Early View

Editorial

Thrombosis and COVID-19 pneumonia: the clot thickens!

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At the end of last year, a novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), resulted in an acute respiratory illness epidemic in Wuhan, China [1, 2]. The World Health Organization (WHO) termed this illness Coronavirus Disease 2019 (COVID-19). The coronavirus family have been shown to enter cells through binding angiotensin-converting enzyme 2 (ACE2), found mainly on alveolar epithelium and endothelium. Activation of endothelial cells is thought to be the primary driver for the increasingly recognised complication of thrombosis. Viral inclusion bodies have been identified in endothelial cells in a variety of organs, from lung to gastro-intestinal tract [3]. The immune dysregulation characteristic of severe COVID-19 infection may be initiated by 'pyroptosis', a particularly pro-inflammatory form of apoptosis initially described in macrophages [4], with rapid viral replication leading to massive release of inflammatory mediators. One of the most consistent findings is that of a raised D-dimer. Although many inflammatory processes can influence D-dimer levels, it almost certainly reflects, to some extent, intra-vascular thrombosis in patients with COVID-19 [5, 6]. In the early studies emerging from China, an elevated D-dimer (>1000 ng/ml) at admission was associated with increased risk of in-hospital death [7]. An elevated D-dimer continues to be one of the most consistent markers of poor outcome [8].

The true prevalence of thrombosis associated with COVID-19 infection is unknown, as most studies to date do not include systematic and comprehensive investigation protocols. It appears that it is also important to consider the stage of the disease course and where the patient is tested, e.g. ward versus intensive care unit (ICU). In addition, both of these factors will influence the amount of anticoagulation a patient may receive, which in turn may influence subsequent thrombosis. Furthermore, most studies refer to pulmonary embolism (PE), whereas there is persuasive evidence to suggest much of this may be "in situ" pulmonary thrombosis [9]. Two recent Dutch studies have reported cumulative incidences of thrombotic events between 48 and 49% respectively in their ICUs in patients with COVID-19 pneumonia [10, 11]. Most thrombotic

events are described as PE, for instance 87% in the paper by Klok *et al.* [12] although it should also be noted that venous and arterial thrombosis is described [10]. In this edition of the *European Respiratory Journal*, Bompard *et al.* refine this further by describing a 50% cumulative incidence of pulmonary embolism (PE), diagnosed by CT-pulmonary angiogram (PA), in COVID-19 patients admitted to ICU in two hospitals of the University of Paris (ERJ ref Bompard). It is important to note that all patients received thromboprophylaxis, which is a common theme in most reports. In fact, in one study 56% of patients already on full anticoagulation were diagnosed with PE [13].

Several studies have compared rates of thrombosis in ICU and non-ICU populations [11, 14](and ERJ ref Bompard). For instance, Middeldorp *et al.*, report a cumulative incidence of any venous thromboembolism (VTE) event (symptomatic or asymptomatic) of 10% at 2 weeks in a non-ICU population versus 48% in ICU, whereas Bompard *et al.* report an 18% cumulative incidence of PE in non-ICU patients versus 50% in ICU. This is further supported by the study of Criel *et al.* also published in this edition of *European Respiratory Journal*, comparing DVT rates between ICU patients (13%) and ward patients (4%) (ERJ ref Criel). One obvious question is whether these rates of thrombosis are higher than we would expect? Two studies have used a retrospective "comparative" non-COVID ICU population and report 3-6 times the expected rates of thrombosis in the COVID populations [15, 16]. There also appeared to be a very high association with thrombosis in SARS, another coronavirus, and higher than H1N1 for instance [17, 18]. However, the described rates are probably consistent with those reported in patients with severe sepsis or shock [19]. It may be that there are just many more very sick patients in this pandemic. However, it is also clear that we are likely to be underestimating the true prevalence.

Several other interesting observations have been described. Firstly, in the study of Lodigiani *et al.*, around 50% of patients were diagnosed with thrombosis within 24 hours of admission, supporting the hypothesis that many patients are probably presenting with PE [14]. Secondly,

Klok *et al.* describe a small population who happened to be on long-term anticoagulation at admission, who appeared to be protected from developing thrombosis, although, interestingly, not overall mortality [12]. Thirdly, there is considerable anecdotal evidence that patients are dying after discharge and that this could be thrombosis related.

