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RESEARCH ARTICLE

Alemtuzumab-Induced Secondary Autoimmune Skin Disorders as Side Effects of Multiple Sclerosis Therapy: Problem That Can Be Influenced or Fate? A Narrative Overview of The Literature
A Neuroprotective Role of Vitamin D?

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ABSTRACT
At present, no cure for multiple sclerosis can be made possible with the pharmaceuticals available, nor can a gradual progression of multiple sclerosis be prevented. In the management of multiple sclerosis, it is therefore necessary to determine existing autoimmune diseases through anamnesis before starting alemtuzumab therapy with disease modifying therapy. With previously existing autoimmune diseases or as prophylaxis, comorbidities can be influenced by vitamin D supplementation. Secured pathophysiological and immunological mechanisms of vitamin D on autoimmunological processes support the daily, individually high-dose oral vitamin D intake. This add-on therapy should positively influence the quality of life of persons with multiple sclerosis in the long term. So that the patients do not reject the highly efficient multiple sclerosis therapy for fear of side effects, this therapy offer is a way of avoiding side effects of alemtuzumab administration, such as alopecia, vitiligo, and thyroid diseases, and of decisively influencing the course of MS. This increases the compliance of the persons with multiple sclerosis in therapy. The pathophysiological mechanisms of alopecia and vitiligo are shown. The immunological mechanisms of vitamin D and its influence on autoimmune diseases are described. The previous use of vitamin D in the therapy of these skin diseases is presented and oral supplementation is put up for discussion. The additional neuroprotective effect is evident. Preventing infection with oral vitamin D supplements, particularly in coronavirus disease 2019, may prevent or mitigate multiple sclerosis exacerbations.

Keywords: Multiple sclerosis, alemtuzumab, autoimmune adverse effects, alopecia, vitiligo, vitamin D supplementation, SARS-CoV-2 infection, neuroprotection

Introduction

Multiple sclerosis (MS) is a complex, autoimmune-mediated central nervous system disease characterized by inflammatory demyelination and axonal/neuronal damage [1]. There is an international (unspoken) consensus that healthcare providers have an ethical obligation to attempt to prevent or alleviate drug-induced secondary autoimmune diseases (SAIDs). Comorbidities affect people with multiple sclerosis (PwMS), especially psychologically if their external appearance is impaired by an additional skin disease (alopecia, vitiligo) and restrict their quality of life. It is not sufficient to educate PwMS only about secondarily induced autoimmune diseases (thyroid, kidney, blood, skin) before starting disease-modifying therapy (DMT) (e.g., alemtuzumab, anti-CD [cluster of differentiation] 52 agent). Alemtuzumab (ALEM) is a humanized monoclonal antibody against CD52 approved for the treatment of highly active relapsing remitting multiple sclerosis (RRMS). However, the high therapeutic efficacy and treatment possibility is accompanied by potentially serious adverse events, of which SAIDs are the most significant for long-term outcomes. Vitiligo is induced by antigen-specific and clonally expanded CD8+ T cells in ALEM-associated vitiligo [2]. Ruck et al. found a dominance of single CD8 T cell clones supporting direct T cell induced pathogenesis after ALEM treatment [1,2]. Alopecia areata (AA), alopecia universalis (AU), alopecia totalis (AT), and vitiligo are rare SAIDs in alemtuzumab therapy (ALEMiAD) in MS [3-6]. AA is an autoimmune disease per se [6,7]. Vitiligo is an autoimmune skin disease defined by the appearance of an achromatic macula [8]. Vitamin D (vitD) deficiency is not only a risk factor in MS, but also in alopecia and vitiligo [9]. Vit D represents a potential player in MS pathogenesis and prevention by ALEMiAD. There are also several of case reports of PmMS developing AA after treatment with daclizumab (anti-CD25-expressing T-cell monoclonal antibody) [10] and ocrelizumab (humanized anti-CD20 monoclonal antibody) [11].

Pathogenesis of alopecia

AA is a polygenic and multifactorial autoimmune disease (AIDs) involving autoreactive CD8+, CD4+, NK (natural killer cells) and pDC (plasmacytoid dendritic cells) and increased cytokine activity [12]. The target is the hair follicles [9]. An association between alopecia and MS has been described, involving poly autoimmunity and particularly autoimmune thyroiditis [5]. Both alopecia and MS share a genetic predisposition, environmental triggers, and similar pathological processes [13].

Alopecia and alemtuzumab therapy

In an overview of alopecia in PwMS treated with DMT, 117 cases with AA after ALEM could be registered. Ruiz et al. report three cases of AA and one case of AT, with one case showing autoimmune hypothyroidism [14]. Since not all cases are published by healthcare providers, the number can be expected to be higher. Because younger MS patients tend to be affected more frequently, preventive oral vitamin D supplementation (VitD Suppl.) should be discussed. This adjuvant VitD administration throughout the 48-month therapy monitoring after ALEM administration has a double benefit. The disease course of MS and alopecia are influenced. Porwal et al. describe the occurrence of poly autoimmunity (MS, two or more autoimmune diseases) [5]. Poly autoimmunity may increase morbidity due to inflammation. Interdisciplinary assessment is beneficial for PwMS [15].

Relationship between vitamin D, vitamin D receptor and alopecia

A growing body of evidence shows that VitD and its receptor (VDR) are responsible for skin homeostasis. VitD unfolds its effect via the VDR. VDR is expressed, inter alia, in epidermal keratinocytes and mesodermal papilla cells [16]. VitD regulates the immune response by, among other things, controlling T and B lymphocytes [9]. It reduces the function, differentiation of T helper 17 cells, downregulates T helper 1 cells, increases the action of Tregs cells, resulting in immune modulation [17-19]. VitD deficiency is inversely correlated with disease severity and duration [9,20-22].

Genetic vitD receptor (VDR) mutations may result in an alteration of the effects produced by the binding of the receptor with $1,25(OH)_2D_3$ in the promoter regions of genes that respond to vitD. Low VitD status and VDR polymorphism are considered important environmental risk factors in the development of autoimmune diseases [23].

Decreased expression of VDRs in alopecia areata lesions ultimately leads to inhibition of proliferation and differentiation of hair follicles and epidermal cells [9]. VDR expression is reduced in AA and histologically inversely correlates with inflammation [22].

Role of VitD in the pathogenesis of alopecia areata

Lin et al. are currently showing the connections between vitD and AA. VitD plays a role in the pathogenesis of AA-related

a) Loss of immune privilege in the hair follicle

b) Autoreactive effector T cells and mast cells
c) nature killer group 2- member d-positive cytotoxic T cells
d) Janus kinase/signal transducer and activators of the transcriptional pathway
e) Regulatory T cells
f) Immune checkpoints ,
g) Oxidative stress [20].

VitD deficiency is a risk factor not only in alopecia and vitiligo, but also in MS [9]. VitD deficiency has been associated with the risk of MS, disease activity and progression [24,25].

Way of a VitD therapy in alopecia: a current and future challenge

Vitamin D status

The circulating concentration of 25(OH)D3 is generally accepted as the best marker for vitD status. The point of contention is the definition of vitD deficiency worldwide. The current National Institute of Clinical Excellence (NICE) guidelines do not define an appropriate VitD status in different AIDs [26]. A major deficit is that there is no international agreement on disease-specific standard values for autoimmune diseases, such as AU. This makes it difficult to compare the large number of studies.

Although vitD deficiency is not the sole etiologic factor in AA pathogenesis; such a deficiency exacerbates the severity of disease, and it is therefore of great advantage to measure serum 25(OH)D levels [27]. Monitoring over time is essential. Mean serum 25(OH)D concentrations were significantly lower in AA than in controls [28].

Although there is no internationally standardized definition of vitD deficiency, [heterogeneous limit values, e.g., vit D deficiency: (< 30ng/mL); and was further categorized into mild: (>21-29ng/mL), moderate (>12-20ng/mL) or severe (≤12ng/mL)] [29], a vitD deficiency was verified in 64.8%. (14.25% severe deficiency, 17.60% moderate deficiency, 32.96% mild deficiency) [29].

