10.1161/CIRCULATIONAHA.120.047549

The Science Underlying COVID-19:

Implications for the Cardiovascular System

Running Title: *Liu et al.; Science & COVID-19*

Peter P. Liu, MD[1,2]; Alice Blet, MD, PhD[1,3,4]; David Smyth, PhD[1,2]; Hongliang Li, MD, PhD[5,6,7]

[1]University of Ottawa Heart Institute and University of Ottawa, Ottawa, Ontario, Canada;

[2]Departments of Medicine and Cellular & Molecular Medicine, University of Ottawa, Canada;

[3]Department of Anesthesiology, Critical Care and Burn Center, Lariboisière - Saint-Louis Hospitals, DMU Parabol, AP–HP Nord, University of Paris, France; [4]Inserm UMR-S 942, Cardiovascular Markers in Stress Conditions (MASCOT), University of Paris, Paris, France;

[5]Department of Cardiology, Renmin Hospital of Wuhan University, Wuhan, China;

[6]Medical Science Research Center, Zhongnan Hospital of Wuhan University, Wuhan, China;

[7]Basic Medical School, Wuhan University, Wuhan, China

Address for Correspondence:
Peter P. Liu, MD
University of Ottawa Heart Institute
40 Ruskin Street, Room H2238
Ottawa, Ontario, K1Y 4W7, Canada
Tel: 613-696-7000 x 19544
Fax: 613-761-4237
Email: peter.liu@utoronto.ca
or pliu@ottawaheart.ca

Abstract

Corona Virus Disease 2019 (COVID-19) pandemic has impacted health and economy worldwide on an unprecedented scale. Patients have diverse clinical outcomes, but those with pre-existing cardiovascular (CV) disease, hypertension, and related conditions incur disproportionately worse outcome. The high infectivity of the SARS-CoV-2 virus is in part related to new mutations in the receptor binding domain, and acquisition of a furin cleavage site in the S spike protein. The continued viral shedding in the asymptomatic and pre-symptomatic individuals enhances its community transmission.

The virus uses the ACE2 receptor for internalization, aided by TMPRSS2 protease. The tissue localization of the receptors correlates with COVDI-19 presenting symptoms and organ dysfunction. Virus-induced ACE2 down regulation may attenuate its function, diminish its anti-inflammatory role, and heightened angiotensin II effects in the predisposed patients.

Lymphopenia occurs early and is prognostic, potentially associated with reduction of the CD4+ and some CD8+ T cells. This leads to imbalance of the innate/acquired immune response, delayed viral clearance, and hyper stimulated macrophages and neutrophils. Appropriate type I interferon pathway activation is critical for virus attenuation, and balanced immune response. Persistent immune activation in predisposed patients, such as the elderly and those with CV risk, can lead to hemophagocytosis like syndrome, with uncontrolled amplification of cytokine production, leading to multi-organ failure and death.

In addition to the airways and lungs, the cardiovascular system is often involved in COVID-19 early, reflected in the release of highly sensitive troponin and natriuretic peptides, which are all extremely prognostic, particularly in those showing continued rise, along with cytokines such as IL-6. Inflammation in the vascular system can result in diffuse microangiopathy with thrombosis. Inflammation in the myocardium can result in myocarditis, heart failure, cardiac arrhythmias, acute coronary syndrome, rapid deterioration and sudden death.

Aggressive support based on early prognostic indicators with expectant management can potentially improve recovery. Appropriate treatment for heart failure, arrhythmias, acute coronary syndrome and thrombosis remain important. Specific evidence based treatment strategies for COVID-19 will emerge with ongoing global collaboration on multiple approaches being evaluated. To protect the wider population, antibody testing and effective vaccine will be needed to make COVID-19 history.

Key Words: Coronavirus; ACE2; severe acute respiratory syndrome; inflammation; myocarditis; vasculitis; heart failure; troponin; acquired immunity; cytokine release syndrome

Non-Standard Abbreviation and Acronyms

ACE	angiotensin converting enzyme
ACE2	angiotensin converting enzyme 2
ARB	angiotensin receptor blocker
BCG	Bacille Calmette-Guerin
BNP	brain natriuretic peptide
CRP	c-reactive protein
COVID-19	corona virus disease 2019
IRAK4	interleukin-1 receptor associated kinase 4

