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REVIEW

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Altered gut microbial metabolites could mediate the effects of risk factors in Covid-19

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Summary

Coronavirus disease 2019 (Covid-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, is now pandemic. While most Covid-19 patients will experience mild symptoms, a small proportion will develop severe disease, which could be fatal. Clinically, Covid-19 patients manifest fever with dry cough, fatigue and dyspnoea, and in severe cases develop into acute respiratory distress syndrome (ARDS), sepsis and multi-organ failure. These severe patients are characterized by hyperinflammation with highly increased pro-inflammatory cytokines including IL-6, IL-17 and TNF-alpha as well as C-reactive protein, which are accompanied by decreased lymphocyte counts. Clinical evidence supports that gut microbiota dysregulation is common in Covid-19 and plays a key role in the pathogenesis of Covid-19. In this narrative review, we summarize the roles of intestinal dysbiosis in Covid-19 pathogenesis and posit that the associated mechanisms are being mediated by gut bacterial metabolites. Based on this premise, we propose possible clinical implications. Various risk factors could be causal for severe Covid-19, and these include advanced age, concomitant chronic disease, SARS-CoV-2 infection of enterocytes, use of antibiotics and psychological distress. Gut dysbiosis is associated with risk factors and severe Covid-19 due to decreased commensal microbial metabolites, which cause reduced anti-inflammatory mechanisms and chronic low-grade inflammation. The preconditioned immune dysregulation enables SARS-CoV-2 infection to progress to an uncontrolled hyperinflammatory response. Thus, a pre-existing gut microbiota that is diverse and abundant could be beneficial for the prevention of severe Covid-19, and supplementation with commensal microbial metabolites may facilitate and augment the treatment of severe Covid-19.

1 | INTRODUCTION

Covid-19 (coronavirus disease 2019) is caused by SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) that has spread worldwide, resulting in a huge impact on public health and the economy. SARS-CoV-2 is much more transmissible than SARS-CoV, with tens of millions of people infected.[1] Although most SARS-CoV-2-infected patients present with mild symptoms or are asymptomatic, severe Covid-19 can be fatal.[2] The common respiratory symptoms are fever, dry cough, fatigue and dyspnoea, with additional manifestations such as increased production of phlegm/sputum, headache and haemoptysis.[3–6] In severe cases, patients present with acute respiratory

Abbreviations: ACE2, angiotensin-converting enzyme 2; ARDS, acute respiratory distress syndrome; CHR, corticotropin-releasing hormone; Covid-19, coronavirus disease 2019; CVD, cardiovascular disease; DIC, disseminated intravascular coagulation; GPR109a, G protein coupled receptor 109a; HDACs, histone deacetylases; isoDCA, isodeoxycholic acid; isoalloLCA, isoallolithocholic acid; NK cells, natural killer cells; 3-oxoLCA, 3-oxolithocholic acid; PPAR-gamma, peroxisome proliferator-activated receptor gamma (PPARAQ7-gamma); RSV, respiratory syncytial virus; S, spike glycoprotein S; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; Treg cells, regulatory T cells; VDR, vitamin D receptor.

distress syndrome (ARDS), sepsis and multi-organ failure. These patients are characterized by hyperinflammation represented by a cytokine storm accompanied by lymphopenia.[7] Clinical studies have repeatedly reported that the risk factors associated with the severity of Covid-19 include age-associated dynamics such as the presence of pre-existing chronic diseases, intestinal SARS-CoV-2 infection, extensive use of antibiotics and stress.[2,8] Understanding how SARS-CoV-2 causes severe Covid-19 is critical to reduce morbidity.

SARS-CoV-2 is a positive-sense single-stranded RNA virus with an envelope. The whole genome sequence of SARS-CoV-2 virus has been mapped.[9,10] In 30-kb nucleotides, at least 10 open reading frames have been identified, which encode multiple proteins for the virus to infect human cells which then progress to replication.[11] These include membrane proteins (spike glycoprotein S, envelope protein E and membrane protein M), nucleocapsid (N), and transcription complex nsps1–10 and nsps12–16.[12] Among them, S and N proteins have been structurally studied.[13] The structure of spike glycoprotein, which can bind to angiotensin-converting enzyme 2 (ACE2) to facilitate the virus to enter host cells, explains why S protein has much higher affinity than that of SARS-CoV for the same receptor.[13,14] Y-shaped spike pairs are present (i.e., two heads with one stem) that render unusual freedom to the spike. N protein has a helical structure wound with RNA with five subunits per turn in a head-to-tail structure allowing the virus to resist environmental and physical challenges.

Recent studies have reported that the gut microbiota is dysregulated in Covid-19.[15–17] Dysregulation of gut microbiota (dysbiosis) could be involved in the pathogenesis of Covid-19 by accelerating hyperinflammation, a clinical characteristic of severe Covid-19. The studies of gut dysbiosis in Covid-19 could provide preventive and therapeutic opportunities. In this review, we summarise the evidence of gut dysbiosis in Covid-19 pathogenesis, discuss the possible mechanisms, explore its links with other risk factors and speculate on clinical implications.

2 | IMMUNE RESPONSES AND CLINICAL CHARACTERISTICS OF COVID-19

Infection with SARS-CoV-2 initiates immune responses. The outcomes of the infection depend on the interaction of the extent of infection of the virus and the containment effect provided by the immune response to the virus. This interaction determines clinical manifestations of Covid-19.[2–4,6,8] The virus enters the body from the respiratory system through binding to ACE2 on the surface of airway cells, particularly alveolar type II pneumocytes. The virus is also able to increase the expression of ACE2 to facilitate the infection.[18] The infection of the virus triggers an initial immune response, in which innate immune cells including monocytes, natural killer cells, macrophages and dendritic cells, as well as secreted cytokines are increased. Dendritic cells and macrophages present antigens to adaptive immune cells to elicit cellular and humoral immune responses. The virus could spread to many organs through the systemic circulation if the virus cannot be restricted to the lungs (Figure 1). As ACE2 is found in many organs, SARS-CoV-2 could potentially infect many sites including the heart, blood vessels, gut, lung, kidney, testis and brain.[19] Damage to major organs could cause severe morbidity and mortality. It has been proposed that SARS-CoV-2 may also initially infect the intestines and spread to other organs but this route has not been verified yet.