In a cross-section study by Lizarondo et al. 34% of patients with AA had vitD deficiency (25(OH)-D levels < 20ng/mL). In this study with 29 patients, the 25(OH)D levels were classified: Deficiency; <20ng/mL; Insufficiency: 21-29ng/mL; Sufficiency: 30-100ng/mL [30].

In a study by Lee et al. 1255 AA subjects had mean 25(OH)D levels 8.5ng/mL lower than controls. The AA patients had a higher probability of vitD deficiency (<20-30ng/mL). The prevalence was 73.8% [21].

In pediatric alopecia areata, the mean 25(OH)D level in 20 patients was 15.47±7.66ng/mL [27]. Decreased serum 25(OH)D levels in AA in comparison to healthy subjects were reported in 8 studies [16].

Conversion of 25(OH)D serum values ng/ml into nmol/l: multiply by a factor of 2.5

Previous therapy strategies

So far, topical application of vitD preparations (calcitriol, paricalcitol, calcipotriol) has been the preferred alternative treatment for AA [17,20,31-33]. The efficiency of oral vitD supplementation (vitD-suppl.) or its analogs is discussed controversially and there are no standard studies that evaluate the oral vitD therapy form. Studies have shown that oral vitD therapy in patients from alopecia has a promising therapeutic effect [29,34,35]. Conic et al. gave their patients in scarring and nonscarring alopecia 50,000IU once weekly for 12 weeks followed by 2000IU daily [29]. Sattar et al. prescribed 200,000IU vitD once every 2 weeks for 3 months with a total of 6 doses and there was a significant improvement in hair growth in diffuse hair loss (telogen effluvium) [35]. Daroach et al. prescribed 60,000IU once weekly for VitD-deficient AA cholecalciferol 12 weeks and registered clinical improvement and VDR upregulation [17,22].

Impressive case reports demonstrate the influence of increased vitD levels on the course of alopecia. Harvey et al. was able to record an excellent therapeutic success by supplying VitD through an optimal diet in an 8-year-old boy with alopecia areata. The intake of vitD requires a high-fat diet. This confirms that a 25(OH)D level > 40ng/mL was also optimal for this child. 5 months after the start of the dietary measures, scalp hair was restored, and eyelashes and eyebrows grew back [36]. In three girls aged 1-5 years, one girl was diagnosed with alopecia totalis (AT), the second girl with alopecia universalis (AU) and the third girl with alopecia focalis (AF) [17]. The cases with AU and AT were treated after 2 years of therapy failure with all available local and systemic pharmaceuticals with cholecalciferol 2000, 4000IU/day and oral calcitriol and its analogue paricalcitol. Success came within 6 months and lasted for years. The third 5-year-old girl with AF received a daily oral high dose of cholecalciferol (8000IU/die) and had complete restitutio ad integrum within 3 months. All three girls were VitD deficient with 25(OH)D levels of 17, 20 and 24ng/mL [17].

A 32-year-old woman with PwMS developed Hashimoto's thyroiditis 6 months later after the second dose of ALEM, followed by AU. Remarkably, a decrease in 25(OH)D levels from 69ng/mL to 8ng/mL was registered before manifestation. The vitD-suppl was given weekly until the onset of the skin disease (dose level not known). After increasing the dose up to 5000 IU/day, the serum vitD levels normalized [37].
At AU developed in a 36-year-old woman after the second ALEM cycle. Temporarily increased TSH levels were registered. The vitD suppl. was subsequently sporadically, the 25(OH)D levels were never tested. Topical therapy with Pimecrolimus and Beclomethasone was tried without success and no hair regrowth had occurred by 26 months [37].

Alemtuzumab induces vitiligo

Reduced 25(OH)D levels have been largely reported in vitiligo, which is an autoimmune skin disorder characterized by the appearance of achromic macules [38]. Active vitiligo foci were observed in patients with 25(OH)D values ≤20ng/mL (deficiency) and 25(OH)D levels of 21-29ng/mL(insufficiency). Patients with vitiligo would reach after 6 months of vitD suppl. 25(OH)D3 level values between 30-100ng/mL, the diseases showed a stable course and a significantly higher repigmentation process [8]. A meta-analysis showed significantly decreased vitD level in vitiligo, as well as in alopecia areata and chronic spontaneous urticaria [39]. There are correlations between vitD levels and interleukin-21 (IL-21) in vitiligo [2]. A significant 25(OH) D deficit was found in patients with vitiligo registered at significantly higher levels of IL-21 [40]. IL-17 was significantly higher in vitiligo patients who had lower 25(OH)D levels [41]. In the two cases described, hypothyroidism, Grave's disease and immunological skin disease occurred simultaneously (Poly autoimmunity) [14].

Gender-specific effects of vitD in autoimmune diseases

VitD plays a different role in women and men. VitD insufficiency has been linked to autoimmune disorders that commonly display significant differences between females and males due to genetic, epigenetic, hormonal, and environmental factors. Notably, estrogen has been demonstrated to enhance VitD function favoring its accumulation, and increasing the expression of VDR, thus resulting in a more potent anti-inflammatory response in females than males [42]. A higher protective effect of vitD-based therapeutic approaches in women, at least in fertile age, than in men, can be assume [42].
So far, the sex-related 25(OH)D levels in AA have been assessed very differently [16].

Discussion

VitD can have a major impact on the development of autoimmune diseases (AIDs) in a disease-prone genotype. CD4+ T cells are involved in the pathogenesis of AIDs. Paracrine calcitriol signaling to CD+4 T cells is probably the main pathway to AIDs. The VitD suppl. dampens pathogenic Th 17 cell-IL-17 synthesis, increases the sensitivity of effector CD4+ T cells to extrinsic cell death signals, and promotes CD4+CD25+Fox P3+Treg cell and CD4+IL-10+FoxP3−Tr1 cell development [43]. A highly dosed VitD suppl. reduces IL-17-producing CD4+ T cells and effector memory CD4+ T cells in PwMS when they have a significant increase in 25(OH)D levels [44]. If 1,25-dihydroxyvitamin D3 lowers the pro-inflammatory cytokines IL-17 and IL-21, [43,45-47] therapy with VitD is indicated for ALEM therapy. VitD suppl. (2000IU/die) for five years reduced AIDs by 22%. If there was a reduction in AIDs in this large primary prevention study (25,000 older adults), PwMS should not be deprived of this add-on therapy, especially since there are no other effective treatments to reduce the occurrence of other AIDs in the sense of poly autoimmunity [48].

Elevated interleukin-21 (IL-21) is commonly found in AIDs. CD4 T cells are the main producer of IL-21. IL-21 acts through the IL-21 receptor on B cells and CD8 T cells [49]. ALEMiAD occurs in up to 48% with a peak after ALEM cycles in 2-3 years. IL-21 serum levels correlate with secondary autoimmunity after ALEM and IL-21 is the driver for antibody-mediated autoimmunity [2,50]. Linked genetic risk factors and genetic associations exist for MS and vitiligo [50]. This proimmune background leads to polyimmunity such as MS, Alopecia areata, vitiligo and thyroid diseases.
$1,25\text{-}(OH)_2D_3$ inhibits the production of pro-inflammatory cytokines such as IL-17 and IL-21 [51]. Thyroid autoimmune diseases (AITD) are the most common ALEMiAD. It could be shown that patients with low 25(OH)D levels had high IL-21 levels. VitD suppl. could inhibit expression levels of thyroid antibodies and IL-21 [52].

