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Vitamin D_3 supplementation and neurofilament light chain in multiple sclerosis

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Objectives: Low circulating vitamin D levels are associated with an increased risk of active MRI lesions and relapses in several cohorts with relapsing remitting multiple sclerosis (RRMS). Randomized controlled supplementation trials are, however, negative on their primary endpoints, while secondary MRI endpoints suggest anti-inflammatory effects. Circulating levels of neurofilament light chain (NfL) are a biomarker of disease activity in RRMS. We explored whether 48-week high-dose vitamin D_3 supplements were associated with lower circulating NfL levels.

Materials & Methods: Of N = 40 Dutch interferon beta-treated participants with RRMS of the SOLAR trial, plasma samples at baseline and 48-week follow-up were available. Of these participants, N = 24 were supplemented with 14 000 IU/d vitamin D_3 and N = 16 with placebo. Twenty-five hydroxyvitamin D_3 (25(OH)D_3) levels were measured with LC-MS/MS, and NfL levels were measured in duplicate with Simoa.

Results: Serum 25(OH)D_3 levels at 48 weeks were increased in the vitamin D_3 when compared to placebo group (median level 281 [IQR 205-330] vs 72 [39-88] nmol/L; $P < .01$). NfL levels at 48 weeks did not differ between the treatment groups (median level 25.4 [IQR 19.6-32.2] vs 25.3 [17.9-30.1] pg/mL; $P = .74$). Higher week 48 NfL level showed a trend toward association with a higher risk of combined unique active lesions on the week 48 MRI scan (OR 2.39 [95% CI 0.93-6.12] for each 10 pg/mL increase; $P = .07$).

Conclusions: Supplementation of high-dose vitamin D_3 for 48 weeks was not associated with lower NfL levels. This study does not support an effect of vitamin D_3 on this biomarker of neuro-axonal injury.

KEYWORDS
25-hydroxyvitamin D, multiple sclerosis, neurofilament light chain, supplementation, vitamin D

1 | BACKGROUND

Low circulating levels of 25-hydroxyvitamin D (25(OH)D) are associated with a higher risk of MRI lesions and relapses in several cohorts with relapsing remitting multiple sclerosis (RRMS).[1] Randomized controlled trials on high-dose vitamin D_3 supplements thus far missed their primary endpoints,[2-8] yet some suggested benefits on secondary MRI endpoints.[2,7,8] In the SOLAR trial, we did not detect an increased proportion of interferon β1α-treated participants with RRMS reaching no evidence of disease activity (NEDA-3) in the high-dose vitamin D_3 supplements vs placebo arm, yet observed a lower number of combined unique active lesions (CUA) and a reduced T2

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volume increase on the 48-week MRI scan.[7] In a sub-study among Dutch SOLAR participants (SOLARIUM), we observed no effect of vitamin D_3 supplements on general markers of lymphocyte homeostasis,[9] but did observe a reduction in circulating anti–Epstein-Barr virus Nuclear Antigen-1 (Ebna-1) IgG antibodies.[10] Anti-Ebna-1 IgG levels have been identified as a biomarker for disease activity of RRMS in several but not all cohorts.[11] Similarly, a Norwegian trial on average-dose vitamin D_3 supplements vs placebo in RRMS did not show an effect on clinical disease parameters,[3] but showed a reduction of anti-Ebna-1 IgG.[12] In the latter study, blood levels of neurofilament light chain (NfL) were measured, but did not show a significant variation.[13] Blood NfL levels have been identified as a biomarker of adverse disease outcomes in RRMS.[14-16] To further explore a possible benefit in taking vitamin D_3 supplements for people with MS, we measured NfL levels in participants of the SOLARIUM cohort. Since serum levels of 25(OH)D were measured with a radioimmunoassay in the SOLAR trial and exceeded the detection limit of the assay, we measured in addition plasma 25(OH)D_3 levels with liquid chromatography-mass spectrometry.

