

CLINICAL STUDY

Low serum vitamin D levels are associated with a low percentage of TREM-2⁺ monocytes in low-grade gliomas and poorer overall survival in patients with high-grade gliomas

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ABSTRACT

INTRODUCTION: Anti-inflammatory effect of vitamin D (VD) could be beneficial in improving the survival of glioma patients. The aim of our study was to analyse the serum levels of vitamin D in glioma patients and to find an association with the prognosis of glioma patients and other investigated parameters.

MATERIAL AND METHODS: The study included 63 patients with gliomas. Percentage of CD14⁺ monocytes, TREM-1⁺ and TREM-2⁺ monocytes were determined by flow cytometry, serum levels of 25(OH)D were evaluated by electrochemiluminescent binding test.

RESULTS: Six patients out of 63 had normal levels of VD. A significant difference in the overall survival (OS) in the patients with severe VD deficiency, VD deficiency and insufficiency in grade IV was found. In grade II and III, the levels of vitamin D positively correlated with the percentage of TREM-2⁺ monocytes, and in grade II also a negative correlation of VD with TREM-1/TREM-2 ratio was observed.

CONCLUSION: Levels of VD could influence the prognosis of patients with high-grade gliomas. Serum level of 25(OH)D in low-grade gliomas positively correlated with the percentage of anti-inflammatory acting TREM-2⁺ monocytes and negatively with TREM-1/TREM-2 ratio. This could be protective against the progression to high-grade glioma, because TREM-2 is associated with protective functions such as: tissue repair, control of local inflammation, or phagocytosis (Tab. 4, Fig. 4, Ref. 79). Text in PDF www.elis.sk

KEY WORDS: inflammation, glioma, prognosis, TREM-2, vitamin D.

Introduction

Gliomas belong to the most frequent primary brain tumours that have the third highest mortality and morbidity rates among cancer in human population. They occur in all age groups, but most of them emerge in adults older than 45 years. According to histological and genetic features defined by WHO (World Health Organization), they are classified into 4 grades (1). Grade I and II are referred to as low grade gliomas, grades III and IV as high-grade gliomas. More than half of patients present a diagnosis of

glioblastoma (GBM, grade IV), the most frequent, aggressive, and lethal form of glioma. Despite an improvement in understanding glioma biology, this disease, especially high-grade forms, still have a bad prognosis. In 2019, Cantrell et al showed that despite improvements in the median and short-term overall survival, the percentage of patients with glioblastoma achieving 5-year overall survival remains very low - 4.6 % (2). The markers of survival prognosis in the patients with gliomas are still evaluated.

Inflammation develops as a defence mechanism to any damage caused by infectious agents, tissue injury or malfunction (3). Both chronic systemic and local inflammation in the tumour microenvironment can support cancer development. Under physiological state, the only cells with immune functions in the central nervous system (CNS) are microglia. However, after blood brain barrier (BBB) disruption induced by trauma, stress or other pathological conditions, the pro-inflammatory molecules and immune cells from the periphery can cross the BBB and enter the CNS (4). As gliomas can also lead to BBB disruption, circulating immune cells not normally found in the CNS – macrophages, various types of T and B cells, regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs) – gain access to tumour areas (5, 6). One of the potential molecules with anti-inflammatory effect is vitamin D (VD).

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Vitamin D is a neuro-hormone regulating bone calcium-phosphate homeostasis, which plays a major role in many aspects of cellular functions and immunomodulation (7–10). However, it has also a direct effect on the function of both innate and adaptive immunity via VD receptor expressed on several immune cells (11–14).

Anti-inflammatory effect of VD in human T cells is partially mediated by inhibitory effect of NF κ B (15). VD also participates in the shifting of T helper (Th) cell response from Th1 (specific cell mediated immunity accompanied by inflammation) to Th2 (specific humoral immunity). Vitamin D inhibits the production of Th1 cytokine IFN- γ and it increases production of Th2 cytokines such as IL-4, IL-5 and IL-10 (16, 17).

VD deficiency is associated with various disorders such as: diabetes, infections, myocardial infarction, autoimmune disease, chronic obstructive pulmonary disease, tuberculosis, and excess mortality in the general population (18–20). Vitamin D deficiency is common worldwide among healthy individuals (21, 22) and particularly among cancer patients (23–27). Laboratory studies demonstrated that 1,25(OH)₂D also has many anti-carcinogenic actions, including: anti-inflammation, anti-angiogenesis, and pro-apoptosis (28, 29). Despite such substantial experimental evidence, there are no formal recommendations for vitamin D supplementation for cancer prevention (30). Nonetheless, screening for vitamin D deficiency and vitamin D supplementation has increased dramatically since the early 2000s.

25-hydroxyvitamin D (25(OH)D) is the major circulating form of VD with a half-life of approximately 2–3 weeks. It is a metabolite of VD that is used to determine whether a patient is VD deficient, sufficient or intoxicated (31, 32). There is no absolute consensus about the normal range for 25(OH)D, but most experts now agree that VD deficiency should be defined as a 25(OH)D level less than 20 ng/mL (50 nmol/L), and levels between 20 and 29 ng/mL (50–74 nmol/L) are classified as insufficiency. Severe VD deficiency is characterized by values below 12 ng/mL. The normal level for 25(OH)D is now recommended to be more than 30 ng/mL (75 nmol/L) (31–34).

