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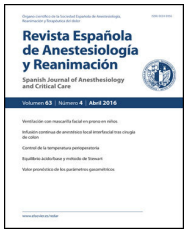

SPECIAL ARTICLE

# TWO KNOWN THERAPIES COULD BE USEFUL AS ADJUVANT THERAPY IN CRITICAL PATIENTS INFECTED BY COVID-19

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**Abstract**

Pneumonia caused by coronavirus, which originated in Wuhan, China, in late 2019, has been spread around the world becoming a pandemic. Unfortunately, there is not yet a specific vaccine or effective antiviral drug for treating Covid-19. Many of these patients deteriorate rapidly and require intubation and are mechanically ventilated, which is causing the collapse of the health system in many countries due to lack of ventilators and intensive care beds. In this document we review two simple adjuvant therapies to administer, without side effects, and low cost that could be useful for the treatment of acute severe coronavirus infection associated with acute respiratory syndrome (SARS-CoV-2). Vitamin C, a potent antioxidant, has emerged as a relevant therapy due to its potential benefits when administered intravenous (IV). The potential effect of vitamin C in reducing inflammation in the lungs could play a key role in lung injury caused by coronavirus infection. Another potential effective therapy is Ozone, It has been extensively studied and used for many years and its effectiveness has been demonstrated so far in multiples studies. Nevertheless, our goal is not to make an exhaustive review of these therapies but spread the beneficial effects themselves. Obviously clinical trials are necessaries but due to the potential benefit of these two therapies we highly recommend adding to the therapeutic arsenal.



## Introduction

The SARS virus belongs to the viral family *Coronaviridae*. which includes two genera, coronavirus and togavirus, each showing similar replication mechanisms and genomic organization but distinct genome lengths and viral architecture. First identified in the 60's, this family identifies itself by large, enveloped, positive-stranded RNA virions. Their appearance is characteristically distinct, with envelopes endowed with host cell membrane-tropic petal shaped spikes (peplomers). The large, amply spaced peplomers on the virion surface suggests a coronal (crown-like) appearance.

Once cell entry is achieved, the virion sheds its envelope to commence its replication in the host cell cytoplasm. It binds to cellular ribosomes and released viral polymerase begins the RNA replication cycle. Newly formed nucleocapsids continue their assembly with the acquisition of new envelopes by means of budding through membranes of the cell's endoplasmic reticulum. Virions are then

released into the general blood and lymphatic circulation, ready to infect new cells, other organ systems, and new hosts. The syndrome progresses to severe disease with respiratory distress and oxygen desaturation requiring ventilatory support in over a third of patients, approximately 8 days after symptom onset. Mortality has been noted to vary according to transmission clusters, ranging from 3 to 20%. This suggests that the etiology of SARS depends upon a heterogenous population of viral quasispecies with variable degrees of virulence.

**Features of severe systemic inflammatory insults**

***Clinical features***

The initial symptoms are usually fever, generally high, chills, headaches, myalgias and dry cough, which may progress to shortness of breath, appearing dyspnea and respiratory distress. Respiratory deterioration is fast, requiring intubation in the first 48 hours after the onset of respiratory symptoms.

***Laboratory hallmarks***

In the early stage of the disease, the total number of white blood cells in the peripheral blood is normal or decreased, and the lymphocyte count reduced. Some patients may have abnormal liver function, and the levels of lactate dehydrogenase, muscle enzyme, and myoglobin increased; Most patients had elevated CRP and ESR levels and normal procalcitonin levels. In severe cases, D-dimer levels are elevated, other coagulation indicators are abnormal, lactic acid levels are elevated, peripheral blood lymphocytes and CD4 + T lymphocytes are progressively reduced, and electrolyte disorders and acid-base imbalances are caused by metabolic alkalosis. Elevated levels of inflammatory cytokines (such as IL-6, IL-8, etc.) can occur during the disease progression stage.