In non-severe Covid-19 patients, adequate immune responses are elicited to eliminate viruses, allowing patients to achieve complete recovery. A case report examined the changes of clinical manifestations and immune responses in a recovered Covid-19 patient.[20] At the early stage of infection, clonally expanded $CD8^+$ T-cells were enriched to clear the cells infected by SARS-CoV-2 viruses. Elicited B-cells by viral antigens secreted antibodies IgG and IgM to neutralize viruses. Other studies showed that these antibodies were mainly against N and S proteins. IgM and IgG can be detected 5–17 and 6–14 days after symptoms appear.[21,22] Liao et al.[23] reported immune adaptations from bronchoalveolar lavage fluid from Covid-19 cases. In moderate cases, inflammatory M1-like macrophages were increased adequately, indicating the inflammatory responses were still under control.

In severe Covid-19 patients, IFN-I and IFN-III are reduced, while neutrophils and macrophages are increased with greatly increased pro-inflammatory cytokines such as TNF-alpha, IL-6, IL-17 and C-reactive protein.[24] This cytokine storm is accompanied by $CD4^+$ T-cell, $CD8^+$ T-cell exhaustion and decreased macrophages. Bronchoalveolar lavage fluid investigations showed increased M1-like macrophages and decreased $CD8^+$ T-cells that were proliferating rather than differentiating.[23] These hyperinflammatory responses are the key features of severe Covid-19, leading to increased mortality. Moreover, the cytokines IL-6 and IL-17 have been correlated with infection severity and mortality.[25] These greatly increased cytokines could cause ARDS and multi-organ failure (Figure 1).[26]

Inflammation of vascular endothelial cells plays a key role in the pathogenesis of severe Covid-19,[27] because it can progress to microvascular thrombosis and disseminated intravascular coagulation. Microvascular thrombosis could present in any organ, which is an important cause for multiple-organ failure.[28] Major organ failure also results from hyperinflammation, leading to fatal damage such as acute heart failure, acute kidney injury/kidney failure and encephalitis/stroke. As the cytokine storm-associated hyperinflammation is a key factor for severe Covid-19, understanding the associated mechanisms and inhibition of the key elements could reduce the severity of Covid-19.

In a recent publication, Leisman et al.[29] compared the cytokine levels in Covid-19 and other cytokine storms. The authors found that IL-6 levels in Covid-19 were much lower than that caused by sepsis, cytokine release syndrome and Covid-19-unrelated ARDS. This arose a controversial opinion about the importance of the cytokine storm in multi-organ failure in Covid-19. Indeed, the multi-organ failure in Covid-19 is caused by various factors such as direct viral infection, microvascular thrombosis and pro-inflammatory cytokines. However, the cytokine storm may play a central role as the formation of vascular thrombosis—a major pathological characteristic found in post-mortem of Covid-19–has inflammation involvement through activation of the coagulation pathway.[7,30] In addition, there are interactions among

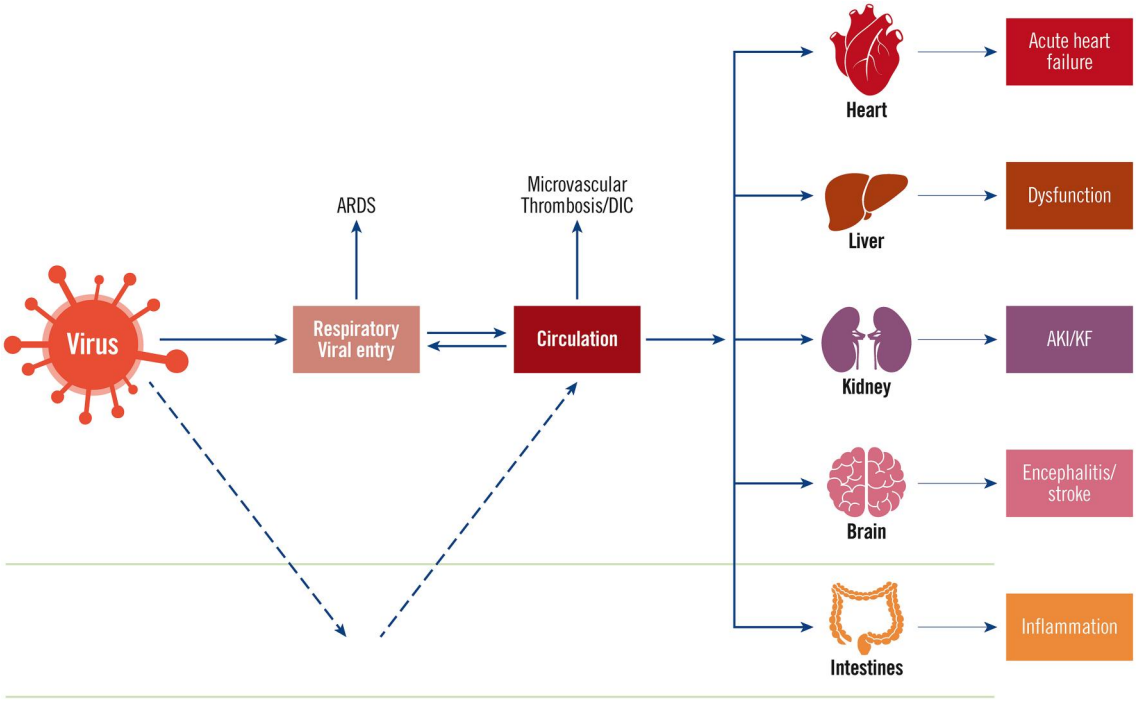

FIGURE 1 Viral entry, dissemination and damage to various organs. Severe acute respiratory syndrome coronavirus 2 virus initially infects respiratory system. If not controlled, it could enter the circulation system and subsequently infect various organs such as heart, liver, kidney, brain and intestines. The virus may also initially gain entry into the digestive system, and could disseminate to other organs. The damage to major organs can cause severe consequences such as acute respiratory distress syndrome, microvascular thrombosis, disseminated intravascular coagulation, acute heart failure, liver dysfunction, acute kidney injury/kidney failure, encephalitis/stroke and intestinal inflammation

cytokines as well as between cytokines and complements, which may have a synergistic effect on an organ.[7] The cytokine storm together with the microvascular thrombosis in Covid-19 could greatly accelerate SARS-CoV-2-caused organ damage. Therefore, even the hyperinflammation has not reached a very high level as sepsis, cytokine release syndrome and Covid-19-unrelated ARDS, it could cause severe disease already.

