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25-Hydroxyvitamin D levels in serum at the onset of multiple sclerosis

M Soilu-Hänninen,[1], L Airas[3], I Mononen[2], A Heikkilä[3], M Viljanen[4] and A Hänninen[1]*
[1]Medicity Research Laboratory, Tykistökatu 6, FIN-20520 Turku, Finland; [2]Turku University Hospital Central Laboratory, Tykistökatu 6, FIN-20520 Turku, Finland; [3]Department of Neurology, University of Turku, FIN-20520 Turku, Finland; [4]Department of Medical Microbiology, University of Turku, FIN-20520 Turku, Finland

Past sun exposure and vitamin D supplementation have been associated with a reduction in the risk of MS. We measured the serum concentration of 25-hydroxyvitamin D (25[OH]D) at the time of MS diagnosis in 40 MS patients and 40 controls. We found no difference in the serum levels of 25(OH)D between MS patients and controls when all samples or samples obtained during winter months were compared, but MS patients had significantly lower serum 25(OH)D concentrations in June to September than the controls. The vitamin D stores were adequate for bone metabolism (>37 nmol/L) in 70% of MS patients throughout the year and within the hypovitaminosis level (<37 nmol/L) in 30% of MS patients at some time of the year. During MS-relapses, 25(OH)D levels were lower than in remission, but mostly within the reference range observed in relation with normal bone metabolism. We conclude that the vitamin D stores in most MS patients are adequate for their normal bone metabolism. However, lower vitamin D levels during MS relapses than in remission suggest that vitamin D could be involved in the regulation of the clinical disease activity of MS. The optimal serum levels of vitamin D for the regulation of immune responses remain to be determined.
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Key words: *EAE; environmental factors; MRI; multiple sclerosis; vitamin D*

Background

Environmental factors that are part of MS pathogenesis, have not been identified. A striking feature of MS epidemiology is its geographical distribution, with dramatic incease in prevalence and incidence with increasing distance from the equator.[1–3] In addition, in Switzerland and Norway, MS rates are lower in high altitudes, where UV light intensity is higher.[4] Moreover, reduced risk of multiple sclerosis in association with past exposure to sun was recently reported in Tasmanian subjects.[3] These epidemiological findings could be explained by UV light catalyzing production of vitamin D3 in the skin. In addition to its role in calcium and bone metabolism, vitamin D regulates cell proliferation and differentiation and can regulate immune responses. Receptors for vitamin D are expressed widely on cells of the immune system.[5] Most notably, the active vitamin D metabolite, 1,25-dihydroxyvitamin D(3), is able to modify dendritic cells so that these important antigen-presenting cells start to promote tolerance instead of vigorous immune responses.[6] In animal models of several autoimmune diseases including experimental allergic encephalomyelitis (EAE), a model of MS, vitamin D and its derivatives have shown disease-moderating effects.[7–10] The most direct evidence so far supporting a protective effect of vitamin D in MS was provided by a recent large prospective epidemiological study which related the intake of vitamin D from multivitamin supplements to a 40% reduction in the risk of MS among female nurses living in the USA.[11]

The serum concentration of 25-hydroxyvitamin D (25[OH]D, calcidiol) is a reflection of the intake of vitamin D in the food and its synthesis from provitamins in the skin under the influence of UV light. Hypovitaminosis D can be defined as a serum level of hydroxyvitamin D, at which serum parathyroid hormone (PTH) concentration starts to increase.[12–14] In severe hypovitaminosis D the serum 25(OH)D concentrations are less than 20 nmol/L, in moderate hypovitamonosis D the levels are 20–37 nmol/L and in persons with adequate vitamin D stores the levels are above 37 nmol/L[12] A marked seasonal variation in 25(OH)D serum levels in the Finnish population has been described.[15] Earlier work have shown a loss of the seasonal rhythm of serum hydroxyvitamin D at the onset of another autoimmune disease, type 1 diabetes mellitus,[16] as well as a striking seasonal variation in multiple sclerosis disease activity.[17] Studies directly measuring the circulating levels of vitamin D in MS are sparse and have mostly included patients with long disease history and severe disability.[18] Therefore, in this cross-sectional study, we have measured serum concentration of hydroxyvitamin D in 40 Finnish patients undergoing diagnostic investigations for MS in comparison with age- and sex-matched non-MS controls, who had given blood at the same time of the year.

