

Vitamin D supplementation and neurofilament light chain in patients with relapsing-remitting multiple sclerosis: A randomized placebo controlled trial

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The effect of vitamin D supplementation in multiple sclerosis (MS) is not established.

Neurofilament light chain (NFL) is a sensitive marker of axonal degeneration, and high levels of 25-hydroxyvitamin D have been associated with low NFL. We have performed a placebo-controlled randomized study of weekly supplementation with 20,000 IU vitamin D3 in 68 patients with relapsing remitting MS (RRMS). There was an unexpected positive correlation between serum levels of 25-hydroxyvitamin D and NFL at baseline ( $p=0.04$ ). Serum levels of 25-hydroxyvitamin D more than doubled in the vitamin D group, but compared to placebo vitamin D supplementation had no overall effect on serum levels of NFL at week 48 ( $p=0.93$ ) or week 96 ( $p=0.56$ ). In a subgroup analysis of patients not receiving disease-modifying therapy, NFL decreased 30.1% (week 48) and 32.6% (week 96) from baseline in the vitamin D group as compared to the placebo group ( $p=0.06$  for both time points). We conclude that with a possible exception for patients not treated with disease-modifying drugs, weekly supplementation with 20,000 IU vitamin D3 did not have a substantial effect on NFL in these RRMS patients.

Epidemiological studies suggest that low levels of vitamin D in blood is a risk factor for development of multiple sclerosis (MS) (1, 2). Observational studies have also shown that low serum concentrations of 25-hydroxyvitamin D are associated with higher inflammatory disease activity in patients with relapsing remitting (RR) MS (3, 4), in some but not all studies even in patients treated with effective immunomodulatory drugs (3, 5). Vitamin D may influence the disease course in MS either through its immunomodulatory effects, as suggested by results obtained in experimental autoimmune encephalitis (6), or alternatively, may exert protective effects directly on resident brain cells (7). The vitamin D receptor and key enzymes needed for activation of vitamin D are overexpressed in normal appearing matter and in chronic active MS lesions (8), and vitamin D supplementation has been shown to promote remyelination independently of immunomodulation in mice (9).

Although epidemiological, observational and preclinical studies support a beneficial role of vitamin D in MS, the effect of vitamin D supplementation has not yet been unequivocally established in clinical trials (10). Whereas some clinical trials using different dosing regimens of vitamin D have shown evidence for a positive effect on clinical or radiological outcomes (11-13) others have not (14, 15). These studies were most likely underpowered, and the effect of vitamin D supplementation in MS is therefore unsettled.

Neurofilament light chain (NFL) is a sensitive marker of axonal damage and degradation (16). The levels of NFL are elevated in the cerebrospinal fluid (CSF) of MS patients (17), and are markedly reduced by effective immunomodulatory treatments such as fingolimod and natalizumab (18, 19). The concentrations of NFL in serum are highly correlated with those in

CSF (20, 21), and do also predict disease activity and reflect treatment effects in patients with RRMS (22). Only one observational study has so far addressed the relationship between vitamin D and NFL in MS, showing that high serum levels of 25-hydroxyvitamin D were associated with low CSF levels of NFL (23). We have previously performed a randomized placebo-controlled trial (RCT) of weekly administration of 20.000 IU vitamin D3 in fully ambulatory patients with RRMS living above the Arctic Circle (15). The aim of the present study was to analyze if high dose vitamin D3 supplementation reduced the serum levels of NFL in this RCT.

#### Materials and methods

The design and clinical results of the RCT have been described previously (15, 24). Briefly, 71 RRMS patients aged 18 – 60 years with Kurtzke expanded disability status scale (EDSS) score  $\leq 4.5$  were randomized to receive either 20.000 IE vitamin D3 (Dekristol™; SWISS CAPS AG, Kirchberg, Switzerland) or placebo for 96 weeks. Participants with estimated calcium intake below 500 mg per day also received 500 mg calcium daily.

