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REVIEW ARTICLE

Improving the quality of life through vitamin D supplementation in people with multiple sclerosis: Pathobiological, immunologically based

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ABSTRACT

Multiple sclerosis as a complex autoimmune-mediated disease requires exhausting all therapeutic options to slow down disease activity and to support synergistic disease-modifying therapy. Based on the known pathophysiological and immunological findings on the effect of daily vitamin D supplementation in MS, reference is to the advantages of vitamin D supplementation in the course of MS, in pregnancy, in the menopause in women, in old age, in comorbidities, on cognition, fatigue, depression, the influence on the gut microbiome and malignancies as side effects of DMTs. In addition, the different response of vitamin D in obesity, the individual reaction to VitD supplementation due to genetic changes is pointed out. There is a discussion on the lack of international agreements regarding the type and dose of vitamin D administration, the 25(OH)D serum levels to be achieved and the different results of vitamin D from studies are presented. The incorporation of vitamin D supplementation into a strategic MS treatment plan through accelerated translation of scientific knowledge into practice is a legitimate demand with the aim of stabilizing the disease or reducing disease activity. This review is intended to present the current knowledge on the experimental and clinical evidence of vitamin D supplementation in autoimmune diseases and in particular in multiple sclerosis in order to reduce the gap of a restrictive prescription in the daily practice of the neurologist.

Keywords: Multiple sclerosis, Immunopathogenese, Vitamin D supplementation, Comorbidities, Ocrelizumab, Obesity

Introduction

An inadequate vitamin D nutritional status is associated with autoimmune diseases, heart disease, deadly types of cancer, neurological disorders and undesirable consequences in pregnancy, amongother things. In a nationally representative sample of adults in Australia on vitamin D (VitD) congestion in the general population, 20% of participants were verified to be Vit D deficient and 43% to have insufficient VitD levels.[1] It is therefore to be welcomed that in 2020 a guideline on VitD supplementation for medical use was created, taking into consideration the opinions of various global institutions.[2]

For more than 15 years there have been reports that vitamin D (VitD) has an immunomodulatory function in multiple sclerosis (MS)[3] and that there is an association between low circulating serum levels of 25-hydroxyvitamin D [s25(OH)D] and an increase in inflammatory activity and impact on quality of life in persons with multiple sclerosis (PwMS).[4,5] Nevertheless, up to the present the "neurological community" has not been able to agree to start a daily add-on therapy using an easy to carry out, inexpensive and low-side effect vitamin D supplementation (VitD suppl.) from the beginning of the diagnosed MS disease or the chronically isolated syndrome (CIS). In the last decade, there has been an accumulation of evidence of immunological and pathophysiological mechanisms that VitD positively influences the course of the disease and should become an eminent factor in therapy.[6] For 13 years, neurologists in Sweden have been informing PwMS about the benefits of VitD-suppl. and given a recommendation for add-on therapy.[7]

Since 2018, the Multiple Sclerosis Society of Canada has recommended for PwMS a serum 25(OH)D level of 20-50mg/mL (50-125nmol/L) through a daily vitamin D supplement (600-4000 IU/day) and as an add-on therapy also to influence bone health (risk minimization for osteoporosis, falls and fractures).[8]

A VitD suppl. as an early adjuvant "basic therapy" could have a positive effect on the course of MS in therapy management for PwMS through multifactorial therapy goals.[9]

1. New aspects of immunopathogenesis - biology of relapsing and non-relapsing MS

Multiple sclerosis (MS) is a complex, autoimmune-mediated central nervous system (CNS) disease characterized by inflammatory demyelination and axonal/neuronal damage.[10] Neuroimmune interactions between glia, neural, and immune cells play important roles in MS pathology.[11]

In the past, specific disease-modifying therapies (DMTs) were preferred in the treatment of (active) MS in order to delay its progression. Current immunopathological findings and special MRI procedures have led to a change in the previous approach to the cause of disability progression and can pave the way for a multi-tracked therapy.

The progression of disability is not only caused by focal inflammatory processes, as previously assumed, (this was the previous creed) and it is defined by acute relapses and changes in the MRI (Gd+enhancing T1 lesions, new and enlarging T2w lesions).

Recent brain MRI scans and neuropathohistological results verified disability progression independent of relapse activity (PIRA, progression independent of

relapse activity) in some PwMS.[12] About 1/3 of PwMS develop PIRA 5 years after the first attack and 50% of PwMS with PIRA showed radiological activity.[13]

The clinical course is insidious and can be viewed as a continuum of simultaneous different pathophysiological processes.[12]

These smoldering pathologic disease processes occur throughout the brain and spinal cord.[14] PIRA begins early in the disease process, it can be observed in all MS phenotypes, and becomes the major cause of disability accumulation in advanced MS.[15] The increase in the EDSS score (expanded disability status scale) was accelerated when both PIRA and relapses occurred. [15]

2. Complex Autoimmune Mechanisms that Trigger Multiple Sclerosis

Although the cause of multiple sclerosis (MS) is not yet fully understood, interactions between B andT cells are discussed in the pathogenesis, such as peripheral escape of B cells from T cell-mediated control, interaction of pathogenic B and T cells in secondary lymph nodes and reactivation of B and T cells accumulating in the CNS (details in[16]). Autoreactive inflammatory cells, including effector T cells (Th1, Th17, CD8 + cytotoxic T cells), activated B cells and plasma cells producing autoantibodies infiltrate the central nervous system (CNS).[17] This T cell migration into the CNS is the key to the pathogenesis of MS. The Treg cells (CD4+ CD25+Foxp3 regulatory cells [Treg]) play an essential role in this. A dysfunction of both the Treg cells and the Tr1 (IL-10-secreting regulatory type 1 cells) has been confirmed.[18,19] Subsets of B cells function as antigen-presenting cells and pro-inflammatory cytokine-producing cells.[20]

3. Pathophysiology of "Smouldering" Multiple Sclerosis"

After the acute phase of inflammatory demyelination in the relapse, 15-30% of the lesions do not heal completely.[21] The chronic lesions can develop differently in further development: 1. chronically active/slowly expanding/smoldering (rim positiv); 2. chronically inactive (rim negative); 3. partially or completely "remyelinated" (without a rim).[22]

These chronic active lesions (CAL) cause tissue damage and can also occur under disease-modifying therapies (DMTs).[22] These chronically active/slowly expanding/smoldering MS lesions (SELs) are markers of an aggressive and progressive disease course. They have been observed in 20-40% of white matter lesions.[14] They do not shrink slowly like other lesions; on the contrary, they typically remain stable or enlarge with ongoing demyelination.[22] Microglial (MG)/macrophage (MK)-mediated inflammation occurs in the CAL, which is a major obstacle to remyelination and accelerates brain atrophy.[23]

Activation of MG and recruited MK are found in acute MS lesions and are also found in chronically active and inactive lesions. Activated MG produces proinflammatory cytokines and inflammatory mediators that cause acute and chronic axon loss.

A new type of lesion, the "broad rim lesions", was discovered histologically, which showed a broad rim with MK and MG around the demyelated areas.[12] Quantitative Susceptibility Mapping (QSM), an imaging technique sensitive to brain iron, was used to detect paramagnetic rim of iron-loaded active MG and MK in a subset of lesions (PRLs) in MS.[24]

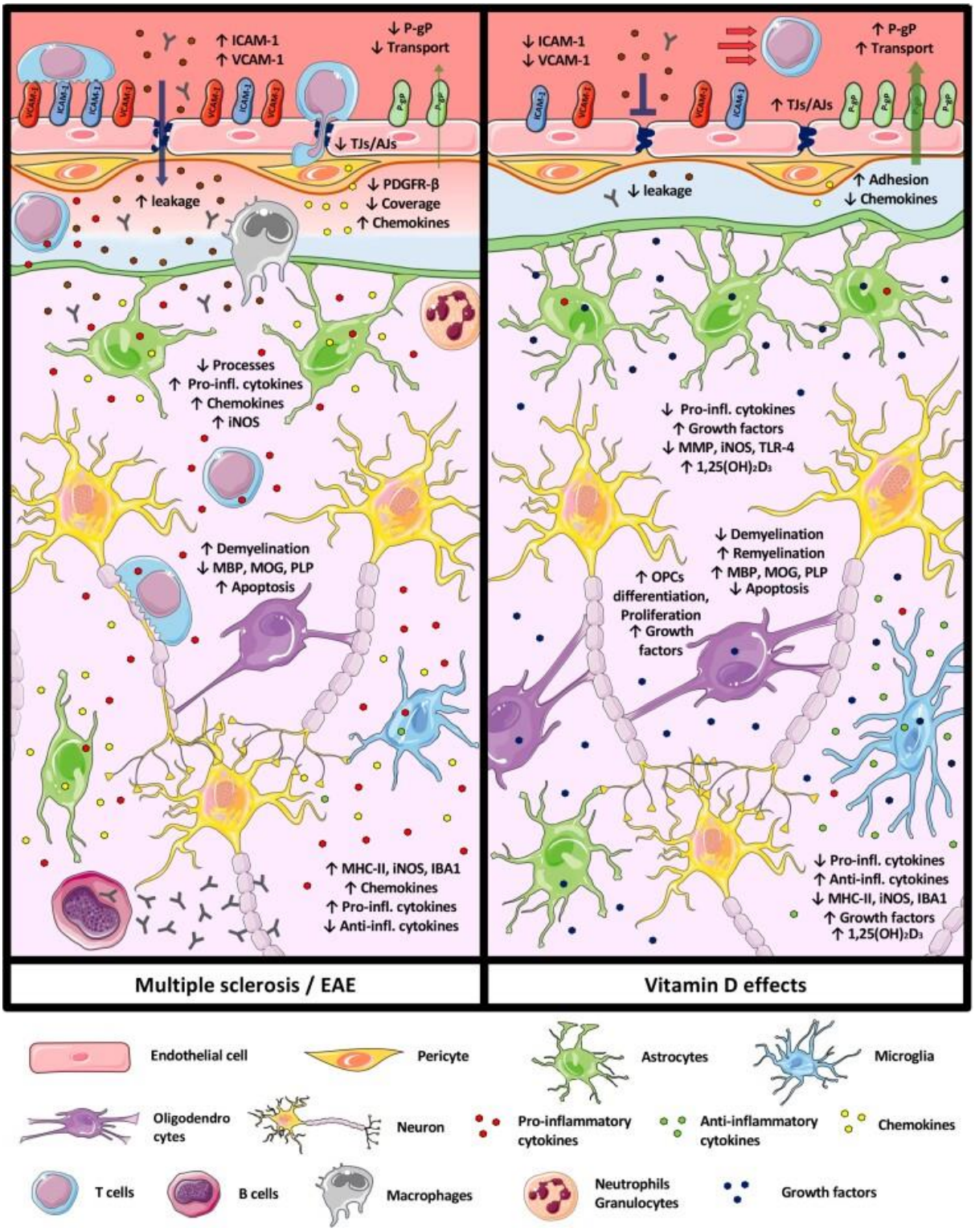

Figure 1: Vitamin D modulates different cellular and molecular mechanisms of CNS-resident cells and oft he blood-brain barrier (BBB) involved in multiple sclerosis pathology. (Left) Schematic representation the cellular and molecular mechanisms involved in MS/EAE [Experimental autoimmune encephalitis] pathology at the level of CNS-residents cells and the BBB. (Right) Schematic representation Vitamin D impact on cellular and molecular mechanisms involved in MS/EAE pathologyat the level of CNS-resident cells and the BBB (↑) Increased; (↓) decreased [39,Oxford University Press].

