Role of nutrients in modulating microbiota and immunity in COVID-19 disease

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Abstract. – COVID-19 is a novel disease with a broad range of clinical patterns. Several patients show dysbiosis in the intestinal tract, with evidence of reduced beneficial bacteria, such as Bifidobacteria and *Lactobacilli*. It is well established that human gut microbiota dysbiosis is associated with several clinical conditions, including respiratory tract diseases due to the gut-lung axis. This narrative review discusses the role of nutrients in the relationship between the gut microbiota and the immune response in SARS-CoV-2 infection. In particular, we will focus on the benefits offered by vitamins and micronutrients on different aspects of COVID-19 disease while also discussing which diets seem to provide the most advantages.

Key Words:
   Inflammation, Microbiota, Immunonutrients, COVID-19 disease.

Introduction

Microbiota extensively colonizes the human body and varies individually and across ethnicities[1]. The common intestinal microbiota comprises four domains, *Firmicutes* (i.e., Clostridia, Bacilli, and *Lactobacilli*), *Bacteroides*, *Actinobacteria* (i.e., Bifidobacteria), and *Proteobacteria*[2]. Disturbance in the intestinal microbiome has been reported[3] as a risk factor for pro-inflammatory conditions and complex disorders, as in the case of sepsis. Patients experiencing a systemic inflammatory response syndrome have shown[4] a lower number of gut anaerobes, such as Bifidobacteria and *Lactobacilli*, and a higher count of opportunistic pathogens.

Dysbiosis of the gut microbiota has also been hypothesized to be associated with the host antiviral immune response: *in vivo* and *in vitro* studies[5] have revealed that the intestinal microbiome could modulate immune responses to viral infections.

The role of the intestinal microbiota in the pathogenesis of several diseases, such as inflammatory bowel[6], chronic kidney, metabolic and cardiovascular diseases[7], has been established[8]. In addition, the gut microbiota has also been shown[9] to play a crucial role in preventing and resolving SARS-CoV-2 infection.

Coronavirus 2019 (COVID-19) disease, caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), was declared a pandemic by the World Health Organization on March 2020. However, COVID-19 continues to spread worldwide, with a great burden in terms of mortality and morbidity[10]. Currently, several vaccines are available, but with the constant emergence of SARS-CoV-2 variants, control of COVID-19 has not entirely been achieved. COVID-19 is a respiratory disease with a broad range of clinical patterns, from asymptomatic or mild complaints with cough and fever to severe pneumonia with multiple

organ failure (MOF) and acute respiratory distress syndrome (ARDS)[11]. ARDS is caused by a cytokine cascade, including interleukin (IL)-1, IL-6, IL-7, IL-10, granulocyte colony-stimulating factor (G-CSF), and tumor necrosis factor-α (TNF-α).

Dysbiosis induces cytokine production and nuclear factor kappa B (NF-κB) mediated local inflammation and is now clearly recognized as a risk factor for developing a more aggressive COVID-19 disease, in addition to old age and comorbidities[12]. Moreover, intestinal dysbiosis facilitates an inflammatory status and a consequent cytokine storm as observed in severe SARS-CoV-2 infections[12]. Finally, dysbiosis causes a decrease in the production of commensal bacterial metabolites responsible for anti-inflammatory functions[13].

Although there are some ongoing studies[14,15], it is known that the use of products such as probiotics and nutraceuticals could be effective in modulating the microbiota during SARS-CoV-2 infection; especially in severe cases, probiotic supplementation seems to have anti-inflammatory and antiviral effects. Particularly, Navarro-López et al[16] conducted a prospective case-control intervention study on COVID-19 patients, aimed to investigate the impact of the probiotic supplementation of the yeast *Kluyveromyces marxianus* B0399 with *Lactobacillus rhamnosus* CECT 30579 for 30 days. They observed in the supplemented group an improvement in the digestive and global symptoms greater than in the control group (respectively, *p* = 0.06 and *p* = 0.03).

Leal-Martínez et al[15] conducted a randomized, blinded, and controlled clinical trial of 80 patients, who were assigned to either a control group with a hospital diet and medical treatment or an experimental group with a hospital diet, medical treatment and nutritional support with vitamins, probiotics, and other nutrients. They[15] observed that survival was significantly increased in the experimental group, and mortality was reduced compared to the control group (*p* = 0.027). In addition, a 10% reduction in mechanical ventilation assistance was noted in the experimental group, with a 15-day reduction in the intubation period and a 38% increase in survival of intubated patients, as well as significant improvements in saturation and oxygen levels, compared to the control group.

This narrative review aims to evaluate the evidence available on the role of nutrients on gut microbiota as to enhance immune system response against SARS-CoV-2 infection and disease, focusing on the interaction among immunity, immunomodulation, and nutrition.

Methods

The literature search for the narrative review was performed on the two scientific literature databases, PubMed or Web of Science, using the following keywords: 'immunology' OR 'microbiota immunomodulation' OR 'immunonutrients' OR 'prebiotic' AND 'COVID-19'. Peer-reviewed research articles, reviews, or editorials, written in English, available in full text, and published mainly between 2000 and 2023 were considered. Studies were selected according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses method.

The Role of Immunity in Infectious Diseases: the Case of SARS-CoV-2

All organisms establish an intricate web of relationships and, from bacteria to humans, an effective immune system is closely associated with evolutionary success[17]. Infectious diseases remain a significant cause of morbidity and mortality worldwide, in spite of advances in vaccination and antimicrobial therapy[18].

In the fight against invading pathogens, mammals developed strong biological systems. In particular, host immunity is based on the cooperation between two closely related systems: innate and adaptive immunity. Innate immunity provides first-line responses, reacting rapidly and non-specifically to pathogens. In contrast, the immunological mechanisms of adaptive immunity are slower but more specific, leading to the generation of long-lived immunological memory[19]. Cell populations involved in innate immunity are myeloid cells, natural killer (NK), innate lymphoid cells, and non-immune cells in specific circumstances. Innate immunity is also mediated by ancient humoral systems, such as complement and defensins[20]. On the other hand, humoral adaptive immunity is based on the immunoglobulin family, while cellular immunity is based on B- and T-lymphocytes[21].

SARS-CoV-2 infection can show several clinical patterns, ranging from asymptomatic or only upper respiratory symptoms or can progress to respiratory failure and ARDS.

The pathogenesis of COVID-19 can be described with three phases: local inflammation, acute systemic inflammation, and low-intensity chronic systemic inflammation[22]. The virus locates the cellular receptors, then suppresses the antiviral response and, in the end, it activates the autoimmune and autoinflammatory processes[23-25].

