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Vitamin D deficiency in patients with myasthenia gravis and improvement of fatigue after supplementation of vitamin D3: a pilot study

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Background: Myasthenia gravis (MG) is a chronic autoimmune neuromuscular disorder. Vitamin D has important roles both in the autoimmune response and in skeletal muscles. We determined the levels of 25-hydroxy vitamin D [25(OH)D] in patients with MG and in healthy subjects to determine whether vitamin D deficiency is present in MG and whether vitamin D supplementation has beneficial effects on fatigue.

Methods: Plasma levels of 25(OH)D were analyzed in 33 patients with MG (22 males; mean age, 58 years) and in 50 healthy age- and sex-matched blood donors, without vitamin D3 medication. MG composite (MGC) score assessed fatigue. Thirteen patients with MG without previous vitamin D3 supplementation were started on vitamin D3 supplementation (cholecalciferol) 800 IU/day, with a follow-up examination after 2.5–10 months (mean, 6 months).

Results: Patients with MG without pre-existing vitamin D3 supplementation ($N = 16$) had a mean MGC of 4.5 and lower plasma 25(OH)D levels (mean, 51 ± 19 nM) than healthy controls (69 ± 21 nM) ($P = 0.017$). Seventeen patients had pre-existing vitamin D3 supplementation, because of corticosteroid treatment, and their mean 25(OH)D was 79 ± 22 nM and mean MGC was 5.5. In the 13 patients who received cholecalciferol, 25(OH)D was overall increased at follow-up with 22% ($P = 0.033$) and MGC score improved by 38% ($P = 0.05$).

Conclusions: Plasma 25(OH)D levels are significantly lower in patients with MG compared with healthy controls. As vitamin D has beneficial effects on the autoimmune response and on fatigue score in patients with MG, we suggest monitoring this parameter in patients with MG and supplementation with vitamin D3 when 25(OH)D levels are low.

Introduction

Myasthenia gravis (MG) is an autoimmune neuromuscular disorder in which autoantibodies are directed against muscle receptors, in most cases the acetylcholine receptors (AChRs; 85%) [1] or muscle-specific tyrosine kinase (MuSK; 10%) [2]. Thymus hyperplasia is common in AChR antibody-seropositive (AChR+) patients with MG, and thus T cells are considered to play an important role in the onset and course of the disease [3]. The immune-regulatory effect of vitamin D is hypothesized to reside in an increased amount of regulatory T cells, and this has been confirmed through recent studies in healthy individuals [4]. In mice with experimental autoimmune myasthenia gravis (EAMG), peripherally circulating regulatory T cells are important for the disease process through controlling self-reactive T cells and thus inhibition of the autoimmune response [5].

Insufficient or deficient levels of vitamin D have been reported in a high percentage of patients with systemic lupus erythematosus (SLE) [6]. Further, vitamin D deficiency is known to correlate with autoimmune activity in several chronic autoimmune diseases, including SLE [6], rheumatoid arthritis (RA) [7], and multiple sclerosis (MS) [8]. One recent randomized, double-blind, placebo-controlled trial with vitamin D3 as addition to treatment with interferon beta in patients with multiple sclerosis (MS) showed a clear reduction in MS disease severity [9]. Nevertheless, there are as yet no general guidelines on a vitamin D supplementation

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regimen for modulating immunological homeostasis in autoimmune disorders. Further, studies on vitamin D status in autoimmune neuromuscular disorders are lacking, even though vitamin D receptors are present on muscles and 25(OH)D deficiency causes muscle weakness and myalgia [10]. Additionally, vitamin D has direct effects on muscle regeneration [11]. Data from a recent study also showed that use of corticosteroids, which is the base treatment in numerous autoimmune disorders including MG, is independently associated with 25(OH)D deficiency [12].

In this study, we wanted to determine whether patients with MG have a deficiency of 25(OH)D and whether their 25(OH)D levels are lower than those found in healthy controls. Further, we aimed at determining the effects on clinical fatigue score before and after therapeutic supplementation with vitamin D3.

Material and methods

Participants

Patients with MG were asked to participate in the study as they came on regular visits to the outpatient neurology clinic during the course of the year, between December 2010 and February 2012. Diagnostic criteria, according to the Myasthenia Gravis Foundation of America (MGFA) [13], included clinical muscle fatigue and neurophysiological evidence of disturbed neuromuscular transmission (repetitive nerve stimulation and/or single-fiber electromyography) in conjunction with the presence of serum antibodies against either AChR or MuSK.

