10^9 cells/L in study and audit patients, respectively) and lymphocytes (mean, 0.80×10^9 cells/L and mean 1.68×10^9 cells/L in study and audit patients, respectively). The values differed statistically, yet without clinical significance, as the values for both groups were at approximately the normal range (neutrophils: $1.50\text{--}8.00 \times 10^9$ cells/L; lymphocytes: $1.0\text{--}5.00 \times 10^9$ cells/L). Unfortunately, no audit data was available for patients with moderate COVID-19 symptoms. Therefore, the duration of hospitalization for the moderate group was compared with that recorded in the severe study group only.

Table 1. Comparing demographics and lab values at admission between study and audit patients at BDF Hospital

| Parameter | Study patients from BDF hospital | | | Audit patients from BDF hospital | | | p-value ¹ |
|--------------------------------------|----------------------------------|---|------------------|----------------------------------|---|------------------|----------------------|
| | mean | N | min; max | mean | n | min; max | |
| Age (years) | 47.5 | 8 | 30.0; 59.0 | 45.8 | 8 | 34.0; 64.0 | 0.76 (T) |
| Sex (male/female) | male | 8 | 0 female; 8 male | male | 8 | 0 female; 8 male | - |
| Oxygen saturation (%) | 94 | 8 | 91; 97 | 95 | 7 | 90; 98 | 0.35 (T) |
| PCR COVID-19 test (CT) | 28.89 | 8 | 23.93; 33.76 | 31.55 | 8 | 21.65; 35.00 | 0.11 (T) |
| Neutrophils ($\times 10^9$ cells/L) | 5.94 | 8 | 3.41; 14.86 | 3.05 | 8 | 1.78; 5.39 | 0.02 (U) |
| Platelets ($\times 10^9$ cells/L) | 204.25 | 8 | 137; 286 | 208.25 | 8 | 134; 303 | 0.89 (T) |
| Lymphocytes ($\times 10^9$ cells/L) | 0.80 | 8 | 0.49; 1.24 | 1.68 | 8 | 1.06; 3.15 | 0.01 (U) |
| Hemoglobin (g/L) | 135 | 8 | 104; 153 | 138 | 8 | 117; 150 | 0.63 (T) |
| Ferritin (ng/mL) | 1119.1 | 8 | 384.1; 4441.0 | 1049.9 | 8 | 149.7; 2117.0 | 0.71 (U) |
| Lactate Dehydrogenase (IU/L) | 493 | 8 | 254; 815 | 473 | 8 | 287; 835 | 0.83 (T) |
| Procalcitonin (ng/mL) | 0.79 | 8 | 0.05; 3.45 | 0.28 | 8 | 0.10; 0.72 | 0.40 (U) |
| D-Dimer ($\mu\text{g/mL}$) | 0.92 | 8 | 0.40; 2.78 | 0.87 | 8 | 0.36; 1.38 | 0.53 (U) |
| CRP (mg/L) | 156.78 | 8 | 41.23; 349.69 | 115.93 | 8 | 28.95; 259.88 | 0.64 (U) |

¹ p-value was calculated using a two-sample t-test (T)/Mann-Whitney U test (U) between the study and audit patients with severe COVID-19 symptoms hospitalized at the Bahrain Defense Force (BDF) Hospital in Bahrain (data were recorded in 2020/2021).

Study product, structure-function relationship, and administration regimen:

The study product, 5-ALA with SFC capsules, was delivered by SBI Pharma in Tokyo, Japan. The BDF managed the import, storage, and supply of the product to the study sites.

The structure-function relationship of the protective abilities of 5-ALA against COVID-19 as discussed in the literature is summarized and visualized in a mechanistic schematic diagram (Figure 3) below:

- Inhibition of viral spike protein binding to Angiotensin Converting Enzyme 2 (ACE2) on the host cell surface by heme and protoporphyrin IX produced from 5-ALA [27, 28]
- Reduction of expression of ACE2 by 5-ALA through heme synthesis and HO-1 induction [29]
- Inhibition of viral gene replication by binding of heme to guanine quadruplex structure in viral RNA [30, 31]

- Induction of HO-1 by 5-ALA leading to expression of anti-viral and anti-inflammatory activities [7]
- Enhancement of mitochondrial activity in immune cells such as T and B cells resulting in enhancement of their immune functions [32].