Serum samples were collected at baseline (January or February) and at week 48 and 96 and frozen at  $-70^{\circ}\text{C}$  until batch analyses. Serum NFL concentration was measured using a Single molecule array (Simoa) assay, as previously described (REF). Intra-assay coefficients of variation (CVs) were 5.3% at 9.0 pg/mL and 1.6% at 128 pg/mL. Serum 25-hydroxyvitamin D concentration was measured by spectroscopy (REF). CVs were 5.3% at 20 nmol/L and 4.0% at 239 nmol/L.

## Statistical analysis

Associations at baseline were analysed with linear regression models, and effects are reported using correlation coefficients. Changes in serum NFL over time were analyzed using linear mixed models with the two follow up time points, study arm, and the interaction between them as predictors. All inferences about changes in NFL concentration and differences between study arms were done using this model. Log transformed NFL concentrations were used, and changes are therefore reported as percentages. All data were analyzed in R and linear mixed models were analyzed using the lme4 package.

## Results

Serum samples were available from 68 patients who completed the study. Baseline characteristics are shown in Table I. The treatment groups were well matched for demographic and disease characteristics and for vitamin D status at baseline.

The relationship between NFL concentrations and baseline characteristics and are shown in Supplementary figure 1. There was a negative correlation between NFL and body mass index ( $r=0.30$ ,  $p=0.01$ ), and a positive correlation with 25-hydroxyvitamin D ( $r=0.25$ ,  $p=0.04$ ).

Baseline levels of NFL did not significantly correlate with EDSS score ( $r=0.23$ ,  $p=0.06$ ) age ( $r=0.14$ ,  $p=0.25$ ) or MS duration ( $r=0.7$ ,  $p=0.55$ ), and they were not significantly associated with smoking (2.2 pg/mL lower in smokers,  $p=0.18$ ) or use of immunomodulatory treatment (0.5 pg/mL lower in treated,  $p=0.76$ ).

As previously reported (REF), the mean serum mean concentration of 25-hydroxyvitamin D was more than doubled in the vitamin D group and was 123.2 nmol/L at week 96, whereas there only was a

minor increase from 57.3 nmol/L to 61.8 nmol/L in the placebo group (25). At week 96 25-hydroxyvitamin D were above 100 nmol/L in 28/35 of the patients in the vitamin D group and 2/33 in the placebo group.

The concentrations of NFL throughout the study are shown in Table II. The effect of vitamin D supplementation versus placebo was analyzed using a linear mixed model with random patient-wise intercepts (Table III). As compared with the placebo group, there was a 1.1% decrease in NFL in the vitamin D group at week 48 and a 7.3% decrease at week 96, but neither of these were statistically significant.

Our previous observational data suggest that the effect of vitamin D could be restricted to patients not receiving interferon beta treatment (3). When analyzing the 29 patients who did not use immunomodulatory treatment throughout the study period separately in the same mixed model, NFL decreased 30.1% in the vitamin D group compared to the placebo group at week 48 ( $p = 0.06$ ) and 32.6 % at week 96 ( $p = 0.06$ ).

## **Discussion**

It is recognized that a causal relationship between vitamin D status and disease activity can only be established in controlled studies (26, 27). In MS, clinical studies with published results so far have been underpowered and inconclusive, but without an overall signal for a clinical effect (28). This is to our knowledge the first randomized controlled study on the effect of vitamin D supplementation on axonal damage in MS. The main result does not support a substantial effect of vitamin D. With the exception of the effect on antibodies against Epstein Barr virus nuclear antigen (29), these results concur with the previously published results on MS disease activity, systemic inflammation and bone turnover from the same study (15, 24, 25, 30).

In this study, we found a positive correlation at baseline between serum NFL and 25-hydroxyvitamin D. This was surprising as it contradicts a previously reported association between high serum 25-hydroxyvitamin D and low CSF NFL (23). It is possible that differences between NFL measurements in serum and CSF have contributed to the discordant results. This is however unlikely as the concentrations in serum and CSF have been shown to correlate strongly (correlation coefficients from 0.75 to 0.97) in patients with diseases of the central nervous system (16). There were also differences between the patient populations in the studies. Sandberg *et al.* also included patients with primary and secondary progressive MS, their patients used several different immunomodulatory drugs that were not used by the patients in the present study, and the vitamin D status of their patients seemed to be lower and more variable (median 44, range 5-333 nmol/L). Although we cannot exclude that these or other differences between the studies contributed to the diverging results, the lack of any overall effect of vitamin D supplementation as compared to placebo in this RCT rather suggests that neither the association previously reported nor the negative correlation between baseline serum concentrations observed by us reflect causal relationships. This notion is supported by the finding that most of the patients in the vitamin D group, and very few in the placebo group, reached the serum levels of 25-hydroxyvitamin D reported by Sandberg *et al.* to be associated with low serum CSF of NFL (23).