How immune responses become uncontrolled is not clearly elucidated although some associated mechanisms have been proposed. Infection of SARS-CoV-2 can attract macrophages, dendritic cells and neutrophils, which secrete cytokines for controlling the viral insult. The binding of virus particles results in endo-phagocytosis and downregulation of ACE2, causing inflammation and thrombosis.[31] Whether increased ACE2 expression by the virus could reduce the effect of ACE2 endo-phagocytosis is not clear.[18] Activation of the complements C5a and C5b-9 is also involved in SARS-CoV-2-caused inflammation.[25] Guo et al.[32] identified a specific type of monocyte, which was responsible for the cytokine storm. The cells were not presented in healthy subjects but accounted for 98.3% of the total monocytes in severe Covid-19 and only 12.1% in the remission of Covid-19 patients. These cells highly expressed pro-inflammatory cytokines, chemokine and inflammasome genes. Other risk factors such as underlying chronic diseases may accelerate already present inflammatory responses, leading to uncontrolled cytokine secretion, but the associated mechanisms are not well explained.

Clinical observations have identified many risk factors which are associated with the severity of Covid-19 such as advanced age, chronic diseases including diabetes, hypertension and cardiovascular disease (CVD).[33] A study with 1482 hospitalized patients showed that among all comorbidities, 49.7% were hypertensive, 48.3% were obese, 34.6% presented with chronic liver disease, 28.3% with diabetes and 27.8% with CVD (https://www.cdc.gov/mmwr). Another study also revealed that hypertension (30%), diabetes (19%) and CVD (8%) adversely affected the clinical outcome of the infection.[34] How these factors are involved in the pathogenesis to cause severe Covid-19 morbidity and mortality is of significant importance in the prevention and treatment of severe Covid-19. We posit that an important plausible mechanism may implicate intestinal dysbiosis in chronic conditions that could accelerate SARS-CoV-2-induced inflammation, leading to hyperinflammation-related morbidity and mortality.

3 | DYSREGULATION OF THE INTESTINAL MICROBIOTA IN COVID-19

Initial evidence of intestinal dysbiosis in Covid-19 emerged from frequently reported gut-associated symptoms.[35–37] Several studies showed that gastrointestinal manifestations including diarrhoea, anorexia and nausea occurred in about 50%–60% of patients.[35,38,39]

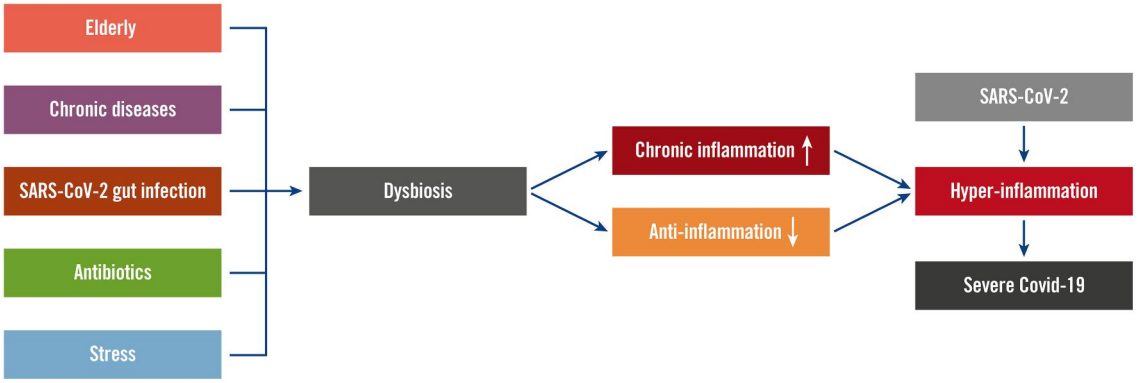

FIGURE 2 Gut dysbiosis in coronavirus disease 2019 (Covid-19). Various risk factors of Covid-19 including elderly, chronic diseases, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) gut infection, use of antibiotics and stress can cause gut dysbiosis. Dysbiosis contributes to hyperinflammation by increased intestinal and systemic chronic inflammation and decreased anti-inflammation mechanisms. These facilitate the form of hyperinflammation after SARS-CoV-2 infection, resulting in severe Covid-19

Subsequently, intestinal dysbiosis was demonstrated in Covid-19.[15] By comparing gut microbiota in 30 hospitalized Covid-19 patients and 30 healthy participants, it was reported that intestinal dysbiosis was associated with the Covid-19 patients.[15] The bacterial diversity was decreased with higher abundance of opportunistic pathobiont bacterial groups such as *Streptococcus*, *Rothia*, *Veillonella* and *Actinomyces*, and lower abundance of commensal bacteria. Zuo et al.[16] also studied 15 hospitalized Covid-19 patients and found persistent alterations of faecal microbiome. Compared to healthy patients, Covid-19 positive patients had increased opportunistic pathogens and decreased beneficial commensal bacteria.

Gut dysbiosis has been associated with the severity of Covid-19. Using a proteomic risk score based on 20 identified blood protein biomarkers, a recent study correlated and hence predicted the progression of severe Covid-19 in older populations.[5] Most of the risk proteins were associated with inflammatory factors including IL-1beta, IL-6, TNF-alpha and high-sensitivity C-reactive protein. Furthermore, gut microbiota was associated with inflammatory factors with *Bacteroides*, *Streptococcus* and *Clostridiales* genera being negatively related to pro-inflammatory factors, while *Ruminococcus*, *Blautia* and *Lactobacillus* genera positively related to pro-inflammatory factors, indicating the involvement of gut dysbiosis in the severity of Covid-19. Zuo et al.[16] showed that the abundance of *Coprobacillus*, *Clostridium ramosum* and *Clostridium hathewayi* were positively correlated with dysbiosis and the severity of Covid-19, while *Faecalibacterium prausnitzii* inversely correlated with the disease severity in 15 hospitalized patients. Indeed, patients with intestinal symptoms were associated with significantly increased risk of admittance to intensive care unit and the rate of mortality than those without gut symptoms.[40]

Various reasons could explain intestinal dysbiosis in Covid-19 (Figure 2). SARS-CoV-2 can also infect enterocytes in the intestines, causing gut dysbiosis and inflammation. Faecal viruses have been detected even in patients without gut symptoms. A recent meta-analysis also showed a detection rate of 43.7% of SARS-CoV-2 RNAs in Covid-19 patients' faecal specimens and much higher detection rates in severely infected patients.[41] Using intestinal organoids, studies demonstrated that SARS-CoV-2 was able to infect enterocytes.[42–44]

Chronic diseases with major organ involvement such as the heart, liver, kidney and brain, linked with diabetes, obesity and hypertension have been identified as risk factors responsible for the severity of Covid-19. Gut dysbiosis has been well demonstrated to play key roles in these metabolic diseases through chronic inflammation.[45–47] Thus, disturbed gut microbiota compositions in these diseases could perpetuate the reported increased severity of Covid-19.