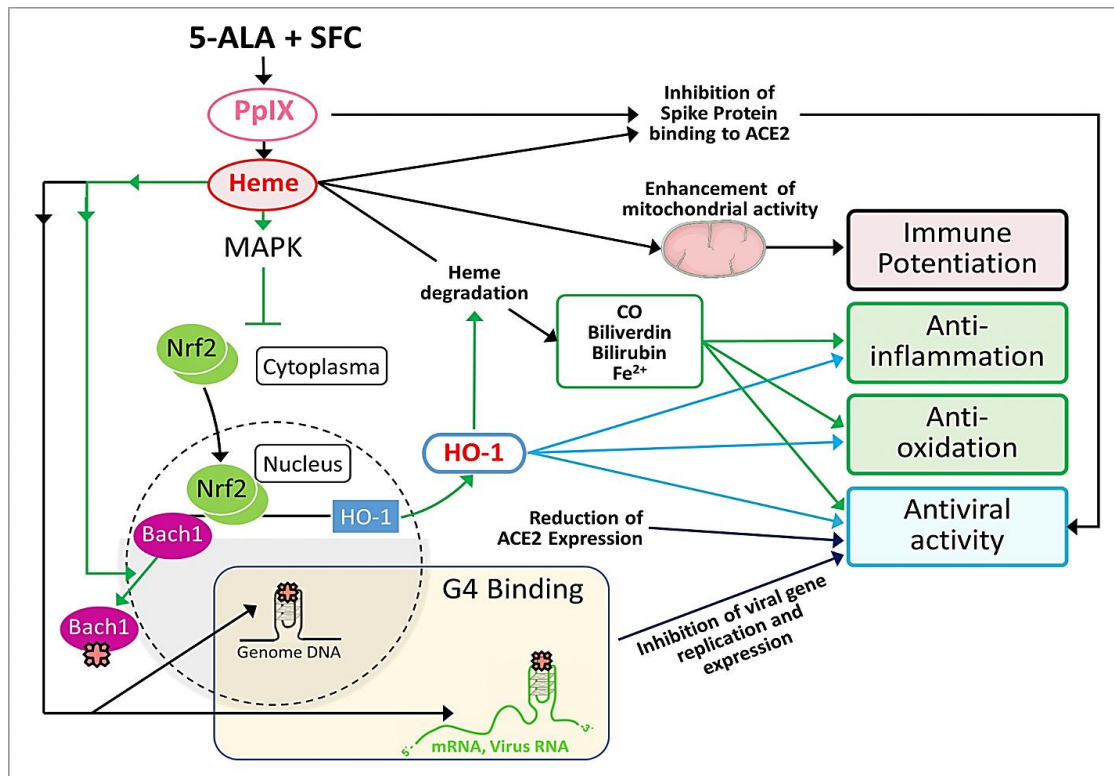


Figure 3. Mechanistic schematic diagram from the journal of the protective abilities of 5-ALA against COVID-19. The heme degeneration process including the inhibition of viral gene expression via binding of heme to guanine quadruplex (G4) was created based on a figure published by Kobayashi, 2021 [31]; refer to the publication for the original figure as well as for a detailed explanation of the process including all abbreviations used. <https://www.ffhdj.com/index.php/ffhd/article/view/838>

