A PORT IN THE STORM: PLASMA ADSORPTION FOR THE DAMPENING OF CYTOKINE STORM IN COVID-19

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Introduction. SARS-CoV-2 (the virus that causes COVID-19) is a positive sense RNA virus which is part of the family of coronaviruses which usually cause the common cold. However, these widely distributed family of viruses can also cause viral pneumonia in patients with comorbidities. In recent years, two novel coronaviruses have emerged (SARS and MERS) which caused epidemics with high mortality rates (9.5% and 36%, respectively)1, with COVID-19 being the third. The newly emergent SARS-CoV-2 (herein referred to as COVID-19) is most closely related to SARS (SARS-CoV-1). Like SARS, it binds via the angiotensin-converting enzyme 2 (ACE 2) receptor located on type II alveolar cells and intestinal epithelia.2

The majority of COVID-19 infections result in mild cold or flu-like symptoms that resolve with no medical intervention.3 However, in patients with advanced age, compromised immune systems, organ or coagulation dysfunction, and /or other significant comorbidities, the disease can result in respiratory compromise, rapid deterioration, and death.4

The primary pathophysiological mechanisms underlying COVID-19 infections are cytokine storm and acute respiratory distress syndrome (ARDS).5 However, as with SARS-CoV-1, for patients that experience acute renal impairment due to the infection (approximately 7%),6 mortality rates increase to 40%.7 Defects in mitochondrial function (the powerhouses of the cell) are key pathophysiological changes that happen during acute kidney injury (AKI). Under physiologically normal circumstances, mitochondria not only provide the vast amount of adenosine triphosphate (ATP) the kidney requires for tubular reabsorption, but they also have been shown to have key roles in downstream processes including activation of the immune response, immunomodulation, and apoptosis and necrosis. Damage to the kidneys increases anaerobic metabolism (fermentation - to compensate for the damaged mitochondria), thereby increasing oxidative stress and runaway inflammatory processes.8

This is also true of patients with preexisting kidney disease. COVID-19 presents particular challenges for patients on dialysis, especially in center hemodialysis. Uremic patients are particularly vulnerable to infection and may exhibit greater variations in clinical symptoms and infectivity, significantly increasing the risk of transmission to medical staff, facility workers, other patients and family members.

ARDS, historically a rare type of severe lung dysfunction that arises acutely 24 – 48 hours after an inflammatory-mediated illness (e.g., pneumonias, influenza) or other injury (such as burn or trauma), can also occur
COVID-19 patients.\textsuperscript{9-12} It affects all or most of both lungs and carries significant morbidity and mortality. Patients present with dyspnea, hypotension and multi-organ failure.\textsuperscript{13} Although the inciting event (e.g., pneumonia [viral or bacterial], sepsis, traumatic injury) may not necessarily be located anatomically within the respiratory system, it can lead to diffuse inflammatory pulmonary infiltrates, and hypoxemic respiratory failure.\textsuperscript{14} Disease severity depends on the degree of alveolar epithelial injury. The risk for developing ARDS depends on the pre-disposing clinical condition and increases with the number of underlying conditions. Sepsis, bacterial pneumonia, multiple trauma, and aspiration pneumonia have been the most common predisposing factors, accounting together for more than 70\% of cases.\textsuperscript{9-12} With the spread of COVID-19, it is likely to become far more prevalent.

The manifestation of ARDS in COVID-19 is characterized by diffuse bilateral alveolar damage (e.g. including hyaline membranes). Pneumocytes with viral cytopathic effect are seen, implying concurrent direct virus damage (rather than a strictly hyper-inflammatory injury).\textsuperscript{15} Concomitant cytokine storm in COVID-19 manifests with similar features of bacterial sepsis or hemophagocytic lymphohistiocytosis, and elevations of C-reactive protein and ferritin, which appear to track with disease severity and mortality.\textsuperscript{16}

