

# Oxygen-ozone autohemotherapy against COVID-19 needs to fit highly experienced, customized, and standardized protocols to succeed

To the Editor,

We read the recent paper by Araimo et al. published in the latest issue of this journal and found some crucial flaws, which raised the comments we are forwarding here.

The authors reported that they treated, with different best available therapies (BATs) and BAT plus oxygen-ozone autohemotherapy (O<sub>3</sub>-AHT), 28 patients (14 + 14), each selected from a cohort of 91 subjects undergoing a “snapshot” analysis, from a larger population of 152 enrolled individuals involved in their probiotics project.<sup>1</sup> They further reported that they have carried out an interventional, randomized, prospective, and double-arm trial, but did not explain their selection approach. Actually, the authors appear to have performed a “cherry picking” randomized selection of 28 patients from the largest cohort of 152 recruited subjects (Clinical Trial NCT04366089), that is, they selected a second cohort of COVID-19 stage-III positive subjects (initially  $n = 91$ , then 63 excluded, so  $n = 28$ ), in which half of them (14 subjects) were treated with BAT plus O<sub>3</sub>-AHT (screening study).<sup>1</sup> We are still wondering if the 152 patients who were hospitalized COVID-19 positive patients, suffering from severe pneumonia or not and if they entered an intensive care unit (ICU). Actually, the same authors stated that they selected 152 patients as an amount necessary to achieve the more correct statistic sample size, considering 76 (non 14) patients for each group ( $\alpha = .05$ , power 80%,  $\delta = -.15$ ).<sup>1</sup> In Araimo et al. paper, the number of patients who were investigated was largely lower than a rigorous sample size calculation from statistics. We are wondering why the authors selected a very narrow cohort of subjects, when their protocol planned a higher number of participants, without providing further good explanations. Moreover, they referred to 85 patients in the Abstract, whereas the number was 91 in the text, without any sound reason. Maybe, the participation in the research paper of such numerous experts, crashed with some difficulty in the communication within the different paper co-authorships.

Again, the primary and secondary endpoints reported by Araimo et al. showed some discrepancy. They evaluated the data of patients undergoing orotracheal intubation despite BAT (primary endpoint) and the data of crude mortality at 7, 14, and 30 days (secondary endpoint); but the authors never went ahead in the therapy protocol, as they stopped O<sub>3</sub>-AHT at 1 week itself without any sound and reliable explanation.<sup>1</sup> In this circumstance, the reader cannot be ensured if carrying out O<sub>3</sub>-AHT further, patients ameliorated their clinical stage, as demonstrated by a previously published paper

from ours.<sup>2</sup> Furthermore, they reported that their primary endpoint was the 15% reduction of COVID-19 positive patients upon admission on ICUs,<sup>1</sup> but then they did not discuss this outcome further, they did not report if reached and how, neither in the text nor in the conclusion.<sup>1</sup> Interestingly, their primary outcome did not deal with major changes in laboratory and clinical markers upon therapy, which left investigators disinterested to follow up patients during their study and have a sound experimental conclusion.<sup>1</sup> In addition, we observed that information about sample enrollment and stratification, as well as statistical confounders and therapeutic regimens used, showed some critical issues.

As a matter of fact, in their recruitment eligibility criteria they selected hospitalized adults undergoing spontaneous breathing, supported either by Venturi's mask or with a high flow nasal cannula or continuous positive airway pressure,<sup>1</sup> but they did not stratify correctly how many patients were spontaneously breathing and how many with assisted ventilation. Doubts, therefore, remain about their correct selection of the COVID-19 clinical stages to be included in the treatment. Patients underwent a statistical homogenization when the authors stated different ventilation needs. These patients were each equally administered with azithromycin 500 mg/day, hydroxychloroquine 200 mg for 2 days, and tocilizumab® 8 mg/kg twice a day and a time lapse of half a day upon admission, without any thorough stratification and differential BAT protocol recommended for each patient, depending on their own clinical stage and pneumonia computed tomography (CT) evaluation.<sup>1</sup> Therefore, we are persuaded that all the 28 patients underwent the same clinical diagnosis and entered the same, homogeneous therapy protocol, without any differential diagnosis and different ages ever reported.<sup>1</sup> Actually, patients' age was not properly reported ( $\geq 18$  years is a poor indicator to have insights about patients' age distribution) and data on chest CT were completely missing.

