COVID-19 autopsies: conclusions from international studies

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COVID-19 autopsies: conclusions from international studies

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Abstract

The rapid pace at which COVID-19 studies are being published is surpassed only by the spread of the virus and the destruction wreaked by the pandemic globally. Therefore, it is likely that, even in the few months prior to this article reaching print, the COVID-19 literature would have moved on. The authors of this article work at a centre for COVID autopsies in London, and the aim of the article is, using their first-hand experience of COVID-19 autopsies, to distil what in their judgement are the most valid and important findings of internationally published COVID-19 autopsy studies. The intention is to provide an illustrated summary of the pathology of the organ systems most often affected by COVID-19, which will be particularly useful to trainee histopathologists and to busy consultant surgical histopathologists who may not have encountered COVID-19 first hand. For the reader who wishes to probe further the question of pathogenesis, a few pertinent references are provided.

Key Words

autopsy, post-mortem, pathology, histology, histopathology, macroscopy, microscopy, COVID-19, SARS coronavirus 2, SARS-CoV-2

<A>Introduction

The human infection caused by the Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) was first recognised in 2019 and named COVID-19 (Coronavirus Disease 2019). It followed the previous High Consequence Infection coronavirus international epidemics, SARS (Severe Acute Respiratory Syndrome) in 2002-2003 and MERS (Middle Eastern Respiratory Syndrome) in 2012. Similar to SARS-CoV-1, SARS-CoV-2 is a 80-120 nm diameter RNA virus, with a central nucleocapsid protein and radiating peripheral spike proteins. The virus enters human cells by the binding of the spike protein to angiotensin converting enzyme 2 (ACE2), but only after the spike protein has been cleaved by surface proteases (transmembrane serine protease 2, TMPRSS2).

COVID-19 reached global pandemic proportions in 2020, with almost 1 million deaths worldwide at the time of writing, but the rapidity of spread being such that international statistics of infection and death rates became outdated on a daily basis. Broadly speaking, approximately 20% of those infected were hospitalised, about 20% of whom needed Intensive Care; about 50-80% those requiring Intensive Care died, giving a 2-4% mortality rate in countries with well-resourced healthcare systems, for an exceptionally contagious infection. The disease has imposed upon global healthcare systems and economies a challenge which is unparalleled in modern times.

With the initial emphasis being on saving lives and not adding to the pressures of the already overburdened mortuary systems, only limited, core biopsy post-mortem studies of a few organs were performed. Although it was anticipated that some information could be adduced from SARS and MERS (see the other article on this topic in this issue), it soon became apparent that there was an urgent need for full post-mortem studies on COVID-19 patients. This was partly driven by clinical need and partly by the research community: the autopsy pathologist was pivotal in providing the interface between the two.

<A>COVID-19 – the autopsy pathologist's tasks and tools

The COVID-19 autopsy pathologist has several tasks to balance: maintaining the safety of the post-mortem team, performing a detailed post-mortem examination, taking histology samples which will satisfy the current and future clinical questions, addressing the needs and wishes of the family, and obtaining samples for the translational research community. Within post-mortem practice gross (macroscopic) findings are striking and easiest to correlate with ante-mortem imaging, but a relatively crude tool. Routine Haematoxylin and Eosin (H&E) staining provides a universal 'open ended question', invaluable in the study of the unchartered disease. However, with an infection like COVID-19 it becomes imperative for the modern autopsy pathologist to be able to detect the virus itself, in order to attempt to answer questions about pathogenesis. This has been achieved using antibodies (immunohistochemical stains) to the nucleocapsid and spike proteins; in-situ hybridisation for the viral RNA; RT-PCR; and electron microscopy. This last method has been fraught with interpretation difficulties in the context of COVID-19 and it

appears that what were claimed to be virions in several of the early publications, are in fact altered cellular components, such as clathrin coated vesicles.[1] An immunohistochemical stain to ACE2 assists in correlating expression of this receptor with demonstrable viral proteins/ RNA.

In the remainder of the article, COVID-19 post-mortem findings are described by organ system.

<A>Cardiovascular system

<B>Blood vessels

Given the importance of diabetes mellitus and essential hypertension as adverse prognostic factors in COVID-19, it is not surprising that all the international COVID-19 post-mortem studies have described the vascular changes of these diseases as prominent findings.