\textbf{Current Treatment Options.}

Recommendations made for managing both ARDS and cytokine storm in patients with COVID-19 are the same as those for the treatment of other viral pneumonias (e.g., influenza). Current management approaches are symptomatic rather than therapeutic, primarily consisting of supportive treatment with lung-protective ventilation and restrictive fluid management. Standard therapy consists of mechanical ventilation, supplemental oxygen, prone positioning, use of paralytics, fluid management and a technique called positive end expiratory pressure (PEEP) to help push the fluid out of air sacs.\textsuperscript{17} These are combined with various antiviral medication cocktails as was done for the treatment of the first SARS outbreak in 2003 and the subsequent MERS outbreak.

Because people with ARDS are less able to fight lung infections, they may develop bacterial pneumonia during the course of the illness. Antibiotics are given to fight the secondary bacterial infection. Also, supportive treatment such as intravenous fluid or nutrition may be needed. If other organ systems become involved (notably the kidneys), measures may be needed to support those organs. Despite advances in critical care and management, mortality rates for ARDS uncomplicated by COVID-19 range between 30-50\%.\textsuperscript{18,19}

There is no currently adopted pharmacological therapy regimen proven to decrease mortality once a disease progresses to ARDS. It is unclear what the mortality rate is for ARDS in the context of COVID-19, however, a retrospective cohort study of 191 COVID-19 patients found that 93\% of non-survivors developed ARDS during the course of their illness.\textsuperscript{20}

\textbf{An Unmet Medical Need.} The mechanisms by which ARDS manifests include vascular leakage, inflammation and, often, infection.\textsuperscript{21} This is often complicated by the underlying cytokine storm that can accompany an active infection such as COVID-19. For a treatment to improve

\textsuperscript{†} https://rarediseases.org/rare-diseases/acute-respiratory-distress-syndrome/
outcomes, it would need to address all four aspects of the pathogenesis.

Additionally, there are no current COVID-19 specific antiviral regimes available today.

No single pharmacological or biologic agents available today can achieve this, and standard of care for these end-stage conditions continues to be associated with high morbidity and mortality.\(^{22}\)

**An old idea made new: Plasma adsorption for removal of inflammatory compounds and toxins.** Extracorporeal charcoal and resin-based blood filtering systems designed to reduce toxin and inflammation burdens have been available in commercial distribution in the US and Europe for over 60 years. Different combinations of blood circulation systems and adsorption columns are used to treat hepatic encephalopathy, cirrhosis, sepsis, and conditions such as severe burns, drug overdose, and ingestion of toxic substances. However, their use and reported outcomes are highly variable due to the lack of standardized protocols, the availability of multiple filtration configurations, the technical complexity of setting up a filtration circuit contributing to the reported serious adverse event rates. Common adverse events include bleeding, thrombocytopenia, leukopenia, coagulopathy, hypocalcemia, hypophosphatemia, hypoglycemia, decrease in fibrinogen, and hemodynamic instability due to complement hemolysis activation (inflammatory response).\(^{23,24}\)

**A CE-marked adsorption column developed by Marker Therapeutics AG.** The D2000 cartridge represents an advance in adsorption technologies with a simpler clinical workflow and multiple concurrently adsorptive materials. The stand-alone cartridge, which can be connected to any compatible plasma separation technology available in the hospital, such as standard CRRT machines or apheresis devices, contains a proprietary patented blend of activated charcoal and resins. The cartridge can remove cytokines, toxins and other inflammatory compounds without changing the ionic balance of blood returned to the patient (unlike therapeutic plasma exchange strategies). After priming the cartridge and assembling the inlet and outlet lines to the plasma separation device, plasma adsorption can be run for 4 hours, to be repeated as needed. It minimizes the need for fluid volume replacement during treatment and is associated with a lower risk of infection and allergic reactions to donor and other substitute fluids.\(^{25}\)