SARS-CoV-2 binds to main host receptors [(membrane angiotensin converting enzyme 2 (ACE 2)] through its spike S-glycoproteins[26,27]. After the binding, the S-protein activates proteolytic processing that determines the fusion of the membranes of the virus to the target cell.

Viral pathogen-associated molecular patterns (PAMPs) start cells' response to infection by downstream signaling molecules [such as mitochondrial antiviral-signaling protein (MAVS), and myeloid differentiation primary response 88 (MyD88), TANK-binding kinases 1 (TBK1), I Kappa B kinase (IKK), transcription factors interferon regulating factor 3 (IRF3), nuclear factor kappa light chain enhancer of activated B cells (NF-κB), which results in high interferon (IFN)-I-III production, which then activates a tyrosine kinase 2 (Tyk2) and Janus kinase/signal transducers and activators of transcription signaling pathway (Jak/STAT)]. The activation of the interferon-stimulated gene factor (ISGF3) induces the transcription of the interferon-stimulated gene (*ISG*). *ISG* expression products, such as 2′–5′–oligoadenylate synthetase, Interferon☐inducible transmembrane, and others, protect cells from SARS-CoV-2 infection by degrading viral RNA and reducing virus penetration[28,29].

In general, SARS-CoV-2 can inhibit all the main stages of the IFN signaling pathway, and the viral non-structural protein 1 (NSP1)[30] and open reading frame 6 (ORF6)[29] have the greatest impact on IFN-I-III production and signaling to *ISG*.

At the same time, the overexpression of IFN is implicated in the pathophysiology of the cytokine storm phenomenon associated with systemic inflammation[23]. In addition, SARS-CoV-2 activates the complement system.

The presence of mannose-rich glycans in the S1 region of SARS-CoV-2 is recognized by mannose-binding-lectin (MBL), which is a component of the complement system, causing its activation, which further induces inflammation and promotes phagocytosis[31].

The adaptive immune system is the second line of the immunological response to SARS-CoV-2.

The virus directly activates CD4+ T cells: consequently, CD4+ differentiates into several helper and effector cell types, particularly virus-specific CD4+ T cells in T helper (Th)1 cells and T follicular helper cells (Tfh). Th1 cells can stimulate the production of IFN-γ and cytokines. These cells are essential for the development of memory B cells and long-term humoral immunity[32].

Specific antibodies are detectable a few weeks after the beginning of the infection; in particular, Immunoglobulin (Ig)M antibodies appear between day 8 and 12, while IgG antibodies appear from day 14[33]. CD4+ T cells also stimulate CD8+ T cell response.

According to Sigal[34], naive CD8+ T cells recognize the viral antigens presented on major histocompatibility complex I (MHC I) through their T-cell receptors (TCRs), and then they secrete TNF, IFN-γ, and other cytokines to kill the virus[35].

An interesting aspect of SARS-CoV-2 is its ability to determine a sustained immune response: in particular, SARS-CoV-2 has been linked[36] to the development of different immunological complications. One of the most studied events that can take place is the development of a cytokine storm as a consequence of the disease[37]. A cytokine storm is characterized by an immune dysregulation, which can present a variety of symptoms and determines systemic inflammation, and multiorgan dysfunction, potentially leading to multiorgan failure if inadequately treated. It was first described[38] in the context of severe infections (e.g., *Yersinia pestis*, influenza A) and afterward as a potential side effect of chimeric antigen receptor (CAR)-T therapy. In the first months of the pandemic, different physicians[39] observed that some patients with SARS-CoV-2 developed a syndrome that seemed to be a cytokine storm. Further studies[40] demonstrated that patients were actually developing a cytokine storm: in a sample of patients from China who were infected by SARS-CoV-2, elevated levels of a plethora of cytokines, such as interleukins, as IL-1β, IL-7, IL-8, IL-9, IL-10, fibroblast growth factors (FGF), granulocyte colony-stimulating factor (GCSF), IFN-γ, interferon gamma-induced protein (IP-10), monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-1 (MIP-1A, MIP1-B), platelet-derived growth factor (PDGF), TNF-α, and vascular endothelial growth factor (VEGF) were observed[40]. In particular, IL-10, IL-6, and TNF-α levels seemed to be directly associated with disease severity. The mechanisms through which patients develop such a disorder are to be found in the abnormal response to the virus triggered by the innate immune system, which can lead to a spike in the levels of IL-10, IL-6, and TNF-α, determining severe alterations in a number of organs and systems, eventually leading to death[41].

Impact of Microbiota on Immunomodulation in COVID-19 Disease

Even though COVID-19 primarily impacts the respiratory system, it is possible that its immunomodulation is related to the gut microbiota.

One explanation may be related to the expression of the angiotensin converting enzyme 2 (ACE 2) receptor also at the level of gastrointestinal cells[42,43]; the virus has been detected even in the feces of infected patients. It can frequently cause gastrointestinal disorders, most commonly nausea, diarrhea, and vomiting[44] even before causing respiratory symptoms[45,46]. On the other hand, patients with gastrointestinal symptoms related to SARS-CoV-2 infection have a more severe respiratory disorder[47].

Recently, systems such as the "gut-liver", "gut-brain", and "gut-heart" axis have been proposed[48]. A "gut-lung" axis has also been suggested to explain the bidirectional interaction between respiratory mucosa and intestinal microbiota, with the ultimate goal of modulating the immune response[49-51]. A healthy lung has its own specific microbiota[52,53,] which is more dynamic and transient and has smaller microbial diversity than the intestinal microbiota[42]. These microorganisms derive mainly from the oral environment, the inhaled air, and the digestive tract (through microaspiration). The main protagonists are *Prevotella, Streptococcus, Veillonella, Fusobacterium*, and *Haemophilus*[52,53]. Although the role of the respiratory microbiota is still poorly understood, it is assumed that, like the intestinal one, it plays a role in regulating the immune response[54]. The loss of lung microbiota diversity causes an immunological imbalance that could impact the genesis of chronic inflammatory respiratory diseases[55].

The existence of a gut-lung axis might explain the influence of intestinal dysbiosis on respiratory diseases[49,56]. Intestinal segmented filamentous bacteria can, for instance, stimulate the production of T helper 17 (Th17) cells, reducing the infection rate and mortality related to *Staphylococcus aureus*[57,58]. Microbial disruption is the most common cause of respiratory infections[59], on the other hand, respiratory infections can modify intestinal functions[60]. The intestine can attenuate the symptoms of respiratory infections by restoring the microbiota, *via* short-chain fatty acids (SCFAs), and modulating immunity[61]. Changes in the composition of the intestinal microbiota are associated with an increase in susceptibility to respiratory tract diseases[14,42,62,63].

