Decreased plasma levels of 25(OH)D in multiple sclerosis patients. Correlation with disease severity expressed by EDSS, MSSS, progression index and Herbert’s scale severity grade

Bucova M1, Durmanova V1, Cudrakova D2, Blazickova S3, Gmitterova K4, Klimova E5, Lisa I4, Kluckova K1, Majernikova B1

Institute of Immunology, Faculty of Medicine, Comenius University Bratislava, Bratislava, Slovakia.
maria.bucova@fmed.uniba.sk

ABSTRACT
OBJECTIVES: Multiple sclerosis is a chronic inflammatory and autoimmune demyelinating disease of the brain and spinal cord. Vitamin D has anti-inflammatory and anti-Th1, Th17 activities, activates the function of regulatory T cells, shifts the immune response towards Th2, so it might be favorable for downregulation of the disease pathogenesis, and if inflammation and Th1 and Th17 immunity are hyperactivated. The aim of our study was to highlight the role of vitamin D in multiple sclerosis pathogenesis.

METHODS: We investigated 178 patients with multiple sclerosis. Plasma levels of 25(OH)D and HMGB1 were investigated.

RESULTS: Despite a regular use of VD by patients, the plasma levels of 25(0H)D were significantly decreased in 57% of them, 14.1% had VD deficiency (level of 25(OH)D < 20 ng/mL) and more than 6% of patients had VD severe deficiency with the plasma level of 25(OH)D < 12 ng/mL. The level of 25(OH)D negatively correlated with the severity of the disease (EDSS, index of progression, duration of the disease) and negative association was found also with Herbert’s six severity grades. HMGB1 levels were higher in patients (p < 0.0001).

CONCLUSION: Our result showed that vitamin D deficiency plays a role in multiple sclerosis pathogenesis. We believe that administration of vitamin D to patients at a sufficient dose providing a physiological level of vitamin D could have a positive effect on the course of the disease. However, regular monitoring of vitamin D levels is required, which should be at least within 30-75 ng/mL, and even more, but below the toxicity limit (Tab. 6, Ref. 66).

KEY WORDS: HMGB1, multiple sclerosis, MS, inflammation, vitamin D.

Introduction

Multiple sclerosis is a chronic inflammatory and autoimmune demyelinating disease of the brain and spinal cord with multifocal areas of focal lymphocytic infiltrations that lead to myelin sheath destruction, oligodendrocyte, axonal and neuronal damage. Pathological processes occur predominantly in white matter, although grey matter infiltrates are also detected (1–3). This leads to a significant disability with deterioration of the motor, sensible, autonomic and neurocognitive functions (1, 4, 5). Neurological symptoms include: sensory disturbances, optic neuritis, loss of vision, limb weakness, ataxia, bladder dysfunction, cognitive deficits and fatigue (6–9).

The cause of MS that affects more than 2.5 million people worldwide, predominantly young adults between 20–40 year, is multifactorial and still unknown (10). It is undeniable, that besides genetic background also environmental factors, stress, immune abnormalities and other factors play a role in MS development. The higher frequency of MS is seen in women, who are affected twice as often as men (11).

It has been long known, that latitude influences MS risk, the prevalence of the disease is minimal at the equator and increases with North or South latitude (12). MS prevalence may change also after migrations that occurred during the second decade of life, with a beneficial effect for people, who migrated from a high latitude region with a higher MS prevalence to a summer, lower latitude region with low MS prevalence (13, 14). Sloka et al (2011) reported that MS prevalence is inversely correlated to the level of solar radiation (15). It was also documented that people, who spent enough time outdoors during childhood and adolescence or people, who prac-
ticed many outdoor activities during their youth, and thus received more UVB, have a significantly lower risk later for MS (16–18).

There are two important key pathogenic events in MS development – autoimmune mechanisms and neuroinflammation. The first one arises from the failure or loss of the tolerance and decreased number or function of regulatory T cells. Th1- and Th17 type of lymphocytes with their production of IL-2, IFN-gama / IL-17 or IL-22 respectively, as well as myelin-reactive CD4+ T cells and autoreactive CD8+ cytotoxic T-lymphocytes play an important role in development of MS (19–26).

In the recent years, the attention has been focused on the role of chronic low-grade inflammation, that accompanies myelin degradation and neurodegeneration and the role of pro-inflammatory cytokines has been studied. The process of inflammation in MS that represent the second key pathogenic mechanism in MS development is promoted by several pro-inflammatory cytokines produced by the immune cells themselves and resident cells like activated microglia (27). Consecutive damaging pathways involve the transmigration of activated B-lymphocytes and plasma cells, which synthesize antibodies against the myelin sheath, boost the immune attack, and result in ultimate loss of myelin (28).

Vitamin D (VD) – a „sunshine“ vitamin was primarily known to regulate the bone calcium-phosphate homeostasis. Later it was found that it plays also a major role in many extra-skeletal metabolic processes, even it can modulate the function of both natural and adaptive immunity.