In addition, the extensive use of antibiotics is a causal factor for gut dysbiosis when prescribed for the prevention of secondary bacterial infections in Covid-19. It is well known that the use of antibiotics can cause gut microbiota alterations and subsequent transluminal mucosa transfer of endotoxins (e.g., lipopolysaccharides [LPS]) from opportunistic pathogens.[48] For example, antibiotics have been associated with *Clostridium difficile* infections, and faecal microbiome transplantation has been used successfully for the treatment of the disease.[49,50]

Psychological distress and stress that can present in the form of depression and anxiety can also be risk factors for the severity of Covid-19. Batty et al.[50] identified mental and cognition to be risk factors for hospitalized Covid-19; lower cognition doubled Covid-19 hospitalization.[50] Adam et al.[51] also reported that mental disorders increased the rate of viral infections such as the common cold by 44%, where the causal agent is another type of coronavirus. An early study by Cohen et al.[52] demonstrated that psychological distress increased coronavirus infections in healthy subjects who were inoculated with either one of five respiratory viruses including coronavirus-type 229E through nasal drops. Gut dysbiosis is well known to exist in chronically stressed individuals,[53] and probiotics have been used to relieve stress in clinical trials.[54]

The risk factors that are causal for intestinal dysbiosis could be associated with increased chronic low-grade gut and systemic inflammation with decreased anti-inflammatory effects (Figure 2).[55]

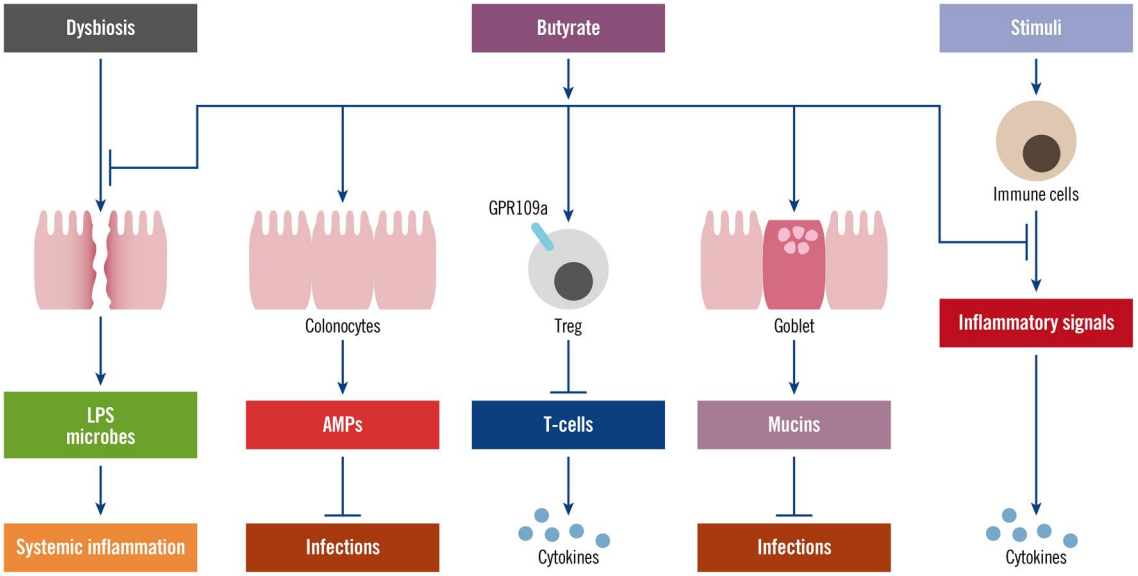

FIGURE 3 Anti-inflammatory effect of butyrate. Butyrate exerts anti-inflammatory effect through multiple mechanisms. Butyrate can reduce dysbiosis-caused gut leakage, thus block the translocation of lipopolysaccharides and microbes, inhibiting systemic inflammation. Butyrate also increases colonocytes to secrete antimicrobial peptides to reduce microbial infections. Butyrate can activate regulatory T-cells, which subsequently inhibit T-cell activation, reducing cytokine production. It can also increase goblet cells to secrete mucins to protect from microbial infections. Through inhibition of multiple pro-inflammatory pathways, butyrate can reduce the cytokine production by immune cells under effects of stimuli

Therefore, in infections with SARS-CoV-2, gut dysbiosis could present with additive effects in respect of inflammation that leads to hyperinflammation.

4 | POSSIBLE MECHANISMS: THE INVOLVEMENT OF BACTERIAL METABOLITES

The effects that could be mediated by the intestinal microbiota may involve various commensal bacterial metabolites. Many bacterial metabolites such as short-chain fatty acids, bile acids and amino acids have been identified and their effects studied on hosts. Among them, butyrate, a four-carbon short-chain fatty acid, is most extensively investigated. Butyrate exerts an important anti-inflammatory effect through multiple mechanisms (Figure 3). It can activate regulatory T-cells (Treg cells) through its receptor G protein coupled receptor 109a (GPR109a) to downregulate cytotoxic T-cells and facilitate M2 macrophage activation to reduce pro-inflammatory cytokines to anti-inflammatory cytokine ratios.[56–58] Thus, given that butyrate participates in immunoregulation biochemical pathways in both the gut and peripheral tissues,[54,58] decreased production of butyrate could facilitate the progression of hyperinflammation. Butyrate can also reduce the activation of multiple pro-inflammatory signalling pathways through inhibition of histone deacetylases (HDACs) and HDAC-independent mechanisms.[59] In addition, butyrate has antibacterial and antiviral effects through promoting the secretion of mucins and antimicrobial peptide defensins.[58] Butyrate also maintains the intestinal barrier and thus prevents the translocation of bacteria/endotoxins, which cause systemic inflammation.[60] Therefore, reduced levels of butyrate could lead to a low-grade inflammation systemically. Other short-chain fatty acids, acetate and propionate, have also been shown to have protective effect on respiratory viral infections.[61,62] Antunes et al.[62] showed that high-fibre diet was able to decrease respiratory syncytial virus (RSV) load in mice and intaking of acetate in drinking water for mice produced a similar effect. Acetate bound to its receptor GPR43, leading to increased production of type I interferon. Lynch et al.[61] found that propionate stimulated Treg cells to reduce RSV infectivity in a mouse model. Their roles in SARS-CoV-2 infection warrant research as well.

