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EDITORIAL

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Dear Readers,
I hope this new issue finds you all well.
Once again, we present important contributions on two topics of great relevance in the field of possible extra skeletal effects of vitamin D: an update on the relationship between vitamin D and muscle and another on its connection with asthma and with respiratory infections, which represent one of the major causes of the exacerbation of asthma; indeed, the latter topic could not but elicit references to the theme on everyone's mind today – the COVID-19 infection.
As you can see from the bibliographical selection of this issue, we deemed it correct to devote some space to the many publications on vitamin D and COVID-19. The debate currently in progress concerns two aspects in particular: is it possible that vitamin D status conditions the risk of COVID-19 infection and/or its clinical manifestations?
As you well know, the rational premises for such a connection are in place. Indeed, the state of our current knowledge allows us to summarize the following points, the first of which are general and the latter more specific:

- general:
  - *in vitro* studies have shown that vitamin D improves the innate immune response, such as the macrophage response, and can increase antiviral defense by stimulating the production of antimicrobial peptides such as cathelicidin and β-defensin;
  - observational studies have provided evidence of an association between low serum levels of 25(OH)D and susceptibility to respiratory infections;
  - a recent meta-analysis has shown that daily or weekly vitamin D supplementation significantly reduces the risk of contracting acute infections of the respiratory tracts, and in particular in subjects with vitamin D deficiency (which is not at all surprising);
  - researchers and institutes, including the AIFA, recognize that vitamin D has an "immunomodulatory" effect; in particular, vitamin D has been shown to be able to attenuate adaptive immune response, especially from cytokines (above all IL-6), by reducing the reaction of the acute post-viral phase, which when pronounced contributes to the pathogenesis of the most serious clinical manifestations of the viral infection (so-called "friendly fire" damage);
  - vitamin D supplementation in patients undergoing mechanical ventilation for various causes has been proven to be able to reduce the duration of hospitalization as well as PCR and IL6 levels;
- specific for COVID-19:
  - higher mortality in the countries of southern Europe (Italy and Spain), which are known for a greater prevalence of vitamin D deficiency, compared to those of northern Europe (Germany, Norway, Finland and Iceland) whose dietary intake of vitamin D is greater, thanks in part to the regular practice of fortifying foods;
  - greater prevalence of COVID-19 infections in the regions of northern Italy, with respect to the sunnier ones of the south or to the greater exposure to the sun of populations below the 35th parallel;
  - a particular incidence and seriousness of the infection in the elderly, in whom hypovita-

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minosis D is notoriously and historically endemic, especially in the months of winter and early spring; hypovitaminosis D is dramatically and chronically present during long-term hospitalization;
- obesity, which is often associated with hypovitaminosis D, has been shown to be a significant risk factor for COVID-19 morbidity and mortality;
- an inverse correlation between serum vitamin levels and incidence of and mortality from COVID-19;
- an inverse relationship between 25(OH) D levels and the severity of systemic inflammation and clinical manifestations in patients hospitalized for COVID-19, even if we must admit here that it is known that phlogosis by itself reduces measurable 25(OH)D levels;
- modulation on the part of vitamin D of the renin–angiotensin system and the expression of the ACE2 receptor, recognized as the entry point of the virus into human cells.

As you see, there is biological plausibility for a protective role of vitamin D with regard to the risk and/or seriousness of the clinical manifestations of COVID-19 infections.
Plausibility, however, is not enough.
Hasty and general proclamations about a protective role of vitamin D which are not supported by appropriate and specific scientific evidence have already led Italy's Ministry of Health to characterize this supposed connection as a hoax. And yet I would not immediately rule out the possibility of benefits because, conversely, we have no scientific evidence proving that vitamin D does not have an effect. While we await the results of the specific trials that are currently underway, to actually discourage safe and cost-effective vitamin D supplementation, especially in the elderly or in those forced to remain at home or in hospital, does not seem to me to be opportune during COVID-19 emergency conditions, in particular if we at least consider its recognized skeletal benefits.
Among other things, I worry about the reduction of over 30% of vitamin D supplementation observed in the first months of the year, including in the elderly, in the wake of AIFA's publication of "note 96", which ignores advanced age as a risk factor for hypovitaminosis D, not taking into consideration the progressive reduction of the skin's ability to synthesize vitamin D from the age of 60 (my age!) and the seasonal nadir. Perhaps it is just an unfortunate coincidence that these two factors played a role in the peak of the COVID-19 infection in Italy. In any case, the question presents us with another reason for worrying about the skeletal health of the elderly.
What do you think?
I hope you enjoy reading this issue.