After assessment for eligibility at screening, the patients in the moderate group received five capsules of 5-ALA with SFC twice daily for the first week (500 mg 5-ALA and 286.8 mg SFC [30.4 mg as Fe]). From day 8 to day 28, the dose was reduced to five capsules once daily (250 mg 5-ALA and 143.4 mg SFC [15.2 mg as Fe]). Patients in the severe group received five capsules thrice daily for the first week (750 mg 5-ALA and 430.2 mg SFC [45.6 mg as Fe] daily). Subsequently, from day 8 to day 28, the dose was reduced to the same level as that administered to patients in the moderate group. Trained personnel on-site dispensed the appropriate number of capsules and ensured that patients swallowed these with

approximately 100 ml of room-temperature water during their hospital stay. For out-of-hospital administration, patients reported their daily intake by phone. During the course of the study, a clinical monitor diligently confirmed patient compliance while also overseeing the accountability of the study product. Furthermore, the clinical monitor ensured the meticulous adherence of patients to the administration protocols.

Statistical analysis: A formal sample size calculation was not performed as this was a pilot study to investigate the overall safety of the test substance when co-administered with SoC treatment and to identify any

| Parameter | Moderate group | | | Severe group | | | Significance ¹ |
|---|----------------|---|--------------|-----------------|----|---------------|---------------------------|
| | Mean | n | min; max | Mean | n | min; max | |
| ALT on Baseline Day 0 [IU/L] | 59.66 | 7 | 19.00; 90.00 | 40.33 | 12 | 15.10; 111.9 | 0.10 (U) |
| ALT at Follow-Up Day 28 (+10) [IU/L] | 34.14 | 5 | 20.00; 49.70 | 28.03 | 9 | 8.80; 60.30 | 0.59 (U) |
| p Value for Change from Baseline ² | 0.13(W) | | | 0.30 (W) | | | |
| COVID-19 Modified Ordinal Scale Baseline Day 0 | 3.29 | 7 | 3.00; 4.00 | 4.00 | 12 | 4.00; 4.00 | 0.001 (U) |
| COVID-19 Modified Ordinal Scale at Follow-Up Day 28 (+10) | 1.00 | 3 | 1.00; 1.00 | 1.00 | 8 | 1.00; 1.00 | >0.99 (U) |
| p Value for Change from Baseline ² | 0.25(W) | | | 0.01 (W) | | | |
| CRP on Baseline Day 0 [mg/L] | 41.50 | 7 | 2.66; 136.03 | 109.42 | 12 | 36.27; 270.45 | 0.04 (U) |
| CRP at Follow-Up Day 28 (+10) [mg/L] | 2.65 | 5 | 0.13; 4.99 | 5.41 | 9 | 0.25; 22.12 | 0.89 (U) |
| p Value for Change from Baseline ² | 0.1250(W) | | | 0.004(W) | | | |
| D-Dimer on Baseline Day 0 [mg/L (DDU)] | 0.69 | 5 | 0.27; 1.09 | 0.59 | 12 | 0.27; 1.53 | 0.49 (U) |
| D-Dimer at Follow-Up Day 28 (+10) [mg/L (DDU)] | 0.31 | 4 | 0.19; 0.46 | 0.44 | 6 | 0.27; 0.65 | 0.16 (U) |
| p Value for Change from Baseline ² | 0.50(W) | | | 0.81 (W) | | | |
| Procalcitonin on Baseline Day 0 [ng/mL] | 0.07 | 7 | 0.01; 0.13 | 0.87 | 12 | 0.01; 7.26 | 0.002 (U) |
| Procalcitonin at Follow-Up Day 28 (+10) [ng/mL] | 0.08 | 5 | 0.05; 0.15 | 0.07 | 9 | 0.05; 0.13 | 0.89 (U) |
| p Value for Change from admission ² | 0.81(W) | | | 0.004(W) | | | |
| IL6 on Baseline Day 0 [pg/mL] | 3.48 | 4 | 2.25; 4.17 | 20.07 | 10 | 2.42; 94.65 | 0.08 (U) |
| IL6 at Follow-Up Day 28 (+10) [pg/mL] | 3.72 | 4 | 1.5; 6.16 | 5.06 | 9 | 1.50; 10.58 | 0.70 (U) |
| p Value for Change from Baseline ² | 0.50(W) | | | 0.02 (W) | | | |
| CD4 on Baseline Day 0 [cells/ μ L] | 450 | 7 | 130; 750 | 250 | 12 | 140; 390 | 0.04 (U) |
| CD4 at Follow-Up Day 28 (+10) [cells/ μ L] | 840 | 5 | 590; 1070 | 880 | 8 | 580; 1260 | 0.94 (U) |
| p Value for Change from Baseline ² | 0.06(W) | | | 0.01 (W) | | | |
| CD8 on Baseline Day 0 [cells/ μ L] | 320 | 7 | 100; 700 | 190 | 12 | 80; 370 | 0.12 (U) |
| CD8 at Follow-Up Day 28 (+10) [cells/ μ L] | 662 | 5 | 460; 900 | 623 | 8 | 290; 1020 | 0.88 (U) |
| p Value for Change from Baseline ² | 0.06(W) | | | 0.01 (W) | | | |