In addition to butyrate, bile acids are well-recognized signalling molecules. They are produced in the liver and further metabolized by the gut microbiota. Recent studies showed that epimerization derivatives of bile acids, isodeoxycholic acid (isoDCA), isoallolithocholic acid (isoalloLCA) and 3-oxolithocholic acid (3-oxoLCA), can modulate inflammatory responses through activation of Treg cells and inhibition of Th17 cells.[63,64] In an in vitro system with co-culture of naïve T-cells and dendritic cells, isoDCA can stimulate differentiation of Treg cells and exhibit an anti-inflammatory effect.[63] The isoDCA reduced the secretion of IL-6 and TNF-alpha by dendritic cells which was stimulated by Toll-like receptor agonists. Treatment of dendritic cells by isoDCA also decreased the expression of genes related to pro-inflammatory factors including *Tlr7*, *Tlr12*, *Nlrc5*, *Stat2*, *Stat6*, *Irf1* and *Irf7*, and increased the expression of genes for suppression of NF-κB, MAPK, cytokine-receptor signalling including *Nfkbia*, *Dusp1*, *Dusp5* and *Socs1*. Hang et al.[65] also found that a derivative of LCA, isoalloLCA, promoted Treg cell differentiation, while 3-oxoLCA inhibited Th17 cell differentiation.[64] Gut microbiota symbionts are necessary to maintain bile-acid-induced Treg differentiation.[65]

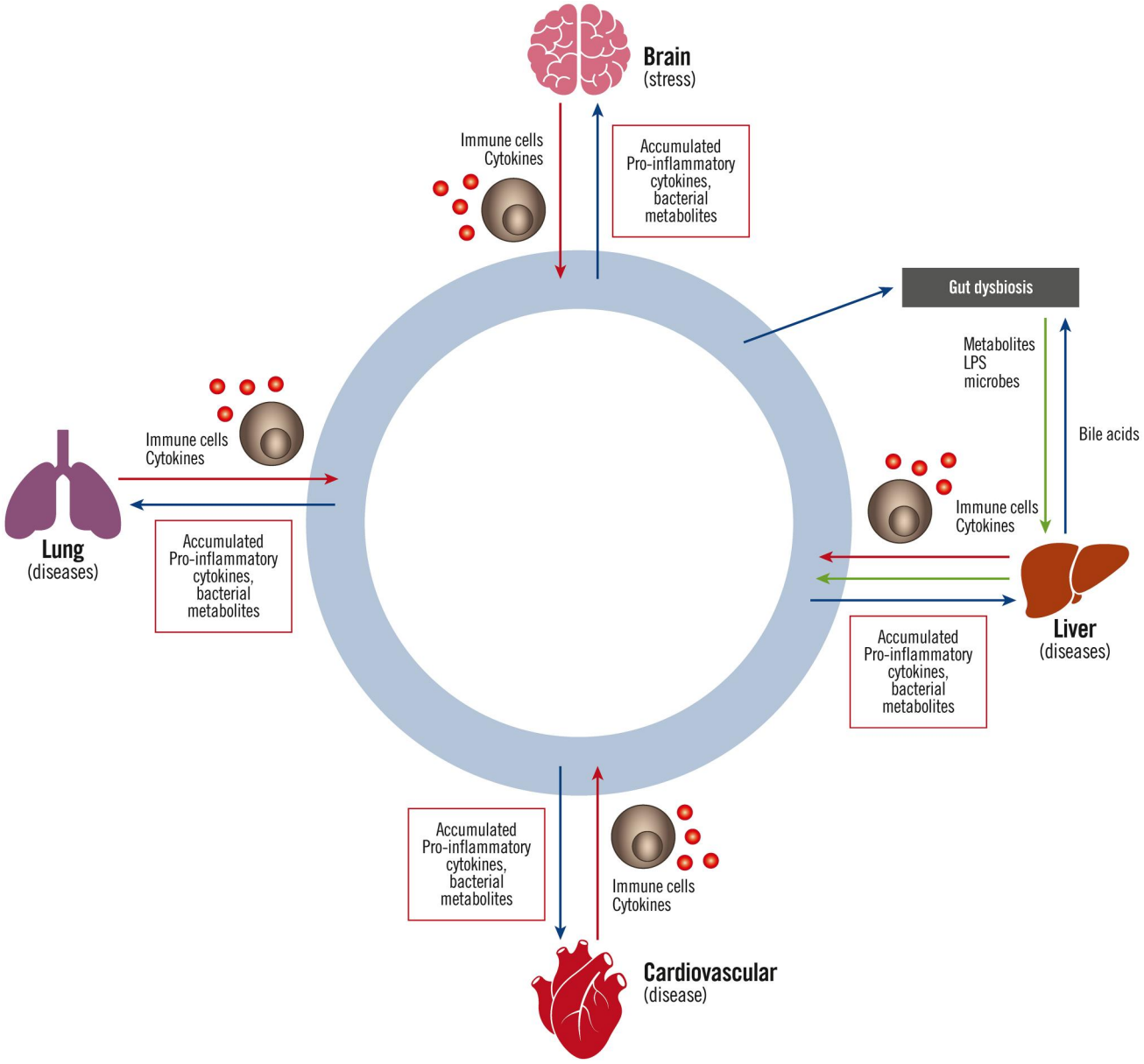

FIGURE 4 Interactions between gut dysbiosis and organs in coronavirus disease 2019. Gut dysbiosis affects multiple organs to form bidirectional gut–organs axes. Bile acids produced in the liver are secreted into gut, which are metabolized by gut bacteria and reabsorbed into the liver to form enterohepatic circulation. Other bacterial metabolites such as butyrate can also be absorbed into the liver. In gut dysbiosis, lipopolysaccharides and microbes are able to cross gut barrier to enter the liver. After processing in the liver, they enter to circulation system. In case of liver disease, there are also activated immune cells and cytokines. All these can be secret into the intestines and other organs. Chronic diseases in cardiovascular system, lung, brain and other organs could also produce and secrete immune cells and cytokine into circulation system

