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REVIEW ARTICLE

Beyond dexamethasone, emerging immuno-thrombotic therapies for COVID-19

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Host immunity is required to clear SARS-CoV-2, and inability to clear the virus because of host or pathogen factors renders those infected at risk of poor outcomes. Estimates of those who are able to clear the virus with asymptomatic or paucisymptomatic COVID-19 remain unclear, and dependent on widespread testing. However, evidence is emerging that in severe cases, pathological mechanisms of hyperinflammation and coagulopathy ensue, the former supported by results from the RECOVERY trial demonstrating a reduction in mortality with dexamethasone in advanced COVID-19. It remains unclear whether these pathogenic pathways are secondary to a failure to clear the virus because of maladaptive immune responses or if these are sequential COVID-19 defining illnesses. Understanding the pathophysiological mechanisms underpinning these cascades is essential to formulating rationale therapeutic approaches beyond the use of dexamethasone. Here, we review the pathophysiology thought to underlie COVID-19 with clinical correlates and the current therapeutic approaches being investigated.

KEYWORDS
anticoagulants, inflammation, randomised controlled trial, translational research, virology

1 | BACKGROUND

In December 2019, coronavirus disease 2019 (COVID-19) caused by the novel severe acute respiratory syndrome coronavirus (SARS-CoV-2), emerged.[1] Unlike other epidemics of novel viruses (e.g. Spanish influenza and human immunodeficiency virus), the rapid spread of SARS-CoV-2 and the commensurate growth in published literature has meant that understanding the clinical and molecular natural history of the disease needs to keep pace with research into treatments that could aid stemming the pandemic.

An early focus within the pandemic has been on repurposing drugs with antiviral properties. This is an attractive approach because such medicines will have prerequisite regulatory approval and can be trialled with greater ease for alternative indications. A vaccine protecting from infection would be the ideal solution although deployment is unlikely prior to 2021, if successfully developed at all. Moreover, a vaccine will not eradicate the disease completely for some time. We propose that in the absence of a vaccine, one strategy could combine the use of medicines targeting viral entry and replication with adjunctive treatments to address complications of serious infection. Such approaches should be subject to high quality trials or, when these are unavailable, clinicians should be encouraged to provide best supportive care where equipoise remains.

Here we review the existing literature aiming to: (i) describe the key pathological phenotypes of severe COVID-19; (ii) describe the molecular pathways underpinning these pathologies; and (iii) describe existing and potential pharmacological interventions to target complications of the disease. We performed a systematic literature search of PubMed from December 2019 to July 2020 with a search strategy including keywords and MeSH terms relating to SARS-CoV-2, COVID-19, immune-thrombotic complications and pharmacological agents tested in COVID-19 patients. We manually searched reference lists of relevant studies. We excluded studies not published as full text articles in English. We also reviewed randomised controlled trials (RCTs) and observational

studies listed on ClinicalTrials.gov pertaining to COVID-19. Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY.

2 | OVERVIEW OF THE CLINICAL COURSE OF THE DISEASE

2.1 | Clinical features

The clinical course of the disease can aid the understanding of the disease pathophysiology. Typically, SARS-CoV-2 results in asymptomatic (15–30%) or pauci-symptomatic (~66%) infection with fever, dry cough, myalgia, fatigue and headache.[2–4] Nonrespiratory features include gastrointestinal symptoms (anorexia, diarrhoea, vomiting and abdominal pain) and anosmia.[5,6] A smaller proportion (4%) deteriorate requiring hospitalisation.[3,4] These patients often exhibit a marked type 1 respiratory failure yet without proportional signs of respiratory distress (the *silent hypoxia* phenomenon).[7] It is from these hospitalised patients that most has been gleaned through routine and experimental sampling.

2.2 | Investigation findings

At this stage, imaging (chest radiographs and computed tomography scans) support occupancy of the alveolar space described either as consolidation (dense opacities with obscuration of underlying vessels) or ground glass opacities (hazy opacities without obscuration of underlying vessels).[8,9] Radiological changes can also be present in asymptomatic individuals; amongst those quarantined from the Diamond Princess Cruise, 39% tested positive, were asymptomatic but had confirmed computed tomography changes.[10] In deteriorating individuals radiological changes may rapidly progress. Pathological correlates from postmortems and lung biopsies indicate that the cell types underlying these infiltrates are likely to be monocytes and macrophages, with minimal lymphocyte infiltration.[11–13]

Blood-based investigations demonstrate early lymphopenia, without a marked systemic inflammatory component indexed by measures of C-reactive protein (CRP). However, deteriorating patients develop elevated CRP and D-dimer.[14] These are thought to represent 2 major complications of COVID-19: hyperinflammation and thrombosis. Less commonly observed abnormalities include elevated troponin, liver enzymes and creatinine; however, these implicate end-organ damage as a result of infection, which may have long-term health implications in survivor cohorts.[14] What drives these changes is not well understood.

2.3 | Current treatment approach

In the absence of specific treatments, the focus of clinical management is supportive care, particularly oxygenation with or without requirement for noninvasive or invasive ventilation.

2.4 | Clinical case examples

Figure 1 shows the typical clinical deterioration of severe COVID-19, associated with the evolution of systemic inflammation and coagulopathy. In contrast, Figure 2 shows a patient with severe COVID-19 who clinically improved, with concomitant down-trending of his CRP and D-dimer.

