Innate Immune Response in COVID-19 and Immunoaugmentive Activity of Ozone Therapy

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Abstract:

SARS-CoV-2, the cause of COVID-19, is the newest member of the Coronaviridae family of viruses and is the third coronavirus crossing animal species barriers to infect human populations. Late stage in severe COVID-19 cases is reported to include a cytokine storm in many cases. This process implies a severe activation of innate immunity. Indeed, the adaptive immune system is employed as specific antibodies are found in COVID-19 patients. However, since innate immunity plays a role in fighting this disease, interventions that involve support of the innate immune response may play an important role in mitigating the disease process. Ozone therapy has a long history of treating infectious diseases, including those caused by viruses. Information of the efficacy of ozone treatment on COVID-19 are emerging. Ozone and ozone-derived reactive oxygen species are reported to be produced by antibodies, indicating that the action of this oxidant may play a vital role in both innate and adaptive immunity. Here we report the history of ozone therapy, its activity against infectious agents, and a case report on 104 patients with advanced COVID-19 treated with ozone therapy. Ozone therapy may be an important adjuvant to more conventional therapies such as vaccines in severe infectious disease and pandemics including that caused by SARS-CoV-2.

Introduction:

SARS-CoV-2, the cause of COVID-19, is the newest member of the Coronaviridae family of viruses and is the third coronavirus crossing animal species barriers to infect human populations. The previous two members of the family are the severe acute respiratory syndrome coronavirus (SARS-CoV), emerging in 2002, and the Middle East respiratory syndrome coronavirus (MERS-CoV), in 2012. Along with the seasonal flu, all can cause severe, even fatal, sudden acute respiratory syndrome or severe cardiopulmonary distress, often accompanied by the cytokine storm syndrome (CSS) is those with severe outcomes or who die from the disease. The SARS-CoV-2, uniquely, is initially reported to attack deoxyhemoglobin reducing the body's ability to carry oxygen.
The preponderance of people with severe COVID-19 symptoms or who die either have comorbid chronic conditions or are amorphously classified as immunocompromised. Young children are considered immunocompromised as their immune systems, rather than failing as is the case in older populations, is developing thus incomplete. Importantly, COVID-19 appears to be sparing the very young when compared to SARS and MERS, the Spanish flu and seasonal influenza. Current data suggest an approximately 1% infection rate between 0 and 19 years.[1] This response is not isolated

to Severe Acute Respiratory Syndromes (SARSs). several infectious diseases are well known to be less severe in children. Paralytic polio occurred in approximately 1 in 1000 infections among infants, in contrast to approximately 1 in 100 infections among adolescents. As compared with young children, teenagers and adults tend to have symptomatic rubella more frequently and have systemic manifestations. One of several, yet unsubstantiated explanations, is that children have a more active innate immune response to compensate for an underdeveloped adaptive immune system.[2,3]

The ability of the innate immune system to respond to microbes may be critical in early life because of the immaturity of the adaptive immune response.[4] Simon et al.[5] state, "The innate immune system provides an early first line of defense against invading pathogens. The cells involved are neutrophils, monocytes, macrophages and dendritic cells, which all interact with the adaptive immune system. These cells develop and mature during fetal life, but at different times, and the function of all components of innate immunity is weak in newborns compared with later life." It is well established that adaptive immune responses are deficient in early life, contributing to increased mortality and morbidity against a broad spectrum of pathogens. New research explains the developmental pattern of cytokine responses, elicited by innate immunity, during early life is age and toll-like receptor specific.[6] Laza-Stanca et al. stated, "The age-dependent decline in regulatory function observed in the present study (SEB-induced IL-10 and percentages of FoxP3+ T regs) was unexpected—we expected to observe age-dependent increases in both parameters. However, high levels of IL-10 production may have an important role early life in regulating the inflammatory response to infections. Greater mitogen-induced production of IL-I0 has been associated with a low risk of severe respiratory infections[7] and deficient IL-10 was associated with greater viral load following in vivo challenge with rhinovirus.[8]" Thus, our knowledge on innate immune development and its modulating factors needs to be expanded, especially in light of COVID-19 early data and the bulk of therapeutic emphasis being placed on supporting adaptive, not innate immunity.[9]