There are two main proposed mechanisms of interaction between the intestine and the lung during infection of the respiratory tract[50]. The first one is that the leaky gut, during dysbiosis, facilitates the gut mucosal translocation of microbiota metabolites[64]. An alternative hypothesis involves the direct transfer of bacteria or bacterial products to the lungs through the blood or the lymphatic system, causing a general or local immunological response that leads to further lung damage[65].

The intestinal microbiota also plays a role in immunomodulation during COVID-19 disease, increasing serum titers of SARS-CoV-2-binding IgG and IgM in 15-30 days[66]. Viral infections cause intestinal dysbiosis, resulting in a general decrease in microbial diversity, enrichment of opportunistic pathogens, and depletion of beneficial diners, such as butyrate-producing bacteria[12,13,67]. These events cause an alteration and subsequent overregulation of the inflammatory response[12,13], which can involve dysregulation of Th17 cells[68,69], mast cells, NK and antigen-presenting cells (dendritic cells in Peyer's plaques, Langerhans cells and macrophages)[70].

To modulate the immune response during COVID-19 disease, the microbiota promotes the activation of specific intestinal immunity cells, with the consequent production of cytokines[71]. The microbiota acts on the immune system also by releasing immunomodulatory signals and metabolites, such as branched-chain and aromatic amino acids, neurotransmitters, carbohydrates, vitamin B6, and SCFAs secreted by commensals, such as *Blautia* species, *Dorea* species, *Eubacterium* species, and *Faecalibacterium prausnitzii*[72].

The involvement of dipeptilpeptidase-4 (DPP4) (originally known as "lymphocyte cell surface protein CD26") in infectious disease processes[73] and its contribution, as coronavirus receptor protein, to the intracellular entry of SARS-CoV-2, have raised considerable interest[74]. Within the immune system, DPP4/CD26 proteins may amplify the signals derived from interactions with an antigen, leading to T-cell activation[73].

The DPP4 is a multifunctional soluble and cell-bound serine protease abundantly expressed in different cellular types, including endothelial and epithelial cells in the lungs, immune cells (CD4+ and CD8+ T cells, B cells, natural killers, macrophages, dendritic cells)[75,76], epithelial tissues including small and large bowel enterocytes. Membrane-associated human DPP4 is also a functional coronavirus receptor, interacting with the virus

through the spike glycoprotein Slb domain. After binding DPP4, S protein is cleaved and activated by transmembrane serine protease 2 (TMPRSS2) or cathepsin L, facilitating viral entry[77,78].

For this reason, DPP4 receptor acts like an ACE2-receptor. ACE2 is a transmembrane carboxypeptidase enzyme and functional coronavirus S1 subunit receptor, expressed in lungs as well as in several extra-pulmonary tissues, including the gut[79]. ACE2 cleaves the last amino acid of angiotensin II, and generates vasodilatory angiotensin (1-7), but can also cleave other metabolically active substrates, including dynorphin A 1-13, apelin-13, neurotensin (1-13) β-casomorphin, des-Arg9-bradykinin and ghrelin[80]. The membrane-associated ACE2 enzyme can be cleaved to a soluble circulating form (sACE2), whose biological importance remains uncertain. The extracellular domain of both ACE2 and sACE2 binds SARS-CoV-2[74,81]. TMPRSS2 predominantly allows SARS spike protein-driven cellular entry[82].

Because coronaviruses enter the cells by binding to DPP4, the hypothesis that DPP4 inhibitors (DPP4is) might affect the clinical outcomes in type 2 diabetes mellitus (T2DM) patients infected by SARS-CoV-2 was proposed[83]. DPP4is have significant systemic anti-inflammatory effects, reducing the concentration of inflammatory cytokines[84]. Furthermore, in mouse models, sitagliptin suppresses lung injury caused by lipopolysaccharide by reducing the release of cytokines and pulmonary hyper-inflammation[85]. Whereas findings from observational studies are heterogeneous, collectively, the available data in the literarure show that DPP4 does not harm patients with both T2DM and COVID-19 disease. One case-control retrospective study[86] described a significant reduction in intensive care unit (ICU) admission and mortality in sitagliptin users *vs.* non-users, however, no other sufficiently large observational study has yet been conducted. While there is no reason to eliminate DPP4 use in patients with T2DM and COVID-19, except for those who are critically ill, there is still insufficient evidence to support the introduction of DPP4is with the intention to improve the prognosis of patients exposed to SARS-CoV-2[76].

An increase in some cytokines, such as IFN-γ, IL-6, and chemokine ligand 2 (CCL2), has been observed[49,87] as a result of increased activation of naïve T helper cells[65] and decreased activation of NK, B cells, memory T lymphocytes, and T regulatory cells (Tregs)[87].

A decrease in bacteria, such as *Lactobacilli* and Bifidobacteria, SCFA-producing bacteria[9,88],

Bacteroides, Faecalibacterium prausnitzii, and *Eubacterium rectale,* has been observed during SARS-CoV-2 infection[89,90].

At the same time, an increase in pathogenic bacteria such as *Clostridium ramnosum, Clostridium hathewayi,* and *Coprobacillus* has also been observed[90]. This increase seems to be correlated with the severity of COVID-19 disease. In contrast, *Faecalibacterium prausnitzii* and *Bacteroides* were inversely correlated to the severity of COVID-19 disease[91].

Furthermore, at the level of the intestinal epithelium, the presence of segmented filamentous bacteria, including bacteria of the genus *Clostridium,* activates the antigen-specific Th17 cells, through IL-23, IL-22, and serum amyloid A proteins[92-94], and promotes IgA synthesis[95,96].

At the same time, the reduction of *Bacteroides fragilis* impairs the development of Tregs through polysaccharide A[94] in favor of the development of Th1 and Th2 cells. In addition, the reduction of SCFA-producing bacteria, powerful activators of Tregs response, is another element through which dysbiosis alters the immune response[92].

An increase in TNF-α, C-X-C motif chemokine ligand 10 (CXCL10), CCL2, and IL-10, whose plasma concentrations are related to the severity of infection has been observed in a study[89] that analyzed the intestinal microbiota in hospitalized patients.

Once intestinal dysbiosis is established, with the consequences on the immune response described above, events may occur that determine the maintenance of the dysbiotic state[93]. In detail, dysbiosis affects the host's immune system through various mechanisms that contribute to the stabilization of the dysbiotic configuration: modulation of inflammasome signaling through microbial metabolites, modulation of toll-like receptor (TLR) signaling, and degradation of the secretory IgAs[92], which explains why dysbiosis can persist even months after SARS-CoV-2 infection is resolved[49].

