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Pathophysiology of infection with SARS-CoV-2—What is known and what remains a mystery

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Abstract

Coronavirus disease 2019 (COVID-19), caused by coronavirus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has caused extensive disruption and mortality since its recent emergence. Concomitantly, there has been a race to understand the virus and its pathophysiology. The clinical manifestations of COVID-19 are manifold and not restricted to the respiratory tract. Extrapulmonary manifestations involving the gastrointestinal tract, hepatobiliary system, cardiovascular and renal systems have been widely reported. However, the pathophysiology of many of these manifestations is controversial with questionable support for direct viral invasion and an abundance of alternative explanations such as pre-existing medical conditions and critical illness. Prior research on SARS-Co-V and NL63 was rapidly leveraged to identify angiotensin-converting enzyme 2 (ACE2) receptor as the key cell surface receptor for SARS-CoV-2. The distribution of ACE2 has been used as a starting point for estimating vulnerability of various tissue types to SARS-CoV-2 infection. Sophisticated organoid and animal models have been used to demonstrate such infectivity of extrapulmonary tissues in vitro, but the clinical relevance of these findings remains uncertain. Clinical autopsy studies are typically small and inevitably biased towards patients with severe COVID-19 and prolonged hospitalization. Technical issues such as delay between time of death and autopsy, use of inappropriate antibodies for paraffin-embedded tissue sections and misinterpretation of cellular structures as virus particles on electron micrograph images are additional problems encountered in the extant literature. Given that SARS-CoV-2 is likely to circulate permanently in human populations, there is no doubt that further work is required to clarify the pathobiology of COVID-19.

KEYWORDS
COVID-19, pathophysiology, SARS-CoV-2, transmission

INTRODUCTION

The spread of coronavirus disease 2019 (COVID-19) across the world has led to an explosion of publications related to COVID-19. Over 65% of these publications were however not based on original data (i.e., viewpoints, editorials, perspectives or expert opinion), with original studies (14.9%), case reports (9.3%) and research letters (10%) comprising the remainder.[1] Sixty percent of published articles have been posted on preprint servers, which have the advantage of easy access, easy feedback and fast dissemination,[2] but this increase in publication has also been associated with increased numbers of articles retracted. Of the top 50 cited publications, there are two related to the clinicopathological aspects of this review—the detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in different specimens and the lung pathophysiology of fatal COVID-19.[3,4]

The intention of this review is to summarize and consolidate the clinical and pathological changes seen in COVID-19; however, one should be mindful that most publications have dealt with hospitalized patients. This is important because this population as a whole has varied admission rates depending on regional, societal, seasonal and political factors, and thus much of what is reported in the medical literature is but the tip of the clinical COVID-19 iceberg.

Another challenge with performing a review is that most of the accessed articles in December to February 2021 were

published in a timeframe based on data collated and obtained from the first 'wave' of the pandemic. Since the emergence of the 'UK', 'South African' or 'Indian' variants of SARS-CoV-2, it remains to be seen to what extent the putative organ dissemination and pathophysiology of the original strain reviewed in most of these publications will be seen in 2021.

PORTALS OF ENTRY

Nasal and oral

The seasonal coronaviruses that are ubiquitous in the general population are associated with upper respiratory tract and nasal symptoms, so it is not surprising that this anatomical site is one of the main portals of entry of coronavirus into the body; however, one of the features that distinguishes COVID-19 from other seasonal coronaviruses has been the relative lack of typical nasal symptoms, such as rhinitis and sneezing, but in contrast to SARS and Middle East respiratory syndrome (MERS) infection, there is a high frequency of anosmia, implying involvement of the olfactory epithelium.[5]

The viral dynamics of COVID-19 in the nasal mucosa will be detailed elsewhere[6] but in general the infected individual can be asymptomatic for up to 5 days after infection, with a high viral load and infectivity in this period. There is a peak at days 5–7 post onset of symptoms.[7] After day 15, the probability of culturing live virus in severe and critically ill or immunocompromised patients is less than 5%, but there may be prolonged shedding in individuals who are of older age, and, or, have medical comorbidities, immunosuppressive conditions, severe disease, delayed hospitalization and are managed with steroids.[8]

The high viral load in the pre-symptomatic phase is in contrast to SARS, and has presented one of the major challenges in reducing and mitigating transmission of viruses from individual to individual. The high frequency of anosmia indicates that viral replication involves the olfactory mucosa, and as this anatomical site is more posterior in the nose, it means that rapid antigen testing will need a more intrusive sampling than just swabbing the anterior nasal cavity. Viral loads are higher in nasal swabs than throat swabs.[9] However, certain nasal pathology such as polyps or deviated nasal septum can lead to a false-negative result, thereby creating an additional challenge for pathologists. The main surface receptor mediating SARS-CoV-2 cell entry is angiotensin-converting enzyme 2 (ACE2) and the serine protease transmembrane protease, serine 2 (TMPRSS2) is important for priming the viral spike protein. As such, expression of ACE2 and TMPRSS2 in various tissues is often taken as evidence that particular organs can potentially support SARS-CoV-2 replication. In an unsolicited review,[10] a high expression of ACE2 and TMPRSS2 was detected in the nasal epithelial cells, and also appeared to be in high expression on the tongue. In the oral cavity, there is a paucity of definitive clinical symptoms with taste alterations, blisters, ulcers and Kawasaki disease reported.[11] A large number of studies have proposed the salivary glands could be potential reservoirs for SARS-CoV-2 based on immunohistochemical detection of ACE2[12–17] or the presence of sialadenitis in early stages of COVID-19 infection.[15] Presently, there are no data to indicate that virus replication can be detected in this site, and ex vivo studies of human explants did not show infection of minor salivary glands.[18]

