Why the Immune Mechanisms of Pulmonary Intravascular Coagulopathy in COVID-19 Pneumonia are Distinct from Macrophage Activation Syndrome with Disseminated Intravascular Coagulation

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Why the Immune Mechanisms of Pulmonary Intravascular Coagulopathy in COVID-19 Pneumonia are Distinct from Macrophage Activation Syndrome with Disseminated Intravascular Coagulation.

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Abstract.
The COVID-19 lung pathology shows significant microvascular thrombosis and haemorrhage linked to extensive alveolar and interstitial inflammation that shares features with macrophage activation syndrome (MAS). We have termed this lung restricted immunopathology as diffuse pulmonary intravascular coagulopathy (PIC) which is distinct from disseminated intravascular coagulation (DIC) in its early stages. Raised D-dimers and cardiac enzymes, respectfully reflecting pulmonary vascular bed thrombosis and fibrinolysis and emergent pulmonary hypertension induced ventricular stress in the face of normal fibrinogen and platelet levels, are key early feature of severe COVID-19 related PIC. Lack of confirmation of COVID-19 viraemia in early disease with extensive immunothrombosis over a wide pulmonary vascular territory, rather than systemic viral infection, explains the adverse impact of male sex, hypertension and diabetes on COVID-19 prognosis. The immune mechanisms that underlie diffuse alveolar and pulmonary insterstitial MAS-like inflammation that triggers immunothrombosis over a wide territory that evolves slowly may unmask subclinical cardiovascular disease and is distinct from MAS and DIC familiar to Rheumatologists.

Introduction

The COVID-19 pneumonia pandemic and earlier coronavirus outbreaks have been associated with adult respiratory distress syndrome (ARDS) and a poorer outcome in older subjects(1, 2). The severity of the systemic inflammation in the human coronavirus family members has features reminiscent of a “cytokine storm” or macrophage activation syndrome (MAS) also known as secondary haemophagocytic lymphohistocytosis (HLH)(3, 4). This has inspired severe COVID-19 pneumonia directed anti-cytokine therapy strategies known to be useful in MAS spectrum disease(4, 5). A key feature of HLH/MAS is haemophagocytosis and an acute consumptive coagulopathy leading to disseminated intravascular coagulation (DIC), which has also been reported in COVID-19 pneumonia, but usually as a pre-terminal event (6, 7).

COVID-19 Pneumonia is distinct from Macrophage Activation Syndrome

We recently described how the MAS-like pulmonary immunopathology of COVID-19 pneumonia is very distinctive from “classical” HLH (8). Haemphagocytosis is a cardinal feature of MAS (9, 10), and has also been reported in SARS(11, 12), where latterly it may also represent phagocytosis of extravascular RBCs consequent to severe lung micro-vascular damage, micro-haemorrhage with physiological haemophagocytosis (Figure 1B), or could possibly occur with very advanced disease with frank MAS-like pathology with DIC evolution (Figure 1).

Extensive macrophage and other immune cell infiltration has been reported in SARS pneumonia with similar changes emerging with COVID-19 infection (12-15) that leads to diffuse alveolar damage (DAD). The extensive nature of COVID-19 viral infection and diffuse inflammation extends to involve the large juxtaposed pulmonary vascular network(8) and this diffuse slowly evolving COVID-19 pneumonia with MAS-like clinical and laboratory picture suggested to us an initial “pulmonary intravascular coagulopathy” (PIC) which is very distinct from DIC (8). Herein, we propose a model for this PIC picture and in particular describe how extensive coronavirus driven infection and age related changes in immunity with diffuse pulmonary immunothrombosis explains cardiovascular mortality (Table 1).

Pulmonary Vascular Pathology in SARS and COVID-19

Acute respiratory infections are associated with a higher risk of cardiovascular related deaths, especially in the weeks immediately after infection with older age, pre-existing cardiovascular disease and pneumonia severity being linked to the risk of death (16) (17). Of particular note, one pathological study demonstrated similar vascular changes in post-mortem tissue obtained from subjects succumbing to non-SARS related bronchopneumonia as those who died from SARS (18), indicating that infection triggered vascular thrombosis rather than the inciting agent itself may be key (Figure 2). However, the tropism of coronavirus family members for ACE2 on type II pneumocytes and the close anatomical juxtaposition of the pulmonary network combined with the severe multifaceted inflammatory reactions likely drives the

generalised pulmonary hypercoagulable state in a pneumocyte-interstitium-pulmonary endothelial/small pulmonary vessel axis(8, 19) (Figure 2)