¹ p-value was calculated using a two-sample t-test (T)/Man Whitney U (U) test between the moderate and severe group.

² p-value for change was calculated using the Wilcoxon signed rank test (W).

One patient in the severe group experienced mild constipation. The patient was not treated, and the symptoms resolved spontaneously. Two patients in the moderate group showed elevated liver values (ALT values up to 230 mg/L, evaluated as mild, and ALT values up to 248 mg/L, evaluated as moderate in severity; Supplementary Table S1) following 5-ALA-Phosphate + SFC treatment, which was stopped on day 7 or day 4, respectively.

Patients who participated in FU on day 28 (+10) showed no elevation in ALT levels. Rather, the values were 34.1 mg/L in the moderate group and 28.0 mg/L in the severe group, which was lower than that at baseline before the start of 5-ALA plus SFC administration (59.7 mg/L in the moderate group and 40.3 mg/L in the severe group; Table 1).

Results of parameters related to COVID-19 infection:

The COVID-19 modified ordinal score decreased from 3.29 to 1.00 in the moderate group ($p = 0.25$), and from 4 to 1.00 ($p = 0.01$) in the severe group. The infection-related biomarker CRP decreased from 41.50 mg/L to 2.65 mg/L in the moderate group and significantly from 109.42 mg/L to 5.41 mg/L ($p = 0.004$). Blood coagulation marker D-dimer levels did not differ between the groups. They decreased from 0.69 mg/L (DDU) to 0.31 mg/L (DDU) in the moderate group and from 0.59 mg/L (DDU) to 0.44 mg/L (DDU) in the severe group. The sepsis

marker procalcitonin showed no significant difference from baseline (0.07 ng/mL) to the FU visit (0.08 ng/mL) in the moderate group; however, in the severe group, the value decreased significantly from 0.87 ng/mL to 0.07 ng/mL ($p = 0.004$). IL-6 did not change in the moderate group from baseline (3.48 pg/mL) to the FU visit (3.72 pg/mL); however, the value decreased significantly from 20.07 ng/mL to 5.06 ng/mL in the severe group ($p = 0.02$). CD4 cells increased from 450 to 840 cells/ μ L in the moderate group and from 250 to 880 cells/ μ L in the severe group ($p = 0.01$). CD8 cells increased from 320 cells/ μ L to 662 cells/ μ L in the moderate group and from 190 cells/ μ L to 623 cells/ μ L in the severe group ($p = 0.01$).

Length of hospitalization compared with historical audit data:

Statistical analysis showed that the length of hospitalization in the severe group in our study (mean, 8 d) was significantly shorter than that in patients with severe symptoms which were included in an audit in the same hospital but treated with SoC only (mean, 16 d; $p < 0.01$ [Figure 4]). The decrease in CRP levels from screening/admission to discharge from the hospital was remarkably higher in the study patients (mean = -139.9; SD = 125.7; $n = 8$) in comparison to the patients from the hospital audit (mean = -108.6; SD = 75.3; $n = 7$). However, owing to the high standard deviation, this change was not statistically significant (Figure 5).