3 | ANTIVIRAL PHARMACOTHERAPY IN COVID-19

The viral life cycle of SARS-CoV-2 has been comprehensively outlined (see Du et al[15]). Briefly, the **viral spike protein** enables viral entry into host target cells via binding to the **angiotensin converting enzyme 2 receptor (ACE2)**.[16] The human type II **transmembrane serine protease (TMPRSS2)** primes the viral spike protein, leading to fusion of the viral and host cell membranes.[17] Virions are assembled within the host cell and released via nonlytic mechanisms. RNA mapping studies reveal highest ACE2 expression in the nose with a decreasing expression gradient in the distal respiratory tract, suggesting a nasal susceptibility to SARS-CoV-2 with subsequent aspiration-mediated virus seeding to the lung.[18] Viral proteins are already the subject of vaccine development and potential repurposing opportunities with existing drugs targeting SARS-CoV-2-human protein interactions have also been identified (e.g. NCT04324606 and work by Krogan and colleagues).[19] Such approaches, if successful and deployed early in infection, may preclude downstream complications. Studies evaluating antiviral agents in COVID-19 has been extensively reviewed elsewhere (e.g.[20]); a summary of existing approaches is outlined in Table 1.

4 | EVIDENCE FOR IMMUNE DYSREGULATION IN COVID-19

4.1 | A brief overview of the antiviral immune response

Innate immune responses form the first line of defence against respiratory viral infections. These include: alveolar macrophage take-up, proinflammatory cytokine (particularly type interferons (IFN-1)) production and natural killer cell activation, which contributes to viral clearance. Presentation of viral antigens by antigen-presenting cells triggers an adaptive immune response: $CD8^+$ T cells kill virally infected cells, and activated $CD4^+$ Tcells promote a germinal centre response leading to expansion of neutralising-antibody producing B-cells.

4.2 | Immune responses in mild COVID-19

In recovered COVID-19 cases these canonical antiviral pathways appear to be conserved.[39] Mild-to-moderate cases display relatively preserved IFN-1 signatures,[40] although blunted IFN-I and III responses to SARS-

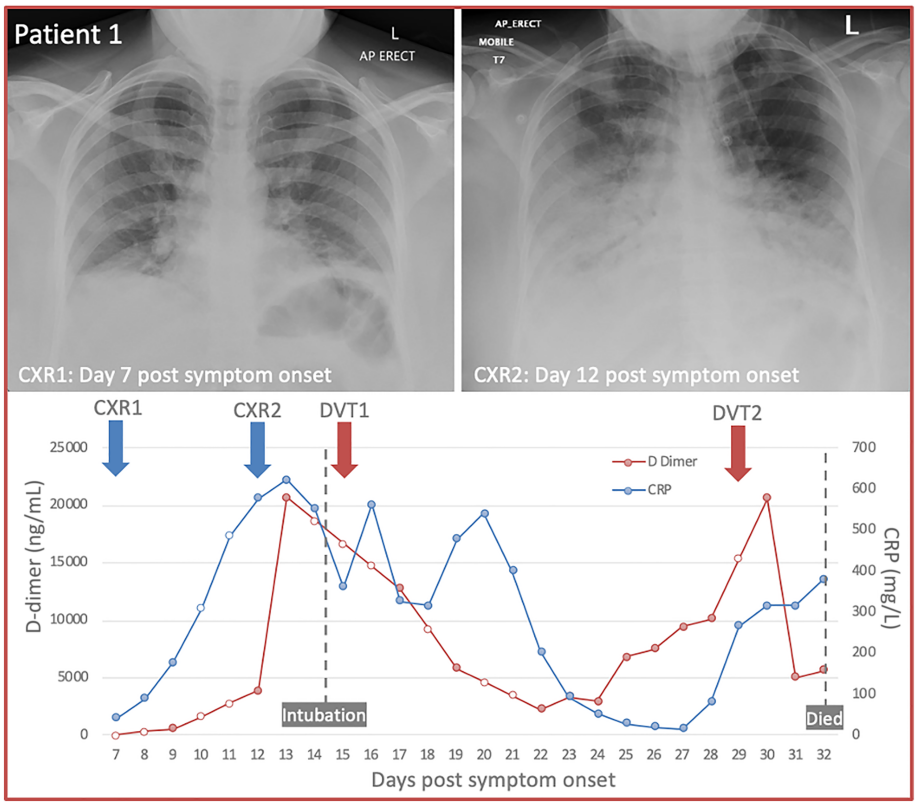

FIGURE 1 Patient case study 1. A patient in their 50s with a history of type 2 diabetes was admitted with a 7-day history of cough, fever and increasing shortness of breath. On admission they were febrile at 38.4°C, oxygen saturations were 98% on room air and the chest X-ray (CXR) showed minor shadowing at the lung bases. COVID-19 was confirmed by polymerase chain reaction. During days 8–12 of the illness, they remained febrile and had increasing oxygen requirements associated with a significant elevation in C-reactive protein (CRP) and D-dimer. A repeat CXR on day 12 showed increased bilateral air space shadowing with a nodular morphology. They deteriorated on day 13, resulting in the need for intubation and ventilation. The patient was found to have an acute below knee deep vein thrombosis (DVT) on day 15 and, despite therapeutic anticoagulation, further bilateral acute DVTs 2 weeks later. They sadly deteriorated further and died 32 days after symptom onset. CRP and D-dimer levels were closely associated throughout their admission and correlated with clinical deteriorations. Interleukin-6 measured during the final days of illness was also elevated (18.5 pg/mL on day 28 and 69.8 pg/mL on day 31). Coloured data points represent laboratory results, white data points represent imputed values for missing data