**Correspondence: Dr M Soilu-Hänninen, MD, PhD, Medicity Research Laboratory, University of Turku, Tykistökatu 6, FIN-20520 Turku, Finland.*
E-mail: mersoi@utu.fi
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Patients and methods

Subjects

Thirty-three female and seven male patients ungergoing diagnostic investigations for MS at the Turku University Central Hospital (Turku, Finland) neurology outpatient policlinic or ward, or Loimaa District Hospital (Loimaa, Finland) neurology outpatient policlinic, between February 2000 and June 2003 were included. The mean age ± SEM of the patients was 36.5 ± 1.3 years. Controls included 26 neurologically investigated patients and 14 healthy persons. Thirty-two of the controls were female and eight were male. Mean age (± SD) of the controls was 34.3 ± 1.3. The diagnoses of the neurological controls were Bell's palsy in one, headache in two, hemiplegic migraine in one, migraine with aura in three, headache after lumbar puncture in two, paresthesia in two, benign positional vertigo in one, dizziness and vertigo in two, scotoma scintillans in three, extrapyramidal syndrome in two, depression in one, seizure in one, unknown paraparesis in one, fibromyalgia in three and panic disorder in one. The study was approved by the joint Commission on Ethics of the Turku University and the Turku University Central Hospital.

Serum and CSF samples

A serum sample was taken from all subjects and a CSF sample from all MS patients and neurologically investigated controls. CSF IgG index and oligoclonal banding were determined at the Turku University Hospital Central Laboratory. An aliquot of each serum and CSF sample was sent to the Department of Medical Microbiology for the determination of Borrelia burgdorferi antibodies and for storage until the 25(OH)D assay. MS patients with positive Lyme serology, or not fulfilling Poser or McDonald diagnostic criteria for definite MS[19,20] by the time of the 25(OH)D analysis, were excluded from this study. None of the patients was on immune modulating therapy at the time of the 25(OH)D sample.

25(OH) D analysis

The serum samples were stored at −20°C and protected from direct exposure to sunlight until the analysis. For the quantitative determination of 25(OH)D in the serum samples, a commercially available 25-hydroxyvitamin D ^{125}I RIA Kit (DiaSorin Catalog No. 68100E, Stillwater, Minnesota, USA) was used according to the instructions provided by the manufacturer. All determinations were performed in the Turku University Hospital Central Laboratory. Two quality control samples were included in each assay series and the specimens and controls were assayed in duplicate.

Magnetic resonance imaging and clinical assessment

T2- and T1-weighted 1.0 Tesla brain MRI scanning with 0.1 mmol/kg gadolinium was performed at the Department of Radiology, Turku University Central Hospital. Mean time from the 25(OH)D serum sample to the MRI was 1.6 months (range −4 to +7 months). Neurological examination for determination of the Kurtzke expanded disability status scale (EDSS) was done at the time of taking the serum sample for the 25(OH)D measurement. A relapse was defined as the appearance of 1 or more new neurological abnormalities or the reappearance of 1 or more previously observed neurological abnormalities for at least 48 hours in the absence of fever or other acute metabolic change. For the patient to be defined as being in remission, the neurological status had to be relatively stable or improving at least for 30 days. The mean ± SEM time in remission before taking of the blood sample for the vitamin D assay was 39 ± 10 months (range 30 days to 13 years). All the ''at relapse'' serum samples were during the peak symptoms of the relapse before the onset of the recovery phase.

Statistical analyses

For group comparisons, a non-paired two-tailed *t*-test was performed using a GraphPad Prism 4-software. To determine whether the seasonal variation of 25-hydroxyvitamin D was altered, the patients we grouped according to the season (June to September considered as summer months and October to May as winter). When indicated, the Bonferroni correction to adjustement of multiple statistical analyses was performed.