The COVID-19 virus and SARS virus genomes are highly homologous and appear to share common clinical and pathological features (20, 21). Initial post-mortem reports from three SARS cases indicated DAD and small pulmonary vessel thrombosis and haemorrhage but also indicated more generalised small vessel thrombosis (22) with a second study of 6 cases reporting vascular pathology in 2 cases (12). A later study of 20 SARS pathological specimens confirmed DAD but found that fibrin thrombi, small vessel occlusion and pulmonary infarction in upwards of 80% of cases (18). A review of aggregated SARS pathology documented vessel wall oedema, inflammatory cell infiltration into the walls of pulmonary microvasculature, significant haemorrhagic necrosis, vessel microthrombus mostly confined to the lung and pulmonary tissue infarction, in the context of septal inflammation and DAD (23). Limited emerging data in COVID-19 pneumonia paints a similar pulmonary vascular picture with blood vessel wall oedema, modest vessel wall immune cell infiltration, hyaline thrombosis, haemorrhagic change and infarction(14, 24).

Lack of Evidence for Coronavirus Myocarditis or Coronary Vasculitis

Myocarditis may occur after severe respiratory viral pneumonia and is comparatively rare but well documented especially in younger females following influenza infection (Table 2). Understandably, raised cardiac enzymes in COVID-19 have been taken to potentially represent myocarditis or virally associated coronary system vasculitis. Experimental and clinical studies suggested that direct cardiomyocyte infection by coronavirus family and interactions with ACE2 receptor may underscore the cardiovascular complications(25) but most evidence points away from cardiac involvement(26, 27). The ACE2 receptor is also expressed on endothelial cells (EC) and in situ hybridisation study suggested pulmonary endothelial infection (28). Other studies showed a complete absence or very low level EC potential infection(26, 27). Moreover, another study in SARS patients, suggested a close link between pneumocytes infection and pro-inflammatory cytokine expression in the same cells(29), but this was not reported for endothelium. Recent publications indicate that COVID-19 RNA was undetectable in the blood in one study and in only 15% of cases in a second study, again arguing for a very pneumocyte centric and adjacent tissue pathology rather than systemic viral infection (30, 31) (Figure 2).

Laboratory Pointers towards early Pulmonary Intravascular Coagulopathy

The key early laboratory observations in COVID-19 pneumonia are an elevated plasma D-dimer in conjunction with elevated cardiac markers including brain natriuretic peptide, creatinine kinase, and troponin-T levels, the elevation of the latter at hospitalisation being inked to a poor prognosis(32). In keeping with this, a previous studies have reported that elevated plasma levels of fibrin degradation products (FDPs) including D-dimers constitute a significant independent biomarker of poor prognosis(6). For example, Zhou *et al* showed that 90% of patients presenting with

COVID-19 had increased coagulation activation marked by elevated D-dimers at presentation (33). Importantly, D-dimer levels > 1μg/ml were associated with an 18-fold increased odds ratio for fatal outcome (33). Furthermore, progressive elevation of D-dimer and FDP were seen in non-survivors(33). However, despite this increase in D-dimers, COVID-19 patients do not typically develop overt systemic DIC. In rare COVID-19 cases where overt DIC does develop, it tends to be restricted to late stage disease. This is reflected in the consistent observation that platelet count and fibrinogen levels were not significantly reduced in COVID-19 patients despite marked increases in D-dimers. Indeed, fibrinogen generally remained elevated in keeping with an ongoing acute phase response.

Extensive Pulmonary Inflammation and thrombosis in COVID-19 Pathology

As mentioned, severe COVID-19 sepsis is associated with a marked MAS type picture with inflammatory markers and ferritin elevation and undoubtedly result in local pulmonary vasculature EC activation. For example, IL-1, IL-6 and TNF have all been shown to trigger acute EC activation (34). Given the critical roles played by EC in maintaining normal haemostasis, regulating fibrinolysis, and determining vessel wall permeability, local EC pulmonary microvasculature dysfunction is likely to play an important role in the thrombo-inflammatory processes that ultimately result in COVID-19 vasculopathy, V/Q mismatch and a refractory ARDS clinical phenotype.