3.1 Paramagnetic Rim Lesions and Severe Disease Course

Recognizing these "rim positive lesions" is meaningful as they shape future disability and neurodegeneration, since rim+ lesions are associated with a more aggressive phenotype of the disease. Iron-loaded MG/MK express pro-inflammatory markers. SELs with paramagnetic rims of activated microglia lead to failure of remyelination, resulting in further destruction of the surrounding parenchyma.[14] Early preclinical cognitive abnormalities in MS could be due to these chronically active, smoldering lesions.[24] The number of perilesional iron margins is also a predictor of long-term clinical disability, especially when more than four such lesions are present.[25] Iron is essentially absent in chronically inactive lesions.

3.2 Optical coherence tomography, Serum Neurofilament Light Chain Levels and Glial Fibrillary Acidic Protein important parameters for assessing activity

Measurement of pRNFL [peripapillary retinal nerve fiber layer] and GCIPL [ganglion cell inner plexiform layer] thicknesses provided further evidence that a higher number of PRLs is associated with pronounced neurodegenerative processes. Lower pRNFL and GCIPL thicknesses could be verified in optical coherence tomography (OTC).[26]

The level of sNfL was higher in RRMS,PPMS, and SMPS than in controls, was associated with gadolinium T1 lesions, and the higher the sNfL levels, the more pronounced the volume loss in the spinal cord and brain.[27]
Serum glial fibrillary acidic protein (sGFAP) will become a specific biomarker for MS progression in the future.[28]

4. Multitherapeutic Approaches

Current knowledge of the pathobiological mechanisms of progression impediment makes it possible to propose several parallel therapeutic approaches in the doctor-patient dialogue for PwMS with acute disease.

1. Anti-inflammatory therapy (DMTs)
2. Therapeutics to promote neuroprotection, remyelination, neurorestoration
3. Anti-aging (immune system)
4. Diet, calorie restriction, infection prophylaxis,
5. Lifestyle change with avoidance of nicotine consumption, ([29], control/avoidance of obesity)
6. Consideration of comorbidities.[14]

5. Inhibition of Macrophage/Microglia Activation by Vitamin D Supplementation

The MS community has made a call to promote neuroprotection, remyelination, and neurorestoration through therapeutics as an add-on therapy to DMTs, which currently cannot effectively combat smoldering MS.[14]

One of the alternative ways would be to achieve early, daily high-dose vitamin D supplementation (VitD suppl.) with s25(OH)D target values of at least 60-80ng/ml (30-130ng/ml).[30,31] Although there is no international consensus on the definition of Vit D deficiency/severe insufficiency, daily doses of 5-10,000 IU/day are associated with benefit in PwMS.[32,33] The effect of vitamin D on the innate and acquired immune system is undisputed and 1,25-dihydroxyvitamin D plays a major role in cell homeostasis in the CNS. 1,25(OH)2 D3 is autocrine and paracrine.[34] 1,25(OH)2D3 also targets macrophages, monocytes, dendritic cells (DC), and T and B cells. VitD regulates the activation of microglia and astrocytes.[35,36] (Figure 2)

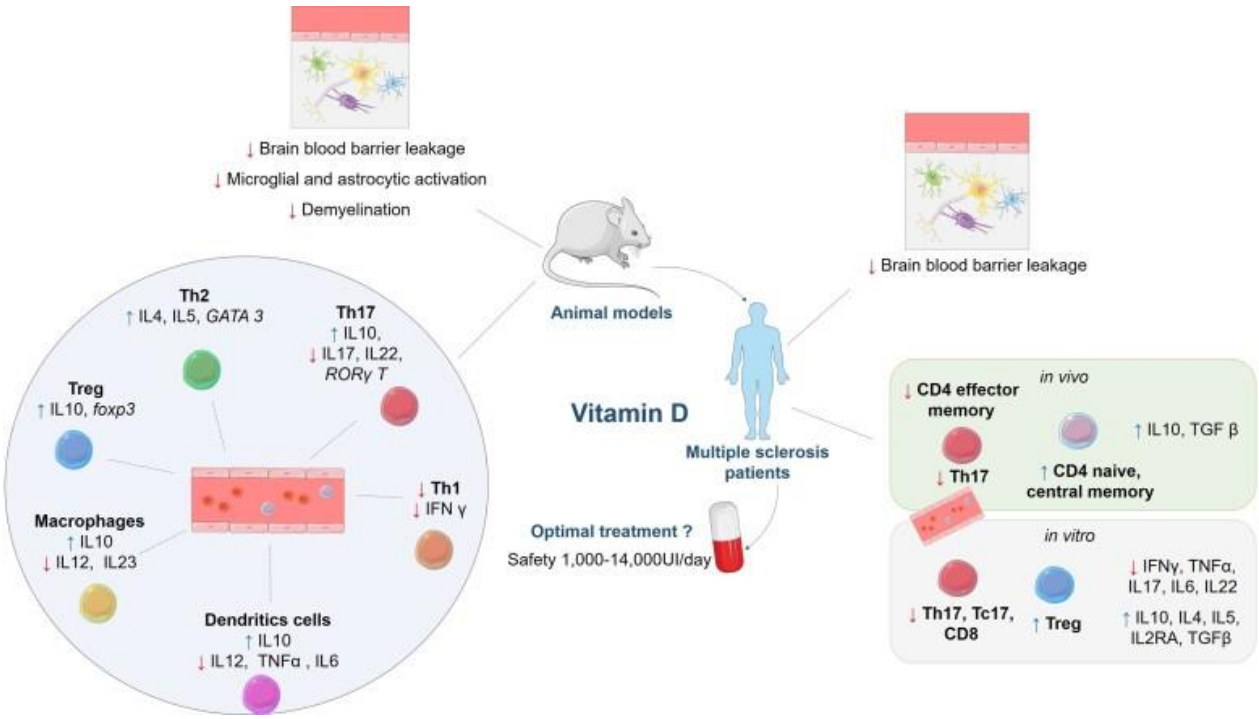

Figure 2: Effects of vitamin D supplementation on PwMS and in animal models. Effect on cytokine production, brain blood barrier and involved cells [39], Oxford University Press]. Increase (↑), decrease (↓)

In DC, Vit D decreases IL-12, TNF alpha and IL-6 production and promotes IL-10 production. The active metabolite of VitD also promotes the development of Forkhead Box Protein 3 (FOXP3+ regulatory T (Treg) cells and IL-10-producing T regulatory type 1 (TR1) cells. 1,25(OH)2D3 blocks B- cell proliferation, plasma cell differentiation and immunoglobulin production.[37,38,39]

Vitamin D exerts its complex immunomodulatory effects on CD4+ T lymphocytes (Th1, Th2, Th17, Treg cells), among others, and the secretion of pro-inflammatory cytokines (IL-2, IL-6, IL-12, IL-17, IFN-γ, TNF-α and TNF-β) is inhibited. It stimulates the production of anti-inflammatory regulatory Th2 cytokines (IL-4, IL-5 and IL-10).[39-42]

The cytokine profile is shifted from a Th1 to a Th2 mediated profile (decrease in IFN-gamma and increase in IL-4)[41]. As a result, vitamin D insufficiency in PwMS must be avoided at all costs.[41, 43]

Not only is vitamin D deficiency associated with MS risk, but s25(OH)D levels are inversely correlated with risk of relapse, CNS lesions, and disability progression. Vit D suppl. reduces the number of new Gd+-enhancing or new/ enlarged T2 lesions on MRI.[41,44]

Activation of MG is the earliest biomarker of CNS inflammatory processes in MS[45]. However, MG/MK-mediated inflammation is a major obstacle to remyelination and accelerates cerebral atrophy.[12,23] The changes in iron homeostasis are a crucial step in the pathophysiology of MS damage. They are based on MG activation and the modification of oligodendrocyte functionality.[46]

The resident MG show inflammatory and anti-inflammatory alternative phenotypes with

other subgroups and in their activation state are divided into the phenotype M1 (pro-inflammatory with production of IL-6, IFN-gamma, IL-23, TNF-alpha) and M2 (anti-inflammatory, expressing IL- 4, IL-10, IL-13).[47,48] Meanwhile, the M2-polarized MK and MG can be divided into subgroups such as M2a, M2b, M2c cells.[48] In contrast, quiescent MG in a stable, normal CNS produce neurotrophic factors, clear myelin debris, serve to maintain oligodendrocyte progenitor cells in the CNS, and modulate neuronal activity and synaptic organization. [47]

Because the pro-inflammatory M1 microglia phenotype is particularly active in the early stage of MS, it is biologically plausible to start VitD supplementation to influence the secretion of inflammatory mediators in order to reduce myelin damage.

Elevated levels of calcitriol lead to a reduction in MG activation, oxidative stress and lower BBB permeability [49] 1,25(OH)2D3 effectively shifts MG from a pro-inflammatory M1 phenotype to a reparative M2 phenotype, particularly in early MS, resulting in limitation of inflammation and demyelination.[47,49] VitD signaling in neurons promotes an anti-inflammatory state in the MG. It is important for normal CNS development.[50] VitD induces anti-inflammatory molecules in primary neurons. In an early state of autoimmunity in the CNS, low VitD levels can enhance autoimmunity.[50]

6. *Future therapeutic avenues*

Bruton's tyrosine kinase inhibitors (BTK) slow down the activity of microglia, macrophages and B cells. The latter produce antibodies and cytokines and are involved in antigen presentation and in the ectopic formation of follicle-like structures in the CNS.[51,52] As a promising target for PwMS, the 6 BTK inhibitors (Evobrutinib, Orelabrutinib, Fenebrutinib, Tolebrutinib, Remibrutinib and BIIB091) are being tested in studie,[53] with widespread use in PwMS in real-world settings still a long way off.