In recent years, the role of the modern inflammatory markers TREM-1 (triggering receptors expressed on myeloid cells) and TREM-2 in tumorigenesis has begun to be studied. The expression of TREM-1 receptor is associated with activated Th1 cell-mediated immunity, which is associated with anti-tumour immunity during the initiation phase of tumour growth. However, the long-lasting presence of this molecule supports the pro-inflammatory state at both systemic and local levels directly in the tumour microenvironment (TME), where it potentiates the tumour growth (35–37). TREM-2 is a negative regulator of inflammatory response. It is expressed in different tissues on dendritic cells, peritoneal and pleural macrophages, and microglia (38). Its expression on microglia is well known, but the exact role and signal pathways are the objects of further investigation (38, 39). The TREM-2 molecule has an anti-inflammatory effect, promotes phagocytosis, and is associated with Th2 immunity and cell-mediated immune suppression that could potentiate the tumour growth (40).

The aim of our study was to find an association of VD serum levels with the survival of glioma patients, with the percentage of pro-

inflammatory TREM-1 positive and anti-inflammatory TREM-2 positive monocytes, TREM-1/TREM-2 ratio.

Subjects and methods

The study group included 63 patients older than 18 years (mean age: 53.29 \pm 14.98 years) with partial or complete resection of CNS tumour. Patients with primary diagnosis and with relapse or progression of residual tumour were analysed. Only patients with histologically proven gliomas of grade II, III and IV were enrolled in our study, all other histological types of tumours or other diagnoses were excluded. Tumours of grade II with signs of grade III were taken as grade III, and one tumour of grade III with signs of glioblastoma was taken as grade IV. The diagnosis was approved by two neuropathologists according to the most recent WHO classification criteria. Blood samples were obtained from the patients, in the morning the day of surgery, before surgical treatment.

All investigations were carried out in accordance with the International Ethical Guidelines and the Declaration of Helsinki. The study was approved by the Ethical Committee of University Hospital in Bratislava, and a written informed consent for enrolling in the study and for personal data management was obtained from all examined cases.

The blood was obtained between the years 2015 and 2018. Percentage of CD14⁺ monocytes (Mo) and TREM-1 and TREM-2 expressions on CD14⁺ monocytes were measured by flow cytometry (Navios, Beckman Coulter France S.A.S). Both percentage and MFI (mean fluorescence intensity) of TREM expressions were analysed by KALUZA analysis software (Beckman Coulter France S.A.S) (antibodies used: CD14-PC7, TREM-1-PE, TREM-2-APC, and isotype controls; all from R&D System, Minneapolis, MN, USA). The TREM-1, TREM-2 analysis that we used in our previous studies (41, 42) was performed in compliance with Flow Cytometry Protocol recommended by the manufacturer. TREM-1 and TREM-2 expressions are presented as the percentage of TREM-1 and TREM-2 positive cells out of all CD14⁺ cells. For each patient, we performed a negative control – sample stained with CD14, and isotype controls without TREM-1 and TREM-2 antibody. In addition, the serum levels of 25(OH)D were evaluated by electrochemiluminescence binding test (Elecsys Vitamin D total-cobas; Roche Diagnostics GmbH, Mannheim, Germany). Survival time was calculated from the time of diagnosis until April 2019 or the time of death. Patients were monitored from the 1th of December 2015 till 30th of April 2019.

Statistics

For the statistical analysis, we used programs InStat and SAS. We used Mann–Whitney test, Cox proportional hazard analysis, Kaplan–Meier survival analysis, Log Rank test, and Spearman correlation. The results were expressed as the median and interquartile range (IQR), mean \pm standard deviation (SD), $p < 0.05$ was considered to indicate the statistical significance.

Results

- 1) Complete characteristics of patients are summarized in Table 1.
- 2) Number of patients with different vitamin D levels in each grade are shown in Table 2.
- 3) Levels of vitamin D were significantly higher in grade II gliomas than in grade III ($p = 0.047$) (Tab. 3). Interestingly, when we compared levels of vitamin D in grade IV with vitamin D in grade III or II, we did not find significant differences. In grade II the median of vitamin D was very similar to that in grade IV (20.4 vs 19.6 $\mu\text{g/L}$), in grade III the median was lower (13.7 $\mu\text{g/L}$), however, the difference was not significant (Tab. 3).

Tab. 1. Characteristics of glioma patients.

Patients	n	Mean age \pm SD
All gliomas	63	53.29 \pm 14.98
Sex (male/female)	38/25	
Grades (male/female)		
G. II	14/5	14/5 (40.47 \pm 12.30)
G. II–III	2/0	2/0 (30.5)
G. III	7/4	7/4 (48.55 \pm 12.57)
G. III–IV	0/1	0/1 (55)
G. IV	15/15	15/15 (64.07 \pm 8.70)
Primary diagnosis	49	
Relapse or progression of residual tumor	13	
Unknown	1	
Diagnosis		
Diffuse glioma II	4	
Oligodendroglioma II	7	
Oligoastrocytoma II	1	
Astrocytoma II	7	
Oligodendroglioma II–III	1	
Astrocytoma II–III	1	
Anaplastic astrocytoma	11	
Anaplastic astrocytoma with signs of GBM	1	
Primary GBM	28	
Unknown GBM	2	
Completely resected		
G. II	4	
G. II–III	0	
G. III	3	
G. III–IV	0	
G. IV	4	
IDH1/2 mutated	25	
G. II, III	24	
G. IV	1	
Steroid treated/untreated		
G. II	7/12	
G. III	9/4	
G. IV	28/3	

n – number of patients, G – grade, GBM – glioblastoma multiforme, IDH – isocitrate dehydrogenase

Tab. 2. Distribution of patients according to serum vitamin D levels.