***Radiology examination;*** The early CT examination show multiple small patches or ground glass shadows. A few days later, the lesions increase showing extensive lungs, multiple ground glass shadows, or infiltrating lesions, some of which showed consolidation of the lungs, often with bronchial inflation signs, and pleural effusions are rare. A small number of patients progressed rapidly, with imaging changes reaching a peak on days 7 to 10 of the course. Typical "white lung" performance is rare. After entering the recovery period, the lesions are reduced, the exudative lesions are absorbed, and some patients' lesions can be completely absorbed.

**Treatment**

Multiples therapeutics scheme have been used but still not successfully. Different combinations as hydroxychloroquine sulfate or chloroquine phosphate with Azithromycin seems to work on

coronavirus. Interferon atomization and inhalation, (interferon κ is preferred) and Tocilizumab a humanized antihuman IL-6 receptor antibody are being used  A cocktail of antiviral drugs is usually being administered in most of the patients, but the efficacy of all antiviral drugs remains to be evaluated in further clinical studies. As a result, we are still doing a support therapy in these patients because not a curative treatment has been found yet.

## POTENTIAL ADJUVANT THERAPIES

### I. INTRAVENOUS VITAMIN C

#### *Introduction*

Vitamin C (ascorbic acid or ascorbate), is a water-soluble vitamin which play a role as a cofactor in many enzymatic reactions, that mediate a variety of essential biological functions. It is considered a powerful antioxidant with antimicrobial properties. Linus Pauling, biochemist and Nobel Prize winner claimed that vitamin C has beneficial effects on cardiovascular health, improves the body's immune function to overcome infections and even aids treatment against cancer [1].

The severity of SARS-CoV-2 infection is being found in lung deterioration. Although the reason for this rapid deterioration remains to be elucidated, we believe that its clinical course is similar to macrophage activation syndrome, a form of secondary hemophagocytic lymphohistiocytosis, which cause hypersecretion of proinflammatory cytokines that damage the lungs, hence the IV administration of vitamin C may be effective given its anti-inflammatory activity [2].

#### *Pharmacokinetics*

Vitamin C exerts most of its biological functions intracellularly and is acquired by cells with the participation of specific membrane transporters The absorption, distribution and retainment of vitamin C is primarily governed by the family of saturable sodium-dependent vitamin C transporters (SVCT). The diverse expression and concentration dependency of these transporters throughout the body has resulted in the highly complex, compartmentalized and non-linear pharmacokinetics of vitamin C at physiological levels. However, the pharmacokinetics of vitamin C appear to change from zero to first order, displaying a constant and dose-independent half-life when administered IV infusion. Following a dose, Vitamin C circulate in the plasma, is freely filtered by the renal glomerulus, and reabsorbed in the proximal tubule through the first sodium-dependent vitamin C transporter (SVCT1). While SVCT1 regulates vitamin C homeostasis throughout the body, a high-affinity, low-capacity sodium-dependent vitamin C transporter SVCT2 protects metabolically active cells against oxidative stress, facilitating accumulation of vitamin C where it is needed [3]. On the other hand, dehydroascorbic acid (the oxidized form of vitamin C) is transported

via glucose transporters family (GLUT) where it is reduced to avoid the irreversible decomposition. In situations like sepsis, there is a diminished absorption into the cells by increased release of cytokines.