CoV-2 infection have been seen in vitro tissue culture and in vivo samples derived from COVID-19 patients.[41] In recovered cohorts, prompt and specific IgG production against SARS-CoV-2 correlates to clinical improvement and falling viral load, and robust $CD4^+$ T-cell responses are important in generating anti-spike IgG titres.[42–45] This is also the premise for trialling sera from recovered patients in trials to treat those infected.[46] Nonetheless the molecular pathology of mild infection remains under-characterised; studies in progress (NCT04318314) will help identify the immune responses predicting seroconversion in asymptomatic/mild cases.[47] The molecular pathophysiology underlying severe COVID-19 phenotypes has received more attention; this is outlined below.

4.3 | Immune dysregulation in severe COVID-19

4.3.1 | Dysregulation in the innate immune system

Severe COVID-19 is characterised by a maladaptive host innate immune response leading to a systemic hyperinflammatory state.[48,49] In a prospective analysis of 41 COVID-19 cases in China, patients requiring intensive care unit (ICU) care had higher levels of systemic proinflammatory cytokines, including granulocyte–macrophage colony stimulating factor, macrophage inflammatory protein-1α, and tumour necrosis factor-α; resembling the *cytokine storm* observed in macrophage activation syndrome (MAS), haemophagocytic lymphohistiocytosis and chimeric antigen receptor T cell-induced cytokine release syndrome.[4,50] Retrospective studies have identified elevated CRP, erythrocyte sedimentation rate and **interleukin-6 (IL-6)** as predictors of poor outcomes.[51–54] In a prospective study of 40 hospitalised inpatients in Germany baseline IL-6 levels accurately predicted respiratory deterioration and the requirement of mechanical ventilation.[55] In multivariate logistic regression models (adjusting for confounding factors), IL-6 was significantly associated with COVID-19 related death.[56] In support of these findings a proteomics screening of blood from 31 patients with COVID-19 observed upregulation of a number of proteins implicated in IL-6 signalling, with the biggest molecular shift occurring when patients first needed oxygen.[57] These findings suggest that a unique profile of innate immunity hyperactivity

(notably IL-6 elevation), distinct from that of bacterial sepsis and influenza, occurs in severe COVID-19, however this requires replication in other datasets.

4.3.2 | Dysregulation in the adaptive immune system

The immune dysregulation in severe COVID-19 also appears to be characterised by hypo-active adaptive immunity, impairing viral clearance. Meta-analysis of 27 studies (2874 patients) confirmed that lymphopenia is a prevalent finding and predicts disease severity.[58,59] In a dataset of 326 COVID-19 cases, low T-cell counts at admission predicted disease progression.[60] Direct virally-mediated adaptive immune injury may be central component of SARS-CoV-2 pathogenesis, as posited for SARS-CoV.[61] The virally-driven *cytokine storm* may induce changes in the differentiation and activity of T cells.[62] Indeed an immune profiling study of 54 COVID-19 patients demonstrated that severe respiratory failure was associated with defects in lymphoid function driven by IL-6-mediated decrease in human leukocyte antigen-D related expression, resulting in $CD4^+$ T-cell lymphopenia followed by B-cell lymphopenia, distinct from bacterial sepsis and influenza.[63] Accordingly severe and fatal COVID-19 is associated with older age which is in turn associated with lower lymphocyte counts and relative immunoparesis.[14,64–67] However, neutralising spike protein- and nucleocapsid-specific antibody levels appear to be higher after severe infection than after mild infection, which as with Middle East respiratory syndrome-CoV may reflect persisting viral RNA.[68,69]

4.4 | Potential causes of immune dysregulation in severe COVID-19

4.4.1 | Age

The mechanism by which this maladaptive immune response emerges remains unclear. Exhausted Tcells may be contributory. COVID-19 patients, particularly those progressing to severe symptomatic stages and requiring ICU, have higher percentages of coexpressing Tim3 + PD-1 + T cells, an established marker of T-cell exhaustion during viral infection.[70,71] Another mechanism at play may be the upregulation of pyroptosis, an inflammatory form of programmed cell death.[72] Emerging data demonstrate that blood level of lactate dehydrogenase, a key pyroptosis-related protein, early in COVID-19 correlates with severe disease progression.[73,74] Consistent with evidence that age is a risk factor for severe COVID-19, these 3 immune phenotypes (inflammatory monocyte infiltration from the blood to the extravascular site of injury, T-cell exhaustion and pryoptosis) are known to be upregulated in the elderly.[75–79] Moreover, postmortem lung specimens and bronchoalveolar lavage fluid in severe COVID-19 cases demonstrate a primarily inflammatory monocyte ($CD14^+CD16^+$) infiltrate, consistent with evidence

