Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Emerging spectrum of COVID-19-related cardiopulmonary pathology in adults

Brian Hanley
Melanie Jensen
Michael Osborn

Abstract

COVID-19 is currently a major cause of morbidity and mortality in adults throughout the world. Given the high infection rate, it is increasingly likely that histopathologists will encounter this disease during their practice. Although COVID-19 is increasingly recognized as a multi-system disease, the lungs and, to a lesser degree, the heart remain the major sites of pathology. This article aims to acquaint the general histopathologist with the main pathological findings in the lungs and heart of adults with COVID-19. It highlights the need for clinicopathological correlation with a discussion of the cardiopulmonary clinical features in COVID-19 and relates those to the pathological findings. In the lungs, diffuse alveolar damage is emphasized with its variety of morphological appearances over time. It concludes with a discussion of the main techniques available to identify the virus in fixed tissues and their potential limitations related specifically to the heart and lungs.

Keywords autopsy; cardiac; coronavirus 2; heart; histology; histopathology; lungs; microscopy; post-mortem; pulmonary; SARS; SARS-CoV-2

Introduction

At the time of writing (January 2021), the coronavirus disease 2019 (COVID-19) pandemic is approaching 100 million documented cases worldwide since its first description in late 2019 with the majority of deaths in adult patients.[1] A vast literature has been amassed on COVID-19 related pathology describing changes across various organ systems. As these reports accumulate, there is an increased awareness of the spectrum of changes seen in this disease. The pathological findings in COVID-19 vary according to many factors, including those related to the host, the virus and the timing of the disease with respect to onset of infection. Given the importance of the thoracic organs in COVID-19, this review focuses on the histopathological findings in the lungs and heart in adult patients with brief mention of the virology and molecular pathology. We will also discuss the limitations of histopathology in COVID-19; such as in dealing with post-mortem tissue and the different techniques for identification of the virus.

As the disease has continued to spread and become a major cause of death in the United Kingdom (UK), histopathologists, particularly those involved in autopsy pathology, are increasingly likely to encounter COVID-19 cases. Thus, this review is aimed at general histopathologists, with the intention of broadly familiarizing the reader with the major pathology in the most affected organs. It is not intended to be exhaustive. In addition, a major question which is often of clinical relevance regards the identification of the virus in tissue sections and we will briefly discuss the variety of techniques available.

SARS-CoV-2 and its cellular receptor

The virus causing COVID-19 is an 80—100 nm, single-stranded, positive sense RNA virus with striking clinical and molecular resemblance to the virus causing the 2002—2003 coronavirus pandemic; severe acute respiratory syndrome coronavirus (SARS-CoV). It was therefore designated as SARS-CoV-2. Middle eastern respiratory syndrome coronavirus (MERS-CoV), SARS-CoV-1 and SARS-CoV-2 are all betacoronaviruses and cause similar human disease. Most other coronaviruses either cause disease exclusively in animals or may cause mild human illness, particularly upper respiratory tract infections. SARS-CoV-2 is defined as a hazard group 3 organism by the advisory committee on dangerous pathogens, indicating that while there is a risk of acquiring potentially severe human disease, this risk may be mitigated through the use of adequate safety precautions.[2]

Like its predecessor, the cellular receptor for SARS-CoV-2 is also angiotensin-converting enzyme-2 (ACE2) which is expressed by the alveolar epithelial cells, bronchial epithelium and endothelium in normal lungs.[3] The surface SARS-CoV-2 spike protein binds to ACE-2 and in presence of various co-receptors (e.g. transmembrane serine proteases), it gains access to the cell.[4] ACE2 is also expressed in the heart (including cardiomyocytes, fibroblasts and smooth muscle cells) rendering it a potential target for direct viral entry.[4]

Clinical manifestations of COVID-19

The clinical features of SARS-CoV-2 infection vary. It is estimated that over 40% of infections are pauci-/asymptomatic.[5] The majority of symptomatic patients experience a mild illness, while few develop severe (~15%) or critical disease (~5%).[6] COVID-19 typically manifests with respiratory symptoms, including cough, shortness of breath and sore throat. Other common symptoms include fever, myalgia, headache and altered smell or taste. Nasopharyngeal symptoms like sneezing and rhinorrhea are less common.[7] Severe disease may involve acute respiratory distress syndrome (ARDS), cardiac arrhythmias, acute cardiac injury, shock, major thromboembolic phenomena and a severe

Brian Hanley *BSc (Hons) MB BCh BAO MSc CHAT, ST4 Histopathology Registrar, Department of Cellular Pathology, Imperial College Healthcare NHS Trust, Northwest London Pathology, London, UK. Conflicts of interest: none declared.*

Melanie Jensen *MBBS MPhil, ST1 Histopathology Registrar, Department of Cellular Pathology, Imperial College Healthcare NHS Trust, Northwest London Pathology, London, UK. Conflicts of interest: none declared.*

Michael Osborn *MBBS FRCPath, Consultant Histopathologist, Department of Cellular Pathology, Imperial College Healthcare NHS Trust, Northwest London Pathology, London, UK. Conflicts of interest: none declared.*

inflammatory response.[7] ARDS typically occurs 7–8 days after symptoms onset.