Finally, according to Hussain et al[97], in COVID-19 patients, a strong correlation was observed between their pathophysiological condition and the microbiome. This suggests that the microbiota could be involved in the pathogenesis of COVID-19 disease.

Moreover, the role of the microbiota in the predisposition of individuals to the development of more severe forms of COVID-19 was observed[98]. Specifically, Gou et al[98] identified 20 proteomic biomarkers through serum analysis of 990 patients with COVID-19 disease, creating a proteomic risk score (PRS) that predicts the severity

of COVID-19. They then looked for a possible correlation between PRS and the gut microbiota, finding it in specific microbial metabolites[98]. Some bacteria, such as *Ruminococcus* and *Blautia* are positively associated with a more severe form of the disease, while *Bacteroidetes* and *Clostridiales* are negatively associated[42].

Role of Immunonutrients on Microbiota in COVID-19 Disease

The quality and quantity of dietary components, both macronutrients (carbohydrates, fats, and proteins), micronutrients (polyphenols and vitamins), as well as minerals and trace minerals (magnesium, iron, selenium, zinc), have shown[99] substantial effects on the intestinal microbiota. Some nutrients have the ability to modulate the innate and acquired immune systems and play a fundamental role in the relationship between immunity, infection, inflammation, injury, or tissue damage with individual nutritional status[100]: interesting results have been observed by Ponzo et al[101], who observed a correlation between different dietary patterns and COVID-19 disease outcomes.

The main nutrients used in the supplementation of the patient with COVID-19, for their function on the immune system and on the microbiota are: arginine (substrate essential for lymphocyte function), glutamine (activator of T lymphocytes), omega-3 fatty acids (ω-3) (with anti-inflammatory activity for the inhibition of NF-κB activity), nucleotides (activators of the phagocytic function of macrophages, useful for a correct synthesis of DNA and RNA), vitamins D, A, E and C, and the trace elements of Selenium, Copper, and Zinc, with anti-inflammatory activity. In particular, Thomas et al[102] have examined the effects of specific supplementation (zinc and vitamin C), but no conclusive results were observed. Moreover, the gut microbiota can be modulated through the administration of symbiotics, as they act synergistically with probiotics and prebiotics, benefitting both small and large intestine[103].

Pimentel et al[104] reported the results of a double-blind clinical trial on 43 COVID-19 patients, assuming 200 ml/day of a standard high-protein normocaloric supplement for 7 days or 200 ml/day of an immunonutrient enriched supplement with L-arginine, nucleotides, and ω-3 essential fatty acids for 7 days. They observed that in the experimental group, there was a greater reduction in C-reactive protein (CRP) levels [23.6 ($\pm$ 7.5) mg/L] than in the control group [14.8 ($\pm$ 12.1) mg/L] ($p = 0.002$).

Arginine, a semi-essential amino acid, is the substrate used for Nitric Oxide (NO) formation by nitric oxide synthase (NOS)[105]. A role for NO as a regulator of apoptosis has been demonstrated[106], and also in infectious diseases, with indirect or direct antiviral activity, to improve immunity, anti-infective, and anti-oxidative responses. Therefore, arginine, through NO, contributes to the formation of peroxynitrite, dinitrogen trioxide, and nitrogen dioxide, which all can have an antiviral effect. Arginine stimulates protein synthesis *via* the mammalian target of rapamycin (mTOR) pathway and improves T cell function, increasing the response in T cell-mediated memory, and reducing susceptibility to viral infections[107]. The proliferation of Th17 cells, and the reduction of cytokines levels, which were related to hyperinflammation during COVID-19, were suppressed by L-arginine supplementation[106], which could offer an interesting therapeutic application, particularly in those populations which present a deficit in this micronutrient. Although low arginine levels have been inversely correlated to the severity of COVID-19, some studies[108] do not recommend its supplementation. Muralidharan et al[109] conducted a parallel-group, triple-blinded, randomized controlled trial on 74 patients with severe COVID-19 pneumonia on oxygen (O_2) support to investigate the effect of 3 g per day of l-Arg supplementation compared to placebo supplementation, until they were off O_2 support, or for a maximum of 10 days. They reported that l-Arg supplementation compared to placebo did not show differences in outcomes. In addition, Matli et al[110] conducted a double-blind, placebo-controlled, randomized clinical trial, on 42 COVID-19 patients who received, on top of medical therapy, either an endothelial protocol also consisting of L-arginine or placebo for up to 14 days. They did not observe statistically significant differences in time to recovery, need for mechanical ventilation or ICU admission, all-cause mortality, or the occurrence of side effects between the two groups.

Glutamine plays a pivotal role in modifying gene expression, cell signaling pathways and helps boost immunity. Among its activities, such as antioxidant, it shows an anti-inflammatory action by blocking NF-κB. Moreover, it stimulates the activation of protein-1, inhibits the CCAAT-enhancer-binding proteins (C/EBP) homology binding protein and plays a role in cell survival, modulating *c-Myc*, and is essential for the proliferation of the immune cells[111]. It was observed that in COVID-19 patients with a respiratory infection, glutamine supplementation significantly

reduced serum levels of high-sensitivity (hs)-CRP, IL-1β and TNF-α and significantly increased appetite, and even affected mortality and need for the ICU[110]. Particularly, Mohajeri et al[112] conducted a case-control study of COVID-19 patients with respiratory infections, of which 230 did not receive any supplementation, while 222 received a supplementation of 10 g of glutamine three times a day for 5 days. They observed that serum levels of IL-1β, TNF-α, and hs-CRP were reduced ($p < 0.05$), and patients' appetite during 5 days of supplementation with glutamine had a significant increase ($p < 0.05$) compared with the control group.

Omega-3 long-chain polyunsaturated fatty acids (LCPUFAs) supplementation, including linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), mitigate the adverse effects of inflammation, acting as mediators in the regulation of inflammatory processes and responses, including resolvins, protectins, and maresins[113]. Conjugates of these substances have been identified, such as resolvin D1 (RvD1), resolvin D2 (RvD2) and maresin 1 (MaR1), and it has been observed[114] that they can interact with various cell types, such as polymorphonucleates (PMNs), eosinophils, dendritic cells, macrophages, innate lymphoid cells, B cells, CD4+ T cells, CD8+ T cells, and γδ T cells. Furthermore, they modulate the levels of proinflammatory mediators, including lipopolysaccharides (LPS) and IL-17. Some authors[115], suggest that omega-3 supplementation could even have the potential to prevent COVID-19, have an impact on reducing the duration of symptoms, decrease the risk of renal and respiratory dysfunction, and increase the patient survival rate.