Muscle weakness and fatigue of patients with MG were assessed according to the MG composite (MGC) scale [14], and each patient was given a total score ranging from 0 (no myasthenic weakness) to maximum 50 points (worst possible myasthenic weakness in all assessed muscles). Medication at the time of study was noted in all patients: 17 patients had pre-existing supplementation with vitamin D and calcium, as osteoporosis prophylaxis owing to concomitant corticosteroid treatment, whereas 16 patients were unsupplemented. Clinicians who performed the MGC status (HA, IN, ARP) were blinded to vitamin D levels at the time of examination and to previous MGC scores. MGC scores were later extracted separately from the patient charts into a database (LH).

Body mass index (BMI) was calculated according to the standard formula (kg/m^2). Normal weight was defined as a BMI of 19–25. Overweight (pre-obesity) was defined as a BMI of 25–30, and obesity was defined as a BMI of 30–35. Further, severe obesity was defined as a BMI of 35–40 and extreme obesity as BMI > 40.

Ethics

All subjects gave their written informed consent to participate in the study, which was approved by the Regional Ethical Review Board of Uppsala (Dnr: 2010/446).

Procedures

Blood sampling and CLIA

Plasma from 50 age- and sex-matched healthy blood donors, without prescribed vitamin D3 medication, 25 females (aged 35–71 years; mean age, 52 ± 11 years) and 25 males (aged 36–74 years; mean age, 51 ± 9 years), was used as reference data in comparison with the patient data of 25(OH)D. All samples were collected at the end of October 2011.

Quantitative determination of 25(OH)D in plasma was performed with a LIASON® analysis (DiaSorin Inc., Stillwater, MN, USA) through chemiluminescence immunoanalysis (CLIA), the standard analysis for vitamin D at Uppsala University Hospital. Severe 25(OH)D deficiency was defined as <25 nM; mild deficiency as 25–74 nM; and optimal level as 75–250 nM (established by DiaSorin). Normal levels of the autoimmune marker interleukin-6 (IL-6) were <3.3 ng/l (Siemens), and normal levels of the following parameters were found in NORIPSs database (http://pweb.furst.no/norip/): calcium, 2.15–2.50 mM; phosphate female, 0.80–1.5 mM; phosphate male, 0.70–1.6 mM; and parathormone (PTH), 1.1–6.9 pM.

Statistical methods

The non-parametric Mann–Whitney test was used for assessment of mean values in continuous variables of independent samples, including seasonal variations in 25(OH)D values. Spearman's rank order correlations were applied to measure associations between two continuous variables. Related samples Wilcoxon signed rank test was applied to calculate differences in 25(OH)D level and MGC before and after therapeutic supplementation with vitamin D3. All analyses were performed using SPSS for Windows (16.0; SPSS Inc., Chicago, IL, USA), and the significance level was set at $P < 0.05$.

Results

Clinical characteristics and treatment status

Thirty-three patients were included in the study, 22 males (mean age, 59 ± 16 years) and 11 females (mean age, 57 ± 12 years). MG composite score in the entire patient group ranged from 0 to maximum 19 points (mean, 5 ± 5 points), and disease duration was 0–37 years (mean,

9.9 ± 9.3 years). The clinical characteristics and therapeutic regimen are displayed in Table 1. Three patients did not have any current MG medication, and four patients had only symptomatic medication, acetylcholinesterase inhibitors (pyridostigmine; Mestinon®), with a daily dose ranging from 240 mg to 480 mg. The remaining patients (n = 26) had chronic immunosuppression, of which 23 patients had daily corticosteroid treatment (Prednisolon® or Deltison®) with a dose ranging from 5 mg to 75 mg (Table 1). Amongst the patients with corticosteroid treatment, six patients additionally had azathioprin (Imurel®), one patient had both azathioprin and cyclosporin (Sandimmun®), and another patient had Takrolimus (Prograf®). Two patients had only azathioprin, and one patient had only cyclosporin. Seventeen patients, the majority on corticosteroid treatment, had pre-existing supplementation therapy with Calcichew-D3-Forte® (calcium carbonate and calciferol; 800 IU/day = 16; 400 IU/day = 1) to avoid calcium and vitamin D deficiency, according to local guidelines.