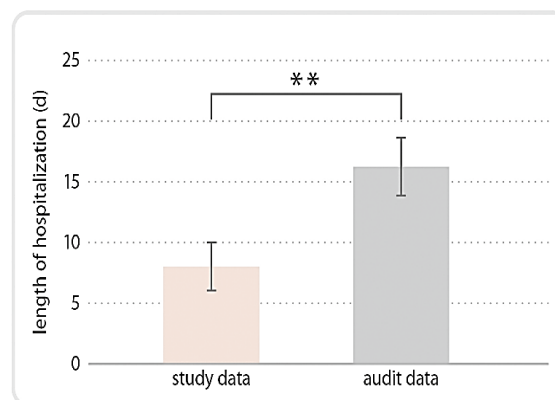


Figure 4. Length of hospital stay from study and audit data. Comparison of the length of hospitalization for study patients with severe COVID-19 symptoms that were included at Bahrain Defense Hospital with those at the same site and time frame in an audit ($n = 8$ in each group). Data are presented as the means \pm SD; $**p < 0.01$ was calculated using a two-sample t-test. The study patients received the same standard treatment against COVID-19 as the audit patients; however, it was received in addition to the study product 5-ALA with SFC.

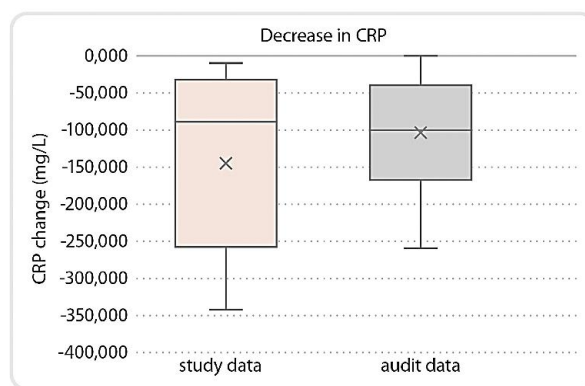


Figure 5. Change in CRP in study and audit patients. The change in CRP from screening/admission to discharge from the hospital for study patients with severe COVID-19 symptoms that were included at Bahrain Defense Hospital ($n = 8$) were compared with those for patients that were included at the same site and time frame in an audit ($n = 7$). The study patients received the standard treatment for COVID-19 and the study product 5-ALA with SFC, whereas the audit patients received the standard treatment only. Boxplot diagram; $p = 0.33$ (two-sample t-test).

DISCUSSION

Many medications currently on the market against COVID-19 and other virus infections have a range of side effects. Supporting such treatments with nutritional supplements that may shorten treatment time would not only benefit patients, but also the healthcare system by reducing costs through shorter hospital stays. A suitable candidate might be 5-ALA with SFC, which naturally occurs in the mitochondria of cells and can induce HO-1, a protein responsible for anti-inflammatory, anti-viral, anti-oxidative and immunological processes [6-19]. The aim of the current investigation was to conduct a pilot study on the safety and tolerability of 5-ALA with SFC when it is co-administered with various medications to patients with COVID-19 with moderate and severe symptoms.

The study was successful as no treatment-emergent adverse events (TEAEs) fulfilled the criteria for CTCAE grades III and IV, which was the primary endpoint of this study. Constipation of mild severity that resolved spontaneously was seen in one patient with severe COVID-19 disease. Two patients in the moderate group had elevated liver enzyme values after administration of 5-ALA with SFC. However, in patient XX08, AST and ALT levels were elevated from 26 and 24 mg/L to 63 and 90

mg/L, respectively, before co-administration of 5-ALA with SFC (Supplementary Table S1).

The treatment in hospital included up to 12 different medications per patient. For example, upon admission, patient XX08 received 1,000 mg paracetamol intravenously, a side effect of which is liver enzyme elevation.