Doaei et al[116] conducted a double-blind, randomized clinical trial study including 101 patients who tested positive for COVID-19 with severe pneumonia, fever, and respiratory distress. The patients received 1,000 mg omega-3 daily for 14 days. Omega-3 supplementation has shown improved renal function with significantly reduced levels of blood urea nitrogen, creatinine, and kalium and increased urine volume in COVID-19 disease.

Another single-blind randomized controlled trial[117] has reported that patients receiving 2 grams of Docosahexaenoic acid plus Eicosapentaenoic acid for 2 weeks showed improved symptoms, such as pain and fatigue, but not olfactory symptoms, reduced levels of CRP and erythrocyte sedimentation rate, but not a reduction of liver enzymes. Moreover, omega-3 acids can interact with the microbiota, varying the types and quantity of the species. Particularly, polyunsaturated fatty acids (PUFAs) are responsible for the decrease in Enterobacteria and the increase in Bifidobacteria, limiting metabolic endotoxemia. An unbalanced ratio of omega-3/omega-6 can alter the presence of *Firmicutes/Bacteroidetes*[118].

Vitamin C (ascorbic acid) is historically regarded as a vitamin that safeguards the integrity of connective tissues and its deficiency causes scurvy[119]. Scurvy was associated with pneumonia in early studies. A large series of animal studies in literature showed that vitamin C plays a role in preventing, alleviating and shortening several infections. Similar effects have been described[120-122] in humans, particularly in pneumonia and in colds. Vitamin C is an efficient water-soluble antioxidant, and it seems to protect host cells against the actions of reactive oxygen species (ROS) released by activated phagocytes during infections. Thomas et al[123] examined the effect of high-dose ascorbic acid and/or zinc compared with usual care in ambulatory patients with SARS-CoV-2 infection. They[123] randomized 214 patients, but the study was stopped for low benefits with no significant difference among the groups. Treatment with high-dose ascorbic acid, zinc gluconate, or a combination of both supplements did not significantly decrease the duration of symptoms compared with standard of care[123]. Other studies[124-126], focusing on vitamin C treatment in SARS-CoV-2 infection, lead to contrasting and often inconclusive results, therefore, more studies, in particular randomized clinical trials, are necessary.

Vitamin D exerts an important antiviral role in inhibiting NF-κB and the following expression of pro-inflammatory cytokines while stimulating immune cells and the expression of catelicidin with antiviral activity. Moreover, vitamin D may improve the IFN-γ-mediated antimicrobial immune response of macrophages[111]. Vitamin D contributes to the maintenance of intestinal homeostasis, acting on the inflammatory state. More specifically, thanks to the binding to its receptor (Vitamin D receptor, VDR), in Th1/Th17 cells, it favors bacterial elimination, restoring the levels of antimicrobial peptides in the mucus and maintaining epithelial integrity. Furthermore, vitamin D favors the cellular suppression of Th1/Th17, promoting Treg cells[127], decreases the expression of proinflammatory cytokines (IL-6), and the activation of TNF-α[128]. It was reported[129] that vitamin D supplementation can counteract SARS-CoV-2 infection by reducing pro-inflammatory cytokines and thus limit mor-

tality associated with acute respiratory distress syndrome in COVID-19 patients. Indeed, an increased risk of pneumonia in patients with low vitamin D levels has been observed[130], thus it might present interesting applications. However, Basstane et al[131] conducted a systematic review and meta-analysis on the relationship between COVID-19 and vitamin D. Of 8,209 patients observed, those with serum 25(OH)vitamin D levels <30 ng/ml showed to have 1.5 times the possibilities to test positive for SARS-CoV-2 respect to subjects with normal 25(OH) vitamin D levels. Unfortunately, the low quality of some studies published, and the lack of strong evidence failed to determine a clear relationship between levels of vitamin D and COVID-19-related outcomes (mortality risk, invasive or non-invasive ventilation, or ICU admission) [131].

Vitamin E, a fat-soluble antioxidant belonging to α, β, γ, δ-tocopherol family and α, β, γ, δ-tocotrienol, protects the cell membrane from radical oxygen species (ROS), neutralizes them, and prevents lipid peroxidation. Vitamin E induces superoxide dismutase, quinone oxidoreductase, glutathione peroxidase, by inhibiting cyclooxygenase (COX)-2, signal transducer and activator of transcription-3 (STAT3), NF-κB, TNF-α, cytokines, and inducible nitric oxide synthase. The recommended daily dose of vitamin E is 200-400 mg[132]. It was observed[132] that a vitamin E deficiency could lead to a greater risk of contracting SARS-CoV-2 infection; at the same time, an excess could lead to worse disease outcomes. While conclusive evidence is not yet available, a study by Erol et al[133] carried out on pregnant women has shown a correlation between vitamin E levels and worse perinatal outcomes, probably due to higher oxidative stress levels, but, again, evidence is not yet conclusive.

Vitamin A maintains mucous membranes, including the intestinal tract, and scavenges free radicals. The recommended daily dose is 300-700 micrograms[134]. Particularly, retinoic acid is known to increase the proliferation of keratinocytes and fibroblasts, the production of extracellular matrix components, such as fibronectin and type I collagen, and decrease levels of matrix-degrading metalloproteinases. Moreover, it can reverse the inhibitory action of anti-inflammatory steroids on wound healing[135]. Furthermore, Vitamin A may improve clinical and paraclinical symptoms in COVID-19 patients[136]. Particularly, Rohani et al[136] conducted a triple-blind controlled study on 182 COVID-19 outpatients to analyze the treat-

ment effects between a group that received 25,000 IU/d of oral vitamin A for 10 days in addition to the standard treatment for COVID-19 and another group that received standard drug treatment alone. In the experimental group, it was observed that clinical symptoms such as fever, body aches, weakness and fatigue, para-clinical symptoms, white blood cell count and C-reactive protein decreased significantly more than with standard treatment alone ($p < 0.05$).