Vitamin D and muscle

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VITAMIN D AND MUSCLE

The last decades have seen increased scientific interest in the extra skeletal effects of the vitamin D system, including its role in muscular function. The hypothesis that this seco-steroid was involved in muscle activity derives from the observation that vitamin D-deficient children affected by rickets experience serious muscular impairment, defined as "myopathia rachitica" [1]. In the first half of the last century, to better understand the pathophysiological mechanisms of such muscular damage, a theory was formulated based on the key role of alterations of phosphoric ester concentrations in muscle tissue [2]. This theory, however, did not take into account the effects on skeletal muscle of secondary hyperparathyroidism due to hypovitaminosis D. In fact, it is known that the hyperparathyroidism leads to significant muscular damage characterized by muscle atrophy due to the loss of mainly type II fibers [3]. This damage is different from pathognomonic alterations of primary myopathy, in which there is degeneration or even necrosis of muscle fibers, together with a proliferation of the endomysial connective tissue.

The discovery of vitamin D receptors (VDRs) is a milestone in the study of vitamin D pleiotropic effects [4]. At the muscular level, VDRs have been identified not only in the myoblasts, myotubes and muscle cells of animal models, but also in human myocytes [5,6]. As a consequence, vitamin D is said to act on multiple cellular components of skeletal muscle during the different stages of an individual's life, starting from embryonic development. In particular, it is involved in tissue repair after injury in all stages of life, until regressive alterations due to aging.

The effects of vitamin D on muscle tissue occur mainly by means of two mechanisms: a long-term one, which involves genomic action, and a short-term one, which entails a non-genomic mechanism [7]. The two mechanisms act in synergy, both on muscle contraction, in response to calcium intracellular fluxes (rapid response), and on muscle strength and mass (long-term response).

Through the first mechanism, vitamin D stimulates the proliferation and differentiation of muscle cells, modulating gene transcription in the myoblasts and thereby increasing synthesis of specific muscle proteins, such as myosin and the calcium-binding protein CBP. This mechanism involves a direct bond of vitamin D activated by the nuclear VDR/retinoid-X receptor (RXR) complex with specific DNA sequences, known as vitamin D response elements (VDREs), with the resulting transcription regulation. In addition to modulating calcium absorption, vitamin D regulates phosphate metabolism at the muscle level to meet structural and energetic cellular needs. Recently, Shirvani et al. [8] have shown that the genomic mechanism activated by vitamin D is proportional to the dosage of vitamin D taken as a supplement. In fact, the expression of 162 genes (86 up-regulated and 76 down-regulated) in peripheral white blood cells were influenced in normal adults who took 600 IU/day for 6 months, while the number of these genes doubles with an intake of 4,000 IU/day and even increases eightfold if the dose reaches 10,000 IU/day.

By means of the short-term mechanism, meanwhile, vitamin D regulates the calcium-dependent action of second messengers, which come into play both in the interaction between cytosol and mitochondria in order to modulate muscle energy metabolism, and in the mechanisms at the base of muscle contraction [9]. Non-genomic mechanisms, which occur following binding between vitamin D with the nuclear VDR (nVDR) and/or the membrane VDR (mVDR) associated with caveolin-1, include the activation of intracellular signal molecules such as PKC, PI3K, MAPK, CaMKII and PLA2 [10]. In its active form, calcitriol influences muscle function by acting on the dependent voltage channels SOC/TRPC3 to regulate calcium intracellular levels and consequently the excitation-contraction coupling of skeletal muscle fibers.