VitD can only unfold its complex immunomodulatory effects if the dose-response relationship is observed. Both for PwMS and adjuvant VitD suppl. as well as against ALEM-induced autoimmune diseases (ALEM-iAD),

pharmacological doses are necessary to achieve long-term success. The benefit of VitD suppl. can only be achieved with a dose of 5000-10,000IU/day and a serum target from above 40-60ng/ml (up to 100ng/ml) .25(OH)D influence MS as well as autoimmune disease. VitD doses up to 10,000 IU/die are safe and tolerable. The daily vitD dose is determined by the inter-individual differences in response to the vitD suppl. PwMS have a diminished serological response to vitD suppl. compared to healthy controls [53,54]. Single nucleotide polymorphisms (SNPs) in genes coding for molecules involved in vitD metabolism have been associated with an increased risk of developing MS [55]. One of many causes may be 4 SNPs associated with 25(OH)D e.g., CYP(Cytochrome) 27B1 variants with loss of function or a CYP24A1 gene variant resulting in a low 1,25dihydroxyvitamin D level [56]. These VitD-related SNPs influence the serological response to high-dose vitD suppl. [57]. In the long-term care of PwMS, the disposition of poly autoimmunity should be pointed out. The case report of a 27-year-old man with MS demonstrates the comorbidity of vitiligo and alopecia areata. [3] Autoimmune thyroid diseases are the most common ALEM-iAD [58]. Depending on the examination criteria, the frequency is given as between 26.4% and 40% [59,60,61].

Dual therapy goal through VitD supplementation

A sufficient VitD suppl. would not only have prophylactic goals to influence ALEM-Induced secondary autoimmune diseases, rather the VitD status also has an influence on the MS risk and course [25]. Low levels of circulating 25(OH)D levels are a widely replicated risk factor for both pathogenesis and disease progression [57]. Serum 25(OH)D is significantly associated with the risk of recurrence after the onset of MS [62]. Serum 25(OH) D levels correlated inversely with risk of relapse, inversely with CNS (central nervous system) lesions, and inversely with disability progression [63]. A vitD effect on delaying brain atrophy is discussed. There is evidence that cognitive performance after VitD suppl. with high doses (10,000 IU/die) and a achieved 25(OH)D level of 49 ± 14.6 ng/mL improves [64]. 1.25-dihydroxyvitamin D increases the efficiency of methylprednisolone pulse therapy in MS relapse. PwMS had significantly low 25(OH)D levels in steroid-resistant MS relapse [63].

The overall effect of vitD supplementation in PwMS leads to a global modulation of inflammation: it decreases the differentiation of effector T and B cells. At the same time, regulatory subgroups are promoted (details of the pathophysiology in [24].

It is hypothesized that the need for high-dose oral vitD supplementation in autoimmune diseases is due to VD resistance. The cause could lie in polymorphisms within genes that affect the vitD system. Low vitD responsiveness paves the way to AIDs. A blockade of the VDR signaling is also discussed. The pathophysiological mechanisms justify the high dose vitD therapy of AIDs [65]. High doses of vitD may compensate for inherited resistance to its biological effects [66].

A current statement by 18 MS specialist`s states that anti-inflammatory DMTs are insufficient to treat and manage smoldering MS [67]. In the MS community it has become reality that PwMS contributes to "brain health" in addition to therapy with DMTs also need alternative strategies. The inclusion of comorbidities, drug-induced disease, prophylaxis of infections and other lifestyle factors is required for neurorehabilitation [67]. An add-on therapy with oral vitD-suppl. should be included in the series of “alternative therapy strategies” because there is ample pathophysiological evidence for a benefit, particularly for the braking effect of vitD on pro-inflammatory cytokines such as IL-17 and IL-2 [51,52].

Direct action of 1,25-dihydroxyvitamin D on the blood-brain barrier (BBB)

Disruption of the blood-brain barrier and cell migration to the CNS - central problem in MS

The integrity and functionality of the CNS is maintained by the BBB and the blood-cerebrospinal fluid barrier. These two barriers are in different CNS compartments, and damage and/or activation of their various components allows for lymphocyte infiltration and subsequent neurodegeneration in MS [68,69]. BBB dysfunction is a major pathophysiological feature of MS. BBB endothelial cells express the vitamin D receptor (VDR). The active form of VitD, $1,25(OH)_2D_3$, prevents disruption of the vulnerable BBB in MS, which exists in both relapsing (RRMS) and secondary progressive (SPMS) forms [70,71]. Specifically, 25(OH)D levels were lower in SPMS than in RRMS -patients or healthy.

The protective effect of $1,25(OH)_2D_3$ on the fragile BBB in PwMS can be explained by upregulation of tight junction proteins and downregulation of adhesion molecules, and the passage of T helper cells (T_H) is slowed down as a result [70]. The migration of autoreactive CD4+ T cells into the CNS is blocked. The activation of macrophages, microglia and astrocytes is

regulated. The preventive administration of VitD is even more successful the earlier the intervention takes place, so that irreversible damage is reduced [72]. The aim of the therapy is to restore the integrity of the brain barriers and thus the CNS homeostasis [71].

$1,25(OH)_2D_3$ also makes it possible to prevent the migration of T_H cells into the CNS parenchyma in a second way. The autoimmune cells are kept in the periphery by slowing migration from the lymph nodes into the CNS parenchyma [73].

The result of the study by Grishkan et al., that the positive effect of the vitD supplement turns into the opposite when the therapy is discontinued, speaks in favor of the daily supplementation of VitD for prevention and reduction of the permeability of the BBB [73,74].

Chronic daily dosing of VitD results in a slow, sustained increase in circulating 25(OH)D that reaches a steady state after 3-4 months, while intermittent or large bolus dosing of Vit D results in a variety of appearance and disappearance rates leads. However, the two modes of dosing differ greatly in their effects on circulating levels of VitD and 25(OH)D. Daily doses of VitD result in stable circulating levels of both compounds, while weekly or longer dosing results in large fluctuations in circulating VitD, but stable concentrations of 25 (OH)D. This may be the key to the diverging results in the VitD supplementation studies with very different application schemes. Variations in Vit-D dosing regimens due to the short circulating half-life of intact vitamin D could have profound implications for clinical trial results [75]. Calcitriol directly protects the BBB [70].

Vitamin D + neuroprotection = benefit for all PwMS through multifactorial mechanisms of VitD supplementation.

Holistic management is currently gaining ground in the therapy of MS. Taking remyelination, neuroprotection and neurorestoration into account, an additional goal, namely the influencing of insidious processes, is aimed at. A growing number of studies show that VitD is crucial for the healthy functioning of neuronal signaling pathways.

VitD deficiency is associated with impaired cognitive function. VitD regulates brain plasticity. (76,77). Preliminary results in this area suggested that VitD signaling affects both the developing and adult brain [78,79]. Epidemiological data suggest that VitD has neuroprotective properties [76,80].

Optimal VitD levels in early life appear to be important to minimize the risk of neurodegenerative diseases [81-83]. Given the fact that VitD protects dopaminergic neurons by upregulating genes, numerous genes are associated with dopaminergic neuron function Studies have also shown that VitD upregulates the synthesis of several neurotrophic mediators, including nerve growth factors and neurotrophins responsible for neurite outgrowth, neuronal growth and survival, neurotransmission and synaptic plasticity are essential [76,81,84].

The neuroprotective properties of VitD act through multiple mechanisms [85]. The direct neuroprotective effect of VitD is associated with the regulation of neurotrophic factors and the reduction of oxidative stress. Only sufficient 25(OH)D serum levels (> 30ng/ml) down-regulate oxidative stress, suboptimal VD levels lead to the opposite [76]. Neurotrophic factors are critical to the differentiation, survival, and maintenance of nerve and glial cells. VitD stimulates the expression of nerve growth factor (NGF), neurotrophic factor (Brain-derived Neurotrophic Factor [BDNF]), glial cell line-derived neurotrophic factor (GDNF [Glia-Neurotrophic Factors]) and Neurotrophin-3 (NT3) and the Neurotrophin receptor $p75^{NTR}$ high [81]. A reduction in VitD-assisted neurotrophic factor expression due to deficiency may result in neurons becoming more vulnerable to vulnerability [76,80,81,84,85].

