Introduction
The global COVID-19 pandemic has triggered the hunt for an effective anti-viral treatment, as well as a race against time in the development of a vaccine. Given the current lack of specific treatment protocols and the high mortality in certain vulnerable groups, researchers are mainly focused on the repurposing of available drugs to develop quick and cost-effective therapeutic strategies, in particular, targeting the hospitalised and critically ill cases. Public health preventative strategies center mainly on social distancing, as well as rapid isolation and containment of identified disease carriers. The individual is encouraged to practice good personal hygiene, be socially responsible, while maintaining health and well-being. Many have sought and tested the spectrum of alternative therapies and dietary modifications available on the market. In this review, we look at the evidence of serum vitamin D levels in relation to COVID-19 infections in retrospective studies, examine the use of vitamin D supplementation in other respiratory tract infections, and describe the anti-viral/microbial and immune-modulatory effects of vitamin D as well as potential adjunctive role in targeting SARS-CoV2.

Vitamin D and the Immune System
The main role of vitamin D in the human body is in calcium homeostasis. However, the vitamin D receptor (VDR) is ubiquitously expressed throughout the body, including on immune cells [1]. The
main form of oral vitamin D supplementation is in the form of 25-hydroxyvitamin D3 \([25(OH)D]\), which is an inactive form of vitamin D. 1α-Hydroxylase converts 25(OH)D to the active form of 1,25-hydroxyvitamin D \([1,25(OH)_{2}D]\). In calcium homeostasis, the parathyroid hormone (PTH) controls the activity of renal 1α-hydroxylase. Extra-renally among immune cells, 1α-hydroxylase is expressed by peripheral blood monocytes, macrophages, B-cells, T-cells as well as dendritic cells [2]. As such, immune cells possessing both VDR and 1α-hydroxylase, control the synthesis of 1,25(OH)\(_{2}\)D in an autocrine fashion. This in turn begs the question of the role of vitamin D in modulating the immune system.

Vitamin D exerts its effects in both the innate and adaptive immune response [3]. In the event of microbial infection, macrophages recognize the lipopolysaccharide (LPS) particles through Toll-like receptors (TLR), hence triggering a cascade of peptide syntheses (cathelocidin and β-defensin4) with potent bactericidal activity [4] (▶ Fig. 1). TLR binding stimulates expression of 1α-hydroxylase and VDR [4]. The binding of 1,25(OH)\(_{2}\)D to VDR is needed for the transcription of cathelocidin and β-defensin-4 [4]. In particular, Liu et al found that the transcription of cathelocidin is dependent on sufficient 25(OH)D levels [4]. In addition, 1,25(OH)\(_{2}\)D inhibits monocyte production of the inflammatory cytokines IL-1, IL-6, IL-8, IL-12, and TNFα (▶ Fig. 1) [5], the fundamental players which build up to a cytokine storm in severe SARS-CoV-2 infection resulting in acute respiratory distress syndrome (ARDS) as well as multi-organ failure.

Furthermore, vitamin D modulates adaptive immunity by inhibiting B cell proliferation, differentiation and secretion of antibodies [6] (▶ Fig. 1). Vitamin D also inhibits T cell proliferation and modulates the T cell phenotype (▶ Fig. 1). It stimulates the development of the suppressive T regulatory cells but inhibits the development of pro-inflammatory Th17 cells [7]. This results in the production of anti-inflammatory cytokines such as IL-10 but decreases the production of inflammatory cytokines such as IL-17 and IL-21 [7]. Hence, possessing adequate levels of 1,25(OH)\(_{2}\)D may have a protective effect against COVID-19 in relation to the immune suppression of cytokine response and possibly reduced severity or risk of ARDS and multi-organ failure. To the best of our knowledge, no randomized controlled study has yet reported vitamin D status and cytokine levels in patients with COVID-19 infection. As the inflammatory marker C-reactive protein (CRP) is a good surrogate of IL-6 [8], we propose to use CRP as a cost-effective marker to estimate the association between vitamin D status and severe COVID-19 infection.

**Vitamin D and its association with respiratory infection**

Multiple studies have demonstrated an association between low serum 25(OH)D concentrations and susceptibility to acute respiratory tract infection (RTI) [9–15]. There are also reports suggesting a reduction in chronic obstructive pulmonary disease [16–18] and asthma [19, 20] exacerbation. These findings however are not consistently demonstrated [21–24]. Research on vitamin D concentration and its association with infections are methodically challenging. The severity of vitamin D deficiency, extent of infection, variation in vitamin D replacement regimens, patient characteristics and coexisting morbidities are some factors that may contribute to the heterogeneity of effect between studies.