Moreover, when remdesivir was used to treat COVID-19, AST/ALT levels increased significantly in four of five patients on the third day of therapy [33]. Patient XX01 was treated with remdesivir upon hospital admission, and ALT levels increased 3–5 d thereafter. In addition, the patient had a history of glucose-6-phosphate dehydrogenase deficiency, which might have interfered with the COVID-19 treatments received during hospitalization.

However, with the conservative safety approach in this study, ALT elevation was considered possibly related to 5-ALA with SFC; therefore, administration was stopped upon SoC treatment in both patients. Notably, such alterations in liver function tests were not observed in patients with severe COVID, even though these patients were administered a higher dose of 5-ALA with SFC. Furthermore, there were no alterations in liver function tests over the remaining 21-day administration period.

The product used in this study is currently on the market in Japan as ALAplus Body Active, a “food with function claim” product that has the same formulation and concentration per capsule; however, it is prescribed at a lower dose. Another marketed product is ALAplus GOLD X10 (same formulation as ALAplus Body Active), which is commercially available in Japan and Vietnam under the category “food.” 5-ALA phosphate has been categorized as food by Japanese authorities since 2013. Other brands with a recommended daily dose of 200 mg 5-ALA-phosphate, with different formulations and concentrations per capsule, are registered in the United Arab Emirates, Kenya, Oman, Cambodia, Mauritius, and Myanmar. No spontaneously reported AEs related to 5-ALA phosphate were observed over the 10-y period.

The safety of the product has been demonstrated in several clinical studies on hyperglycemic subjects and patients with type 2 diabetes mellitus. Exposure ranged from 1 d to 12 weeks, and the doses administered ranged from 5 to 1,500 mg of 5-ALA and 2.87 mg to 2,351 mg SFC [34-37]. However, safety information for COVID-19 is limited, especially for patients with moderate-to-severe symptoms.

Our open-label, interventional, and exploratory pilot study confirmed the results of previous studies for COVID-19 indication, even when the product was co-administered with various drugs primarily prescribed for acute treatment (e.g., remdesivir, corticosteroids, paracetamol, and vitamin D). Furthermore, our results indicated long-term safety of 5-ALA with SFC at significantly higher concentrations than when typically used as a food or food supplement (750 mg 5-ALA and 430.2 mg SFC daily for 7 d, and 250 mg 5-ALA and 143.4 mg SFC once daily for the next 21 d). No other studies with a standardized dose have been conducted for this indication to date. In the case reports of six patients with severe symptoms of COVID-19 published by Kaketani and Nkajima in 2021, the doses differed significantly day by

day, ranging from 100 to 2,250 mg of 5-ALA per day [26]. Our safety results for the fixed-dose regimen will be valuable for planning future studies with patients exhibiting moderate-to-severe symptoms of COVID-19 and other severe infections, such as influenza, which is reasonable considering the mode of action of 5-ALA with SFC (Figure 1 in the introduction).

The secondary objective of this study was to detect signals indicating the supportive potential of 5-ALA with SFC when co-administered with the SoC. One of the endpoints was the duration of hospitalization, which was 7.4 d shorter in the patient group with severe symptoms of COVID-19 than the historical data from a hospital audit, where the mean length of 16.3 d was reported. Although the audit data were not part of our study, they were suitable for comparison because they were collected at one of the sites concurrently to conducting the study. The principal investigator at the site and the co-author of this article confirmed that these patients received the same SoC as those with severe symptoms included in the study. Therefore, these data were suitable for evaluating the duration of hospitalization compared to the data in literature.

As described in the Introduction, the administration of 5-ALA with SFC leads to a strong induction of HO-1 expression, which is a key regulator of antiviral, anti-inflammatory, antioxidant, and immunological processes.