As we age and change, so do our immune systems. Most experimental data on immune changes with aging show a decline in many immune parameters when compared to young healthy subjects. The bulk of these changes is termed immunosenescence.[10] Fewer cells are produced for the adaptive immune response, in which pathogens are precisely identified, targeted and "remembered" for a swift, thorough response if the same or similar pathogen is encountered in the future. And while there's an expansion of cells in the innate, non-specific immune response as we age, they lose efficiency and effectiveness. That's why older people tend to present with higher levels of inflammatory biomarkers like C-reactive protein, uric acid, sedimentation rates, and white blood cell counts. The overall effect is reduced function and greater vulnerability to infection which often manifest in vascular and other chronic conditions. These changes contribute to the decreased response to vaccines seen in many older adults, and morbidity and mortality from infection. Infections (e.g., influenza, pneumonia and septicemia) appear among the top ten most-common causes of death in adults in the USA aged 55 years and older.[11]

Despite the universal understanding that the innate and adaptive immune systems are interrelated and interdependent, the preponderance of medical interventions for infectious and chronic conditions ignore support of innate immunity. Vaccines bolster adaptive immunity and most chronic diseases are managed at the "symptoms" level, which has little to no benefit to underlying immunity. And, in this delivery system, innate response is often considered hostile, with immunosuppressant drugs seeing wider use to control the manifestations of the response, namely upregulation of cytokines and inflammation. Yet there are no substantial studies showing a mortality benefit from these treatments but many existing showing an increase in early mortality

risk.[12] We believe a parallel track to improve patient outcomes and reduce morbidity and mortality must include interventions that support innate immunity, before escalating cytokine levels cause collateral damage. This approach may obviate the need for the symptomatic or suppressing approaches in many cases.

Ozone Therapy Supports Innate and Adaptive Immunity

Ozone therapy offers a low-cost and potentially highly effective method for augmenting innate and adaptive immunity and reducing coronavirus-induced cytokine storms when used early in the infectious process before exponential proliferation of infection and concomitant cytokine production. Bona fide COVID-19 pharmaceuticals are not proven and the human immune response is incapable of abating severe disease is vulnerable populations since normal T-cell activation is known to be sluggish due to the body's naiveté relative to novel SARS antigen. This delayed response time arguably leads to severe or fatal disease. Reduction of viral load, through ozone treatment, is posited to offer a plausible emergency viral abatement strategy. This approach may also be applied in disease prevention in both the uninfected and infected asymptomatic individuals, amelioration of disease severity, or curing current disease in non-emergency cases.
Medical ozone therapy is used to disinfect and has a 150-year history of successful use to treat infections, wounds and multiple diseases. It has been used to disinfect drinking water before the turn of the last century. Ozone was known to treat many inflammatory and infectious diseases.[13] During the first world war doctors applied ozone topically to infected wounds and discovered it remedied infection and hemodynamic and anti-inflammatory properties.[14] Subsequently, types of disease treated with ozone include circulatory disorders, geriatric conditions, macular degeneration, dental infections, viral diseases, rheumatoid arthritis, cancer, SARS and HIV/AIDS. Even though the ozone therapies may not have the potency to cure cancer or AIDS, they could be a very useful tool if they are used as an adjuvant for established medical procedures if their effect is additive. [15] Ozone, like any "medication" offers both a therapeutic and toxic effect. When used based on established guidance, toxic effects are avoided.**Error! Bookmark not defined.**,[16]