Within the olfactory mucosa, there has been much attention focused on the sustentacular cells as a target for viral entry.[19] ACE2 has been identified on the motile cilia of the airway epithelial cells,[20] but the use of angiotensin-converting enzyme inhibitors or receptor blockers did not increase susceptibility to infection in humans. The direct infection of the epithelium by the virus has been blamed for the loss of smell,[21] and in 25% of patients this anosmia may fail to resolve.[9] In the laboratory setting, despite a large concentration of viral antigen in the olfactory mucosa of hamsters,[22] there is no involvement of the olfactory nerve as a potential route of transmission to the central nervous system. In hACE2 transgenic mice infected with SARS, there was involvement of the olfactory bulb, but this was not seen in infection with SARS-CoV-2, even though infection of the sustentacular cells was documented.

Ocular

The importance of the eye as a site for viral entry, spread to the upper respiratory tract via the lacrimal duct or transmission to other individuals has been the subject of a number of well-written and comprehensive reviews.[23–26] Ocular involvement of other respiratory viruses, including the seasonal coronaviruses has been well documented,[27] but there have been conflicting results on whether the conjunctiva or other ocular tissues express ACE2.[28–30] Patients with clinical COVID-19 have presented with folliculitis, keratoconjunctivitis, ocular pain and discharge,[31] but these are thought to reflect a publication bias. Ex vivo conjunctival tissues were reported to be susceptible to infection[18]; however, it was commented in a reply to this article that the study was an artificial laboratory setting which may not exist in the in vivo setting. Sampling of tear fluid for virus is reportedly unreliable.[26] The conclusion from most of these reviews is that the ocular route of entry is low, with insufficient evidence to provide a conclusive statement on this portal. Despite this low potential risk, professional bodies such as the American Academy of Ophthalmology advise the use of goggles and face shields.[27] In fact, the use of face shields has become widespread clinically.

Respiratory

At the beginning of the COVID outbreak in 2020, the main concern was how severe this emerging disease was. In particular, would this outbreak have the same manifestation and pathology to that seen in two of the three recently