Copper is an essential cofactor for enzymes involved in redox reactions, including catalase, superoxide dismutase (SOD), and cytochrome oxidase, in metabolic reactions, in oxygen transport. In addition, it has antibacterial, antifungal, antiviral, and anti-inflammatory effects, enhancing the host's immune system response against pathogens, by inducing autophagy and apoptosis and leading to the formation of autophagic vacuoles that maintain the cell's antiviral defense. Copper is known[137] to inhibit RNA polymerase activity by more than 60%, also activating several potentially antiviral mechanisms of action. The recommended daily dose is 0.3-1.6 mg[134]. Furthermore, copper has been observed[138] to mediate the activation of DNA viruses, such as SARS-CoV-2. Indeed, copper nanoparticles embedded in the active site of SARS-CoV-2 S-glycoprotein have the potential to inactivate it, by blocking the key enzyme that aids virus replication. Indeed, copper supplementation could have useful outcomes for COVID-19 patients, also restoring the IL-2 secretion and activity in deficiency state[138]. Moreover, copper has been shown to alter the phylum *Firmicutes* and Verrucomicrobia, the families *Lactobacillaceae*, *Eubacteriaceae*, *Ruminococcaceae*, *Erysipelotrichaceae* and *Verrucomicrobiaceae*, and the genera *Lactobacillus*, *Coprococcus*, *Oscillospira*, *Allobaculum* and *Akkermansia*[139]. Finally, according to Hackler et al[140], serum copper levels contribute to a good prediction of survival. Furthermore, in case of deficiency, copper supplementation can positively influence the course of COVID-19, as it contributes to survival and plays a key role in the immune response and antioxidant defense systems.

Selenium, as an integral part of selenoproteins, plays a key role as a cofactor of antioxidant enzymes, such as glutathione peroxidase, catalase, superoxide dismutase, and thioredoxin reductase. Acting through antioxidant pathways, it is able to increase the number of T-cell and IL-2 cytokine secretion, enhance NK-cell activity and mitogenic lymphocyte responses, and reduce the risk of infection[140]. In addition, it increases the production of

IFN-γ and is involved in the production of immunoglobulins, lipid biosynthesis, cell cycle, calcium regulation, and ultimately protein folding. It was observed that selenium and its compounds could be effective in preventing COVID-19 disease[141]. According to Hackler et al[140], serum selenoprotein P (SELENOP) levels contribute to a good prediction of survival and supplementation of selenium in case of deficiency is recommended in order to positively influence the course of COVID-19. The recommended daily dose is 19-70 mcg[134]. Furthermore, it was documented that the administration of Selenium-enriched *Streptococcus thermophilus* and *Enterococcus faecium* improved antioxidant status[139].

Zinc is an essential component of transcription factors involved in DNA binding, RNA packaging, activation of transcriptional and translational factors, regulation of apoptosis, and protein folding. Zinc has been shown[111] to inhibit viral RNA polymerase activity, inducing the production of interferon, IFN-α, and IFN-γ. The recommended daily dose is 7.5-12-7 mg. Zinc is also the mediator of the signaling of TLR4 receptor, and increases the release of cytokines, contributing to antiviral defense. COVID-19-related symptoms, such as lower respiratory tract infections, may also decrease with zinc supplementation[130,142]. Moreover, zinc-enriched probiotic supplementation was observed[143] to increase serum levels of IL-2, IL-6 and IFN-γ and decrease IL-10, improving immune function. However, in deficient subjects, a zinc-enriched diet was observed to alter the gut microbiota and decrease resistance to *Clostridium difficile* infection[144]. On the other hand, according to Heller et al[145], biomarkers of zinc and SELENOP status in the reference ranges indicate high survival probabilities in COVID-19 and imply that zinc or selenium supplementation in case of deficiency can favor convalescence.

It is also worth noting that, while these findings are of some interest, studies need to be carried out to actually understand whether or not these micronutrients could be of use in clinical applications and in what context. Most of the available literature did not focus on specific groups of patients or on specific COVID-19 variants, thus, the findings are varied, and the possible applications are yet to be understood.

The impact of immune nutrients on the immune system and microbiota is summarized in Figure 1.
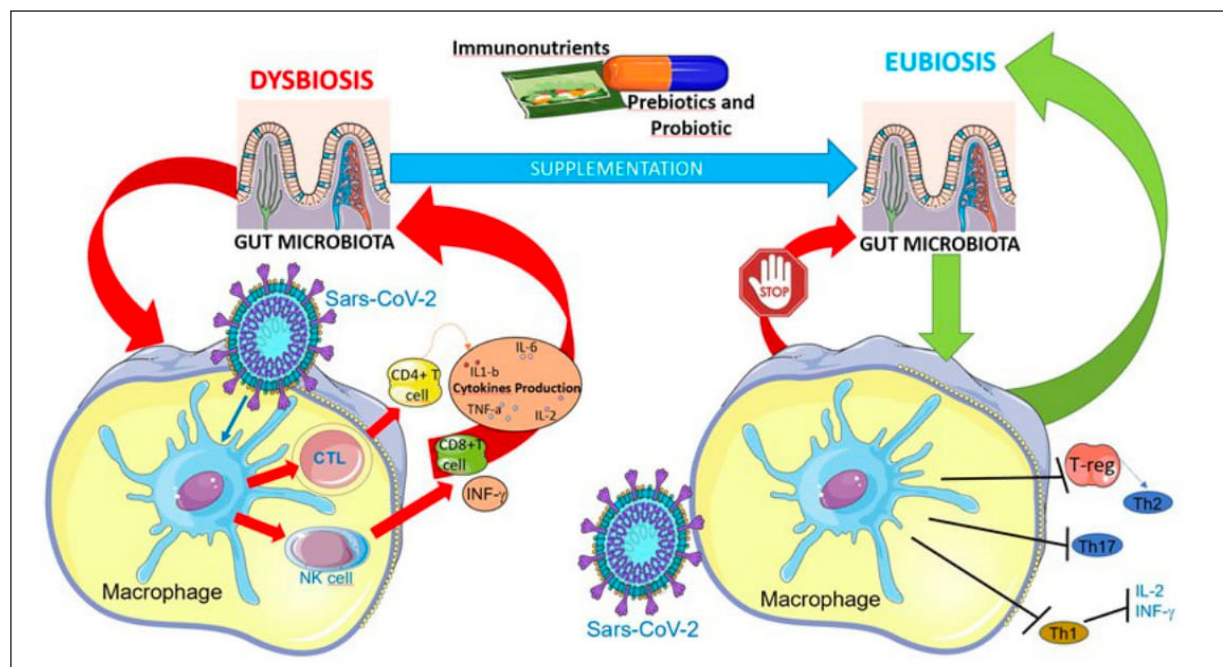

Figure 1. SARS-CoV-2 can elicit a number of complex reactions from our immune system, which can sometimes, though, determine a systemic inflammatory response. A healthy gut microbiota, modulated through immune nutrients and probiotics, can help to reduce inflammation, promoting anti-inflammatory mechanisms which can balance the overall inflammatory status of the organism. TNF-α, Tumor necrosis factor; IL, Interleukin; INF-γ, Interferon-gamma; CTL, cytotoxic T lymphocytes; Ig, immunoglobulin; NK, natural killers; Th, T-helper lymphocyte; Treg, T regulatory cell.