ACE2 is widely expressed on endothelial cells (a notable exception being in the renal glomerulus), so this provided a potential explanation for the finding that COVID-19 was associated with hypercoagulability, deep vein thrombosis and pulmonary arterial thrombo-embolism (Figure 1). Although most hospitalised COVID-19 patients are anti-coagulated, post-mortem studies have confirmed venous thrombo-embolic disease in a significant proportion of patients.[2]

What took a little longer to emerge, from post-mortem studies and translational research, was the concept of microvascular injury and 'immunothrombosis' (including the role of complement activation and Neutrophil Extracellular Traps, NETs) being the basis for the multi-system pathology of COVID-19 (Figure 2). [3,4,5,6]

<B>Heart

Similarly, the pre-existing cardiac pathologies (predominantly hypertensive and ischaemic heart disease) of these patients have been highlighted by the autopsy studies.

In addition to the microvascular injury and thrombosis described above, with increased numbers of circulating megakaryocytes, a finding ascribed to COVID-19 has been necrosis of individual myocytes.[7] The issue of myocarditis has been a controversial one, and in our opinion the overwhelming evidence is that COVID-19 is not associated with a true myocarditis according to Dallas criteria; mild and patchy interstitial infiltrates of mononuclear cells, particularly at the epi-myocardial interface, have been described in several papers, but without the mandatory associated myocyte destruction.[3] The early clinical hypothesis of precipitation of atherosclerotic plaque rupture and coronary artery thrombosis has not been supported by post-mortem studies.

<A>Respiratory system

<B>Upper airways

Severe tracheo-bronchitis, manifest as aphthous ulcers[8] and mononuclear inflammation[9] have been described, and claimed to be due to the virus itself, and to be unassociated with mechanical ventilation or superimposed bacterial infection.

<B>Lungs

Faced with large numbers of COVID-19 patients in respiratory failure and requiring mechanical ventilation, the lungs were the focus of the international medical community from the outset.[8,10]

The most obvious manifestation of COVID-19 in the lungs is diffuse alveolar damage (DAD), with its congestion, proliferation and organising phases paradoxically juxtaposed, giving a picture of 'temporal heterogeneity' (Figures 3a, 3b)[8,10,11], and correlating macroscopically with very heavy and solid but wet lungs (Figure 3c). An Acute Fibrinous Organising Pneumonia (AFOP) pattern of fibrosis, with intra-alveolar fibrin balls being replaced by Masson bodies, may also be seen. In the proliferative phase of DAD there is an interstitial infiltrate of lymphocytes and florid, atypical type 2 pneumocyte hyperplasia (within the cytoplasm of which some authors have demonstrated viral inclusions/ protein/ RNA), sometimes associated with squamous metaplasia. As in the myocardium, circulating megakaryocytes and microthrombi are prominent in the lungs[12], as well as thrombo-emboli (associated with infarction if sufficiently large), depending on anti-coagulation. Most of the published post-mortem studies have shown a moderate incidence of secondary lung infections – bacterial pneumonia, invasive aspergillus (particularly in patients treated with ExtraCorporeal Membrane Oxygenation, ECMO) and, less commonly, viral pneumonias.[1]

It is important for autopsy pathologists, particularly those examining community deaths, to note that in the early stages of COVID-19 lung disease the DAD changes may be focal and subtle, and easily masked by a superimposed bacterial bronchopneumonia: the absence of DAD does not exclude COVID-19, and if SARS-CoV-2 PCR swabs had not been taken during life or as part of the post-mortem examination but clinical or pathological suspicion is high, material from formalin fixed paraffin wax embedded (FFPE) post-mortem lung tissue can be submitted for PCR testing.

<A>Brain

Few published series have examined whole brains, and those that have, have in a subset of cases demonstrated widespread microthrombi, microinfarcts and microhaemorrhages; global or watershed anoxic injury; and a very focal infiltrate of T lymphocytes and microglia. [3,13]

<A>Kidneys

The primary pathology in the kidneys has been that of pre-existing diseases: diabetic and hypertensive nephropathy. Not unexpectedly, changes of Acute Tubular Injury and myoglobin casts have also been commonly reported.[14]

Noteworthy for its absence in most of the published studies has been glomerular capillary thrombi/ evidence of thrombotic microangiopathy (TMA), diverging from the findings in typical 'disseminated intravascular coagulation' (DIC).