***Biological effects***

Vitamin C is an electron donor and therefore a reducing agent. All known physiological and biochemical actions of vitamin C are due to its action as an electron donor. Vitamin C has immunostimulatory effects, antioxidant properties and possible antimutagenic effects [4,5]. Vitamin C has been shown to enhance neutrophil chemotaxis, phagocytosis, and therefore microbial clearance [6,7]. In addition, it promotes the proliferation of T cells and natural killer cells modulating their functions [8]. Vitamin C is also necessary for the catecholamine synthesis (formation of epinephrine from dopamine by dopamine beta-hydroxylase enzyme [9,10], and adrenal steroidogenesis [11]. Vitamin C improves the synthesis of norepinephrine both by recycling tetrahydrobiopterin, a critical cofactor in catecholamine synthesis, and by increasing tyrosine hydroxylase expression [12]. Furthermore, it is also a cofactor for peptidyl-glycine alpha-amidating monooxygenase that is required for endogenous synthesis of vasopressin [13]. One study in cardiac surgical patients has suggested that preoperative administration of Vitamin C mitigates the adrenal suppression induced by the anesthetic Etomidate [14]. Thus, there has been significant interest in using vitamin C for the management of hemodynamically unstable patients because Vitamin C-dependent synthesis of the vasopressors norepinephrine and vasopressin may play an important role in supporting cardiovascular function during severe infections and septic shock [15]. In a recent article Nabzdyk et al. has reviewed the use of vitamin C in critical care management and its biological effects [16].

***Experience with Critical Burns Patients***

Traditionally, vitamin C has been used with burn patients. Increased capillary leakage, with an important extravasation of fluid and proteins, and free radical generation are feature of burn injuries. Free radicals have emerged as important mediators for burn injury at the cellular level. Continuous vitamin C infusion appears to be a useful adjunct in minimizing the effects of free radical injury and reduces fluid resuscitation requirements among burn patients [17,18]. High doses of vitamin C appear to improve microvascular barrier dysfunction, without affecting leukocytes activation [19]. In a study with dogs suffering burn injuries, the administration of vitamin C (14 mg/kg/hour) decreased lipid peroxidation, and microvascular protein and fluids [20]. A randomized, double-blinded study in sheep demonstrated a significant reduction in net fluid balance and plasma lipid peroxidation among sheep sustaining a 40% total body surface area (TBSA) burned

who were resuscitated with fluid in conjunction with a high-dose infusion of ascorbic acid [21]. Another randomized, prospective study in this case in burn patients with greater than 30% TBSA burn, investigators found that administration of vitamin C (1,584 mg/kg/day) was well tolerated, and reduced fluid volume requirements along with an overall improvement in pulmonary function, demonstrated by a significant reduction in mechanical ventilation days [22].

***Sepsis***

Recently there has been an increase in interest with the use of vitamin C as an adjuvant treatment for sepsis. This was due to the results of the study by Marik et al [23] in which they administered a cocktail of vitamin C (1.5 g IV every 6 h), hydrocortisone (50 mg IV every 6 h) and thiamine (200 mg IV every 12 h) to 47 with sepsis admitted to the ICU. Patients treated with this regimen had an absolute reduction of 30% in mortality despite similar comorbidities and mortality risk prior to treatment. Currently, there are multiple ongoing randomized controlled trials including VICTAS, ACTS and HYVCTTSSS, which aim to confirm the beneficial effects of vitamin C and supplements in critically ill patients with sepsis [24-26].

***Pneumonia and Acute Respiratory Syndrome (ARDS)***

ARDS is usually accompanied by uncontrolled inflammation, oxidative injury, and the damage to the alveolar-capillary barrier. Unfortunately, there are very few studies in critically ill patients with ARDS who have reported the use of IV vitamin C as adjuvant therapy. In animal studies, Vitamin C has been shown to increase resistance to infection caused by coronavirus, also modifying the susceptibility to the infection [27]. In patients, Nathens et al. administered 1 gram of ascorbic acid every 8 hours combined with oral vitamin E for 28 days in 594 surgically critical patients and found an instances of significantly less acute lung injury and multiple organ failure [28]. In a clinical study described by Sawyer et al., large IV doses of ascorbic acid and others were used antioxidants (tocopherol, N-acetylcysteine and selenium), in patients with established ARDS and showed a 50% reduction in mortality [29]. Bharara et al, administered 50 mg/kg each 6 hours for 96 hours to treat recurrent ARDS with good results and no effect secondary [30]. Fowler et al. described the case of a 20-year-old woman with viral ARDS (Rhinovirus and enterovirus D68) who received IV vitamin C successfully [31]. In another study IV vitamin C was administered in patients with severe pneumonia, the treated patients had significantly less hospital mortality [32]. Prior preclinical and subsequent clinical research performed at Virginia Commonwealth University (VCU) have revealed that high plasma levels of vitamin C act in a “pleiotropic” fashion to attenuate systemic inflammation and correct sepsis-induced coagulation abnormalities, while simultaneously attenuating vascular injury [CITRIS-ALI; clinicaltrial identifier NCT02106975]. These critically ill