Quantitative MRI measurements (WB-MTR - [Magnetization transfer ratio of the whole brain] and normal-appearing gray matter MTR (NAGM-MTR)] and vitamin D level investigations showed in progressive MS that vit D can play a protective role for the myelin content. The 25(OH)D values are > 30ng/ml. In the MS patient group examined, the vitD levels were between 30 and 50ng/ml in over 70% [86]. Tabular overview of the practical procedure of vit D supplementation as an adjunctive therapy in all forms of MS, starting as early as possible (Table 1).

Table 1: Key points for optimizing vitamin D supplementation in multiple sclerosis

- A daily continuous oral vitD-suppl. leads to a constant 25(OH)D level, absorption is favored by a high-fat meal. A bolus administration leads to strong fluctuations in the concentrations of 25(OH)D. High circulating concentrations lead to chronic dysregulation of the activity of enzymes responsible for the synthesis and degradation of 1,25(OH)2D3. The result is reduced concentrations of this metabolite in the extra-renal tissue. The protective effect of vitD on MS and infections is reduced [105,110-112].
- For an optimal immunomodulatory effect, a mean 25(OH)D level of over 40-60ng/ml (minimum 30ng/ml, maximum 100ng/ml [up to 130ng/ml] is necessary and up to 100ng/ml is safe [45,53,64,107,113-118]. The argument that 25(OH)D levels > 80ng/ml (in animal studies) could increase MS disease activity is incorrect [119]. Exacerbation seemed to be mediated primarily by vitD induced hypercalcemia rather $1,25(OH)_2D_3$ itself because hypercalcemia induced the activation of T cells leading to the migration of activated myeloid, Th1, and Th17 cells into the CNS [63].
- The daily dose of VitD varies greatly depending on the individual response to achieve an effective 25(OH)D level. Individuals can be classified as high, mid, and low responders to vitD [120].
- PwMS have less robust responses to the vitD suppl. [115].
- An initial intake of 10,000IU/die will generally result in a sufficient 25(OH)D level in 8 weeks. The dosages vary between 5000 and 10,000IE/die when taken continuously. Calcium substitution should be strictly avoided to avoid hypercalcemia and hypercalciuria. By determining 25(OH)D serum levels, Ca and Phosphorus, initially every 3 months, later every 6 months, monitoring of calcium metabolism is ensured [115]. Hypercalcemic diseases can be ruled out based on the history (anamnesis).
- The blood-brain barrier (BBB) limits immune cell trafficking to activated T cells. Dysfunction of the blood-brain barrier is a hallmark of MS [70,121]. VitD reduced the transport of immune cells at the level of the BBB (Fundamental details on further vitD immunomodulation currently in [24]). Treatment with 1,25 $(OH)_2D_3$ prevents disruption caused by both RRMS and SPMS (secondary progressive MS) through upregulation of tight junction proteins [70].
- It is becoming increasingly clear that there is a "time window" for high-dose add-on therapy with vitD for PwMS [81]. Because the regenerative capacity of the CNS is limited, early therapeutic intervention can limit irreversible damage [81]. Adjuvant therapy given in the early phase of MS will be most effective in influencing disease severity [81, 82, 83].

Expansion of the therapeutic use of vit-D supplementation for infections, especially with SARS-COV-2

PwMS with comorbidities, severe disability, and methylprednisolone flare therapy are at higher risk of infection and increased risk of severe COVID-19 [1,87]. Therefore, developing strategies to prevent infections or to react to them in a forced manner is of eminent importance. Particular attention should be paid to those PwMS that are treated with DMTs. Infections (SARS-COV-2) can increase the symptoms of MS (pseudo relapses) or cause real relapses. [88] In the post-COVID syndrome (long-COVID), every 8th patient has symptoms after at least 3 months, e.g., paresthesia in the limbs, the MS-typical symptoms could also be exacerbated. [89] There is evidence that elevated serum levels of pro-inflammatory cytokines are associated with poorer outcome of COVID-19 disease. Neurological complications and severe courses could be caused by the cytokine storm. Interleukin-6 (IL-6) increases the permeability of the blood-brain barrier resulting in further damage to the CNS [90], which is severe in PwMS. VitD inhibits the proinflammatory pathways and inhibits the production of proinflammatory cytokines like IL-6 (TNF-α, IL-2, IFNγ). [91] One of many risk factors is a vitD deficiency. The anti-inflammatory effect of VitD as an IL-6 immunomodulator is a reason for add-on therapy with vitD. [92] Low 25(OH)D levels were inversely correlated with high IL-6 levels and were independent predictors of COVID-19 severity and mortality. [93] The early start of therapy for vitD-suppl. is crucial to influence influenza and COVID-19 infections. [94] Low 25(OH)D levels are associated with higher risk of infection, severity of COVID-19, severity of respiratory distress, length of hospital stays, mortality. Acute sequelae (long-COVID) occur more frequently [95-100]. The importance of Vit D metabolism as a potential prophylactic, immunoregulatory, and neuroprotective treatment for infections (COVID-19) is becoming more and more common in clinical

practice as part of a multitherapeutic approach. [85, 100,101]The relationship between Vit D and COVID-19 has been discussed in over 200 publications and is continuously updated. Over 85 vit D COVID-19 treatment studies demonstrate a benefit. In high-risk groups (approximately over 50 years of age, comorbidities), an optimal 25(OH)D level (40-60ng/mL) after COVID-19 vaccination can improve the immune response via complex immunological signaling pathways. [102-104] At present, there are no consensus guidelines proposing an adequate concentration of serum 25(OH)D to prevent COVID-19 or reduce its morbidity and mortality. The practical approach could be to take 10,000 IU vitD/die over 4 weeks ("saturation" of the 25(OH)D serum value) to quickly reach serum values of 40-60ng/mL. The "maintenance dose" depends individually on the achieved 25(OH)D serum levels and could be 4000-5000 IU/day (dose-response effect). [105] The highest upper daily dose of vitD would be 10,000IU. Hypercalcemia is hardly to be expected up to this dose level. [63,106,107] However, in the case of severe respiratory infections (COVID-19) or sepsis, a single oral bolus dose (100,000 to 500,000IU) or divided doses of 50,000IU are administered to ensure rapid (within 3-5 days) adequate intracellular supply of calcitrol to the to make available. [108] 25(OH)D values of at least 30ng/mL (preferred range 40-60ng/mL) are required to achieve the effectiveness of VitD-suppl. against severe infections [pathophysiological details in [109]].

To correct the vit D deficiency without acute illness, a weekly Vit D administration with 50,000IU over 8 weeks is suggested to achieve a 25(OH)D serum concentration of over 30ng/mL. These 25(OH)D levels were also achieved with a single oral dose of 200,000-600,000IU. [109]The suggestion of a single oral dose of Calcifediol (0.014mg/kg body weight) allows for rapid levels within 4 hours. [108] 1,25(OH)2D3 (calcitriol) doses are associated with marked intestinal calcium absorption, which can result in transient hypercalciuria and hypercalcemia. [109]

Update 2023- New aspects on the clinical course of MS.