Other bacterial metabolites such as tryptophan may also be involved in inflammation. In gut dysbiosis, the metabolism of tryptophan into kynurenine is increased due to activated indoleamine 2,3 dioxygenase, a rate-limiting enzyme in the kynurenine pathway. Kynurenine is a pro-inflammatory metabolite of the amino acid L-tryptophan, and kynurenine/tryptophan is used to indicate the inflammatory states.[66,67] Indeed, it has been shown that altered tryptophan metabolism is involved in the pathogenesis of Covid-19 and correlated with IL-6 levels.[68]

5 | GUT DYSBIOSIS LINKED TO RISK FACTORS OF SEVERE COVID-19

The intestinal microbiota and commensal bacterial metabolites play important roles in regulating pro- and anti-inflammatory actions. Dietary factors, antibiotics and other medications (e.g., proton-pump inhibitors), ageing, chronic diseases and lifestyle stressors are risk factors associated with gut dysbiosis.[69] Therefore, gut dysbiosis and altered bacterial metabolites can explain, at least in part, how

intestinal microbiota dysbiotic shifts could mediate risk-factor-increased severe Covid-19 (Figure 4).

5.1 | Ageing

Advanced age is almost a universally reported risk factor from Covid-19 reports. Individuals in the older age bracket have been identified as a key factor associated with mortality rates.[34] With increasing age, Covid-19-caused mortality has an increased prevalence.[70] In a recent publication, Verity et al.[71] established a model to estimate the mortality rates in different age groups.[71] It was estimated that the Covid-19 mortality rate for those aged less than 60 years was 0.32%, for those aged between 60 and 80 years, sharply increased to 6.4%, while for those aged more than 80 years reached a prevalence of mortality of 13.4%. Du et al.[72] reported the clinical features of 85 Covid-19 death cases. The report revealed that the average age was 65.8 years; most had comorbidities including hypertension, diabetes and coronary heart disease.

Ageing has been closely associated with chronic, low-grade, sterile inflammation–inflammaging.[73] In inflammaging, innate immune cells such as macrophages are continuously activated with increased secretion of pro-inflammatory cytokines including IL-6, IL-8 and IL-1β, as well as a balance towards immunosenescence.[73] The compositions of Treg cells are changed in an aged population with increased natural Treg cells but decreased inducible Treg cells.[74,75] The dysfunctions of older Treg cells are indicated by increased incidences of excessive immune diseases such as autoimmune diseases and cancer. Therefore, SARS-CoV-2 infections in aged individuals may accelerate inflammaging, leading to hyperinflammation.

A dysregulated gut microbiota has been recognized to play a central role in inflammaging.[73] A review that investigated the available evidence with regard to ageing, inflammaging and the intestinal microbiota adverse shifts, reported that an *aged-type* gut microbiota could be correlated with inflammaging.[76] With increasing age, gut microbiota present with deficits of beneficial inputs. Aged individuals most probably display increased proteobacteria and decreased butyrate-producing bacteria such as *Faecalibacterium prauznitzii*, and thus present with a chronic deficit of intestinal microbiome-elaborated butyrate.[73] Increased proteobacteria is positively correlated with pro-inflammatory cytokines IL-6 and IL-8.[76] The low levels of butyrate in aged individuals may contribute to chronic low-grade inflammation.

5.2 | Diabetes and obesity

Both diabetes and obesity are factors that can predispose individuals for severe Covid-19.[77,78] Therefore, diabesity (i.e., diabetes with concomitant obesity) presents a higher risk factor compared to either diabetes or obesity alone. Diabetes is the third most prevalent comorbidity of Covid-19, increasing the adverse outcomes by two to three folds, and mortality by more than three folds.[78] Diabetes increases susceptibility to infections due to reduced immune responses by poor glycaemic control.[79] A recent study showed that glucose increased SARS-CoV-2 viral replication in monocytes and stimulated production of pro-inflammatory cytokines IFN-alpha, IFN-beta, IFN-lamda, IL-6 and IL-1beta.[18] This has demonstrated to be caused by increased glycosis through increased expression of HIF-1alpha target genes *GLUT-1*, *PFKFB3*, *PKM2* and *LDH-A*, which are responsible for glucose transport and glycolytic pathway.[18] Co-culture of SARS-CoV-2-infected monocytes with T-cells or pulmonary epithelial cells caused dysfunction of T-cells and apoptosis of epithelial cells, which was reversed by inhibition of HIF-alpha, revealing impairment of T-cells and epithelial by high levels of glucose.[18] Impaired immunity in diabesity also reduces systemic viral clearance. In addition, increased ACE2 expression by elevated glucose levels and anti-diabetic agents such as thiazolidinedione[18,79] could facilitate infection with SARS-CoV-2. Importantly, in diabesity, there is chronic inflammation with increased IL-6 and TNF-alpha,[80] which increases the susceptibility to SARS-CoV-2-induced hyperinflammation. In obesity, low-grade chronic inflammation in visceral adipose tissue has been associated with increased inflammatory immune cells and cytokines including $CD8^+$ T-cells, macrophages, neutrophils and natural killer cells.[81,82] In addition, Treg cells and M2 macrophages have been reported as decreased.[83] Manipulation of peroxisome proliferator-activated receptor gamma to increase Treg cell number could have therapeutic effects on obesity and insulin resistance.[84]

Inflammatory responses in diabetes and obesity have been linked to gut dysbiosis.[85,86] Colonization of the gut with an *obese microbiota* in germ-free mice markedly increased the body weight.[85] The inflammation that accompanies intestinal dysbiosis in diabetes can be reduced through the administration of probiotic lactic acid bacteria through increased butyrate production.[86] The administration of probiotics can also reduce the blood levels of glucose and increase glucose tolerance.[86,87] Butyrate not only exerts anti-inflammatory effect but also regulates glucose metabolism. Butyrate is necessary for the secretion of glucagon-like peptide-1 (GLP-1), which promotes insulin secretion but has a short half-life of 2 min[88] Thus, butyrate could be effective for the treatment of obesity and diabetes.[89] Administration of butyric acid normalized hyperglycaemia in a diabetes mouse model.[90] The effect of metformin has been associated with increased butyrate-producing bacteria.[87,91] In diabetes, gut microbiota is dysregulated that causes reduced butyrate production.[92,93] Administration of metformin restores the bacteria with increased *Akkermansia muciniphila*, *Subdoligranulum variabile*, *Escherichia* spp and decreased *Intestininibactor bartlettii*, as well as increased butyrate production.[94–96] Therefore, metformin not only decreases the glucose levels but also reduces inflammation through increasing butyrate production. However, the outcomes of clinical studies are controversial. Several studies reported the beneficial effects of metformin on diabetic Covid-19 patients,[97–99] while other studies showed no effects or even worse outcomes.[100,101] The discrepancy could be caused by the selection of patients such as difference in blood glucose levels.[101] It could be important to have the same blood

glucose levels for selected patients for comparison as glucose greatly increases ACE2 expression to enhance viral infectivity. A prevention strategy of diabetes by *Faecalibacterium prausnitzii* transplantation has also been proposed.[102] However, whether probiotics and butyrate could reduce the adverse effect of diabetes or diabesity in severe Covid-19 is not well studied.