patients often have reduced concentration of antioxidants. Therefore a positive effect of vitamin C can be expected. IV vitamin C is already being employed in China against COVID-19 coronavirus. Peng Zhiyong Director of Intensive Care at Zhongnan Hospital of Wuhan University has registered a clinical trial to verify the efficacy of IV vitamin C in the treatment of severe 2019-nCoV infected pneumonia [identifier: NCT04264533], patients has receiving 24 g of IV vitamin C per day for 7 days, and the Shanghai Coronavirus Disease Clinical Treatment Expert Group has strongly recommended including high daily doses of vitamin C in critically ill patients affected by SARS-CoV-2, due to it is use appears to achieve a significant improvement in the oxygenation index [33]. Other hospitals are giving already IV vitamin C and Zinc (220 mg oral) in the treatment protocol plus azithromycin and hydroxychloroquine.

**Administration Protocol** (Table 1)

- Central venous catheter is preferred for administration, overall for high doses but peripheral access is acceptable but infusion has to be slower.
- Obtain previously: blood count, renal function, electrolytes, iron, ferritin and G6PD (glucose-6-phosphate dehydrogenase). Hemolysis can occur in patients with G6PD deficiency. Measurement of the serum ferritin level may be a useful indicator of therapy response, and prognosis.
- It is preferred to administer in its salt form; sodium ascorbate. The dose varies from 0.1 to 1 g/kg. Our recommendation is to start with a dose of 0.2 g/kg of vitamin C diluted in 250 or 500 mL of water solution sterile for injection or, alternatively in Plasmalyte or Ringer's Lactate. The solution bag should be covered with a black bag to prevent light-induced auto-oxidation.
- Osmolarity will depend on the administered dose. Doses of 100 g should be diluted in 1 L for infusion since the mOsm /L will thus be 1085 mOsm /L.
- Infusion rate; around 0.25-0.5 g/min, for example; 15 grams in 30 minutes, 25 grams in 1 hour, 50 grams in 2 hours, 75 grams in 2 hours and a half hours, and 100 grams in 4 hours.
- Monitor calcium and magnesium since the chelating effect of vitamin C can cause hypocalcemia and hypomagnesemia. Correct if develops.
- Frequency of administration: It will depend on the severity and response of the patient to treatment. We recommend starting daily and rest on the weekend. If very sick dosing could be twice daily.
- During rest days, it is recommended to administer by nasogastric tube, 4 to 6 grams per day of vitamin C.

- Vitamin C crosses the placenta and is distributed into breast milk so high doses of vitamin C is contraindicated in pregnancy.
- Cautions must be taken in patients with renal failure (creatinine >175 μmol/L (1,98 mg/dL).