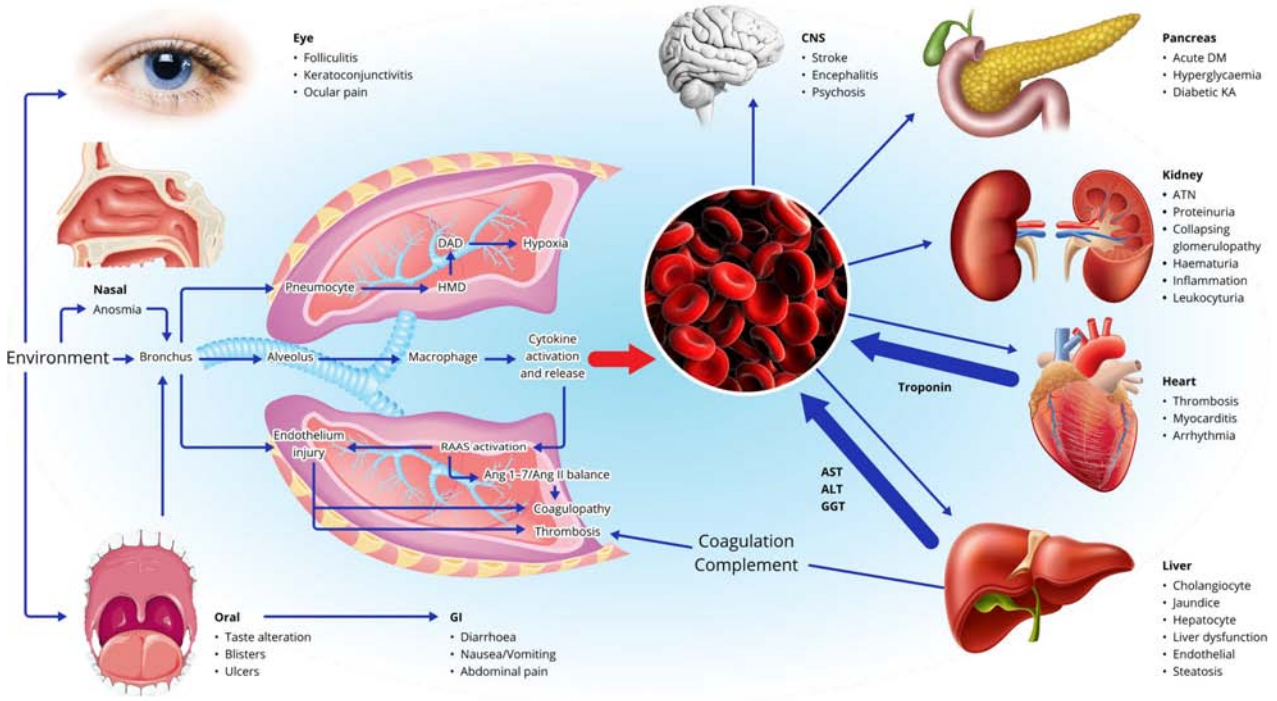

FIGURE 1 Simplified schematic of proposed pathological changes in severe acute respiratory syndrome coronavirus 2 infection. The three main portals of entry into the respiratory tract are through the eye, nasal cavity and oral route, with the latter also leading to infection of the gastrointestinal tract. In the respiratory tract, infection of pneumocytes leads to exudation of fibrinogen and hyaline membrane formation, followed by diffuse alveolar damage with hypoxia. Stimulation of macrophages and bronchiolar epithelial damage causes cytokine release into the alveolar spaces and into the blood. Either virus infection of endothelium or cytokine release activates the renin–angiotensin–aldosterone system, producing a pro-thrombotic tendency, with the formation of thrombi, mainly in the pulmonary vasculature. Either viraemia or cytokinaemia in the systemic circulation damages the brain, pancreas, kidneys, heart and liver producing a number of organ-specific changes, in addition to the increased thrombotic tendency. The multi-system damage is manifest by elevated troponin and liver enzymes in the blood, and the release of factors aggravates the pro-thrombotic tendency

described coronavirus infections in the past 20 years—SARS and MERS, or follow a similar pattern to the third emerging coronavirus NL63, with mild respiratory disease? Initially, it was thought that infection was limited to the respiratory tract, but with the rapidly increasing number of cases, it became evident that this was a respiratory virus with multi-system complications (Figure 1) As lung biopsies were rarely performed in the SARS outbreak, the respiratory pathology was based on post-mortem/autopsy material. Three chronological histological patterns were thus identified: an exudative phase characterized by hyaline membrane formation and pulmonary oedema, followed by a proliferative phase of Type II pneumocyte hyperplasia, with or without giant cell formation, then an organizing phase of fibrosis and vascular proliferation, together with squamous metaplasia in the conducting airways.[32] As the standard treatment in many institutions in the SARS outbreak was the use of steroids to reduce the 'cytokine storm', patients with prolonged disease had secondary bacterial or fungal infection.

Once antibodies to SARS-CoV nucleoprotein were available, these demonstrated antigen in the first 14 days, but little evidence of positive staining after that, indicating that the proliferative phase and organizing phase were secondary to viral-induced pneumocyte damage.[33] Review of the respiratory pathology caused by H1N1, H2N2, H7N9 and H5N1 influenza viruses showed that there were similar morphological changes in the lung in these conditions to that seen in SARS. Thus, it was proposed that there were no unique morphological changes seen in SARS infection compared to severe influenza, and that what was seen was a similar pattern of cellular damage followed by tissue repair.[34]

The first reports of COVID lung pathology appeared in February and March 2020 in two publications involving seven patients,[35] but these were based on limited autopsy material. These reports confirmed that the early changes were very similar to SARS, with the presence of hyaline membranes in the alveolar spaces with a variable degree of oedema present.

As the pandemic spread to involve more countries and the long-term temporal course of disease was better appreciated, there were seven autopsy publications in April 2020, and 12 more in May, in total involving 97 patients, where the early changes of oedema and diffuse alveolar damage progressed to an organizing pneumonia and repair within the lung.[35] However, in this period, two features emerged which would be different from that reported in SARS. The first was that, owing to the lessons learned from the SARS outbreak, there was less use of steroid treatment, and so secondary opportunistic infection was not as prevalent. The second, and probably more important feature from a clinical

point of view, was a reporting of the presence of fibrin and organizing thrombi in the vessels in the pulmonary vasculature,[36] raising the possibility that there was damage to the pulmonary endothelium, with the pulmonary thromboemboli detected in a number of post-mortem and antemortem cases.[37–39] This thromboembolism was noted in the SARS cases, but was not as pronounced as that seen in COVID infection, possibly because of the limited numbers of fatalities with autopsies performed in SARS.[35] Once antibodies that were reliable for formalin-fixed tissues became available, studies performed by the US Centers for Disease Control and Prevention[40] and National Institutes of Health[41] showed positive staining in alveolar epithelium, hyaline membranes and epithelial cells in the conducting airways. Although co-localization of viral antigen with the endothelial marker CD31 has been reported, detection of virus in endothelial cells has been challenging, with occasional reports showing co-localization of viral antigen with endothelial markers, but in these positive areas there is no thrombosis or vascular damage noted.[42,43]

The lung pathophysiology model which was developed after SARS proposed that Type I pneumocytes would become infected and damaged, leading to a loss of the integrity of the basal layer, fibrin exudation and hyaline membrane formation. The Type II pneumocytes would then differentiate to replace the Type I pneumocytes. Since that time, there has been the emergence of new concepts of lung repair and regeneration, where even though the Type II pneumocytes may replace damaged Type I pneumocytes, there exists a population of Krt-5-positive basal cells which may also replace the Type I pneumocytes.[44,45] If the SARS paradigm is relevant for COVID-19, then the initiating event would therefore be demonstration of viral antigen in Type I pneumocytes. Unfortunately, antibodies that are definitive for Type I pneumocytes rather than Type II pneumocytes (such as caveolin) have not been routinely used in the autopsy studies for COVID infection, and thus many publications have based tropism on cellular morphology combined with immunohistochemistry (IHC)/ISH-positive cells. One publication showed co-localization of SARS-CoV-2 antigen with Thyroid transcription factor 1 (a marker of Type II pneumocytes and club cells) and also in macrophages of the lung, in p63-positive basal cells and ciliated cells, but not MUC5AC-positive mucus-secreting cells.[46] In this study, there was no report of endothelial cell tropism. Occasional reports of intracytoplasmic viral-like inclusions are mentioned,[47] but these tend to be the exception rather than the rule. Giant cells have also been reported,[48] but these can also be seen in non-COVID-associated diffuse alveolar damage. There have also been the occasional reports of the presence of the 'viral type particles' in bronchiolar epithelial cells and alveolar epithelial cells.[49]