Results

Seasonal variation of 25-hydroxyvitamin D

There was no difference in the serum levels of 25(OH)D between MS patients and controls when all samples (50 ± nmol/L in MS and 57 ± nmol/L in the controls, $P = 0.202$) or samples obtained during winter months (41 ± 5 nmol/L in the MS versus 44 ± nmol/L in the controls, $P = 0.659$) were compared, but during the summer months MS patients had significantly lower 25(OH)D levels than the controls (58 ± nmol/L in MS versus 85 ± nmol/L in controls, $P = 0.022$ (Figure 1). Use of the Bonferroni correction for multiple statistical analyses in comparing the summer and winter seasons separately did not abolish the statistical significance in the 25(OH)D values between MS patients and controls during the summer (corrected *P*-value 0.044 for the summer season).

During the summer, two MS patients but none of the controls suffered from a moderate hypovitaminosis D [25(OH)D levels 20–37 nmol/L]. During the winter, one MS patient and one control had a severe hypovitaminosis D [25(OH)D levels below 20 nmol/L] and eight MS patients and eleven controls had a moderate hypovitaminosis D.

Serum 25-hydroxyvitamin D and clinical disease activity

Twenty-one of the patients were seen at relapse and 17 in remission. Two patients were classified as primary progressive (Table 1). At the time of MS relapses, 25(OH)D levels were significantly lower than at remission (Figure 2). MS patients in relapse and in remission were similar in terms of age (36.0 [range 18–53] years in relapse and 36.5 [range 25–50] years in remission), gender (M/F 3/21 in relapse and M/F 1/17 in remission), disease type

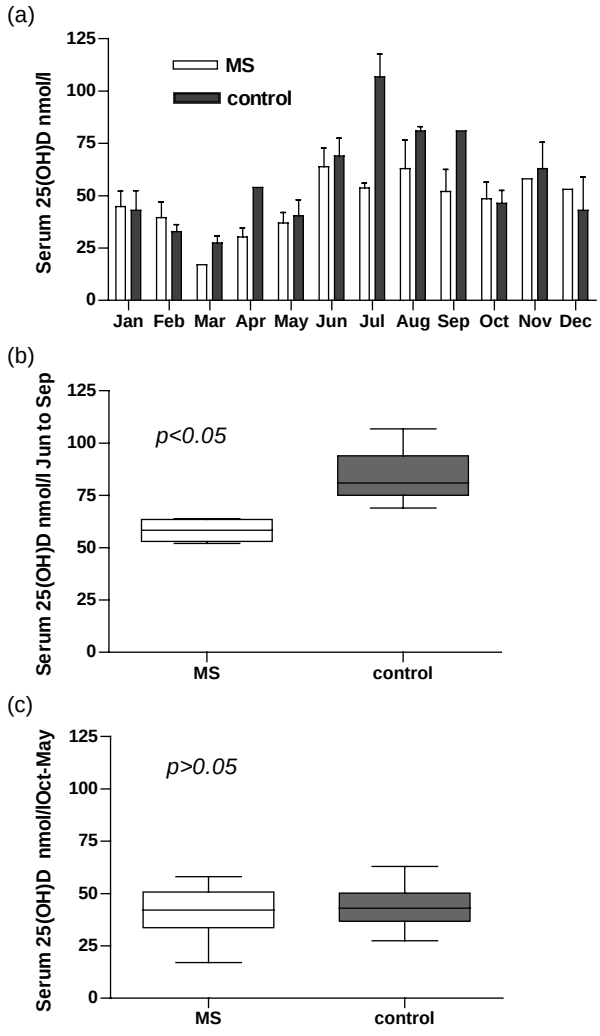

Figure 1 *Seasonal variation of circulating 25-hydroxyvitamin D is altered in MS. Serum 25(OH)D was determined at the onset of MS in 40 patients and compared with 40 age and sex-matched controls, who had given blood at the same time between February 2000 and June 2003. (A) Each bar represents Mean ± SEM of serum 25(OH)D in the patients and in the controls from whom the blood sample had been taken at the indicated month of the year. Mean ± SEM of serum 25(OH)D in the MS patients was 50 ± 3 nmol/L and in the controls 57 ± 6 nmol/L (P = 0.202 in unpaired two-tailed* t*-test, non significant). (B) Bars represent Mean ± SEM of serum 25(OH)D in the summer (June to September). MS patients had significantly lower 25(OH) D levels than the controls (58 ± 3 versus 85 ± 8 nmol/L, P = 0.022). (C) Bars represent Mean ± SEM of serum 25(OH)D in the winter (October to May). There was no difference in the serum 25(OH)D between the MS patients and the controls (41 ± 5 in the MS group versus 44 ± 4 in the controls, P = 0.659).*

(relapsing–remitting in all, since the two progressive patients were excluded from this analysis) and EDSS (mean ± SEM EDSS 1.5 ± 0.2 in both groups). The average disease duration (time from the first MS symptom to the time of taking the blood sample for vitamin D) in the relapse group was 1.9 years [range 3 days–13 years] and in the remission group 3.4 years [range 2 months–13 years], not statistically significant, $P = 0.3585$ in unpaired t-test).