6. Serum Neurofilament Light Chains and Glial Fibrillary Acidic Protein Essential for Assessing Activity

Essential biomarkers for the course of the disease and individual patient management as well as the motivation for VitD suppl. are the determination of sNfL and sGFAP. Both parameters indicate pathological processes in the CNS and are medium- to long-term prognostic biomarkers in serum for the development and progression of MS.[54-57] The supplement to the MRI and the clinic is not only convenient and cost-effective for the prognosis assessment in the long-term care of PwMS, but also very useful.[58] In pathological processes on axons, neurofilament light proteins are released and the level of sNfL values reflects the acute disease activity of MS.[59]

The determination of the sNfL at intervals of about six (three to four) months can determine the therapeutic procedure. Elevated values, adjusted for age, body weight and a z-score above 1.5 for example, give a predictive indication of a relapse in the next few months to a year with damage to the spinal cord and brain.[60,61] A gradual axonal loss and breakthrough activity can be detected with this biomarker and integrated into the therapy strategy.[58] High sNfL values correlate with the number of T2 lesions in the MRI, indicate brain atrophy and also correlate with cognitive impairment.[62]

The appearance of sNL in serum secondary to activated microglia, particularly in the

perilesional normal-appearing white matter and at the chronic lesion margin, contribute to the detection of neuroaxonal damage. This enables CNS damage to be identified without clinical signs of relapsing activity due to a smoldering disease process. Monitoring with MRI (Gd+ lesions) and observing clinical symptoms (relapse) is not sufficient to assess activity.[63]

Glial fibrillary acidic protein (GFAP) is an intermediate cytoskeletal protein of astrocytes, and astrocyte activation is a pathologic hallmark of advanced MS.[59] SGFAP levels increase when astrocytes are activated or increasingly degraded. GFAP is a prognostic biomarker for future PIRA, i.e., chronic, smoldering disease processes, and, in combination with the sNfL determination, the individual course of the disease can be assessed more precisely.[64] Especially in inactive patients, higher sGFAP levels are a warning sign of later progression. [59]

7. Aging and Multiple Sclerosis in Disease Management

The clinical course of MS is closely related to aging, especially chronologically, but also connected to biological and epigenetic aging and senescence (state of cells in which they cannot replicate but can produce and secrete pro-inflammatory molecules).[65] In order to halt this non-relapse-related progression, an important part is the integration of aging into the management of MS.

Three factors worsen VitD insufficiency in the course of MS disease:

i) The Uthoff phenomenon (sensitivity to heat leads to avoidance of solar radiation) and its increase as the disease progresses

ii) Reduction of outdoor activities due to disability in locomotion, and the

III) VitD synthesis is physiologically reduced with age.[66]

Senescent MG promote chronic secretion of inflammatory cytokines. They create a climate that inhibits remyelination because they have reduced phagocytic activity. [12]

A lower s25(OH)D level is associated with a lower brain volume.[67,68], sufficient 1,25(OH)2D3 levels are associated with neuroprotective effects.[69] VitD promotes autophagy, slows down the aging process in which dysfunctional mitochondria are removed, reduces oxidative stress and inflammation, and influences calcium signaling, epigenetics, DNA damage and telomere shortening.[70]

The association of a sufficient VitD serum level with delayed aging has been demonstrated by studies. [71,72]

Low VitD levels are associated with the risk of cognitive impairment [73] and with smaller volumes of brain tissue, white matter, and hippocampus.[74] Higher concentrations of s25(OH)D in the brain were associated with better cognitive function before death.[75]

8. Menopause in People with Multiple Sclerosis

Menopause, which is linked to biological, hormonal and immunological changes, has a significant impact on women's quality of life, especially in women with MS.[76]

Gender differences in concentrations of serum concentrations of total 25(OH)D, 24.25(OH)2D, free 25(OH)D and free 1.25(OH)2D are present and resulted in lower values in female PwMS.[77]

9. Vitamin D and Cognitive Functions

MS is associated with cognitive decline in 34-65% of PwMS.[78] Because this symptom can also occur in the early stages of MS and is associated with a deficit in quality of life and stress in the family environment, early registration is required.[79]

Even in adolescents who did not have MS, there was reduced visual memory with VitD insufficiency, which improved with those with higher and sufficient 25(OH)D values. Thus, the interest in the VitD application should definitely exist with PwMS.[80]

It is interesting to note that PwMS, the course of which was classified as "benign MS" (PwBMS), still have a cognitive impairment in 38% compared to PwMS with "non-benign MS" in 66%. In PwBMS, the lowest impairment rate was found in the area of verbal functioning (18%) and the highest impairment rate in the area of information processing (32%). In addition, fatigue was found at 78% and depression at 55%. The EDSS score commonly used in practice does not record these impairments.[81]

There is ample evidence that VitD plays a crucial role in overall brain health and morphology, and its deficit is associated with neurological disorders because essential brain functions are impaired.[82] Higher 25(OH)D3 concentrations in the brain (neuropathology) were associated with a 25% to 33% lower likelihood of dementia or mild cognitive impairment before death.[75] It could also be shown that a low 25(OH) D level was associated with a reduction in the brain volume of the olfactory functional areas.[82] Furthermore, in patients with CIS and a low 25(OH) D serum level, poorer cognitive performance and neuronal integrity could be registered in an observation over 11 years.[83] There was an association with serum neurofilament light chains (sNfL), which is a biomarker of neuroaxonal damage. High or rising NfL values in longitudinal studies are an indication of active disease or impending MS relapses.[61] A higher serum calcidiol level predicted better cognitive performance. A 50 nmol/L higher mean 25(OH)D level in the first 2 years was associated with a 65% lower likelihood of poorer PASAT-3 (Paced Auditory Serial Addition Test) performance at year 11. NfL concentrations in the 11th year of observation showed that a 50 nmol/L higher mean 25(OH)D level in the first 2 years was also associated with a 20% lower sNfL.[83]

In a systematic review and meta-analysis, an association was found in a measurement of the biomarkers sNfL and vitamin D in correlation with cognitive impairment (IPS: slower information processing speed).[78]

For 10 years there has been evidence that there is a connection between s25(OH)D and IPS. Higher levels of calcidiol by supplementation resulted in long-term neuroprotection and consequently inhibition of cognitive impairment.[84,85]

10. Dual Therapy: Synergism of 25(OH)D and Disease-Modifying-Therapies - The Beginning of Personalized Medicine?

Ocrelizumab (humanized monoclonal antibody) is approved for the treatment of relapsing and progressive MS. It attaches to B lymphocytes (pre-B cells, mature B cells, and memory B cells) that carry the CD20 antigen and decreases the number of circulating CD20-positive B lymphocytes to slow disease

activity. In addition to the T cells, the B cells are assigned another key role in the therapy of MS.[86]

Sufficient 25(OH)D levels suppress B cell proliferation and immunoglobulin production, resulting in better MRI results.[87,88] Recognizing that further doses of anti-CD20 treatment are necessary to deplete CD20-expressing T cells, therapy should not be stopped prematurely due to falsely insufficient effect. Early repopulating B cells exhibit an anti-inflammatory transition phenotype and are unlikely to be associated with early MS disease activity following anti-CD20 therapy.[89] The immune reconstitution therapy will probably only work optimally if long-term therapy is carried out. [90] Because it is currently accepted that MS is a disorder of the immunoregulatory network and not a disease of a single cell subgroup,[90] the supportive administering of VitD would be a tile in the mosaic of MS therapy.

Ocrelizumab therapy in RRMS (relapsing remitting MS) and PPMS (primarily progressive MS) reduced the number of new MRI lesions and annualized relapse rate (ARR) and reduced the progression of disability. A greater benefit was shown for lighter PwMS (lower body weight) than for heavier PwMS. For ocrelizumab 600 mg treatment, body weight was identified as the most influential covariate on ocrelizumab pharmacokinetics. Greater B cell depletion and less B cell repletion was achieved with higher doses.[91] The higher the body weight, the lower the available blood level of vitamin D, because the vitamin D accumulates in the adipose tissue and the daily VitD-suppl. dose has to be adjusted, with the sufficient s25(OH)D level being the constant parameter.[92] It would be biologically plausible to increase the dose of VitD suppl. to adjust to BMI (body weight/m^2).

10.1 Concordant mechanisms of anti-CD-20/ Disease-Modifying-Therapies and vitamin D

Mechanisms between anti-CD-20 treatment and the effect of VitD are also evident. The IFN-gamma, TNF-alpha and IL-6 are increased before an anti-CD20 treatment and are reduced and IL-10 increased after treatment. VitD supplementation achieves the same effect (see above).

A Vit D suppl. in PwMS treated with ocrelizumab delayed early B cell recurrence, with 25(OH)D levels above 30 ng/mL affecting B cell kinetics and radiological activity of MS. [93] A reduction in radiological activity was also observed with additive therapy with vitamin D in treatment with disease-modifying therapies (DMTs), how interferon (IFNß), glatirameracetet, natalizumab, fingolimod and rituximab.[41,94]

An interferon-ß group with higher s25(OH)d levels showed reduced risk of relapse in MS.[95] Many mechanisms of VitD on the immune processes are similar to those that have also been described for interferon-beta.[66,95]

A daily Vit-D suppl. showed improved MRI results in terms of percent change in brain volume and fewer new/enlarging T2 lesions and a trend toward less depression when added to fingolimod.[96]

The additive administration of VitD to rituximab therapy achieved low inflammatory activity with higher 25(OH)D levels.[97]

The combination of vitamin D and siponimod improved the remyelination process. The M1 microglia phenotype was attenuated such that a VitD-suppl. was used to strengthen the siponimod effect in PwMS.[98]

It would be biologically plausible that Vit D suppl. could also have a positive effect on inflammation in the choroid plexus (PC) in MS, demonstrated by MRI and clinically relevant.[99] The stroma density of CD8+ T cells and granulocytes was increased in the PC in patients with progressive MS, as was a high number of macrophages and dendritic cells in the PC as a sign of inflammation in the PC.[100] Increased inflammation in the choroid plexus may already occur in the pre-symptomatic MS stage and the increase in volume could be due to increased macrophage/microglia infiltration.[101]

11. Prevention/Reduction of DMTs-induced Secondary Autoimmune Psychologically Stressful Skin Diseases in PwMS (Alopecia, vitiligo)

The potential role of vitamin D in autoimmune diseases is recognized and vitamin D deficiency has been described in a variety of autoimmune diseases, in particular the tendency towards polyautoimmunity in PwMS.[102-107]