The assessment of the disease course and therapy of MS focused on the categorization into RRMS (relapsing remitting MS), SPMS (secondary chronic MS) and PMS (progressive MS). Recent brain MRI scans and neuropathohistological results verified disability progression independent of relapse activity (PIRA [progression independent of relapse activity]) in some PmMS. [123] The clinical progression is insidious and can be viewed as a continuum of simultaneous different pathophysiological processes. [123] In the future, the multiple progressive mechanisms and treatment options of this insidious manifestation of PIRA should be explored in clinical practice. [123] PmMS exhibiting RRMS and PIRA showed accelerated brain atrophy because they were associated with increased diffuse neuroaxonal loss, particularly of the cerebral cortex. [123] Regardless of lesion activity, smoldering activity leads to progression. [124] In chronic active lesions (CAL), microglia/macrophage (MG/MK)-mediated inflammation occurs in 56% of PmMS. [125] These CAL, previously verified only autoptically, could be imaged by 7-tesla MRI and 3-tesla susceptibility-based MRI in vivo as non-gadolinium (Gd+) enhancing lesions with paramagnetic rim. [125] Activation of MG is the earliest biomarker of CNS inflammatory processes in MS. [126] However, MG/MK-mediated inflammation is a major impediment to remyelination and accelerates brain atrophy. [123,124]

Resident MG exhibit inflammatory and anti-inflammatory alternative phenotypes and are classified in their activation state with other subgroups into M1 (pro-inflammatory with production of IL-6, IFN-gamma, IL-23, TNF-alpha)) and M2 (anti-inflammatory, express IL-4, Il-10, IL-13) phenotype.[127,128] In contrast, quiescent MG in a stable, normal CNS produce neurotrophic factors, remove myelin debris, serve to maintain oligodendrocyte progenitor cells in the CNS, and modulate neuronal activity and synaptic organization. [127]

Because the pro-inflammatory M1 microglia phenotype is active, especially in the early stages of MS, it is biologically plausible to initiate VitD supplementation to influence the secretion of inflammatory mediators to reduce myelin damage. Increased calcitriol levels result in decreased MG activation, oxidative stress, and lower BBB permeability. [76] 1,25(OH)2D3 effectively shifts MG, especially in early-stage MS, from a pro-inflammatory M1 phenotype to a reparative M2 phenotype resulting in limitation of inflammation and demyelination [76,127]. VitD signaling in neurons promotes an anti-inflammatory state in MG. It is important for normal CNS development. [129] VitD induces anti-inflammatory molecules in primary neurons. In an early state of autoimmunity in the CNS, low levels of VitD may enhance autoimmunity. [129]

Conclusion:

In the MS community, it is becoming more and more common that the therapy of MS should not only be reduced to effective pharmaceuticals (DMTs), but also that a combination therapy (add-on therapy) potentiates the success of the therapy. 1. Secondary autoimmune diseases induced by alemtuzumab, such as alopecia and vitiligo, could be influenced or prevented by high-dose, oral, daily supplementation with vitD. 2. Vitamin deficiency has been associated with the risk of multiple sclerosis, disease activity, progression, brain atrophy and progression of the EDSS (Expanded disability status scale). The daily vitD dose is individual and it is becoming more and more common to recognize clinical normal ranges of 40-100ng/mL. The overall effect of vitD supplementation in PwMS results in a global modulation of inflammation. 3. Vitamin D is involved in neuroprotection. 4. For the prevention of acute infections, especially with COVID-19, there is sufficient evidence to reduce the risk of COVID-19 infection and to prevent severe outcomes, including mortality. Withholding this simple, inexpensive, relatively low-risk add-on therapy PwMS with quadruple therapy goals is currently difficult to justify, considering countless publications.

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References

1. Wiendl H, Gold R, Berger T, et al. Multiple Sclerosis Therapy Consensus Group (MSTCG): position statement on disease-modifying therapies for multiple sclerosis (white paper). *Ther Adv Neurol Disord*. 2021;14:1-39
2. Ruck T, Barman S, Schulte-Mecklenbeck A, et al. Aemtuzumab-induced immune phenotype and repertoire changes: implications for secondary autoimmunity. *Brain*. 2022;145(5):1711-1725
3. Alcalá C, Pzére-Miralles F, Gascón F, et al. Recurrent and universal alopecia areata following alemtuzumab treatment in multiple sclerosis: A secondary autoimmune disease. *Mult Scler*. 2019;27;406-408
4. Dikeoulia E, Neufeld M, Pawlitzki M, Böhm M. Alemtuzumab-induced Alopecia areata-a case report and systematic literature review of adverse events associated with alemtuzumab. *JDDG*. 2021;19(8):1159-1164
5. Porwal MH, Salter A, Patel D, Obeidat AZ. Alopecia in Multiple Sclerosis patients treated with Disease Modifying Therapies. *J Cent Nerv Syst Diseas*. 2022;14:1-10
6. Chan JK, Traboulsee AL, Sayao A-L. Case of alemtuzumab-related alopecia areata management in MS. *Neurol Neuroimmunol Neuroinflamm*. 2019:6(1):e516
7. Rajabi F, Abdullahimajd F, Jabalameli N, Nasiri Kashani M. Firooz A. Immunogenetics of Alopecia Areata. Adv in Exp Med Biol. 2022,1367:19-59,
8. Colucci R, Conti R, Dragoni F et al. Evidence of a possible therapeutic role of vitamin D in a cohort of adult Caucasian vitiligo patients. *Int J Vitam Nutr Res*. 2020; 90(3-4):200-204
9. Siddappa H, Kumar YHK, Vivekananda N. Evaluation of Association of Vitamin D in Alopecia Areata: A Case-control Study of 100 patients in a Tertiary Rural Hospital of Southern India. *Indian Dermatol Online J*. 2019:10(1):45-49
10. Oh J, Saidha S, Cortese I, et al. Daclizumab-induced adverse events in multiple organ systems in multiple sclerosis. *Neurology*. 2014;18;82(11):984-988
11. Chin LD, AbuHilal M, Ocrelizumab-induced alopecia areata-A series of five patients from Ontario, Canada: A case report. *SAGE Open Med Case Rep*. 2020; 8:2050313X20919614
12. Simakou T, Butcher JP, Reid St, Henriquez FL. Alopecia areata- Multifactor autoimmune disease, *J Autoimmun*. 2019:98, 74-85
13. Rossi A, Muscianese M, Federico A et al. Associations between alopecia areata and multiple sclerosis: A report of two cases and review of the literature. *Int J Dermatol*. 2020;59(4):490-493
14. Ruiz RL, Fernàndez, Ruiz de Arcos M, et al. Skin autoimmunity Secondary to Alemtuzumab in a Tertiary Care Spanish Hospital. *Neurology Clin Prac*. 2022;12(1):29-35
15. Bliddal S, Nielsen CH, Feldt-Rasmussen U. Recent advances in understanding autoimmune thyroid disease: the tallest tree in the forest of polyautoimmunity. *F1000Res*. 2017:6:1776
16. Gerkowicz A, Chyl-Surdacka K, Krasowska D, Chodorowska G. The Role of Vitamin D in