Ozone therapy is documented to have cytoprotective activity based on an assessment of 74 peer-reviewed original articles.[17] Use of ozone in viral infections, clinically, decreases damage by modulating inflammation and oxidative stress. Ozone therapy contributes to homeostasis through modulation of the NF-κ B/Nrf2 pathways and IL-6/IL-1β expression. Increased numbers of NK cells upon a single dose of ozone was observed in vitro culture experiments with samples obtained from healthy subjects.[18] These cells are important components of the innate immune system and are known to participate in the early detection and elimination of viral infections and also play a role in tumor immune surveillance.[19,20] Ozone rectal insufflation was applied to 18 children with compromised phagocyte cell-mediated immunity. Positive increases in neutrophil counts occurred in all cases and leukocyte phagocytic function showed substantial improvement in all but one case, as determine 1 month after a 3-month course of treatment.[21]

Significantly, researchers at Scripps Research Institute clearly demonstrated that antibodies can destroy pathogens by producing localized concentrations of ozone gas.[22] According to the authors, "The ozone may be part of a previously unrecognized killing mechanism that would enhance the defensive role of antibodies by allowing them to subject pathogens to hydrogen peroxide and participate directly in their killing. Previously, antibodies were believed only to signal an immune response." This landmark finding illustrates the elegance and interconnectedness of the innate and adaptive immune system. It also supports the thesis that exogenous ozone, when used at physiologically safe levels, can be a natural, safe, and effective treatment of pathogens.

Results:

At our outpatient primary care office in West Bloomfield, MI, we have currently treated 104 patients presenting with COVID-19. Michigan has been particularly hard hit by SARS-CoV-2 compared to the rest of the U.S. with a high relative death rate currently (5/10/2020) estimated to be 9.6%. [23] The demographics for the patients are provided in Table 1. All patients were provided with basic oral supplements to support innate immunity including: vitamins A, C, D, and iodine. Patients were instructed to follow label instructions with the exception of vitamin C where our recommendation was 4g of ascorbate/day. Patients with more severe symptoms that included: shortness of breath and fever, were pre-treated with intravenous dosages of vitamins including vitamin C.

Average age	53.7 yrs.	
Age Range	12-88 yrs.	
Sex	Male 27 (26%)	Female 77 (74%)

Table 1: Demographics of 104 Patients Treated for COVID-19

Although not a formal clinical study, all procedures performed in the program involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. All data was acquired in strict conformance with health data privacy laws by medical personnel and all stored data were contained on a HIPAA compliant cloud.

The main course of treatment for the two case studies consisted of intramuscular injections of ozone. 20cc of 18 gamma ozone was given as in intravenous push over 1-2 minutes in each buttock. The ozone was produced with an ozone generator (Promolife O3 Elite, single stage ozone generator) and 100% pure oxygen. In a small minority of patients, this treatment was duplicated within a day or two if symptoms did not resolve. Based on Michigan statistics, our population had an expected death rate of 10 patients. All of our patients survived and, as of 5/11/2020, all have recovered and are symptom-free. One patient was hospitalized but has since recovered and did not require mechanical ventilation while hospitalized. We present two typical case studies that provide greater detail to our approach.

Case 1: AD is a 37-year-old female who presented on April 15, 2020 with two-day-old complaints of fever (temperature fluctuating between 100-102 degrees Fahrenheit), cough, loss of sense of taste and smell, gastrointestinal disturbances including two episodes of diarrhea and having an upset stomach, body aches, and shaking as well as severe fatigue. The patient was offered a test for coronavirus but refused. A diagnosis of COVID-19 was given based on patient symptoms and PCR testing. The diagnosis of COVID-19 was initially and primarily made based on symptoms. The Centers for Disease Control and Prevention relaxed the criteria for diagnosing COVID-19. COVID-19 may be diagnosed without laboratory confirmation if the physician suspects the illness based on symptomology. The CDC states the most common symptoms of COVID-19 are: fever, cough, fatigue, anorexia, shortness of breath, sputum production, and myalgias, of which this patient had most.