To limit potential confounders, meta-analysis of primary randomized controlled trials (RCTs) has been conducted in more recent
Vitamin D deficiency and possible links with COVID-19

There have been several associations observed that may link vitamin D deficiency to the occurrence and severity of COVID-19 infections. The geographical distribution of COVID-19 cases worldwide has led to hypotheses regarding vitamin D status and number and severity of infection. A study has linked increasing latitude above 35 degrees North to higher mortality, suggesting vitamin D deficiency over winter could be linked to SARS-CoV-2 becoming rampant [26]. Seeming to be outliers, Nordic countries have relatively low rates of COVID-19 infections and mortality despite having low sunlight exposure during winter. However, the prevalence of vitamin D deficiency is low in Nordic countries due to the widespread use of cod liver oil and supplementation [27], leading one to speculate about the role of vitamin D supplementation in the outcome of COVID-19 infections.

A negative correlation was found between mean vitamin D levels in each country versus their number of COVID-19 cases and mortality rates (▶Table 2 and ▶Fig. 2), which corresponds to that found in another study [28]. Notably there is significant scatter, as other factors such as government policies and availability of healthcare services may significantly influence outcome as well, however an interesting pattern is observed.

There is also growing evidence that COVID-19 tends to disproportionately affect African Americans in the United States [29, 30], and that those of black and ethnic minority origins have more severe disease [31]. Due to reduced vitamin D production from skin pigmentation, there is a higher prevalence of vitamin D deficiency in dark-skin individuals [32].

Many proposed confounders that link ethnic minority groups to increased COVID-19 infections such as socioeconomic status, access to healthcare and comorbidities are insufficient to explain the disparity in COVID-19 cases and death rates. In models accounting for socioeconomic factors and significant comorbidities, Black race remains an independent factor for COVID-19 cases and deaths in the United States [33]. These findings are mirrored in a British cohort: after correcting for population density, deprivation level, socioeconomic status, education, self-reported health and occupational exposure, increased COVID-19 mortality remained significant in Black, Indian, Bangladeshi and Pakistani populations as compared to a White cohort [34]. Biological and genetic factors may play a role in the ethnic disparity observed, nonetheless the possible effect of low vitamin D status in these ethnic groups cannot be excluded. The effect of confounding variables particularly socioeconomic status on vitamin D levels was reported using data from the UK Biobank, where vitamin D levels and presence of vita-

<table>
<thead>
<tr>
<th>Authors</th>
<th>Publication year</th>
<th>Number of trials</th>
<th>Number of participants</th>
<th>Adjusted Odds Ratio (95% CI)</th>
<th>Test for heterogeneity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Charan J et al. [11]</td>
<td>2012</td>
<td>5</td>
<td>1868</td>
<td>0.582 (0.417–0.812), ( p = 0.001 )</td>
<td>( I^2 = 24.7% )</td>
</tr>
<tr>
<td>Bergman P et al. [10]</td>
<td>2013</td>
<td>11</td>
<td>5660</td>
<td>0.64 (0.49–0.84), ( p = 0.0014 )</td>
<td>( I^2 = 72% )</td>
</tr>
<tr>
<td>Zittermann A et al. [25]</td>
<td>2016</td>
<td>16</td>
<td>7421</td>
<td>0.65 (0.50–0.85), ( p = 0.005 )</td>
<td>( I^2 = 74% )</td>
</tr>
<tr>
<td>Martineau AR et al. [9]</td>
<td>2017</td>
<td>25</td>
<td>11 321</td>
<td>0.88 (0.81–0.96), ( p = 0.003 ) NNT 33 (20–101)</td>
<td>( p &lt;0.001 )</td>
</tr>
</tbody>
</table>

CI: Confidence interval; NNT: Number needed to treat. In all 4 studies, vitamin D was shown to be protective against RTI. There was, however, significant heterogeneity in 2 of the 4 studies.
Vitamin D deficiency were significant risk factors for COVID-19 infection, however this significance was negated when adjusted for confounders such as comorbidities, deprivation status and disability [35]. In interpreting data from the UK Biobank, we are mindful that vitamin D levels were collected more than a decade ago, and evidence suggests that an increasing awareness of vitamin D deficiency particularly among ethnic groups has led to an increase in testing and supplementation in the UK in recent years [36]. Due to conflicting evidence from retrospective studies, data from randomised controlled trials are urgently needed to elucidate the role of vitamin D supplementation in COVID-19 infections.