Non-Scarring alopecia. *Int J Mol Sci*. 2017;18(12):2653
17. Papadimitriou DT, Bothou Ch, Dermitzake E, Alexopoulos A, Masstorakos G. Treatment of alopecia totalis/universalis/focalis with vitamin D and analogs: Three case reports and a literature review. *World J Clin Pediatr*. 2021;10(6):192-199
18. da Costa DS, Hygino J, Ferreira TB, et al. Vitamin D modulates different IL-17-secreting T cell subsets in multiple sclerosis patients. J *Neuroimmunol*. 2016; 299:8-18
19. Tsai, TY, Huang Y-C. Vitamin D deficiency in patients with alopecia areata: a systematic review and meta-analysis. *J Am Acad Dermatol*. 2018;78(1):207-209
20. Lin X, Meng X, Song Z. Vitamin D and alopecia areata: possible roles in pathogenesis and potential implications for therapy. *Am J Transl Res*. 2019;11(9):5285-5300
21. Lee S, Kim BJ, Lee CH, Lee WS. Increased prevalence of vitamin D deficiency in patients with alopecia areata: a systematic review and meta-analyses. *J Eur Acad Dermatol Venerol*. 2018;32(7):1214-21
22. Daroach M, Narang T, Saikia UN, Sachdeva N, Kumaran MS. Correlation of vitamin D and vit D receptor expression in patients with alopecia areata: a clinical paradigm. *Int J Dermatol*. 2018;57(2):217-222
23. Bizzaro G, Antico A, Fortunato A, Bizzaro N. Vitamin D and Autoimmune Diseases: Is Vitamin D Receptor (VDR) Polymorphism the Culprit. *Isr Med Assoc*. 2017:19(7):438-443
24. Galoppin M, Kari S, Doldati S, et al. Full spectrum of vitamin D immunomodulation in multiple sclerosis: mechanisms and therapeutic implications. *Brain*. 2022;4(4), fcac171.
25. Pierrot-Deseilligny Ch, Souberbielle J-C. Vitamin D and multiple sclerosis: An update. *Mult Scler Rel Disord*. 2017; 14:35-45
26. Harrison StR, Li D, Jeffery LE, Raza K, Hewison M. Vitamin D, autoimmune Disease and Rheumatoid Arthritis. *Calcif Tissue Int*. 2020;106(1):58-75
27. Unal M, Gonulalan G. Serum vitamin D level is related to disease severity in pediatric alopecia areata. *J Cosmet Dermatol*. 2018:17(1):101-104
28. Gao Y, Huo S; Sun M, et al. Evaluation of several immune and inflammatory indicators and their association with alopecia areata. *J Cosmet Dermatol*. 2022;7,2995-3001
29. Conic RRZ, Piliang M, Bergfeld W, Atanaskova-Mesinkovska N. Vitamin D status in scarring and nonscarring alopecia. *Am Acad Dermatol*. 2021;85(2): 478-480
30. Lizarondo FPF, Gervasio MKR, Chamberlin ChVS, Gnilo CMS, Silva CY. Determination of serum 25-hydroxyvitamin D levels in patients with alopecia areata and their comparison with levels in healthy controls: a cross-sectional study. *JAAD International*. 2021;5:78-84
31. Cerman AA, Solak SS, Altunay L, Kücükünal NA. Topical Calcipotriol Therapy for Mild-to-Moderate Alopecia Areata: A Retrospective Study. *J Drugs Dermatol*. 2015;14(6):616-20
32. Molinelli E, Campanati A, Brisigotti V, Sapigni C, Paolinelli M. Offidani A. Efficacy and Safety of Topical Calcipotriol 0.005% Versus Topical clobetasol 0.05% in the Management of Alopecia Areata: An Intrasubject Pilot Study. *Dermatol Ther. (Heidelb.)*. 2020;10(3): 515-521
33. Narang T, Daroach M, Kumaran MS. Efficacy and safety of topical calcipotriol in management of alopecia areata: A pilot study. *Dermatol Ther*. 2017;30(3): e122464
34. Afvari S, Kazlouskaya M, Cline A. Reply to "Vitamin D status in scarring and nonscarring alopecia". *JAAD online*, 2022;87(2): E89-E90
35. Sattar F, Almas U, Ibrahim NA, Akhtar A, Shazad MK, Akram S et al. Efficacy of oral Vitmain D3 Therapy in Patients suffering from Diffuse Hair Loss (Telogen Effluvium). *J Nutr Sci Vitaminol*. 2021;67:68-71
36. Harvey C. Combined Diet and Supplementation Therapy Resolves Alopecia Areata in a Paediatric patient: A Case Study. *Cureus*. 2020;12(11):e11371
37. Boriello G, Ianiello A, Toosy AT. Alopecia Universalis Occuring after Alemtuzumab Treatment for Multiple Sclerosis. A Two-Year Follow-Up of Two Patients. *Int J Environ Res Public Health*. 2021;18(14):7338
38. Varikasuvu SR, Aloori S, Varshney, S, Bhonir AV. Decreased circulatory levels of vitamin D in vitiligo: a meta-analysis. *A Bras Dermatol*. 2021;96(3):284-294.
39. Tuchinda P, Kulthanan K, Chularojanamontri L et al. Relationship between vitamin D and chronic spontaneous urticaria. A systematic review. *Clin Transl Allergy*. 2018;8:51
40. Amer A, Amer M, Khater EMG, Marei A M, Firjani BAA. Correlation between Vitamin D and Interleukin-21 in Patients with Vitiligo. *J Clin Dermatol Ther*. 2019;5:039 Doi.10.24966/CDT-8771/100039.
41. Aly D, Mohammed F. Sayed K, Gawdat H, Mashaly H, Hay RA et al. Is there a Relation between Vitamin d and Interleukin-17 in

Vitiligo? A Cross-Sectional Study. *Dermatology*. 2017;233(6):413-418
42. Dubuis ML, Pagano MT, Pierdominici M, Ortona E. The role of vitamin D in autoimmune diseases: could sex make the difference? *Biol Sex Differ*. 2021:12:12
43. Hayes CE, Hubler SL, Moore JR, Barta LE, Praska CE, Nashold FE. Vitamin D Actions on CD4(+) T Cells in autoimmune disease. *Front Immunol*. 2015;6:100
44. Bhargava P, Sotirchos E, Eckstein Ch, et al. High-dose vitamin D supplementation reduces IL-17 producing CD4+ T –cells and effector-memory CD4+ T-cells in multiple sclerosis patients (S38.001). *Neurology*. 2015:84 (14 Suppl.)
45. Charoenngam N, Holick MF. Immunologic Effects of Vitamin D on Human health and Disease. *Nutrients*. 2020;12(7):2097
46. Martens PJ, Gysemans C, Verstuyf A, Methieu AC. Vitamin D's Effect an immune function. *Nutrients*. 2020;12(5):1248.
47. Bishop EL, Ismailova A, Dimeloe S, Hewison M, White JH. Vitamin D and Immune Regulation: Antibacterial, Antiviral, Anti-Inflammatory. *NBMR Plus*. 2020;5(1):e10405
48. Hahn, J, Cook NR, Alexander EK, et al. Vitamin D and marine omega 3 fatty acid supplementation and incident autoimmune disease: VITAL randomized control trial. *BMJ*. 2022:376e066452
49. Ren HM, Lukacher AE, Rahman ZSM, Olsen NJ. New developments implicating IL-21 in autoimmune disease. *J Autoimmun*. 2021;122:102689
50. Ruck T, Pfeuffer S, Schulte Mecklenbeck A, et al. Vitiligo after alemtuzumab treatment: Secondary autoimmunity is not all about B cells. *Neurology*. 2018;91(24):e2233-e2237
51. Jefferey LE, Burke F, Mura M, Zheng Y, et al. 1,25-dihydroxyvitamin D3 and interleukin-2 combine to inhibit T cell production of inflammatory cytokines and promote development of regulatory T cells expressing CTLA-4 and FoxP3. *J Immunol* 2009;183(9):5458-5467
52. Feng Y, Qiu T, Chen H, et al. Association of serum IL-21 and vitamin D concentrations in Chinese children with autoimmune thyroid disease. *Clin Chim Acta*. 2020;507:194-198.
53. O'Conell K, SulaimaniJ, Basdea SA, et al. Effects of vitamin D3 in clinically isolated syndrome and healthy control participants: a double-blind randomized controlled trial. *Mult Scler J Exp Trans Clin*. 2017;3(3):2055217317727296.
54. Bhargava P, Steele SU, Waubant E, et al. Multiple sclerosis patients have a diminished serologic response to vitamin D supplementation compared to healthy controls. *Mult Scler*. 2016;22(6):753-760
55. Agnello L, Scazzone C, Sasso BL, et al. Role of Multiple Vitamin D-Related Polymorphisms in Multiple Sclerosis Severity: Preliminary Findings. *Genes*. 2022;13,1307
56. Malhotra S, Midaglia L, Chuquisana O, et al. The CYP24A1 Gene Variant rs2762943 is Associated with low Serum 1,25-DihydroxyvitaminD levels in Multiple Sclerosis Patients. *J Neuroinflamm*. 2021, Posted Date Dec 17th 2021.
57. Mimpen M, Rolf L, Poelmans G, et al. Vitamin D related genetic polymorphisms affect serological response to high-dose vitamin D supplementation in multiple sclerosis. *PLoS One*. 2021;2;16(12): e0261097
58. Goischke H-K. AlemtuzumabTreatment-Induced Thyroid Dysfunction in RRMS: a Varied Clinical Picture in an Interdisciplinary Terrain. *Akt Neurol*. 2017;44:1-8
59. Goischke H.K. Vitamin D supplementation for the prevention or depletion of side effects of therapy with alemtuzumab in multiple sclerosis. *Therapeutic Clin Risk Man*. 2019;15:891-904
60. Rauma I, Mustonen T, Sepp Ä JM, et al. Safety of alemtuzumab in a nationwide cohort of Finnish multiple sclerosis patients. *J Neurol*. 2022;269(2):824-853
61. Rotondi M, Molteni M, Leporati P, Capelli V, Marino M, Chiovato L. Autoimmune Thyroid diseases in Patients Treated with Alemtuzumab for Multiple Sclerosis: An Example of Selective Anti-TSH receptor Immune Response. *Front Endocrinol (Lausanne)* 2017;8:254
62. Vandebergh M, Dubois B, Goris A. Effects of Vitamin D and body Mass Index on Disease Risk and Relapse Hazard in Multiple Sclerosis: A Mendelian Randomization Study. *Neurol Neuroimmunol Neuroinflamm*. 2022;9(3)e1165
63. Miclea A, Bagnoud M, Chan A, Hoepner. A Brief Review of the Effects of Vitamin D on Multiple Sclerosis. *Front Immuno*. 2020;11:781.
64. Sintzel M. Rametta M, Redler AT. Vitamin D and Multiple Sclerosis: A Comprehensive Review. *Neurol Ther* 2018;7(1):59-85
65. Lemke D, Klement RJ, Schweiger F, Schweiger B, Spitz J. Vitamin D resistance as a possible Causa of Autoimmune disease: A Hypothesis confirmed by a therapeutic high-dose vitamin D protocol. *Front Immunol*. 2021;7,12, 655739