The psychological burden of PwMS, in which additional conspicuous skin diseases develop as a result of drug therapy, should not be underestimated.[108] Depression is a frequent psychiatric comorbidity in patients with chronic dermatosis.[109] After therapy with DMTs such as alemtuzumab (alemtuzumab delivery after previous fingolimod therapy), ocrelizumab, daclizumab, natalizumab, teriflunomide, dimethyl fumarate, fingolimod, interferon beta-1a, interferon beta-1b, glatiramer acetate, siponimod, cladribine and rituximab, autoimmune-related alopecia was described in 8,759 cases with a total of 44,114 side effects (AEs) due to DMTs up to the year 2020.[110-116] Vitiligo case reports have also been published.[111,117-119] Both diseases are multifactorial autoimmune diseases. In patients with alopecia and vitiligo, serum BDNF (brain-derived neurotrophic factor) and serum VitD were significantly lower than in controls and correlated positively with each other. There was also an inverse association of serum BDNF and VitD with depression.[109]

Although secondary skin diseases induced after alemtuzumab administration are rare and the pathogenic mechanisms are not fully understood, the vitamin D serum levels in alopecia were lower than in healthy subjects [120] and inversely correlate with the severity of the disease.[107,121] Serum VitD levels are negatively associated with the concentration of pro-inflammatory cytokines (IL-21, IL-17). These cytokines have been shown to induce Th17 cells and inhibit redifferentiation of regulatory T cells (Treg), which have an important function in autoimmune diseases.[122] IL-21 levels are associated with the development of secondary autoimmune diseases after alemtuzumab in MS.[122] On the other hand, through a high-dose VitD-suppl., a reduction in IL-17 and IL-21 was achieved [123,124] and the neutrophins (BDNF) increased.[125]

If there are already indications that depressive symptoms/depression are more likely to occur in healthy people with hypovitaminosis D and less frequently in people with high vitamin D levels, it is biologically plausible to offer endangered PwMS a permanent VitD supplement. High single doses do not achieve the desired goal.[126,127]

12. High-dose VitD-supplement as a Replacement for an Interrupted Therapy with Disease-Modifying-Therapies During Pregnancy

The desire to have a child was made possible by effective therapy with 20 different immune-modulating drugs. However, because some

DMTs have to be discontinued for safety reasons, a VitD suppl. high-dose is possible as a "DMT replacement therapy" to reduce the activity of MS in all pregnancy periods and postpartum with high safety.[128] The example of stopping fingolimod, a sphingosine-1-phosphate receptor modulator that is used to treat relapsing MS, shows how seriously stopping DMT can affect a mother's health. Because of the risk of teratogenicity and adverse effects on the fetus, the drug must be discontinued prior to conception. One study showed that 31% of women relapsed during pregnancy (mainly in the second trimester) and 44.6% in the postpartum year after delivery. More than 6% had a major relapse (SRDCS [Compound Severe Relapse Score]) one year after delivery, resulting in permanent severe disability.[129]

A study in women (without MS) showed that the probability of pregnancy and a live birth increased if the VitD level (>= 75 nmol/L) was sufficient before conception.[130] There are numerous indications that an optimal VitD serum level has a positive influence on the course of MS and that a VitD suppl. can be used during pregnancy as well as during breastfeeding.[131,132,133]

For women who wish to have children, within the scope of prevention for the course of pregnancy, it is crucial both for the health of the mother and the child to start a VitD supplement at least 2 months before conception (before placentation and trophoblast invasion) with around 5,000IU/day, to reach an optimal s25(OH)D level (above 100nmol/l). The effect of VitD on the placental gene expression and on the inflammation within the placenta creates an optimal initial condition for the fetus.[132,133,134]

If VitD supplementation is necessary even for "non-MS persons" to ensure an optimal VitD status of mother and infant[135], supplementation for mothers with MS in the management of the disease should be a matter of course.

On the other hand, an insufficient s25(OH)D level increases the risk of preeclampsia and preterm birth and can lead to a lower birth weight.[136] In pregnant women without MS, VitD-suppl. reduced risk of shortened gestational age was achieved and was associated with improved infant growth. [137]

As little as 2,000 IU/day during pregnancy can reduce the risk of fetal or neonatal mortality.[137] An excess of VitD metabolites in the fetus is unlikely if the maternal VitD value is within the normal range. Up to 4,000 IU/day were supplemented during pregnancy, resulting in sufficient 25(OH)D values without an increased risk of toxicity for the women or for the newborns. Serum levels of 25(OH)D above 125 nmol/l should not be reached.[138] The Endocrine Society (UK) recommends a safe upper limit for VitD intake during pregnancy of 10,000 UI/day for women over 19 years of age who are VitD deficient.[139] In mothers with insufficient VitD levels (<30 nmol/L [<12ng/mL]) in early pregnancy, a 2-fold increased risk of the offspring developing MS was verified.[140] This result is consistent with the observations that insufficient s25(OH)D levels in utero lead to an increased risk of developing MS later in life. The neonatally measured s25(OH)D values in later-life MS patients were 33 nmol/L and 35.9 nmol/L below healthy control persons.[141] Single nucleotide polymorphisms (SNPs) in or near genes in the VitD metabolic pathway appear to modify the reaction to the VitD suppl. during pregnancy.[142] Compared to healthy

subjects, PwMS had a lower admission rate when supplemented with VitD.[143]

A relapse prophylaxis with vitamin D should be considered during pregnancy, especially in the first trimester.

A pathophysiological explanation as to why immunological tolerance increases during pregnancy was found by Engler et al.[144] In MS, it has been generally reported that regulatory T cells (Tregs) have a reduced suppressive potential.[144] The authors demonstrated that progesterone greatly increases Tregs via interaction at the glucocorticoid receptor in T cells.[144] However, the enrichment of the Tregs could also be caused by a VitD suppl. and contribute to relapse prophylaxis, because Tregs are essential for suppressing autoreactive reactions.[123] Jeffery et al. were able to show that 1,25-dihydroxy-vitamin D3 (1,25(OH)2D3= calcitriol) has a significant and direct effect on the generation of FoxP3+-CTLA-4+ Tregs, which are capable of strong immunosuppression.[123] A recent study points to the need for closer surveillance of women with MS and (planned) pregnancy. A higher risk of elective caesarean sections, instrumental delivery, maternal infections and antenatal bleeding/placental detachment has been observed. There was also a higher risk of preterm birth, medically indicated preterm birth, and lower birth weight.[145] In order to spare MS patients a caesarean section, a sufficient VitD suppl. > 30ng/mL (> 75nmol/L) may be helpful and may also reduce the risk of postpartum bleeding.[146,147]

13. Benefit of a VitD Supplement for Symptoms/Comorbidities

It is undisputed that growing interest in the influence of comorbidities is required in the management of MS. The presence of comorbidities can delay MS diagnosis, increase the rate of hospitalization and disability progression, reduce quality of life, and increase the risk of death.[148]

13.1 Migraine

Reports on the prevalence of migraines in PwMS vary widely, ranging from around 31% to between 24% and 43%, depending on the continent.[149] A primary headache is in PwMS at about 56%.[150] VitD suppl. is controversially discussed in the literature. VitD deficiency or insufficiency is indicated at between 45 and 100% in migraine and headache patients. In studies, the VitD level correlated negatively with the frequency of headaches. A VitD suppl. with a dose of 1000-4000 IU/day reduced the frequency of attacks in migraine sufferers.[151,152,153] Genetic increased VitD levels were associated with a reduced risk of migraines.[154] Advantages of a combination therapy of vitamin D and simvastatin could be used in personalized medicine [155] because a benefit of this combination therapy has a positive effect both on acute optic neuritis and the course of SPMS.[156]

13.2 Fatigue and Depression as Comorbidities in Multiple Sclerosis

Depression as a comorbidity occurs in up to 50% of PwMS and significantly impairs the quality of life and is also reported by PwMS (PROs/patient-reported outcomes].[157-161]

Fatigue is present in 35-97% of PwMS, which also severely impacts quality of life.[162-164] Fatigue already manifests itself at the beginning of MS, which worsens as the disease progresses and was also associated with increased

depression manifestations. In PwMS who received an injectable DMT, the increase in fatigue plays an important role.[165] Over the past decade, knowledge of the pathophysiology and immunology of MS and depression, and the complex links to vitamin D balance, has increased rapidly. Both diseases are characterized by an imbalance of pro-inflammatory and anti-inflammatory cytokines, increased serum neurofilament light chains, disruption of the blood-brain barrier, abolition of the physiological function of the various types of microglia, decreased calcidiol [25(OH)D] levels, disorders of the gut microbiome in combination with hyperactivity of the HPA axis/microbiome gut brain axis.[166-178]

13.3 Detection of inflammatory biomarkers for personalized intervention in depression

Tackling inflammation is being increasingly discussed as a strategy to treat (treatment-resistant) depression. The inflammatory biomarkers focus on hsCRP, IL-6, TNF alpha, IL-1ß.[179,180,181] 21.2% of the patients with depression had hsCRP levels > 3 mg/L, where a higher BMI (body mass index) is a reinforcing factor.[179,182]

In depression, stress initiates cellular and molecular changes in the brain via increased cortisol release in the HPA axis. Microglial activation and neuronal damage as well as dysregulation of neuroplastic and neurotrophic factors complete the spectrum of pathological damage. It is discussed that gut dysbiosis leads to increased gut permeability, which favors endotoxemia and ultimately paves the way to systemic inflammation.

With the help of nuclear magnetic resonance spectroscopy (NMR), it was possible to prove that for depression there are close connections between the intestinal microbiome and blood metabolome and that the intestinal microbiome requires increased attention in the management of depression.[183]

A VitD supplement could bring the microorganisms in the gut back into balance and reduce the inflammatory processes at various levels.

VitD promotes Treg cell proliferation, inhibits expression of Th1 and Th17 immune cells, and inhibits pro-inflammatory IL-17. 1,25(OH)2D3 reduces the secretion of IFNγ, TNFα by TH 17.1 cells. Increased levels of calcitriol lead to a reduction in MG activation, oxidative stress and lower BBB permeability.[184-188]

An early, long-term, daily sufficient VitD supplement as an add-on therapy under control of the 25(OH)D serum values is an essential therapeutic instrument to slow down the disability of MS and thereby also primarily the stress and subsequently to prevent or reduce the manifestation of depression. In the future, the therapeutic successes/failures will be able to be mapped through the continuous measurement of the sNfL and s25(OH)D levels. Early determination of the sNfL values ("troponin of the psychiatrist") and inclusion in the management of PwMS will have a decisive influence on the fate, in that a highly effective therapy is offered to this group of people in particular with elevated sNfL values.