In addition, the COVID-19 associated MAS-like picture will trigger EC expression of active tissue factor (TF) as well as TF expression on activated infiltrating macrophages and neutrophils (34). The net effect will be presentation of local sources of blood-borne TF within the lungs, which will further amplify coagulation cascade activation. Importantly, EC disruption, TF expression and coagulation cascade activation will all be progressively exacerbated by development of local hypoxia (35), establishing a deleterious positive feedback thrombo-inflammatory loop within the small vessels of the lungs with thrombosis and haemorrhage (Figure 3).

Hypoxaemia development secondary to COVID-19 induced ARDS may be capable of activating the coagulation cascade and could be important in endothelial dysfunction beyond the capillary network and play a role in adjacent small pulmonary vascular thrombosis (36, 37). Other factors including mechanical ventilation in patients progressing to ARDS may be contributory to this picture. The role of vascular microrthrombi formation or “immunothrombosis” in containment of bacterial infection and spread is well established but its role in viral infection remains less well established (38). Likewise, the role of local pulmonary intravascular immunity and its impact in the PIC phenotype is completely unknown (39). Nevertheless, adenoviral access to the circulation in an artificial model system triggers a MAS type picture with DIC(40) .

Finally, it has also been demonstrated in experimental SARS that normal aged primates have a similar degree of viral replication as younger primates but that aged

primates have more pulmonary damage, and have lower type 1 interferon responses gene pathway activation(41). At the molecular level, what was described as as exacerbated innate immune responses was associated with NF-kB gene pathway activation including elevated IL-8 and also elevated expression of Tissue factor-the key extrinisic clotting pathway protein (41). It is well established that type 1 interferon responses in man decrease with age (42). Following respiratory viral infection, type 1 interferon responses are reduced but at what age this transition occurs needs better definition(43). Viral or age related impairment in the "first wave" of type 1 interferon cytokines appears to be associated with a "second wave" of pro-inflammatory cytokines and tissue factor expression that substantially contribute to the PIC picture.

Implications of PIC

The role of anti-coagulation in the COVID-19 PIC setting is of considerable interest. Expert recommendations for the use of anti-coagulants have already been published reflecting the recognition of clotting dysregulation(44). The potential relevance of anti-cardiolipin antibodies in the COVID-18 critical care setting is also recognised but is of uncertain significance(45). However, minimal data are available which are mostly derived from prospective non-randomised cohort studies. In a study of nearly 450 COVID-19 cases, LMWH did not confer an overall survival advantage but in the group with a high sepsis induced coagulopathy (SIC) score there was a substantial survival advantage (33). The role and timing of anticoagulation in this extensive virally related immunothrombosis especially where pulmonary haemorrhaging is occurring needs very careful consideration. Analogous to DIC where thrombosis and bleeding may occur simultaneously, the same scenario appears to also arise in PIC (Figure 3).

Given the MAS-like pathology the question arises whether anti-cytokine therapy will ameliorate the diffuse immunothrombosis process associated in severe COVID-19 pneumonia. In the translational setting, blockade of canakinumab, an IL-1 beta blocker is associated with a decreased risk of all cause cardiovascular mortality(46), with this benefit higher when serum IL-6 fell to the greatest magnitude(47). Unlike low grade arterial inflammation without overt infection, it remains to be seen whether the severe COVID-19 associated MAS with PIC will be successfully targeted using these strategies, but ongoing viral infection might represent a major hurdle(8).

The most critical question is whether the emergent early activation of coagulopathy and fibrinolysis in COVID-19 pneumonia is purely due to an appropriate immune response to the virus, or whether there is a degree of excessive inflammation that could be targeted to help prevent PIC progression. The potential survival advantage of drugs pioneered to treat inflammatory and hyper-inflammatory states needs to be viewed through the lens of severe diffuse pulmonary immunothrombosis. This PIC model has implications for understanding mortality in the current pandemic and for deciphering whether currently used anti-thrombotic or immunomodulatory therapy or both, or neither, have a role in the face of the COVID-19 pneumonia epidemic. It is also important to determine to what degree COVID-19 mediated diffuse alveolar

damage with ARDS development in the absence of coagulopathy may also contribute to outcomes.