Although the primary manifestations of COVID-19 are respiratory, multi-system organ involvement is now well-recognised. In particular, cardiac symptoms, ranging from the clinically silent (e.g. asymptomatic pericarditis) to the potentially fatal (e.g. acute myocardial ischaemia) are emerging. The increased risk of clinical acute myocardial ischaemia associated with COVID-19 was observed early in the pandemic. In a large COVID-19-positive Danish cohort, the incidence of acute myocardial ischaemia was 5 times higher during the 2 weeks post-diagnosis compared to the control interval, with heightened risk persisting up to 1-month post-diagnosis.[8] It is well-established that patients with risk factors for atherosclerosis are at increased risk of acute coronary syndromes during acute infections. Indeed cardiac injury in COVID-19 is more common in those with pre-existing hypertension, diabetes, coronary heart disease, heart failure, and higher baseline troponin and pro-B-type natriuretic peptide (BNP) levels.[9]

Cases have also emerged of COVID-19 patients with non-ischaemic cardiac injury. Pericardial manifestations ranging from a pauci-symptomatic pericardial effusion to life-threatening cardiac tamponade have been described.[10] Typically these have occurred sub-acutely and concurrent with respiratory manifestations, although reports exist of acute pericarditis as the primary presentation of COVID-19.[10] Sub-acute myocardial involvement has also been noted; in a case series of 68 fatal COVID-19 cases, five patients died of fulminant myocarditis 2–3 weeks after disease onset.[11] Even in the absence of myocarditis, cardiac troponin levels are significantly elevated in severe COVID-19 infection, reflecting direct or indirect myocardial injury. Alongside the acute cardiac manifestations described, cardiac complications may linger long after viral clearance.

Although the exact incidence is uncertain, it is clear that a subset of COVID-19 patients develop superimposed infections (predominantly bacterial and fungal).[12] The estimated case fatality rate varies across studies. While several early Italian studies estimated fatality rates above 7%, as the rates of asymptomatic testing have increased, more recent studies made estimations below 1%.[13] As of January 29 2021, the ECDC has documented 99,727,853 cases of COVID-19 and 2,137,670 COVID19-related deaths.[1] It is difficult to be certain about the exact case fatal rate because both asymptomatic and fatal cases may go unrecognized. Furthermore, it is possible that certain causes of death may be incorrectly ascribed to COVID-19, when other co-existing pathologies predominate.

The majority of COVID-19 deaths occur in older patients and those with medical co-morbidities. Chief among these are cardiovascular disease, diabetes mellitus, chronic pulmonary conditions, cancer, chronic kidney disease and obesity.[6] The mortality rate appears to be higher among men and non-white races in the UK. Certain peripheral blood markers have been correlated with worse outcomes and include lymphopaenia, thrombocytopenia, acute kidney injury and elevations in various inflammatory markers, troponin, creatinine kinase, D-dimers, prothrombin time, lactate dehydrogenase and liver enzymes. Severe COVID-19 has also be associated with higher viral loads on nasopharyngeal swabs and the identification of SARS-CoV-2 RNA outside the respiratory tract.[14]

Access to cardiopulmonary tissues in COVID-19

Access to tissue has been a significant limitation in developing our understanding of COVID-19 pathophysiology. Live patients with COVID-19 are unlikely to undergo tissue biopsy. Where live lung samples have been taken from patients with COVID-19, it often represents mild or early stage disease.[15–17] Lung tissue from live patients until recently was only reviewed in the context of resections indicated for reasons other than COVID-19 (e.g. cancer resections), however a recent study has reported the use of cryobiopsies in live patients with COVID-19.[15–17] The majority of histopathological studies of COVID-19 have come from post-mortem examinations, however these generally describe late or severe disease. Autopsies allow multi-organ sampling, targeting of samples to macroscopic abnormalities and the exclusion of various pathologies, however autolysis is an acknowledged limitation of autopsy histology. In our centre we have been able to describe a range of informative pathologies, even with relatively extended post-mortem intervals (see Figure 1f, acute fibrinous and organizing pneumonia in a patient whose post-mortem interval was nine days). Similarly, in our centre we have been able to identify small amounts of SARS-CoV-2 RNA using quantitative RT-PCR in a patient who died thirteen days after onset of symptoms and with a post-mortem period of nine days.[18] Furthermore, while autopsy samples typically display late stage disease, samples from SARS-CoV-2 swab positive patient who died from other causes are likely to be another valuable resource in studying early stage or mild disease. Unfortunately, for various reasons including the significant burden of COVID-19 on mortuaries and the National Health Service in general, only limited numbers of hospital autopsies were performed on confirmed COVID-19 patients during the first wave of the pandemic. We have described previously the safe performance of COVID-19 autopsies and will not discuss their performance further.[2]

The spectrum of findings in the lung

The lung is the primary organ affected in COVID-19, however the pathological findings in the lung are variable and depend on patient factors and the stage of the disease. Although most histopathological studies in COVID-19 come from autopsy series[18–26] and necessarily represent the severe end of the spectrum of COVID-19 lung disease, occasional descriptions of early disease in live patients have been made. Tian et al.[15] described the lung histology in two patients who underwent lung cancer resections and were found to have COVID-19 post-operatively. They describe early and non-specific changes including pulmonary oedema, multinucleated giant cell infiltrates and pneumocyte hyperplasia. Both patients subsequently developed severe COVID-19 and only one survived the hospital admission. Zeng et al. showed similar features in lung tissue of a 55 year-old woman who had undergone a lobectomy for a benign lung nodule and was later discovered to have had early phase COVID-19 at the time of surgery.[17] More recently, an Italian group[16] has performed lung biopsies on a series of live COVID-19 patients. In addition to the pneumocyte hyperplasia noted by previous groups, they showed vascular changes including capillary and venular enlargement and perivascular $CD4^+$ T cell infiltrates. Furthermore, they demonstrated strong nuclear expression of