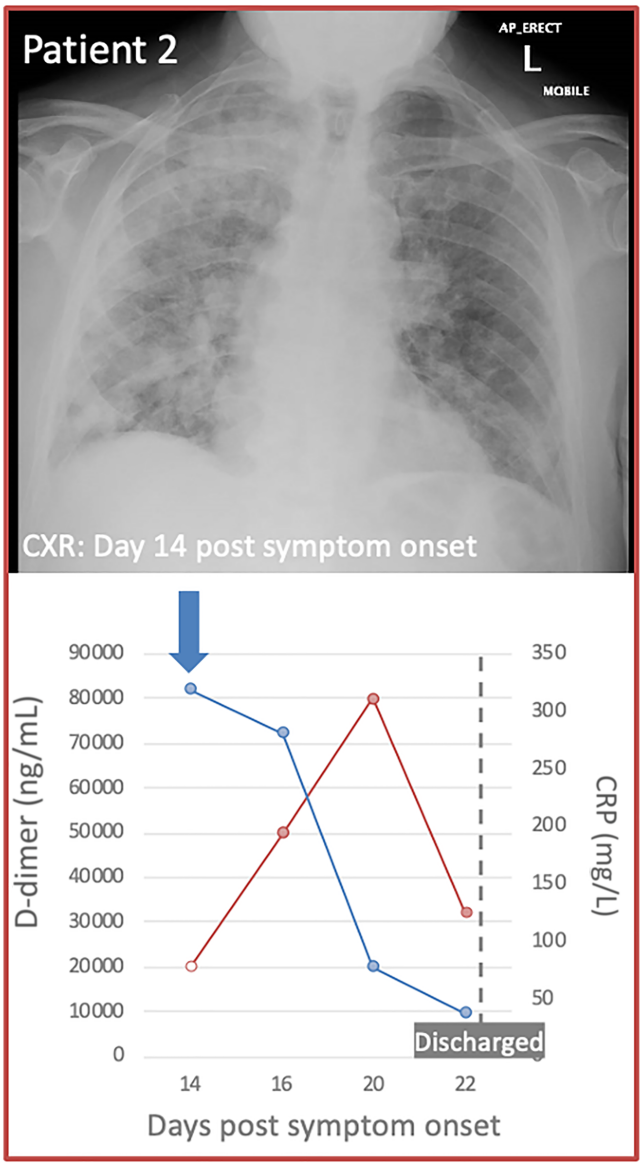

FIGURE 2 Patient case study 2. A patient in his 80s was admitted with a 2-week history of dry cough and a 1-week history of increasing shortness of breath. On admission he was febrile at 38.8°C, oxygen saturations were 85% on room air and the chest X-ray (CXR) showed extensive opacification within the right lung and more subtle changes on the left. COVID-19 was confirmed by polymerase chain reaction. He initially required high-flow oxygen (60% FiO_2); however, over the course of 8 days he clinically improved and was successfully weaned off oxygen. His C-reactive protein (CRP) and D-dimer, both of which were markedly elevated fell in line with his clinical improvement before discharge

that the number of circulating $CD14^+CD16^+$ monocytes are higher in the elderly.[11,80,81] Accordingly, severe COVID-19 in children is rare (the death rate declines to ~0.002% in children aged 9 y and under), although rare Kawasaki-like phenotypes are emerging.[82,83] Whether paediatric populations exhibit a unique protective mechanism remains unclear; it has been postulated that high ACE2 concentrations and constitutional high lymphocyte counts in children may contribute.[84]

TABLE 1 Repurposing drugs with antiviral properties for COVID-19

Pharmacological agent	Original indication	Mechanism of action	*In vitro* studies in SARS-CoV-2	*In vivo* studies in SARS-CoV-2
Hydroxychloroquine and chloroquine	Malaria	Interfere with the glycosylation of ACE2 receptors required for binding of the SARS-CoV-2 spike protein and increase endosomal pH necessary for viral/host cell fusion.[21]	Chloroquine potently blocks SARS-CoV-2 infection at low concentrations and with a high selectivity index.[21]	Positive signals have been noted in open-label studies and a preprint RCT, although case series have failed to replicate the protective effect of hydroxychloroquine.[22–25] A retrospective observational analysis in 96 032 COVID-19 patients suggested increased mortality (~30% excess deaths) with hydroxychloroquine treatment compared to the control group but the paper was subsequently withdrawn due to concerns over the validity of the primary data sources.[26,27] An RCT testing hydroxychloroquine as postexposure prophylaxis for in 821 participants revealed no difference in the incidence of COVID-19 between participants receiving hydroxychloroquine (11.8%) and those receiving placebo (14.3%).[28] Interim findings of the RECOVERY trial (NCT04381936) reported no significant difference in the primary endpoint of 28-day mortality for hospitalised COVID-19 patients randomised to hydroxychloroquine (*n* = 1542) *vs* standard of care (*n* = 3132; hazard ratio 1.11, 95% CI 0.98–1.26) so have halted enrolment to the hydroxychloroquine arm. Similar disappointing interim results have led the SOLIDARITY trial to discontinue the hydroxychloroquine arm (NCT04321616).
Remdesivir	Ebola disease	Interferes with the action of viral RNA-dependent RNA polymerase	Remdesivir inhibits SARS-CoV-2 replication *in vitro* and improves respiratory disease and lung viral loads in a rhesus macaque model of SARS-CoV-2.[21,29,30]	Positive signals were initially noted with use of remdesivir in 53 patients who received remdesivir on a compassionate basis.[31] A subsequent RCT failed to find significant benefit of remdesivir in 235 patients in Wuhan, China; however, the trial failed to recruit its target of 453 patients and was underpowered.[32] A recent highly-powered RCT in 1063 hospitalised COVID-19 patients found that remdesivir shortened recovery time from 15 to