More recently, a third mechanism has been suggested through which vitamin D presum-

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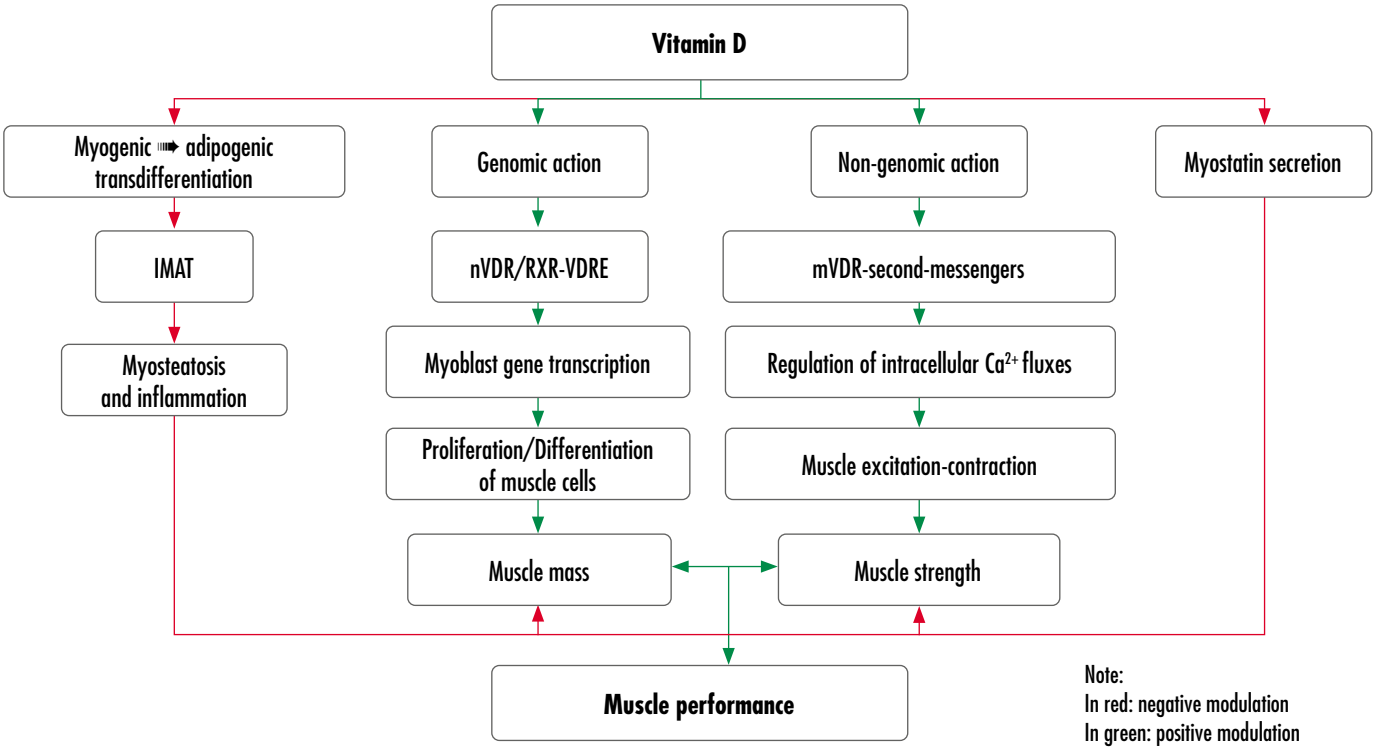

FIGURE 1.
Biological mechanisms of vitamin D effects on skeletal muscle.

ably exerts beneficial effects on muscle function, namely by inhibiting the trans-differentiation of myogenic precursors in adipocytes, thereby reducing intra- and inter-muscular adipose tissue accumulation (IMAT) [11]. *In vitro* studies on myoblasts exposed to calcitriol [12] demonstrate another of its hypothetical indirect effects: vitamin D inhibits the secretion of myostatin, a key negative regulator of muscle mass (Fig. 1).

From a clinical point of view, the evidence that vitamin D can play a key role on muscle function mainly derives from the many studies performed on the controversial association between hypovitaminosis D and risk of falls, particularly during the aging.

As it is commonly known, older people, especially when institutionalized, show reduced VDR expression in muscle and lower serum concentrations of 25(OH)D [13]. It has been suggested that vitamin D deficiency plays a key role in the age-related muscle mass loss and that muscle impairment precedes the onset of biochemical aspects of osteomalacia [14].