AD was treated with p.o. dosing of vitamin A (10,000 U/day), vitamin D (50,000 IU/day), Vitamin C (10,000mg/day) and iodine (50mg/day). All oral therapies were to be taken for four days, then stopped. She was also treated with a dilute (0.04%) hydrogen peroxide (3cc in 250 cc of sterile, normal saline) solution along with 1 drop of 5% Lugol's iodine given via a nebulizer. She was instructed to nebulize 3 cc of the mixture every hour while awake and to gradually reduce the frequency as she improved. Finally, she was treated with ozone given as an intramuscular injection with 20 cc of 18 gamma ozone being injected into each buttock.
On day 3 of her illness and one day after the start of treatment the patient reported marked improved in all symptoms. After the ozone injection at the office, she stated that her fever began lowering in 60 minutes and three hours later was resolved. The body aches, shaking and fatigue began to improve when the fever resolved. On day 4, 2-days after first treatment, the patient stated that her sense of smell and taste was improving. On day 5, the patient reported that all symptoms were gone except for minor fatigue which resolved a few days later.

Case 2: CP is a 58-year-old white male who became ill 14 days before contacting our clinic. His initial symptoms were severe fatigue, sore throat and body aches. After a few days, they progressed to fevers fluctuating between 99.5- and 102-degrees Fahrenheit. He tested positive for SARS-CoV-2 antibodies seven days into his illness. He was instructed to self-quarantine at home and go the emergency room when his symptoms became unbearable. Over the next week, his symptoms worsened and new symptoms developed included loss of taste and smell and shortness of breath. The fatigue worsened to the point where he could not get out of bed to reach a glass of water. On day 17 of his illness we had a phone consult with the patient. CP was instructed to orally take: vitamin A (10,000 IU\day); vitamin D (50,000 IU/day); vitamin C (1,000 mg of ascorbic acid each hour until bowel tolerance and then take 75% of that amount daily, over 3 doses, morning, noon, and evening until further notice; and iodine (50 mg/day of Lugol's solution 5%). He was also advised to nebulize with a dilute hydrogen peroxide solution (0.04%) along with 1 drop of Lugol's 5% solution every waking hour until symptoms improved. After the second nebulized treatment CP reported he could start to feel the improvement in his breathing. "I could finally take a deep breath of air for the first time in many days. I no longer felt like I was going to die," he stated. At day 3 of treatment, he fever was still present but much lower, not going over 100.5 degrees Fahrenheit. The next day he was treated, in office, with an intramuscular injection of 20cc of 18 gamma ozone. After the ozone shot, he reported a dramatic improvement. The fever resolved within two hours and did not return. Later that day, his muscle aches and pains resolved. He made a full recovery over the next few days.

Discussion:

Supplements and Hydrogen Peroxide to Pre-Treat Patients with Viral Infections

Vitamin A has been shown to modulate the immune system's response to pathogens and can down regulate the secretion of specific cytokines such as TNF- α and Il-6 by macrophages which both are pro-inflammatory cytokines that can contribute to cytokine storm. Similarly, Vitamin D has been shown to modulate cellular immunity and reduce cytokine storm by reducing the production of proinflammatory cytokines including TNF-α and interferon γ as well as increasing the anti-inflammatory cytokines produced by macrophages.[24] Vitamin C has been used in hospitalized COVID-19 patients in many countries including China and the US. A Chinese report of intravenous vitamin C infusion on 50 moderate-to-severe COVID-19 subjects found all

patients improved and able to be discharged from the hospital. The subjects were given between 10 and 20 g of IVC per day over a period of 8-10 hours.[25] Iodine is one of the most powerful antimicrobial agents known. It is broad spectrum and considered an antiseptic with both antiviral and antibacterial effects. Iodine supplementation has been shown to increased IgG synthesis in human lymphocytes.[26]

Ozone in Treating Infectious Disease

Ozone is often strong anti-pathogenic and importantly is pathogen-agnostic, displaying similar oxidative biological properties to innate immune cells, thus has theoretical and practical attributes to make it a viable candidate as a COVID-19, MERS, and SARS viral load-reducing agent. Most research efforts on ozone's viricidal effects have centered upon its efficacy at breaking apart lipid molecules at sites of multiple bonds. When the lipid envelope of the virus is fragmented, its DNA or RNA core cannot survive. Ozone can also destroy capsid proteins of non-enveloped viruses. However, the enveloped viruses are usually more sensitive to oxidative action compared to naked virions. The novel coronavirus is an enveloped virus thus likely has great susceptibility to clinical ozone therapy due to the fragility of their lipid-rich envelopes.