<A>Liver

All the published studies have noted changes of pre-existing diseases, predominantly obesity and diabetes mellitus. The explanation for the deranged liver function tests seen in many patients appears to be simply severe passive congestion with centrilobular necrosis and collapse. An occasional study has noted Kupffer cell activation in all of their patients[15]; since this is difficult to appreciate on an H&E stain, particularly in autolysed post-mortem liver, use of a CD68 (PGM1) or other immunostain to highlight the Kupffer cells may be required to highlight this feature (Figure 4).

<A>Lymph nodes, spleen and bone marrow

Post-mortem autolytic change is particularly challenging in the interpretation of the histology of the reticulo-endothelial system.

White pulp atrophy of the spleen has been a common finding.[15,16,17] Lymphoid depletion has also been documented in lymph nodes, with some studies finding reactive plasmablastic proliferation[9] and haemophagocytosis.[1,3] One of these studies found haemophagocytosis to also be a feature in the bone marrow, whereas other studies have reported only non-specific reactive marrow changes.

<A>Skeletal muscle

Providing an explanation for the severe myalgia experienced by many patients, those who have looked at skeletal muscle have found a mononuclear myositis associated with myocyte necrosis.[17]

<A>Other organs

Some studies have noted focal necrosis in the testis, but this is likely to just represent a manifestation of the systemic microvascular changes.[17] Although ACE2 is expressed by intestinal enterocytes, and diarrhoea is a well recognised symptom of COVID-19, bowel changes are not described in most published post-mortem studies, likely due to a lack of obvious abnormalities on H&E staining and bowel autolysis rendering assessment difficult. Saliva has been identified to contain a high viral load, but salivary gland findings are also not described. Skin manifestations of

COVID-19 are now well established and their histopathology documented, but as these have been by means of biopsy material from living patients, they are not addressed in this article.

<A>Practice points

- Autopsies and pathologists play a vital role in the fight against the COVID-19 global pandemic.
- Current evidence points to systemic microvascular injury and 'immunothrombosis' (with activation of the haematolymphoid system) as playing a key role in the pathogenesis of COVID-19.
- The lung is the organ which is worst affected in COVID-19, with diffuse alveolar damage in all its phases paradoxically juxtaposed, being the characteristic pathology, and correlating with the common clinical finding of respiratory failure.
- The other organs showing potentially fatal COVID-19 pathology are the heart and brain.
- The kidneys and liver show secondary changes due to COVID-19, as well as manifestations of underlying disease

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Figure 1 (a) Thrombosis of external iliac vein (arrowhead). (b) Pulmonary arterial thrombo-emboli (arrowhead), associated with haemorrhagic infarction of the lung lobe.

Figure 2 Thrombus in intramyocardial arteriole (arrowhead), with surrounding sub-acute microinfarct.

Figure 3 (a) Diffuse alveolar damage - congestion / proliferation phases, with lymphocytic infiltration of alveolar septa. (b) Diffuse alveolar damage – organising phase with paradoxical hyaline membranes (arrowhead). (c) Typical macroscopic appearance of COVID lung.

Figure 4 Kupffer cell activation and haemophagocytosis (arrowhead) (PGM1 immunohistochemistry).

Multiple choice questions

1) Which of the following is not a recognised feature of COVID-19 autopsy lung pathology?

A. Diffuse alveolar damage
B. Superimposed bacterial pneumonia
C. Neuroendocrine tumourlets
D. Thrombi
E. Thrombo-emboli

Answer: C. Neuroendocrine tumourlets

2) Which of the following is not commonly seen in COVID-19 autopsy hearts?

A. Myocarditis
B. Left ventricular hypertrophy
C. Coronary artery atherosclerosis
D. Individual myocyte necrosis
E. Microthrombi

Answer: A. Myocarditis

3) Which of the following is not commonly seen in COVID-19 autopsy kidneys?

A. Arteriosclerosis
B. Acute Tubular Injury
C. Myoglobin casts
D. Diabetic nephropathy
E. Thrombotic microangiopathy

Answer: E. Thrombotic microangiopathy

4) Which of the following liver pathologies in COVID-19 autopsies is ascribed to the SARS-CoV-2 itself?

A. Centrilobular necrosis
B. Kupffer cell activation
C. Hepatocyte nuclear glycogenation
D. Steatosis
E. Fibrosis

Answer: B. Kupffer cell activation