Serum 25-hydroxyvitamin-D and paraclinical disease activity

The mean EDSS of all the patients was 1.5 ± 0.1, range 0–3.5). Only five patients had an EDSS value of 3.0 or 3.5, which would indicate mild disability. Most of the patients had minimal to no disability. There was no correlation between the 25(OH)D concentrations and the EDSS, the magnitude of elevation of the CSF IgG index, detection of oligoclonal bands in the CSF or enhancing lesions in the brain or spinal cord MRI (data not shown).

Discussion

An inverse correlation between brain MRI activity in MS patients and serum 25(OH)D levels in the general population living in the same area in Southern Germany has previously been shown.[21] Although we could not show a correlation between brain MRI activity and vitamin D levels in our material, such a correlation could still exist. This is because MRI scans were obtained up to 4 months before or up to 7 months after the serum sample for vitamin D. Thus, changes in vitamin D level and disease activity that would have occurred within this time could have obscured a potential (inverse) correlation between vitamin D levels and brain MRI activity.

However, in our study, serum levels of 25(OH)D were lower at the time of MS relapses than in remission, which suggests that vitamin D could be involved in regulating the clinical disease activity. Even during the relapses of MS the mean values of serum 25(OH)D were within the range found optimal for the role of vitamin D in calcium and bone metabolism.[12] It is of note that the optimal levels of vitamin D that are needed for the modification of immune responses are not known. It cannot be ruled out that the levels of vitamin D that are needed to promote immunological tolerance are higher than those needed for bone mineralization. This view is indirectly supported by a recent large prospective epidemiological study, which demonstrated the protective effect of vitamin D in MS patients, who had obtained supplemental vitamin D, mostly from multivitamins.[11] Indeed, a previous work has shown that immunological effects are induced in MS patients by oral administration of 1000 IU of vitamin D daily for 6 months. This lead to increased serum levels of the immunoregulatory cytokine TGF-beta, while the proinflammatory cytokines tumor necrosis factor-alpha and interferon-gamma remained unchanged.[22] The usefulness of vitamin D in the treatment of MS is hampered by its potential toxicity, but a curative treatment of vitamin D-deprived EAE rats with a non-toxic 1,25-dihydroxyvitamin D(3) analogue (MC1288)[23] raises hope for use of such compounds in the management of multiple sclerosis.

Finland is situated in Northern Europe between latitudes 60° and 70°. There is evidence indicating that serum levels of vitamin D vary with latitude.[24–27] Finland also belongs to a high-risk region for multiple sclerosis, with prevalences from 100 to 200 per 100 000 population in different areas.[28] The incidence of MS has been still increasing in the original high risk area Seinäjoki, located inland in central Finland, from early 1970s to the early