Hydrogen peroxide is produced throughout the human body. Every cell in the body is exposed to some level of hydrogen peroxide.[27] The lungs are known to produce hydrogen peroxide.[28] Nebulized hydrogen peroxide has been shown to have antiviral activities.[29] Hydrogen peroxide can activate lymphocytes[30] which are known to be depleted in COVID-19.

Clearly, some viruses are more susceptible to ozone's action than others and a differentiating feature is the lipid-enveloped viruses, like SARS-CoV-2, which are the most sensitive.[31,32] This group also includes hepatitis B and C, herpes 1 and 2, Epstein-Barr, HIV 1 and 2, influenza A and B, West Nile virus, Togaviridae, Eastern and Western equine encephalitis, rabies, and Filiviridae (Ebola, Marburg). For hepatitis C, ozone therapy significantly improved clinical symptoms, with normalized ALT and AST levels achieved in most patients. [33] In this study, ozone therapy was associated with elimination of HCV RNA from the serum of 25-45% of patients. In mice infected with influenza A virus, exposure to ambient ozone levels of 0.5ppm improved the pathogenesis of the respiratory infection. [34] "The ozone-mediated alteration in viral antigen distribution was consistent with significantly reduced influenza disease mortality and prolonged survival time." A Hong Kong hospital admission study showed a relationship between ambient ozone and influenza, with a conclusion that transmissibility reduced with elevated ambient ozone.[35]

Ozone as an Adjuvant Treatment of SARS-CoV-2

Ozone is reported to activate the immune system in infectious diseases, to improve the utilization of oxygen and stimulate release of growth factors and other mediators that may re-activate the immune system and reduce ischemia in vascular disease, now known to be a significant contributor to adverse Covid-19 outcomes.[36,37,38] According to the International Scientific Committee of Ozone Therapy,[39] "systemic ozone therapy can be 'potentially' useful in SARS-CoV-2. The rationale and mechanism of action has already been proven clinically with other viral infections similar to SARS-CoV-2. The mechanism of action is as follows: 1) The induction of adaptation to oxidative stress, hence a re-equilibration of the cellular redox state. 2) The induction of IFN-gamma and proinflammatory cytokines. 3) The increase of blood flow and tissue oxygenation to vital organs (i.e. renal, pulmonary and cardiac circulation). 4) It has the potential to act as an auto-vaccine when administered in the form of minor autohemotherapy."

Ricevuti et al.[40] present information on the potential for an oxygen-ozone immunoceutical therapeutic approach in response to the COVID-19 outbreak. They cite emerging, yet anecdotal "initial positive" results using this approach in Lombardy, Italy hospitals. Baeza-Noci et al.[41] suggest a specific protocol for ozone therapy of COVID-19 sufferers that includes:
Day 1: 100 mL at 30 mcgr/mL concentration.
Day 2: 150 mL at 30 mcgr/mL concentration.
Day 3 – 14: 200 mL at 30 mcgr/mL concentration.
In-hospital patients: each 12 hours application for minimum 14 days.

This can be achieved via a number of technologies that have long been the purview of pioneer physicians. Most experience has been gleaned from methods utilizing the serial treatment of blood aliquots with oxygen/ozone gaseous mixtures, known as autohemotherapy (AHT). More comprehensive methods—although more sophisticated but ones that have greater potential to succeed in Covid-19 culling—involve the treatment of the total blood and lymph volumes via techniques called extracorporeal blood oxygenation ozonation—EBOO.[42,43,44]

In the technique of ozone autohemotherapy (AHT), an aliquot of blood (50 to 500 ml) is withdrawn from a virally afflicted patient, anticoagulated, interfaced with a calibrated ozone/oxygen mixture, then reinfused. This process is repeated serially, in a manner consonant with treatment protocols until viral load reduction and symptom abatement are observed. In EBOO, the entire blood volume is treated using an ozone-resistant hollow-fiber oxygenator-ozonizer, much in the model of dialysis intervention.[45] This technique appears superior to AHT for management of substantial pathogenic infections. For the present time, however, AHT offers simpler interventions but unproven due to a lack or randomized clinical trials, in Covid-19.