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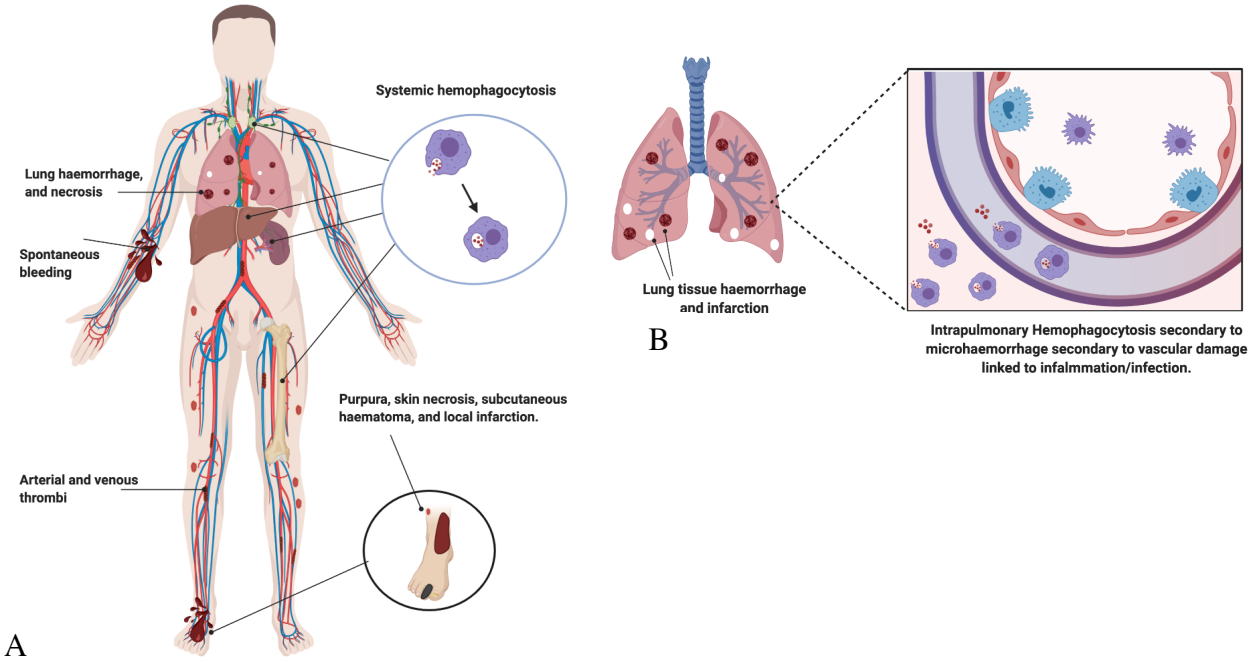
Table 1-Differences and Similarities Between DIC and PIC

Clinical Features	Disseminated Intravascular Coagulation (DIC) linked to HLH/MAS	Pulmonary Intravascular Coagulopathy (PIC) linked to COVID-19
Onset	Acute	Subacute
Hepatosplenomegaly	+++	
Adenopathy	++	
Pulmonary Involvement	50%	100%
Thrombosis	Multi-organ clotting	Lung only (with severe ARDS evolves into DIC)
Bleeding	Generalised	Intrapulmonary micro-haemorrhage
Active Infection Considerations	Yes- for primary HLH Secondary HLH may not have driving infection	Thought to be ongoing alveolar infection
Laboratory Parameters		
Liver Function	Raised Transaminase +++	+/-
Anaemia	+++	-
Thrombocytopenia	+++	Normal/low
Immune Cell Cytopenia	++	No- but lymphopenia a feature of COVID-19 in general
Creatanine Kinase	+ (skeletal and cardiac origin)	+ (worse prognosis)
Troponin-T	+	++ with higher levels associated with worse outcome
Haemophagocytosis	Generalised to marrow, liver and other sites detectable in 80%+	Occasional intrapulmonary and regional lymph node haemophagocytosis reported
Coagulation and Immunology		
Elevated PT/ APTT	+++ / +++	+/normal
Fibrinogen levels	Decreased	Normal/slight increase
FDPs/D-Dimer	Increased	Increased
CRP	Elevated	Elevated
Ferritin Elevation	+++	Elevated
Hypercytokinaemia	+++	++