Low 25(OH)D levels in unsupplemented patients with MG, whereas supplemented patients with MG have significantly higher 25(OH)D

To avoid treatment bias, the patient group with MG was subdivided into patients with pre-existing D3 substitution (N = 17; calcium carbonate and calciferol) and those without D3 supplementation (N = 16). Disease duration did not differ between the groups: in the

Table 1 Clinical characteristics, month of sampling, treatment status and vitamin D [25(OH)D] levels

Patient no.	Sex/age	Dur (years)	MGC score	25(OH)D (nM)	Month of sample	BMI	MG medication	Pred (mg/day)	Vit D3 (IU/day)
1	M/68	4	0	78	Dec	26.8	M, C	5	800
2	M/30	11	0	75	Dec	–	M	0	0
3	M/44	2	14	38	Dec	27.5	M, Aza, C	10	800
4	M/70	24	2	90	Dec	24	M, C	5	800
5	M/76	9	2	52	Jan	–	M, C	25	800
6	M/76	11	0	61	Jan	33.3	M, Aza, C	5	0
7	M/73	2	2	28	Jan	41.8	M, Aza, C	10	0[a]
8	M/74	1	0	85	Jan	26.5	Aza, C	10	800
9	M/63	1	0	43	Sep	31.6	C	10	800
10	M/65	6	7	57	March	27.4	C	5	0
11	M/53	23	4	102	March	32	M, C, Aza, Cyc	5	800
12	M/36	6	0	66	Aug	26.3	Aza	0	0
13	M/78	4	2	54	March	–	–	0	0
14	M/63	12	13	32	March	32.5	Cyc	0	0
15	M/49	25	3	31	April	22.8	M	0	0
16	M/49	1	8	43	Sep	31.6	M, C	50	0
17	M/77	1	3	82	Oct	27.4	C, Aza	10	800
18	M/48	4	9	34	Oct	22.9	M, C	75	800
19	M/65	5	2	52	Aug	32	M, C	10	800
20	M/27	1	2	29	Dec	32.8	C	7.5	0
21	M/61	4	0	48	Jan	33.2	Aza	0	800
22	M/55	0	4	49	Jan	27.6	–[b]	0	0
23	F/71	16	5	27	Oct	25.6	M	0	0
24	F/40	10	0	62	Sep	23.1	M	0	0
25	F/73	7	1	70	Sep	20.8	–	0	0
26	F/67	37	6	95	Sep	36	M, C	7.5	0
27	F/54	13	6	72	Dec	27.2	M, C	12.5	800
28	F/53	1	0	117	Feb	34.8	M, C	10	800
29	F/46	31	19	95	March	32.2	C, Takrol, Solu	50	800
30	F/65	6	18	87	May	25.6	C, Solu	50	800
31	F/52	9	6	46	April	–	M, C	5	0
32	F/37	14	0	71	Oct	40.4	M, C, Aza	7.5	400
33	F/56	9	8	86	Oct	41.0	M, C	5	800

Sex: M, male; F, female. 25(OH)D, 25 hydroxylated vitamin D in plasma (nM). Severe deficiency, <25 nM; mild deficiency, <75 nM; optimal level, 75–250 nM. Dur, disease duration (years); MGC score, MG composite score; BMI, body mass index. MG treatment, M, acetylcholinesterase inhibitors pyridostigmine bromide (Mestinon®); C, corticosteroids (Prednisone® or Deltison®); Aza, azathioprin (Imurel®); Cyc, cyclosporine (Sandimmun®); Solu, regular high-dose corticosteroid infusion (Solumedrol®); Takrol, takrolimus (Prograf®); Pred, daily dose of prednisone (mg). VitD3, supplementation of vitamin D3 (IU/day).

[a]The patient was prescribed vitamin D3 (calciferol) in the dose 800 IU/day but did not follow this regimen.