Most patients using disease-modifying drugs in this study received interferon beta, which has been suggested to exert immunomodulatory effects in interaction with vitamin D (31). In MS patients followed with frequent MRI, we have previously found that 25-hydroxyvitamin

D was associated with disease activity before, but not during interferon beta treatment (3).

We therefore analyzed the effect of vitamin D supplementation in untreated patients separately, showing a trend for a beneficial effect in this subgroup of patients. This concurs with our previous findings suggesting that there may be an effect of vitamin D in untreated RRMS patients, but that immunomodulatory treatment, even with low efficacy drugs such as interferon beta, suppress inflammatory disease activity to the same levels independently of vitamin D status (3, 32). It should be underscored that this subgroup analysis included a restricted number of observations. The result was not significant and must be interpreted with caution.

This study has limitations. First, it is limited by the restricted number of participants, particularly for the analysis of the effect in untreated MS patients. Second, the patients had low disease activity as measured by relapse rate, and their vitamin D status was generally quite good at baseline. The patients were allowed to continue use of vitamin D supplements, and more than 50% of the patients in the placebo group reported a vitamin D intake exceeding 7.5 µg/day. This concurs with the generally favorable vitamin D status in the placebo group throughout the study. Third, it is possible that daily supplementation of vitamin D could be superior to weekly dosing as used in this study (33). The results could therefore be different in patients with more aggressive inflammatory activity, progressive forms of MS, poorer vitamin D status, or by using other vitamin D-dosing regimens. The strength of the study includes the randomized design minimizing the risk of selection bias, the stringent follow up of patients with 25-hydroxyvitamin D measurements ensuring adherence to the study drug.

In conclusion, this RCT does not support a substantial effect of weekly administration of high dose vitamin D3 on axonal damage in patients with RRMS. However, given the low cost and risk of vitamin D and the possible signal towards a beneficial effect in patients not receiving conventional disease modifying drugs, the effect of vitamin D on NFL should be studied in a larger clinical trial.

Table 1. Baseline characteristics

		Vitamin D group (N; 35)	Placebo group (N; 33)
Females	N	24	24
Age (years)	Mean (SD)	40 (8)	41 (6)
EDSS score	Median (95% CI)	2.5 (2.5-3.5)	2.0 (2.0-3.0)
Body mass index	Mean (SD)	25.9 (4.5)	26.4 (4.9)
Smoking	N	15	14

Immunomodulatory treatment	N	17 <sup>a</sup>	17 <sup>b</sup>
Disease duration (years)	Mean (SD)	11 (7)	10 (7)
Annual relapse rate <sup>c</sup>	Mean (SD)	0.11 (0.22)	0.15 (0.31)
25-hydroxyvitamin D (nmol/L)	Mean (SD)	55.6 (29.0)	57.3 (21.8)

<sup>a</sup> 16 patients on IFN- $\beta$  and 1 patient on glatiramer acetate; <sup>b</sup> 15 patients on IFN- $\beta$ , one on glatiramer acetate and one on natalizumab; <sup>c</sup> Determined by relapses leading to hospitalization last 24 months; EDSS: Kurtzke's Expanded Disability Status Scale

Table 2. Mean (SD) neurofilament light chain levels (pg/mL) during the study

	Treatment group	N	Week 0	Week 48	Week 96
All patients	Vitamin D	35	8.8 (4.3)	8.5 (4.3)	7.9 (4.1)
	Placebo	33	10.6 (8.5)	9.7 (7.5)	10.4 (8.9)
Immunomodulatory treatment	Vitamin D	17	8.3 (3.9)	8.9 (3.7)	8.1 (4.1)
	Placebo	17	10.5 (9.8)	7.5 (2.5)	7.7 (5.1)