There are three major immune activities of this vitamin that can have an impact on MS pathogenesis. VD has 1) a direct anti-inflammatory activity; 2) suppresses the Th1 and Th17 differentiation and thus the Th1 and Th17 immunity; on the contrary: 3) VD promotes the activation of regulatory T cells, that also contributes to downregulation of Th1 and Th17 immunity and inflammation (29, 30). So, vitamin D may inhibit all most important pathogenic mechanisms that can potentiate the development of MS.

As we have mentioned above, chronic inflammation plays a great role in MS development and its progression. In our study, we decided to investigate the plasma level of 25(OH)D (25-hydroxyvitamin D), as a factor influencing many immunological mechanisms, alterations of which can potentiate the MS development. Moreover, we tested also the plasma levels of a modern inflammatory marker HMGB1 (high mobility group box 1 protein). It is a vital intranuclear protein with many intranuclear functions. However, after getting to extracellular milieu it has a strong pro-inflammatory activity and can be passively secreted by necrotic cells or actively produced by activated monocytes, macrophages and also microglia (31). Finally, the aim of our study was to find an association between the evaluated plasma levels of 25(OH)D and MS, severity of MS defined by EDSS (expanded disability status scale) (32), MSSS (multiple sclerosis severity score) (33), PI (progression index) (34) and Herbert's six severity grades H1 – H6 (35).

Subjects and methods

The study was performed at the Institute of Immunology, Faculty of Medicine Comenius University in Bratislava in collaboration with three Slovak specialized neurological centers for multiple sclerosis therapy (two in Bratislava – the 1st Department of Neurology, Faculty of Medicine, Comenius University and University Hospital, the 2nd. Department of Neurology, Faculty of Medicine Comenius University and University Hospital in Bratislava and one in Presov – Centre for Therapy and Diagnosis of Multiple Sclerosis at Faculty Hospital of Jan Adam Raiman in Presov, Slovakia.

The blood samples were obtained during diagnostic procedures from 178 patients into a 9 mL tube with EDTA (ethylendiamine tetra acid) to prevent coagulation. Immediately after that, the blood was centrifuged (3000 rpm for 10 min), subsequently the plasma was sucked off and distributed in small Eppendorf tubes and after that the plasmas and Buffy coats (for DNA isolation) were frozen in a deep freezer box (–80 °C), where they were stored until analysis. The study was approved by the local Ethics Committee for Research of the Faculty of Medicine Comenius University and University Hospital Bratislava, Slovakia and each patient signed an informed consent.

178 patients were enrolled in the study (125 women (70.22 %) and 53 men (29.78 %). 162 of them had relapsing remitting form of MS (RR-MS), one primary progressive form (PP MS) and 15 patients had secondary progressive form of MS (SP MS). The diagnosis of MS was based on the revised McDonald criteria (36).

Patients underwent a complex clinical investigation, laboratory and MRI (magnetic resonance imaging) investigation and corresponding physicians from above mentioned specialized MS centers were responsible for exact diagnosis and therapy of MS patients. The degree of neurological disability at the time of examination was quantified using Kurtzke`s extended disability status scale (EDSS) (32). EDSS is used to express the disability of MS patients and MSSS is used to express the severity of the disease using disability and disease duration (33). In addition, a progression index (PI) is used, that indexes progression, calculated as disability grade divided by duration of the disease (34). The latest MS classification system includes a patient's disability scale according to the severity of the course, relative to the MSSS score – Six degrees of disease severity, Herbert's six severity grades: H1–H6 (35).

The plasma level of 25(OH)D was evaluated by electrochemiluminescent binding test (Elecsys Vitamin D total-cobas; Roche Diagnostics GmbH, Mannheim, Germany) in Laboratoria Piestany Ltd., Piestany, Slovakia.

Moreover, in the cohort of 178 MS patients (125 women (70.22 %) and 53 men (29.78 %) the plasma level of HMGB1 (high mobility group box 1) protein was determined by a sandwich enzyme-linked immunosorbtent assay (ELISA; Shino-test Corporation, Japan/IBL Technology USA) according to the manufacturer's protocol. Finally, the obtained results concerning 25(OH)D were compared within subgroups of patients and correlation analysis with EDSS, MSSS, PI, H1-H6 and plasma levels of HMGB1 were calculated.

31 healthy subjects (20 women and 11 men) were also enrolled into the study as a control group to test the plasma levels of HMGB1. All of them were healthy, without MS and any other neurological diseases, without autoimmune, allergic or oncological diseases and without any acute or chronic sickness.
Statistical analysis

The INSTAT 3 program (GraphPad Software Inc., San Diego, CA, USA) was used to statistically evaluate the measured parameters. The one-sample Kolmogorov–Smirnov test was used to determine whether the investigated population followed a normal distribution. Mean and medians were determined by an unpaired t-test with Welch correction, or non-parametric Mann–Whitney test. One-way ANOVA and Kruskal–Wallis test were used when comparing the median of three or more variable values. For correlation analysis, the Pearson’s test (linear relationships), or non-parametric Spearman’s test were used. A significant difference was assumed if p value was < 0.05.