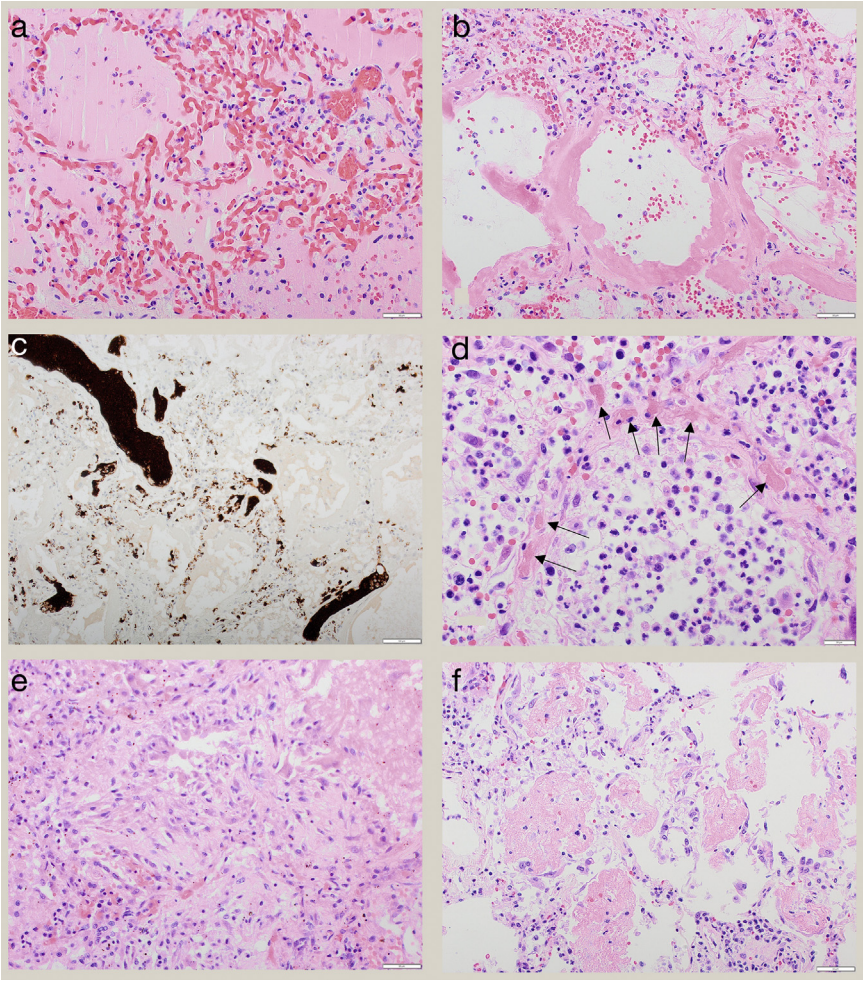

Figure 1 The spectrum of histopathological features in COVID-19 lungs. **a**: Pulmonary oedema and vascular congestion in a 69-year-old woman who died from COVID-19. These are features of "pre-exudative phase" DAD but note that she had features of exudative phase DAD elsewhere in the lung. **b**: Hyaline membranes indicative of exudative phase DAD in a 24-year-old man. **c**: CD61 stain highlights extensive microthrombi in pulmonary arteries, arterioles and capillaries in a 61-year-old man who died with exudative phase DAD. **d**: Microthrombi (arrows) in the capillaries surrounding an alveolus a 64-year-old man with COVID-19. **e**: Fibroblastic proliferation, alveolar collapse and architectural remodelling in a 79-year-old man in organizing phase of DAD. **f**: "Fibrin balls" indicative of AFOP in a 64-year-old man with COVID-19. Scale bar 20 μm in D. Scale bar 50 μm in A, B, E and F. Scale bar 100 μm in C.

phosphorylated STAT3 in type II pneumocytes and endothelial cells which they postulate may be related to cytokine activation.[16]

The pathological findings in the lung in fatal COVID-19 are typically more marked and are characterized by diffuse alveolar damage (DAD), which is consistent with the previous SARS and MERS epidemics. DAD was identified as the major lung pathology in most large post-mortem series including at least 87% of 47 autopsies[25] 73% of 40 autopsies[23,26] and 100% of 38 cases.[20] This characteristic involvement of the lower respiratory tract may represent the high expression of ACE2 in pneumocytes. Although, various histological patterns were initially described in COVID-19 lungs, it is important to note that the features of DAD vary over time. Therefore, it is critical to correlate pathological findings clinically with the timing of onset of symptoms. DAD is usually widespread and bilateral, with the result that the post-mortem lungs are generally heavy, oedematous and may show petechial haemorrhage on the surface. However, normal lung weights do not exclude COVID-19, especially in its early stages. DAD may occasionally be localized and widespread sampling of the lungs is warranted in certain cases. Similarly, the absence of evidence of DAD does not completely exclude the possibility of COVID-19, particularly if the patient had a poor physiological reserve and may have died in the acute phase of the disease. For example, in our practice we identified a patient with near normal lung weights, however on imaging and histologically the DAD was limited to the right lower lobe. Gross purulent consolidation may be seen, although this should alert the pathologist to the possibility of a secondary infection and microscopy and culture may be considered.[24]

Oedema and intra-alveolar haemorrhage are typically the earliest morphological appearances in DAD (Figure 1a). This is sometimes referred to as the pre-exudative phase, which is subsequently followed by the exudative phase. The exudative phase of DAD is characterized by the classic eosinophilic hyaline membranes, mild interstitial inflammation, interstitial oedema, microthrombosis and pneumocyte hyperplasia (Figure 1b–d). The interstitial inflammatory cells are mostly lymphocytic with fewer neutrophils and plasma cells. The hyperplastic

pneumocytes are mostly type II and may show reactive features and high proliferation. Squamous metaplasia of the respiratory epithelium may occur and may also show marked reactive features. Foamy alveolar histiocytes are also usually present which is are often accompanied by intra-alveolar haemorrhage. The morphological appearances of DAD may be more marked or extensive in young patients and those with better physiological baselines, given that they may survive until a stage at which the disease is further progressed.[24] Although this is not a universal feature, we have noted it in our centre in several cases.[18]

After the exudative phase, DAD may develop into the organizing phase that displays a proliferation of fibroblasts and myofibroblasts, continued hyperplasia of alveolar epithelial cells, alveolar collapse and progressive re-modelling (Figure 1e). The onset of the organizing phase is typically more than one week after the onset of COVID-19. Complete histological resolution of DAD has been reported after exudative phase and even organizing phase DAD. ARDS survivors may similarly show minimal ongoing respiratory compromise, although many encounter ongoing pulmonary dysfunction.