2 | METHODS

2.1 | Study design

Regrettably, no biomaterials were available from the SOLAR trial to study NfL levels. Therefore, the effect of vitamin D_3 supplements on blood NfL levels was explored as post hoc measurements in the Dutch SOLARIUM study,[9,10,17] a sub-study of the SOLAR trial (NCT01285401).[7] In short, participants were interferon β1α-treated RRMS patients, with a first clinical event within the previous 5 years, but no relapse 30 days before inclusion. Patients were randomized to a placebo or vitamin D_3 group following procedures described elsewhere.[7] Patients in the vitamin D_3 group received cholecalciferol drops (Vigantol Oil, Merck) 7000 IU/d in the first 4 weeks, followed by 14 000 IU/d up to week 48. As part of the SOLAR study protocol, presence of CUA at the week 48 MRI scan (new or enlarging T2 lesions when compared to the baseline MRI scan and/or gadolinium-enhancing MRI lesions, Y/N) was registered.[7] Written informed consent was acquired, and the SOLARIUM study was approved by the Ethical Committee METC-Z (Heerlen, the Netherlands).

2.2 | Vitamin D and NfL measurements

Plasma samples were collected at baseline and week 48 and stored at −80°C. Plasma levels of 25(OH)D_3 were measured using liquid chromatography-mass spectrometry/ mass spectrometry (LC-MS/MS) following earlier published procedures.[18,19] Coefficients of variation (CV) were 7.4% at 36 nmol/L, 4.0% at 88 nmol/L, and 3.1% at 124 nmol/L, respectively. Lower limit of quantification was 1 nmol/L. From the SOLAR dataset, serum 25(OH)D levels were available as determined using the DiaSorin immunoassay method.[7] Plasma levels of NfL were determined with a single molecule array (Simoa) analysis in duplicates following earlier published procedures.[16] Median intra-assay CV was 5.1% (interquartile range 3.0%-7.1%; min-max range 1%-18%).

TABLE 1 Cohort characteristics

	Placebo (N = 16)	Vitamin D_3 (N = 24)
Female sex (N [%])	11 (69)	17 (71)
Age (y; Med [IQR])	40 (33-47)	37 (31-42)
Disease duration (mo; Med [IQR])	5.7 (4.1-8.2)	6.5 (4.5-11.1)
≤1 relapse previous 2 y (N [%])	2 (13)	9 (37)
Time since last relapse (mo, Med [IQR])	8.0 (6.6-9.3)	6.5 (4.0-9.9)
BMI ≥ 25 (N [%])	9 (56)	12 (50)

2.3 | Statistical analysis

Statistical analyses were conducted with GrahPad Prism (GraphPad Software) and SPSS (SPSS Inc, version 20.0). Descriptive statistics are provided for continuous variables as median and corresponding interquartile range (IQR), for proportions as number with corresponding percentage. Non-parametric paired (Wilcoxon signed ranks) or non-paired (Mann-Whitney *U*) statistics were applied, and correlations were tested with the Spearman correlation coefficient. Prediction of 25(OH)D_3 levels reached in the vitamin D_3 arm by BMI corrected for age was analyzed in a linear regression model, and prediction of week 48 CUA by NfL levels corrected for age was analyzed in a logistic regression model. A *P*-value <.05 was considered significant.

3 | RESULTS

Of the SOLARIUM sub-study, material could be retrieved of N = 40/53 participants (75%), of which N = 24/30 (80%) were allocated to the vitamin D_3 arm, and N = 16/23 (70%) to the placebo arm. These two groups were balanced for most variables, except a larger proportion of placebo-treated patients experiencing 2 or more relapses the previous 2 years (Table 1). The characteristics of this subgroup were not different from those of the total SOLARIUM cohort (data not shown).

Plasma 25(OH)D_3 levels were significantly elevated in the vitamin D_3 group, with a median level of 81.80 nmol/L (IQR 58.56-123.17) at baseline and 281 nmol/L (IQR 205.29-330.40) at week 48 ($P < .01$). The serum levels in the placebo group remained stable, 76.7 nmol/L (IQR 53.58-91.42) vs 71.9 nmol/L (38.95-87.87), respectively ($P = .42$). Plasma 25(OH)D_3 levels as measured with LC-MS/