(Continues)

TABLE 1 (Continued)

Pharmacological agent	Original indication	Mechanism of action	*In vitro* studies in SARS-CoV-2	*In vivo* studies in SARS-CoV-2
				11 days relative to placebo, with no significant differences in survival.[33] Other trials investigating the efficacy of remdesivir in COVID-19 remain underway (NCT04292899; NCT04292730; NCT04321616).
Lopinavir–ritonavir	HIV	Protease inhibitors	Lopinavir displays antiviral activity against SARS-CoV-2 in vitro, however the coronavirus protease does not contain the C2 symmetrical pocket, which is the target of HIV protease inhibitors, leading to debate regarding the likely efficacy of HIV protease inhibitors in COVID-19.[34,35]	An open-label RCT in 199 hospitalised COVID-19 patients revealed no benefit in time to clinical improvement with lopinavir-ritonavir beyond standard of care.[36] In June 2020, the RECOVERY trial Steering Committee concluded that there was no beneficial effect of lopinavir–ritonavir in patients hospitalised with COVID-19 and closed randomisation to the treatment arm (1596 patients randomised to lopinavir–ritonavir compared with 3376 patients randomised to usual care alone; 28-day mortality 22.1% lopinavir–ritonavir *vs* 21.3% usual care).[37] The SOLIDARITY trial has also discontinued the lopinavir/ritonavir arm following interim results showing minimal reduction in the mortality of hospitalised COVID-19 patients compared to standard-of-care (NCT04321616). Recent evidence that SARS-CoV-2 uses several cellular proteases as entry activators suggests that a combination of multiple protease inhibitors will probably be needed to achieve clinical benefits.[38]

4.4.2 | Sex

Sex differences may also influence immune responses to SARS-CoV-2. Men are more likely to progress to severe illness and death (for example data from Wuhan shows that 75% of those who died were men, and 70% of critically ill patients admitted to ICUs in New York have been male).[85,86] Although this discrepancy may be explained in part by the higher burden of cardiovascular comorbidities in men (which in themselves are risk factors for severe COVID-19), immune differences may contribute.[86]

Male mice are more susceptible to severe SARS-CoV infection and biochemical oophorectomy of female mice using oestrogen receptor antagonists increases mortality, which may indicate a protective effect for oestrogen receptor signalling.[87] The degree of sex bias in SARS-CoV infection in this mouse model increased with advancing age, suggesting that age and sex may be synergistic risk factors.[87] In a preprint study of 93 hospitalised COVID-19 patients, male patients had higher plasma levels of innate immune cytokines and more robust induction of nonclassical monocytes, whereas female patients displayed more robust T-cell activation.[88] Differences in X-chromosome gene expression (for example toll-like receptor 7, a molecule critical for viral recognition, which escapes lyonization in some immune cells) and sex hormones may also play a role (although the latter is less plausible given that sex biases in COVID-19 mortality persists

postmenopause).[89] Humoral immunity may also differ between sexes: in clinical settings, females exhibit more robust antibody responses to vaccines, but whether sex influences antibody responses in SARS-CoV-2 infection remains unclear.[90] Nonimmune factors may also contribute to the observed sex bias: it has been hypothesised for example that lower oestrogen-mediated baseline expression of ACE2 in women may limit the spread of SARS-CoV-2 beyond the upper respiratory tract.[91]

4.4.3 | Viral load

If an inflammatory storm is established, it is important to understand first if it persists in the presence and therefore as a result of the virus and second the mechanisms by which it accelerates mortality. Regarding the first query, there are few studies that reliably measure ongoing viral infection in patients through their clinical course. One study found that COVID-19 patients requiring ICU displayed persistently high viral loads, peaking 10 days post-symptom onset.[92] In contrast, late respiratory deterioration despite decreasing nasopharyngeal viral RNA has been observed, but viral RNA load in the lungs may not reflect nasopharyngeal viral shedding.[93,94] Ongoing studies are needed to correlate persistent viral load as the key driver for the complications described. This is important as, if true, clearance of the virus itself should afford a degree of resolution of inflammation. Regarding the second query: proinflammatory cytokines increase capillary leak in the systemic circulation, enabling inflammatory mediators to reach the extravascular sites of infection. Indeed, the specific cytokines that are elevated in severe COVID-19 (namely granulocyte–macrophage colony stimulating factor, macrophage inflammatory protein-1α, tumour necrosis factor-α and IL-6) increase capillary permeability.[95–97] The resulting exudates increase extravascular pressures and reduce tissue perfusion. Such vascular shunting in the pulmonary circulation can result in hypoxaemia; consistent with clinical data that COVID-19-affected lungs are physiologically compliant in most, and hypoxaemia is driven by deficits in perfusion rather than alveolar ventilation.[98,99]

Immune profiling is limited in COVID-19 to small and retrospective cohorts. Cytokine profiling is not part of routine blood work and is thus reliant on appropriate laboratories being open and available for contemporaneous investigation of biological markers and molecular phenotypes. Moreover, whilst there is a clear hyperinflammation consequence, it remains unclear if these cytokines are causal.[100] If cytokine excess is a consequence of high viral loads, targeting cytokines alone without virus clearance may not be beneficial, although this remains unproven. Moreover, the exact timing of administration in the clinical course, the dose and frequency of administration of anti-cytokine agents are key considerations that may depart from the usual use of drugs used as repurposed entities. For example earlier treatment with immunosuppressives may avoid the need for ICU although treatment once on ICU may increase the risk *vs* benefit ratio as, by definition, patients are already critically unwell and more prone to side-effects e.g. life-threatening infective complications.