13.4 Influence of Vit D Supplementation on the Comorbidity Osteopenia/Osteoporosis

The indication for a VitD suppl. cannot only be established from the results of studies on the activity of MS but must include the overall

effect on the immune system in autoimmune diseases, anti-inflammatory, antimicrobial potency and the effect on skeletal homeostasis.[189] There is evidence that there is a significantly increased prevalence of osteoporosis in men and women with MS. 80% of PwMS had reduced bone mass, with osteopenia observed in 43% and osteoporosis in 17-37.5%. 21% had vertebral, rib or extremity fractures. The proportion of male PwMS with reduced bone mass is high and the association with MS disease progression has been noted and bone health monitoring in PwMS over 40 years of age is considered necessary.[190,191]

A risk score was developed because the risk of osteoporosis in PwMS is increased with the risk of bone fractures with associated morbidity and mortality. Recognizing this comorbidity enables personalized medicine with recommendations for early prevention of osteoporosis, with it becoming apparent that an EDSS score of 4.5 is to be regarded as a relevant limit value for increased alertness. Osteoporosis was more common in postmenopausal PwMS and male PwMS compared to healthy subjects.[192]

Individuals without MS aged 60-84 years received 60,000 IU/month VitD and showed a reduction in the incidence of total fractures over a 5-year follow-up.[193]

The results on the comorbidity of osteoporosis/osteopenia in MS speak for a daily VitD suppl. PwMS who underwent bone mineral density testing (DEXA) had lower bone mineral density and a higher prevalence of osteoporosis (up to 29%) compared to those without MS. A two-fold increased likelihood of osteoporosis existed with low bone mineral density (DEXA) in PwMS.[194]

In a large study, the prevalence of osteoporosis was found to be 17% and that of osteopenia 43% in PwMS.[191] The knowledge of the effect of inflammatory processes on bone loss expands the spectrum of VitD-Suppl. Osteoclastic bone resorption is promoted by increased circulating pro-inflammatory cytokines. CD4+ T cells play a central role in inflammation-induced osteoporosis. Th-17 cells are known to produce IL-1, IL-6, IL-17, RANKL (nuclear factor kappa B[NF-kB) ligands and TNF, as well as IFN-gamma, which promote osteoclastogenesis. In contrast, the Treg cells have an anti-osteoclastogenic effect,[195] which is promoted by VitD supplement. Treg cells can regulate osteoclasto genesis by secreting TGF-ß and IL-10 as well as IL-4 cytokines and thus suppress the differentiation of osteoclasts.[196]

If high doses of VitD improve fracture healing outcomes in healthy non-osteoporotic patients,[197] this VitD suppl. should not be withheld from the PwMS. 75% of healthy adult fracture patients (age 18-50) had an s25(OH)D <30ng/mL.[197]

14. Disorders of the Gut Microbiome in Multiple Sclerosis

A disrupted bacterial and viral gut microbiota is thought to be part of the pathogenesis of MS, mediated by an altered gut-microbiota-brain axis. The composition of the gut microbiota affects the production of serotonin in the gut, which in turn affects the serotonin-mediated regulation of systemic immune function.[198]

In a systematic review of gut microbiota composition in 286 PwMS up to 2019, most studies found no difference in gut microbiota diversity. However, taxonomic differences from controls were verified and immunomodulatory

drugs showed individual taxonomic differences.[199] A recent study showed that inflammatory markers (blood leukocytes, CRP, blood cell gene expression of IL-17A and IL-6) were positively associated with a group of bacteria that are more frequent in MS.

Bacterial species that were more common in disease-active, treatment-naïve MS were also positively associated with the plasmacytokines IL-22, IL-17A, IFNß, IL-33 and TNFalpha, some of which are targeted by vitamin D influence.[200] Because high-dose VitD-suppl. at 10,400 IU/day reduced IL-17-producing CD4+ T cells and effector memory CD4+ T cells and was associated with a concomitant increase in the proportion of central memory CD4+ T cells and naïve CD4+ T cells, a VitD supplement should be used as a potential therapeutic agent.[185] After 6 months, the 25(OH)D level increased by 34.9 ng/ml,[201] demonstrating that a high-dose supplement is necessary for an immunological effect. The active form of vitamin D3, 1,25(OH)2D3, inhibits IL-22 production and can be viewed as an adjuvant therapeutic to regulate IL-22 production.[202] The two pro-inflammatory cytokines (IL-22, IL-17) correlate with active brain lesions in MS.[203] The bacterial species richness of treatment-naïve MS cases was associated with the number of relapses, which is a surprising result.[200]

15. Efficacy of Vitamin D3 Supplementation on Cancer Mortality

The association between MS and cancer has been examined in many studies and the results have diverged.[204] It is being discussed whether PwMS as an autoimmune disease already carry a higher risk of developing certain malignant diseases.[205] It is also theoretically possible that activation of the immune system in MS leads to a protective effect against cancer by increasing immune surveillance. On the other hand, chronic inflammation and therapy with immunosuppressants could lead to loss of immune protection against cancer or activation of the immune system.[205] Cancer incidence differs by examination before and after the treatment era. In the period 1953-1995, PwMS had a similar incidence of cancer as controls, but an increased incidence of cancer was noted in endocrine glands.[206] In 1993, the first disease-modifying therapies (DMTs) began to appear. In the period 1996-2017, after the start of the introduction of DMTs, the incidence of cancer in PwMS was higher, especially in those older than 60 years.[206] By 2023, 20 different immunosuppressants will be used to treat MS.

The type of medication used in MS is crucial. There is general agreement that immunosuppressive therapy with azathioprine, cyclophosphamide and mitoxantrone are associated with an increased risk of cancer.[205,207] On the other hand, DMTs have previously been used to treat cancer (rituximab, cladribine, methotrexate).[205] The potential for carcinogenesis is specifically linked to the individual DMT and is definitely mentioned in the monitoring program by the individual pharmaceutical manufacturers. (Detail in publication in [205]].

15.1 Melanoma - Easy to monitor risk?

Using the example of melanoma development, a longer observation period of treated PwMS with DMTs shows how frequent the frequency of melanomas is with alemtuzumab,[208-211] natalizumab,[212-214] ocrelizumab,[205,215]

fingolimod,[216-218] siponimod,[219] and ponesimod,[219] In the case of cladribine,[205] experimental cell studies did not indicate a significant risk of melanoma.[220]

Sphingosine 1-phosphate receptor modulators (fingolimod, siponimod, ozanimod, ponesimod) are oral DMTs. There is a suspicion that the occurrence of melanoma could be increased. An increased proliferation of melanoma cell lines in vitro at therapeutic doses could be registered. In VigiBase (VigiBase is the unique WHO global database of reported potential side effects of medicinal products) up to November 2022, 418 cases of melanoma were recorded, 412 of them with fingolimod and 6 with siponimod.[219]

In the case of ofatumumab, the first complete human monoclonal anti-CD20 antibody (mAb), a malignant melanoma in situ and basal cell Ca (n=4) have been observed to date.[221] Monitoring of skin lesions, which is easy to perform, should be recommended to PwMS on a regular basis. Basal cell carcinoma and Bowen's disease (dermatosis praecancerosa) could also be discovered as possible malignant diseases.[219] In therapy with ozanimod, malignant diseases were associated with older age (> 50 years).[222]

15.2 Role of VitD in melanoma pathogenesis

As part of the "basic therapy" of vitamin D in PwMS, a VitD suppl. can be accompanied as prevention of skin cancer, because an increased incidence of cutaneous melanoma and VitD deficiency (s25(OH)D < 20ng/mL) could be verified in a meta-analysis.[223]

At the time of melanoma diagnosis, s25(OH)D was inversely correlated with tumor thickness. Deeper Breslow thickness, higher stage and earlier distant metastasis were associated with lower s25(OH)D value.[223,224] In primary invasive melanoma and reduced VitD serum levels, there was a significant correlation with the tumor mitotic rate (more than 1 mitosis/mm²), tumor ulceration, a tumor thickness of more than 1 mm and BMI.[225]

Evidence from experimental and clinical studies suggests that vitamin D signaling disorders are associated with i) melanoma development,[226] ii) progression and iii) disease-free survival of patients. [227,228] The melanoma cells are able to convert 25(OH)D into 1,25(OH)2D3.[229] The active metabolite of VitD, 1,25(OH)2D3, shows relevant anticancer effects on melanoma cells. It has an antiproliferative, differentiating and proapoptic effect on malignant cell invasion and metastasis.[230] In addition to VitD-induced growth arrest and apoptosis in tumor cells or their non-neoplastic precursors, there are also mechanisms such as the improvement of DNA repair, anti-oxidative protection and immune modulation.[231]

15.3 Vitamin D deficiency may play a survival role in melanoma

For 40 years the potential of VitD suppl. as an adjuvant therapy for melanoma[227,228,232] has been emphasized. There is evidence that patients with cutaneous malignant melanoma could benefit from a daily VitD suppl.[226] Low VitD levels are associated with poorer overall survival.[233] Although the association between VitD and risk of melanoma is still a matter of debate, an s25(OH)D value above 30-40ng/ml may have a protective effect.[224,234,235] Although there are no generally applicable strategies due to conflicting results from different studies,

it is becoming clear that serum levels of s50-80ng/mL 25(OH)D should be aimed for in the long term in diagnosed melanoma.[229,230] Among other things, single nucleotide polymorphisms (SNPs) in a VitD suppl. can contribute to variability of the study results.[236] A report on an increased risk of melanoma with increased VitD intake in men promotes a differentiated approach, while a protective effect was seen in women.[237]

However, when a malignant melanoma manifests, high-dose to ultra-high-dose vitamin D supplementation is under discussion.[208] High circulating s25(OH)D levels were associated with reduced melanoma progression and improved survival.[235]

15.4 Basal cell carcinomas

The basal cell carcinomas (BCC) observed with sphingosine-1-phosphate receptor modulator therapy could also benefit by VitD suppl. It has been shown that there is a significant association between lower s25(OH)D levels in primary and recurrent BBC. The BBC recurrence rate was significantly reduced with 25(OH)D values >25ng/ml.[238]

The complexity of assessing s25(OH)D levels in BCC and melanoma is demonstrated by a dose-response meta-analysis where skin cancer risk was analyzed. In this study, a possibly high VitD status showed an increased risk for melanoma and BCC but could also be distorted by increased sun exposure.[239]

In studies of people who did not have MS, regular use of VitD was associated with fewer cases of melanoma compared to no use.[240]

15.5 Gut microbiome and melanoma

The gut microbiome has been recognized as a potential new player in the pathogenesis and therapy of malignancies.[241] Gut microbiota composition has been noted to change in early-stage melanoma from in situ to invasive and then metastatic disease.[241] VitD deficiency compromises the integrity of the gut barrier and the composition of the gut microbiome community. A Vit D supplement protects the intestinal barrier and positively changes the intestinal microbiota.[242] It is also hypothesized that radiotherapy alters the composition of the gut microbiota, which in turn negatively affects the response to radiation therapy.[242] It has also emerged that the gut microbiome can modulate the response to immune checkpoint inhibitors (ICIs) via the immune system.[243]

A recent study of a VitD supplement on cancer mortality in the general population showed a 12% lower cancer mortality with daily VitD suppl. Daily VitD intake is decisive in contrast to bolus administration, in which no reduction in mortality was observed. Subjects who started VitD therapy before cancer diagnosis seemed to benefit most from this daily substitution,[244] which supports prevention in PwMS.