The most striking bias emerged from reading this study with regard to O<sub>3</sub>-AHT.

Their conclusion was that 28 patients on a total of 91 recruited subjects, undergoing O<sub>3</sub>-AHT associated with BAT (14 patients), did not show any significant difference respect to controls (BAT solely), despite the fact that only one single death for each group was reported.<sup>1</sup> The authors did not elucidate the question if O<sub>3</sub>-AHT finally ameliorated the ratio of patients undergoing oro-tracheal intubation, limiting their conclusion to the observation that O<sub>3</sub>-AHT had an impact on the need for forced ventilation but without being ensured

by a statistical significance. Moreover, O<sub>3</sub>-AHT did not show any change in inflammatory markers.

The correct dosage of blood hemo-transfused oxygen-ozone, the different ratio oxygen/ozone, and the rate (or speed) of re-infusion, are major parameters for warranting a successful outcome of O<sub>3</sub>-AHT in targeted patients, usually suffering from inflammatory pathologies.<sup>3–6</sup> Araimo et al. used a dose of ozone (30 µg/ml), which is too scanty, in our expert opinion, to address the inflammatory state caused by COVID-19, including deep microvascular thrombosis, an evidence that can explain why O<sub>3</sub>-AHT did not result in a significant difference in blood inflammatory markers and the primary outcomes of ventilation need.<sup>1</sup> The authors treated subjects for a week only, they did not follow anymore their clinical course by investigating fundamental markers of COVID-19 evolution, no inflammatory, thrombotic (D-dimer), and respiratory indicators were changed, leaving the questionable issue if ozone therapy did not work for having applied a biased O<sub>3</sub>-AHT protocol, that is, having treated subjects with O<sub>3</sub>-AHT without achieving the primary endpoint. Reporting data about the therapeutic use of O<sub>3</sub>-AHT, should query for a sound and detailed description of protocols (doses, times, and patients' phenotypes) and technology (devices, machines, and blood sterile bags),<sup>2</sup> all items quite completely missing or reported in a poorly way in the paper by Araimo et al.<sup>1</sup>

Both the O<sub>3</sub>-AHT group and the control one, reported one single death at 30-day follow up, whereas plasma biomarkers showed poorly reproducible differences between the O<sub>3</sub>-AHT group and controls ( $p > .05$ , not significant), and therefore, those deaths cannot be statistically associated with differential therapy. Aside from two deaths occurred at 7 and 14 days for both cohorts ( $n = 14$ ), that is, O<sub>3</sub>-AHT + BAT and BAT, the calculated lethality at day 30 was perfectly similar (7.1%) and cannot be used to conclude for a differential outcome between BAT solely and BAT + O<sub>3</sub>-AHT. As a matter of fact, reading the paper by Araimo et al. makes particularly hard to discriminate the effect of O<sub>3</sub>-AHT from the effect of BAT, that is, patients' outcomes cannot be associated with O<sub>3</sub>-AHT alone, aside from some change in the lymphocyte subsets, which yet are particularly sensitive to intra- and interindividuals' variability.<sup>2</sup>

On the contrary, Franzini et al. recently showed the ability of O<sub>3</sub>-AHT to restore health and reduce hospitalization in elderly COVID-19 positive patients (mean age = 75 years) undergoing sub-intensive therapy.<sup>2</sup> Contrary to the report by Araimo et al. treating these subjects with 45 µg/ml ozone in O<sub>3</sub>-AHT for at least 3 weeks, improved notably the inflammatory markers (CRP, IL-6), thrombo-hemolytic markers (D-dimer), and respiratory indexes of oxygenation.<sup>2</sup> Therefore, we sought to elucidate how O<sub>3</sub>-AHT failed in proving effective in COVID-19 patients with severe pneumonia, despite the existence of positive outcomes in the field.<sup>2</sup> According to our opinion, major flaws in the clinical application of O<sub>3</sub>-AHT regarded the methodology and the technology used to administrate the correct O<sub>2</sub>/O<sub>3</sub> concentration ratio in patients, the time of O<sub>3</sub>-AHT used and patients' follow-up and the more correct ozone amounts depending on the different clinical phenotype considered for therapy. The fundamental data retrieved by the authors referred to the simple 7-day treatment,

no more ozone therapy was further performed. This is the major bias retrieved by reading Araimo et al.'s report.