IL-1β	interleukin-1 beta
IL-6	interleukin 6
IRF-3	interferon regulatory factor 3
IRF7	interferon regulatory factor 7
MERS	Middle East respiratory syndrome
NK	natural killer
NLRP3	NOD-like receptor protein 3
PAI-1	plasminogen activator inhibitor-1
RAS	renin-angiotensin system
ROS	reactive oxygen species
SARS	severe acute respiratory syndrome
SARS-CoV	SARS coronavirus
SARS-CoV-2	SARS coronavirus 2 (causative agent of COVID-19)
TMPRSS2	transmembrane protease serine 2
TRAF6	TNF receptor associated factor 6
WHO	World Health Organization

The coronavirus infection COVID-19 first presented as an outbreak of atypical pneumonia in Wuhan, China on December 12, 2019[1, 2]. Since then it has spread globally to infect over 1,963,943 individuals and killed more than 123,635 in over 200 countries as of April 14, 2020. This infection has impacted health and the economy worldwide on an unprecedented scale.

Whereas COVID-19 is primarily a respiratory infection, it has important systemic effects including on the cardiovascular and immune systems. Patients with pre-existing cardiovascular conditions represent large proportions of patients with symptomatic infection, and experience disproportionately worse outcomes at between 5-10 fold increase in mortality (World Health Organization, WHO)[3].

While we are learning constantly about the changing epidemiology, the rapidly evolving underlying science, together with insights from previous coronavirus infections, such as SARS, can help us to better understand COVID-19, and in turn diagnose and treat our patients more insightfully.

Clinical Spectrum of Cardiovascular Involvement in COVID-19

In addition to increased propensity and worse outcomes for COVID-19 in patients with pre-existing cardiovascular diseases (Table 1), patients with new COVID-19 infections can also develop cardiovascular complications, such as heart failure, myocarditis, pericarditis, vasculitis, and cardiac arrhythmias[4, 5].

Between 8-28% of patients with COVID-19 infections will manifest troponin release early in the course of the disease, reflecting cardiac injury or stress[6-9]. The presence of troponin elevation, or its dynamic increase during hospitalization, confers up to 5 times the risk of requiring ventilation, increases in arrhythmias such as VT/VF, and 5 times the risk for mortality

(Figure 1)[5]. A similar proportion of patients also manifest elevations of natriuretic peptides (BNP). Troponin and BNP, together with the presence of underlying cardiovascular diseases or cardiovascular risk factors, are highly prognostic of requirement for ICU admission, ventilation and death.

Unique Properties of the Virus and the Disease Phenotype

SARS-CoV-2, the virus causing COVID-19, is a novel betacoronavirus (large RNA virus) that shares 80% sequence homology with the earlier SARS-CoV virus that caused the SARS outbreak in 2003[1]. The coronavirus surface features multiple spike glycoproteins (S) consisting of homotrimers protruding far from the viral surface, giving it a halo like appearance (or corona). The spike S protein is used by the virus to engage its target cell receptor, angiotensin converting enzyme 2 (ACE2) (Figure 2). The S protein has 2 subunits: S1 and S2, facilitating target cell internalization.

SARS-Cov-2 has evolved several features that make it a more efficient virus for infection than SARS-CoV. The most critical receptor binding domain of SARS-CoV-2 preserved the overall configuration of the SARS-CoV binding domain, including 8 of the 14 residues being *completely identical*[10]. However, the 3D structure of the SARS-CoV-2 binding site shows that it is more compact, has improved binding stability, and potentially enhanced ACE2 receptor binding affinity[11].

Another difference is that SARS-Cov-2 contains a polybasic (furin) cleavage site inserted at the boundary of the S1/S2 subunits of the spike S-protein[12]. This furin binding site is unique, can enhance the virus' ability to internalize into cells, and is a feature shared by several recent highly pathogenic, viruses including avian influenza.

Generally speaking, RNA viruses are prone to higher mutation rates, and viruses are

known to continue to mutate during an epidemic. Viruses continue to adapt to local environments to facilitate their transmission. The ability to track changes over geography and time can help us to better understand the disease pathogenesis, clinical phenotype variation, and its molecular epidemiology[13].

Clinical Implications

The high replication rate of the virus, especially in the human oral pharynx and upper airway where the ACE2 receptors are located, likely enhances its ability for efficient person to person transfer[14]. This allows shedding of the virus just with normal speaking or singing, without the need for coughing or sneezing, which may partially explain its infectivity.

The ability of the virus to proliferate and shed in completely asymptomatic individuals, including children and young adults, and also before symptoms occur (presyptomatic shedding), further increase its ability to transmit between individuals (Figure 1)[15]. In a series of 191 inpatients from Wuhan with COVID-19, the median duration of viral shedding was 20 days in survivors, with the longest viral shedding being 37 days, beyond symptom resolution, and SARS-CoV-2 was detectable until death in non-survivors[16].