COVID-19, Cytokine Storm Syndrome, and the Impact of Ozone Therapy of Inflammatory Biomarkers

Cytokine Storm Syndrome (CSS) is common in severe viral and bacterial infections. CSS involves a complex interplay of various cytokines, some of which are more piqued during disease. Acute Respiratory Distress Syndrome (ARDS) and multiorgan dysfunction are among the leading causes of death in critically ill patients with COVID-19. Elevated inflammatory cytokines, noted in COVID-19 cases, suggest CSS may play a major role in the pathology of COVID-19. This pathway suggests reaction by the innate immune system as the primary physiological defense mechanism against the novel coronavirus.

Quantitatively measuring physiological health is an important component in determining aspects of immunity involved in controlling viral replication. It is also crucial in identifying and staging sufferers when resources are consumed as in periods of pandemics. Initial serology on severe COVID-19 cases shows adverse levels for the following markers that are current used to risk stratify hospitalized patients at the Harvard Medical School affiliated Massachusetts General Hospital: CBC with diff (trend total lymphocyte count), Complete metabolic panel, CPK (creatine kinase), D-dimer, Ferritin, ns-CRP, ESR, LDH, Troponin, viral serology, and baseline ECG.[46,47,48]

Currently there are few studies on ozone therapy that demonstrate positive changes in biomarkers of physiological health. In future studies, particularly those focused on controlling pathogenic diseases, those markers elevated in severe COVID-19 should be obtained. Ozone therapy for treatment of presumed non-infectious disease historically demonstrates modest improvement in phycological markers know to be elevated in CSS and hospitalized COVID-19 patients. In a study of

osteoarthritis, pre- and post-treatment levels of CRP and ESR were recorded. The intervention was performed on 33 patients and included 4 sessions (1 session/week) of an intra-articular infiltration of a medical mixture of Oxygen-Ozone (95% to 5%) at 20 ug/mL concentration. C-reactive protein (CRP) diminished from 0.33 ± 0.32 mg/dL to 0.25 ± 0.23 mg/dL (P = 0.0456). Erythrocyte sedimentation rate (ESR) decreased from 15.06 ± 12.09 mm/h to 11.81 ± 8.32 mm/h (P = 0.01). [49] Sloan performed an extensive study on the impact of ozone on blood components. Erythrocyte sedimentation rate reduced in a statistically significantly with ozone therapy, with more pronounced effects at low (1-2mm/hr.) compared to high (4mm/hr.) rates of ozone introduction with a similar trend being noted for the reduction in CRP levels.[50] CRP and MMP-3 levels, measured in synovial fluid of osteoarthritis patients consistently showed important reductions after ozone treatment.[51]

Summary and Conclusions:

The primary approach to treating viral infections is through vaccines. This approach supports the adaptive immune system only. Further, long-term vaccine development is required for each new pandemic-capable virus. Available interventions, that are relatively pathogen agnostic, must be tested and, if proven efficacious, be available as a primary or adjuvant treatment while more traditional therapies are developed. Thus, in parallel, research also needs to center on finding new methods of relieving the biological stress caused by onslaughts of viremic invasions that are common to many families of pathogenic viruses. The coronaviruses are a case in point, as they all possess lipid envelopes susceptible to structural modifications by ozone.

Considerable existing evidence shows that ozone augments the action of innate immunity. Further, COVID-19, through CSS, challenges innate immunity. Also, ozone appears to be a natural defense mechanism against infection, the production of which is stimulated by antibodies. Ozone has favorable safety profiles and therapeutic benefits.

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