Without immunomodulatory treatment	Vitamin D	18	9.2 (4.7)	8.0 (5.0)	7.5 (4.3)
	Placebo	16	10.7 (7.9)	11.5 (9.7)	13.1 (11.0) <sup>a</sup>

<sup>a</sup>One value missing

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Table III. Effect of vitamin D on neurofilament light chain levels (whole study population)

		Mean (SE) change from baseline (%),			
Treatment group	N	week 48	p-value	Week 96	p-value
Vitamin D	35	-5.2 (8.6)	0.55	-12.0 (8.6)	0.16

Placebo	33	-4.1 (8.8)	0.64	-4.8 (8.9)	0.16
Vitamin D versus placebo		-1.1 (1.2)	0.93	-7.3 (12.4)	0.56

#### Reference List

- (1) Munger KL, Levin LI, Hollis BW, Howard NS, Ascherio A. Serum 25-hydroxyvitamin D levels and risk of multiple sclerosis. *JAMA* 2006 Dec 20;296:2832-2838.

- (2) Salzer J, Hallmans G, Nystrom M, Stenlund H, Wadell G, Sundstrom P. Vitamin D as a protective factor in multiple sclerosis. *Neurology* 2012 Nov 20;79:2140-2145.
- (3) Loken-Amsrud KI, Holmoy T, Bakke SJ, et al. Vitamin D and disease activity in multiple sclerosis before and during interferon-beta treatment. *Neurology* 2012 Jul 17;79:267-273.
- (4) Simpson S Jr, Taylor B, Blizzard L, et al. Higher 25-hydroxyvitamin D is associated with lower relapse risk in multiple sclerosis. *Ann Neurol* 2010 Aug;68:193-203.
- (5) Ferre' L, Clarelli F, Sferruzza G, et al. Basal vitamin D levels and disease activity in multiple sclerosis patients treated with fingolimod. *Neurol Sci* 2018 May 13.
- (6) Colotta F, Jansson B, Bonelli F. Modulation of inflammatory and immune responses by vitamin D. *J Autoimmun* 2017 Dec;85:78-97.
- (7) Wrzosek M, Lukaszkiwicz J, Wrzosek M, et al. Vitamin D and the central nervous system. *Pharmacol Rep* 2013;65:271-278.
- (8) Smolders J, Schuurman KG, van Strien ME, et al. Expression of vitamin D receptor and metabolizing enzymes in multiple sclerosis-affected brain tissue. *J Neuropathol Exp Neurol* 2013 Feb;72:91-105.
- (9) Nystad AE, Wergeland S, Aksnes L, Myhr KM, Bo L, Torkildsen O. Effect of high-dose 1.25 dihydroxyvitamin D3 on remyelination in the cuprizone model. *APMIS* 2014 Dec;122:1178-1186.
- (10) Shoemaker TJ, Mowry EM. A review of vitamin D supplementation as disease-modifying therapy. *Mult Scler* 2018 Jan;24:6-11.
- (11) Kimball SM, Ursell MR, O'connor P, Vieth R. Safety of vitamin D3 in adults with multiple sclerosis. *Am J Clin Nutr* 2007 Sep;86:645-651.
- (12) Burton JM, Kimball S, Vieth R, et al. A phase I/II dose-escalation trial of vitamin D3 and calcium in multiple sclerosis. *Neurology* 2010 Jun 8;74:1852-1859.
- (13) Soilu-Hanninen M, Aivo J, Lindstrom BM, et al. A randomised, double blind, placebo controlled trial with vitamin D3 as an add on treatment to interferon beta-1b in patients with multiple sclerosis. *J Neurol Neurosurg Psychiatry* 2012 Feb 22.
- (14) Stein MS, Liu Y, Gray OM, et al. A randomized trial of high-dose vitamin D2 in relapsing-remitting multiple sclerosis. *Neurology* 2011 Oct 25;77:1611-1618.
- (15) Kampman MT, Steffensen LH, Mellgren SI, Jorgensen L. Effect of vitamin D3 supplementation on relapses, disease progression and measures of function in persons with multiple sclerosis: exploratory outcomes from a double-blind randomised controlled trial. *Mult Scler* 2012 Feb 21.
- (16) Zetterberg H, Blennow K. From Cerebrospinal Fluid to Blood: The Third Wave of Fluid Biomarkers for Alzheimer's Disease. *J Alzheimers Dis* 2018 May 11.
- (17) Lycke JN, Karlsson JE, Andersen O, Rosengren LE. Neurofilament protein in cerebrospinal fluid: a potential marker of activity in multiple sclerosis. *J Neurol Neurosurg Psychiatry* 1998 Mar;64:402-404.