DAD has many causes apart from SARS-CoV-2, which are beyond the scope of this article. However, common causes include trauma, various inhalants, drugs, toxins, radiation, shock and sepsis. The aetiology of DAD generally cannot be identified based on the morphology alone, however viral infections are an exception to this, where the virus can be detected in fixed tissues. It should also be noted that the causes of DAD are not necessarily mutually exclusive. For example, DAD may also be seen in mechanically ventilated patients (particularly where high O_2 concentrations were used) and this has been termed ventilator-associated lung injury.[27] Thus, a mechanically ventilated COVID-19 patient may have multiple reasons for DAD. However, similarly because mechanical ventilation itself can cause DAD, care must be taken when ascribing these findings to SARS-CoV-2 without clinical and molecular correlation.

Although not specific to COVID-19 related DAD, thromboembolic disease is a common feature in COVID-19 post-mortem series. Thrombi have been identified in many vessels including pulmonary arteries, arterioles and capillaries. Pulmonary embolism was the direct cause of death in 4 of 12 autopsies in one series,[24] and we demonstrated pulmonary microthrombi in most COVID-19 cases in our series (Figure 1c—d).[2] Larger series have now demonstrated thromboemboli in 80—90% of COVID-19 lungs at autopsy.[25] We have found CD61 immunohistochemistry particularly useful for the purpose of demonstrating microthrombi. Several hypotheses exist to explain these prominent thrombotic phenomena. Ackerman et al. demonstrate marked vascular damage, angiogenesis and thrombosis in seven COVID-19 lungs compared with the changes associated with influenza.[22] This vascular damage is further supported by lymphocytic cuffing of small blood vessels. This vascular damage could be related to direct viral infection of endothelial cells as suggested by some authors and supported by the expression of ACE2 by pulmonary epithelium. Another possibility is that the downregulation of ACE2 may lead to excess angiotensin II activity, which has been associated with vascular damage. A procoagulant state related to the systemic inflammatory response may also play a role. Importantly, these different pathways may also function simultaneously and with additive effect.

Acute fibrinous organizing pneumonia (AFOP) is another similar pattern which is described in COVID-19 and characterized by "fibrin balls" within alveoli associated with organizing pneumonia (Figure 1f). Although AFOP is a distinct pattern, it is also part of the spectrum of lung injury in relation to time and is classically considered a subacute finding.[28] Finally, while the major pathological cause of respiratory failure in COVID-19 is DAD, there have also been occasional reports of encephalitides involving the brainstem structures in autopsy examinations.[29] This suggests the possibility that central cardiorespiratory depression may contribute to respiratory failure in certain COVID-19 patients.

While the organizing fibrosis noted in at autopsy, highlights that a proportion of patients who survive severe COVID-19 are likely to develop pulmonary fibrosis, it must be noted that these features indicate the severe end of the spectrum and long-term follow-up data are not available. Data from the previous SARS epidemic are informative and demonstrate that although long-term respiratory impairment was common, this impairment was mild in most cases.[30,31] However, care must be taken in extrapolating from these data as most of the patients included were young and had few comorbidities which is unrepresentative of many COVID-19 patients.

The spectrum of pathological findings in the heart

Although initial pathologic studies focused on the respiratory sequelae of COVID-19, case reports and case series are increasingly describing ante- and post-mortem cardiac pathologic changes. Many features have been described; ranging from those of uncertain clinical significance (e.g. cardiac amyloid deposition) to the fatal (e.g. myocardial infarct). Herein we focus the discussion to those most likely to be of clinical consequence.

A recent systematic review of 277 post-mortem COVID-19 cases identified acute myocardial infarction in 4.7% of cases, with early/micro ischaemic injury in 13.7% of cases.[32] In line with clinical data, predisposing risk factors for coronary artery atherosclerotic disease, including advancing age, diabetes, obesity, and pre-existing coronary artery disease, are common in COVID-19 post-mortem cohorts so it is unclear the extent to which these changes are virus-mediated.[32] Although certain cases showed concomitant acute coronary thrombus formation (Figure 2a), many did not. Alternative mechanisms of injury have been postulated, including hypoxia-drive myocardial infarction and myocardial infarction caused by intramyocardial venous thrombosis (in line with the hypercoagulable state well-described in COVID-19).[33]

Non-occlusive microthrombi are a common post-mortem finding in COVID-19; occurring in 10—80% of examined hearts with estimates varying between case series (Figure 2b).[18,32,34] They appear to occur more commonly in active COVID-19 cohorts, compared to those who had cleared the virus at the time of death, deaths due to influenza, and non-virally mediated deaths.[34] In keeping with this, diffuse small-vessel microangiopathy and widespread small vessel micro-thrombi have been noted in multiple organs across several post-mortem COVID-19 studies.[35] The microthrombi constituents appear to be distinct from those of intramyocardial thrombi from COVID-19-negative subjects; with higher fibrin and terminal complement C5b-9 levels.[36] Given the shared features with the better