Because of SARS-CoV-2's efficiency in viral transmission, aggressive control measures are critical for attenuating the COVID-19 pandemic. But with 25%-50% of infections being asymptomatic, traditional containment measures based on symptoms alone are less effective. Contact tracing based on accurate and rapid diagnostic tests will be necessary. When deployed effectively, these measures can avert hundreds of thousands of cases[17].

Patients with cardiovascular diseases are more prone generally to viral illness, and thus constitute already a high risk group. This is consistent with documented increases of acute myocardial infarction following influenza epidemics[18,19], even though this trend has not been

consistently observed with COVID-19.

Whether the virus can directly proliferate in the heart is unknown. There are very few pathology studies on COVID-19 patients. Previous analysis of human hearts in patients who died from SARS demonstrated that 7 of 20 (35%) hearts harbored virus in the myocardium[20]. Direct viral entry into the myocardium and blood vessels can certainly enhance the risk for myocardial injury, as well as subsequent inflammatory response. It is not known whether the observed cardiac damage is due to viral injury, or due to an immunological response impacting the myocardium and related structures, such as the pericardium and conduction system.

The Viral Receptor ACE2 and TMPRSS2 Contribute to the Disease Phenotype

ACE2 has been confirmed recently as the SARS-CoV-2 internalization receptor causing COVID-19[21], in concert with the host's TMPRSS2 membrane protease that primes the spike S protein of the virus to facilitate its cell entry[22]. ACE2 is the same functional receptor of the earlier SARS-CoV (SARS coronavirus) [23]. The presence of TMPRSS2 significantly enhances viral infectivity [24]. Protease inhibitors against TMPRSS2 appear to block effectively viral entry and infection of lung cells *in vitro*.

ACE2 is a type 1 transmembrane protein, with its enzymatic domain located on the external surface of cells where it carries out its function of converting angiotensin II (1-8) to angiotensin 1-7[25, 26]. The latter is a vasodilator, and a counter-regulator of the renin- angiotensin system (RAS). In stress states or pro-inflammatory conditions, membrane-bound ACE2 protein can be cleaved by the transmembrane dysintegrin ADAM17, releasing ACE2 into the interstitium or circulation, without depleting intracellular ACE levels[27]. The latter conditions are found more commonly in patients with heart failure and diabetes. Loss of ACE2 enhances susceptibility to heart failure, and increasing ACE2 levels prevent and reverse the heart failure

phenotype. In established heart failure, expression of ACE2 is downregulated, though the function may be upregulated. There is reduced expression on the cell surface but increases in circulating ACE2 levels.

ACE2 is expressed in the airway and type 2 pneumocytes in the lung. In models of diabetes, ACE2 can be found to be increased in renal tubules[28]. In humans, circulating ACE2, shed from endothelial cells is a biomarker of hypertension and heart failure[29, 30], as well as in diabetes[31], reflecting increased ACE2 activity (Figure 2).

Additionally, ACE2 has important immune modulation roles, acting through at least two mechanisms. ACE2 can directly interact with macrophages in the setting of vascular and lung inflammation[32], as demonstrated by genetic manipulation in a model of SARS, as well as by the salutary anti-inflammatory effects of infusion of recombinant ACE2[33, 34]. In addition, ACE2 reduces the levels of angiotensin II, which is directly pro-inflammatory and pro-oxidant. Therefore, ACE2 is important in controlling excess inflammation in the presence of danger signals[34].

Clinical Implications

TMPRSS2 and ACE2 facilitate SAR-CoV-2 entry, and the co-presence of these two molecular entities in tissues to a large extent explains the tropism of viral proliferation. TMPRSS2 and ACE2 are co-expressed in lung, heart, gut smooth muscle, liver, kidney, neurons and immune cells[35]. Their distribution may help to explain patient symptoms or laboratory findings in COVID-19 [Table 2].

Interestingly, circulating ACE2 levels in patients is sex dependent, being 50% higher in males than in females in HF[29, 30]. Whereas circulating levels do not directly reflect tissue levels, ACE2 is shed as part of the tissue response to stress thereby up-regulating ACE2 function. Of

note, the ACE2 gene is located on the X chromosome, such that females have two copies of the ACE2 gene, compared to a single copy in males. Whether this ACE2 gene polymorphism is functionally relevant is unknown. Another intriguing association is the fact that in COVID-19 infections the death rate of males compared to females is much higher (Table 3), despite adjustment for differences in risk factor profiles[36].