The differentiation of the effect of a VitD suppl. was shown in a study in the general population, where the success of a supplement depending on body weight was examined. It was shown that with a normal body weight (BMI [body mass index] <25.0; calculated as weight in kilograms divided by height in meters squared) the incidence of cancer was significantly reduced by 24% and mortality was reduced by 42% with a daily intake of 2000 IU VitD.[245] A 22% lower incidence of autoimmune diseases was also registered.[246,247]

16. PROs as Patient-centered Therapy Management- current MS reality?

The presence of personalized medicine is becoming more and more important. Patient-

reported-outcome measurements (PROs) have been shown to be effective in assessing treatment outcomes but are time-consuming.[248] This is all the more important since the assessment of the effectiveness of interventions in MS from the patient's perspective is different than that from the therapist's perspective. There is a significant discrepancy between the physicians' and the patients' assessments of drug side effects and the reduction in their quality of life (QoL) in PwMS.[249,250]

The motivation of the practitioners of PwMS for VitD suppl. should also be reinforced by attention to QoL. Fatigue occurs in about 50-60% of PwMS, affects about 80% of them and contributes significantly to lowering QoL.[251,252,253]

There are increasing indications that high-dose VitD-suppl. alone or in combination with DMTs is associated with improvements in mood, mental health, physical health and fatigue.[250,254-257]

17. Pharmacokinetics of Vitamin D

A significant problem in assessing the results of supplementation studies is that the VitD dose is administered as a bolus dose rather than daily. Orally ingested vitamin D appears in the blood with a maximum after 12 hours and the half-life is around 12-24 hours. Because of this short half-life, large bolus doses of vitamin D of 50,000 to 100,000 IU are cleared from the circulation within a week. Long-term daily supplementation of Vit D leads to a slow, sustained increase in 25(OH)D and reaches a steady state after 3-4 months. Bolus doses of VitD lead to oscillating rates of appearance and disappearance with an impact on the immunological effects and also on the results of VitD supplement studies.[258] Such large fluctuations have an adverse effect on the activity of enzymes responsible for the synthesis and degradation of the active vitamin D metabolite, 1,25-dihydroxyvitamin D. This dysregulation leads to reduced concentrations of the metabolites in the extrarenal tissue.[259,260] In randomized controlled studies on Vit-D supplementation in respiratory diseases, it was shown that intermittent high-dose bolus administration is ineffective, although sufficient s25(OH)D values have been achieved.[261,262] One explanation could be that the bolus administration induces 24-hydroxylase activity and leads to an inactivation of vitamin D3 via the formation of 24,25(OH)2D3. This effect persisted for at least 28 days after the bolus.[263]

Large studies have shown that bolus administration even increases bone resorption and the risk of falls, particularly in older people.[264-268]

Even if there are still no international recommendations for PwMS for a VitD supplement as to the dose level in order to achieve optimal immune modulation, it is becoming increasingly clear that a continuous VitD suppl. under control of s25(OH)D levels is necessary for proper permanent restoration of immune homeostasis.[39] Approximate daily VitD doses are between 7000 to 20,000IU/day in the so-called "high dose" range. The safety range of s25(OH)D serum values is between 30-100ng/ml. A daily dosage of <15,000 IU/day is interpreted as "physiological dosage" and as "safe". The additional control of the Ca-phosphate homeostasis and additionally the parathyroid hormone creates further security.[39,42,44,269,270-280]

18. The Role of Obesity in MS and its Relationship to s25(OH)D Levels

A meta-analysis of Mendelian randomization (MR) studies on genetically predicted BMI (Body Mass Index) provides evidence for a causal association of excessive obesity and an increased risk of MS .[281] In both childhood and adulthood, BMI was positively associated with MS and s25(OH)D was inversely associated with MS.[282] In particular, obesity has been identified for the development of CIS (clinical isolated syndrome) and MS in children, particularly in adolescent girls.[283,284] Because obesity is associated with lower VitD levels [247] and thereby the rate of MS recurrence in childhood is increased.[285] addressing childhood obesity through nutritional intervention in the management of MS is inevitable. Eight years ago, an MR study demonstrated that a genetically reduced s25(OH)D value (four responsible SNPs) is strongly associated with an increased susceptibility to MS.[286] The generally accepted definition of BMI for non-Asian is normal = 18.5-24.9, overweight = 25-29.9, obese grade I = 30 to 34.9, grade II/III = ≥ 35.0 kg/m²; Asian: underweight<18.5, normal 18.5-22.9, overweight 23-27.4, obese 27.5+.[287] The VitD deficiency rate ranged from 23.3% in obesity class I to 33.5% in obesity class II/III in Australian adults.[288]

To what extent this definition can still be accepted in the future is revealed by a study where the BMI alone is not sufficient to verify overweight/obesity. The BMI underestimates the "real" obesity, which was determined by DEXA scan in obese.[289] People who were assigned 36% to the obese group by measuring the BMI showed 74% obesity in the DEXA measurement (National Health and Nutrition Examination Surveys.[289] Obesity is the cause of low s25(OH) D levels [247] and the prevalence of VitD insufficiency and VitD deficiency exists in overweight and obese.[247]

Several theories exist as to why obesity is associated with low s25(OH)D levels. In addition to the sequestration in the adipose tissue and the associated lower amount in the circulation, a volumetric dilution of VitD over a larger fat mass is postulated. A disturbed hepatic 25-hydroxylation in non-alcoholic fatty liver (downregulation of the activity of CYP2R1) and the influence of an increased parathyroid hormone level in vitamin D deficiency could also come into question .[247,290-292]

Obesity in newly diagnosed PwMS is associated with greater disease severity and poorer prognosis. The risk of reaching EDSS 3 over a period of 6 years was significantly increased in PwMS with a BMI ≥30 kg/m² compared to patients <30 kg/m² .[293]

In a cross-sectional study, PwMS (RRMS) were shown to have increased central inflammation associated with obesity. The pro-inflammatory molecules IL-6 and leptin and a reduced concentration of the anti-inflammatory cytokine IL-13 were found in the CSF. At the same time, a higher clinical disability was registered.[294] Low s25(OH)D levels were associated with elevated serum IL-6 levels.[295]

18.1 Improvement of the clinical outcome by vitamin D supplementation?

A VitD suppl. lowers serum IL-6 levels.[39,296,297] VitD may be able to redirect the pro-inflammatory IL-6 function in activated human T helper cells to the production of anti-inflammatory IL-10 via autocrine vitamin D signaling.[298] A high VitD suppl. with the consequence of rising 1,25-(OH) 2D3 serum levels increases the IL-13 level.[299,300]

This would be beneficial as increased levels of IL-13 in the brain can effectively limit lesions

and myelin loss. Among other things, this cytokine improves the properties of the BBB through increased tight junction components.[301]

18.2 Weakened reaction of a vitamin D supplementation in obesity – altered Vitamin D metabolism?

In a supplementation study (VITAL), mean total s25(OH)D levels were significantly lower (38.6 vs. 45.9 ng/mL) at the same Vit D dose after one year of follow-up in obese compared to normal-weight participants (2000IU/day).[302] It is highly probable that the recording of the VitD suppl. is reduced in the overweight and obese. It would need to be clarified whether resistance to vitamin D activity occurs at the target tissue or organ levels in obese individuals.[303] In VitD Suppl. studies, this would explain the negative outcome in PwMS without differentiation between normal-weight and obese patients. An optimal dose/day or the s25(OH) level to aim for in obesity cannot currently be determined. There are various causes that could explain a reduced serological reaction to Vit D suppl. It has been discussed that PwMS may have altered gut motility [304] and disturbances in the uptake of dietary fat [305] and changes in the microbiota could alter bile acid metabolism,[306] which could interfere with the uptake of Vit D as a fat-soluble vitamin.[143,307] The daily dose varies greatly, depending on the individual response, to achieve an effective 25(OH)D level. Individuals can be high, mid, and low responders to Vit D.[308] Uniform data on the daily dose for all PwMS are not possible because various specific genetic errors in the VitD metabolism determine the serum VitD level.

For example, the CYP24A1 rs2762943 polymorphism for MS may be associated with susceptibility, disease progression, increased pro-inflammatory environment and lower 1,25(OH)2D3 levels.[309] A study with high doses of Vit D of 14,000 IU/day proved that the Vit D-related genetic background influences the serological reaction and that this must be considered in supplementation studies in order to avoid misinterpretations.[310, 311] The role of multiple Vit D -related polymorphisms in MS severity is controversial.[312]

Furthermore, the hypothesis of a VitD resistance due to a blockage of the VitD receptor (VDR) is discussed. 25% of the population does not respond adequately to conventional doses of VitD. The cause could be a different response to VitD at the molecular and biochemical level.[308,313-318]

19. Socio-medical Aspects as an Indication for a Daily "Vitamin D Basic Therapy"?

Since the effects on reduced professional performance, especially in the early stages of MS, receive less attention, even small positive influences on the quality of life of PwMS should be used. The early onset of the disease, sometimes with the start of vocational training, with manifestations of fatigue, depressive phases, cognitive deficits, reduced visual memory, concentration disorders are drastic experiences.[79,81]

Loss of social status with abandonment of a higher-skilled occupation to a less demanding occupation as a result of information processing impairment,[81] unemployment, family concerns in what may be the most productive years of life, and the impact on the family situation are factors of note for therapeutic management and should be realized in the supervision.[319,320,321] The possible potentiation of a DMT therapy

by vitamin D supplementation opens up the chance of a clinically stable course and a reduced risk of loss of earnings or an insufficient disability pension.