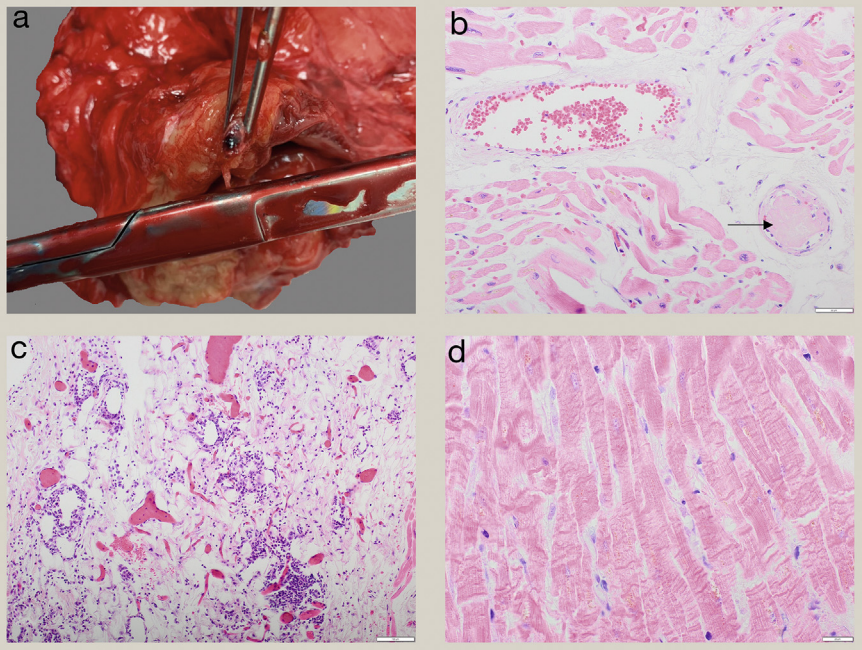

Figure 2 The spectrum of histopathological features in COVID-19 hearts. **a**: Acute right coronary artery thrombus in a 61-year-old man with COVID-19. **b**: Microthrombi in the left ventricle of a 97-year-old man with COVID-19. **c**: Acute inflammation in the epicardial fat of a 79-year-old man who did not undergo cardiorespiratory resuscitation and who died from COVID-19. **d**: Contraction band necrosis in a 64-year-old man who died from COVID-19 and had spent six days in the intensive care unit. Scale bar 20 μm in D. Scale bar 50 μm in B. Scale bar 100 μm in C.

known complement-mediated thrombotic microangiopathy (TMA) syndromes, a 'COVID-19 TMA pathogenic model' has been proposed, in which ineffective clearance of SARS-CoV-2 instigates complement-initiated vessel damage, with inflammatory cells inducing a cytokine 'burst', perpetuating a bidirectional immune-coagulation axis.[35] The purported microvascular dysfunction may explain why disorders characterized by microarteriopathy, namely diabetes and hypertension, are risk factors for COVID-19 mortality.

Albeit rare, a number of post-mortem studies have noted pericarditis as an autopsy finding in COVID-19 (Figures 2c and 3a). This was first described as a novel finding in 2 of the 9 COVID-19 patients examined in a UK case series: one patient showed a microscopic acute pericarditis, whilst the second patient showed an extensive fibrinous pericarditis containing fungal hyphae.[18] A subsequent focused cardiac autopsy study of 9 patients who had died because of cardiogenic shock subsequent to COVID-19, observed a pericardial effusion in 6 patients, typically co-occurring with a fibrous pericarditis with lymphocytic infiltrates.[37] In the latter study molecular studies did not identify SARS-CoV-2 genomes in cardiac tissue, despite SARS-CoV-2 PCR positivity in lung tissue, suggesting an immune-mediated pathogenesis over direct viral pericardial tropism.[37] Ante-mortem analyses of the pericardial fluid corroborate this: most cases have revealed a virus-negative polymorphonuclear or lymphocyte-predominant inflammatory exudate, except in two cases where RT-PCR testing for SARS-CoV-2 was positive in pericardial fluid.[10]

Initial review of post-mortem findings indicated that myocarditis was a common finding in COVID-19 (~7% of examined hearts).[32] Closer examination of reported information however, revealed that many cases had classified small foci of myocardial lymphocytes, which are not uncommon in elderly hearts, as myocarditis. Instead the true prevalence of functionally significant myocarditis, evidenced by diffuse or multifocal inflammatory infiltrate alongside myocyte injury, is low if indeed it can be directly related SARS-CoV-2 infection.[32] Given the absence of formal pathologic criteria, care must be taken to avoid over-interpretation of minor, non-specific myocardial inflammatory infiltrates in COVID-19 autopsies.

The presence of at least one cardiac pathology is a common finding in COVID-19 autopsies (one recent review puts the estimate at 47.8% of hearts).[32] Whether these can be directly attributed to SARS-CoV-2 pathogenesis remains unclear. The variable detection of SARS-CoV-2 in cardiac tissue suggests that these pathologies may not all be a direct consequence of viral cardiac tropism. The timing and nature of a number of cardiac pathologies (e.g. lymphomononuclear myopericarditis, endothelial changes in small vessels, micro-thrombi), suggests pathology secondary to para-infectious phenomena, specifically the systemic hyperinflammatory and hypercoagulable syndromes; well-described features of COVID-19. Alternatively, cardiac pathology identified at autopsy could represent iatrogenic changes secondary to prolonged critical care. Indeed certain findings (e.g. the contraction band necrosis described in several cases; Figure 2d) could relate to inotropic medication received in the intensive care.[18] Where non-acute cardiac pathology has been identified, the direction of effect must also be considered. In one case series, cardiac amyloidosis was noted in 25% of autopsied COVID-19 hearts, much higher than the typical 3.7% occurrence at the centre.[34] Given the chronicity of amyloid build-up, it is more likely that pre-existing cardiac amyloidosis is either a risk factor for COVID-19 mortality or unrelated to death entirely. Finally, certain common cardiac pathologic findings (e.g. ventricular hypertrophy, myocardial fibrosis) can be attributed to comorbidities