- (18) Gunnarsson M, Malmestrom C, Axelsson M, et al. Axonal damage in relapsing multiple sclerosis is markedly reduced by natalizumab. *Ann Neurol* 2011 Jan;69:83-89.
- (19) Novakova L, Axelsson M, Khademi M, et al. Cerebrospinal fluid biomarkers of inflammation and degeneration as measures of fingolimod efficacy in multiple sclerosis. *Mult Scler* 2017 Jan;23:62-71.
- (20) Kuhle J, Barro C, Disanto G, et al. Serum neurofilament light chain in early relapsing remitting MS is increased and correlates with CSF levels and with MRI measures of disease severity. *Mult Scler* 2016 Oct;22:1550-1559.
- (21) Novakova L, Zetterberg H, Sundstrom P, et al. Monitoring disease activity in multiple sclerosis using serum neurofilament light protein. *Neurology* 2017 Nov 28;89:2230-2237.
- (22) Varhaug KN, Barro C, Bjornevik K, et al. Neurofilament light chain predicts disease activity in relapsing-remitting MS. *Neurol Neuroimmunol Neuroinflamm* 2018 Jan;5:e422.
- (23) Sandberg L, Bistrom M, Salzer J, Vagberg M, Svenningsson A, Sundstrom P. Vitamin D and axonal injury in multiple sclerosis. *Mult Scler* 2015 Oct 13.
- (24) Steffensen LH, Jorgensen L, Straume B, Mellgren SI, Kampman MT. Can vitamin D(3) supplementation prevent bone loss in persons with MS? A placebo-controlled trial. *J Neurol* 2011 Mar 13.
- (25) Holmoy T, Lindstrom JC, Eriksen EF, Steffensen LH, Kampman MT. High dose vitamin D supplementation does not affect biochemical bone markers in multiple sclerosis - a randomized controlled trial. *BMC Neurol* 2017 Apr 4;17:67.
- (26) Jorde R. RCTS are the only appropriate way to demonstrate the role of vitamin D in health. *J Steroid Biochem Mol Biol* 2018 Mar;177:10-14.
- (27) Damoiseaux J, Smolders J. The Engagement Between Vitamin D and the Immune System: Is Consolidation by a Marriage to Be Expected? *EBioMedicine* 2018 Apr 18.
- (28) Zheng C, He L, Liu L, Zhu J, Jin T. The efficacy of vitamin D in multiple sclerosis: A meta-analysis. *Mult Scler Relat Disord* 2018 May 12;23:56-61.
- (29) Rosjo E, Lossius A, Abdelmagid N, et al. Effect of high-dose vitamin D3 supplementation on antibody responses against Epstein-Barr virus in relapsing-remitting multiple sclerosis. *Mult Scler* 2016 Jun 20.
- (30) Rosjo E, Steffensen LH, Jorgensen L, et al. Vitamin D supplementation and systemic inflammation in relapsing-remitting multiple sclerosis. *J Neurol* 2015 Oct 1.
- (31) Stewart N, Simpson S Jr, van dM, I, et al. Interferon-beta and serum 25-hydroxyvitamin D interact to modulate relapse risk in MS. *Neurology* 2012 Jul 17;79:254-260.
- (32) Rosjo E, Myhr KM, Loken-Amsrud KI, et al. Vitamin D status and effect of interferon-beta1a treatment on MRI activity and serum inflammation markers in relapsing-remitting multiple sclerosis. *J Neuroimmunol* 2015 Mar 15;280:21-28.

- (33) Hollis BW, Wagner CL. Clinical review: The role of the parent compound vitamin D with respect to metabolism and function: Why clinical dose intervals can affect clinical outcomes. *J Clin Endocrinol Metab* 2013 Dec;98:4619-4628.