MS were overall higher than serum 25(OH)D levels reported with the radioimmunoassay (median 91.55 nmol/L [IQR 62.5-161.78] vs 70 nmol/L [IQR 46-119.75], resp., $P < .01$), but showed a strong positive correlation within the measurable range (<250 nmol/L; $R = .917$, $P < .01$). Participants in the vitamin D_3 arm with a higher BMI had numerically lower 25(OH)D_3 levels, BMI < 25 median 304.1 nmol/L (IQR 276.61-334.33) vs BMI ≥ 25 median 237.9 nmol/L (IQR 158.62-287.42; $P = .143$). In these participants, 25(OH)D_3 levels reached were best predicted in a linear model (overall model fit $R^2 = .330$; $P = .02$) containing BMI category (β −96.37 [95% CI −162.73 to −30.00]; $P < .01$) and age (β 4.54 [95%CI −0.431 to 9.503]; $P = .07$).

Average plasma NfL levels were at baseline and week 48 equally low in placebo (median level 32.8 pg/mL [IQR 24.58-34.4] vs 25.3 pg/mL [IQR 17.85-30.05], resp.) and vitamin D_3 arm (median levels 23.7 pg/mL [IQR 19.8-33.65] vs 25.35 pg/mL [19.55-32.13], resp.) without any significant or relevant differences between timepoints or treatment arms (Figure 1A). Baseline NfL levels correlated positively with age (Spearman R .356; $P = .03$), but not with other baseline variables. Participants with CUA (n = 8) on their 48-week MRI scan had numerically slightly higher week 48 NfL levels when compared to participants without CUA (n = 29, median 28.2 [IQR 22.7-38.4] vs 22.6 [18.3-28], resp., $P = .12$) (Figure 1B). Corrected for age, higher NfL levels corresponded with a trend toward an increased risk of CUA (OR 2.39 [95% CI 0.93-6.12] for each 10 pg/mL increase; $P = .07$). Of the eight participants with CUA, six participants had N = 1 new T2 lesion on their 48-week MRI scan, one participant had N = 2 new T2 lesions (NfL level 26.5 pg/mL), and one participant had N = 3 new T2 lesions (NfL level 40.8 pg/mL). No participants had gadolinium-enhancing MRI lesions on the week 48 MRI scan.

4 | DISCUSSION

High-dosed vitamin D_3 supplements in interferon beta 1a–treated RRMS did not result in lower plasma NfL levels. This adds to the negative findings of this intervention on clinical endpoints in this study.[7] It is also in line with another study, where moderate doses of vitamin D_3 supplements did not affect both clinical and NfL endpoints.[3,13] These negative results are paralleled by a positive effect of supplements on MRI endpoints.[2,7] Therefore, despite these data, vitamin D_3 supplementation in RRMS remains a controversial issue.

Median blood NfL levels in our sample were in the same range as reported for instance in the FREEDOMS (27.1 pg/mL) and TRANSFORMS (24.1 pg/mL) studies by the same laboratory.[16] However, our data set showed only two samples with NfL levels exceeding 60 pg/mL (baseline 86.3 and 64.3 pg/mL, 2.53 and 3.42 months, respectively, after a clinical attack), where the range of NfL levels in these previous studies was up to 372.7-589.5 pg/mL.[16] These data support the general notion of inclusion of patients without severe inflammatory activity in the SOLAR trial, which may be attributable to the introduction of many new disease-modifying therapies at the time of the SOLAR study.[7] Treatment with interferon β1α did result in a drop of circulating NfL in an earlier study.[20] Since the association between MRI activity and 25(OH)D levels was also abrogated by treatment with interferon β in this same cohort,[21] an effect of vitamin D_3 supplements on plasma NfL levels may be masked by this treatment. However, the trend toward an association with the presence of combined unique active lesions on week 48 MRI suggests that NfL may also reflect relevant inflammatory disease activity in these low ranges. There are some limitations of the data presented. A relevant effect of vitamin D_3 supplements on blood NfL levels in cohorts with a more pronounced disease activity cannot be excluded. Most importantly, the small sample size and exploratory nature of analyses performed carry a high risk of false-negative results. Regrettably, no biomaterials of the parental SOLAR trial are available for analysis. The samples of the SOLARIUM cohort were therefore the best to explore any effect of high-dosed vitamin D_3 supplements in the context of this trial. The Dutch MS cohort is likely to show a small seasonal fluctuation of 25(OH)D_3 levels.[22] This fluctuation was not taken into account in this study, but is unlikely to have substantially confounded the large variation in 25(OH)D_3 levels induced by highly dosed vitamin D_3 supplements. The strengths of our data include the double-blind, placebo-controlled, and randomized design, and the extensive monitoring of participants during follow-up in a clinical trial.