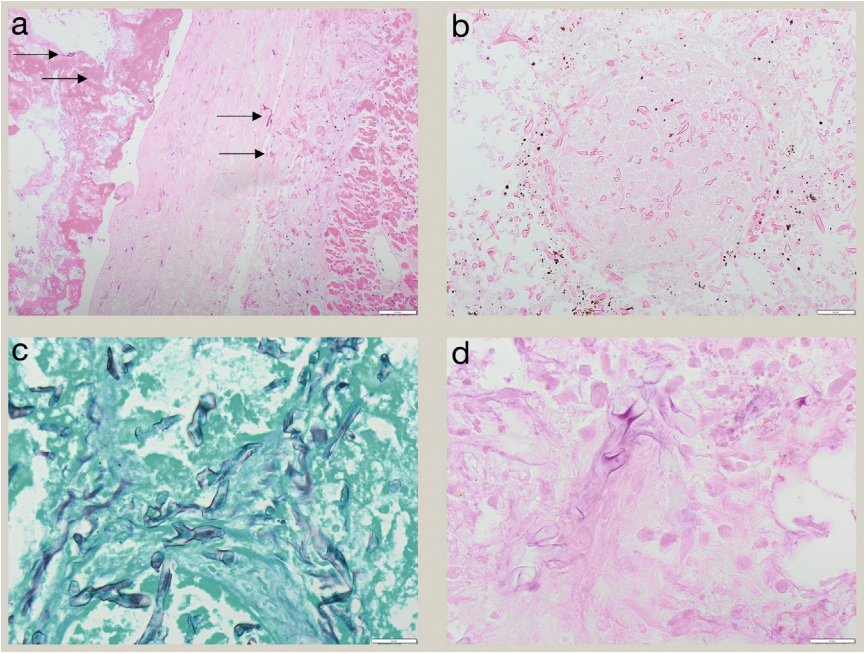

Figure 3 Secondary disseminated mucormycosis in a patient with COVID-19. a-d are from a 22-year-old man with COVID-19 who died after 22 days in the intensive care unit. **a**: Fibrinous pericarditis with pericardial fibrosis and interspersed fungal hyphae (arrows). **b**: Angioinvasive fungal hyphae in an area of lung showing infarct-type necrosis. **c** (Grocott Silver Stain) and **d** (Periodic Acid Schiff Stain): show broad, branching, non-septate, ribbon-like fungal hyphae indicative of mucormycosis. This was confirmed with Mucorales-specific PCR. Scale bar 20 μm in C and D. Scale bar 50 μm in B. Scale bar 100 μm in A.

such as hypertension which commonly co-occur in COVID-19-positive cohorts. Unpinning which cardiac pathologies can be attributed to i) direct viral cardio-invasion, ii) para-infectious phenomena, and/or iii) pre-existing comorbidities, requires ongoing clinicopathological correlation. Thus identifying endo-carditis/pericarditis at autopsy, as recently described in one case series, does not imply SARS-CoV-2 as the causative agent.[18]

Identification of the virus

It is now well accepted that SARS-CoV-2 is identifiable in fixed lung tissue. However, the viral load is higher during early infection and it may not be identifiable later in the disease. The issue of whether the virus infects the heart and is directly responsible for cardiac damage is an area of ongoing research. There are numerous ways to identify the virus in fixed tissues.

Quantitative real time PCR (qRT-PCR) is the most sensitive test for identifying the virus. The largest case series to date identified a viral load above 1000 copies per μg RNA in 24 of the 39 (41%) hearts examined.[38] In contrast a recent cardiac autopsy report of 9 patients, all with myocarditis, failed to detect SARS-CoV-2 in the myocardium despite consistent positivity in lung tissue.[37] Other studies have reported variable, predominantly low, SARS-CoV-2 RNA copy numbers in the heart in a subset of their cohort.[24] A major limitation of qRT-PCR is that it does not provide any spatial localization for the virus. For example, the identification of viral RNA alone by qRT-PCR may simply reflect viraemia (particularly when dealing with extra-pulmonary tissue). It is possible to design probe that indicate that actively replicating virus is present, as described previously.[18] The tissue site is also important. For example, one study has identified patients with a negative upper respiratory tract swab but virus present in the lung on microscopic examination.[39] Thus, a negative swab does not necessarily exclude the diagnosis, and where there is clinical and pathological evidence to support diagnosis COVID-19, multiple body sites may be examined.

Although less sensitive, in-situ techniques for identification of viral proteins (e.g. immunohistochemistry) and viral RNA (e.g. in-situ hybridization) are available.[16,25] These can layer spatial resolution on top of qRT-PCR and inform the investigator about which cell types are infected to allow for direct comparison with the patterns of tissue injury. Electron microscopy can also identify which cells are infected and provide information about the subcellular localization of the virus. However, many subcellular structures (e.g. cross cut microvilli and clathrin coated pits) have appearances resembling virions and input from an experienced electron microscopist, or correlation with immuno-electron microscopy is likely to be beneficial in this scenario.[40]

Secondary infections in the heart and lung in COVID-19

Secondary lung infections have also been described in both clinical and autopsy series in COVID-19. Observed pathogens include various types of bacterial pneumonia and invasive fungal infections including aspergillosis and mucormycosis (Figure 3a−d). Secondary or co-existent viral infections have also been noted albeit less commonly. Fungal infections in particular, may be seen in patients who have spent significant time on a ventilator or patients who received extracorporeal membrane oxygenation. Notably, secondary infections may have different tissue reactions and hence show histological appearances other than DAD. Thus, unexpected patterns in COVID-19 such as widespread acute bronchopneumonia, should raise the possibility and their associated patterns of injury may completely replace