It should also be recognized that most autopsy studies have failed to distinguish whether the tissue was from people who died with COVID-19, or of COVID-19, or whether the clinical and pathological changes are secondary to intensive care management. Goligher et al. posed this clinical question on whether severe COVID was a typical or atypical form of ARDS,[50] and concluded that in classical ARDS the hypoxia was a result from atelectasis and consolidation, with an increased physiological shunt fraction, but that COVID-19 was different in that the hypoxaemia was out of proportion to the lung parameters and was more vasocentric in keeping with the pro-thrombotic tendency. Their suggestion was that apart from more anticoagulation there should not be other major changes in management and the goal should be to avoid unsafe lung stress and strain.

On the pathological findings to investigate if the changes seen in the lung were secondary to intensive care management, an early report of COVID-19 patients who died without hospital admission showed diffuse alveolar damage with hyaline membrane formation, Type II hyperplasia and interstitial lymphocytic inflammation.[51] Intriguingly, of the nine cases studied, there was no evidence of microthrombi formation and no myocarditis was seen. This thromboembolic tendency was seen in another clinical study that showed 18 of 370 patients admitted with COVID had computed tomography (CT) evidence of thrombosis.[52] A review of most of the autopsy publications shows a confirmation bias of histological changes of diffuse alveolar damage but more attention should be given to images which show areas of positive antigen, but no significant other morphological changes,[46,53] suggesting that not all virus infection of the functional lung unit leads to massive damage and that the cytokine storm is only seen in a minor proportion of cases.[54] The problems with understanding human lung pathophysiology and COVID-19 are illustrated in two cases of fatal COVID-19 in which post-mortem was performed. In the first case involving a patient with cardiovascular morbidities who died after 3 days, typical features of hyaline membrane disease are identified (Figure 2A), and yet IHC for virus was negative (Figure 2B). In a second case (Figure 2C–F), occurring in a patient who died after 10 days, there is antigen in macrophages and desquamated epithelial cells, but not in endothelial cells but as this is autopsy tissue accurately determining if the antigen is in Type 1 or Type 2 cells is not possible. In most countries, there is a delay of 3–10 days from death until autopsy, during which tissue breakdown occurs, thus accurate understanding of the nature of disease in future will probably rely on laboratory animal models, which may not faithfully replicate human pathophysiology.

Gastrointestinal

The potential role of the gastrointestinal tract as a route of replication and transmission was proposed soon after there was evidence of viral spread from China to other parts of the world.[55,56] This hypothesis was initially based on historical evidence of the finding of virus in intestinal samples during the 2003 SARS outbreak,[57] as well as the documented spread of the virus through wastewater systems in the Amoy Gardens housing complex in Hong Kong, leading to a large

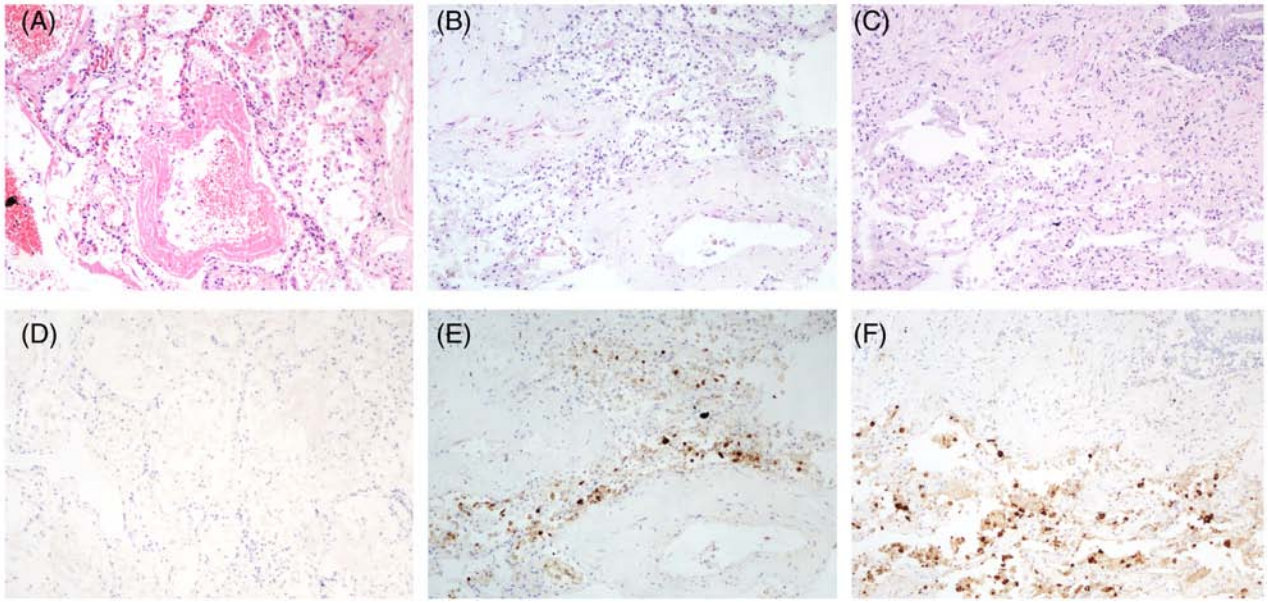

FIGURE 2 (A–C) Haematoxylin and eosin-stained sections of two fatal cases of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and (D–F) corresponding immunohistochemistry using a polyclonal rabbit antibody to SARS-co-V N-protein. Magnification ×100

community outbreak (reviewed in 2006[58]). The MERS outbreak in 2012 documented one quarter of patients having gastrointestinal symptoms, and viral RNA was detected in the gastrointestinal tract.[59]