Results

Characteristics of baseline demographic and clinical data of investigated patients

We investigated 178 MS patients, the most represented form was the relapsing-remitting form of MS. The duration of the disease was higher in women (11.1±6.6; p = 0.03) than in men (7.9±6.59). The EDSS score was significantly higher in women with MS (EDSS: 3.10±1.29 vs 2.50±1.28, p = 0.0364). The MSSS score did not differ. The progression index was higher in men (p = 0.04932), with a shorter, but more severe duration of the disease (p = 0.03) (Tab. 1).

25(OH)D sufficiency, insufficiency, deficiency and severe deficiency in MS patients. Comparison of plasma level of 25(OH)D vitamin in men and women

Despite a regular use of VD by MS patients, only approximately 43 % of all investigated patients were VD sufficient (the plasma level of 25(OH)D ≥ 30 ng/mL), 57 % had decreased plasma level of 25(OH) vitamin D – 36.7 % had VD insufficiency (level of 25(OH)D between 20–29 ng/mL), 14.1 % had VD deficiency (level of 25(OH)D < 20 ng/mL) and more than 6 % of MS patients had VD severe deficiency with the plasma level of 25(OH)D < 12 ng/mL (Tab. 2). Men had higher levels of 25(OH)D than women, however, the difference was not statistically significant. We did not find the difference neither between individual forms of MS (Tab. 3), probably also due to uneven representation of the tested subgroups. The most represented form was the RR MS (162 patients), the others were less represented, which could affect the result.

Comparison of plasma level of 25(OH)D with disease severity according to Herbert’s six severity grades H1-H6 in MS patients

The Kruskall–Wallis test revealed a statistically significant negative association between the plasma level of 25(OH)D and disease severity according to Herbert’s six severity grades (p = 0.0446) (Tab. 4). Patients with the most severe disease grades (H5 and H6) had significantly lower levels of vitamin D than the patients with H1-H4 (p = 0.0032). The level of 25(OH) D was lower in all grades of severe disease compared to the remaining grades (p < 0.05) (Tab. 4).
Vitamin D – the „sunshine“ vitamin, was primarily known as a micronutrient regulating bone calcium-phosphate homeostasis. Later it was found that it is a neurohormone that plays also a major role in myriad extra-skeletal metabolic processes, such as glucose metabolism, and in many aspects of cellular functions and immunomodulation (37–40). It has a direct effect on the function of both innate and adaptive immunity via VD receptor expressed on several immune cells (41–44). VD inhibits the maturation and function of DC, downregulates the presentation of antigen and development of adaptive immune response (45, 46). 1,25(OH)2D increases the number of Tregs and directly affects CD4+ T cells (47–50).

Vitamin D deficiency is associated with various disorders such as: autoimmune diseases, infections, cardiovascular diseases, asthma bronchiale, chronic pulmonary diseases, severity and mortality of sepsis, and excess mortality in the general population (30, 51–55).

MS is the most common chronic autoimmune neuroinflammatory demyelinating disease affecting central nervous system (CNS) of young adults worldwide characterized by demyelination and axonal damage in CNS and spinal cord (1–3). Except inflammation, the loss of tolerance and increased Th1/Th17 immune response and downregulated regulatory T cells play a role in MS pathogenesis. Chronic inflammatory processes that continuously disturb neuroaxonal homeostasis drive neurodegeneration, so the clinical outcome probably depends on the balance of stressor load (inflammation) and any remaining capacity for neuronal self-protection (56).

There are three major immune activities of vitamin D, that have an impact on MS pathogenesis: suppression of inflammation, suppression of Th1 and Th17 immunity and activation of regulatory T cells, that also contributes to downregulation of Th1 and Th17 immunity and inflammation (29, 30). Downregulating Th1 immunity and increasing the activity of regulatory T cells, VD participates also in the shifting of T helper (Th) cell response from Th1 (adaptive cell mediated immunity accompanied by inflammation) to Th2 (adaptive humoral immunity). The production of Th1 cytokine IFN-γ together with proinflammatory cytokines is inhibited, the production of Th2 cytokines IL-4, IL-5 and IL-10 is elevated, which leads to the limitation of the potential tissue damage associated with excessive Th1 cellular immune responses associated with hyperinflammation (57, 58). VD has also been proven to promote self-tolerance (38, 59, 60). While mechanisms of adaptive Th1/Th17 immunity are depressed, mechanisms of natural immunity are potentiated (29, 61, 62).