4.5 | Pharmacological approaches to managing immune dysregulation COVID-19

Clinical studies targeting hyperinflammation have now begun to report their results. Most promising of these have been the results from the RECOVERY trial, a multiarmed adaptive-design RCT comparing 2104 hospitalised patients with COVID-19 receiving 6 mg of oral or intravenous **dexamethasone** for 10 days to 4321 patients receiving standard of care. A 30% reduction in mortality was observed in patients with COVID-19 on mechanical ventilation (from 40.7% in the standard of care arm) and a 20% reduction of mortality was observed in patients with COVID-19 on supplemental oxygen.[101] No mortality benefit was seen in patients not requiring respiratory support.[101] These findings are concordant with the hypothesis that hyperinflammation is a key contributor to mortality in severe COVID-19. Another treatment arm of the RECOVERY trial is evaluating the impact of **azithromycin**, a commonly used antibiotic with cellular and humoral immunomodulatory effects, on mortality outcomes (NCT04381936). **Colchicine**, commonly used in autoinflammatory disorders and gout, counteracts inflammasome assembly, thereby reducing the release of a range of proinflammatory cytokines, is being assessed in 2 phase II trials (NCT04326790; NCT04322565).

The above anti-inflammatories are blunt tools for dampening hyperinflammation; given evidence for pathological upregulation of specific immune pathways in COVID-19, therapeutics that inhibit specific proinflammatory pathways are underway. Early studies targeting cytokines have begun, and these may give an early and important steer of the risk benefit ratio of such strategies. In an observational study of 210 patients with COVID-19 requiring ICU, those who received **tocilizumab** (an IL-6 blocker) had reduced mortality (hazard ratio 0.64, 95% CI 0.47–0.87).[102] A recent retrospective cohort study in 1351 patients admitted with severe COVID-19 found that 73 (20%) of patients in the standard care group died, compared with 13 (7%; $P < .0001$) patients treated with tocilizumab.[103] Retrospective reports have however raised concerns that IL-6 blockade may increase the risk of secondary bacterial infections.[104] However, these observational findings contrast with early indications of IL-6 blockade in COVID-19 where an initial trial has reported that the primary endpoint was not reached; however, the full report is awaited as are results of other trials.[105] Other anti-inflammatory approaches targeting different cytokine pathways are also under investigation. **IFN-β** (an agent licensed for multiple sclerosis that shifts cytokine networks in favour of an anti-inflammatory axis) has shown promising signals in in vitro and in vivo studies in Middle East respiratory syndrome-CoV and SARS-CoV, and RCTs in COVID-19 patients are in progress (ISRCTN identifier: 83971151, EudraCT: 2020–001023-14).[106] A small prospective study of **mavrilimumab**, a monoclonal antibody targeting granulocyte–macrophage colony-stimulating factor, a cytokine which activates proinflammatory pathways in macrophages and neutrophils and increases secretion of proinflammatory cytokines including IL-6, trialled in 13 patients with COVID-19 and hyperinflammation has shown positive signals.[107] Appropriately powered RCTs assessing the effect of interleukin

blockade are necessary and underway (NCT0431022853, NCT0430670552, ChiCTR200002976549, ChiCTR200003019650, ChiCTR200003044251).

Dysregulation in the renin–angiotensin aldosterone system (RAAS) has been identified as an inflammatory hallmark of severe COVID-19, with skewing of the ACE/AngII axis hypothesised to amplify inflammatory organ damage. For example in a study of 12 hospitalised SARS-CoV-2 patients in China, plasma angiotensin II (AngII) levels were markedly elevated and linearly associated to lung injury.[108] RAAS inhibition is being explored in several RCTs and may have a role in treating severe COVID-19.[109]

A key consideration is the optimal time-point to initiate an immunomodulatory approach. RECOVERY highlighted an incremental response to dexamethasone with disease severity and longer duration of symptoms. Similarly the case outlined in Figure 1 illustrates that there may be a window of opportunity to dampen the hyperinflammatory response, which may be more efficacious than the same approach employed at other disease stages. It has been suggested that severe COVID-19 may be characterised by a stage subsequent to hyperinflammation: *viral breakthrough* in which T lymphocytes become exhausted, viral replication rises, and the effect of immunosuppressives wanes.[110] As outlined above, lung-resident and circulating T lymphocytes from severe COVID-19 patients exhibit transcriptional hallmarks of exhaustion.[70,71] Accordingly, therapeutics attempting to minimising T-lymphocyte exhaustion, such as IL-7 and anti-PD-1, have been shown to promote antiviral immunity in other viral infections, and may warrant exploration in severe COVID-19 not responding to dexamethasone.[110–112] Trials investigating IL-7 and anti-PD1 are underway (NCT04379076; NCT04407689; NCT04356508). A phase II trial of N-acetylcysteine, an antioxidant thought to restore T-lymphocyte function, is also in progress (NCT04374461). Similarly, as demonstrated by the RECOVERY trial, immune-dampening in early-stage disease may prove less beneficial. It has been hypothesised that, at this stage, boosting the IFN response to promote viral clearance before hyperinflammation ensues may be beneficial, in keeping with observations that IFN-I and III responses are blunted in response to SARS-CoV-2 infection.[41] Trials testing IFN-III amplification in patients with mild–moderate disease are underway (NCT04388709; NCT04354259).