Table 1 Patient characteristics and serum 25(OH)D levels in 40 MS patients

Sex/age	*Time of 25(OH)D sample*	*Time from first MS symptoms to sample*	*MS diagnosis*[a] *MS type*	*CSF IgG index OB*[d] *+/−*	*MRI positive/ Gd-enhancing*	*EDSS*[b]	*25(OH)D nmol/L*
1. F/46	2/00	1 year	11/00 RR	0.91/OB+	Yes/−	1.0	47
2. F/36	4/00[c]	3 days	7/00 RR	1.21/OB+	Yes/+	1.0	39
3. F/36	7/00[c]	4 months	9/00 RR	1.39/OB+	Yes/+	1.0	57
4. F/39	7/00[c]	3 months	7/00 RR	0.62/OB+	Yes/+	1.0	55
5. F/25	8/00	6 months	12/01 RR	1.13/OB+	Yes/−	1.5	68
6. F/32	9/00	3 years	9/00 RR	4.82/OB+	Yes/−	3.5	43
7. F/48	9/00[c]	6 years	9/00 RR	1.13/OB+	Yes/−	2.5	45
8. M/39	12/00[c]	3 days	2/02 RR	0.89/OB+	Yes/−	2.5	53
9. M/47	1/01	5 years	2/01 RR	1.1/OB+	Yes/−	1.0	29
10. M/18	1/01[c]	5 days	12/01 RR	0.96/OB+	Yes/ND	1.0	35
11. F/37	1/01	13 years	2/01 RR	1.1/OB+	Yes/−	1.0	44
12. F/35	1/01	2 months	2/01 RR	0.79/OB+	Yes/+	1.5	73
13. F/24	4/01[c]	8 months	4/01 RR	1.55/OB+	Yes/+	2.0	27
14. F/37	6/01	1 year	4/03 RR	0.51/OB−	Yes/−	0	44
15. F/34	6/01[c]	13 years	7/01 RR	2.09/OB+	Yes/−	1.0	59
16. F/29	7/01	6 years	7/01 RR	2.09/OB+	Yes/+	2.0	56
17 F/45	8/01[c]	6 months	8/01 RR	0.6/OB−	Yes/−	1.0	29
18. F/27	9/01	2 years	8/00 RR	1.54/ND	Yes/−	0	93
19. M/46	9/01	6 months	12/01 PP	0.82/OB+	Yes/+	3.0	33
20. F/36	10/01	9 years	10/01 RR	1.38/OB+	Yes/−	1.5	48
21. F/27	10/01	3 years	10/01 RR	2.14/OB+	Yes/−	1.5	51
22. M/35	10/01	8 years	10/01 PP	0.82/OB+	Yes/−	3.0	28
23. F/31	1/02[c]	7 years	1/02 RR	0.62/OB−	Yes/−	2.5	59
24. F/30	1/02[c]	1 year	2/02 RR	0.65/OB+	Yes/ND	0	29
25. M/18	3/02[c]	3 months	4/02 RR	2.34/OB+	Yes/+	1.0	17
26. F/30	4/02[c]	1 week	9/02 RR	0.5/OB−	Yes/−	2.0	25
27. F/45	5/02	4 years	5/02 RR	0.75/OB+	Yes/−	3.0	36
28. F/41	5/02[c]	10 days	8/02 RR	1.4/OB+	Yes/−	1.0	29
29. M/39	5/02[c]	10months	5/02 RR	0.48/OB+	Yes/+	1.0	46
30. F/41	6/02[c]	2 weeks	10/02 RR	0.67/OB−	Yes/−	1.5	47
31. F/50	6/02	5 years	6/02 RR	0.52/OB−	Yes/−	3.0	87
32. F/53	7/02[c]	6 months	7/02 RR	0.74/OB+	Yes/+	1.5	47
33. F/37	8/02[c]	2 weeks	11/02 RR	0.81/OB+	Yes/+	2.0	95
34. F/46	8/02[c]	5 years	9/02 RR	0.9/OB+	Yes/+	1.0	60
35. F/52	9/02[c]	5 years	10/02 RR	1.25/OB+	Yes/ND	2.0	46
36. F/41	10/02	5 months	6/03 RR	1.12/OB+	Yes/+	1.0	67
37. F/43	11/02	10months	9/02 RR	0.4/OB−	Yes/−	1.5	58
38. M/20	2/03[c]	6 weeks	3/03 RR	0.99/OB+	Yes/+	2.0	32
39. F/36	6/03	9 months	3/03 RR	1.15/OB+	Yes/+	1.0	95
40. F/28	6/03	16 months	12/02 RR	1.3/OB+	Yes/−	1.0	51
(mean ± SEM) 36.5 ± 1.4	21/40 at relapse	2.6 ± 0.5 years	2 PP, 38 RR	(mean ± SEM) 1.11 ± 0.1	15/40 Gd enhancing	(mean ± SEM) 1.5 ± 0.1	(mean ± SEM) 50 ± 3

[a] McDonald or Poser criteria for definite MS.[13,14]
[b] At the time of serum sampling for serum 25(OH)D analysis.
[c] Relapse at the time of sampling.
[d] Oligoclonal banding, ND = not defined.