Other poor prognostic factors for COVID-19 include older age and underlying comorbidities such as hypertension, diabetes, obesity, and ischemic heart disease [38–40], which are also risk factors for vitamin D deficiency [41–45]. Indeed, two retrospective preprint studies have found that serum 25(OH)D levels were lowest in critical cases and highest in mild cases of COVID-19 [46, 47], and was significantly correlated with positivity for SARS-CoV-2 [48].

A retrospective cohort study which explored the role of combined vitamin D, vitamin B12, and magnesium supplementation in COVID-19 hospitalised patients found a significant protective effect against clinical deterioration after correction for age and hy-

### Table 2: Vitamin D status of populations worldwide and their corresponding COVID-19 case numbers and mortality.

<table>
<thead>
<tr>
<th>Country</th>
<th>COVID-19 cases/mil</th>
<th>COVID-19 deaths/mil</th>
<th>Vit D/nmol/l</th>
<th>Population, years</th>
<th>n</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Americas</strong></td>
<td></td>
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<tr>
<td>USA</td>
<td>4974</td>
<td>295</td>
<td>49.7 ± 21.2</td>
<td>Age &gt; 20</td>
<td>4495</td>
<td>Forrest et al. 2011 [81]</td>
</tr>
<tr>
<td>Canada</td>
<td>2187</td>
<td>166</td>
<td>58.3 ± 0.9</td>
<td>Age 3–79</td>
<td>11 336</td>
<td>Sarafin et al. 2015 [82]</td>
</tr>
<tr>
<td>Brazil</td>
<td>1565</td>
<td>99</td>
<td>63.9 ± 28.6</td>
<td>Age 2–95</td>
<td>39 004</td>
<td>Eloi et al. 2016 [83]</td>
</tr>
<tr>
<td><strong>Europe</strong></td>
<td></td>
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<tr>
<td>Russia</td>
<td>2237</td>
<td>22</td>
<td>54.8 ± 0.7</td>
<td>Age 18–75</td>
<td>1544</td>
<td>Karonova et al. 2016 [84]</td>
</tr>
<tr>
<td>UK</td>
<td>3747</td>
<td>536</td>
<td>Men 37.0 (95% CI 36.8–37.2), Women 38.7 (95% CI 38.6–38.8)</td>
<td>Age &gt; 18</td>
<td>210 502</td>
<td>Crowe et al. 2019 [36]</td>
</tr>
<tr>
<td>Germany</td>
<td>2146</td>
<td>100</td>
<td>45.6</td>
<td>Age 18–79</td>
<td>6995</td>
<td>Rabenberg et al. 2015 [85]</td>
</tr>
<tr>
<td>Netherlands</td>
<td>2620</td>
<td>338</td>
<td>48.4</td>
<td>Age 0–98, winter</td>
<td>2503</td>
<td>Boonman-de Winter et al. (2015) [86]</td>
</tr>
<tr>
<td>Norway</td>
<td>1538</td>
<td>43</td>
<td>58.9 ± 23.1</td>
<td>Age 19–55</td>
<td>2505</td>
<td>Larose et al. 2014 [87]</td>
</tr>
<tr>
<td>Finland</td>
<td>1180</td>
<td>55</td>
<td>65 (95% CI 65–66)</td>
<td>Age &gt; 30</td>
<td>4051</td>
<td>Jääskeläinen et al. 2017 [27]</td>
</tr>
<tr>
<td><strong>Asia</strong></td>
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<tr>
<td>Japan</td>
<td>131</td>
<td>6</td>
<td>49.9 ± 18.4</td>
<td>Age 40–74</td>
<td>9084</td>
<td>Nakamura et al. 2015 [88]</td>
</tr>
<tr>
<td>Hong Kong</td>
<td>142</td>
<td>0.5</td>
<td>70.6 ± 27.0</td>
<td>Age &gt; 50</td>
<td>382</td>
<td>Wat et al. 2007 [89]</td>
</tr>
<tr>
<td>China</td>
<td>58</td>
<td>3</td>
<td>Median 52.9 (IQ 42.4–62.4)</td>
<td>Age 20–89</td>
<td>2588</td>
<td>Lu et al. 2012 [90]</td>
</tr>
<tr>
<td><strong>Oceania</strong></td>
<td></td>
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<tr>
<td>Australia</td>
<td>279</td>
<td>4</td>
<td>69.2 ± 26.4</td>
<td>Age &gt; 24</td>
<td>2413</td>
<td>Gill et al. 2014 [91]</td>
</tr>
<tr>
<td>New Zealand</td>
<td>312</td>
<td>4</td>
<td>63 ± 1.5</td>
<td>Age &gt; 15</td>
<td>4721</td>
<td>NZ Ministry of Health, 2012 [92]</td>
</tr>
<tr>
<td><strong>Africa</strong></td>
<td></td>
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<td></td>
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<tr>
<td>South Africa</td>
<td>340</td>
<td>7</td>
<td>69.0 ± 23.8</td>
<td>Age 0–90</td>
<td>3112</td>
<td>Mogire et al. 2020 [93]</td>
</tr>
<tr>
<td>Egypt</td>
<td>155</td>
<td>7</td>
<td>74.3 ± 24.7</td>
<td>Age 0–90</td>
<td>1851</td>
<td>Mogire et al. 2020 [93]</td>
</tr>
<tr>
<td>Zimbabwe</td>
<td>3</td>
<td>0.3</td>
<td>100 ± 27.1</td>
<td>Age 0–90</td>
<td>63</td>
<td>Mogire et al. 2020 [93]</td>
</tr>
<tr>
<td>Kenya</td>
<td>22</td>
<td>0.9</td>
<td>70.3 ± 92.1</td>
<td>Age 0–90</td>
<td>385</td>
<td>Mogire et al. 2020 [93]</td>
</tr>
<tr>
<td>Ghana</td>
<td>209</td>
<td>1</td>
<td>74.1 ± 8.5</td>
<td>Age 0–90</td>
<td>595</td>
<td>Mogire et al. 2020 [93]</td>
</tr>
<tr>
<td><strong>Middle East</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iran</td>
<td>1570</td>
<td>87</td>
<td>Median 35.2 (IQ 24.0–72.4)</td>
<td>Age &gt; 30</td>
<td>251</td>
<td>Hosseinpanah et al. 2011 [94]</td>
</tr>
<tr>
<td>Turkey</td>
<td>1834</td>
<td>51</td>
<td>37.9 ± 22.0</td>
<td>All ages</td>
<td>35 667</td>
<td>Solak et al. 2018 [95]</td>
</tr>
<tr>
<td>Jordan</td>
<td>69</td>
<td>0.9</td>
<td>Men 183 ± 73.1 Women 99.3 ± 51.7</td>
<td>Age &gt; 19</td>
<td>4590</td>
<td>Batieha et al. 2011 [96]</td>
</tr>
</tbody>
</table>