Previous investigations of ACE2 polymorphism in SARS susceptibility or outcomes did not reveal significant linkages. However, this attribute is still unknown in the setting of COVID-19 infection. There appears to be different population outcomes observed to date (e.g. higher mortality in Italy vs lower mortality in Germany), but this may be related to many other factors, such as age, health system organization, virus testing, social behaviour, etc. Whether this may be related to different distributions of functional ACE2 polymorphism in different populations, awaits further studies [37].

Virus-Receptor Interaction and Potential Consequences

During virus engagement of the ACE2 receptor in the presence of TMPRSS2, the virus can enter the target cell through endocytosis or membrane fusion. The positive strand viral RNA is then transcribed by the host cell ribosome while also being transported to the endoplasmic reticulum to mediate transcriptional activation and production of viral component proteins. These are ultimately assembled into intact viruses and discharged from the cell. This process can disable or destroy the host cell, leading to the release of potential danger signals to activate the host's innate immune responses.

There is also evidence in preclinical models using the SARS-CoV virus of significant downregulation of ACE2 in the heart as a result of virus engagement of the ACE2 receptor during infection[20]. This is likely part of the host defense mechanism in response to the infection,

to limit continued viral proliferation. However, the potential consequence of this interaction is that the biologically essential role of ACE2 is also significantly diminished. This can lead to unopposed angiotensin II effects, including pro-inflammatory, prothrombotic and pro-oxidant risks.

This virus does proliferate, likely at low levels, in the host's heart, possibly involving inflammatory responses, as troponin can be released very early during disease presentation and portends poor prognosis[7]. This may lead to further release of potential danger signals to the immune system to trigger downstream exaggerated responses. However, no replicable SARS-CoV-2 virus has been recovered in blood, thus the quantity of replicated virus in the circulation is not high, in contrast to that present in the oral and respiratory systems[14].

There has been much controversy regarding the role of RAS-interfering agents, such as ACE inhibitors or angiotensin receptor blockers (ARB's), on the levels of ACE2 expression, hence susceptibility to SARS-CoV-2 infection. There have been good review articles on this controversy and will not be repeated here[38, 39].

Clinical Implications:

The initial phase of this virus infection can be marked by evidence of cardiac injury with the release of troponin. This portends a poor prognosis. Whereas the release of troponin is relatively modest, this may be an indication of either viral or immune-mediated cardiac injury. The release of "danger signals" from the heart in patients with heightened immune response can further amplify myocardial damage. Patients with continued increases in biomarker release are usually marked by an amplified inflammatory response and worse outcomes. Thus, patients with cardiac injury or stress marker release warrant more careful monitoring and institution of cardioprotective agents to minimize ongoing damage.

Down-regulation of ACE2 with viral infection may predispose to relatively unopposed angiotensin II effects, such as hypertension, enhanced inflammation and thrombosis (Figure 2)[34]. Whereas there are no definitive data on the risk versus benefit of ACE inhibitors or ARBs in COVID-19, most cardiac professional organizations have recommended continuation of RAS inhibitors in patients who have been prescribed them. In fact, there is cautious emerging data to suggest that the benefit may outweigh the risk in patients with COVID-19 and hypertension when prescribed ACEi or ARB. However, ongoing randomized trials will help to provide definitive answers to these questions.

The virus-receptor interaction step also provides many opportunities for potential intervention, and a number of therapeutic trials are currently ongoing. Infusion of recombinant human ACE2 may act as a decoy to interfere with viral replication. Chloroquine or hydroxychloroquine may interfere with cellular endocytosis of the virus, and viral proliferation can be blocked at multiple stages, including inhibition of RNA polymerase with remdesivir, amongst many others.

Virus-Receptor and the Immune System Interactions

The SARS-CoV-2 infection can generate a diverse range of responses in patients, ranging from completely asymptomatic virus shedding to a severe inflammatory response including cytokine storm-like outcomes that are accompanied by high mortality. Current epidemiology suggests that 81% of infected individuals have mild symptoms, 14% have severe symptoms requiring hospitalization, while 5% become critically ill requiring ventilation. The differences in response are likely the result of degree of viral load, host immune response, age of the patient and presence of co-morbidities. This is similar to many types of respiratory infections including the flu.

In terms of cardiac manifestations of infection, the spectrum is also similar to conditions such as viral myocarditis. After infection with common RNA viruses such as coxsackievirus, most exposed patients may experience only a transient viral syndrome with no significant cardiac dysfunction. However, those with an exuberant immune response can manifest acute myocarditis with heart failure or cardiogenic shock, accompanied by hypercytokinemia and inflammatory cell infiltration of the heart[40]. With support the patients can recover, but some persist with inflammatory cardiomyopathy[41].