	VD severe deficiency ($<12 \mu\text{g/L}$)	VD deficiency ($12 - 19.99 \mu\text{g/L}$)	VD insufficiency ($20 - 29.99 \mu\text{g/L}$)	Normal VD ($\geq 30 \mu\text{g/L}$)
G. II	1	7	9	2
G. II–III and III	2	8	2	1
G. III–IV and IV	5	11	12	3

G – grade, VD – vitamin D

Tab. 3. Comparison of serum 25(OH)D levels between different grades of gliomas.

Vitamin D	Gliomas G II	Gliomas G III	Gliomas G IV
n	19	13	31
Min	8.1	6.15	6.84
Max	61.5	35.1	43.4
Median	20.4	13.7	19.6
IQR	9.28	11.64	12.09
Mann-Whitney test	$p=0.047$ (II vs III)		

G – grade, n – number of patients, IQR – interquartile range

Tab. 4. Correlations of serum 25(OH)D level with percentage of TREM-2⁺ monocytes and TREM-1/TREM-2 ratio.

25(OH)D in gliomas		Spearman r	0.5083
G. II	% of TREM-2 monocytes	p	0.0312
	TREM-1/TREM-2	Spearman r	-0.4681
G. III	% of TREM-2 monocytes	p	0.0581
	TREM-1/TREM-2	Spearman r	0.6648
G. IV	% of TREM-2 monocytes	p	0.0390
	TREM-1/TREM-2	Spearman r	NS

G – grade

- 4) In grade II and III gliomas, a significant positive correlation of vitamin D level with percentage of TREM-2⁺ monocytes was found (G. II: $p = 0.0312$, G. III: $p = 0.0390$) (Tab. 4).
- 5) In grade II glioma patients, we saw a trend to a negative correlation of vitamin D level with TREM-1/TREM-2 ratio ($p = 0.0581$) (Tab. 4).
- 6) In the Cox proportional analysis, serum level of vitamin D showed no association with overall survival in any grade. However, grade IV glioma patients with vitamin D lower than 20 $\mu\text{g/L}$ survived significantly shorter time than the patients with vitamin D higher than 20 $\mu\text{g/L}$ ($p = 0.0027$) (Fig. 1).
- 7) When we divided the patients into the three subgroups: 1, with vitamin D $< 12 \mu\text{g/L}$; 2, VD level between 12 and 20 $\mu\text{g/L}$ and 3, VD level $> 20 \mu\text{g/L}$, we observed that patients with severe vitamin D deficiency ($<12 \mu\text{g/L}$) and vitamin D deficiency (12–20 $\mu\text{g/L}$) survived significantly shorter time than patients with vitamin D higher than 20 $\mu\text{g/L}$ ($p = 0.0099$) (Fig. 2). Three patients had VD in normal range – more than 30 $\mu\text{g/L}$ (Tab. 2).
- 8) When we omitted the patients with normal levels of vitamin D in grade IV, we observed significant differences in the overall survival with very similar p as shown in results 6 and 7 (Figs 3 and 4).
- 9) We did not see significant differences in VD levels comparing the primary diagnosed glioma patients with the patients with relapse or progression of residual tumour (primary diagnosis: median: 19.6 $\mu\text{g/L}$; median 23.6 $\mu\text{g/L}$ in relapses or progression of residual tumour).
- 10) Regarding steroid therapy, we did not see differences in vitamin D levels and the percentage of TREM-2⁺ monocytes.

Discussion

VD deficiency is common among the general population. It is also observed in up to 76 % of critically ill patients (43). In recent years, there has been a great deal of enthusiasm regarding the potential role of vitamin D in the primary and secondary prevention of cancer (44).

The serum/plasma level of 25(OH)D vitamin is the best indicator of overall vitamin D status, because it reflects the total vitamin D from dietary intake and sunlight exposure (45).

In our study we observed, that only 6 patients out of 63 had normal levels of vitamin D. Blood samples of our glioma patients were collected after diagnosis of CNS tumour was proven and many of the patients were at the time of blood collection under steroid treatment. However, we did not observe differences in 25(OH)D serum levels between steroid treated and steroid not treated patients. Patients with relapses were also included in our study, however, we did not observe differences in vitamin D levels between glioma patients with primary diagnosis and patients with relapse or progression. In grade IV, 5 patients out of 31 suffered from severe VD deficiency. In grade II, there was only one patient and in grade III there were two patients.

Low VD levels were associated with a higher risk of various types of cancer – colorectal (46–48), breast (49, 50), pancreatic (51), ovarian (52), and skin cancer (53). Many of these analyses highlight the importance of prospective studies with blood collected years in advance of diagnosis and treatment. Studies of different designs examined the level of 25(OH)D and cancer mortality/survival and are included in several reviews and meta-analyses. Blood samples were collected years prior to diagnosis, at the time of diagnosis or in some cases after diagnosis or treatment (54–59). In literature, we did not find studies about vitamin D levels and prognosis of gliomas, however, we found one study that investigated the association between vitamin D levels and glioma risk. Zigmont et al in 2015 published the first study in which they evaluate a potential association between pre-diagnostic serum vitamin D and glioma risk. They found that men older

than 56 years with higher levels of serum 25(OH)D had a reduced risk of glioma (60).