5 | EVIDENCE FOR HYPERTHROMBOTIC STATES AND MICROVASCULAR DYSFUNCTION

5.1 | Evidence from clinical and biochemical data

Descriptions of critically ill COVID-19 patients describe a procoagulant state; commonly clotting off arterial lines and dialysis catheters and with a markedly high incidence of thrombotic complications, including pulmonary and deep vein thrombosis (DVTs), often in the absence of other major predisposing factors.[113,114] Indeed, the patient outlined in Figure 1 developed a DVT despite prophylactic low molecular weight heparin (LMWH) and further acute DVTs despite 2 weeks on full treatment dose. Emerging reports suggest an increased incidence of large-vessel thrombotic stroke in COVID-19 (7-fold increase in 1 centre), a complication which is not typically associated with bacterial sepsis or other viral infections.[115] Biochemical data supports a COVID-associated coagulopathy. A retrospective cohort study of 191 COVID-19 cases showed increasing odds of death with a D-dimer above 1 μg/mL which persisted in multivariable regression accounting for potential confounders including age.[54] In a further study coagulopathy, defined as a 3-second extension of prothrombin time or a 4-second extension of activated partial thromboplastin time, was present in 50% of nonsurvivors compared to 7% of survivors.[116] A prospective analysis of haemostatic parameters in 183 patients in Wuhan identified that prolonged prothrombin time and activated partial thromboplastin time, and significantly elevated D-dimer and fibrin degradation product levels were present on admission in nonsurvivors compared to survivors.[117] Further evidence suggests that raised D-dimer is associated with the development of acute respiratory distress syndrome in COVID-19 and progression from acute respiratory distress syndrome to death.[56]

5.2 | Evidence from pathological data

Postmortems and minimally invasive autopsies reveal extensive pulmonary microvascular thrombosis in critically ill COVID-19 patients.[118–121] Although some studies have revealed coexistent DVTs (58% in the German autopsy series), this was felt to be a late effect following prolonged admission, with the primary pathology being pulmonary thrombosis driven by extensive pulmonary microthrombi.[121] This is consistent with clinical observations that *in situ* pulmonary thrombi are more common in severe COVID-19 than pulmonary emboli from peripheral veins.[122] An autopsy series of 67 patients revealed, alongside pulmonary microthrombi, extrapulmonary microthrombi in organs with high endothelial ACE2 expression, for example the brain.[118] Moreover prominent haemophagocytosis and an haemophagocytic lymphohistiocytosis-like phenotype was identified in the lymphoid tissue of a subset of patients with concomitant elevation in cytokines and inflammatory markers, paralleling findings of MAS.[118] In a clinicopathological case series of 14 people who died from COVID-19 in the USA, although the primary pathology observed in all patients was of diffuse alveolar damage, 4 patients showed focal pulmonary microthrombi and 2 patients had evidence of central pulmonary arterial thrombi.[123] Together these findings point towards a thrombotic microangiopathy syndrome and associated procoagulant state, driven by ACE2 tropism and a MAS-type hypercytokinaemia.[118]

5.3 | Potential causes of hypercoagulability and microvascular dysfunction

The drivers of hypercoagulability in COVID-19 remain unclear. Given the high incidence of venous thromboembolism (VTE) in severe

COVID-19, risk factors may extend beyond those classically associated with critical illness and/or ICU admission (namely immobility and the haemodynamic impact of prolonged mechanical ventilation with high pressures). The hyperinflammatory state is likely to be contributory; it is well established that profound infection leads to activation of the coagulation cascade, impaired fibrinolysis and disruption of the endothelial barrier. Indeed the CRP and D-dimer were closely associated throughout the admission of the patient in Figure 1. The *MAS-type* intrapulmonary inflammation may also predispose to severe local vascular dysfunction including microthrombosis and haemorrhage leading to lung-centric intravascular coagulopathy.[124] Given the higher incidence of VTE in severe COVID-19 compared to bacterial sepsis (17–27% *vs* 3.2%), viral-specific factors may also drive thrombotic complications.[113,125,126] Receptors on the host cell surface that facilitate entry of SARS-CoV-2 (the critical ACE2, but also TMPRSS2, sialic acid receptors and extracellular matrix metalloproteinase inducer) are expressed on endothelial cells.[127–130] A single-cell atlas study has also revealed ACE2 receptor expression on pericytes, perivascular mural cells with key regulatory roles on the microcirculation, such that pericyte injury due to virus infection could result in capillary endothelial cell dysfunction.[131] SARS-CoV-2 can also directly infect engineered human blood vessel organoids *in vitro*.[132] A recent postmortem analysis of 3 patients with severe COVID-19 found evidence of direct viral infection of endothelial cells and diffuse endotheliitis.[133] As such, COVID-19-endotheliitis could drive impaired microcirculatory function, providing a rationale for trialling therapies that stabilise the endothelium.[133]