Microbiota, Probiotics, and COVID-19 Disease

Probiotic supplements contain beneficial bacteria that can protect the gut epithelium blocking pathogens by competing for specific receptors[146]. Furthermore, probiotics can block viral internalization, enhance the production of antiviral substances, and immunomodulate the antiviral response[147]. After oral administration, probiotics are believed[148] to modulate the relationship between commensal microorganisms and mucosal immunity in response to viral infections.

The intestinal dysbiosis established by SARS-CoV-2, which persists beyond the disappearance of the symptoms, depends on an increase in bacterial strains with inflammatory activity, such as *Coprobacillus*, *Clostridium ramnosum* and *Clostridium hathewayi*, *Burkholderia cepacian complex* (BCC), *Staphylococcus epidermidis* and *Mycoplasma spp*, and those that modulate the expression of ACE2, *Bacteroides dorei*, *Bacteroides massiliensis*, *Bacteroides ovatus* and *Bacteroides thetaiotaomicron*, with simultaneous reduction of *Lachnospiraceae*, *Roseburia*, *Eubacterium* and *Faecalibacterium prausnitzii*, which favors an anti-inflammatory microenvironment[90]. Moreover, *Clostridium hathewayi*, *Enterobacteriaceae*, *Enterococcus*, *Actinomyces viscosus*, and *Bacteroides nordii* increase during COVID-19 and cause bacteremia[71].

It was observed[149] that the severity and course of COVID-19 could be influenced by dysbiosis related to SARS-CoV-2 infection, leading to a dysfunctional immune response. Several studies[149] have in fact shown alterations in the serum and microbiome of COVID-19 patients, with elevated levels of peptidoglycan, fatty acid-binding protein-2 and lipopolysaccharide, markers of intestinal permeability, and with an enrichment of *Actinobacteria spp.* and an underrepresentation of *Bacteroides spp.* with an increased ratio of *Firmicutes/Bacteroidetes*. A reduction of *Faecalibacterium prausnitzii*, *Eubacterium rectale* and Bifidobacteria was also observed in fecal samples up to 30 days after the disease course. These species correlate negatively with the severity of COVID-19. Particularly, an abundance of the microbial species was observed in patients with severe associated conditions, such as acute respiratory distress syndrome and acute kidney damage. In addition, the abundance of *Clostridium innocuum*, *Ruthenibacterium lactatiformans* and *Alistipes finegoldii* was correlated with blood inflammatory markers, such as CRP, white blood cells and procalcitonin, and disease progression. Reduced levels of *Faecalibacterium prausnitzii*, *Blautia luti*, *Alistipes putredinis*, *Gemmiger formicilis* and *Dorea longicatena* were observed[149] in severe and fatal cases.

Thus, the dysbiosis state in COVID-19 patients leads to a decrease in beneficial bacteria, such as *Bifidobacterium*, and an increase in deleterious bacteria related to bacteremia or sepsis, such as *Brevibacterium* and *Pantoea*[149].

Therefore, administering probiotics to patients with COVID-19 could restore intestinal eubiosis. Indeed, probiotics can be used to maintain intestinal microecological balance and reduce secondary bacterial infections[150].

Prebiotics and probiotics could have antiviral effects also against COVID-19 disease and could positively impact host immune functions[151]. The commensal bacteria interact with the gut mucosa to stimulate the immune response and perform competitive action to protect the intestinal epithelium from pathogens[152].

As reported by Zhao et al[153], the intake of *Bifidobacterium* improves the response of vaccines against SARS-CoV-2. Moreover, probiotics could support pharmacological therapies to treat the inflammatory processes underway during COVID-19, thanks to remodeling the microbiota of the lung-intestine axis[150].

Bifidobacteria are used to help and treat several intestinal conditions[154]. The prerequisite for Bifidobacteria colonization of the gastrointestinal tract is the adhesion to the intestinal mucosa by binding the receptors of intestinal epithelial cells[155], with subsequent inhibition of the colonization by pathogenic microorganisms[156].

In a study[157] including 70 COVID-19 patients, the oral bacterial treatment achieved a reduced risk of respiratory failure and mortality. Another retrospective study[158] showed decreased *IL-6* levels and lower mortality rates in COVID-19 patients treated with Bifidobacteria.

In some COVID-19 patients, the intestinal Bifidobacteria and *Lactobacilli* were significantly reduced, which could be linked to weakened immunity[159]. In a randomized, double-blind, placebo-controlled trial[160] it was observed that treatment within 96 hours of COVID-19 diagnosis with a throat spray of the probiotics *Lacticaseibacillus casei* AMBR2, *Lacticaseibacillus rhamnosus* GG and *Lactiplantibacillus plantarum* WCFS1, reduced the SARS-CoV-2 titer, improving the prognosis.

Probiotics based on *Lactobacillus rhamnosus* GG were able to determine a reduction in diarrheal symptoms in patients hospitalized for COVID-19[161].

Recent studies[162] indicated that *Lactobacillus gassier* could be used as a supplementary nutritive option to reduce viral replication and as an additional treatment for COVID-19. Yet, it is worth noting that the transfer of sufficient numbers of bacteria to the gut mucosa is crucial for the successful treatment of COVID-19 patients.

Moreover, the common medications used to manage COVID-19 disease (antiviral, steroids, and antibiotics) have a role in altering gut microbiota and enhancing dysbiosis; beneficial bacteria and dietary controlled regimens may offer additional protection to minimize gastrointestinal and systemic symptoms[48].

Lactobacillus plantarum strains and *Pediococcus acidilactici* taken for 15 days significantly ($p < 0.001$) improve some systemic symptoms of COVID-19, such as fever, body aches, or shortness of breath[67].

In a randomized, parallel, quadruple-blinded, placebo-controlled study[67] on 239 COVID-19 patients, treated with supplementation with *Lactiplantibacillus* plantarum KABP033 (CECT30292), *Lactobacillus plantarum* KABP022 (CECT7484), *Lactobacillus plantarum* KABP023 (CECT7485) and *Pediococcus acidilactici* KABP021 (CECT7483), in a ratio of 3:1:1:1 colony-forming units (CFU), respectively, and a total dose of ≥2×109 total CFU, higher titers of spike-binding IgM and IgG, compared to placebo were observed. Although no significant compositional changes of the basal enterotype were observed, the probiotic miscellaneous may have directly interacted with the host immune system.