In the early reports of clinical presentation in COVID-19, the symptoms were, as expected, non-specific with diarrhoea, nausea and vomiting reported.[60] The gastrointestinal symptoms were more frequent in patients after hospitalization, rather than on admission.[61]

The presence of ACE2 has been detected in the oesophageal epithelium and enterocytes of the ileum and colon,[62,63] and intriguingly the colonic organoid system developed in the rat model showed upregulation of ACE2 in organoids derived from hypertensive rats.[63] Of note, ACE2 has been detected in the intestines of cats and tigers, in which sporadic reported cases of infection have occurred,[64] leading to concern that these animals may be reservoirs for virus mutation.[65] Even though human ex vivo explants have been used to study tropism,[18] gastrointestinal explants have not been successful, and as a result some centres are using organoid cultures derived from human pluripotent stem cells (hPSC). Organoid systems act as multicellular composites that faithfully replicate the characteristics of the native epithelium. The first report of this system from the United States[66] showed multiple colonic cell types, including enterocytes that were susceptible to infection with expression of ACE2 detected.

CT abnormalities in the abdomen have been identified in 18.1% of patients admitted with COVID-19, with fluid-filled colon and pericolic stranding reported as the most common features[67]; however, these appearances did not correlate with abdominal symptoms. There have been isolated case reports of colonic ischaemic change,[68,69] in line with the increased thromboembolic tendency seen in more severe cases of COVID-19.

As with other systems reviewed here, the challenge has been to detect actual virus replication in human gastrointestinal tissues, and unfortunately the hard data supporting this are lacking. There have been two case reports documenting the presence of virus in human tissues. The first, from Germany,[70] was from a 43-year old male who 6 weeks after admission for COVID-19, developed large bowel obstruction requiring resection, in which electron microscopy reported isolated viral particles, but this was not confirmed by ISH, IHC or PCR, and the isolated particles appeared to have a hollow core. A second report from a patient with colonic carcinoma,[71] who was positive for viral RNA, also claimed to find viral particles; however, close examination shows that these isolated particles are 150 nm in diameter, larger than the spectrum of coronavirus particles seen in other studies, and most likely represent other structures.

Despite the lack of definitive sites of replication of SARS-CoV-2 in the gastrointestinal site there has been a great deal of interest in the detection of viral genetic material in waste water from a number of countries and regions to screen for virus prevalence in the community setting. This has been recently reviewed in a number of multi-regional publications.[72–75] From these studies, it can be concluded that the viral load is extremely variable in waste water treatment plants depending on stages of the outbreak, and not surprisingly that viral RNA does not imply infectiousness. This finding of RNA in waste water is important as it has been proposed that in regions where there is poor hand hygiene, open defecation, squat toilets and lack of water sealing U-traps, the possibility of environmental transmission from an infected individual to another should be considered.[76]

DISSEMINATION AND TRANSMISSION

Viraemia

One of the crucial clinical questions asked about COVID-19 is whether this is a condition limited to the respiratory and gastrointestinal sites, or whether there is dissemination to other organs. In the 2003 SARS outbreak, there were two publications which found evidence of viral RNA in the blood,[77,78] and one publication demonstrating viral RNA in 33% of patients with MERS-CoV infection[79]; however, there has been no published information on the other human coronaviruses. Although there has been interest in exploring the multi-organ tropism of this virus,[80] in view of the time frame of organs sampled from 20 to 81 days after infection correlation with damage due to direct virus interaction needs to be examined more closely.

With clinical COVID-19, there have been a number of publications on viral RNA in the blood with the first publication using data from the early 2020 Wuhan outbreak.[81,82] Since then, more extensive publications from Spain,[83,84] Germany,[85,86] United States[87–89] and Norway[90] have been published using outpatients, hospital ward patients and critically ill patients. The positive detection rate is between 28% and 32% of hospitalized patients; however, those in intensive care have rates up to 78%. Only a few studies have looked at the temporal changes,[88,91,92] and it appears the peak of viral load is in the first 10 days with plasma levels declining after that. It is noteworthy that this decline is regardless of outcome.[92] One early study found that the RNA level correlated with IL6 levels,[82] and other inflammatory chemokine levels.[83] A review of the presence of viral RNA in the blood[93] commented that in only two of the 23 published studies was there an attempt to grow virus in culture (i.e., to distinguish RNAaemia from true viraemia) and both of these failed to grow virus. Another study[92] involving 71 patients stated that additional studies were needed to confirm that the plasma 'viraemia' actually represented infectious virions. This distinction is important not only in understanding the clinical spectrum of disease, but also whether virus can be transmitted by blood, as a number of centres have been using convalescent plasma for the management of severe disease. It was also noted in the review of RNAaemia that in most studies reporting positive RNA, the level was close to the limit of detection with a cycle threshold (Ct) value on quantitative PCR greater than 30 in most cases. The results of virus culture in blood have been recently summarized in a preprint review,[94] with no publications demonstrating virus growth from PCR-positive blood samples. Thus, the conclusions from another review of virus tropism[95] have concluded that there was no firm evidence that there could be virus infection of other organs via the bloodstream, and in view of the correlation of viral RNA with severe disease, the RNA in the blood could be a manifestation of viral RNA released from damaged epithelial cells.