Ongoing studies on vitamin D_3 supplements in early MS and clinically isolated syndrome (CIS)[1] may be able to retrieve samples from a larger cohort of participants with more active disease for NfL

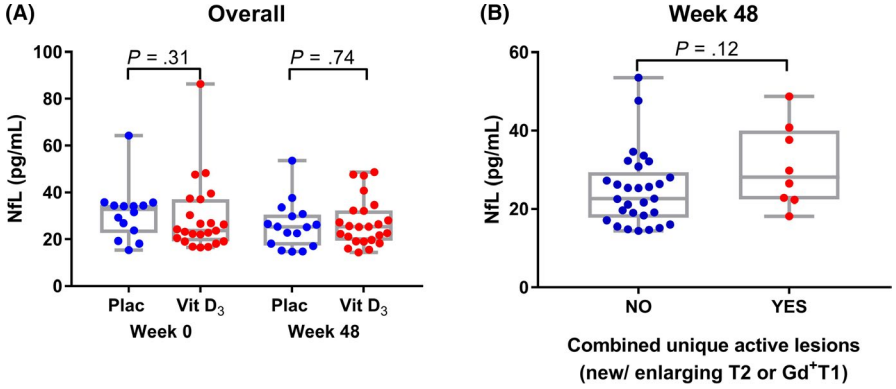

FIGURE 1 Plasma NfL levels. A, NfL levels stratified for treatment arm and timepoint. Plac, placebo arm; Vit D_3, Vitamin D_3 arm. Difference between treatment arms was tested with the Mann-Whitney *U* test. B, Week 48 NfL levels stratified for presence of combined unique active lesions (NO/YES). Significance was tested with the Mann-Whitney *U* test

measurement. Biomarkers as NfL and MRI are important to reveal biologically relevant effects of vitamin D_3 supplements. These may be insufficiently captured in clinical endpoints in relatively small trial cohorts. Upcoming randomized controlled trials and subsequent meta-analyses of published trials may bring an end to the controversy surrounding vitamin D_3 supplementation in MS.

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CONFLICT OF INTEREST

JS received consultancy and/or lecture of Novartis, Sanofi-Genzyme, Merck, and Biogen; MM, JO, JD, and JvdO had no conflicts of interest to declare; RH received fees for advisory boards and lectures and institutional research grants from Sanofi-Genzyme, Merck and Biogen; and JK received travel support, research support, speaker fees, and/or served on advisory boards by ECTRIMS, Swiss MS Society, Swiss National Research Foundation (320030_160221), University of Basel, Teva, Roche, Protagen AG, Novartis, Merck, Genzyme, Biogen, and Bayer.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