The purported microvascular dysfunction may also explain why disorders characterised by microarteriopathy are risk factors for severe COVID-19. In a recent major systematic review of 30 retrospective observational studies (530 000 COVID-19 patients), factors predicting severe disease and death included diabetes, hypertension and cardiovascular disease.[134] In a meta-analysis of 2552 COVID-19 patients, hypertension was associated with a ~2.5-fold increased risk of disease severity and mortality, and there was weak evidence from meta-regression suggesting that hypertension may be a clinical predictor of severity in patients over the age of 60 years.[135] RAAS inhibitors are frequently prescribed in this cohort, leading to concerns that ACE inhibitors and **angiotensin-receptor blockers (ARBs)** may be harmful in patients with COVID-19.[136] Animal studies have also shown that ACE inhibitors and ARBs can upregulate ACE2 expression, which may increase target availability for SARS-CoV-2.[137] Several large observational studies, however, found that RAAS inhibitor use was not associated with increased risk of severe COVID-19 or the risk of in-hospital death, and may even reduce the risk of COVID-19.[138–142] A retrospective study of 7335 COVID-19 cases in China reported that type 2 diabetes independently increased the risk of all-cause mortality.[143] People of black ethnicity may be at increased odds of mortality from COVID-19.[144] The effect appears to be only partially attributable to pre-existing clinical risk factors and deprivation.[145,146] Interest has also focussed on ethnic differences in the expression of ACE2, with a preliminary report noting significantly higher ACE2 expression in East Asian individuals compared to other ethnic groups.[147] To understand susceptibility to severe COVID-19 in groups where comorbidities and social determinants of health (including health-seeking behaviour) cluster these associations need to be examined on a larger scale.

5.4 | Pharmacological approaches to managing coagulopathy in COVID-19

What remains unclear is the strategy for thrombo-prophylaxis and/or anticoagulation of hospitalised patients or high-risk ambulatory patients with COVID-19. Anecdotal evidence, such as that presented in Figure 1, suggests a significant failure rate of standard prophylactic regimens. Indeed in the absence of clear data, certain groups (e.g. Imperial) are initiating treatment-dose LMWH in patients with D-dimer levels >3000 μg/L.[148] The debate regarding appropriate anticoagulation has not been assessed in large-scale studies and bleeding risk is currently unknown. Clinical trials evaluating the impact of anticoagulation with usual, subtherapeutic although greater than prophylactic doses, and therapeutic dose LMWH and/or unfractionated heparin on survival are in progress (NCT04345848; NCT04344756). Drugs which may counter both endothelial dysfunction and procoagulopathy, for example ARBs via modulation of the ACE2/angiotensin/MAS and angiopoietin/Tie-2 signalling axis, have also been hypothesised as 1 approach to treating patients with severe COVID-19.[149] Natural history cohorts will also be important in determining outcomes where blood-based measures, radiologically-proven thrombosis, and long-term outcomes can be determined.

6 | RECOMMENDATIONS

By reviewing what is known about the clinical and molecular pathophysiology of COVID-19 we have outlined a framework to understand existing therapeutic endeavours. Rational efforts to repurpose existing drugs can be understood in the context of the molecular pathways outlined—from upstream targets (entry via ACE2 or viral replication) to downstream targets (modulating the hyperinflammatory state and/or the coagulopathy). We therefore propose that 1 therapeutic approach could be viral clearance by either small molecular entities or preventative approaches when vaccines are available. However, advanced cases where immunological and thrombotic complications are present may require a combination approach, targeting both viral clearance and adjunctive treatment to address the key complications of serious infection (hyperinflammation and coagulopathy). The benefit of antivirals as adjunctive treatments in severe COVID-19 requires a better understanding of the degree to which viral persistence contributes to deterioration, requiring further studies exploring the relationship between viral RNA load kinetics and disease severity.

To move forward, it is essential to analyse the clinical phenotypes by collecting data on patient demographics, comorbidities, medication history, disease severity, and progression towards surrogate and clinical endpoints. We also require detailed laboratory data, including virology parameters (viral load, acute and convalescent serology) and

inflammatory markers (including cytokine profiling), ideally with both real-time systemic and intrapulmonary monitoring. Genome-wide association studies, RNA and proteomic analyses will be crucial in evaluating the pathogenic mechanism behind intrinsic risk factors (for example sex and ethnicity), and approaches including Mendelian randomisation may steer towards causal pathways prioritising drugs for repurposing. Once the backlog of coronial autopsies is processed, research autopsies on COVID-19 positive patients must be prioritised. The histology from postmortem studies, as well as the cytopathology from bronchoalveolar lavage, will be crucial in elucidating the mechanisms of mortality. Across all of these data domains, large cohorts will need to be analysed before conclusions can be drawn. To better understand the molecular pathways at play, efforts should also be made to elucidate the clinical and molecular phenotype of asymptomatic and mild SARS-CoV-2 cases.

COVID-19 has demonstrated relevance of clinical pharmacology from discovery of new molecular entities and updating trial design to tackle the disease and has produced results at pace. Moreover, whilst we have outlined here that there is still much to learn, it is important to note that dexamethasone itself has a large treatment effect, is widely available and cheap, and therefore accessible to all health economies. Whilst we continue to learn about the exact pathways of disease and the place in therapy of emerging treatments, this success is important to acknowledge and celebrate.

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COMPETING INTEREST

There are no competing interests to declare.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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