**Table 1. Administration protocol**

| INTRAVENOUS VITAMIN C PROTOCOL FOR COVID-19 INFECTION |
|---|
| • Central venous access preferible for very high doses (> 50 g) |
| • Check: blood count, renal function*[1], electrolytes, and G6PD |
| • Check IL-6, ferritin levels may be a useful indicator of therapy response and prognosis |
| • Use sterile water, Plasmalyte or Lactated Ringer's for mixture or Dextrose 5%-10% |
| • Doses: 0.2-0.5 g/kg vitamin C*[2] |
| • Administer daily until improvement, then every 2 days |
| • Infusion rate: adjust for 0.25-0.5 g/min (usually between 1 to 4 h according to the dose) |
| • Supplement with calcium and/or magnesium IV if necessary |
| • If possible add Zinc sulfate 220 mg/24h *[3], thiamine (400 mg/d), vitamin D 1000-3000 IU/24h, vitamin E 1600 IU/48 h oral/NG |

*[1] caution with doses and frequency of administration

*[2] if patient in critical condition we suggest administration dose recommended twice a day (every 12 hours)

*[3] 220 mg of zinc sulfate contains 50 mg of elemental zinc.

**Conclusion**

Vitamin C may be an effective therapy in the treatment of SARS-CoV-2 given its antioxidant and immune system enhancement, and antiviral properties. Furthermore, vitamin C can also help to eliminate alveolar fluid accumulate in the ARDS by preventing the activation and accumulation of neutrophils, and reducing alveolar epithelial water channel damage. Given the fact that its administration is safe by IV infusion to maximum doses of 1.5g/kg as long as the described precautions are taken, healthcare professionals should take a close look at this opportunity. Obviously, well-designed clinical studies are absolutely needed to develop standard protocols for bedside use.

**OZONOTERAPIA:**

***Introduction***

There is much scientific evidence on the clinical use of ozone, however ozone therapy has not yet been fully accepted. We cannot forget that ozone is a gas made up of three oxygen atoms of oxygen ($O_3$). Ozone generators produces it from pure oxygen passing through a high voltage gradient (5–13 mV) according to the reaction:

$$3O_2 + 68.400 \text{ cal} \longrightarrow 2O_3.$$

Ozone therapy (OT) utilizes 1-5% ozone in 95-99% oxygen as a gas. A medical ozone generator produces concentrations of ozone from 1 to 100 μg/mL but for medical purposes concentrations of 10 to 40 μg /mL are commonly used. Ozone therapy is characterized by the simplicity of its application, its great effectiveness, good tolerance, and by the virtual absence of side effects.

Russia, Cuba, Germany have recognized it in their legislation; In Spain it is regulated in more than 76% of the autonomous regions of Spain. Italy, China, and some South America have specific regulations

The effectiveness of ozone against pathogens is well recognized and ozone appears to be the best agent for sterilization of water. Due to its biological properties ozone therapy may play a possible role in the therapy of SARS, as an adjunct to standard treatment regimens. Owing to the excess energy contained within the $O_3$ molecule, it is theoretically likely that $O_3$, unlike organism-specific antiviral options available today, will show effectiveness across the entire genotype and subtype spectrum of SARS [34].

***Pharmacokinetics***

Ozone physically dissolves in pure water according to Henry's law regarding temperature, pressure and ozone concentration. Unlike oxygen, ozone reacts immediately as soon as it dissolves in any biological fluid. In the body, it has a half-life of milliseconds due to its high affinity for carbon double bonds. Ozone reacts rapidly with biomolecules possessing this double bond causing a rapid oxidation or breakdown reaction with consequent formation of secondary molecules which are the causes of its therapeutic action. The therapeutic efficacy of ozone therapy may be partly due the controlled and moderate oxidative stress produced by the reactions of ozone with several biological components.

***Biological Effects***

When blood is treated with ozone, ozone instantly reacts with electron rich bonds and creates longer living downstream metabolites called ozonides: reactive oxygen species and lipid oxidation products, inclusive of peroxides. These molecules appear to act as messengers for the key biochemical and immune modulating effects of the therapy.