Cardiovascular system

Initially, the clinical spectrum of COVID-19 was thought to be a respiratory disease, similar to SARS-CoV; however, as the pandemic evolved, multi-organ involvement was seen and the heart was no exception. Initial autopsy studies from the United States,[96–99] Germany[100–102] and later other countries such as Netherlands,[103] UK,[104] Belgium,[105] Italy,[106] Switzerland,[107] Austria[108] and China[109] have shown that in patients who have died with COVID-19 there have been a range of cardiac findings including lymphocytic myocarditis, pericardial damage, dilated cardiomyopathy, hypertrophic cardiomyopathy, hypertensive cardiomyopathy,[96] septal myocardial infarction,[97] perivascular lymphocytic inflammation,[110] right ventricular damage[105] and even amyloidosis.[99] A number of studies have highlighted the presence of a prothrombotic state, characterized by the identification of thrombo-emboli in a number of organs,[104] including non-occlusive fibrin microthrombi.[99]

Although these autopsy series would initially indicate that clinical COVID-19 can have widespread cardiac involvement, a number of review publications published 10 months after the outbreak have pointed out a number of challenges with these reports.[111] The first is a disconnect between autopsy studies and clinical myocarditis, pointing out that the diagnosis of clinical myocarditis is a challenge, requiring a number of distinct and distinguishing investigations including ECG, non-invasive technologies like echocardiography and cardiac MRI. Although a definitive diagnosis of myocarditis needs endomyocardial biopsy (EMB), there have actually been very few published studies in which EMBs have been performed.[112] Even if EMB is performed, the sensitivity is between 10% and 22%, because all autopsy data have demonstrated that myocardial involvement is patchy and there is significant interobserver variability.[113]

The second problem with autopsy data is that mortality from COVID-19 is increased in patients with cardiovascular diseases such as heart failure, diabetes, hypertension and atherosclerosis. Therefore, whether the histological findings in the heart can be attributed to direct viral damage, or pre-existing disease has to be carefully detailed. A review of cardiovascular pathology[108] came to the conclusion that even though 7.2% of patients had histological evidence of myocarditis, most of these cases would likely not be functionally significant, and reduced the true prevalence to less than 2%, suggesting that the patients with autopsy were dying 'with' myocarditis rather than 'of' myocarditis. A similar conclusion was reached by another group of researchers in a review article[111] indicating that distinct European Society of Cardiology criteria should be used before a diagnosis of myocarditis was made The pathogenesis of myocardial dysfunction is probably multifactorial (see below).

In patients who died from SARS, autopsy data showed evidence of an increased thrombotic tendency,[33] and many studies from the COVID-19 patients have shown that patients admitted to hospital had increased tendency towards thrombosis, including deep vein thrombosis,

pulmonary embolism[100] and microthrombi[98,102] despite anticoagulation.[97] These thromboembolic features were seen in multiple organs[104] and have resulted in many authors proposing the term endothelial dysfunction. This is taken to imply a change of the endothelium from an anti-thrombotic state to a pro-thrombotic one, with a consequent increase in vascular permeability and a change to a large number of biomarkers including prothrombin time, C-reactive protein, ferritin, IL6 and plasma creatinine.

The mechanisms for this endothelial dysfunctional have been extensively reviewed,[114] and have been attributed to interference with the renin–angiotensin–aldosterone system (RAAS), oxidative damage, cytokine storm, immune damage, disseminated intravascular coagulation and even by a recently described process called ferroptosis,[110] an iron-dependent form of regulated cell death where there is a loss of glutathione peroxidase activity leading to excessive peroxidation of polyunsaturated fatty acids.

In most models of the mechanisms of endothelial dysfunction, the initiating trigger has been proposed to be direct viral infection of myocytes or endothelial cells, causing cellular damage, disturbances of intercellular junctions and exposure of the subendothelial collagen leading to the prothrombotic tendency. It has been previously acknowledged using IHC that endothelial cells express ACE2 (the SARS-CoV-2 receptor), and thus should be a likely target for viral entry and replication; however, the data supporting this mechanism are by no means conclusive. Distribution of ACE2 was investigated after the SARS 2003 outbreak by a number of publications using ACE2 antibodies, with the distribution found in many organs,[115] thus implying that SARS could be a systemic disease; however, the in situ hybridization studies done in the fatal SARS patients found no evidence of signal in endothelial cells. A number of structural proteins apart from ACE2 have been proposed to serve as viral attachment factors such as CD147 (BSG) which is expressed on the basal surface of the endothelium in culture in one study,[116] but this has not been confirmed by another study,[117] with a further publication showing that ACE2 decreases with age, but BSG actually increased.[118]

Most review articles on whether there is direct endothelial infection cite two articles in which 'virus-like particles' were identified in endothelial cells using electron microscopy.[119,120] These two articles have been quickly followed by a number or short communications challenging these findings,[121,122] attributing these ultrastructural changes to rough endoplasmic reticulum, ribosomes, clathrin-coated vesicles, or multi-vesicles—mimics of virus particles.[123] In a laboratory setting, human microvascular endothelial cells showed a low level of viral replication,[124] with no electron microscopy performed. Unfortunately, another human primary lung microvascular endothelial cell line was not able to replicate this finding.[125] RNA-seq studies on vascular arterial venous and microvascular beds also failed to find ACE2, although pericytes were found to be positive.[126] Similarly, a report of finding virus within endothelial cells in the skin from a series of paediatric patients presenting with chilblains[127] was challenged in a number of correspondence replies[128–130] regarding the veracity of the virus particles in the endothelium.