In our study, in grade IV glioma patients, we observed significant differences in overall survival of patients with severe VD deficiency, VD deficiency and VD insufficiency; with increasing levels, the overall survival was better. This finding was not observed in grade II or III of gliomas.

Higher 25(OH)D status in cancer patients at the time of diagnosis has generally been reported in reviews to be associated with an improved survival for most malignancies – breast, colorectal, stomach, lung, prostate and head/neck cancers (54–57). The majority of studies included in these reviews measured the levels of 25(OH)D in patients after diagnosis. Only three studies measured the 25(OH)D in pre-diagnostic blood samples and their conclusion was similar – the reduced risks of mortality from colorectal and prostate cancer with higher levels of 25(OH)D (61, 62, 48). Maalmi et al conducted a meta-analysis of colorectal and breast cancer survival and reported a lower overall and disease-specific mortality with higher vitamin D status. 25(OH)D was measured in blood samples taken after diagnosis for all of the breast cancer studies and three of the five colorectal cancer studies (63). Other published data are more mixed in their findings and conclusions. For example, higher pre-diagnostic vitamin D status was associated with a significantly lower lung cancer mortality in two Danish cohorts, but not with colorectal cancer mortality (64). High post-diagnostic level of 25(OH)D was associated with a lower colorectal cancer mortality (65, 27), ovarian cancer mortality (66), and Merkel cell carcinoma mortality (although not significant) (67). This was observed also in one study concerning pancreatic cancer (68).

Vitamin D levels measured after diagnosis were not associated with a prostate cancer mortality in two studies (69, 70), however, a higher pre-diagnostic 25(OH)D status was associated with a lower prostate cancer mortality in the Swedish Malmo cohort (71) and the Finnish Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study cohort (72). In 2014, Salomón et al observed an improved overall survival associated with vitamin D receptor expression in human glioblastoma tissues (73).

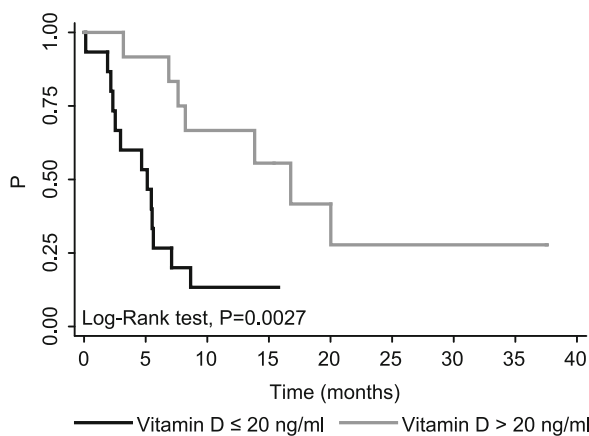


Fig. 1. Kaplan-Meier survival curves of patients with different serum vitamin D levels in grade IV (two subgroups)

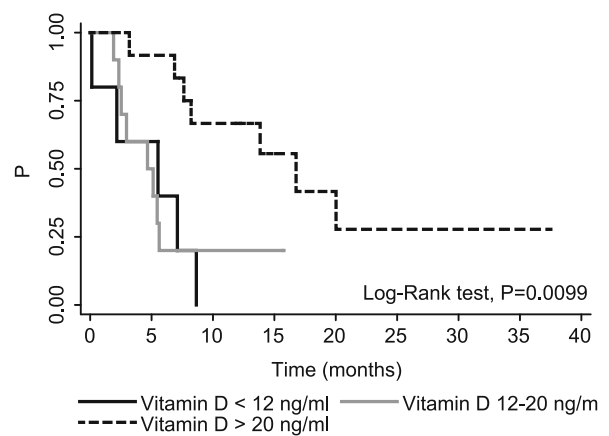


Fig. 2. Kaplan-Meier survival curves of patients with different serum vitamin D levels in grade IV (three subgroups)

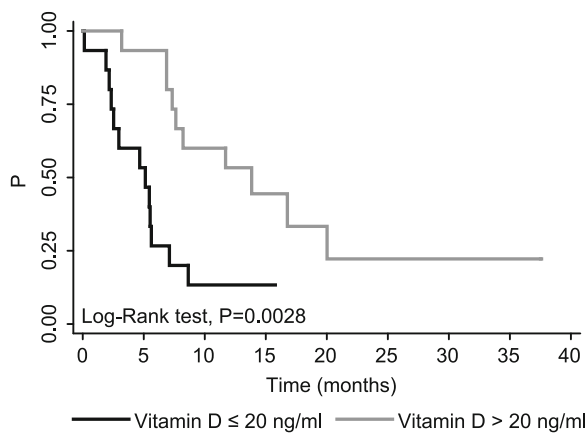


Fig. 3. Kaplan-Meier survival curves of patients with different serum vitamin D levels in grade IV (two subgroups, patients with normal VD omitted)

The expression of TREM-1 receptor is associated with activated Th1 cell-mediated immunity, which is associated with anti-tumour immunity during the initiation phase of tumour growth. However, the long-lasting presence of this molecule supports the pro-inflammatory state and it potentiates the tumour growth (35–37). The TREM-2 molecule has an anti-inflammatory effect, promotes phagocytosis, what might have a positive impact in the prevention of tumour growth, however, it is also associated with Th1 cell-mediated immune suppression and activation of Th2 immunity, what in the case of longer lasting state also to our opinion could potentiate the tumour growth (40).