Analysis of the inflammatory response to SARS-CoV-2 is relatively limited. A consistent finding is lymphopenia that occurs in over 80% of patients. The degree of lymphopenia is a very important prognostic indicator early in the course of infection. Among the most prominent findings in early analyses of patients succumbing to COVID-19 are marked reductions in circulating levels of CD4+ and CD8+ T lymphocytes, and a relative dominance of mononuclear cells (monocytes and macrophages) in target injury tissues, where the lung was primarily assessed (Figure 1)[42, 43],

In a parallel with the SARS infection, in which lymphopenia was also observed to be highly prognostic, reports showed an early reduction in T cells, in particular a reduction in CD4+ more than CD8+ T cells[44]. Recovery of lymphocyte count coincided with clinical improvement. Pathology in select SARS patients revealed the spleen featuring atrophic white pulp with lymphoid depletion (without local viral signatures), whereas the bone marrow appeared to show normal activity[45]. This suggests that T cells (possibly CD4+) are selectively destroyed, possibly by the immune system, though one cannot rule out the possibility of direct viral infection of T cells.

The important role of CD4+ T cells was further delineated in a primary infection model

with SARS-CoV in senescent mice. CD4+ T cells were found to enable production of neutralizing antibodies and a balanced immune response. Without CD4+ T cells, there was much more severe interstitial pneumonitis. When both CD4+ and CD8+ T cells were depleted, there was a predominance of neutrophils and innate immune macrophages in the lesions[46].

Accompanying the loss of CD4+ T cells, there is an unusual macrophage predominance in SARS lung infiltration. This can be accompanied by *hemophagocytosis* in lung and spleen, compatible with severe immune cytokine dysregulation[47]. This syndrome results from the ineffective activation of cytotoxic CD8+ T lymphocytes and Natural Killer (NK) T lymphocytes, resulting in ineffective viral clearance and weak antibody production. This, in turn, stimulates further macrophage activation and the loop of self-amplification can be uncontrolled, leading to cytokine-storm syndrome and multi-organ failure. Indeed, patients who died from COVID-19 infection show continued virus shedding at the time of death[16].

Other indications that SARS-Cov-2 induces a relatively mild immune response is the fact that there is prolonged virus shedding in many individuals. Virus proliferation is extremely rapid in COVID-19, yet many patients are asymptomatic. This suggests that while the immune system is mounting a response, it is not adequate to attenuate viral replication potential. In one study of serial viral and immunological monitoring in hospitalized patients, the virus was capable of inducing IgM and IgG. But viral proliferation is not affected, suggesting lack of neutralizing effect and ineffective early viral clearance[14].

To gain insight into the successful attenuation of the virus without severe disease, we may search for clues in nature. In bats, where the virus may have originated, it is able to reside at low levels chronically without severe disease. The bat immune system has much lower NLRP3 inflammasome activation, thus limiting an excessive inflammatory response, with lower

levels of IL-1β. It also has an NK cell repertoire with dominant inhibitory signaling. Furthermore, bats manifest an enhanced response to infection with 22 interferon-ω genes[48], in coordination with a continuously activated IRF3 leading to IRF7 up-regulation[49], providing adequate amount of type I interferons. This overall general anti-inflammatory milieu while effectively controlling infections has been invoked to explain the extraordinary lifespan of bats (30-40 years) for their body size.

Type I interferon production is important for innate defense against SARS-CoV, as demonstrated by protection in a rhesus macaque infection model[50] as well as in limited clinical studies. Several studies of SARS and related Middle East Respiratory Syndrome (MERS), also the result of a coronavirus infection, have illustrated the importance of type I interferon to potentiate effective antiviral immunity. Murine modeling and clinical evidence suggest interferon alpha production, mainly by plasmacytoid dendritic cells, is necessary for control of viral infection[51]. In MERS studies, type I interferons were found to be rapidly upregulated[52], whereas successful clearance of MERS has been suggested to be dependent on appropriately rapid induction of high levels of Type I IFNs[53].