Table 2- Cardiovascular Disease, Myocarditis and Infection

	Findings	Ref.
Cardiac Injury not specific to COVID-19	Avian Influenza (H7N9) Cardiac injury in 60%+ Also linked to history of cardiovascular disease but not male sex or diabetes (Note myocarditis well documented with influenza in other studies summarised below)	(48)
	pandemic (H1N1) 2009 virus 46% Cardiac Injury Mean age 34 Female more common than male Presumed Myocarditis- No histology/ No post mortem data	(49)
COVID-19 Viral Access to Heart	No Viraemia in 9 cases in COVID-19	(50)
	"RNAemia in only 15%" Cardiac injury linked to ICU admission and not RNAaemia	(31)
Endothelial cells	Express ACE2 but cytopathic changes that are seen in pneumocytes not reported	(29)
Cardiac Myocytes in Human	With SARS no cytopathic change or viral protein detection	
Community Acquired pneumonia Bronchopneumonia	Increased risk of cardiovascular death	(16, 17)
Other factors linked to cardiac Pathology	Pre-existing disease Hypoxia from ARDS development Emergent DIC late disease	
Viral Myocarditis	H1N1 Influenza A Virus. Rare link to Fulminant Myocarditis Reported	(51)
Viral Myocarditis	47 Cases of Fatal Influenza Virus, Children/ Female predominant Myocarditis deemed to be cause of death in 5 cases	(52)

Figure 1



	Early MAS with DIC Picture	Early COVID-19 Picture
Clinical	Hepatosplenomegaly, lymphadenopathy	Pneumonia
Cardiac	Usually Normal	Raised Troponin-T and Brain Natriuretic Peptide
Blood	Anaemia, low platelets	Normal
Clotting	Low Fibrinogen/ high FDPs	Normal fibrinogen/High FDPs
Inflammatory Markers	CPR/ESR Elevated	CPR/ESR Elevated
Bleeding	Diffuse clotting and bleeding	Clotting and haemorrhage confined to pulmonary vasculature
Immuotherapy	Treat Cause e.g. EBV Suppression of inflammaiton	No effective anti-viral therapy Inflammation Suppression?
Anti-Coagulation	Suppression of inflammation	Role of anti-coagulation?
Evolution		May evolve into DIC