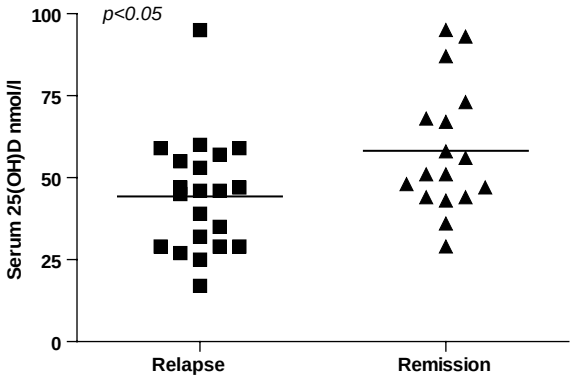

Figure 2 *Serum 25-hydroxyvitamin D (25[OH]D) levels at MS relapse and remission. Twenty-one of the 40 MS patients in the study were undergoing a relapse at the time when the blood specimen was taken for the 25(OH)D analysis, and 17 were stable. The two primary progressive patients were not included in this analysis. 25(OH)D at relapse was 44±4 nmol/L and at remission 59±5 nmol/L (statistically significant,* P = *0.0259).*

1990s. During the same period of time in Vaasa, located on the coast of central Finland, the incidence has been decreasing from intermediate to low level.[29] This points to recent changes in environmental factors, since the diagnostic practices of MS have remained the same. One recent environmental change is a change in the dosage recommendation of vitamin D supplementation for infants, which was 4000–5000 IU until 1964, 2000 IU until 1975, 1000 IU until 1992, when it was reduced to the current levels of 400 IU/day.[30] A previous study from Norway[4] reported diverging incidences between a coastal and an inland town similarly as between Vaasa and Seinäjoki in Finland. It was speculated that this could be due to differences in the UV index or higher rate of consumption of fish oil rich in vitamin D on coast.[4]

Vitamin D supplementation is safe only in persons in whom the supply of vitamin D from food or sunlight is suboptimal. Moderate vitamin D deficiency is very common among young Finnish men in the winter, affecting 38.9% of recruits of the Finnish army in January 2000, but very uncommon in the summer, affecting only 0.9% of the same population in July 2000.[31] Moderate hypovitaminosis D is also very common among Finnish medical patients affecting 70% of female and 61% of male inpatients and 44% female and 37% of male outpatiens in the winter.[14] There is no available data on the occurrence of hypovitaminosis D in Finnish medical patients during the summer. In this study, 10% of the MS patients but none of the controls had a moderate hypovitaminosis D during summer and 43% of MS patients and 48% of controls had moderate or severe hypovitaminosis D during the winter. Thus hypovitaminosis D among MS patients is as common as it is among Finnish medical outpatients and healthy population in general in the winter, but more common than in healthy population in the summer. This could be explained by the well-known heat intolerance affecting most MS patients, which is likely to limit the time spent in sunlight during the summer. Physical disability limiting the mobility of the patients and thus keeping them at home or in hospital is not a likely explanation for the difference, since the patients were at very early stages of the disease and the mean EDSS score of 1.5 even during the relapses indicated minimal to not existing physical disability. An alternative explanation is that the alteration of the seasonal rhythm of serum 25(OH)D in MS could be related to the immune-mediated nature of the disease process itself, since a similar phenomenon has been shown at the onset of another autoimmune disease, type-I-diabetes.[16]

To our knowledge, this is the first study of vitamin D levels during early phase of MS and during its remission and relapse. The small sample size and the cross-sectional design of our study as well as lack of knowledge of potential confounding factors such as smoking habits and use of multivitamin supplements cause important limitations and prevent from drawing conclusions for treatment and public policy recommendations. Nevertheless, our study suggests that although the vitamin D levels are optimal for their bone metabolism throughout the year in 70% of MS patients, they are lower at the time of MS relapses than during remission, suggesting that vitamin D could be involved in the regulation of the clinical disease activity in MS. Further prospective longitudinal studies measuring monthly MRI activity and monthly serum vitamin D levels combined to careful clinical and immunological assessment, and ultimately, controlled clinical trials will be needed to evaluate the full therapeutic and preventive potential of vitamin D and its analogues in MS.

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