If finding virus in endothelial cells had been challenging, finding virus in the myocardium and thus attributing the myocarditis to direct viral damage have been in general unrewarding. Despite many autopsy findings of PCR-positive results in the myocardium, in no cases has virus been able to be cultured from the myocardium, and in only a few cases have possible single viral particles been identified,[131] with no groups of viruses or replication complexes observed.[132] The recent report of fulminant myocarditis with positive IHC[133] is confounded by the fact that the N-protein antibody used is not considered reliable in paraffin-embedded tissue.[134]

It should also be noted that small animal and primate studies have also failed to detect viral antigen or show significant myocardial damage.[22,135] The negative IHC, and EM findings despite a lymphocytic myocarditis is not unique to SARS-Co-2 infection, and is more likely to be immune mediated (rather than direct viral damage). It should also be mentioned that this immune-mediated damage has been documented in other virus-associated myocarditis and has been attributed to an exaggerated immune response, with increased levels of serum cytokines and TNFα detected.[136] This is supported by evidence that cardiac troponin is higher in patients with more severe infection with non-ischaemic cardiomyopathy with limited evidence of direct infection.[137]

It therefore appears that the cardiac damage is not a direct viral damage,[138] nevertheless the recognition of myocarditis is important as it leads to an increased risk of in-hospital mortality with adverse long-term clinical outcome, with current management being supportive unless cardiogenic shock occurs.

Liver

There are a number of excellent reviews on liver involvement in clinical COVID-19.[139–142] As with other organs system reviews, these are biased towards an inpatient cohort, often with severe disease, so the extent by which there may be hepatic involvement in mild disease has not been properly studied. Liver involvement can be subdivided into damage to hepatocytes, leading to changes in biochemical processes and coagulation, changes to the cholangiocytes leading to jaundice and changes to the hepatic sinusoids resulting from an endotheliitis.

It has been postulated that hepatocyte damage may be drug induced, direct viral damage or resulting from endotheliitis, coagulopathy, with damage to cholangiocytes by the cytokine storm, and drug-induced liver injury.[139] The extent of biochemical liver dysfunction increases with the severity of disease in patients and is associated with older age and male sex,[143] and the liver injury correlates with the severity of pulmonary disease.[144]

Many of the patients who develop severe disease have coagulopathy; therefore, it has been difficult to determine pathological changes in the inpatient setting using liver biopsy because of the risk of bleeding. However, one study which did perform liver biopsies found steatosis, Kupffer cell activation, luminal thrombosis and portal fibrosis.[145]

From a virological point of view, a major question (similar to other organs) has been whether the damage seen in clinical disease is due to direct viral infection or whether the liver is an 'innocent bystander'.[146] In studies done on macaques by the Erasmus group, there was no evidence of extrapulmonary virus spread[135]; conversely, a number of studies have commented that ACE2 is expressed in the liver,[147] and this expression can be increased by activation of the RAAS.[148] In vitro, liver cancer cell lines appear susceptible to infection, and indeed a HuH7 has been used as a positive control for a number of virus replication studies. Furthermore, use of liver organoids that have been derived from human pluripotential stem cells are able to support virus replication.[149]

A number of autopsy studies detailing the liver pathology in COVID-19 have been reported in the United States,[142,150] Belgium[103,146] and Netherlands,[103] in which a common feature is steatosis (often microvesicular), platelet fibrin microthrombi, lobular inflammation, Zone 3 haemorrhage and ischaemic type hepatic necrosis. The challenge with these finding is that hepatic pathologies such as steatosis can be seen in patients who have a high risk of developing severe disease, that is, diabetes, obesity and patients with cardiovascular disease. One report showed interesting 'atypical basophilic sinusoidal structures' in which detailed IHC was not able to elucidate the nature of these structures.[142]

Immunohistochemical analysis[150] and electron microscopy[151] for antigen and virus, respectively, have claimed to find positive staining; however, subsequent correspondence by Philips et al.[152] challenged these findings and proposed that these particles were not of viral origin and could represent cholesterol crystals. Most reviews to date have come to the conclusion that apart from the prothrombotic tendency which is seen in severe disease, possibly by endothelial dysfunction, most of the changes are non-specific and secondary to factors such as hypoxaemia, drug induced, ischaemia or result from systemic inflammation.[139–142,153]

Kidney and urine

One of the first series of articles investigating the renal involvement in clinical COVID-19 was a multicentre US analysis from February to May 2020 involving 3993 hospitalized patients with clinical COVID.[154] Of these patients, 46% developed acute kidney injury (AKI) and 19% of this cohort required dialysis. The main renal manifestations were proteinuria (84%), haematuria (81%) and leukocyturia (60%). The frequency of this AKI varies significantly in a number of review articles from 0.5% to 80%, with factors such as geography, race/ethnicity and sex accounting for this variation.[155] Similar to other organ systems reviewed, the AKI correlated with disease severity and morbidity.[156] Other review articles indicated that the main pathology was acute renal tubular damage.[157] Similar to other organs discussed in this review paper, there has been much discussion on whether the changes are due to direct viral damage or secondary to hypoxaemia and hypercoagulability.[158]