Conversely, in the ageing immune system, there is progressive lymphopenia with CD4+ T cell attrition and decreased regulatory T-cell function, leading to homeostatic lymphocyte proliferation, with propensity for autoimmune and excessive inflammatory responses[54]. This can be compounded by decreased capacity to phagocytose apoptotic cells by senescent macrophages, leading to a general pro-inflammatory state. The imbalanced aged immune system is then exacerbated by infection such as COVID-19, which further exacerbates the depletion of CD4+T cells and inflammatory macrophage response. The net consequence of inadequate interferon response and inefficient viral clearance therefore is detrimental to the patient, with development

of inappropriate cytokine storm, inadequate sustained immune response and lack of effective formation of immunologic memory. Consistent with this phenomenon, studies in an aged mouse model showed that mice produced increased levels of immunosuppressive prostaglandin D2, leading to impairment of dendritic cell recruitment and reduced T cell function.

Clinical Implications

In patients with COVID-19 infection, in addition to lymphopenia, there appears to be heightened level of IL-1β inflammatory response, particularly in those with a poor prognosis. As the infection progresses, building on IL-1β elevation, there is also increasing production of IL-6, which can presage an impending cytokine storm.

In patients with potential dysfunctional immune responses there are early warning signals, such as lymphopenia, troponin release, elevated BNP, rising inflammatory markers such as cRP, IL-1β and IL-6; these patients should be followed closely, monitored for organ failure, with efforts made to restore immune balance. If viral proliferation is still continuing, strategies to attenuate the virus may be critical. The ability to restore immune balance, with approaches such as type I interferon, immunoglobulin, and recovered serum, may be considered. Utility of anti-inflammatory strategies, whether anti-IL-1 or anti-IL-6 approaches, will be best determined in time through randomized trials. However, intervention will likely need to be instituted early, before the immune amplification process is fully underway.

Impact of Advanced Disease on the Microvascular System and Coagulation

Vascular smooth muscle has both ACE2 receptor and TMPRSS2 protease to facilitate local viral entry and proliferation. Pathological evaluation of lung tissue and other affected organs has uncovered evidence of microvascular inflammation together with microvascular thrombi. There have also been clinical observations of distal vasculitis with acrosyndrome and dyshydrosis in

terminal digits of patients with COVID-19. These cutaneous vasculitis signs have been discussed as early indications of SARS-CoV-2 infection.

Activated macrophages can release cytokines, including IL-1β and IL-6, which will promote expression of adhesion molecules for endothelial activation, inflammatory cell infiltration and vascular inflammation. This may be locally enhanced if there is smooth muscle cell harboring of viral proliferation and cellular damage. Endothelial cells release pro-inflammatory cytokines that contribute to propagation of microcirculatory lesions[55]. The dysfunctional endothelium becomes pro-adhesive and pro-coagulant[56]

Localized macrophages can also release pro-coagulant factors such as plasminogen activators. With the retreat of ACE2 and activation of angiotensin II, the production of downstream of PAI-I is also enhanced. This combination further accelerates vascular inflammation, and enhances a prothrombotic state. This is often seen in patients with advanced disease with laboratory evidence of increases in IL-6 together with d-dimer elevation.

The presence of microangiopathy and microthrombi can also predispose the patient to micro-infarcts within multiple organs, such as the liver, heart or kidney, further exacerbating the state of multi-organ injury and failure.

Clinical Implications

The presence of vasculitis and prothrombotic state can lead to increased frequency of pulmonary embolism which worsens hypoxemia by increasing shunting in these already highly hypoxemic patients with ARDS. This, in combination with systemic inflammatory or cytokine storm, can worsen cardiac injury, heart failure and the prognosis.

Patients with clinical evidence of vasculitis, or laboratory indicators of progressive inflammation, such as rising IL-6 and/or d-dimer levels, should be considered early for anti-

inflammatory measures. Many of these patients should also be considered for full anticoagulation, such as heparin, depending on individual risk versus benefit. Patients can also be enrolled in ongoing clinical trials evaluating different mitigation strategies.

Impact of COVID-19 on the Heart

Between 8-28% of patients with COVID-19 infections show evidence of cardiac injury with elevated troponin[7]. In a series of patients from Seattle, the first major COVID-19 center in the U.S., several patients presented with cardiomyopathy[57]. Patients with evidence of cardiac involvement had a marked increase in mortality, confirming the major impact of the cardiovascular system in the prognosis of these patients.

Many patients with COVID-19 infections die from cardiac arrest, likely as a result of a combination of primary cardiac involvement, or manifestation of systemic involvement such as severe hypoxia, multi-organ dysfunction syndrome, or systemic inflammatory response syndrome, amongst others.

In the 2003 SARS outbreak, Oudit from our group demonstrated SARS-CoV viral presence in 7 of 20 SARS patient hearts on autopsy[20]. Viral proliferation was likely enabled by ACE2 and TMPRSS2 receptor expressions. However, there was also reduced expression of ACE2 following infection, likely contributing to the enhanced inflammation.

