Histological evidence of pulmonary micro-thrombosis and vasculitis in life-threatening respiratory virus diseases

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ABSTRACT

Pulmonary micro-thrombosis and vasculitis occur in fatal COVID-19. To determine if these processes occur in other life-threatening respiratory virus infections we identified autopsy studies of fatal influenza(n=455 patients), SARS(n=37), MERS(n=2), adenovirus(n=34) and RSV(n=30). Histological evidence of thrombosis was frequently present in adults with fatal influenza and SARS, with vasculitis also reported.

Key words: Influenza; SARS Coronavirus; Acute Respiratory Distress Syndrome; Thrombosis; Pulmonary Thromboembolism; Vasculitis

INTRODUCTION

Thrombotic complications occur with high frequency in coronavirus disease 2019 (COVID-19), involving pulmonary thrombo-emboli (PTE), deep vein thrombosis and catheter-related thrombosis, despite thromboprophylaxis with low molecular weight heparin (LMWH).[1] Histologically, pulmonary micro-thrombi are frequent autopsy findings in fatal COVID-19, even in the absence of macro-vascular PTE, and in a sub-group of patients are likely due to immunothrombosis distinct from conventional PTE.[2] This occurs irrespective of receipt of LMWH thromboembolism prophylaxis.[2] There is also mounting evidence of a spectrum of pulmonary vasculitis in COVID-19: neutrophilic capillaritis[3,4], lymphocytic endotheliitis[5], lympho-plasma cellular arterial vasculitis[6], obliterating endarteritis involving C5aR1^{+} macrophages[7] and MRP8^{+} mononuclear cell vasculitis.[2] Together, these findings suggest pulmonary vasculitis and immunothrombosis could be treatable traits contributing to respiratory failure in sub-groups of patients.[8] The established clinical benefit of corticosteroids in COVID-19 supports a causal role for inflammation in severe disease, and trials of anticoagulation are ongoing (NCT04344756, NCT04406389).[9] We reviewed human autopsy data from other life-threatening respiratory virus infections to determine if thrombosis and pulmonary vasculitis occur in other viral infections.

METHODS

We searched the PubMed (MEDLINE) database using the following keywords: 'Adenovirus' OR 'Rhinovirus' OR 'Metapneumovirus' OR 'Parainfluenza' OR 'Bocavirus' OR 'Influenza' OR 'Severe Acute Respiratory Syndrome' OR 'Middle East Respiratory Syndrome' OR 'Respiratory Syncytial Virus' AND 'autopsy' OR 'post-mortem' (NOT 'COVID-19'). H.W.D. and C.D.R. independently reviewed the search results to select relevant studies. Studies were included that reported pulmonary histopathological findings from post-mortem examinations of humans infected with the viruses of interest. We excluded animal studies, studies not written in English, studies potentially reporting overlapping patient cohorts and studies conducted exclusively in transplant recipients or patients

with cancer or immunodeficiencies. For all viruses except RSV and adenovirus, only studies of adult patients were included. Case reports of influenza were excluded but those of other viruses were included due to the paucity of otherwise available data. Pulmonary histological features were recorded using a standard pro-forma (*Supplementary Data*).

RESULTS

From 1224 search results we identified reports for patients with fatal influenza (n=455 patients; 24 studies), SARS (n=37; four studies), MERS (n=2; two studies), Adenovirus infection (n=34; nine studies) and RSV infection (n=30; five studies). The specific histological parameters reported by each study varied and it was not possible to determine the proportion of patients receiving thromboprophylaxis (*Supplementary Data*).

