

Title: Weathering the cytokine storm in susceptible patients with severe SARS-CoV-2 infection

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High risk patients requiring hospitalisation for severe SARS-CoV-2 infection are those over 60 years old with common comorbidities including hypertension, cardiovascular disease, diabetes and chronic lung disease.<sup>1</sup> In one meta-analysis, the relative risk for experiencing severe versus non-severe COVID-19 disease was 58%, 59% and 71% higher in patients with hypertension, respiratory disease and cardiovascular disease respectively.<sup>1</sup> Use of angiotensin converting enzyme (ACE) inhibitors and angiotensin receptor blockers for hypertension, heart failure and diabetes up-regulates expression of ACE2, the latter being involved in the binding of SARS-CoV-2 to lung epithelium.<sup>2</sup> It remains unclear whether the increased risk of COVID-19 infection is attributable to hypertension or age related comorbidity per se. Upstream therapeutic strategies revolve around the use of antivirals. One study disappointingly showed no benefit in using lopinavir plus ritonavir over standard of care for hospitalized adult patients with severe COVID-19 pneumonia even though the study was likely underpowered.<sup>3</sup> This in turn perhaps infers that using upstream anti-viral therapy alone may not be successful later on in the illness once the downstream cytokine avalanche has been triggered, resulting in subsequent lung damage. Having said that compassionate use of iv remdesivir in 53 patients with severe COVID-19 infection resulted in 68% having improved oxygenation status and 18% mortality among those receiving invasive ventilation <sup>4</sup>.

Blocking viral host cell entry with either hydroxychloroquine or is another potential upstream modality. Hydroxychloroquine and chloroquine are both used for malaria with the former being safer and more potent in terms of in vitro SARS-CoV-2 suppression.<sup>5, 6</sup> Hydroxychloroquine acts via ACE2 and increases endosomal pH to attenuate SARS-CoV-2 endocytic host cell entry into the lung epithelium. Hydroxychloroquine has shown some promising preliminary clinical results in terms of attenuating in vivo SARS-CoV-2 viral load<sup>7</sup> and has been included as a treatment arm in the WHO SOLIDARITY trial as well as the NIHR RECOVERY and PRINCIPLE trials. Hydroxychloroquine may also exhibit downstream immunosuppressive effects by reducing IL6 production from T cells and monocytes,<sup>8</sup> which may explain in part its use in autoimmune conditions such as rheumatoid disease and SLE. Bromhexine is an over-the-counter cough remedy that blocks receptor mediated viral cell entry via the transmembrane protease, serine 2 (TMPRSS2) enzyme.<sup>9</sup> Theoretically, there could be potential synergy in terms of more effectively blocking viral host cell entry using combined hydroxychloroquine and bromhexine (NCT04340349), but at present this only remains speculative. It remains to be seen whether such agents are more likely to be effective early on in COVID-19 disease given that they prevent viral entry into host cells.

Little consideration appears to have been given to susceptible patients with obstructive airways disease with regards to COVID-19 infection. In particular, elderly COPD patients have impaired respiratory reserve and a plethora of comorbidities, which potentially confers both pathophysiological and pharmacological susceptibility. Such individuals are at high risk of adverse outcomes as a result of severe SARS-CoV-2 infection. COPD patients taking inhaled corticosteroid (ICS) combination therapy have an increased pneumonia risk, especially with lipophilic drugs such as fluticasone furoate<sup>10</sup> due to its prolonged lung retention and associated local immunosuppression in the presence of altered lung microbiome and impaired mucociliary clearance.<sup>11</sup> Moreover, suppression of interferon by fluticasone propionate is associated with an increased bacterial load following rhinovirus infection.<sup>12</sup> Corticosteroids may also attenuate production of the antibacterial protective peptide cathelicidin in the lung epithelium.<sup>13</sup> Thus, secondary bacterial infection might contribute to the cumulative inflammatory burden in addition to viral pneumonia. Caution should be exercised in extrapolating from COPD to asthma, even though up to 20% of COPD patients have a corticosteroid responsive eosinophilic component. Hence, in COPD patients with blood eosinophils  $\geq 300$  cells/ $\mu$ l the benefit of fluticasone furoate in reducing severe exacerbations outweighs its risk in inducing severe pneumonia.<sup>14</sup> Nevertheless, one Canadian cohort study of asthma patients demonstrated that current ICS use was associated with a 45% relative increased risk of pneumonia, amounting to an excess of 1.44 cases per 1,000 patient years.<sup>15</sup>

Interestingly, ciclesonide and mometasone, but not budesonide, beclomethasone or fluticasone, exhibit in vitro suppression of SARS-CoV-2 replication to a similar degree as lopinavir.<sup>16</sup> For ciclesonide, its target on viral replication appears to be non-structural protein 15 (NSP15). Pointedly, asthmatics taking ICS are 49% less likely to have a severe outcome following hospitalization for influenza A infection, perhaps inferring a generic protective ICS class effect. In the meantime, the key message for our asthma patients is to adhere to their ICS controller therapy as this is likely to offer the best protection against any viral insult including SARS-CoV-2. At this juncture, no evidence can support switching asthma patients to inhaled ciclesonide or mometasone on the basis of putative protection against SARS-CoV-2. A study evaluating ciclesonide in South Korea will look at the rate of SARS-CoV-2 eradication in patients with mild COVID-19 infection (NCT04330586).