Cumulative COVID-19 data as of 23rd May 2020 [37]. Vitamin D is represented as mean levels ± standard deviation, unless otherwise stated. IQ: Interquartile range, CI: Confidence interval. Vitamin D population data were limited in Latin America, Asia, and Africa. Studies in selected groups, e.g., pregnancy, infants, elderly and the institutionalised were avoided as they were deemed to be not representative of the overall population.
rapid clinical response within days of supplementation is not specified, it provides a promising base for future controlled trials.

While evidence from retrospective studies and associations cannot prove causality, there are plausible mechanisms regarding the role of vitamin D deficiency and its modulation of the renin-angiotensin system (RAS) and immune response that may influence the outcome of COVID-19 infection. Together, they give sufficient grounds to investigate the role of vitamin D deficiency further with prospective randomised trials.

Severe COVID-19 results in ARDS, mediated by a cytokine storm, which can cause multi-organ failure and death. Several pro-inflammatory cytokines such as IL-1β, IL-2, IL-6, IFN-γ, IL-7, IL-10, G-CSF, IP10, MCP1, MIP1A, and TNF-α have been implicated in the pathogenesis of cytokine storm in COVID-19, favouring a Th1 and Th-17 cytokine profile [50–52]. 1,25-Dihydroxyvitamin D3 was able to suppress the pro-inflammatory Th1 and Th17 cytokine profile in mice and human trophoblasts [53, 54]. A meta-analysis found that vitamin D supplementation significantly reduced hs-CRP but not TNF-α and IL-6 levels in type 2 diabetes mellitus [55]. The negative results about suppression of chronic inflammation in chronic disease may not be generalizable to the possible effects of vitamin D on a cytokine storm induced by acute illness. More evidence is needed in critically ill patients.