1. Amato MP, Derfuss T, Hemmer B, et al. Environmental modifiable risk factors for multiple sclerosis: report from the 2016 ECTRIMS focused workshop. *Mult Scler J*. 2017;24(5):590-603.
2. Soilu-Hänninen M, Aivo J, Lindström B-M, et al. A randomised, double blind, placebo controlled trial with vitamin D3 as an add on treatment to interferon β-1b in patients with multiple sclerosis. *J Neurol Neurosurg Psychiatry*. 2012;83(5):565-571.
3. Kampman MT, Steffensen LH, Mellgren SI, Jørgensen L. Effect of vitamin D $_3$ 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 J*. 2012;18(8):1144-1151.
4. Stein MS, Liu Y, Gray OM, et al. A randomized trial of high-dose vitamin D2 in relapsing-remitting multiple sclerosis. *Neurology*. 2011;77(17):1611-1618.
5. Golan D, Halhal B, Glass-Marmor L, et al. Vitamin D supplementation for patients with multiple sclerosis treated with interferon-beta: a randomized controlled trial assessing the effect on flu-like symptoms and immunomodulatory properties. *BMC Neurol*. 2013;13(1):60.
6. O'Connell K, Sulaimani J, Basdeo SA, et al. Effects of vitamin D_3 in clinically isolated syndrome and healthy control participants: a double-blind randomised controlled trial. *Mult Scler J Exp Transl Clin*. 2017;3(3):205521731772729.
7. Hupperts R, Smolders J, Vieth R, et al. Randomized trial of daily high-dose vitamin D_3 in patients with RRMS receiving subcutaneous interferon β-1a. *Neurology*. 2019;93:1-19.
8. Camu W, Lehert P, Pierrot Deseilligny C, et al. Cholecalciferol in relapsing-remitting MS. A randomized clinical trial (CHOLINE). *Neurol Neuroimmunol Neuroinflamm*. 2019;6(5):e597.
9. Muris A-H, Smolders J, Rolf L, Thewissen M, Hupperts R, Damoiseaux J. Immune regulatory effects of high-dose vitamin D_3 supplementation in a randomized controlled trial in relapsing remitting multiple sclerosis patients receiving IFNβ; the SOLARIUM study. *J Neuroimmunol*. 2016;300:47-56.
10. Rolf L, Muris A-H, Mathias A, et al. Exploring the effect of vitamin D_3 supplementation on the anti-EBV antibody response in relapsing-remitting multiple sclerosis. *Mult Scler J*. 2017;24:1280-1287.
11. Kvistad S, Myhr K-M, Holmøy T, et al. Antibodies to Epstein-Barr virus and MRI disease activity in multiple sclerosis. *Mult Scler J*. 2014;20(14):1833-1840.
12. Røsjø 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*. 2017;23(3):395-402.
13. Holmøy T, Røsjø E, Zetterberg H, et al. Vitamin D supplementation and neurofilament light chain in multiple sclerosis. *Acta Neurol Scand*. 2018;139:172-176.
14. Khalil M, Teunissen CE, Otto M, et al. Neurofilaments as biomarkers in neurological disorders. *Nat Rev Neurol*. 2018;14(10):577-589.
15. Barro C, Benkert P, Disanto G, et al. Serum neurofilament as a predictor of disease worsening and brain and spinal cord atrophy in multiple sclerosis. *Brain*. 2018;141(8):2382-2391.
16. Kuhle J, Kropshofer H, Haering DA, et al. Blood neurofilament light chain as a biomarker of MS disease activity and treatment response. *Neurology*. 2019;92(10):e1007-e1015.
17. Rolf L, Muris A-H, Theunissen R, Hupperts R, Damoiseaux J, Smolders J. Vitamin D_3 supplementation and the IL-2/IL-2R pathway in multiple sclerosis: attenuation of progressive disturbances? *J Neuroimmunol*. 2017;314:50-57.
18. van den Ouweland JMW, Beijers AM, van Daal H. Overestimation of 25-hydroxyvitamin D3 by increased ionisation efficiency of 3-epi-25-hydroxyvitamin D3 in LC–MS/MS methods not separating both metabolites as determined by an LC–MS/MS method for separate quantification of 25-hydroxyvitamin D3, 3-epi-25-hydroxyvitamin D3 and 25-hydroxyvitamin D2 in human serum. *J Chromatogr B*. 2014;967:195-202.
19. Rolf L, Smolders J, van den Ouweland J, Hupperts R, Damoiseaux J. Correlation of different cellular assays to analyze T cell-related cytokine profiles in vitamin D3-supplemented patients with multiple sclerosis. *Mol Immunol*. 2019;105:198-204.
20. Varhaug KN, Barro C, Bjørnevik K, et al. Neurofilament light chain predicts disease activity in relapsing-remitting MS. *Neurol Neuroimmunol Neuroinflammation*. 2018;5(1):e422.
21. Loken-Amsrud KI, Holmoy T, Bakke SJ, et al. Vitamin D and disease activity in multiple sclerosis before and during interferon- treatment. *Neurology*. 2012;79(3):267-273.
22. Muris A-H, Smolders J, Rolf L, et al. Vitamin D status does not affect disability progression of patients with multiple sclerosis over three year follow-up. *PLoS ONE*. 2016;11(6):e0156122.

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