66. Finamor DC, Sinigaglia-Coimbra R, Neves LC, et al. A pilot study assessing the effect of prolonged administration of high daily doses of vitamin D on the clinical course of vitiligo and psoriases. *Dermatoendocrinol*. 2013;5:222-34
67. Giovannoni G, Popescu V, Wuerfel J, et al. Smouldering multiple sclerosis: the 'real MS'. *Ther Adv Neurol Disord*. 2022;15,1-8
68. Alvarez JI, Cayrol R, Prat A. Disruption of central nervous system barriers in multiple sclerosis. *Biochim Biophys Acta*. 2011;1812:252–264
69. Mapunda JA, Tibar H, Regragiu W, Engelhardt B. How Does the Immune System Enter the Brain? *Front Immunol*. 2022;13:805657
70. Takahashi S, Maeda T, Sano Y, Nishihara H, Takeshita Y, Shimizu F et al. Active form of vitamin D directly protects the blood-brain barrier in multiple sclerosis. *Clin Exp Neuroimmunol*. 2017;8(3):244-254. Doi.org/101111/cen3.12398
71. Nishihara H, Engelhardt B. Brain Barriers, and Multiple Sclerosis: Novel Treatment Approaches from a Brain Barriers Perspective. *Handb Exp Pharmacol*. 2022:273:295-329
72. De Oliveira et al. Calcitriol Prevents Neuroinflammation and Reduces Blood-Brain Barrier Disruption and Local Macrophage/Microglia Activation. *Front Pharmacol* 2020;11:161. Doi. 10.3389/fphar.2020.00161
73. Grishkan IV, Fairchild AN, Calabresi PA and Gocke AR. 1,25-Dihydroxyvitamin D3 selectively and reversibly impairs T helper-cell CNS localization. *PNAS*. 2013;110(52):21 101-21 106. Doi.org/10.1073/pnas.1306072110
74. Cantorna MT, Hayes CE, DeLuca HF. 1,25-Dihydroxyvitamin D3 reversibly blocks the progression of relapsing encephalomyelitis, a model of multiple sclerosis. *Proc Natl Acad Sci USA* 1996;93(15):7861-4 doi: 10.1073/pnas.93.15.7861.
75. Hollis BW, Wagner CL. The Role of the Parent Compound Vitamin D with Respect to Metabolism and Function: Why Clinical Dose Intervals Can Affect Clinical Outcomes. *J Clin Endocrinol Metab*. 2013;98(12):4619-28 Doi.org/10.1210/jc.2013-2653
76. Gombash SE, Lee PW, Sawdai E, Lovett-Racke EE. Vitamin D as a Risk Factor for Multiple Sclerosis: Immunoregulatory or Neuroprotective? *Front Neurol*. 2022;13:796933
77. Mayne PE, Burne THJ. Vitamin D in Synaptic Plasticity, Cognitive Function, and Neuropsychiatric Illness. *Trends Neurosci*. *2019*;42(4).293-306
78. Holick MF. Vitamin D and brain health: the need for vitamin D supplementation and sensible sun exposure. *J Intern Med*. 2015;277(1)90-3
79. Eyles DW. Vitamin D: Brain and Behavior. *JBMR Plus*. 2020;5(1)/e10419
80. AlJohri R, AlOkai M, Haq SH. Neuroprotective role of vitamin D in primary neuronal cortical culture. *Neurological Sci*. 2019;14:43-48, doi. 10.1016/j.ensci.2018.12.004
81. Zorzella-Pezavento SFG, Mimura LAN, Denadai MB et al. Is there a window of opportunity for the therapeutic use of vitamin D in multiple sclerosis? *Neurol. Regeneration Research*. 2022;17(9):1945-1954, DOI: 10.4103/1673-5374.335139
82. Boltjes R, Knippenberg S, Gerlach O, Hupperts R, Damoiseaux J. Vitamin D supplementation in multiple sclerosis: An expert opinion based on the review of current evidence. *Expert Rev Neurother*. 2021;21(6):715-725
83. Smolders J, Torkildsen O, Camu W, Holmoy T. An Update on vitamin D and disease Activity in Multiple Sclerosis. *CNS Drugs*. 2019;33(12):1187-1199
84. García Menéndez S, Giménez VMM, Holick MF, Barrantes FJ, and Manucha W. COVID-19 and neurological sequelae: Vitamin D as a possible neuroprotective and/or neuroreparative agent. *Life Sci*. 2022 May 15; 297: 120464.Published online 2022 Mar 7. doi: 10.1016/j.lfs.2022.120464
85. Xu Y, Baylink DJ, Chen CH-S, et al. The importance of vitamin D metabolism as a potential prophylactic, immunregulatory and neutroprotective treatment for COVID-19. *J Transl Med* 2020;18:322
86. Abbatemarco JR, Fox RJ, Li H, and Ontaneda D. Vitamin D and MRI Measures in Progressive Multiple Sclerosis. Mult Scler Relat Disord. 2019; 35: 276–282. Doi.10.1016/j.msard.2019.08.014
87. Longinetti E, Bower H, McKay KA, Englund S, Burman J, Fink K et al. COVID-19 clinical outcomes and DMT of MS patients and population-based controls. ACTN 2022, First publ. 22 August
88. Cauchi M, Willis M, Adrews A, et al. Multiple sclerosis, and the risk of infection: association of British Neurologists consensus guideline. *Pract Neurol*. 2022