In our study we showed that vitamin D levels positively correlated with the percentage of TREM-2⁺ monocytes in grade II and III glioma patients. Moreover, in grade II gliomas a negative correlation with TREM-1/TREM-2 ratio was found. The positive correlation of plasma vitamin D with percentage of TREM-2 positive monocytes and the negative correlation with TREM-1/TREM-2 ratio could be protective. While TREM-1 molecule acts pro-inflammatory and could potentiate the tumour growth, TREM-2 positive monocytes/macrophages are associated with alternative type of inflammation, act anti-inflammatory and have potentiated phagocytic activity. It means that vitamin D potentiated cell mediated innate immunity might account for immune protection in low grade gliomas. These TREM-2 positive cells are important not only in control of local inflammation, phagocytosis, but also in tissue repair (74).

As only a few studies (75, 76, 42) analysed the effect of vitamin D level on anti-inflammatory TREM-2 receptor expressions, we cannot compare in a broader sense our results with the findings of other authors. Bucova et al observed an increase of TREM-2 receptor expression with VD serum level in pulmonary sarcoidosis (42). Zhao et al in 2018 investigated the effect of active vitamin D on the expression of TREM-1, but not TREM-2 in the renal tissues of diabetic nephropathy (DN) rats. Their results demonstrated that VD could suppress macrophage adhesion and migration by reducing the expression of TREM-1 (77). Addula et al in 2018 investigated the effect of VD on TREM-1 and TREM-2 expression in

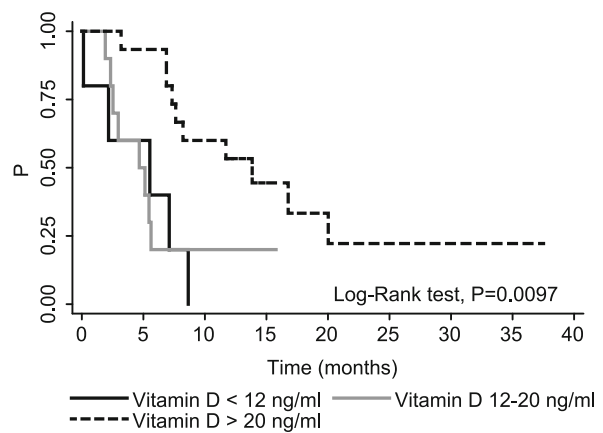


Fig. 4. Kaplan-Meier survival curves of patients with different serum vitamin D levels in grade IV (three subgroups, patients with normal VD omitted)

inflammatory bowel diseases in 8 cases and 11 healthy controls. They concluded that insufficient VD levels were associated with an increased inflammatory state, which was accompanied by an increase of TREM-1 (pro-inflammatory molecule) and decrease of TREM-2 (anti-inflammatory) expressions (78). Results of Kim et al in 2013 showed that 1,25(OH)₂D₃ could affect the innate and inflammatory responses by up-regulating TREM-1 expression and suggested the possibility that 1,25(OH)₂D₃ might function as an enhancer of innate immune response in chronic inflammatory conditions (79).

Conclusion

The levels of vitamin D could influence the prognosis of patients with high-grade gliomas. We observed significant differences in the overall survival of grade IV glioma patients with severe VD deficiency, VD deficiency and VD insufficiency; with increasing levels, the overall survival was better. Supplementation of VD might be helpful in improving OS and quality of life of these patients. Vitamin D in plasma correlated positively with the percentage of anti-inflammatory TREM-2⁺ monocytes and negatively with TREM-1/TREM-2 ratio in low grade gliomas, what might account for potentiated phagocytosis, decreased inflammation, and tissue repair.

References

- Villa C, Miquel C, Mosses D, Bernier M, Di Stefano AL. The 2016 World Health Organization classification of tumours of the central nervous system. *Presse Med* 2018; 47 (11–12 Pt 2): e187–e200.
- Cantrell JN, Waddle MR, Rotman M, Peterson JL, Ruiz-Garcia H, Heckman MG et al. Progress Toward Long-Term Survivors of Glioblastoma. *Mayo Clin Proc* 2019; 94 (7): 1278–1286. DOI: 10.1016/j.mayocp.2018.11.031.
- Galvão RP, Zong H. Inflammation and Gliomagenesis: Bi-Directional Communication at Early and Late Stages of Tumor Progression. *Curr Pathobiol Rep* 2013; 1 (1): 19–28.