[b]First visit, at disease onset.

supplemented group, this was 9.3 ± 9.4 years, and in the non-supplemented group, 10.1 ± 9.4 years. Mild vitamin D deficiency (25–74 nM) was found in 14 (88%) of patients without pre-existing vitamin D3 supplementation, compared with 62% of the healthy controls. The mean value of 25(OH)D was 51 ± 19 nM (range, 27–96 nM) in the non-supplemented patient group with MG, which was significantly lower than in the healthy controls, where the mean value was 69 ± 21 nM (range, 29–133 nM) ($P = 0.017$; Fig. 1).

Calcium and PTH, which are influenced by vitamin D levels, were measured.

The mean value of calcium in the non-supplemented patient group was 2.30 nM, and none of the patients had calcium deficiency. PTH ranged from 3.7 to 7.8 nM, and four patients with concomitant mild vitamin D deficiency had increased PTH levels ranging from 6.9 to 7.8. Phosphate was normal in all patients. The mean value of IL-6 was 0.8 ng/l, and two patients (nos 6 and 7) had elevated IL-6. The patient with highest IL-6 (8.2 ng/l) also had the lowest 25(OH)D level (28 nM). Clinical MGC scores in the non-supplemented patient group ranged from 0 to 13 with a mean score of 4.5. No correlation was observed between myasthenic weakness, as measured by MGC score, and 25(OH)D level (Spearman rank = −0.30; $P = 0.80$). Further, no correlation was found between age and vitamin D (Spearman rank = 0.02; $P = 0.91$). No between-season sampling differences were observed for the 25(OH)D levels in the patients with MG: winter ($N = 14$), 66 ± 26 nM; spring ($N = 9$), 62 ± 27 nM; summer ($N = 1$), 66 nM; and fall ($N = 9$), 58 ± 23 nM ($P = 0.40$).

In the group of 17 patients who had pre-existing vitamin D3 supplementation, this had been initiated at least 6 months before blood sampling. Clinical MGC score ranged from 0 to 19 with a mean value of 5.5, and six patients (35%) were identified with mild vitamin D deficiency, despite prescribed vitamin D3 supplementation. The mean value of 25(OH)D in this group was 79 ± 22 nM (range, 38–117 nM), which had a trend of being higher than the healthy control group ($P = 0.058$). However, the treated patient group with MG had significantly higher values of vitamin D than the non-supplemented patient group with MG ($P = 0.001$; Fig. 1).

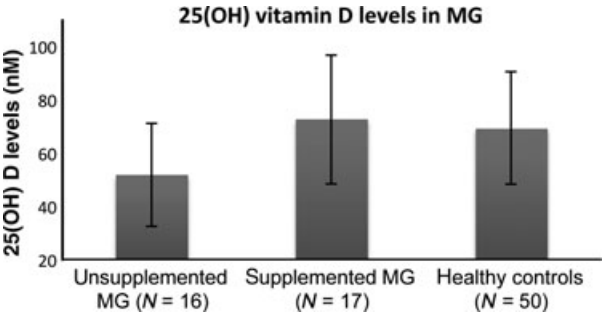

Figure 1 Graph of 25(OH)D vitamin values on the *y*-axis (nM) in age- and sex-matched control persons ($n = 50$), non-supplemented patients with MG ($n = 16$), and patients with MG supplemented with calcium carbonate and calciferol (800 IU/day, $n = 16$; 400 IU/day, $n = 1$). The 25(OH)D values were significantly lower in the non-supplemented patient group, as compared with the other groups. Results are presented as mean ± SD. *$P < 0.05$.

Body mass index (BMI) was measured, because it is known that vitamin D is fat soluble and therefore bound in its inactive form in the adipose tissues, which in turn inhibits the transformation to the active form of vitamin D [15]. We were able to measure BMI at follow-up visits in 29 patients (one patient was abroad, one patient had died, and two patients were unavailable). In this cohort, a total of 24 patients (83%) had a BMI > 25. Ten patients (36%) were considered overweight (BMI, 25–30), 10 patients (34%) were defined as obese (BMI, 30–35), one patient (3%) as severely obese (BMI, 35–40), and three patients (10%) patients as extremely obese. Only five patients (17%) were considered to have normal BMI (BMI, 19–25; Table 1). Nevertheless, there was no correlation between BMI and 25(OH)D levels (Spearman $R = -0.2$; $P = 0.51$).