DAD. The clinical course of a COVID-19 patient in ITU can also be exacerbated by other infective cardiac complications; for example a case of *S. aureus* infective endocarditis complicating COVID-19 pneumonia has been described, along with other secondary infections.[12,41]

Conclusion

Although COVID-19 is increasingly recognized as a multi-system disease, the thoracic organs remain the major site of pathology. Infection rates remain high in the UK and it is likely that practicing pathologists will come into contact with cardiopulmonary tissues from COVID-19 patients. This is particularly the case for autopsy pathologists, but also potentially for those practicing surgical pathology in these organs. This article has described the major cardiopulmonary histopathological findings in COVID-19. In the lung, DAD is the most prominent pattern of injury, however the morphological appearances vary greatly depending on timing of disease. A wide range of cardiac pathology findings have also been described, however it is not clear in many cases whether these relate to direct viral cardiac tropism, para-infectious phenomena or pre-existing conditions. Tissue access is likely to be a limiting factor in the ability to understand this disease as the pandemic progresses and histopathologists can play an important role in this. ◆

REFERENCES

1 European Centre for Disease Prevention and Control. Threats and outbreaks: COVID-19. 2020, https://www.ecdc.europa.eu/en/novel-coronavirus-china (accessed 30 January 2021).
2 Hanley B, Lucas SB, Youd E, Swift B, Osborn M. Autopsy in suspected COVID-19 cases. *J Clin Pathol* 2020; **73:** 239–42.
3 Hoffmann M, Kleine-Weber H, Schroeder S, et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell* 2020; **181:** 271–80.
4 Nicin L, Abplanalp WT, Mellentin H, et al. Cell type-specific expression of the putative SARS-CoV-2 receptor ACE2 in human hearts. *Eur Heart J* 2020; **41:** 1804–6.
5 Oran DP, Topol EJ. Prevalence of asymptomatic SARS-CoV-2 infection: a narrative review. *Ann Intern Med* 2020; **173:** 362–7.
6 Wu Z, McGoogan JM. Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: summary of a report of 72 314 cases from the Chinese center for disease control and prevention. *Jama* 2020; **323:** 1239–42.
7 Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 2020; **395:** 497–506.
8 Modin D, Claggett B, Sindet-Pedersen C, et al. Acute COVID-19 and the incidence of ischemic stroke and acute myocardial infarction. *Circulation* 2020; **142:** 2080–2.
9 Shi S, Qin M, Shen B, et al. Association of cardiac injury with mortality in hospitalized patients with COVID-19 in Wuhan, China. *JAMA cardiol* 2020; **5:** 802–10.
10 Sauer F, Dagrenat C, Couppie P, Jochum G, Leddet P. Pericardial effusion in patients with COVID-19: case series. *Eur Heart J Case Rep* 2020; **4:** 1–7.
11 Ruan Q, Yang K, Wang W, Jiang L, Song J. Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China. *Intensive Care Med* 2020; **46:** 846–8.
12 Rawson TM, Moore LSP, Zhu N, et al. Bacterial and fungal co-infection in individuals with coronavirus: a rapid review to support COVID-19 antimicrobial prescribing. *Clin Infect Dis* 2020; **71:** 2459–68.
13 World Health Organisation. Estimating mortality from COVID-19. 2020, https://www.who.int/publications/i/item/WHO-2019-nCoV-Sci-Brief-Mortality-2020.1 (accessed 25 August 2020).
14 Xu D, Zhou F, Sun W, et al. Relationship between serum SARS-CoV-2 nucleic acid(RNAemia) and organ damage in COVID-19 patients: a cohort study. *Clin Infect Dis* 2020 Jul 28;ciaa1085. https://doi.org/10.1093/cid/ciaa1085. Online ahead of print.
15 Tian S, Hu W, Niu L, Liu H, Xu H, Xiao S-Y. Pulmonary pathology of early phase 2019 novel coronavirus (COVID-19) pneumonia in two patients with lung cancer. *J Thorac Oncol* 2010; **15:** 700–4.
16 Doglioni C, Ravaglia C, Chilosi M, et al. Covid-19 interstitial pneumonia: histological and immunohistochemical features on cryobiopsies. *Respiration* 2021; **100:** 369–79.
17 Zeng Z, Xu L, Xie X-Y, et al. Pulmonary pathology of early-phase COVID-19 pneumonia in a patient with a benign lung lesion. *Histopathology* 2020; **77:** 823–31.
18 Hanley B, Naresh KN, Roufosse C, et al. Histopathological findings and viral tropism in UK patients with severe fatal COVID-19: a post-mortem study. *Lancet Microbe* 2020; **1:** e245–53.
19 Menter T, Haslbauer JD, Nienhold R, et al. Post-mortem examination of COVID19 patients reveals diffuse alveolar damage with severe capillary congestion and variegated findings of lungs and other organs suggesting vascular dysfunction. *Histopathology* 2020; **77:** 198–209.
20 Carsana L, Sonzogni A, Nasr A, et al. Pulmonary post-mortem findings in a series of COVID-19 cases from northern Italy: a two-centre descriptive study. *Lancet Infect Dis* 2020; **20:** 1135–40.
21 Calabrese F, Pezzuto F, Fortarezza F, et al. Pulmonary pathology and COVID-19: lessons from autopsy. The experience of European Pulmonary Pathologists. *Virchows Arch*, 2020; 1–14.
22 Ackermann M, Verleden SE, Kuehnel M, et al. Pulmonary vascular endothelialitis, thrombosis, and angiogenesis in Covid-19. *N Engl J Med* 2020; **383:** 120–8.
23 De Michele S, Sun Y, Yilmaz MM, et al. Forty postmortem examinations in COVID-19 patients. *Am J Clin Pathol* 2020; **154:** 748–60.
24 Wichmann D, Sperhake JP, Lütgehetmann M, et al. Autopsy findings and venous thromboembolism in patients with COVID-19: a prospective cohort study. *Ann Intern Med* 2020; **173:** 268–77.
25 Borczuk AC, Salvatore SP, Seshan SV, et al. COVID-19 pulmonary pathology: a multi-institutional autopsy cohort from Italy and New York City. *Mod Pathol* 2020; **33:** 2156–68.
26 Bradley BT, Maioli H, Johnston R, et al. Histopathology and ultrastructural findings of fatal COVID-19 infections in Washington State: a case series. *Lancet* 2020; **396:** 320–32.
27 Slutsky AS, Ranieri VM. Ventilator-induced lung injury. *N Engl J Med* 2013; **369:** 2126–36.
28 Nicholson AG, Osborn M, Devaraj A, Wells AU. COVID-19 related lung pathology: old patterns in new clothing? *Histopathology* 2020; **77:** 169.
29 Al-Sarraj S, Troakes C, Hanley B, et al. Invited Review: the spectrum of neuropathology in COVID-19. *Neuropathol Appl Neurobiol* 2020; **47:** 3–16.
30 Ngai JC, Ko FW, Ng SS, To KW, Tong M, Hui DS. The long-term impact of severe acute respiratory syndrome on pulmonary