4. **Wilson EH, Weninger W, Hunter CA.** Trafficking of immune cells in the central nervous system *J Clin Invest* 2010; 120 (5): 1368–1379.
5. **Chen P, Hsu WH, Chang A, Tan Z, Lan Z, Zhou A et al.** Circadian regulator CLOCK recruits immune suppressive microglia into the GBM tumor microenvironment. *Cancer Discov* 2020; 10(3): 371–381
6. **Giering A, Pszczolkowska D, Walentynowicz KA, Rajan WD, Kaminska B.** Immune microenvironment in gliomas. *Lab Invest* 2017; 97 (5): 498–518.
7. **Adams JS, Hewison M.** Update in vitamin D. *J Clin Endocrinol Metab* 2010; 95 (2): 471–478.
8. **Bivona G, Agnello L, Ciaccio M.** The immunological implication of the new vitamin D metabolism. *Cent Eur J Immunol* 2018; 43 (3): 331–334.
9. **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.
10. **Holick MF.** Vitamin D deficiency. *N Engl J Med* 2007; 357 (3): 266–281.
11. **Beard JA, Bearden A, Striker R.** Vitamin D and the anti-viral state. *J Clin Virol* 2011; 50 (3): 194–200.
12. **Bikle DD.** Vitamin D and the immune system: role in protection against bacterial infection. *Curr Opin Nephrol Hypertens* 2008; 17 (4): 348–352.
13. **Mahon BD, Wittke A, Weaver V, Cantorna MT.** The targets of vitamin D depend on the differentiation and activation status of CD4 positive T cells. *J Cell Biochem* 2003; 89 (5): 922–932.
14. **Veldman CM, Cantorna MT, DeLuca HF.** Expression of 1,25-dihydroxyvitamin D (3) receptor in the immune system. *Arch Biochem Biophys* 2000; 374 (2): 334–338.
15. **Yu XP, Bellido T, Manolagas SC.** Down-regulation of NF-kappa B protein levels in activated human lymphocytes by 1,25-dihydroxyvitamin D3. *Proc Natl Acad Sci U S A* 1995; 92 (24): 10990–10994.
16. **Hewison M.** Vitamin D and the immune system: new perspectives on an old theme. *Endocrinol Metab Clin North Am* 2010; 39 (2): 365–379.
17. **Lemire JM, Archer DC, Beck L, Spiegelberg HL.** Immunosuppressive actions of 1,25-dihydroxyvitamin D3: preferential inhibition of Th1 functions. *J Nutr* 1995; 125 (6 Suppl): 1704S–1708S.
18. **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.
19. **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.
20. **Sebekova K, Krivosikova Z, Gajdos M, Podracka 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.
21. **Holick MF.** Vitamin D deficiency. *N Engl J Med* 2007; 357 (3): 266–281.
22. **Zgaga L, Theodoratou E, Farrington SM, Agakov F, Tenesa A, Walker M et al.** Diet, environmental factors, and lifestyle underlie the high prevalence of vitamin D deficiency in healthy adults in Scotland, and supplementation reduces the proportion that are severely deficient. *J Nutr* 2011; 141 (8): 1535–1542.
23. **Crew KD, Shane E, Cremers S, McMahon DJ, Irani D, Hershman DL.** High prevalence of vitamin D deficiency despite supplementation in premenopausal women with breast cancer undergoing adjuvant chemotherapy. *J Clin Oncol* 2009; 27 (13): 2151–2156.
24. **Fakih MG, Trump DL, Johnson CS, Tian L, Muindi J, Sunga AY.** Chemotherapy is linked to severe vitamin D deficiency in patients with colorectal cancer. *Int J Colorectal Dis* 2009; 24 (2): 219–224.
25. **Shanafelt TD, Drake MT, Maurer MJ, Allmer C, Rabe KG, Slager SL et al.** Vitamin D insufficiency and prognosis in chronic lymphocytic leukemia. *Blood*; 2011 117 (5): 1492–1498.
26. **Vrieling A, Hein R, Abbas S, Schneeweiss A, Flesch-Janys D, Chang-Claude J.** Serum 25-hydroxyvitamin D and postmenopausal breast cancer survival: a prospective patient cohort study. *Breast Cancer Res* 2011; 13 (4): R74.
27. **Zgaga L, Theodoratou E, Farrington SM, Din FV, Ooi LY, Glodzik D et al.** Plasma vitamin D concentration influences survival outcome after a diagnosis of colorectal cancer. *J Clin Oncol* 2014; 32 (23): 2430–2439.
28. **Giovannucci E.** The epidemiology of vitamin D and cancer incidence and mortality: a review (United States). *Cancer Causes Control* 2005; 16 (2): 83–95.
29. **Holick MF.** Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease. *Am J Clin Nutr* 2004; 80 (Suppl 6): 1678S–1688S.
30. **Institute of Medicine Dietary Reference Intakes for Calcium and Vitamin D.** Washington, DC: The National Academies Press; 2011
31. **Holick MF.** High prevalence of vitamin D inadequacy and implications for health. *Mayo Clin Proc* 2006; 81 (3): 353–373.
32. **Holick MF.** Resurrection of vitamin D deficiency and rickets. *J Clin Invest* 2006; 116 (8): 2062–2072.
33. **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.
34. **Holick MF.** Vitamin D status: measurement, interpretation, and clinical application. *Ann Epidemiol* 2009; 19 (2): 73–78.
35. **Scheurer ME, Amirian ES, Davlin SL, Rice T, Wrensch M, Bondy ML.** Effects of antihistamine and anti-inflammatory medication use on risk of specific glioma histologies. *Int J Cancer* 2011; 129 (9): 2290–2296.
36. **Badie B, Schartner J, Klaver J, Vorpahl J.** In vitro modulation of microglia motility by glioma cells is mediated by hepatocyte growth factor/scatter factor. *Neurosurgery* 1999; 44 (5): 1077–1083.
37. **Pelham CJ, Agrawal DK.** Emerging roles for triggering receptor expressed on myeloid cells receptor family signaling in inflammatory diseases. *Expert Rev Clin Immunol* 2014; 10 (2): 243–256.
38. **Sharif O, Knapp S.** From expression to signaling: roles of TREM-1 and TREM-2 in innate immunity and bacterial infection. *Immunobiology* 2008; 213 (9–10): 701–713.
39. **Takahashi K, Rochford CD, Neumann H.** Clearance of apoptotic neurons without inflammation by microglial triggering receptor expressed on myeloid cells-2. *J Exp Med* 2005; 201 (4): 647–657.
40. **Turnbull IR, Gilfillan S, Cella M et al.** Cutting edge: TREM-2 attenuates macrophage activation. *J Immunol* 2006; 177 (6): 3520–3524.
41. **Suchankova M, Bucova M, Tibenska E, Tedlova E, Demian J, Majer I et al.** Triggering receptor expressed on myeloid cells-1 and 2 in bronchoalveolar lavage fluid in pulmonary sarcoidosis. *Respirology* 2013; 18 (3): 455–462.
42. **Bucova M, Suchankova M, Tibenska E, Tedlova E, Demian J, Majer I et al.** TREM-2 Receptor Expression Increases with 25(OH)D Vitamin Serum Levels in Patients with Pulmonary Sarcoidosis. *Med Inflamm* 2015; 2015: 181986104
43. **Dickerson RN, Van Cleve JR, Swanson JM, Maish GO, 3rd, Minard G, Croce MA et al.** Vitamin D deficiency in critically ill patients with traumatic injuries. *Burns Trauma* 2016; 4: 28.
44. **Mondul AM, Weinstein SJ, Layne TM, Albanes D.** Vitamin D and Cancer Risk and Mortality: State of the Science, Gaps, and Challenges. *Epidemiol Rev* 2017; 39(1): 28–48.