In the accompanying editorial, Huertas *et al.* elegantly describe the early pathogenesis in COVID-19 pneumonia defined by a widespread endotheliilitis affecting multiple organ systems (ERJ ref Huertas). As such, viral inclusion bodies are observed within endothelial cells accompanied by apoptosis, inflammatory cell infiltration and microvascular thrombosis [20]. At the same time, systemic inflammation is commonly observed, with elevated levels of CRP, fibrinogen and cytokines such as IL-6 [21]. Although the exact mechanisms of COVID-19 induced thrombosis have not been elucidated, at least some of the well-described mechanisms associated with infection/inflammation are likely to be relevant [22]. These include the increased production of tissue factor and amplification of the coagulation cascade, resulting in increased production of thrombin and consequently fibrin. Small studies describing thromboelastography in patients with COVID-19 suggest clot formation is extremely rapid and also resistant to breakdown [23]. This, in turn, is likely to relate to reduced fibrinolysis due to increased production of PAI-1, again as a result of inflammation. Deposition of components of the complement system such as C5b-9 in damaged vessels of COVID-19 patients indicate that this may be another important pro-thrombotic mechanism as it has been observed in other conditions associated with micro-thrombosis such as anti-phospholipid syndrome [24]. Furthermore, neutrophil extracellular traps (NETs) have also been observed in vessels in autopsy specimens of patients with COVID-19 [25]. These are associated with high circulating levels of cell-free DNA and histones, which in turn can activate pro-thrombotic pathways leading to increased thrombin production [26]. In addition to ACE2 mediated SARS-CoV-2 viral entry, recent reports of affinity of the SARS-CoV-2 spike protein and CD147, a membrane glycoprotein and extracellular matrix metalloproteinase inducer expressed on a variety of haematopoietic cell lines, suggest another potentially novel mechanism of thrombosis and inflammation in the arterial and venous circulations [27]. Finally,

the profound hypoxaemia that is often observed is a likely driver of vasoconstriction, inflammation and thrombosis. Hypoxaemia will result in activation of hypoxia-inducible factors (HIFs), which in turn will activate cytokines, tissue factor and PAI-1 [28, 29].

Overall, the high rate of pulmonary thrombosis in COVID-19 conceivably lies in the confluence of three processes: firstly, the intense endothelial inflammation described above, leading to "in situ" thrombosis, including microvascular thrombosis; secondly, altered pulmonary blood flow in response to the parenchymal process, disturbing Virchow's triad within the lung; and thirdly, classical DVT to PE transition, which may, in fact, be the minor partner [10].

As the recognition of the thrombotic complications of COVID-19 increases, more guidelines are emerging on their prevention, investigation and management [30, 31]. As already stated, we are likely to be missing thrombotic complications in many patients. Most guidance recognises the importance of keeping the balance between providing quick, pragmatic advice to worried physicians keen to do the best for their patients, with the recognition that evidence for full anticoagulation is of course lacking in this population. Ideally, therefore, wherever practical, we believe the diagnosis of VTE in patients with COVID-19 should be made by traditional imaging modalities; CTPA and/or in the case of clinical suspicion of DVT, by compression venous Doppler of the lower limb [31]. There should be a high suspicion for thrombotic complications and low threshold for definitive imaging, where possible. Traditional D-dimer cut offs are unlikely to be useful in determining who should go on to have a CTPA due to an unacceptably low specificity which will be exacerbated by admission to ICU [5, 32]. However, for many reasons imaging may be impossible. D-dimer has been used in some institutions to guide anticoagulation, even in the absence of imaging detected VTE [31, 33]. The risks and benefits of an approach aimed at targeting both imaging detected VTE as well as "presumed thrombosis" is yet to be determined but excess bleeding is an almost inevitable consequence [34]. Whether this excess risk is offset by improvements in overall survival is yet to be seen. Anticoagulation is clearly not the only treatment needed in patients with COVID-19 and in many patients, on the