Discussion

Vitamin D – the „sunshine“ vitamin, was primarily known as a micronutrient regulating bone calcium-phosphate homeostasis. Later it was found that it is a neurohormone that plays also a major role in myriad extra-skeletal metabolic processes, such as glucose metabolism, and in many aspects of cellular functions and immunomodulation (37–40). It has a direct effect on the function of both innate and adaptive immunity via VD receptor expressed on several immune cells (41–44). VD inhibits the maturation and function of DC, downregulates the presentation of antigen and development of adaptive immune response (45, 46). 1,25(OH)2D increases the number of Tregs and directly affects CD4+ T cells (47–50).

Vitamin D deficiency is associated with various disorders such as: autoimmune diseases, infections, cardiovascular diseases, asthma bronchiale, chronic pulmonary diseases, severity and mortality of sepsis, and excess mortality in the general population (30, 51–55).

MS is the most common chronic autoimmune neuroinflammatory demyelinating disease affecting central nervous system (CNS) of young adults worldwide characterized by demyelination and axonal damage in CNS and spinal cord (1–3). Except inflammation, the loss of tolerance and increased Th1/Th17 immune response and downregulated regulatory T cells play a role in MS pathogenesis. Chronic inflammatory processes that continuously disturb neuroaxonal homeostasis drive neurodegeneration, so the clinical outcome probably depends on the balance of stressor load (inflammation) and any remaining capacity for neuronal self-protection (56).

There are three major immune activities of vitamin D, that have an impact on MS pathogenesis: suppression of inflammation, suppression of Th1 and Th17 immunity and activation of regulatory T cells, that also contributes to downregulation of Th1 and Th17 immunity and inflammation (29, 30). Downregulating Th1 immunity and increasing the activity of regulatory T cells, VD participates also in the shifting of T helper (Th) cell response from Th1 (adaptive cell mediated immunity accompanied by inflammation) to Th2 (adaptive humoral immunity). The production of Th1 cytokine IFN-γ together with proinflammatory cytokines is inhibited, the production of Th2 cytokines IL-4, IL-5 and IL-10 is elevated, which leads to the limitation of the potential tissue damage associated with excessive Th1 cellular immune responses associated with hyperinflammation (57, 58). VD has also been proven to promote self-tolerance (38, 59, 60). While mechanisms of adaptive Th1/Th17 immunity are depressed, mechanisms of natural immunity are potentiated (29, 61, 62).

Vitamin D has anti-inflammatory and anti-Th1, -Th17 activities, activates the function of regulatory T cells, shifts the immune response towards Th2, so it might be favorable for downregulation of MS pathogenesis, where inflammation and Th1 and Th17 immunity are hyperactivated.

Tab. 5. Correlation of serum vitamin D level with MSSS and PI.

| STUDY GROUP | Number of patients | 25(OH) D Median (ng/ml) | Correlation analysis p value |
|-------------|--------------------|-------------------------|----------------------------|
| MSSS        | 178                | 24.24                   | Pearson’s test p=0.0292     |
| PI          | 178                |                         | Spearman’s test p=0.0144    |

MSSS – multiple sclerosis severity score, PI – progression index

of both innate and adaptive immunity via VD receptor expressed on several immune cells (41–44). VD inhibits the maturation and function of DC, downregulates the presentation of antigen and development of adaptive immune response (45, 46). 1,25(OH)2D increases the number of Tregs and directly affects CD4+ T cells (47–50).

Vitamin D deficiency is associated with various disorders such as: autoimmune diseases, infections, cardiovascular diseases, asthma bronchiale, chronic pulmonary diseases, severity and mortality of sepsis, and excess mortality in the general population (30, 51–55).

MS is the most common chronic autoimmune neuroinflammatory demyelinating disease affecting central nervous system (CNS) of young adults worldwide characterized by demyelination and axonal damage in CNS and spinal cord (1–3). Except inflammation, the loss of tolerance and increased Th1/Th17 immune response and downregulated regulatory T cells play a role in MS pathogenesis. Chronic inflammatory processes that continuously disturb neuroaxonal homeostasis drive neurodegeneration, so the clinical outcome probably depends on the balance of stressor load (inflammation) and any remaining capacity for neuronal self-protection (56).

There are three major immune activities of vitamin D, that have an impact on MS pathogenesis: suppression of inflammation, suppression of Th1 and Th17 immunity and activation of regulatory T cells, that also contributes to downregulation of Th1 and Th17 immunity and inflammation (29, 30). Downregulating Th1 immunity and increasing the activity of regulatory T cells, VD participates also in the shifting of T helper (Th) cell response from Th1 (adaptive cell mediated immunity accompanied by inflammation) to Th2 (adaptive humoral immunity). The production of Th1 cytokine IFN-γ together with proinflammatory cytokines is inhibited, the production of Th2 cytokines IL-4, IL-5 and IL-10 is elevated, which leads to the limitation of the potential tissue damage associated with excessive Th1 cellular immune responses associated with hyperinflammation (57, 58). VD has also been proven to promote self-tolerance (38, 59, 60). While mechanisms of adaptive Th1/Th17 immunity are depressed, mechanisms of natural immunity are potentiated (29, 61, 62).