The relationship between VitD deficiency (calcidiol levels [s25(OH)D]) and poorer performance in measurements of spatial working memory and visual memory has been investigated for 10 years.[78] Nonverbals (visual memory) benefit from higher doses of a VitD supplement (4000IU/day) at 25(OH)D levels around 52ng/mL.[322]

Depression can be associated with reduced cognitive functioning.[323,324] The molecular biomarkers sNfL and vitD play an important diagnostic role in cognitive impairment in PwMS, namely to predict long-term cognitive performance.[83,84] A slowed down information processing speed (IPS) has been verified in PwMS. However, the IPS could be maintained with a Vit D supplement (and Vit B12) through long-term neuroprotection.[78,83,272,325]

It has recently been confirmed that high doses of VitD improve quality of life and fatigue.[250] If comorbidity depression and cognitive dysfunction occurs, the determination of the peripheral biomarkers TNF-alpha, hsCRP and 4-hydroxynonenal could contribute to the assessment of the state of health.[326] The patient-reported outcomes (PROs) of the therapy and the assessment of their quality of life are to be included as a building block for the therapy strategy.[248,327] (Figure 3)

20. Discussion

The goal of delaying or halting disability in PwMS and improving quality of life requires consideration of the extreme complexity of the immunological mechanisms. Genetic, epigenetic, hormonal, nutritional, geographic, and cultural influences must be incorporated into the strategy.[328]

In the scientific community, the VitD -Suppl. has been discussed controversially for decades, not always to the advantage of PwMS, because this means that recommendations for a "basic therapy with VitD supplement" are pushed to the future.[329] Recommending this simple, inexpensive add-on therapy for PwMS with few side effects[330] met with little response.[279] There is general consensus that MS is a multifactorial disease, with genetic predisposition and adverse environmental factors playing major roles. However, the reduction of a risk factor, that of vit-D deficiency, should not be neglected as an easily changeable variable.[331]

20.1 No international agreement on target values for s25(OH)D values

For 30 years there have been around 9 preclinical, and over 15 clinical, studies on VitD-Suppl. Since then, there has been great controversy about the results, because international studies cannot agree on exact definitions of the "normal values for s25(OH)D in autoimmune diseases". The normal values of healthy people cannot be the benchmark for patients with autoimmune diseases because immune homeostasis is disturbed in this patient group. In particular, the duration of VitD administration varies in each study and rarely exceeds 96 weeks. Almost every publication gives different values for "Vit-D deficiency", "Vit-D insufficiency", "ideal VitD level" and "upper safety limit of the VitD level".[332]

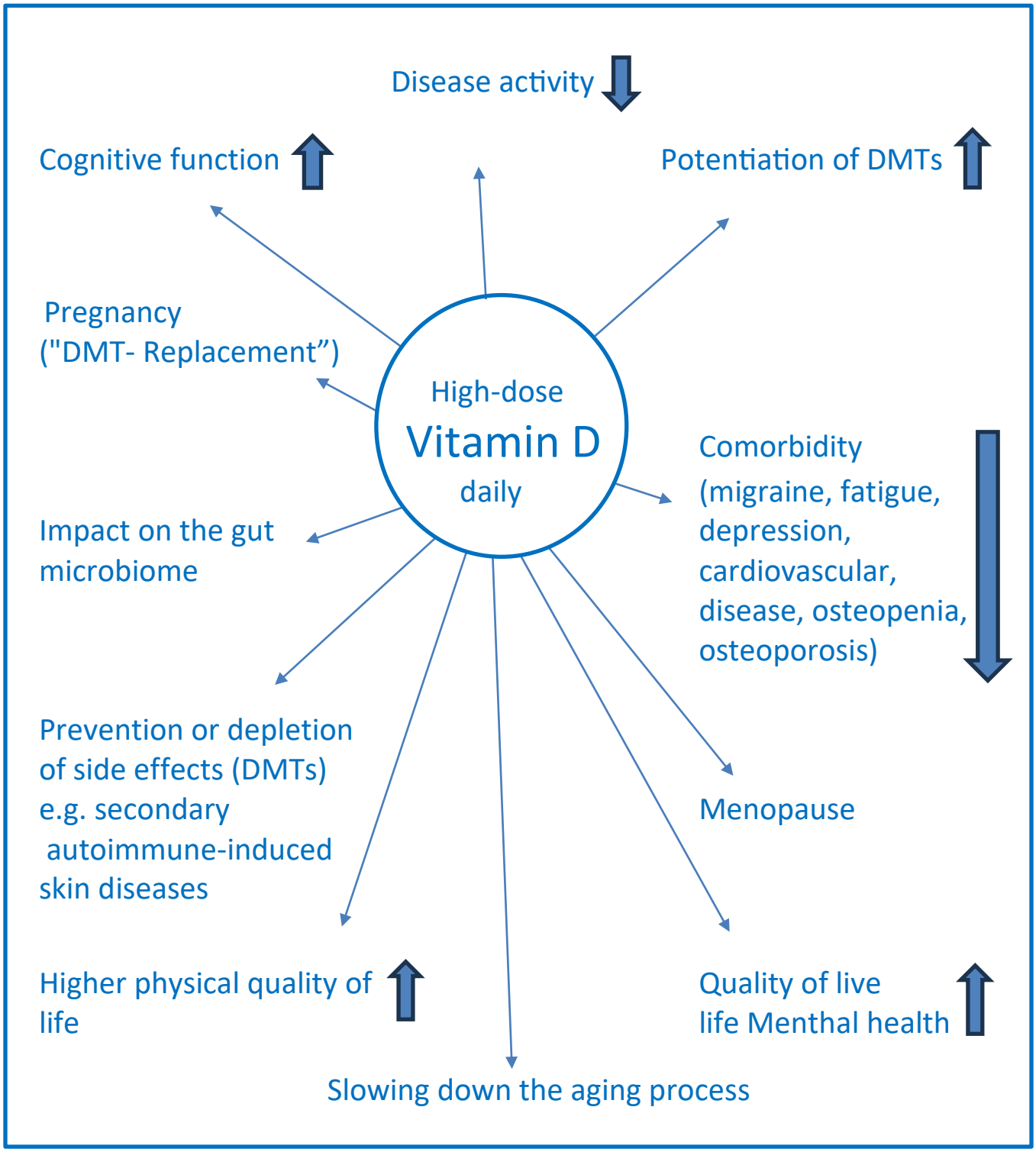

Figure 3: Spectrum of effects of vitamin D supplementation in PwMS Increase (↑) decrease (↓)

Likewise, there is no recommendation for daily high-dose VitD administration (which is pharmacologically justified), which must also be individually based on the absorption rate of the PwMS and the serum control values of 25(OH)D. Compared to controls, PwMS show reduced absorption and altered metabolism to a Vit-D supplement, which reflects different VitD pharmacokinetics in PwMS.[143]

20.2 Vitamin D s25(OH)D levels and genetics

The VitD level is influenced by genetic factors.[312]

VitD-related genetic polymorphisms affect the serological response to high-dose VitD supplement (e.g., 14,000IU/day for 48 weeks). The VitD-binding protein (DBP), for example, with the high-risk allele *rs 7041*, showed a reduced serological reaction.[310] *Cyp27B1* (1-alpha-hydroxylase) catalyzes the hydroxylation of the inactive 25(OH)D to the active 1,25(OH)D2 D3. *Cyp27B1*-linked high-risk alleles also influence the serological response and the course of MS.[310] Each SNP was associated with an independent and significant negative impact on serum 25(OH)D3 and its effect was additive.[328] SNPs of *CYP24A1 rs 2762939* are associated with reduced VitD levels and could also influence the severity of MS.[312]

20.3 Different study designs - serious weaknesses

Another factor in the study design is the differing duration, which is sometimes far too short and only extends over a few months and is rarely designed to last several years.[332]

In a recent study /VIDAMS [Vitamin D to Ameliorate MS]), a daily VitD dose of 5000 IU/day for 96 weeks as add-on therapy to glatiramer acetate with MRI monitoring over 2 years was carried out with 89 patients initially and, among other things, the recurrence rate, the number of relapses requiring treatment, the number of new or enlarging T2 lesions, changes in the EDSS scale (Expanded Disability Status Scale), changes in the MSFC score (Multiple Sclerosis Functional Composite), changes in visual acuity, changes in brain parenchymal volume, changes in cortical thickness and changes in health-related quality of life.[333]

The evaluations show no significant benefit from a VitD supplement. with regard to relapse, MRI endpoints and clinical condition at a dosage of 5000IE/day and "possibly a VitD suppl. does not appear beneficial".[334] This raises the question of whether the dosage was high enough (dose-response relationship). Significant limitations are moderated by the authors on the outcome of the study.

However, the i) too few study participants, ii) too short duration of the study and iii) possibly too low 25(OH)D level aimed at are discussed as causal factors for the negative result.[334]

However, due to the lack of a second DMT comparison group, this study could not make any direct statements about a possible effect modification.[335]

Another point of criticism is the exclusion in this study of PwMS, who showed a vitamin D deficiency (25(OH)D <15ng [37.5nmol/L). Therefore, it should be discussed to substitute especially PwMS with VitD deficiency as a target group. In the study by Cassard et al.[334] 5,000IU/day are also referred to as "high dose", while on the other hand 14,000IU/day or doses from 7,000IU to 20,000IU/day are

classified as "high dose" and have been used in therapeutic studies.[39,310]

An essential criterion for VitD suppl. was not taken into account when characterizing the subjects, namely body weight (BMI). BMI is associated with an altered response to VitD supplementation, and this may explain the decreased results observed in supplementation studies.[247, 303] It is biologically plausible that the in vivo effects on serum or plasma cytokine levels and on the proportions of key immune cell populations implicated in MS [39] are dose dependent. In order to achieve an optimal therapeutic range of s25(OH)D, physiological doses of up to 15,000 IU/day are not associated with increased undesirable side effects.[39] The safety range is between 30 and 100ng/mL s25(OH)D values.[39]

With supplementation of 14,007 IU/day cholecalciferol (SOLAR study) for 48 weeks, the number of new gadolinium (Gd+) -enhancing or new/enlarging T2 lesions on MRI was significantly reduced by 32%, but there was a non-significant trend towards a lower annual relapse rate (ARR).[44] With a dose reduction to 7,143 IU/day in another study with a duration of 96 weeks, no significant reduction in ARR was verified with 129 PwMS, with a further 90 PwMS a significant reduction in ARR was described after 96 weeks.[269]

Rather, there are indications that "supraphysiological doses" and early administration of vitamin D are a prerequisite for therapeutic efficiency in order to achieve 1,25-dihydroxyvitamin D serum values for controlling the neuroinflammatory processes.[9]

The PREVANZ study of vitamin D supplementation in high-risk CIS examined whether VitD supplement delays the time to new clinical or radiological activity. It was shown that VitD monotherapy alone is not an effective treatment for preventing the development of relapsing-remitting MS. However, the study only lasted 48 weeks and only 49 patients received a dose of 10,000 IU/day. Only a few patients had VitD deficiency (less than 50 nmol/l). It took 12 weeks to reach a steady state of 25(OH)D levels, so only 36 weeks allowed VitD to show its optimal effect. A similarly designed study has recently been completed in France, where 100,000 IU/Vit D every 14 days was studied for a maximum of 24 months until conversion from CIS to full MS. (Details in NCT01817166.[336]