5.3 | Cardiovascular disease

The heart is a major organ that is susceptible to assault by the SARS-CoV-2 and CVD increases the severity of Covid-19. In a meta-analysis, it was shown that CVD increased the rate to develop severe disease by five times.[103] SARS-CoV-2 infection can cause myocardial injury indicated by increased troponin. ACE2 is highly expressed in the heart. Although it is lower than that in the kidney and small intestine, it is higher than that in the lung.[104] Chen et al.[3] examined eight types of cells in the heart that included cardiomyocytes, endothelial cells, macrophages, fibroblasts, pericytes, smooth muscle cells, T-cells and neuron-like cells, and found that ACE2 was highly expressed in the pericytes, which accounted for about 10% of the total cells in the heart.[104] The pericytes are located outside the endothelial cells of capillaries and thus infections by SARS-CoV-2 could promote insufficient blood supply to cardiomyocytes, which in turn may increase the expression of ACE2. Studies have revealed that Ischaemic cardiomyopathy increases ACE2 expression by a factor of 1.8-fold compared to non-diseased hearts.[105,106] Increased expression of ACE2 by therapeutic agents for CVD has also been reported.[107]

ACE2 is also expressed in endothelial cells, and SARS-CoV-2 infection causes endotheliitis.[27,108] In an *in vitro* experiment, SARS-CoV-2 was demonstrated to infect engineered human blood vessel organoids.[109] The dysfunction of endothelial cells could cause coagulation abnormality, which affects multiple organs. Endothelial cell dysfunction usually leads to vasoconstriction and thus, organ ischaemia, inflammation and tissue oedema.[110] Inflammatory cytokines produced by other infected organs may accelerate the endothelial cell dysfunction (Figure 4).

Gut microbiota has been well demonstrated to play a critical role in heart disease through the *gut–heart* axis (Figure 4).[111] A study has shown that CVD patients with SARS-CoV-2 infections have a higher rate of gut leakage and inflammasome activation.[112] Gut dysbiosis has also been considered as a pathogenic factor in hypertension through bacterial metabolites, sympathetic nervous system stimulation and endotoxaemia.[113] Plasma butyrate levels are inversely correlated with hypertension,[114,115] while oral supplementation of butyrate decreases endothelial dysfunction and macrophage activation in a mouse atherosclerotic model.[116] Butyrate can bind GPR41 and GPR43 receptors on endothelial cells to increase functionalities.[117] Decreased production of butyrate is a characteristic of CVD.[118] Other bacterial metabolites have also been reported to be involved in the pathogenesis of CVD.[118] Therefore, dysbiosis in CVD may cause inflammation and decreased anti-inflammatory capability, which facilitates the formation of hyperinflammation in Covid-19.

5.4 | Chronic pulmonary disease

Gut dysbiosis could explain chronic pulmonary diseases as a risk factor of severe Covid-19. The link of lung diseases with gut microbiota is also well recognized, giving rise to the gut–lung axis (Figure 4).[119,120] Gut dysbiosis is a common occurrence in many pulmonary conditions and diseases and is involved in the pathogenesis of allergies, asthma, cystic fibrosis, lung cancer and chronic obstructive pulmonary disease.[121] A dysbiotic gut promotes inflammatory profiles in lung conditions and reduces the regulation of pro- and anti-inflammatory activities.[121] Alternatively, lung inflammation such as viral infections can disturb the gut microbiota that progresses to dysbiosis by releasing pro-inflammatory cytokines into systemic circulation and subsequently the intestines.

This bidirectional interaction may also exist between SARS-CoV-2 lung infections and gut dysbiosis. Pre-existing gut dysbiosis can cause low-grade systemic inflammation, which could then accelerate the inflammation in the lung caused by SARS-CoV-2 lung infection. The pro-inflammatory cytokines released from lung inflammation may transfer to the gut through the systemic circulation, accelerating gut dysbiosis. Therefore, it forms a feed-forward regulation. It has been proposed that targeting the gut–lung axis could be used for anti-inflammation therapy in Covid-19.[122–124]

5.5 | Chronic liver disease

The investigation about the overall effect of chronic liver disease on the severity of Covid-19 has resulted in controversial outcomes. By comparing Covid-19 with liver disease and non-liver disease in 2780 patients, Singh et al.[125] found that pre-existing chronic liver disease, particularly cirrhosis, increased the severity and mortality from Covid-19.[126] Several other studies have also shown that liver disease is associated with severe Covid-19.[127] Even simple hepatic steatosis can increase the severity of Covid-19.[128] However, in a pooled study, Lippi et al.[129] showed no association between chronic liver disease and the severity and mortality from Covid-19 infections. This contrast could be explained by the selection of patients studied. Different liver diseases may have various impacts. As shown in Singh's study,[125] the effects of cirrhosis and non-cirrhosis on Covid-19 is significantly different.[126] Simple hepatic steatosis and non-alcoholic steatohepatitis may also need to be distinguished as pre-existing inflammatory conditions could be important in the severity of Covid-19. A recent meta-analysis confirmed that liver injury was common in Covid-19, reaching 25%, with a worsening clinical outcome.[130]

The effect of chronic liver disease on the severity of Covid-19 could be explained by the gut–liver axis (Figure 4). Intestinal dysbiosis is reported in chronic liver disease, which leads to altered levels of bacterial metabolites such as butyrate and primary and

secondary bile acids. Therefore, there is increased risk of local and systemic low-grade inflammation and decreased anti-inflammatory capacity in the gut, which increases the severity of Covid-19. In non-alcoholic fatty liver disease, butyrate is decreased, leading to increased inflammation both in the liver and intestines.[131]