Influenza virus

Diffuse alveolar damage (DAD), alveolar haemorrhage and bronchiolitis were the most commonly reported findings in fatal influenza, with neutrophilic bronchopneumonia suggestive of secondary bacterial infection also common (*Supplementary Data Table 1*). DAD was specifically reported in every study, present in 75% of patients. Where data were available, 56% of patients received invasive mechanical ventilation (IMV; 150/270). The presence or absence of histological evidence of thrombosis was reported in 14/24 studies, with thrombosis present in patients from 12 of these studies and 66/317 (21%) patients where quantified. These were most commonly described as fibrin microthrombi. Pulmonary vascular inflammation was less commonly sought by investigators but was present in 5/6 studies where it was specified (15/79 cases where quantified, 19%). In four studies this was described as perivasculitis and where examined, inflammatory cells involved were $CD8^+$ T-cells (n=7) or mononuclear cells (n=1). One study reported inflammatory infiltrate in the intima of medium vessels and endotheliitis (inflammatory cell type not stated). Vascular involvement in fatal influenza A H1N1 and Covid-19 was compared directly in one study [10]. Although prevalence within

the cohort was not reported, an infiltrate of $CD3^+$ T-cells associated with pre/post-capillary walls was described in both diseases. Capillary micro-thrombi were seen in all cases from both diseases, but were substantially more prevalent in Covid-19.

SARS-CoV and MERS-CoV

DAD was present in all fatal cases and where stated all patients had received IMV. In SARS, thrombosis was reported in 3/4 studies and where quantified was present in 18/26 patients (specified as involving small veins/vessels in 17/18 patients). Vasculitis was reported as being present in two studies, though its prevalence was not stated (*Supplementary Data Table 2*). One study described a small vessel pulmonary vasculitis (inflammatory cell type not stated), and another reported vasculitis of small pulmonary veins, involving monocytes, neutrophils and lymphocytes, with fibrinoid necrosis (in addition to vasculitis of small veins in the heart, liver, kidney, adrenal gland and muscle, involving monocytes, lymphocytes and plasma cells). Only two case reports of autopsies in fatal MERS were identified, one of which reported a $CD4^+$ lymphocytic pulmonary artery vasculitis.

Respiratory Syncytial Virus

There were no reports of pulmonary thrombosis in four studies of infants with fatal RSV infection, often in the context of sudden infant death (*Supplementary Data Table 3*). Vascular inflammation was described in one study, with a predominantly mononuclear and occasionally eosinophilic infiltrate surrounding bronchial arteries.

Adenovirus

Nine studies of fatal adenovirus infection were identified with infrequent reports of thrombosis (2 patients) and vasculitis (2 patients), which was described as necrotising pulmonary vein vasculitis in one case and due to giant cells in the other (*Supplementary Data Table 4*).

DISCUSSION

Histological data from human autopsy studies of multiple patient cohorts indicate that pulmonary thrombosis and vasculitis occur in sub-groups of adult patients with fatal influenza and SARS. Whilst thrombosis is likely to be quantitatively greater in COVID-19[10], it is not unique to this disease. This appears distinct from fatal RSV infection in infants where thrombosis has not been reported and adenovirus infection, where there were very infrequent reports. Overall, these histological features do not appear to have been frequently sought systematically by investigators in the past and could be under-appreciated features of viral lung injury. In the only study directly comparing influenza A with COVID-19, where thrombosis was specifically sought, it was observed in all influenza cases, though confirmation bias is also a possible explanation for this.[10] Similar appearances have also been described in animal models of influenza and SARS in a variety of species.[11-13]

The acute respiratory distress syndrome (ARDS) is the final common pathway of life-threatening viral lung injury. DAD is found in open lung biopsy and autopsy studies of around 50% of patients with a clinical diagnosis any-cause ARDS (cf. 75% of patients with fata influenza in this study).[14] When present, DAD is associated with greater illness severity in ARDS.[14] Pulmonary vascular histological findings are not commonly reported in ARDS autopsy studies but have been reported in two studies including 32 patients with a clinical diagnosis of ARDS (non-selected aetiology), with micro-thrombi present in 28/32.[15,16] These two reports, from 1983 and 1976, would include patients not receiving thromboembolism prophylaxis with heparins which is now routine. A leucocytoclastic pulmonary vasculitis was reported in one cohort (7/22 patients), but only in association with bacterial, viral or fungal super-infection in patients who died >10 days after intubation.[16] A more recent histological study of ARDS, utilising open lung biopsy, did not report on the presence of micro-thrombi or vasculitis, with DAD representing the most common finding, as expected.[17]