Finally, criticism arose while reading the whole experimental setting performed by Araimo and colleagues. The study was included in a wider project called PROBIOZONOVID, including probiotics (SivoMixx<sup>®</sup> 200 billion), yet the authors did not report any data about the effect of this probiotics on patients.<sup>1</sup> The introduction of probiotics is a statistic confounder for O<sub>3</sub>-AHT, as probiotics may affect the immune response of treated subjects, despite the role of O<sub>3</sub>-AHT.<sup>7</sup> Furthermore, the authors did not specify when probiotics were administered in their paper. In the registered NIH trial NCT04366089, they reported that probiotics was included with O<sub>3</sub>-AHT, that is, SivoMixx<sup>®</sup> 200 billion (six sachets twice a day), but never in their paper. Treated subjects probably underwent a significant modification in their immune response due to probiotics, as 30% of control patients underwent probably COVID-19 caused dysbiosis, reporting diarrhea.

While we can explain why O<sub>3</sub>-AHT did not show encouraging outcomes, the introduction of a multistrain probiotics mixture is completely incomprehensible for the full meaning of the study, as it acted most probably as a statistic confounder. As a matter of fact, they refer to ozone rectal insufflation while talking about probiotics but as they did not perform this practice, the consideration about probiotics is clumsy. Fundamentally, they did not report any evaluation about the effective role of probiotics in their O<sub>3</sub>-AHT research project.

Taken together, the results described by Araimo and colleagues cannot shed further light on the role of O<sub>3</sub>-AHT in COVID-19 therapy and may discredit the several positive results currently emerging in the literature. It is very difficult to fully ascertain the effect of O<sub>3</sub>-AHT if protocols used are not fully defined. The correct use of ozone against COVID-19 is particularly burdensome to achieve a positive outcome in patients affected by severe pneumonia due to SARS-CoV2 infection. Ozone cannot be used as a natural compound, a nutraceutical or a probiotics-like therapy; its application in treating COVID-19 is particularly sensitive and pushes research toward standardized, accustomed, and highly expertised protocols, particularly in COVID-19 treatment.

## PEER REVIEW

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Salvatore Chirumbolo<sup>1</sup> 

Marianno Franzini<sup>2,3</sup>

Vincenzo Simonetti<sup>4</sup>

Luigi Valdenassi<sup>2,3</sup>

Giovanni Ricevuti<sup>2,3</sup>

Dario Bertossi<sup>5</sup>

Sergio Pandolfi<sup>2,3</sup>

<sup>1</sup>Department of Neurosciences, Biomedicine and Movement Sciences, University of Verona, Verona, Italy

<sup>2</sup>SIOOT, High School in Oxygen Ozone Therapy,  
University of Pavia, Pavia, Italy

<sup>3</sup>SIOOT International, Communian Clinic, Gorle Bergamo, Italy

<sup>4</sup>Oxygen-Ozone Therapy Scientific Society (SIOOT), Gorle, Italy

<sup>5</sup>Department of Surgery, Dentistry, Pediatrics and Gynaecology Unit of  
Maxillo-Facial Surgery,  
University of Verona, Verona, Italy

#### Correspondence

Salvatore Chirumbolo, Department of Neurosciences,  
Biomedicine and Movement Sciences, University of Verona, Strada  
Le Grazie 9, 37134 Verona, Italy.  
Email: [salvatore.chirumbolo@univr.it](mailto:salvatore.chirumbolo@univr.it)

#### ORCID

Salvatore Chirumbolo  <https://orcid.org/0000-0003-1789-8307>

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