Some viruses are more susceptible to ozone's action than others. It has been found that lipid-enveloped viruses are the most sensitive and coronavirus is on of them. Coronavirus is rich in cysteine, which residues must be intact for viral activity [35]. Cysteine is highly vulnerable to oxidation to disulfide or other residues, which effect will cripple its biochemical activity in proteins, altering their three-dimensional structure. Enzymes may become inactive when reduced thiols are oxidized, sulfhydryl groups are vulnerable to oxidation and ozone is one of the molecules with the highest oxidizing potential, capable of reacting with a large of organic and inorganic compounds [36].

The effects of ozone administered in infected blood for virus may recruit a variety of mechanisms.; ozone disrupts viral proteins, lipoproteins, lipids, glycolipids, or glycoproteins. Ozone proper, and the peroxide compounds it creates, may alter structures on the viral envelope that are necessary for attachment to host cells. Peroxides created by ozone administration show long-term antiviral effects that may serve to further reduce viral load. Deprived of an envelope, virions cannot sustain nor replicate themselves. The creation of dysfunctional viruses by ozone offers unique therapeutic possibilities. In leukocytes, ozone can enhance phagocytic activity of neutrophils. Within monocytes and lymphocytes, peroxide hydrogen obtained by ozone activates a tyrosine kinase with the consequent phosphorylation of the IκB kinase, one of the resting trimeric components of the nuclear factor kappa-B (NF-κB) with the consequent synthesis of different proteins [37]. NF-κB plays a key role in regulating the immune response due to infection [38,39]. Of great importance is also that ozone-induced also release of cytokines (IFNγ, TNFα and IL-8 and IL-2) and several acute phase proteins [40], which may constitute an avenue for the reduction of circulating virions and an attenuation of lung inflammation.

***Major Autohemotherapy***

Major autohemotherapy (MAH) consists of drawing blood from the venous system, normally between 50 and 225 mL, which is mixed with a volume of oxygen-ozone at concentrations of 10 to 40 μg/mL. It is then reinfused into the body intravenously to cause its effects. Administration must

be done very slowly for it to allow for rapid dissolution. The ways to express concentration and dose in ozone therapy are shown below and Table 2:

Concentration: µg / mL; (MAH) = µg/mL /mL of blood

Dose: Ozone concentration by volume (µg/mL x mL = µg $O_3$)

The total dose is simply calculated by multiplying the ozone concentration with the gas volume. As an example, if we ozonate a blood volume of 150 mL with 150 mL of gas (1:1 ratio) with an ozone concentration of 30 µg/mL, the total dose is equivalent to 4.5 mg of ozone.

**Table 2. Concentration and doses of ozone for MAH**

| | **Low** | **Medium** | **High** |
|---|---|---|---|
| **concentration*[1] (µg/mL)** | 5-10 | 15-35 | 40-70 |
| **Dose (mg)** | 0,25-1,0 | 1,12-3,5 | 4-8,75 |

***1** Ozone concentrations for systemic uses range from 10 µg/mL to 40 µg/mL, concentrations higher than 80 µg/mL, due to the increased risk of hemolysis, the reduction of 2.3 DPG should be avoided, and antioxidant values with consequent inability to activate cells immunocompetent.

The side effects that can be observed are minimal. It should be noted that given their beneficial effects may require adjustment of adjuvant medication, e.g., medication antidiabetic at lower doses, or antihypertensive medication at lower doses.

***Critical Care experience***

There are multiples articles about ozono therapy and its effectiveness against virus but it is not the aim to make a full review of the literature. Cespedes et al. treated patients with chronic hepatitis B for one year with MAH**,** patients showed negativization of the surface antigen, antibody positivity against the surface antigen, significant decrease of viral load to undetectable values and normal values of the transaminases demonstrating the functional recovery of the disease associated with favorable immunological response [41]. They also treated patients with HIV-AIDS for two years and it was a significant decrease in viral load to undetectable values and an increase of CD4 and CD8 [42].