In COVID-19, circulating markers of thrombosis and endothelial injury (D-dimer, angiopoietin-2, endothelin-1 and von Willebrand Factor A2) increase in a stepwise fashion with disease severity

(along the WHO ordinal severity scale) with equivalent concentrations in patients who require IMV and survive compared to patients who die.[18] Similar D-dimer changes occur in patients with H1N1 influenza A infection; which, combined with the increased risk of radiologically-diagnosed PTE, supports thrombosis being a relevant process in pathogenesis and not a non-specific post-mortem artefact.[19-21]

Biological mechanisms further support the contribution of these processes to pathogenesis. Engulfment of influenza virions by platelets activates TLR7 signalling, leading to pro-thrombotic neutrophil DNA release and aggregation.[22] Platelet degranulation and neutrophil pro-thrombotic proteomic signatures have been identified in blood from patients with COVID-19 ARDS and low density neutrophils from these samples aggregate with platelets.[23] TLR7 is a ssRNA sensor, also involved in the host response to SARS-CoV-2[24], and activation is likely to occur during infection with other single-stranded RNA viruses. A TLR7/8 agonist upregulates healthy neutrophil Mac-1 platelet binding complex, as see on neutrophils from COVID-19 ARDS patients.[23] Platelet-endothelial adhesion also occurs *in vitro* in response to influenza and in people with COVID-19, circulating platelets display a hyper-reactive transcriptional response and aggregate with leucocytes.[25,26] In ARDS, platelets interact with endothelial cells, immune cells, neutrophil extracellular traps and pathogens, and their activation can lead to immunothrombosis.[27]

Evidence from multiple sources supports the role of myeloid recruitment to the lung in COVID-19 which could link inflammation, vasculitis and immunothrombosis and identify therapeutic targets. In a genome wide association study of critical illness in COVID-19, a CCR2 variant predicted to increase expression in the lung was identified.[28] Similarly, the chemoattractant C5a and myeloid growth factor GM-CSF are associated with COVID-19 severity[7,29]. The C5a-C5aR1 axis has also been associated with H1N1 influenza.[30]

Immunothrombosis and vasculitis were identified in cohorts of adult patients whereas they were infrequent in children. Increased innate immune activation in older adults could contribute to

susceptibility to these processes, or alternatively it may relate to clinical differences: most of the children had died suddenly whereas most adults had received IMV and would have had a more protracted illness.[31]

The majority of the identified studies, including COVID-19 and those from the 2009 H1N1 pandemic, have been conducted at a time when critically ill patients routinely receive thromboembolism prophylaxis with LMWH.[32] Thrombosis could therefore be occurring largely independently of the intrinsic pathway in some patients. Whilst the results of trials of therapeutic anticoagulation in COVID-19 are awaited, we suggest that a sub-group of patients with immunothrombosis, especially with vasculitis, may be more responsive to immunomodulatory therapy, distinct from conventional PTE responsive to therapeutic anticoagulation alone. An alternative or potentially complementary approach would be to therapeutically protect the endothelium, as recently discussed in the context of COVID-19.[33] Such approaches could include administration of nitric oxide, endothelin receptor antagonists, VEGF antagonists or other anti-proliferative drugs.

Overall, we contend that further investigation of the role of immunothrombosis and pulmonary vasculitis in patients with life-threatening respiratory virus infections is warranted, and autopsy studies will have an important role in this. These pathophysiological features could represent treatable traits in sub-groups of patients, with implications for prioritising investigational therapeutic interventions and enriching clinical trials.

POTENTIAL CONFLICTS OF INTEREST

H.W.D., P.M.D.P., A.W-S., S.C., J.E.M., J.K.B., D.A.D., C.D.L., C.D.R. – no conflict.

PATIENT CONSENT STATEMENT

This study did not include any work requiring patient consent.

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