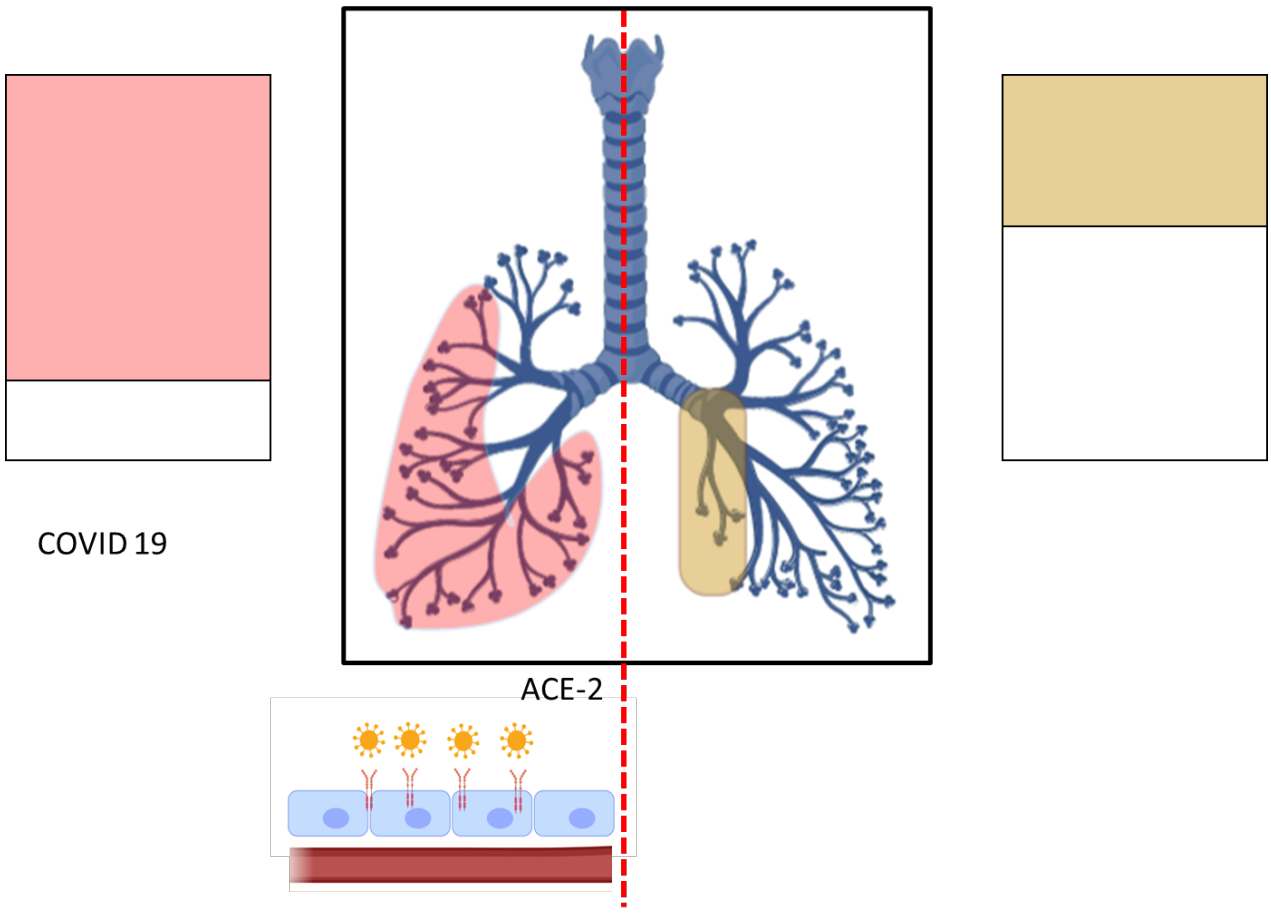
Legend for Figure 1

Figure 1A. Secondary HLH/MAS is associated with organomegaly, thrombocytopenia and haemophagocytosis and disseminated intravascular coagulation (DIC) with pulmonary involvement in half the cases(53). Activation of bone marrow, lymphoid organ, hepatic Kupffer cells and circulating mononuclear cells leads to a severe consumptive coagulopathy with low fibrinongen levels and increased fibrinogen degradation. In addition, liver dysfunction exacerbates the consumptive coagulopathy. A rapid onset DIC pattern with hyperferritinaemia reflecting generalised haemophagocytosis with erythrocyte degradation, sequestration and export with diffuse clotting and bleeding.

Figure 1B. Pulmonary involvement without generalised lymphoid organ hyperplasia is typical of COVID-19 pneumonia. Haemophagocytosis, albeit intrapulmonary has also been reported in coronavirus family infection(12). But in the early stages the systemic coagulopathy is not a feature. Such intrapulmonary haemophagocytosis and that in regional nodes indicates activated macrophage mediated removal of extravascular red blood cells secondary to vascular

injury. A DIC picture may also develop late in the course of COVID-19 pneumonia in cases with ARDS development.