Treatment with vitamin D3 improves 25(OH)D levels and MGC score

In the group of patients without initial vitamin D3 supplementation, medication with Calcichew-D3 Forte®, containing 800 IU of vitamin D3/day, was initiated. Follow-up examination of MGC score and plasma levels of 25(OH)D was performed in 13 of 16 patients, after a treatment period ranging from 2.5 to 10 months (mean, 6 months). The mean level of vitamin D before treatment in this group was 51 ± 20 nM, and after treatment, the mean value of 25(OH)D was 62 ± 12 nM. Vitamin D levels were significantly increased in 10 of 13 patients (Table 2), with an overall increase of 21% ($P = 0.033$). The mean MGC score before treatment was 4.5 (range 0–13), and at follow-up, MGC score had a mean of 2.5 (range, 0–9) (Table 2). Thus, this resulted in an overall improvement (reduction) of MGC score in five patients, unchanged best possible MGC (MGC, 0) in three patients, and unchanged clinical status in four patients. A slight worsening was seen in one patient. The overall improvement in this group of MGC was 38% ($P = 0.05$). Amongst the five patients who improved their MGC score, patients 7, 10, 14, and 15 had unchanged medication for at least 1 year. Patient no. 18 had started high-dose corticosteroids (Deltison

Table 2 Vitamin D [25(OH)D] levels and clinical MGC score before and after supplementation with vitamin D3

Patient no.[a]	Vitamin D after (before)	MGC after (before)	Diff 25(OH)D	Diff MGC (inverse)	Follow-up time (months)
6	55 (61)	0 (0)	−6	0	6.0
7	51 (28)	1 (2)	+23	+1	5.0
10	67 (57)	0 (7)	+10	+7	7.0
12	67 (66)	0 (0)	+8	0	2.5
13	64 (54)	2 (2)	+10	0	9.0
14	49 (32)	9 (13)	+17	+4	6.0
15	73 (31)	0 (3)	+42	+3	6.0
18	55 (34)	0 (9)	+21	+9	3.0
23	44 (27)	5(5)	+18	0	3.0
24	50 (62)	0 (0)	−12	0	5.0
25	78 (70)	2 (1)	+8	−1	3.0
26	86 (95)	7 (7)	−9	0	10.0
31	62 (46)	6 (6)	+16	0	8.0

Measured levels of 25-hydroxy vitamin D [25(OH)D] and clinical myasthenia gravis composite (MGC) score in 13 patients before and after treatment with 800 IU/day of vitamin D3. The difference in 25(OH)D before and after supplementation is shown in nM. The difference in MGC is inverse, that is, an improved MGC status is shown as + and worsening in MGC status as −.

[a]The patients are numbered identically in Table 1.

75 mg/day) just before the initial examination, and at the time of follow-up 3 months later he had Deltison in a dose of 50 mg/day.

Discussion

This is the first report on vitamin D deficiency in patients with MG. Vitamin D could have dual effects in MG through (i) regulating the autoimmune response and (ii) maintaining muscle function through effect on the vitamin D receptor in muscles [16]. The metabolically active form of vitamin D, 25(OH)D, is believed to exert its immune-regulatory effect by increasing the amount of regulatory T cells [4]. The number of CD4+/CD25+ T_{reg}s in the thymus of patients with MG is unchanged; however, these MG thymus cells possess severe functional defect in their regulatory activity [17]. Experimental studies have revealed that 25(OH)D is able to skew the T-cell compartment into a more anti-inflammatory and regulated state, with inhibition of Th1 and Th17 cells and promotion of Th2 and T_{reg} cells [18]. In patients with MS, compromised T_{reg} function is believed to be critically involved in the disease process, and in these patients high 25(OH)D levels are associated with an improved T_{reg} function and with skewing of the Th1/Th2 balance toward Th2 [19]. Hence, we speculate that 25(OH)D might have an important role in the function of T_{reg}s also in patients with MG, because MG is considered a CD4+ T-cell-dependent disease.

Despite the presence of vitamin D receptors on muscles, 25(OH)D levels did not correlate with myasthenic weakness, as measured by MGC score, although this score may not be detailed enough to discover subtle correlations. Another issue when performing clinical fatigue examinations on patients with MG is the variation of fatigue between different times of the day. As the MGC score does not directly correlate with autoimmune activity, we analyzed IL-6, which has been found to be a crucial factor for the induction of the EAMG disease phenotype in mice [20]. However, in our cohort of MG patients, IL-6 was not generally increased, with the exception of two patients with concomitant vitamin D deficiency. A possible explanation for the low IL-6 levels could be that the vast majority of patients were on chronic immunosuppression at the time of analysis.