Support of antiviral processes: HO-1-dependent molecular mechanisms inhibit the replication of RNA and DNA viruses. The supportive role of HO-1 in antiviral processes has been reported for several viruses, such as HIV, Zika, Ebola, dengue, herpes simplex, and hepatitis B [9-15]. Thus, it can be assumed that this mechanism also works for other viruses such as SARS-CoV-2 and influenza and that the administration of 5-ALA with SFC supports this via HO-1 activation. A possible mode of action is that

5-ALA with SFC directly and indirectly suppresses ACE2 expression via HO-1 in host cells, which may reduce the binding of SARS-CoV-2 [38]. The therapeutic potential of 5-ALA with SFC for viral diseases is highlighted here [39]. Another mode of action was mentioned for the anti-viral activity of COVID-19. Porphyrins, the metabolic products of 5-ALA, bind guanine quadruplexes, which are a part of the SARS-CoV-2 genome [40] and may therefore reduce viral replication. Moreover, *in vitro* experiments with different SARS-CoV-2 strains showed that even low concentrations of 5-ALA with SFC inhibited replication of the Wuhan, alpha, and delta strains. Higher concentrations were effective against the beta and gamma strains [41].

Support of anti-oxidant processes: HO-1 degrades heme into the potent antioxidants, biliverdin/bilirubin, CO, and Fe [7, 17-19]. This could serve as a plausible explanation for the significant reduction in inflammatory markers, including CRP, procalcitonin, and IL-6, observed in patients initially experiencing severe symptoms. Similar findings have been reported in case reports where individuals with COVID-19 received elevated doses of 5-ALA with SFC, particularly demonstrating the effect on CRP [26]. Sharif-Askari et al. [42] examined metabolites in the saliva and blood plasma of patients with COVID-19 using different severity grades. They detected a strong negative correlation between the CRP and 5-ALA levels. This suggested that substitution with 5-ALA leads to a decrease in CRP. This negative correlation was highly significant in the severe symptom group, which is consistent with the results of our study (see above). Moreover, the decrease in the CRP level from screening/admission to discharge from the hospital was markedly higher for study patients with severe symptoms who were included at Bahrain Defense Hospital than those included at the same site and time frame in an audit. Although the difference was not statistically

significant, it can be evaluated as a signal that confirms the hypothesis that the food supplement 5-ALA with SFC positively supports treatment with standard drugs against COVID-19 to enable faster recovery.

Similar functional foods and bioactive compounds against COVID-19:

The overarching goal of the pilot study was to evaluate whether further development of 5-ALA with SFC as a functional food product for the indication of COVID-19 is desirable. The study results, although based on a very small number of patients, lead to a positive answer to this question. Many other similar functional foods and bioactive compounds are found to be supportive for the treatment of COVID-19 and other infections like influenza. A comprehensive overview including the assumed mechanisms provides recently published review articles [43-46]. Farzana et al. listed seven different vitamins (vitamins A, B6, B12, C, D, E, and Folate) found in different foods that were identified to have beneficial properties for the treatment of COVID-19 [43]. Haslberger et al. presented a table with plant extracts (quercetin e.g., from onions, capers, chives; curcumin from turmeric; epigallocatechin gallate from green tea; etc.) for which study results show at least a potential for supportive effectiveness against different viruses [46]. It would be interesting to conduct studies with our product and such substances administered as comparator to test superiority or administered together with 5-ALA with SFC to evaluate whether it makes sense to develop combination products.

CONCLUSIONS

This pilot study aimed to demonstrate the safety of 5-ALA phosphate with SFC when administered together with SoC in patients with moderate and severe COVID-19 symptoms. The major limitation of this study was the small sample size, which appeared to be sufficient to

demonstrate that 5-ALA administration is safe and tolerable. The statistical power was too low to conduct relevant outcome hypothesis tests for the secondary outcomes. Moreover, the comparison group was not initially included in the study design and therefore, not all data required for the study were recorded, which limits the data quality and comparability. Nevertheless, the good safety results, the suggestion that hospital stay was shortened and the infection marker, CRP, was markedly decreased in comparison to the historical audit data, permit the start of a research program for 5-ALA with SFC on this or comparable indications, such as influenza. The excellent safety profile together with its supportive properties in antiviral, antioxidant, and anti-inflammatory processes and the low cost, promise a wide range of applications for the food supplement 5-ALA with SFC.