function, exercise capacity and health status. *Respirology* 2010; **15:** 543–50.
31 Zhang P, Li J, Liu H, et al. Long-term bone and lung consequences associated with hospital-acquired severe acute respiratory syndrome: a 15-year follow-up from a prospective cohort study. *Bone Res* 2020; **8:** 8.
32 Halushka MK, Vander Heide RS. Myocarditis is rare in COVID-19 autopsies: cardiovascular findings across 277 postmortem examinations. *Cardiovasc Pathol* 2021; **50:** 107300.
33 Rapkiewicz AV, Mai X, Carsons SE, et al. Megakaryocytes and platelet-fibrin thrombi characterize multi-organ thrombosis at autopsy in COVID-19: a case series. *EClinicalMedicine* 2020; **24:** 100434.
34 Bois MC, Boire NA, Layman AJ, et al. COVID-19-Associated nonocclusive fibrin microthrombi in the heart. *Circulation* 2021; **143:** 230–43.
35 Merrill JT, Erkan D, Winakur J, James JA. Emerging evidence of a COVID-19 thrombotic syndrome has treatment implications. *Nat Rev Rheumatol* 2020; **16:** 581–9.
36 Pellegrini D, Kawakami R, Guagliumi G, et al. Microthrombi as A major cause of cardiac injury in COVID-19: a pathologic study. *Circulation* 2021; **143:** 1031–42.
37 Del Nonno F, Frustaci A, Verardo R, et al. Virus-negative myopericarditis in human coronavirus infection: report from an autopsy series. *Circ Heart Fail* 2020; **13**. CIRCHEARTFAILURE120007636-CIRCHEARTFAILURE.
38 Lindner D, Fitzek A, Bräuninger H, et al. Association of cardiac infection with SARS-CoV-2 in confirmed COVID-19 autopsy cases. *JAMA Cardiol* 2020; **5:** 1281–5.
39 Barton LM, Duval EJ, Stroberg E, Ghosh S, Mukhopadhyay S. Covid-19 autopsies, Oklahoma, USA. *Am J Clin Pathol* 2020; **153:** 725–33.
40 Roufosse C, Curtis E, Moran L, et al. Electron microscopic investigations in COVID-19: not all crowns are coronas. *Kidney Int* 2020; **98:** 505–6.
41 Spinoni EG, Degiovanni A, Della Corte F, Patti G. Infective endocarditis complicating COVID-19 pneumonia: a case report. *Eur Heart J Case Rep* 2020; **4:** 1–5.

Practice points

- Diffuse alveolar damage is the predominant histopathological finding in COVID-19 lungs and its morphological appearances vary over time
- Diffuse alveolar damage may be seen in the pre-exudative, exudative and organizing phases.
- Thrombotic events are a major cause of morbidity and mortality in COVID-19 and may be identified in various organs including the heart.
- There is a paucity of evidence on the histological findings in early or mild COVID-19.
- Pre-existing cardiac disease, particularly hypertension and diabetes-related, is a common finding in COVID-19 hearts
- Secondary infections are not uncommonly identified and should be considered where the macroscopic and microscopic appearances are not typical of COVID-19

Multiple choice questions

1. Which of the following is not typically seen in COVID-19 lungs?
A. Microthrombi
B. Hyaline membranes
C. Organizing pneumonia
D. Bronchiolar squamous metaplasia
E. Necrotizing Granulomas

Answer: E - Necrotizing granulomas.

2. Which of the following may mimic SARS-CoV-2 on electron microscopy?
A. Cross-cut microvilli
B. Rough endoplasmic reticulum
C. Clathrin-coated pits
D. Microvesicular bodies
E. All of the above

Answer: E - All of the above.

3. Which of the following is true with regard to autopsy in COVID-19?
A. Myocarditis is a major determinant of death
B. Thromboembolic events are a major determinant of death
C. The absence of SARS-CoV-2 on tissue sections excludes COVID-19 as a cause of death
D. SARS-CoV-2 is often identified in cardiomyocytes
E. Evidence of viral RNA in heart sections by qRT-PCR means that the virus is infecting the heart

Answer: B - Thromboembolic events are a major determinant of death.