45. Zhang Y, Leung DY, Richers BN, Liu Y, Remigio LK, Riches DW et al. Vitamin D inhibits monocyte/macrophage proinflammatory cytokine production by targeting MAPK phosphatase-1. *J Immunol* 2012; 188 (5): 2127–2135.
46. Yan Y, Gong Z, Xu Z. Vitamin D supplementation and colorectal cancer prognosis. *Med Oncol* 2019; 36 (8): 69.
47. Lee JE. Circulating levels of vitamin D, vitamin D receptor polymorphisms, and colorectal adenoma: a meta-analysis. *Nutr Res Pract* 2011; 5(5): 464–470.
48. Fedirko V, Mandle HB, Zhu W, Highes DJ, Siddiq A, Ferrari P et al. Vitamin D-Related Genes, Blood Vitamin D Levels and Colorectal Cancer Risk in Western European Populations. *Nutrients* 2019; 11 (8): 1954.
49. O'Brien KM, Sandler DP, Taylor JA, Weinberg CR. Serum Vitamin D and Risk of Breast Cancer within Five Years. *Environ Health Perspect* 2017; 125 (7): 077004.
50. O'Brien KM, Sandler DP, Xu Z, Kinyamu HK, Taylor JA, Weinberg CR. Vitamin D, DNA methylation, and breast cancer. *Breast Cancer Res* 2018; 20 (1): 70.
51. van Duijnhoven FJB, Jenab M, Hveem K, Siersema PB, Fedirko V, Duell EJ et al. Circulating concentrations of vitamin D in relation to pancreatic cancer risk in European populations. *Int J Cancer* 2018; 142 (6): 1189–1201.
52. L'Espérance K, Datta GD, Qureshi S, Koushik A. Vitamin D Exposure and Ovarian Cancer Risk and Prognosis. *Int J Environ Res Public Health* 2020; 17 (4): 1168.
53. Slominski AT, Brożyna AA, Zmijewski MA, Józwicki W, Jetten AM, Mason RS et al. Vitamin D signaling and melanoma: role of vitamin D and its receptors in melanoma progression and management. *Lab Invest* 2017; 97 (6): 706–724.
54. Pilz S, Kienreich K, Tomaschitz A et al. Vitamin D and cancer mortality: systematic review of prospective epidemiological studies. *Anticancer Agents Med Chem* 2013; 13 (1): 107–117.
55. Chowdhury R, Kunutsor S, Vitezova A, Oliver-Williams C, Chowdhury S, Kieffe-de-Jong JC et al. Vitamin D and risk of cause specific death: systematic review and meta-analysis of observational cohort and randomised intervention studies. *BMJ* 2014; 348: g1903.
56. Autier P, Boniol M, Pizot C, Mullie P. Vitamin D status and ill health: a systematic review. *Lancet Diabetes Endocrinol* 2014; 2 (1): 76–89.
57. Robsahm TE, Schwartz GG, Tretli S. The Inverse Relationship between 25-Hydroxyvitamin D and Cancer Survival: Discussion of Causation. *Cancers (Basel)* 2013; 5 (4): 1439–1455.
58. Schöttker B, Jorde R, Peasey A, Thorand B, Jansen EH, Groot LD et al. Vitamin D and mortality: meta-analysis of individual participant data from a large consortium of cohort studies from Europe and the United States. *BMJ* 2014; 348: g3656.
59. Yin L, Ordóñez-Mena JM, Chen T, Schöttker B, Arndt V, Brenner H. Circulating 25-hydroxyvitamin D serum concentration and total cancer incidence and mortality: a systematic review and meta-analysis. *Prev Med* 2013; 57 (6): 753–764.
60. Zigmont V, Garrett A, Peng J, Seweryn M, Rempala GA, Harris R et al. Association Between Prediagnostic Serum 25-Hydroxyvitamin D Concentration and Glioma. *Nutr Cancer* 2015; 67 (7): 1120–1130.
61. Ng K, Meyerhardt JA, Wu K, Feskanich D, Hollis BW, Giovannucci EL et al. Circulating 25-hydroxyvitamin d levels and survival in patients with colorectal cancer. *J Clin Oncol* 2008; 26 (18): 2984–2991.
62. Fang F, Kasperzyk JL, Shui I, Hendrickson W, Hollis BW, Fall K et al. Prediagnostic plasma vitamin D metabolites and mortality among patients with prostate cancer. *PLoS One* 2011; 6 (4): e18625.