89. Ballering AV, van Zon SKR, Hartman TC, Rosmalen J GM et al. Persistence of somatic symptoms after COVID-19 in the Netherland. an observational cohort studies. *Lancet*. 2022;400:452-61
90. Gerhard A, Prüß H, Franke C. Manifestations of the central nervous system after COVID-19. *Nervenarzt*. 2022;93:769-778
91. Orry B, Szekeres-Bartho J, Bizzarri M, Spiga AM, Unfer V. Inhibitory effects of Vitamin D on inflammation and IL-6 release. A further support for COVID-19. *Eur Rev Med Pharmacol Sci*. 2020;24:8187-8193
92. Silberstein M. COVID-19 and IL-6: Why vitamin D (probably) helps but tocilizumab might not. *Eur J Pharmacol*. 2021;899:174031
93. Campi I, Gennari L, Merlotti D, et al. Vitamin D and COVID-19 severity and related mortality: a prospective study in Italy. *BMC Infectious Diseases*. 2021;21:566
94. Malaguanera L. Vitamin D3 as potential treatment adjuncts for CORONA-19. *Nutrients*. 2020; 12(11) 3512
95. Gönen MS, Alaylioglu M, Durcan E, et al. Rapid and Effective Vitamin D Supplementation May Present Better Clinical Outcomes in COVID-19 (SARS-COV-2) Patients by Altering Serum INOS1, IL1B, IFNg, Cathelicidin-LL37, and ICAM1. *Nutrients*. 2021;13(11):4044
96. Garjani A, Middleton RM, Nicholas R, Evangelou N. Recovery From COVID-19 in Multiple Sclerosis. *Neurology: Neuroimmunology and Neuroinflammation* 2022;9,e1118
97. Glinsky, G. Tripartite Combination of Candidate Pandemic Mitigation Agents: Vitamin D, Quercetin, and Estradiol Manifest Properties of Medicinal Agents for Targeted Mitigation of the COVID-19 Pandemic Defined by Genomics-Guided Tracing of SARS-CoV-2 Targets in Human Cells. *Biomedicines*. 2020; 8(5):129
98. Chiodini I, Gatti D, Soranna D, Merlotti D, Mingiano Ch, Fassio A et al. Vitamin D Status and SARS-CoV-2 Infection and COVID-19 Clinical Outcomes. *Front Public Health*. 2021;9:736665
99. Petrelli F, Luciani A, Perego G, et al. Therapeutic and prognostic role of vitamin D for COVID-19 infection: A systematic review and meta-analyses of 43 observational studies. *J Steroid Biochem Mol Biol*. 2021;211:105883
100. Fiorino S, Zippi M, Gallo C, et al. The rationale for a multi-step therapeutic approach based on antivirals, drugs and nutrients with immunomodulatory activity in patients with coronavirus-SARS2-induced disease of different severities. *Br J Nutr*. 2020; online 24 July 2020
101. Peng M-Y, Liu W-Ch, Zheng J-Q, et al. Immunological Aspects of SASRS-CoV-2 Infection and the Putative Beneficial Role of Vitamin-D. *Int J Mol Sci*. 2021;22(10):5251
102. Chiu SK. Putative Role of Vitamin D for COVID-19 Vaccination. *Int Sci. J Mol*. 2021:22(16):8988
103. Goncalves-Mendes N, Talvas J, Dualè C, et al. Impact of Vitamin D Supplementation on Influenza Vaccine Response and Immune Functions in Deficient Elderly Persons. *Front Immunol*. 08 Febr 2019
104. Chillon TS, Demircan K, Heller RA, et al. Relationship between Vitamin D Status and Antibody Response to COVID-19 mRNA Vaccination in Healthy Adults. *Biomedicines*,18 Nov. 2021
105. Grant WB, Lahore H, McDonell SL, et al. Evidence that Vitamin D Supplementation Could Reduce Risk of Influenza and COVID-19 Infections and Death. *Nutrients*. 2020;12(4):988
106. Holick MF. The vitamin D deficiency pandemic: approaches for diagnosis, treatment, and prevention. *Rev Endocr Metab Disord*. 2017;18:153-65
107. Dobson R, Cock HR, Brex P, Giovannoni G. Vitamin D supplementation. *Pract. Neurol*. 2018;18:35-42
108. Wimalawansa S. Rapidly Increasing Serum 25(OH)D Boosts the Immune System, against Infections-Sepsis and COVID-19. *Nutrients*. 2022;14(14):2997
109. Bae JH, Choe HJ, Holick MF. Lim S. Association of vitamin D status with COVID-19 and its severity: Vitamin D and COVID-19: a narrative review. *Rev Endocrine Disord* 2022;23(3):579-599
110. Martineau AR, Jolliffe DA, Greenberg L, et al. Vitamin D supplementation to prevent acute respiratory tract infections: individual participant data meta-analysis. *Health Technol Assess*. 2019;23(2):1-44
111. Helde-Frankling M, Björkhem-Bergman L. Vitamin D in Pain Management. *Int J Mol Sci*. 2017;18:2170.
112. Hollis BW, Wagner CL, The Role of the Parent Compound Vitamin D with Respect to Metabolism and Function. Why Clinical Dose Intervals Can Affect Clinical Outcomes. *J Clin Endocrinol Metab*. 2013;98:4619-4628

113. Feige J, Moser T, Bieler L. Schwenker K, Hauer L Sellner J. Vitamin d Suoolementation in Multiple Sclerosis: A Critical Analysis of Potentials and Threats. *Nutrients*. 2020;12(3):783
114. Calton EK, Keane KN, Newsholme P, Soares MJ. The impact of Vitamin D levels on Inflammatory Status: A systematic Review of Immune Cell Studies. *PLoS* One 2015;10(11):e0141770
115. Shoemaker TJ, Mowry EM. A review of vitamin D supplementation as disease-modifying therapy. *Mult Scler*. 2018;24(1).6-11
116. Wesnes K, Myhr K-M, Riise T, Kvistad SS, Torkildsen O, Wergeland S et al. Low vitamin D, but not tobacco use or high BMI, is associated with long-term disability progression in multiple sclerosis. *Mult Scler Relat Disord*. 2021;50:102801
117. Jetty V; Lueck CJ, Wang P, et al. Safety of 50,000-100,000 Units of Vitamin D3/Week in vitamin D-Deficient, Hypercholesterolemic patients with reversible Statin Intolerance. *N Am J Med Sci*. 2016;8(3):156-162
118. Sotirchhos ES, Bhargave P, Eckstein C, Van Haren K, Baynes M, Nitranos A. Safety, and immunologic effects of high- vs low-dose cholecalciferol in multiple sclerosis. *Neurology*. 2016;86(4):382-390
119. Häusler D, Torke S, Peelen E, Bertsch T, Djukic M, Nau R et al. High dose vitamin D exacerbation central nervous system autoimmunity by raising T cell excitatory calcium. *Brain*. 2019;142:2737-55
120. Carlberg C, Haq A. The concept of the personal vitamin D response index. *J Steroid Biochem Mol Biol*. J 2018;175,12-17
121. Mapunda JA, Tibar H, Regragui W, Engelhardt B. How Does the Immune System Enter the Brain? *Front Immunol*. 2022;13:805657
122. Engelhardt B, Comabella M, Chan A. Multiple sclerosis: Immunopathological heterogeneity and its implications. *Eur J Immunol*. 2022;52(6):869-881
123. Kuhlmann J. et al. Multiple sclerosis progression: time for a new mechanism – driven framework. Lancet Neurol 2022, publ. online. 18.Nov.2022
124. Cagol A et al. Association of brain atrophy with disease progression independent of relapse activity in patients with relapsing multiple sclerosis. JAMA Neurology 2022;79(7):682-692
125. Absinta M et al. Association of chronic active multiple sclerosis lesions with disability in vivo. JAMA Neurol 2019;76(12):1474-1483.
126. Spanier JA et al. Vitamin D3-mediated resistance to a multiple sclerosis model disease depends on myeloid cell 1,15-dihydroxyvitamin D3 synthesis and correlates with increased CD4+ T cell CTLA-4 expression. J Neuroimmunology 2019:338:577105
127. Radandish M et al. The role of distinct subsets of macrophages in the pathogenesis of MS and the impact of different therapeutic agents on these population. Front Immunol 2021;12:667705
128. Wang J et al. Targeting microglia and macrophages: A potential treatment strategy for multiple sclerosis. Front Pharmacol 2019;10:286
129. Lee, PW et al. Neuron-specific vitamin d signaling attenuates microglia activation and CNS autoimmunity. Front Neurol 2020;11:19