There are bidirectional interactions between the gut microbiota and the liver (Figure 4).[132] The bile acids produced in the liver are important for the maintenance of a balanced microbial ecosystem in the gut. The detergent effect of bile acids can inhibit bacterial overgrowth and as such bile acids control pathobiont proliferation. Furthermore, the metabolism of bile acids by gut bacteria is an important factor that maintains a normal bile acid pool. Bile acids are signalling molecules, which regulate bile acid biosynthesis in the liver as well as the physiological processes of other organs. The intestinal dysbiosis in chronic liver disease affects the composition of the bile acid pool, which in turn further disrupts the gut microbiota. How the anti-inflammatory bile acids, namely isoDCA and isoalloLCA, change in chronic liver disease and how they contribute to the effect of chronic liver disease on the severity of Covid-19 at present remain unknown.

An important function of the liver is to detoxify environmental and other ingested chemicals that have been absorbed from the intestines. Liver detoxification processes could be impaired in various chronic liver diseases that could progress the accumulation of pro-inflammatory chemicals such as LPS in the systemic circulation, which subsequently enter various end organs (e.g., heart and brain).

5.6 | Stress

The gut–brain axis exhibits a bidirectional flow of interactions in neurological diseases between the brain and gut microbiota (Figure 4). Lifestyle stressors could increase the severity of Covid-19[133] as the adverse effect of stress on the common cold has shown.[52] Moreover, these effects could be mediated by gut dysbiosis.

Stress can increase mast cell secretion of pro-inflammatory cytokines such as IL-6 and TNF-alpha through increasing production of hypothalamic and amgydala corticotropin-releasing hormone.[134] This causes gut dysbiosis with a concomitant reduced level of butyrate production. The effect of gut dysbiosis causes adverse effects on Covid-19 similar to other unfavourable conditions. In addition, Covid-19 could cause stress, which accelerates the severity of Covid-19, giving rise to a feed-forward loop. Therefore, lifestyle stressors could be a critical risk factor for severe Covid-19 that warrants further investigation.

6 | PREVENTIVE AND THERAPEUTIC IMPLICATIONS

Understanding the important roles of the gut microbiota in the pathogenesis of Covid-19 could have important implications in the prevention and treatment of the disease. A healthy gut microbiota can maintain an immune system that is in equilibrium ready to neutralize Covid-19 viral assaults. Hence a pre-existing balanced pro- and anti-inflammatory gut of microbial metabolites could potentially avoid hyperinflammation after Covid-19 and thus prevent severe Covid-19. Various approaches which could improve the gut microbiota could be used beneficially, particularly in the vulnerable populations. The aged or those with underling chronic diseases may greatly benefit from a gut microbiota that may be improved with the administration of probiotics, prebiotics and synbiotics.

Improvement in gut microbiota profiles could be useful in reducing the possibility to trigger hyperinflammation in those Covid-19 patients presenting with advanced age. As noted previously, gut microbiota profiles that produce higher levels of butyrate and bile acid derivatives that stimulate Treg cells and M2 macrophages will provide an efficient *immune brake* in the prevention of cytokine storms. The roles of the gut microbiota and butyrate in the prevention and treatment of hyperinflammation in Covid-19 warrant dedicated focused studies.

The approaches to improve gut microbiota profiles for the treatment of Covid-19 could be difficult in those patients who have dysbiotic gut caused by SARS-CoV-2 infections of intestinal cells and who have extensively administered antibiotics. Alternatively, microbial metabolites could be used directly such as the administrations of butyrate and ursodeoxycholic acid.[135–137] Given that butyrate has an anti-inflammatory effect through various mechanisms, in Covid-19, butyrate may reduce hyperinflammation. Studies have shown that high levels of gut butyrate-producing bacteria are associated with reduced respiratory viral infections in kidney transplant recipients.[138] However, butyrate is metabolized in the human body rapidly, leading to low bioavailability. Therefore, specific dosages to be used could be of importance for achieving an anti-inflammatory effect while avoiding weakening the antiviral effect of immune responses. Although as yet to be studied, IsoDCA could also be used in the treatment of Covid-19.

Gut microbiota and bacterial metabolites could also be involved in the prevention and treatment efficacy of other agents used in the treatment of Covid-19, such as vitamin D. Low levels of vitamin D are now known to be associated with the severity of Covid-19; low concentrations of vitamin D and its metabolite 25-hydroxy vitamin D are inversely correlated with the severity of Covid-19.[139,140] Gut microbiota can affect the blood levels of 25-hydroxy vitamin D and vitamin D receptor (VDR) expression.[139] After binding of vitamin D, VDR regulates genes to increase innate immunity and secretion of antivirus defensin, which cleave virus membrane.[141] Activation of VDR also increases ratios of Th2/Th1 and Treg/Th17 to facilitate anti-inflammatory effects.[142] In animal experiments, vitamin D metabolite 1,25-dihydroxyvitamin D binds to VDR to reduce renin–angiotensin system, and thus decrease the inflammatory status.[143] On the other hand, activated VDR affects gut microbiota composition.[144] It also increases antimicrobial peptide expression and gut barrier integrity.[139] Increased expression of antimicrobial peptides by vitamin D in the respiratory tract protects the lungs from viral infections.[130] Vitamin D in combination with magnesium and vitamin

B12 are able to reduce oxygen support and intensive care in Covid-19.[131] Combination use of vitamin D and bacterial metabolites may warrant controlled clinical studies.

7 | CONCLUSIONS

Intestinal dysbiosis is common in patients presenting with high risk factors of severe Covid-19. Intestinal dysbiosis could mediate, at least partially, the pathogenesis of severe Covid-19. The gut commensal bacterial cohort elaborated metabolites are important in maintaining a regulated pro- and anti-inflammatory states in the host. Loss of equilibrium in immunity with partial dependence on a eubiotic gut microbiota could facilitate the occurrence of hyperinflammation, leading to severe Covid-19 and increased mortality. Improvement of intestinal microbiota profiles and administrations of commensal bacterial metabolites could prevent severe Covid-19 and lead to novel therapeutic strategies.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

Jiezhong Chen drafted the original manuscript with assistance from Luis Vitetta. All authors contributed to further improvement of the manuscript and approved the final version of the manuscript.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable for this narrative review.

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