Figure 2

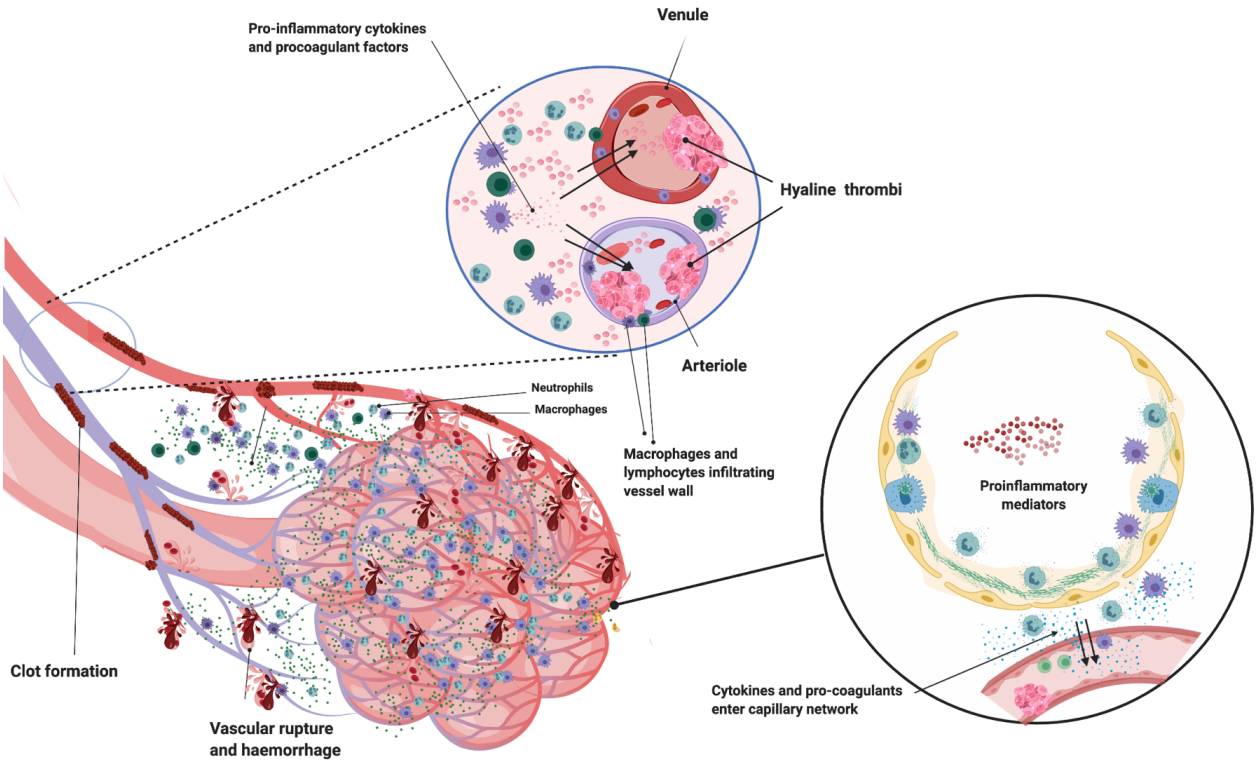


Legend for Figure 2

Figure 2A. Coronavirus family members gain access to the lungs via the ACE-2 receptor that expressed on type II pneumocytes. Diffuse CT scan determined alveolar changes that are distinct from bronchopneumonia show how COVID-19 interfaces with a large area of the pulmonary microvasculature and hence diffuse pathology. Indeed, extensive CT changes associating with a worse prognosis(33) with diffuse CT disease also a feature of subjects who succumbed to SARS infections(28).

Figure 2B. Segmental bronchopneumonia including bacterial and influenza induced more typically hasa different lung distribution, with prominent airway involvement including haemorrhagic destruction of trachea and large airways(52) and generally more patchy alveolar network disease and overall less infection driven immunothrombosis. As shown in Figure 2B there may be larger areas with normal perfusion. The slow evolution of COVID-19 infection with alveolar hypoxia and micro thrombosis may result in pulmonary arterial hypertension and the cardiac picture that mostly occurs with hypoxaemia and raised D-dimers. The scale of alveolar and microvascular inflammation rather than systemic viral infection per se determines the cardiovascular pattern of disease.

Figure 3



Legend for Figure 3

Scheme showing how extensive COVID-19 lung involvement with large anatomical interfacing between infected type II pneumocytes, extensive interstitial immunocyte MAS-like activation and the extensive pulmonary microvascular network, triggers diffuse pulmonary bed extrinsic inflammation with immunothrombosis leading to a microthrombotic immunopathology that leads to right ventricular stress and contributes to mortality.

Diffuse type II pneumocyte centric pathology with extension into the intestituim leads to extensive pulmonary macrophage recruitment and activation leading to a local MAS-like picture. Pro-inflammatory and pro-coagulants gain access to the capillary network (lower circle). The low pressure nature of the vascular system and thin vessel walls in and proximal to the alveolar network triggers immunothrombosis by a variety of mechanisms including local elevations in pro-inflammatory cytokines, vessel wall tissue damage with tissue factor production and direct injury to small vessels. Vigorous fibrinolytic activity (detected early by D-dimer elevation) may not keep check with the extensive microthrombi formation and lead to the evolution of COVID-19 inflammation driven pulmonary infarction, haemorrhaging and pulmonary intravascular coagulopathy induced pulmonary hypertension. Risk factors for cardiovascular disease may thus increase the likelihood of death in severe COVID-19 inflammation.