Corticosteroids may be considered as a rather blunt tool for dealing with the cytokine cascade in COVID-19 infection as they exhibit a broad-spectrum suppressive effect on a wide variety of cytokines. Systemic corticosteroids are part of the routine

management of acute viral exacerbations of asthma and COPD and are effective at treating the eosinophilic component of type 2 inflammation.<sup>17</sup> Corticosteroids may also suppress host innate immune responses and increase viral replication which is reversed by adjuvant interferon.<sup>18</sup> A study with inhaled interferon-beta-1a (SNG001) will evaluate whether up-regulating lung antiviral defenses is effective in COVID-19 illness, while other trials will evaluate subcutaneous interferon-beta-1a with lopinavir/ritonavir (NCT04315948).

One might postulate that nebulized interferon-beta-1a may not achieve adequate alveolar drug concentrations in severe COVID infection since by the same token nebulized antibiotics are not effective in bacterial pneumonia.

SARS-CoV-2 infection may induce a profound downstream cytokine cascade involving IL1, IL6, IL12 and TNF- $\alpha$  (Figure).<sup>19</sup> This release of cytokines is followed by rapid development of lung tissue damage resulting in ARDS, sepsis and organ failure, which may require assisted ventilatory support and extracorporeal membrane oxygenation. One study in severe COVID-19 infection found that risk of respiratory failure in patients with circulating IL6 levels >80pg/ml was 22 fold higher with a median time to mechanical ventilation of 1.5 days.<sup>20</sup>

A more selective approach is therefore required to address the downstream cytokine storm. Recent attention has centered around the possibility of therapeutic intervention with anti-IL6 drugs such as tocilizumab and sarilumab which are indicated for rheumatoid disease. There is emerging evidence that they may also be useful when repurposed for severe SARS-CoV-2 infection in terms of dampening the downstream cytokine response and the associated hyperinflammatory syndrome, the latter primarily characterized by secondary hemophagocytic lymphohistiocytosis.

A compassionate use study from China in 21 patients infected with SARS-CoV-2 using the anti-IL6 agent tocilizumab showed a rapid reduction in fever, C reactive protein (CRP) and oxygen requirement along with improved radiological appearances and normalization of lymphocyte counts within 5 days of administration of a single 400mg dose.<sup>21</sup> Moreover 90% of patients were discharged within a mean hospitalization period of 13.5 days after tocilizumab. All patients already had routine treatment for 1 week including lopinavir and methylprednisolone prior to receiving tocilizumab. A significant limitation may therefore be survival bias in that sicker patients would be expected to have rapidly deteriorated within the first week of hospitalised illness. Evidently these data need urgent replication ideally in controlled trials. A study is now under way recruiting hospitalized patients with SARS-CoV-2 (NCT04315298) using the anti-IL6 agent sarilumab, while three other studies are evaluating tocilizumab alone: COVACTA (NCT04320615) and TOCOVID

(NCT04322773), or in combination with favipiravir (NCT04310228). We would advocate that patients with severe COVID-19 infection should be screened for biomarkers of hyperinflammation including rising CRP, ferritin, D-dimer, plasma viscosity and cytopenia including falling platelet count, as these may highlight at-risk patients where IL6 suppression could be beneficial.

There are cogent reasons to suggest that a combined treatment modality might be required to obviate upstream SARS-CoV-2 lung tissue binding with hydroxychloroquine or bromhexine and downstream IL6 blockade by sarilumab or tocilizumab (Figure). With this in mind we believe studies are urgently warranted to investigate such combination therapy in older susceptible individuals with comorbidities who are at high risk for developing severe COVID-19 pneumonia to assess if this might reduce duration of hospital stay, especially when the reality is that such patients are less likely to be offered assisted ventilation.

#### **Key learning points:**

- Severe SARS-CoV-2 infection with poor outcomes occurs in older susceptible patients with multiple comorbidities including hypertension, cardiovascular disease, diabetes and chronic lung disease.
- Patients with eosinophilic asthma and COPD should continue to use ICS containing therapy to maintain optimal control and protect against viral insults including SARS-CoV-2 infection.
- Antiviral therapy such as lopinavir-ritonavir may not be successful unless used early in the course of COVID-19 infection.
- More severe COVID-19 infection may produce a cytokine storm associated with hyperinflammatory syndrome and hemophagocytic lymphohistiocytosis, with IL6 levels being highly predictive of respiratory failure.
- Screening for biomarkers of hyperinflammation such as cytopenia, CRP, D-dimer, plasma viscosity, and ferritin may identify at-risk patients where cytokine suppression may be beneficial.
- Clinical trials are urgently warranted to evaluate a combined therapeutic strategy to target upstream (hydroxychloroquine or bromhexine) and downstream (sarilumab or tocilizumab) pathways in severe COVID-19 disease.

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## Figure legend

Depicts the cytokine cascade resulting from acute severe SARS-CoV-2 infection, with downstream IL-6 activation considered to be a hallmark feature in terms of progression of COVID-19 pneumonia to ARDS. Also shown are the putative mechanisms of action for bromhexine and hydroxychloroquine in attenuating upstream SARS-CoV-2 tissue binding, the effect of antivirals on replication, together with Tocilizumab and Sarilumab's effect downstream as an IL-6 blocker.

ACE2 – angiotensin converting enzyme 2

ACEI – angiotensin converting enzyme inhibitor

ARB – angiotensin II receptor blocker

ARDS – acute respiratory distress syndrome

CD4 – cluster of differentiation 4

ECMO – extracorporeal membrane oxygenation

IL-6 – interleukin 6

RNA – ribonucleic acid

SARS-CoV-2 – severe acute respiratory syndrome coronavirus 2

TMPRSS2 – transmembrane protease, serine 2