sicker end of the spectrum, may be too late to alter outcome. The intimate association between inflammation and thrombosis would suggest an anti-inflammatory/anti-viral therapeutic approach should be considered in parallel to anticoagulation. Furthermore, as the fibrinolytic potential of blood from patients with COVID-19 is extremely low, even with anticoagulation, immediate consequences of thrombus may not be treated. We may just be preventing further thrombus. Indeed, case reports describe the use of fibrinolytics in selected patients with short-term benefits [35], and trials are on-going. But how long will this beneficial effect last in the procoagulant COVID-19 milieu? Histology from autopsy specimens demonstrate platelet aggregates obstructing the microvasculature. Thromboelastography and platelet function tests reveal that patients with COVID-19 have activated platelets [36], suggesting that there may be a role for anti-platelet therapy. Here, the potential to do benefit may be theoretically greater but so is the chance of doing harm.

Clearly results of ongoing trials are urgently needed to further inform clinical practice on the use of anticoagulation. This includes both patients admitted to ICUs and those at risk on the general ward. With regards to non-ICU patients, questions to be asked include: what are the triggers for more intense anticoagulation where imaging is not available? Can this be done using D-dimer alone and what is the threshold to use? What do you do with patients when they are discharged? In the meantime, local and national advice is available and being constantly updated [21]. Finally, with certainty, we can say that the more we know about COVID-19, the more the clot thickens!

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Figure 1: Hypothesis of the origin of Covid-19-associated pulmonary emboli and lung microcirculatory thrombotic disease: Interaction of inflammation and coagulation

When coronavirus 19 infects cells expressing the surface receptors angiotensin-converting enzyme 2 (ACE2), active replication and release of the virus may cause the host cell to undergo pyroptosis (pro-inflammatory apoptosis) and release damage-associated molecular patterns (DAMPs), activating oxidant stress, and generating pro-inflammatory cytokine and chemokine release from nearby epithelial cells, endothelial cells and alveolar macrophages. These proteins in turn attract inflammatory cells to the site of infection, promoting a pro-inflammatory feedback loop.

Tissue factor, usually hidden on the subendothelium, is upregulated on platelets, leucocytes and EC during inflammation, leading to activation of both the extrinsic and intrinsic coagulation pathways to make thrombin. Complement activation is also relevant. Thrombin binds to protease-activated receptors (e.g. PAR-1) to promote the formation of fibrin from fibrinogen, the activation of platelets and subsequent clot stabilization, also propagating further inflammation. Natural anticoagulants and fibrinolytics may also be reduced in Covid 19 infection. Occluded small pulmonary blood vessels are likely to contain fibrin, platelets and coagulation factors, as well as neutrophils that become trapped in 'NETS' as they pass through the lung. Ongoing inflammation provides a positive feedback loop. Additional procoagulant stimuli include lung hypoxia, for example via upregulation of PAI-1 through suppression of fibrinolysis.

In this prothrombotic pneumonitis or ARDS, whether similar mechanisms promote both microthrombosis as well as larger vessel pulmonary embolic disease is not known.

Abbreviations: ACE2 angiotensin receptor type 2, EC endothelial cell, ARDS acute respiratory distress syndrome, NETs neutrophil extracellular traps, PAR proteinase-activated receptor, PAI plasminogen activator inhibitor

DVT source

Disturbed flow

1. Large vessel Pulmonary embolism

2. Distal pulmonary microvascular occlusion

Shunt

Raised PVR?

VQ mismatch

Viral spike attachment to ACE2 receptor

? CD147

Viral replication in cell

MMPs VEGF

Pyroptosis ?

DAMPs Oxidative stress

IL-6

Cytokine release from neighbouring epithelial cells, endothelial cells, alveolar macrophages

Pro-inflammatory feedback loop: Cytokines and chemokines attract monocytes, macrophages, T cells..

Complement activation

EC activation

TF upregulated on platelets, monocytes, macrophages, & EC in response to cytokines

Inflammatory vascular injury induces thrombin formation

Downregulation of fibrinolytic pathways

Downregulation of anticoagulant pathways

Platelet activation

Prothrombotic state → 1+2

Lung hypoxia

Propagation of inflammation

PAI-1

RBC

Fibrin strands

Activated platelets

Deformed Neutrophils, NETS