Moreover, a randomized, placebo-controlled, double-blind study[163] in 200 elderly volunteers showed that serum IgG, IgA and TGF-β levels were significantly higher in subjects treated with the probiotic *Loigolactobacillus coryniformis* K8 CECT 571, compared to placebo.

A mixture of multi-strain probiotics (2,400 billion bacteria/day) based on *Lactobacillus acidophilus*, *Lactobacillus helveticus*, *Lactobacillus paracasei*, *Lactobacillus plantarum*, *Lactobacillus brevis*, *Bifidobacterium lactis* and *Streptococcus thermophilus* given for seven days as adjuvant therapy to drugs administered to COVID-19 patients, hospitalized and in oxygen therapy, resulted in a significant decrease in the estimated risk of respiratory failure, modulating disease progression and mortality[157].

Recent results obtained[164] in 58 COVID-19 patients, 24 of which received oral probiotics (including *Bifidobacterium*, *Lactobacillus*, and *Streptococcus*) during hospitalization, and 34 not supplemented with oral bacteria, showed that patients taking probiotics had increased serum arginine, asparagine, and lactate, which could prevent at least in part the development of chronic fatigue by better-exercising glucose and energy pathways.

Using a homology computational modeling and molecular docking system, Nguyen et al[49] demonstrated the antiviral activity of plantaricin E and plantaricin F, metabolites of *Lactobacillus plantarum* Probio-88, against the non-structural protein helicase of SARS-CoV-2.

As secondary infections may be a serious issue in COVID-19, the specific types and schedules for probiotic use, need to be studied in future clinical trials. The formation of high-affinity bonds between the plantaricins and the viral helicase would block the binding of the ssRNA to the helicase, and thus, the penetration into the host and the viral replication.

Personalized and specific probiotic administration regimens may be necessary because empirical supplementation of probiotics may not produce desired effects on the mucosa[165]. Studies[166] have shown that 108-109 colony-forming units (CFU)/g is an ideal probiotic dose for health benefits. Yet, some strains of lactic acid bacteria modulate innate and acquired immunity when administered at a dose of 109 CFU for a day[167].

The long-term effects of probiotics are unknown and need to be studied in randomized controlled trials. Further exploration is needed into different bacterial strands to determine their effectiveness[168]. Scientists and clinicians around the world are also studying the relationship between the gut microbiome and vulnerability to SARS-CoV-2 infection and assessing the best probiotic strains for reducing viral load through different mechanisms.

The role of probiotics in restoring intestinal eubiosis and the gut microbiota profiles are summarized in Figure 2.

The reported results, particularly the ones detailing the presence of dysbiosis in patients infected with SARS-CoV-2, are mostly applicable to the first and second wave, particularly to the alpha and the delta variants. Results concerning omega variants, for instance, are not as widely available. It is possible that, given its milder symptoms, it may also be associated to milder dysbiosis, yet it is only a hypothesis.
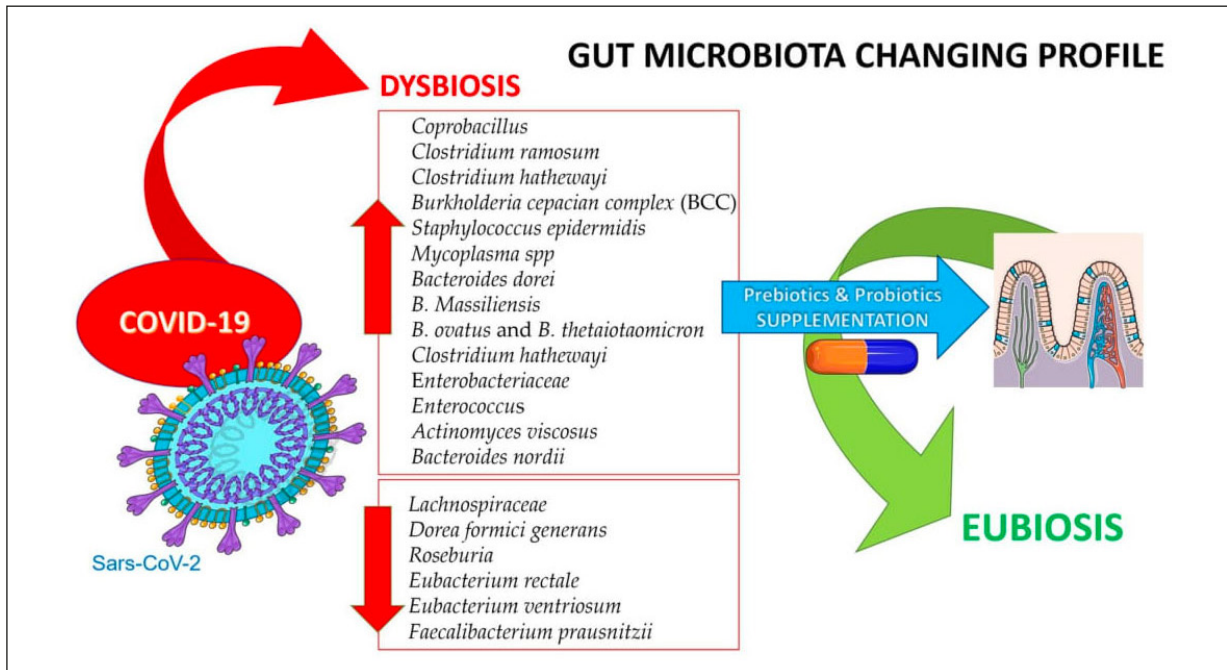
Figure 2. Gut microbiome features in patients with COVID-19. Role of probiotics in restoring intestinal eubiosis and increasing immunity. Data were collected from published literature[137-161].

Conclusions

The current review highlights the link among COVID-19 disease, microbiota, immunomodulation, and nutrients. Yet, a gap in knowledge is present. While the mechanisms of immunity in infectious diseases and SARS-CoV-2 infection are becoming more evident, very little is known about the role of immunonutrition in restoring eubiosis in COVID-19 patients. Moreover, the available evidence is not yet exhaustive in terms of viral variants and different populations. There is an important gap in evidence concerning the questions of whether different variants determine different kinds of dysbiosis, thus needing to be treated differently; whether different micronutrients may be more effective than others depending on the different variants; and, finally, whether different populations are more or less responsive to prebiotics and probiotics, and micronutrients.

Overall, in this review, we offer an overview, but further clinical studies are needed to identify the best immune nutrients, including prebiotics, probiotics, postbiotics, and polyphenols, that may be effective against dysbiosis during COVID-19 disease.

Conflicts of Interest

The authors declare no conflict of interest.

Ethics Approval

Not applicable.

Informed Consent

Not applicable.

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