Hypovitaminosis D is closely associated with both the reduction of muscle mass and with the worsening of appendicular muscle strength – in particular with regard to antigravity muscles – and physical performance [15,16]. It is interesting to note that the same myopathic pattern present in rickets, with a prevalent reduction of type II fibers, is found in elderly people affected by long-term vitamin D deficiency. This pattern is also evident in sarcopenia, a disease characterized by a reduction of muscle mass and function. Such deficiency increases the risk of falls and fragility fractures [17], which in turn initiates a vicious circle of reduced mobility and autonomy and an exacerbation of hypovitaminosis D status because of fewer outdoor activities and less exposure to sunlight [18].

By means of its many receptors and enzymes ubiquitously present in the human body, the vitamin D system contributes to modulating muscle performance by regulating various functions related to physical performance, androgen synthesis, cognitive status and neuroprotection. Depletion of active vitamin D needed to carry out the organism's physiological functions triggers a series of pathological events which compromise muscle function (Fig. 2).

Although hypovitaminosis D and altered muscle functions are more common later in life, we should emphasize that vitamin D also plays a key role in the physical and cognitive performance of younger subjects. In fact, a recent study suggests the use of vitamin D supplementation in specific populations which require high-level physical and psycho-emotional performance, such as that demanded of soldiers in wartime situations. Wentz et al. [19] claim that vitamin D supplementation given to soldiers affected by hypovitaminosis D represents a noninvasive and low-cost measure for improving combat performance.

Among the many extra skeletal actions attributed to the vitamin D system, that which regards striated muscles undoubtedly represents an intriguing element in understanding the mechanisms at the basis of its complex biological function. Pre-clinical, clinical and observational studies appear to confirm a close relationship between serum 25(OH)D and muscle activity, in particular in improving functional performance and in reducing the risk of falls and disability.

At the moment, however, there is no agreement about the optimal serum levels of 25(OH)D to reach, the dosage to give as supplement, or the frequency of administration which would lead to the attainment of potential beneficial effects of vitamin D on muscle function.

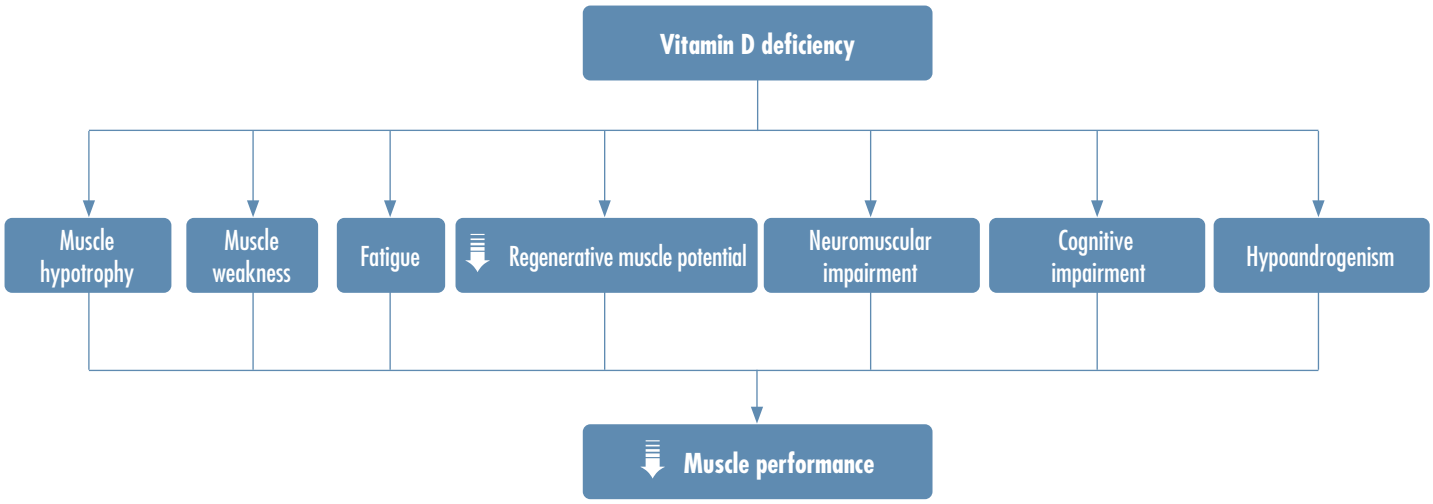

FIGURE 2.
Pathologic mechanisms of hypovitaminosis D in compromising muscle performance.