63. Maalmi H, Ordóñez-Mena JM, Schöttker B, Brenner H. Serum 25-hydroxyvitamin D levels and survival in colorectal and breast cancer patients: systematic review and meta-analysis of prospective cohort studies. *Eur J Cancer* 2014; 50 (8): 1510–1521.
64. Afzal S, Brøndum-Jacobsen P, Bojesen SE, Nordestgaard BG. Genetically low vitamin D concentrations and increased mortality: Mendelian randomisation analysis in three large cohorts. *BMJ* 2014; 349: g6330.
65. Wesa KM, Segal NH, Cronin AM, joberg DD, Jacobs GN, Coletton MI et al. Serum 25-hydroxy vitamin D and survival in advanced colorectal cancer: a retrospective analysis. *Nutr Cancer* 2015; 67 (3): 424–430.
66. Walentowicz-Sadlecka M, Grabiec M, Sadlecki P, Gotowska M, Walentowicz P, Krintus M et al. 25(OH)D3 in patients with ovarian cancer and its correlation with survival. *Clin Biochem* 2012; 45 (18): 1568–1572.
67. Samimi M, Touzé A, Laude H, Le Bidre E, Arnold F, Carpentier A et al. Vitamin D deficiency is associated with greater tumor size and poorer outcome in Merkel cell carcinoma patients. *J Eur Acad Dermatol Venereol* 2014; 28 (3): 298–308.
68. Cho M, Peddi PF, Ding K, Chen L, Thomas D, Wang J, Lockhart AC et al. Vitamin D deficiency and prognostics among patients with pancreatic adenocarcinoma. *J Transl Med* 2013; 11: 206.
69. Holt SK, Kolb S, Fu R, Horst R, Feng Z, Stanford JL. Circulating levels of 25-hydroxyvitamin D and prostate cancer prognosis. *Cancer Epidemiol* 2013; 37 (5): 666–670.
70. Gupta D, Trukova K, Popiel B, Lammersfeld C, Vashi PG. The association between pre-treatment serum 25-hydroxyvitamin D and survival in newly diagnosed stage IV prostate cancer. *PLoS One* 2015; 10 (3): e0119690.
71. Brändstedt J, Almquist M, Manjer J, Malm J. Vitamin D, PTH, and calcium in relation to survival following prostate cancer. *Cancer Causes Control* 2016; 27 (5): 669–677.
72. Mondul AM, Weinstein SJ, Moy KA, Männistö S, Albanes D. Circulating 25-Hydroxyvitamin D and Prostate Cancer Survival. *Cancer Epidemiol Biomarkers Prev* 2016; 25 (4): 665–669.
73. Salomón DG, Fermento ME, Gandini NA, Ferronato MJ, Arévalo J, Blasco J et al. Vitamin D receptor expression is associated with improved overall survival in human glioblastoma multiforme. *J Neurooncol* 2014; 118 (1): 49–60.
74. Hsieh CL, Koike M, Spusta S, Niemi E, Yenari M, Nakamura MC et al. A Role for TREM2 Ligands in the Phagocytosis of Apoptotic Neuronal Cells by Microglia. *J Neurochem* 2009; 109 (4): 1144–1156.
75. Hewison M, Freeman L, Hughes SV, Evans KN, Bland R, Eliopoulos AG et al. Differential regulation of vitamin D receptor and its ligand in human monocyte-derived dendritic cells. *J Immunol* 2003; 170 (11): 5382–5390.
76. Hewison M. Vitamin D and the immune system: new perspectives on an old theme. *Endocrinol Metab Clin North Am* 2010; 39 (2): 365–379.
77. Zhao Y, Guo Y, Jiang Y, Zhu X, Zhang X. Vitamin D suppresses macrophage infiltration by down-regulation of TREM-1 in diabetic nephropathy rats. *Mol Cell Endocrinol* 2018; 473: 44–52.
78. Addula M. Association of Novel inflammatory markers TREM 1 & TREM 2 with Vitamin D Levels in Inflammatory Bowel Disease. *Am J Gastroenterol* 2018; 118 (10): A125
79. Kim TH, Lee B, Kwon E, Choi SJ, Lee YH, Song GG et al. Regulation of TREM-1 expression by 1,25-dihydroxyvitamin D3 in human monocytes/macrophages. *Immunol Lett* 2013; 154 (1–2): 80–85.

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