In bench testing, the Depuro D2000 Plasma Cartridge has been specifically shown to remove statistically significant proportions of IL-3, IFN-gamma, IL-10, IL-1B, IL-6, IL-8, MCP-1, and TNF-alpha as compared to control. This ability translates to improved clinical outcomes for patients in an ICU setting, particularly for those with pulmonary complications due to viral infections and/or sepsis. In an early case series of nine ICU patients with sepsis, use of the D2000 cartridge once daily for four
hours (average number of treatments was 3.3, range 2-7), there was an average decrease in APACHE II score of 5 points. Elevated levels of CRP and cytokines before treatment initiation were also significantly reduced, and all patients remained hemodynamically stable during the treatments, though some did require inotropic support.

This case series included subjects with pulmonary complications (1 case of pulmonary edema, 2 cases of ARDS [1 secondary to H1N1 infection, the other secondary to sepsis] 1 cases of lung infection secondary to sepsis, 2 cases of pneumonia) and all patients stabilized and improved clinically.

A second patient, a 43-year old male with sepsis and ARDS, bilateral multifocal pneumonia, shortness of breath, high grade fever and uncontrolled diabetes showed material improvement after 3 D2000 cycles and was discharged from ICU.

A third patient, a 55-year old male with Severe ARDS secondary to H1N1, acute respiratory distress, pneumonia, diffused chest haziness, desaturated status and a history of hypotension showed material improvement after 5 D2000 cycles. The rest of the treated patients (n=7) were all reported to have improved markedly after 2-5 treatment cycles.

There were no adverse events related to plasma filtration for any of the treated subjects.

**Conclusions.** SARS-CoV-2 is a newly emergent coronavirus which has reached pandemic spread. This means that most people will be exposed to it, and, with no specific innate immunity from past similar infections in the population, older and compromised individuals may experience significantly worse clinical outcomes. Current estimates of the $R_0$ (infection rate) are ~2.5-2.9 which is slightly higher than seasonal influenza.$^{26,27}$

The pandemic is especially concerning, because, similar to other RNA viruses, SARS-CoV-2 continues to recombine and mutate. These mutations may allow the virus to become more virulent over time, posing challenges in medical management and vaccine development, and altering the course of transmission and recovery.$^{28}$ RNA

• If $R_0 = 1$, then epidemic will continue at a steady pace.
• If $R_0 > 1$, the epidemic will increase exponentially.

†† $R_0$ is the average number of people that an infected person transmits the virus to
If $R_0 < 1$, the epidemic will burn out.
virus populations contain closely related but different genomic sequences infecting an individual host. Further, RNA viruses are known to evolve rapidly and efficiently in the presence of antiviral medications. Specifically in the coronavirus family, mutations that allow for resistance have been shown to emerge quickly during viral replication. Because host immune responses act on clouds (groups) of mutants rather than single sequences, these viruses have the ability to escape detection and elimination, and can develop existing drug resistance. Virulence and transmission will shift over time in ways which cannot be predicted. Currently, there is evidence of two distinct haplotypes of SARS-CoV-2.

Therefore, and exacerbated by the current lack of specific antivirals, instituting new treatment modalities to manage the severe respiratory manifestations of infection with these viruses will be critical for saving lives, especially given the potential for viral mutations and increased virulence over time. Clinical evidence suggests that if the D2000 were to be used at an early stage of cytokine storm response to COVID-19, the reduction of cytokines and endotoxins could reduce the inflammatory response in the various organs and systems. In particular, this reduction in the lungs and kidneys would reduce the onset of respiratory distress and ARDS, a pathology that has been observed in the majority of deaths in COVID-19 positive patients.

The use of the D2000 cartridge presents an extremely low risk and a high probable benefit based on its ability to reduce elevated cytokine levels, which strongly correlate with poor clinical outcomes.

Use of the D2000 may significantly reduce morbidity and mortality in the newly emergent COVID-19 pandemic.

Disclosures. Dr. Antonio Belli is a founder of Marker Diagnostics, a Marker, AG company.
Literature Cited

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