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Vitamin D and asthma

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Asthma is a complex respiratory disorder of inflammatory origin, whose onset and progression are influenced by numerous elements, such as genetic, environmental and ethnic factors as well as socio-economic conditions [1]. The characteristics of asthma vary from person to person; it is manifested by different responses to a variety of triggers and therapies. Recognizing the heterogeneity of asthma and its relative characterization aids in determining an appropriate and specific treatment therapy for each patient [2].

Different hypotheses have been put forth to explain the increasing diffusion of asthma and other allergic diseases in western countries since the 1970's: some regard hygiene and intestinal microbiota, while others propose a connection between vitamin D status and the development of asthma, wheezing, allergic rhinitis, food allergies and atopic dermatitis [3]. The International Study of Asthma and Allergies in Childhood (ISAAC) analyzed the connections between the role of westernization – a lifestyle in which we spend more time indoors with resulting vitamin D deficiency – and the increase of cases of asthma and allergies. The study found a very high incidence of asthma symptoms in countries such as the UK, Australia, New Zealand and Ireland [4,5]. Some studies carried out in several Chinese cities with different socio economic profiles have shown a prevalence of asthma and allergic symptoms in Hong Kong, the most westernized city among those analyzed [6].

In recent years, vitamin D has been considered as a new factor possibly able to inhibit inflammation of respiratory tracts by virtue of its immunomodulatory properties through the regulation of the functions of the innate and adaptive immune systems. Indeed, vitamin D deficiency has been linked to an increase of respiratory tract inflammation, to compromised pulmonary function and to an increase of exacerbations and unfavorable prognoses in patients with asthma [7,8]. In particular, interest in a possible immuno-modulatory function of vitamin D in people affected by asthma has emerged from the fact that vitamin D receptors (VDRs) are present on immune cells and on various respiratory tract tissues. VDR receptors are located on epithelial cells of the respiratory tract and on immune cells (B cells, T cells, macrophages and monocytes), where the active form of vitamin D (1,25(OH)2D3) produces its physiological effects by binding to the VDR receptors [9,10].

EFFECTS OF VITAMIN D ON THE IMMUNE SYSTEM

The most known biological functions of vitamin D are calcium homeostasis and bone metabolism. However, because the vitamin D receptor, as a member of the nuclear receptor's family, has been localized in many tissues and cells of the human body, including the antigen-presenting dendritic cells (DC), it is reasonable to speculate that vitamin D is active in many ways [11,12]. Such actions take place in the immune system, given that vitamin D plays a very precise role in the course of autoimmune diseases, in which it inhibits the response and proliferation of T helper 1 and 17 cell lymphocytes [13]. Vitamin D plays a key role in the differentiation of T regulatory cells (Treg) [14]. Several studies show the positive effects of vitamin D in pathologies that lead to a hyperactivation of T helper 1 cell lymphocytes, such as rheumatoid arthritis, multiple sclerosis and psoriasis [15].

A beneficial effect of vitamin D has been observed on the progression of those allergic diseases in which the T helper 2 lymphocytes play an essential role, regardless of the underlying pathogenic mechanisms [16]. In one study, Pichler et al. examined the effect of 1,25(OH)2D3 on naïve CD4+ T helper and CD8+ cytotoxic T lymphocytes found in cultured human cells isolated from the umbilical cord. The study found that 1,25(OH)2D3 had inhibitory effects on the production of both IFN-γ promoted by IL-12 and on the production of IL-4 and IL-13 promoted by IL-4 in naïve cells [17]. In addition, vitamin D is able to inhibit IL-17A and to prevent the conversion of CD8+ T lymphocytes from cells which pro-

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duce IFN-γ to cells which produce IL-13, as documented in asthmatic patients resistant to corticosteroids [18-20].
Regarding the possible role of vitamin D in the eosinophil immune response, it was found that those cells expressing the vitamin D receptor had longer survival rates [11]. Moreover, vitamin D reduces eosinophil necrosis and the cytolytic release of peroxidase. In addition, reduced production of immunoglobulin E (IgE) and an increase of IL-10 expression have been observed [21].
Filho et al. analyzed a non-selected population, that is, one in which allergic patients were not predominant, and found that patients affected by vitamin D deficiency had higher blood eosinophil counts. An inverse correlation of vitamin D has been observed between serum circulating levels of basophils and neutrophils [22].