When you administered ozone in blood, it will be an improvement of the oxygenation in vital organs and in ischemic areas, in addition to supporting respiratory, cardiac and kidney. If the patient's metabolic conditions do not deteriorate excessively, in 3-4 days of treatments with

ozonated autohemotherapy, increased enzyme synthesis antioxidants and hemoxygenase-1 induction can reduce oxidative stress caused simultaneously due to infection-inflammation-tissue necrosis and dysmetabolism. Bocci et al, reported a patient that in the postoperative period of an aortic dissection, development ARDS and was treated with ECMO, he reported improved after administration of MAH for 3 days, starting therapy at dose of 40 μg/mL with successive concentrations of 25 μg/mL in the following days [43].

***Treatment frequency:***

The number of treatment sessions and the ozone dose administered will depend on the general condition of the patient, age and the main disease. The treatment can be administered daily if necessary.

**Administration Protocol (Table 3)**

Venous access is required, a butterfly at least number 19 G, is preferred rather than a cannula. The MAH can be applied with variable intervals, from daily to weekly and even monthly. The total dose of ozone to be applied in each session will vary according to disease. Some groups advise that the concentration of ozone in the $O_3/O_2$ gas mixture does not exceed 80 μg/mL due to risk of hemolysis. Blood volume at use varies between 50 mL and 225 mL, since higher blood volumes can only cause hemodynamic changes so no higher volumes are required. For example, for collection safe blood we can apply a volume of 1.2 mL/kg to 1.3 mL/kg, so that for a 70 kg person should draw 84 mL of blood (1.2 x 70 = 84). The gas volume must always add in a 1: 1 volume ratio. We recommend using low doses when starting, with an initial concentration of 10-25 μg/mL per mL of blood, mix the gas with the blood for 1-5 minutes to prevent foaming is enough to complete the reaction to ozone before reinfusion of ozonated blood into the donor that can be reinfuse in about 10-15 minutes. In some cases, the use of up to 80 μg/mL which has been shown to be safe and with greater induction capacity of cytokines.

For anticoagulation use heparin sodium 20 IU/mL of blood. Sodium citrate 3.8% 10 mL per 100 mL of blood or failing citrate dextrose Solution A, USP (2.13% free citrate ion) from 7 mL -10 mL per 100 mL of blood are other alternatives. Frequency of application recommended is daily with up to 3 passes per day according to clinical state.

Once the treatment is done, we recommend administering 500 mg of N-Acetyl cysteine IV with 0.5-1 g of vitamin C to pass in 2 hours immediately after ozone therapy, and therefore 5-6 hours before the next session, given the oxidative stress state of these patients.

**Table 3.** Protocol for major autohemotherapy administration

| MAJOR AUTOHEMOTHERAPY PROTOCOL FOR COVID-19 INFECTION |
|---|
| • Peripheral venous access is preferible |
| • Use a butterfly or cannula |
| • Blood removal: 1,3 mL/kg of blood |
| • Use of heparin sodium as an anticoagulant: heparin sodium 15-20 IU /mL of blood. |
| • Adjust ozone/oxygen mixture in 1: 1 ratio |
| • Initial dose: 25 μg/mL of ozone per mL of blood. Increase of dose is acceptable in following days (max: 80 μg/mL) |
| • Shake the bottle gently once ozone is mixture with the blood and during administration |
| • Number of sessions: daily until improvement |
| • Number of pass per session: up 3 daily |
| • After therapy: N-Acetyl cysteine 500 mg IV plus vitamin C 0.5-1 g is recommended, administer over 2 hours |

***Conclusion***

Ozone has biological properties suggesting a possible role in the therapy of SARS-CoV2. Coronaviruses have abundant cysteine in their spike proteins that may be easily and safely exploited with ozone therapy. Cysteine residues are also abundant in viral membrane proteins and must be "conserved" for viral cell entry. Conserved cysteines seem functionally important for virus production. MAH has proven to be an effective and safe therapy in multiple patients, so in this situation where there is no cure for this terrible infection, we highly recommend adding it to the current treatment protocols.

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