20.4 Early Vitamin D supplementation increases the benefit

It is usually not possible to determine how long the CIS symptoms existed before the diagnosis and the start of the study. Hypothetically, the pathologic process may have been smoldering for years because unrecognized demyelinating events often precede the clinical onset of multiple sclerosis (MS). It has been demonstrated that sNfL levels were elevated 6 years prior to the onset of clinical MS, suggesting that MS can have a prodromal phase lasting several years and that neuroaxonal damage already occurs during this phase.[337] Several years before diagnosis, MS patients presented various clinical manifestations such as anxiety, depression, migraines and reduced cognitive function.[338,339] A benefit of an early VitD-suppl. is plausible since PIRA is involved early in the disease process. Smoldering MS occurs in all MS phenotypes.[15]

The willingness of the therapists to take a daily, high-dose VitD -suppl. including it in the

therapy spectrum could be facilitated by the reassessment of the clinical course of MS by the "International Advisory Committee on Clinical Trials in Multiple Sclerosis". In the future, the focus will be on personalized treatment, taking into account the individual pathology and knowledge, since the progressive biology from the onset of the disease and also neurodegenerative processes are involved.[12]

20.5 Indication for Vitamin D supplement includes comorbidities

The indication for VitD-suppl. can not only refer to the outcome of RCT with selected clinical and MRI criteria on the course of MS, but must also consider comorbidities and symptoms. Comorbidities must be of great interest to physicians caring for PwMS as they may be partially preventable or treatable. Furthermore, comorbidities are associated with greater physical and cognitive impairments, a lower health-related quality of life and increased mortality.[340] For example, there is an increased risk of cardiovascular disease.[341] A VitD suppl. can reduce the risk of cardiovascular events.[342]

The era of personalized medicine becomes more urgent with the recognition of the smoldering processes in MS from the start, with the consequent progressive accumulation of disability. The holistic approach for therapy management, for combination therapies and alternative strategies receives the "holy water".[14] Finally, the general question may be raised as to whether, given the proven complex effect of Vit D on the immune system in autoimmune deseases, PwMS should be offered “basic therapy” with a high-dose daily vitamin D supplement, especially in the early stages. This low-cost, easy to-implement medication with few side effects should not be denied for ethical reasons. The questions addressed in the individual RCTs are always just a selection of the diverse therapeutic questions and cannot always answer all the problems affecting the course of the disease.[343]

20.6 VitD suppl. not only make you dependent on relapses of MS

If MS alone is associated with a persistent EBV-specific immune response,[344-350] and the decrease in circulating anti-EBNA1 antibodies in PwMS with VitD3-suppl. is a very consistent finding (humoral response to anti-EBNA-1 is associated with more advanced cortical atrophy and lesion burden), why is Vit D suppl. rejected when the indication is made?[5,157,278,343,344,347-356]

A sufficient s25(OH)D value improves cognitive function[67,78,83,84,85] and requires a VitD supplement straight forward.

20.7 Patient care - an essential element of vitamin D supplementation

The ethics of clinical research have so far only been marginally included under the aspect of patient care.[357,358,359] The obligatory determination of the serum neurofilament light chains (sNfL) immediately after diagnosis of MS and in the future also of sGFAP could set the course for a highly effective treatment for those PwMS. High sNfL values within the first year of illness correlated with a long-term worsening of disability.[57] In this initial time of a possibly difficult fate, there is for the VitD -suppl. a time window for the early use of a "complementary basic therapy", because the best therapy results can be expected at this point in time, both experimentally and from a clinical point of view.[9,47,49,125,360]

A vitD suppl. could also be used to prevent colorectal cancer. Overall survival and cancer-specific survival in the early phase after colorectal cancer diagnosis were lower in PwMS than in those without MS.[361] There was an inverse association between circulating s25(OH)D levels and the risk of developing colorectal cancer in people without MS. This correlation was particularly evident in young patients (<50 years) and in people ≥ 50 years.[362]

20.8 Outlook into the future

If one favors the increasing understanding of the immune pathogenesis of MS through therapeutic intervention as an accepted paradigm in the future and this leads to faster and more efficient implementation of preclinical research into practical clinical application (translational/reverse medicine) [89, 363] there is also the add-on therapy with a VitD suppl. an option in therapy with DMTs (ocrelizumab). Long-term treatment with ocrelizumab leads to a reduction in CD4+CD25+FOXP3+ regulatory cells. [364] These Treg cells are essential for maintaining self-tolerance and their impairment has been linked to autoimmunity and MS.[365,366] Even before ocrelizumab therapy, CD4+FOXP3+ Treg cells were lower in PwMS than in healthy controls.[364] In order to restore homeostasis, high-dose adjuvant vitD suppl. promote CD4+CD25+FoxP3+ Treg cell and CD4+IL-10+FoxP3− Tr1 cell development and suppressive function.[184]

Lymphopenia with ocelizumab therapy is associated with an effect on CD8 T cells and a greater decrease in this subpopulation was shown.[367] As a result, the antiviral function of CD8 T cells can be weakened and upper and lower respiratory tract infections and viral influenza as well as herpes virus related infection could develop. However, infections of the urinary tract have also been observed with ocrelizumab.[367] Despite indications of the heterogeneity of studies on vitD suppl. there are complex pathophysiological mechanisms as a preventive measure and therapy, such as the protective effect of 1,25(OH)2D3 on the mucosal homeostasis of the respiratory and urogenital tract and at the same time promotes the killing of pathogens via the induction of antimicrobial peptides.[368] The tight junction proteins, adherens junction proteins (ZO-1, Occludin, Claudin-10, ß-Catenin, VE-Cadherin) play an important role in maintaining the integrity of the lung barrier.[369]

A daily VitD suppl. can prevent and therapeutically influence the risk of acute respiratory infections and infections of the urinary tract.[370-383] Systemic infections can lead to MS relaps[384] and practicing neurologists may have a tendency to recommend PwMS less DMTs.[385]

During treatment with ocrelizumab, learning the immunopathogenesis through therapeutic interventions,[89] shows that the CD8+ T cells can be assigned an essential role in the development of MS disease activity.[386]

In addition to helper T cells (CD4+ cells predominantly in the perivascular space), cytotoxic CD8+ T cells in the parenchyma have also been described in MS lesions.[387] Intensive accumulation of CD8+ T cells in acute and chronic plaques is well documented.[388,389,390]

CD8+ T cells have higher expression of VDR than CD4+ T cells.[391] Lysandropulus et al. found that CD8+ T cells treated with 1,25(OH)D2D3 secreted lower amounts of the pro-inflammatory cytokines IFN-gamma and TNF-alpha and

more anti-inflammatory cytokines such as IL-5 and TGF-ß.[360]

Ocrelizumab markedly enriched CD8+CD28- regT cells with decreased CD4+FOX P3 regT cells.[364] The latter are reduced in PwMS anyway (especially in children)[364,392] so that it is possible to remedy this relative deficiency. The regulatory T-cells (Treg cells) are considered to be of major importance in the pathophysiology of MS.[125] Vit D suppl. on the other hand, promotes CD4+CD25+FoxP3+Treg cell and CD+4 IL-10+FoxP3-Tr1 cell development.[184,393] A simultaneous optimal Vit D suppl. to ocrelizumab therapy could exert a synergistic effect with the aim of reduced disability progression in PwMS.

Mathias et al. observed under ocelizumab therapy a loss in memory CD8+CD20+ and central memory CD8+ T cells.[394] The loss of memory CD8+T cells correlated with lower CXCR expression and CNS-related LFA-1 integrin expression as well as a reduced antiviral cellular immune response. This constellation could be the cause of infection in 18.4% of ocrelizumab -treated patients.[394] This "gap" in the immunological defense could be closed by vitD suppl. Avoiding infections could also promote long-term adherence to therapy with DMTs in PwMS.

The current continuing risk of severe infection from COVID-19 infection should be a dual motivation for daily high-dose vitamin D administration.[395,396] An s25(OH)D level of 40-60ng/mL should be achieved by dosing up to 6000IU/day.[397] A daily intake of 10,000IU/day vitD for 4 weeks would result in a more rapid optimal s25(OH)D level in "status nascendi" infection.[398] The further daily VitD dose will depend on the s25(OH) values. However, in the case of severe respiratory infections (COVID-19) or sepsis, a single oral bolus dose (100,000 to 500,000IU) or divided dosis of 50,000IU are administered to ensure rapid (within 3-5 days) adequate intracellular supply of calcitrol to he to make available.[382]

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The current continuing risk of severe infection from COVID-19 infection should be a dual motivation for daily high-dose vitamin D administration. An s25(OH)D level of 40-60ng/mL should be achieved.

In order to slow down the progression of MS and to maintain a good quality of life from PwMS in the long term, all available therapeutic options based on currently recognized scientific knowledge and experience must be offered.

VitD supplementation is a factor in modulating the course of the disease. This add-on therapy should be used in orchestral interaction, especially with DMTs by potentiating their effect from the beginning of the MS disease. Since the manifestation of MS mostly coincides with the peak of productive age, the multifactorial effect of vitamin D and the broad mode of action when taken daily must be presented to the PwMS through intensive education in order to ensure their adherence. The treatment goal of daily Vit D supplementation is to stabilize or reduce disease activity, delay disability and avoid or reduce comorbidities. A synergistic effect in therapy with DMTs, the replacement of DMTs during pregnancy, the preservation of cognitive function should be considered in everyday clinical practice and

this adjuvant therapy with a broad spectrum of effects should not be denied to PwMS. In order to prophylactically counteract the risk of infections caused by DMTs, an essential therapeutic goal is to reduce inflammatory cytokines, especially IL-6. Adherence to disease -modifying therapies could be supported by vitamin D supplements.

The" plures effectus" of vitamin D on a wide variety of pathophysiological mechanisms recommends vitamin supplementation for all people with MS as part of a comprehensive health concept [330].

The question asked over 6 years ago as to whether the connection between vitamin D and the immune system will ever lead to a solid marriage[399] can now be answered in the affirmative due to the accumulation of positive results with high-dose, daily vitamin D administration - at least until "Evidence-based reasons for divorce", such as damage caused by a vitD suppl., become known.

Abbreviations

BMI Body mass index
CAL chronic active lesions
CIS clinically isolated syndrome
CNS Central nervous system
DMTs disease modifying therapy
DEXA dual energy X-ray absorptiometry
EDSS-Scores Expanded Disability Status Scale
GFAP Glial fibrillary acidic protein
IL interleukin
MS multiple sclerosis
MG microglia
MK macrophages
MRI magnetic resonance imaging
OCT optical coherence tomography
PIRA progression independent of relapse activity
PwMS Persons with multiple sclerosis
SNP single nucleotide polymorphisms
sNfL Serum neurofilament light chains
sGFAP glial fibrillary acidic protein
s 25(OH)D serum-25(OH)D
Treg cells T-regulatory cells
1,25(OH)D2D3 1,25-dyhydroxy-vitamin D
VitD Vitamin D
VitD suppl. Vitamin D supplementation
VDR Vitamin D Receptor

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