GENETIC MODULATION OF VITAMIN D

The binding of vitamin D and its analogs to the VDR receptor regulates the expression of several genes associated with inflammation and immunomodulation [23] (Fig. 1). The biological effects of vitamin D can be altered by single nucleotide polymorphisms (SNPs) of the VDR gene. Specifically, many studies have suggested that SNPs in the vitamin D receptor VDR gene, such as rs2228570 (FokI), rs731236 (TaqI), rs1544410 (BsmI), and rs7975232 (ApaI), may represent risk factors for the onset of asthma. Masoud Hassanzadeh Makoui et al. observed a statistically significant correlation between the FokI and TaqI SNPs and asthma risk [24, 25]. Moreover, in the same study subgroup analysis was conducted to assess a potential ethnicity-specific effect, revealing that the presence of the FokI SNP in an African population decreases the risk of asthma below the dominant (OR = 0.60) and allelic models (OR = 0.54). The tt genotype of the TaqI SNP was associated with an increased risk of asthma in an Asian population (OR = 2.94) and with a decreased risk in American populations (OR = 0.64). This difference in outcome among ethnicities might originate from differences in dietary patterns and geographic locations, as well as from a significant influence of ethnicity on VDR gene expression and serum vitamin D levels [25,26]. Each of these four SNPs can disturb the stability of VDR mRNA, which in turn causes an imbalance between Th1 and Th2, with a resulting As a consequence, there will be a reduction in the production of IL-12 and of interferon gamma (IFN)-γ, which will lead to a production of mainly Th2 cytokines, such as IL-4 and IL-13 [25].

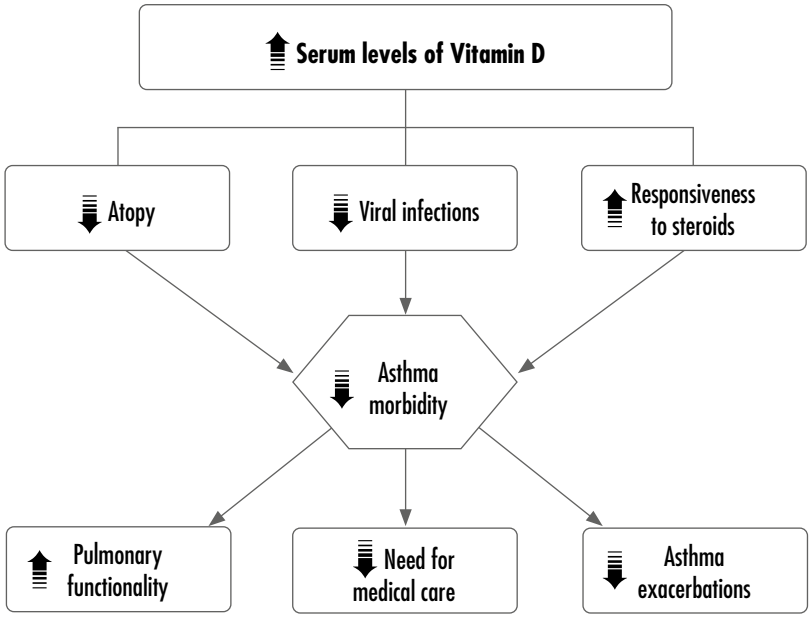

FIGURE 1.
Vitamin D and its network

ROLE OF VITAMIN D AND RESPONSE TO THERAPY IN PATIENTS WITH ASTHMA

Vitamin D may also influence the response to anti-inflammatory therapy, especially to glucocorticoids (GC), in patients with asthma [27]. Studies to evaluate the response to GC in patients with asthma suggest that up to 50% of these patients may not respond well to inhaled corticosteroids (ICS), while up to 25% of patients who have difficulty controlling asthma may not respond well to oral glucocorticoids, with high morbidity and a potentially life-threatening progression of the disease [28,29]. In patients with steroid-resistant asthma, defects in GC induced gene transcription of anti-inflammatory mediators such as IL10 and mitogen-activated protein kinase phosphatase-1 (MKP-1) may play a role [30,31].
In confirmation of this hypothesis, Xystrakis et al. have found that the addition of vitamin D and dexamethasone (Dex) to cultures of CD4+ regulatory T cells (Treg) in patients with steroid-resistant asthma increased IL-10 synthesis to levels observed in cells of steroid-sensitive patients cultured with Dex alone [30]. Zhang et al. have confirmed that vitamin D increased the GC induction of MKP-1 and IL-10 in peripheral blood mononuclear cells of children with asthma [32].