Vitamin D deficiency has been identified in patients with a variety of autoimmune diseases such as SLE [6], RA [7], MS [8], and narcolepsy [21]. Previous studies in patients with RA and SLE have shown the important effects of vitamin D both as a marker of disease activity and as treatment. Additionally, a recent randomized, double-blind, placebo-controlled trial with vitamin D3 as addition to treatment with interferon beta-1b in patients with MS showed a clear reduction in disease severity [9]. Similarly, vitamin D supplementation in our cohort of patients with MG improved MGC score in most patients. The considerable improvement of MGC amongst the patients where vitamin D3 supplementation was initiated in this study indicates a beneficial response to active vitamin D in myasthenic muscle fatigue. This finding should be viewed in the context that it was a non-blinded, non-randomized study with no placebo treatment and that follow-up was not performed after exactly the same time interval in all patients. Nevertheless, the clinicians who performed the MGC status were not aware of previous MGC scores or 25(OH)D levels at the time of the clinical examination.

Considering that cholecalciferol is harmless in therapeutic levels, compared with many other medications, supplementation in cases of low vitamin D in patients with MG and in patients with neuromuscular disorders is recommended. Today, there is no biomarker in MG that correlates with disease amongst patients; therefore, the thought prior to this study was that 25(OH)D could potentially serve as a biomarker in MG. Nevertheless, as this study did not reveal an obvious correlation between myasthenic fatigue and 25(OH)D, further placebo-controlled clinical studies would have to be performed to confirm the potential role of 25(OH)D as a biomarker in MG.

Mild vitamin D deficiency was strikingly common in the Swedish healthy control population, and one has to take into account the sun exposure as an important factor when interpreting data of 25(OH)D. On the basis of the study design, patients were sampled as they attended regular visits to the neurology clinic, and hence the season of analysis ranged from December 2010 to February 2012. With the current study design, regular visits to the outpatient clinic were more common in autumn and winter seasons. Hence, we chose to sample the healthy blood donors in late autumn, at the end of October. When calculating de-seasonalized vitamin D levels by regressing the measured 25(OH)D levels, this plot revealed comparable levels throughout the months in the patient group with MG. From previous studies [22], we know that from 25 μg (1000 IU/day) 25(OH)D values increase at approximately 25 nM in healthy males. Intriguingly, a therapeutic substitution of 800 IU/day vitamin D3 in the MG patient population was not sufficient to obtain significantly higher 25(OH)D levels than those in the non-supplemented healthy controls. One explanation would be that active vitamin D is needed to keep the autoimmune response in balance by increasing the T_{reg} function of self-reactive T-cell destruction.

A high BMI has been shown to correlate with low levels of 25(OH)D [15], because inactive vitamin D is bound to the adipose tissue. Intriguingly, 82% of patients in our study had a BMI > 25, which could in part contribute to low vitamin D levels [23]. Nevertheless, there was no correlation between 25(OH)D levels and BMI in our MG cohort. As the majority of patients with MG are on corticosteroid treatment, the risk of osteoporosis is considerably higher in the case of low vitamin D, and thus it seems reasonable to take into account the vitamin D status in particular in these patients. This is supported by a previous study showing that the odds of having 25(OH)D deficiency were 2-fold higher in those subjects who reported steroid use compared with those without corticosteroids, and thus steroid use is independently associated with vitamin D deficiency [12]. Taken together, this suggests the need for screening of 25(OH)D and repletion with vitamin D3 in patients on chronic steroid treatment in autoimmune neuromuscular disorders, including patients with MG.

In conclusion, this is the first report on vitamin D deficiency in patients with MG. We recommend monitoring of vitamin D status in patients with MG to avoid direct negative effects on the muscles or autoimmune response. Additionally, vitamin D3 supplementation can serve as a symptomatic treatment to optimize MG status, as well as minimizing future risk of osteoporosis secondarily to vitamin D deficiency.

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Disclosure of conflict of interest

The authors declare that they have no conflicts of interest.

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