Besides its effects on blood pressure and sodium retention, activation of the RAS has more recently been linked to autocrine and paracrine effects that are involved in tissue remodelling and fibro-
s in the lung [56], and vitamin D has been shown to regulate the RAS. Angiotensin converting enzyme (ACE) and ACE2 have opposing actions to up and downregulate angiotensin II, respectively [57]. Angiotensin II, in addition its hypertensive effects, mediates pro-inflammatory responses via an increased production of reactive oxygen species and leukocyte recruitment, and induces a fibro-proliferative response in the lungs [58, 59].

ACE2 is the entry receptor via which SARS-CoV-2 infects human host [60], and is expressed in endothelial cells and arterial smooth muscle cells in almost all organs, including lung alveolar epithelial cells [61, 62]. While increased ACE2 acting as viral entry points may theoretically be a risk factor for infection of SARS-CoV-2, there is currently no evidence for this. Diabetes and hypertension are risk factors for COVID-19 infection, even though ACE2 levels are reduced in these conditions [63, 64]. On the contrary, upregulation of the ACE2-Ang-1(1–7)-Mas axis appears to have an anti-inflammatory role and is protective against acute lung injury [65, 66], while blocking ACE2 can exacerbate SARS-CoV-induced pulmonary injury [65, 67]. Binding of the viral spike protein to ACE2 downregulates ACE2 and increases angiotensin II-mediated lung inflammation [67]. In mice and in vitro studies, vitamin D suppresses renin synthesis and is a negative regulator of the RAS via inhibition of cAMP response element in the renin gene promoter [68, 69]. Calcitriol increased ACE2, Ang-1(1–7) and Mas levels and reduced ACE/ACE2 ratio [69, 70]. Conversely, vitamin D deficiency is associated with downregulation of the ACE2 pathway, activation of the RAS and higher circulating angiotensin II levels [71]. A schematic diagram is illustrated in Fig. 3.

Vitamin D deficiency has been described to be associated with the development of acute respiratory failure [72, 73], with type II alveolar cells displaying an upregulation of multiple genes associated with cell growth, differentiation and response to wounding in response to vitamin D treatment [72]. In critically ill patients with severe vitamin D deficiency, administration of high dose vitamin D was found to reduce hospital mortality [74] and increase ventilator-free days among mechanically-ventilated patients [75]. Besides its role in modulating the RAS and suppressing cytokine storm in ARDS, calcitriol has also been thought to attenuate lung injury by increasing surfactant production, stimulating epithelial repair, and inhibiting alveolar cell apoptosis [76, 77]. We await the results from the VITADLIZE study about the effect of high dose vitamin D3 supplementation to critically ill patients with severe vitamin D deficiency [78]. Similarly, prospective studies are needed to evaluate if vitamin D administration will be beneficial to reduce the development of ARDS induced by SARS-CoV-2.

Vitamin D supplementation is cheap and safe, with low risk of toxicity [79]. Target levels for optimal immune function is unknown, although experts suggest higher levels of 25(OH)D above 75 nmol/l (30 ng/ml) may be required for non-skeletal benefits [80], which is greater than what is frequently defined for bone health of levels above 50 nmol/l (20 ng/ml). Practically, given that it can take weeks to months to raise serum 25(OH)D levels, a population-based preventive strategy can be undertaken for at-risk groups. Its use can be seen as an adjunctive role for reducing overall population risk rather than an acute treatment strategy.

Conclusion

We have discussed the role of vitamin D and its analogues in the modulation of the innate and adaptive immunity which may protect against several respiratory tract pathogens, suppression of cytokine storm in ARDS and modulation of the renin-angiotensin system favouring the ACE2 axis. There are some epidemiological associations between vitamin D deficiency and the prevalence and severity of COVID-19 infection which may be explored further. Prospective interventional studies are necessary to establish whether population-based vitamin D supplementation as an adjunct to other anti-viral therapies will be helpful in reducing severity and risk of COVID-19 infection.

Conflict of Interest

The authors declare that they have no conflict of interest.

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