We have demonstrated previously that viral infection of the heart, modeled with another RNA virus, coxsackievirus, can activate the intrinsic innate immune system very rapidly[58, 59]. However, the balance of the immune response determined the ultimate outcome[60-62]. Downstream activation of toll-like receptor signal regulators, such as IRAK4 leading to TRAF6-NF-kB activation, can modify monocyte migration and accelerate myocarditis[63, 64]. On the other hand, early enhancement of IRF3-IRF7-type I interferon production can mediate more viral

attenuation while reducing overall inflammation[64]. This can also be enhanced by augmenting the regulatory T cell population, while allowing adequate interferon production[65]. Therefore, the balance of NF-kB- vs IRF3-related signal amplification, and that between the innate and acquired immunity, are critical to outcome and recovery[64].

Clinical Implications

The early presence of cardiac injury and stress, as evidenced by biomarkers of elevated troponin and/or natriuretic peptides, are important to ascertain especially in higher risk patients, and appropriate expectant treatments to monitor and prevent cardiac and systemic complications are warranted. Enrolling these high risk patients into therapeutic trials will be the vital next step.

A small proportion of patients may have direct cardiac involvement including cardiomyopathy, myocarditis or heart failure. Again these features have a major influence on the patient's overall outcome. As the inflammatory response is self-amplifying, the early recognition and attenuation can have potentially important impact on outcomes.

In patients with suspected acute myocarditis or inflammatory cardiomyopathy, appropriate investigations, such as magnetic resonance imaging where feasible and safe to perform, can be helpful[4, 66]. In patients with heart failure, appropriate heart failure medications, including RAS inhibitors, should be considered.

Arrhythmias, such as atrial fibrillation, are also more frequent in COVID-19 cardiomyopathy, occurring in up to half of patients admitted to an ICU, as inflammation is a substrate for atrial arrhythmias[67]. Ventricular arrhythmias are also observed and may accompany cardiac arrest in these patients. QT prolongation will need to be monitored, as this may occur as a combination of myocarditis and also concomitant side effects from medications such as chloroquine and hydroxychloroquine. Appropriate use of anticoagulants evaluating risk vs

benefit will be important.

Potential Therapeutic Opportunities

Since COVID-19 is a new disease, there are currently no proven treatments. There are good evidence-based websites collating up-to-date information: [https://covdb.stanford.edu/search/?study=clinical-studies&virus=SARS-CoV-2]. The medical community, biotechnology and pharmaceutical partners have already come together and proposed over 500 ongoing clinical trials to date, such as [https://www.transparimed.org/single-post/2020/03/27/COVID-19-clinical-trials-information-sources] or https://clinicaltrials.gov.

The most important public health solution is an effective vaccine for the broad population remaining at risk. The spike glycoprotein S of SARS-CoV-2 is an ideal antigenic target for vaccine development, and there are multiple candidates now beginning to enter clinical trials.

A very partial list of potential targeted processes, and examples of candidate agents now entering clinical trials is provided in Table 4.

Potential Long Term Consequences and Remaining Questions

While the pandemic is evolving its course, there will be a growing population of recovered patients. The majority will do well. However, many unanswered questions remain. Will exposed patients have adequate long-term immunity? Does the IgG produced have adequate neutralization capacity?

Those with complications may have a more challenging course of recovery and long term sequelae. The immune activation and dysfunction can lead to target tissue fibrosis and microangiopathy, as was observed in some patients following SARS. This can affect long-term lung function, or if the heart was involved, residual cardiomyopathy. There was also increased cardiometabolic risk reported in patients who recovered from SARS, possibly related to steroid

treatment and ongoing RAS imbalance[68].

From an epidemiological point of view, an important question is how many people in the population ultimately acquire immunity to SARS-CoV-2 as antibody testing becomes more available. Will there be an effective vaccine for the remaining unexposed population? Will there be continued mutation of the virus? Does the virus have a natural reservoir? Are there enough asymptomatic carriers that can restart another infectious cycle?

Global Collaboration to Advance Science and Medicine on COVID-19

These are unprecedented times in health and medicine worldwide. However, the pandemic has also brought together the medical community to share information and seek rapid solutions for so many patients. It has also underscored the critical importance of science and data-driven decision making in times of uncertainty.

Much more will be learned about COVID-19 in ensuing months and years. We are only at the beginning of this journey together.