Vitamin D has anti-inflammatory and anti-Th1, -Th17 activities, activates the function of regulatory T cells, shifts the immune response towards Th2, so it might be favorable for downregulation of MS pathogenesis, where inflammation and Th1 and Th17 immunity are hyperactivated.

Tab. 6. Plasma levels of HMGB1 in patients with MS and healthy controls.

| STUDY GROUP | Number of patients | Median (HMGB1) (ng/ml) | Confidential interval | Mann-Whitney test (2-tailed) p value |
|-------------|--------------------|------------------------|-----------------------|------------------------------------|
| Controls    | 31                 | 2.999                  | 1.686-9.844           |                                    |
| MS (all patients) | 165              | 13.529                 | 2.330-113.45          | p<0.0001                           |
| MS women   | 111 (67.27%)       | 15.098                 | 2.330-113.45          | p>0.05                             |
| MS men     | 54 (32.73%)        | 12.571                 | 2.557-99.725          |                                    |
| RR MS      | 159                | 15.351                 | 2.330-113.45          | p=0.6872                           |
| SP MS      | 6                  | 13.935                 | 3.255-65.608          | p>0.05                             |

HMGB1 – high mobility group box 1; MS – multiple sclerosis; RR – relapsing remitting form of multiple sclerosis; SP – secondary progressive form
25(OH)D is the major circulating form of vitamin D, that has a half-life of approximately 2–3 weeks. It is a summation of both WD produced from sun exposure and WD intake and it is just that vitamin D metabolite used to determine whether a patient is vitamin D deficient, sufficient or intoxicated (63). There is no absolute consensus about normal range for 25(OH)D, but most experts now agree that WD deficiency should be defined as a 25(OH)D level of <20 ng/mL (50 nmol/L). WD insufficiency is classified as a serum 25(OH)D level between 20 and 29 ng/mL (50–74 nmol/L). The preferred level for 25(OH)D is now recommended to be >30 ng/mL (75 nmol/L) (63–65).

The aim of our study was to find association of WD plasma levels with MS and MS severity expressed by EDSS score, MSSS, PI and Herbert’s severity scale H1–H6 and proinflammatory cytokine HMGB1. In our study, we investigated 178 MS patients, in which the most represented form was the relapsing-remitting form of MS (162), which also reflects the real representation in the population. The duration of the disease was higher in women than in men (p = 0.03), the EDSS score was significantly higher in women (p = 0.0364), the progression index was higher in men (p = 0.04932), with a shorter, but more severe duration of the disease (p = 0.03) (Tab. 1).

Despite a regular use of WD by MS patients, only approximately 43% of all our investigated patients were WD sufficient (the plasma level of 25(OH)D ≥30 ng/mL), 57% had a decreased plasma level of 25(OH)D; 36.7% had WD insufficiency (level of 25(OH)D between 20–29 ng/mL), 14.1% had WD deficiency (level of 25(OH)D < 20 ng/mL) and more than 6% of MS patients had WD severe deficiency with the plasma level of 25(OH) D < 12 ng/mL (Tab. 2).

A statistically significant negative association between the plasma level of 25(OH)D and disease severity expressed according to Herbert’s six severity grades H1–H6 was found in MS patients (p = 0.0446) (Tab. 4). Patients with the most severe disease levels (H5 and H6) had significantly lower levels of vitamin D compared to patients with H1–H4 (p = 0.0032). The level of 25(OH) D was the lowest in two most aggressive disease groups (H5, H6), with the highest levels in the group of patients with mild disease. The levels of 25(OH)D were the most significantly negatively correlated also with a disease progression – lower levels of vitamin D3 were associated with a more serious course of the disease – higher MSSS scores (p = 0.0292) and progression index (p = 0.0144) (Tab. 5). We believe that administration of vitamin D to patients with MS at a sufficient dose providing a physiological level of vitamin D could have a positive effect on the course of the disease. However, regular monitoring of vitamin D levels is required, which should be at least within 30–75 ng/mL, and even more, but below the toxicity limit.

The recent systematic review of Berezowska et al (2019) also supports our results and opinion. They selected ten studies, one trial found a significant effect on EDSS score, three demonstrated a significant change in serum cytokines level, one found benefits to current enhancing lesions and three studies evaluating the safety and tolerability of vitamin D reported no serious adverse events. Disease measures improved to a greater extent overall in those with lower baseline serum 25(OH)D levels (66).

Plasma levels of the late proinflammatory cytokine HMGB1 in MS patients were statistically significantly higher than in healthy subjects (p < 0.0001) (Tab. 6) and confirmed ongoing inflammatory process. Our results concerning higher HMGB1 levels in MS patients as compared to healthy controls were demonstrated also by other authors, but they were not correlated with the level of 25(OH)D.