For future studies, the statistical power should be increased by inclusion of at least 30 patients in each group and should include a placebo group and further dosage groups. Additionally, a longer application period of up to 12 weeks would be highly recommended. This would help to determine the optimum time of consumption and dosage of the product.

Abbreviations: (m)ITT: (modified) intention-to-treat; (S)AE:(serious) adverse event; 5-ALA: 5-aminolevulinic acid phosphate; ALT: alanine aminotransferase; AST: aspartate aminotransferase; BDF: Bahrain Defense Hospital; CD4: Cluster of Differentiation 4; CD8: Cluster of Differentiation 8; CO: carbon monoxide; COVID-19, corona virus disease of 2019; CRP: C-reactive protein; CT, computed technology; CTCAE: common toxicity criteria for adverse events; CV, coefficient of variation; DDU: D-dimer units; ECMO: extracorporeal membrane oxygenation; Fe: Iron; FiO₂: fraction of inspired oxygen; FU: follow-up; HBV: hepatitis B virus; HCV: hepatitis C

virus; HO-1: heme oxygenase-1; IL-6: interleukin 6; ISO: International Organization for Standardization; PaO₂: arterial partial pressure of oxygen; qSOFA: quick Sequential Organ Failure Assessment; R&D: research and development; RNA: ribonucleic acid; (SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; SFC: sodium ferrous citrate; SoC: standard of care; SpO₂: saturation of peripheral oxygen; TEAE: treatment emerged adverse events; ULN, upper limit of normal; WHO: World Health Organization.

Competing Interests: Andrea Ebeling, Riyadh Rehani, Marcus Stocker, and Norbert Berenzen are employees of photonamic GmbH and Co. KG. However, none of these authors have further financial arrangements (e.g., consultancies, stock ownership, equity interests, patent-licensing arrangements, research support, or major honorary). Motowo Nakajima is an employee of SBI Holdings and a shareholder of SBI ALA Pharma Japan; the study product was provided by SBI Pharmaceuticals, which is part of SBI Holdings. Walter Stummer is a consultant for SBI ALA Pharma and receives fees for consultancy. The remaining authors declare no conflicts of interest.

Author Contributions: Motowo Nakajima, Andrea Ebeling, Riyadh Rehani, Walter Stummer, Marcus Stocker, Norbert Berenzen, and Stephen Atkin designed and supervised the study. Abdulla Darwish and Abdulrahman Almadani were investigators at BDF Hospital, Jameela Alsalman was an investigator at Salmaniya Medical Complex. Mariam Murad was responsible for project administration. Riyadh Rehani, Stephen Atkin, and Norbert Berenzen wrote the manuscript. Norbert Berenzen was primarily responsible for final content and editing. All the authors have read and approved the final version of this manuscript.

Acknowledgements and Funding: This study was funded by photonamic GmbH and Co. KG, Pinneberg, Germany. We thank Ph. Yaser Sakheer Alameeri, Head of Medical Supplies and Directorate of Maintenance & Material Supplies at the Bahrain Defense Force Royal Medical Services, Riffa, Kingdom of Bahrain, for his support with the import, storage, and supply of the study product to sites. We also thank Aslam Mohamed Sadiq for clinical monitoring and ensuring patient safety and data integrity. Moreover, we thank Vedic Lifesciences for the statistical analysis and evaluation of the study results as well as Edi Berentzen for designing and artistic realization of the figures included. The study products were provided by SBI Pharmaceuticals Co. Ltd. (Tokyo, Japan). We would like to thank Editage (www.editage.com) for English language editing.

Ethical Approval: This open-label, interventional, and exploratory pilot study was approved by the ethical committees of the Bahrain Defense Hospital, National Health Regulatory Authority and COVID-19 Committee of Bahrain. This study was conducted following good clinical practice and in accordance with the guidelines of the Declaration of Helsinki. All patients provided written informed consent. Data integrity and quality were regularly checked by a clinical monitor during on-site monitoring visits.

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