Acknowledgments

We thank the team members of Ottawa Heart Institute Heart Function Lab for key contributions to this manuscript, including Dr. Gauri Akolkar, Dr. Mohammad Al-Khalaf, Dr. Qiujiang Du, Dr. Hanbin Lin, Erin Liu, and Dr. Liyong Zhang.

Sources of Funding

This work is partially supported by research grants from the Canadian Institutes of Health Research, the Heart & Stroke Foundation and Research Funds from the University of Ottawa of Heart Institute.

Disclosures

There are no conflicts of interest to declare.

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Table 1. Death rate to date of patients with COVID-19 infection and specific pre-existing conditions (WHO Data)

PRE-EXISTING CONDITION	DEATH RATE
Cardiovascular disease	**10.5%**
Diabetes	**7.3%**
Chronic respiratory disease	**6.3%**
Hypertension	**6.0%**
Cancer	**5.6%**
no pre-existing conditions	**0.9%**

Table 2. Distribution of ACE2 and TMPRSS2 in organs, and symptoms of COVID-19 (percentage indicate estimated frequency in COVID-19 patients).

ACE2/TMPRSS2 Distribution	**Symptoms/Lab Findings**
Lymphocytes/Dendritic Cells	Fever (>99%), fatigue (70%), myalgia, lymphopenia
Lung (type 2 pneumocytes, bronchial epithelium)	Dyspnea (31%), dry cough (60%), respiratory failure
GI Smooth Muscle	Nausea (30%), Diarrhea
Myocardium	Myocarditis, heart failure, arrhythmias
Vasculature (smooth muscle)	Vasculitis, thrombosis, microangiopathy
Neurons	Anosmia, hypogeusia, encephalopathy, seizures, myopathy
Liver	Abnormal liver function
Kidney	Renal dysfunction

Table 3. Mortality in confirmed cases, or in all cases, according to sex distribution in COVID-19 patients.

SEX	DEATH RATE confirmed cases	DEATH RATE all cases
Male	**4.7%**	**2.8%**
Female	**2.8%**	**1.7%**

Table 4. Potential processes in COVID-19 infection amenable to therapeutic targeting, with examples of candidate agents

Potential Targeted Process	Candidate Agent
Antiviral/anti-inflammatory general	Convalescent serum (COVID-19 patients), type I Interferon, immunoglobulins, mesenchymal stem cells
ACE2 entry	Soluble recombinant ACE2
TMPRSS2 Protease S Priming	Protease inhibitor (camostat mesylate)
Receptor endocytosis	Chloroquine or hydroxychloroquine
RNA polymerase for replication	Remdesivir, favipiravir
Viral proteases	Lopinavir/vitonavir
Importin nuclear transport	Ivermectin
IL-1 excess activation	Anakinra, canakinumab, colchicine
Angiotensin II excess	ACE inhibitors / ARB, recombinant ACE2
Cytokine storm	Torcizumab, sarilumab or siltuximab (IL-6 inhibitors) or baricitinib (JAK inhibitor), Lenzilumab (GM-CSF inhibitor)
Oxidative stress	Deferoxamine, Vitamin C
Fibrosis	Nintedanib
Bacterial infection/Inflammation	Azithromycin
Coagulopathy	Normal or high dose anticoagulation regimen
SARS-CoV-2	Multiple vaccine candidates, including BCG

Figure Legends

Figure 1. Clinical course of COVID-19 infection. The incubation period averages 7 days, but can be up to 14 days. There can be asymptomatic, presymptomatic or postsymptomatic viral shedding, likely contributing to its rapid transmission. Cardiac biomarkers such as high sensitivity troponin (hsTroponin) can be detectable in patients at symptom onset and is prognostic. Continued increases in troponin together with rising cytokines predict need for ICU stays, ventilation, and vascular complications. Together with cytokine rise, NTproBNP rise can predict risk of myocarditis or heart failure. Lymphopenia, with suppression of T cells and inefficient viral clearance, set the stage for over-stimulated macrophages, cytokine amplification, and hemophagocytosis with organ failure, including the heart (CRP=c-reactive protein, BNP=brain natriuretic peptide).

Figure 2. SARS-Cov-2 uses the ACE2 internalization receptor, facilitated by TMPRSS2 protease. ACE2 can be shed in the circulation, and ACE2 is increased in patients with hypertension, heart failure, or diabetes. ACE2 can be down-regulated following viral entry. This partial decrease in ACE2 function leads to dominant angiotensin II effects, including enhanced inflammation, vasoconstriction and propensity for thrombosis. This can also worsen heart failure (TMPRSS=transmembrane protease, serine 2, ROS=reactive oxygen species).