Conclusion

Vitamin D has anti-inflammatory and anti-Th1, Th17 activities, activates the function of regulatory T cells, shifts the immune response towards Th2, so it might be favorable for downregulation of MS pathogenesis, where they are hyperactivated. Our results showed elevated levels of proinflammatory cytokine HMGB1, which points to an ongoing inflammation. Despite a regular use of WD by MS patients, the plasma levels of 25(OH)D were significantly decreased in 57% of them, 14.1% had WD deficiency (level of 25(OH)D < 20 ng/mL) and more than 6% of MS patients had WD severe deficiency with the plasma level of 25(OH) D < 12 ng/mL. The level of 25(OH)D negatively correlated with severity of the disease expressed as EDSS, index of progression, duration of the disease, and negatively associated with a disease the severity expressed according to Herbert’s six severity grades. We believe that the administration of vitamin D to patients with MS at a sufficient dose providing a physiological level of vitamin D could have a positive effect on the course of the disease. However, regular monitoring of vitamin D levels is required, which should be at least within 30–75 ng/mL, and even more, but below the toxicity limit.

References

1. Compston A, Coles A. Multiple sclerosis. Lancet 2008; 372 (9648): 1502–1517.
2. Pierrot-Deselligny C, Souberbielle JC. Vitamin D and multiple sclerosis: An update. Mult Scler Relat Disord 2017; 14: 35–45.
3. Lassmann H, Brück W, Lucchetti C. The immuno-pathology of multiple sclerosis: an overview. Brain Pathol 2007; 17: 210–218.
4. Kalatha T, Arnaoutoglou M, Koukoulidis T et al. Does cognitive dysfunction correlate with neurofilament light polypeptide levels in the CSF of patients with multiple sclerosis? J Int Med Res 2019; 47 (5): 2187–2198.
5. Mayo CD, Miksche K, Attwell-Pope K, Gawryluk JR. The relationship between physical activity and symptoms of fatigue, mood, and perceived cognitive impairment in adults with multiple sclerosis. J Clin Exp Neuropsychol 2019; 41 (7): 715–722.
6. Hughes AJ, Dunn KM, Chaffee T. Sleep disturbance and cognitive dysfunction in multiple sclerosis: a systematic review. Curr Neurol Neurosci Rep 2018; 18 (1): 2.
7. Hughes J, Jokubaitis V, Lugaresi A et al. Association of inflammation and disability accrual in patients with progressive-onset multiple sclerosis. JAMA Neurol 2018; 75 (11): 1407–1415.
8. Razazian N, Yavari Z, Farnia V et al. Exercising impacts on fatigue, depression, and paresthesia in female patients with multiple sclerosis. Med
Sci Sports Exerc 2016; 48: 796–803.

9. Sadeghi Bahmani D, Kesselring J, Papadimitriou M et al. In patients with multiple sclerosis, both objective and subjective sleep, depression, fatigue, and paresthesia improved after 3 weeks of regular exercise. Front Psychiatry 2019; 10: 265.

10. Koch-Henriksen N, Sorensen PS. The changing demographic pattern of multiple sclerosis epidemiology. Lancet Neurol 2010; 9: 520–532.

11. Kurtzke JF. Epidemiology of multiple sclerosis. Does this really point toward an etiology? Lectio doctoralis. Neurol Sci 2000; 21: 383–403.

12. Simpson Jr. S, Blizzard L, Otahal P, Vander Mei I, Taylor B. Latitude is significantly associated with the prevalence of multiple sclerosis: a metaanalysis. J Neurol Neurosurg Psychiatry 2011; 82: 1132–1141.

13. Handel AE, Gavin Giovannoni G, George C, Ebers GC, Sreeram V. Ramagopalun. Environmental factors and their timing in adult-onset multiple sclerosis. Rev Artic Nat Rev Neurol 2010; 6: 156–166.

14. McLeod JG, Hammond SR, Kurtzke JF. Migration and multiple sclerosis in immigrants to Australia from United Kingdom and Ireland: a reassessment. I. Risk of MS by age at immigration. J Neurol 2011; 258 (6): 1140–1149.

15. Slota S, Silva S, Pryse-Phillips W, Patten S. A Quantitative Analysis of Suspected Environmental Causes of MS. Can J Neurol Sci 2011; 38 (1): 98–105.

16. Bjørnevik K, Riise T, Casetta I et al. Sun exposure and multiple sclerosis risk in Norway and Italy: The EnvlMS study. Milt Scler 2016; 20: 1042–1049.

17. Laursen JH, Sondergaard HB, Sorensen PS, Sellebjerg F, Oturai AB. Association between age at onset of multiple sclerosis and vitamin D level-relatedfactors. Neurology 2016; 86: 88–93.

18. van der Mei IAF, Ponsonby AL, Dwyer T, Blizzard L et al. Past exposure to sun, skin phenotype, and risk of multiple sclerosis: case-control study. BMJ London 2003; 327 (7410): 316.