ROLE OF VITAMIN D IN COMBATTING RESPIRATORY TRACT INFECTIONS

Vitamin D also plays a crucial role in protecting against respiratory tract infections and therefore in the prevention of exacerbations of asthma. Observational studies have found a correlation between low serum concentrations of 25(OH)D and a susceptibility to acute respiratory tract infections and to exacerbations in patients with asthma [33,34]. In particular, *in vitro* studies of epithelial cell lines and of primary cultures of respiratory epithelial cells infected by a Rhinovirus-family virus demonstrate that vitamin D can increase antiviral defenses, generating an improvement of antimicrobial peptides (AMPs), such as cathelicidin and β-defensin [35,36]. Activation of innate immunity pathogen recognition receptors (PRRs) on respiratory tract epithelial cells upregulates antimicrobial peptide secretion through epithelial cells, programmed cellular death, and other intracellular responses, releasing proinflammatory mediators such as cytokines and chemokines. Vitamin D can interfere with many functions of PRRs [37,38].

Several studies report that oral supplementation of vitamin D in children suffering from recurrent respiratory tract infections (RRTIs) reduces the number of infections, consequently lessening their global socio-economic impact by assuming a prevention role [39]. Using an individual participant data (IPD) meta-analysis of randomized controlled studies, Martineau et al. found that vitamin D supplementation induces a reduction of the risk of contracting at least one acute respiratory infection. Daily or weekly vitamin D administrations brought significant benefits to those patients with serious baseline vitamin D deficiency; moreover, vitamin D administrations were also beneficial for patients with higher 25(OH)D concentrations [34]. Many studies show a correlation between vitamin D deficiency and a higher susceptibility to and greater severity of tuberculosis (TB) [40].

VITAMIN D AND EXACERBATIONS

Several meta-analyses have highlighted the role of vitamin D supplementation in reducing the rate of exacerbations, mainly in patients with vitamin D deficiency [41]. In fact, moderately acute recurrences were found after treatment with vitamin D supplementation only in patients with baseline 25(OH)D levels less than 25 nmol/L, but not in those patients with higher levels of circulating 25(OH)D [33].

Following treatment with $1,25(OH)_2D_3$, an inhibition of the production of cytokines expressed by Th 17 cells (IL-17 and IL-22) was observed in studies performed on the peripheral blood mononuclear cells (PBMCs) in patients affected by severe asthma. The importance of this finding is evident: given the inability of corticosteroids to inhibit the cytokines expressed by Th 17 cells, it suggests that vitamin D could improve the response to steroid therapy in patients with asthma [33,42].

VITAMIN D AND SARS-COV-2

In light of the protective role of vitamin D in many diseases associated with pneumonia, hypercytokinemia and acute respiratory distress syndrome (ARDS) – and therefore considering its antiviral effects that directly interfere with viral replications – it is legitimate to suppose that vitamin D could have important effects on the SARS-CoV-2 infection. Initially, the SARS-CoV-2 virus adopts mechanisms to evade immune defense, triggering immune hyper-action and a cytokine storm [43-46]. Preventive treatment with vitamin D has positive documented effects in ARDS animal models. Such effects consist in reducing pulmonary permeability, modulating the activity of the renin-angiotensin system, and reducing expression of the ACE2 receptor, which is known as the entry point of the SARS-CoV-2 virus in human cells [47,48].

Therefore, the possible use of vitamin D as adjuvant treatment or as a prophylaxis should be evaluated [49].

CONCLUSIONS

In light of the different mechanisms which are activated in respiratory diseases, the diverse pathways that can affect the individual capacity to produce adequate vitamin D concentrations at the local level, and the variability of this "beneficial" serum in each patient (including the side effects of vitamin D supplementation, such as hypercalcemia, hypercalciuria and kidney stones), the main message here is the importance of diagnosing, preventing and treating vitamin D deficiency. These observations should encourage us to view vitamin D not so much as a "universal" and independent factor for asthma, but as an important "regulator" of our immune system.

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