As people who contract COVID-19 and require hospitalization often have disturbed coagulopathy, renal biopsies have not been routinely performed except for other conditions.[159,160] These biopsies have shown AKI with podocytopathy and collapsing glomerulopathy (a form of focal segmental glomerulosclerosis). The first large multicentre renal biopsy study was from the United States.[161] This confirmed the presence of acute tubular injury and collapsing glomerulopathy, but in keeping with disturbed coagulopathy seen in many inpatients reported endothelial injury and a thrombotic microangiopathy. The AKI was predictive of multi-organ dysfunction.[162] As ACE2 is expressed in the renal tubules, it was expected that this region would be a site of viral damage; however, it was reported that though the tubules might express ACE2, there was a lack of expression of TMPRSS2.[163]

The first autopsy study detailing the renal findings was from China which described finding virus-like particles in six of 2626 cases with positive IHC,[164] although others have described the renal findings as non-specific.[165] Another article by Farkash et al. also reported finding virus-like particles,[166] but the EM conclusions of these articles was challenged by two consecutive letters, which proposed that the structures were either endocytic vesicles coated with clathrin or multivesicular bodies (MVBs), as the putative viral particles lacked electron-dense nucleocapsid material.[167,168] Calomeni et al. furthermore reported that when they examined 10 renal biopsies from the pre-COVID era they were able to identify the same MVBs,[169] and a subsequent review of the challenges of identifying coronaviruses indicated that confirmation of these 'virus-like structures' needed immunoelectron microscopy.[170] Puelles et al.[171] also came to the same conclusion and also commented that for the cases of PCR-positive renal material obtained at autopsy, the RNA levels were low, and that the increased numbers of endocytic vesicles could be seen in patients with proteinuria. There have been a number of publications using IHC to investigate viral antigen in the tubules, most have found no positive staining,[172,173] and in the ones finding some positive findings, the antibodies used (against the spike protein or nucleoprotein) were not reliable in formalin-fixed tissues.[134,174,175]

If there was supposed to be widespread replication of virus in the kidney, especially the tubules, then one would expect to find a high frequency and detection of virus or RNA in the urine. In one study from China using droplet digital PCR of the urine, 5.41% of patients tested positive,[176] with no detection found in 74 recovered patients.[177] Other studies have varied from region to region[95,177–179] with

review studies finding overall rates of less than 10%, correlating with severe disease status, but similar to the viraemia studies, in quite a few cases this was a low concentration with a high cT values.[180] Only one case from China has reported positive virus culture from urine, and in another series from South Korea 'contagious' virus was found in two of 247 samples. Not surprisingly, this was reported as being at a very low level. The general conclusion on the urine therefore is that a positive finding is rare, and this tends not to be associated with renal disease.[181]

Pancreas

COVID-19 has been implicated as a cause of acute pancreatitis in several case reports, but a causal link is yet to be established.[182–184] In a retrospective observational study of a large inpatient population in New York, 32 of 11,883 (0.27%) patients hospitalized with COVID-19 had acute pancreatitis according to the Revised Atlanta Classification.[185] Furthermore, pancreatitis in COVID-19 patients was less likely to be attributable to other causes such as gallstones or alcohol, increasing the possibility of a causal link between SARS-CoV-2 infection and pancreatitis.[185] Hyperglycaemia, insulin-dependent diabetes mellitus and diabetic ketoacidosis have been reported as complications of SARS-CoV-2 infection.[186–188] Whether this is due to infection-induced islet cell dysfunction is debatable as acute stress responses, glucocorticoid treatment and underlying poorly controlled diabetes are all plausible explanations for these clinical observations.

Autopsy series have reported a spectrum of involvement including microscopic inflammation, focal pancreatitis and necrotic-haemorrhagic pancreatitis in some deceased COVID-19 patients.[104,189] SARS-CoV-2 RNA has been detected in pancreatic tissue from COVID-19 deceased patients, albeit at lower viral loads than respiratory tissue.[190,191] Virus-infected cell clusters (identified by immunohistochemical staining) were mostly in the exocrine compartment in one study, but with proximity to the islets of Langerhans. Viral RNA has also been documented in the endothelium of pancreatic blood vessels.[190] Both exocrine (acinar and ductal cells) and endocrine cell populations in the pancreas have been reported to express ACE2 and TMPRSS2, although there may be significant inter-individual variations in distribution of these entry factors within the various pancreatic cell populations.[191,192] Endocrine cells and hPSC-derived β-cells are susceptible to SARS-CoV-2 infection in vitro .[11,13,149,191] Interestingly, recent evidence suggests that SARS-CoV-2 infection might decrease insulin-positivity in β-cells driving them to degranulation and dedifferentiation in patients with severe COVID-19.[191]

CONCLUSION

At the time of writing, just over 1 year has passed since a novel coronavirus entered the community in China. From there, it rapidly spread throughout the world. Even though a number of vaccines have been developed in a short period of time, with initiation of mass population vaccination, it is acknowledged that the virus will not be eradicated and most likely will become one of the circulating seasonal viruses. SARS-CoV-2 infection will continue to cause human disease, and despite the large number of clinical cases and excess mortality, to date, owing to the limited number of autopsies and restricted availability of clinical samples, it is disappointing that the progress made in vaccinology has not been matched by progress in understanding many aspects of the pathobiology of this disease.[193] Unlike the first wave of the pandemic in which healthcare settings were overwhelmed with untreated cases and deaths occurring in early stages of the disease resulting in most of the publications cited in this review, with worldwide vaccination strategies in effect in 2021, plus improved testing and therapeutics, there will be a reduced ability to understand the natural, untreated course of disease and many aspects of this disease in terms of pathophysiology will continue to be a mystery.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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