19. Buc M. Role of regulatory T cells in pathogenesis and biological therapy of multiple sclerosis. Mediators Inflamm 2013: 963748.

20. Jaddidi-Niaragh F, Mirshahi R et al. The deviated balance between regulatory T cell and Th17 in autoimmune. Immunopharmacol Immuno toxicol 2012; 34 (5): 727–739.

21. Mohiuddin IH, Pillai V, Baughman EJ et al. Induction of regulatory T cells from memory T-cells is perturbed during acute exacerbation of multiple sclerosis. Clin Immunol 2016; 166–167: 12–8.

22. Muls N, Jnaoui K, Dang HA et al. Upregulation of IL-17, but not of IL-9, in circulating cells of CIS and relapsing MS patients. Impact of corticosteroid therapy on the cytokine network. J Neuroimmunol 2012; 20: 1042–1049.

23. Kleinewietfeld M, Hafler DA. Regulatory T cells in autoimmune neuroinflammation. Immunol Rev 2014; 259 (1): 231–244.

24. Käröncü M, Tüzün E, Türkoglu R et al. Effect of short-term interferon-β treatment on cytokines in multiple sclerosis: significant modu-lation of IL-17 and IL-23. Cytokine 2012; 59 (2): 400–402.

25. Skundric DS, Cruikshank WW, Montgomery PC, Lisak RP, Tse HY. Emerging role of IL-16 in cytokine-mediated regulation of multiple sclerosis. Cytokine 2015; 1043–4666(15)00009-5.

26. Waisman A, Hauptmann J, Regen T. The role of IL-17 in CNS dis-eases. Acta Neuropathal 2015; 129 (5): 625–637.

27. Pérez-Cerdá, Sánchez-Gómez MV, Mutue C. The link of inflammation and neurodegeneration in progressive multiple sclerosis. Mult Scler Demyel Disorder 2016; 1: 9.

28. Lubetzki C, Stankoff B. Demyelination in multiple sclerosis. Handb Clin Neurol 2014; 122: 89–99.

29. Iruretagoyena M, Hirigoyen D, Naves R, Burgos PI. Immune Response Modulation by Vitamin D: Role in Systemic Lupus Erythematosus. Front Immunol 2015; 12 (6): 513.

30. Olejarova M, Dobisova A, Suchankova M et al. Vitamin D deficiency – a potential risk factor for sepsis development, correlation with inflammation markers, SOFA score and higher early mortality risk in sepsis. Bratisl Med J 2019; 120 (4): 284–290.

31. Naglova H, Bucova M. HMGB1 and its physiological and pathologi-cal roles. Bratisl Lek Listy 2012; 113 (3): 163–71.

32. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology 1983; 35 (11): 1444–1452.

33. Roxburgh RH, Seaman SR, Masterman T et al. Multiple Sclerosis Severity Score: using disability and disease duration to rate disease severity. Neurology 2005; 64 (7): 1144–1151.

34. Cendrowski WS. Progression index and disability status in multiple sclerosis: a resurvey of 207 patients in central Poland. Swiss Arch Neurol, Psychiatry Psychoth 1985; 137 (4): 5–13.

35. Charlson R, Herbert J, Kistler I. Severity Grading in Multiple Sclerosis. Int J MS Care 2016; 18 (5): 265–270.

36. Polman CH, Reingold SC, Banwell B et al. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. Ann Neurol 2011; 69 (2): 292–302.

37. Adams JS, Hewison M. Update in vitamin D. J Clin Endocrinol Metab 2010; 95 (2): 471–478.

38. Bivona G, Agnello L, Ciaccio M. The immunological implication of the new vitamin D metabolism. Cent Eur J Immunol 2018; 43 (3): 331–334.

39. Holick MF. Vitamin D deficiency. N Engl J Med 2007; 357 (3): 266–281.

40. Holick MF. Vitamin D: importance in the prevention of cancers, type 1 diabetes, heart disease, and osteoporosis. Am J Clin Nutr 2004; 79 (3): 362–371.

41. Beard JA, Bearden A, Striker R. Vitamin D and the anti-viral state. J Clin Virol 2011; 50 (3): 194–200.

42. Bikle DD. Vitamin D and the immune system: role in protection against bacterial infection. Curr Opin Nephrol Hypertens 2008; 17 (4): 348–352.

43. Mahon BD, Wittke A, Weaver V, Cantorna MT. The immunological implication of the new vitamin D metabolism. J Clin Endocrinol Metab 2010; 95 (2): 471–478.

44. Veldman CM, Cantorna MT, DeLuca HF. Expression of 1,25-dihydroxyvitamin D(3) receptor in the immune system. Arch Biochem Biophys 2010; 500 (2): 334–338.

45. Barragan M, Good M, Kolls JK. Regulation of Dendritic Cell Function by Vitamin D. Nutrients 2015; 7 (9): 8127–8130.

46. Ferrer R, Verbergh L, Verstuyf A, Mathieu C, 1,25-Dihydroxyvitamin D3 and its analogs as modulators of human dendritic cells: A comparison dose-titration study. J Steroid Biochem Mol Biol 2013; 136: 160–165.
47. Cantorna, MT, Snyder L, Lin YD, Yang L. Vitamin D and 1,25(OH)2D regulation of T cells. Nutrients 2015; 7: 3011–3021.
48. Adorini, L. Tolerogenic dendritic cells induced by vitamin D receptor ligands enhance regulatory T cells inhibiting autoimmunlue diabetes. Ann N Y Acad Sci 2003; 987: 258–261.
49. Van Halteren AGS, Tysma OM, van Etten E, Mathieu C, Roep BO. 1,25-Dihydroxyvitamin D3 or analogue treated dendritic cells modulate human autoreactive T cells via the selective induction of apoptosis. J Autoimmun 2004; 23 (3): 233–239.
50. Ferreira GB, Gysemans CA, Demengeot J et al. 1,25-Dihydroxyvitamin D3 promotes tolerogenic dendritic cells with functional migratory properties in NOD mice. J Immunol 2014; 192: 4210–4220.
51. Akdere G, Efe B, Sisman P, Yorulmaz G. The relationship between vitamin D level and organspecific autoimmune disorders in newly diagnosed type I diabetes mellitus. Bratisl Lek Listy 2018; 119 (9): 544–549.
52. Bucova M, Suchankova M, Tibenska E et al. TREM-2 Receptor Expression Increases with 25(OH)D Vitamin Serum Levels in Patients with Pulmonary Sarcoidosis. Mediators Inflamm 2015; 2015: 181986.
53. Gondim F, Caribé A, Vasconcelos KF, Segundo AD, Bandeira F. Vitamin D Deficiency Is Associated with Severity of Acute Coronary Syndrome in Patients with Type 2 Diabetes and High Rates of Sun Exposure. Clin Med Insights Endocrinol Diabetes 2016; 9: 37–41.
54. Lee P, Nair P, Eisman JA, Center JR. Vitamin D deficiency in the intensive care unit: an invisible accomplice to morbidity and mortality? Intensive Care Med 2009; 35 (12): 2028–2032.
55. Sebekova K, Krivosikova Z, Gajdos M, Podrcka L. Vitamin D status in apparently healthy medication-free Slovaks: Association to blood pressure, body mass index, self-reported smoking status and physical activity. Bratisl Lek Listy 2016; 117 (12): 702–709.
56. Friese MA, Schattling B, Fugger L. Mechanisms of neurodegeneration and axonal dysfunction in multiple sclerosis. Nat Rev Neurol 2014; 10 (4): 225–238.
57. Hewison M. Vitamin D and the immune system: new perspectives on an old theme. Endocrinol Metab Clin North Am 2010; 39 (2): 365–379.
58. Lemire JM, Archer DC, Beck L, Spiegelberg HL. Immunosuppressive actions of 1,25-dihydroxyvitamin D3: preferential inhibition of Th1 functions. J Nutr 1995; 125 (Suppl 6): 1704–1708.
59. Colotta F, Jansson B, Bonelli F. Modulation of inflammatory and immune responses by vitamin D. J Autoimmun 2017; 85: 78–97.
60. Vanherwegem AS, Gysemans C, Mathieu C. Regulation of Immune Function by Vitamin D and Its Use in Diseases of Immunity. Endocrinol Metab Clin North Am 2017; 46 (4): 1061–1094.
61. Vim S, Dhawan P, Ragunath C, Christakos S, Diamond G. Induction of cathelicidin in normal and CF bronchial epithelial cells by 1,25-dihydroxyvitamin D3. J Cyst Fibros 2007; 6: 403–410.
62. Chun RF, Liu NQ, Lee T et al. Vitamin D supplementation and antibacterial immune responses in adolescents and young adults with HIV/AIDS. J Steroid Biochem Mol Biol 2015; 148: 290–297.
63. Holick MF. High prevalence of vitamin D inadequacy and implications for health. Mayo Clin Proc 2006; 81 (3): 353–373.
64. Bischoff-Ferrari HA, Giovannucci E, Willett WC, Dietrich T, Dawson-Hughes B. Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. Am J Clin Nutr 2006; 84 (1): 18–28.
65. Holick MF. Vitamin D status: measurement, interpretation, and clinical application. Ann Epidemiol 2009; 19 (2): 73–78.
66. Berezowska M, Coe S, Dawes H. Effectiveness of Vitamin D Supplementation in the Management of Multiple Sclerosis: A systematic Review. Int J Mol Sci 2019; 20 (6): pii: E1301. doi: 10.3390/ijms20061301.

Received July 1, 2019.
Accepted July 15, 2019.