Original Research Paper

Supplementary medication in multiple sclerosis: Real-world experience and potential interference with neurofilament light chain measurement

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Abstract

Background: As vitamins and dietary supplements are obtainable without prescription, treating physicians often ignore their intake by patients with multiple sclerosis (MS) and may therefore miss potential adverse effects and interactions.

Objective: We aimed to assess the spectrum and intake frequency of supplementary medication in a cohort of MS patients and to analyse the effect of biotin intake on measurement of serum neurofilament light chain (sNfL), an emerging marker of disease activity.

Methods: MS patients visiting our neurology outpatient clinic completed a questionnaire on their past or present use of vitamins or dietary supplements. In addition, the impact of two different doses of biotin (10 and 300 mg/day) on sNfL was studied in healthy volunteers.

Results: Of 186 patients, 72.6% reported taking over-the-counter vitamins or dietary supplements currently or previously. Most frequently used was vitamin D (60.0%), followed by biotin. Female patients and patients with primary progressive MS tended to use supplements more frequently. Biotin intake did not interfere with sNfL measurement by single molecule array (Simoa).

Conclusions: The use of vitamins and dietary supplements is frequent among patients with MS. Thus, treating physicians should be aware of the pitfalls of supplementary treatment and educate their patients accordingly.

Introduction

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS) characterised by cumulative disability and progressive neurodegeneration. Treatment with immune-modulating therapies reduces relapse rates and disease progression, but often does not stop disease activity completely and has significant side effects. Furthermore, therapeutic options for patients with progressive forms of disease are limited. Therefore, many patients are interested in alternative or supplementary treatment strategies.

Potential beneficial effects of vitamins and dietary supplements in MS have been discussed for many years.[1] At present, evidence to justify routine supplementation is only available for vitamin D, and only in cases of confirmed deficiency.[2,3] Caution is required, however, when it comes to high-dose supplementation of vitamin D, as this may lead to severe hypercalcaemia and acute renal failure.[4] In animal models of MS, experimental autoimmune encephalomyelitis, high-dose vitamin D even worsens disease symptoms due to overactivation of T cells.[5]

In recent years, increasing attention has been paid to the potentially positive effects of biotin (vitamin B7 or H) on disease progression. In 2015, an

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uncontrolled, non-blinded pilot study reported an improvement in disability and progression in patients with chronic progressive MS treated with high doses of biotin (100–300 mg per day).[6] A subsequent placebo-controlled study with 154 patients showed sustained reversal of MS-related disability in a subset of patients with progressive MS upon intake of 300 mg biotin per day.[7] On the other hand, two prospective studies reported no benefit of high-dose biotin on disability in progressive MS.[8,9] Failure to meet primary and secondary endpoints was also preliminarily reported from a large Phase III trial in progressive MS (NCT02936037).[10]

In the meantime, as at least low-dose biotin is available over-the-counter as a supplement for hair and nails, self-medication is discussed frequently in the media and internet forums for patients with MS. Far from being a harmless vitamin supplement, however, biotin (at least in high doses) not only causes side effects such as myopathy,[11] but also interferes with a considerable number of common laboratory assays. These include analysis of thyroid function via thyroid-stimulating hormone (TSH),[12] hepatitis B and C viral serologies,[13] and cardiac troponin.[14] In 2017, the US Food and Drug Administration (FDA) published a safety communication after the death of a patient due to falsely low troponin test results after intake of high-dose biotin.[15] In 2019, pharmacovigilance information on biotin interference was released by the European Medicines Agency.[16]

Neurofilament light chain in serum (sNfL) is a marker for neuronal damage and is emerging as a parameter for disease activity in neurodegenerative diseases. In MS, sNfL correlates with relapse rates and treatment response.[17,18] As analysis of sNfL by single molecule array (Simoa technology) is a biotin–streptavidin-based assay, we assumed a potential risk of interference with biotin, which would be highly relevant for the interpretation of study results on disease activity in MS.

As vitamins and dietary supplements are obtainable without a prescription, treating physicians often ignore the additional intake and may therefore miss potential adverse effects and interactions. Literature on their actual use among MS patients is scarce.[19] Therefore, we aimed to assess the frequency and spectrum of the intake of vitamins and dietary supplements in a cohort of MS patients, with a focus on vitamin D and biotin, and to analyse potential interactions of biotin with measurement of sNfL.

Methods

Participants
From February to August 2019, patients attending our University Medical Center's outpatient department for inflammatory CNS diseases were asked to voluntarily complete a questionnaire (please see Supplementary material) on their past or present use of vitamin D, biotin and other vitamins or dietary supplements. Answers were used by the treating physicians to discuss and advise on supplementary medication. Retrospectively, data from all patients diagnosed with MS according to 2017 McDonald criteria and aware of their diagnosis were collected and analysed for this study. All data were anonymized prior to evaluation in accordance with all applicable national and institutional guidelines.

In addition, five healthy volunteers took two different doses of biotin for 1 week each. The first week's dosage (10 mg/day) was chosen based on the most common supplements available online or in pharmacies. For the second week, the dosage used in pharmacological studies for treatment of progressive MS (300 mg/day) was selected.[6–9] Blood samples were taken at baseline and after 1 and 2 weeks. Biotin concentration in serum was analysed by an external laboratory. Serum was also frozen at –80°C for further analysis. Cerebrospinal fluid (CSF) from three patients with relapsing–remitting MS was used to analyse neurofilament light chain (NfL) after addition of biotin in doses of 500, 1000 and 1500 µg/l. These doses were chosen based on the results from the serum study in the healthy volunteers. Informed consent was obtained from all participants included in the study.

Analysis of neurofilament light chain (NfL)
The frozen serum and CSF samples were allowed to come to room temperature and sNfL measurement was performed in a single centre at a Simoa HD-1 (Quanterix, Billerica, MA) machine with a standardized protocol and a single lotusing the NF-Light Advantage Kits (Quanterix) according to manufacturer's instructions. Resorufin-β-D-galactopyranoside (RGP) was incubated at 33°C for 60 min prior to running the assay. Samples were measured in duplicates. The coefficient of variation (CV, as a percentage) of each sample was obtained by dividing the standard deviation of both replicates by the mean of both replicates multiplied by 100. We obtained a mean intra-assay CV of 3.9% by averaging all individual sample CVs. Low and high controls, consisting of recombinant human NfL antigen, were run in

duplicates together with the samples. Mean sNfL concentration of both controls (low: 4.0 pg/ml; high: 144.8 pg/ml) were in the lot-specific validity range depicted in the certification of analysis. sNfL measurements were performed in a blinded fashion without information about sample data.

Analysis

Excel software (Microsoft, Redmond, WA) was used to combine and analyse data. Graphics were created using GraphPad Prism version 7.04 for Windows (GraphPad software, La Jolla, CA). Statistical analyses were performed with GraphPad Prism; significance was defined as $p < 0.05$.

Results

Patient characteristics

Of 203 patients who completed the questionnaire, 186 fulfilled the criteria to be enrolled in further analysis (see Supplementary Figure 1 for participant flow chart). Of these, there were 166 patients with relapsing–remitting MS, 11 patients with primary progressive MS and 9 patients with secondary progressive MS. Some 68.3% of patients (127/186) were female; 49.5% of patients were born before and 50.5% on or after 1 January 1980. Patient characteristics including disease-modifying therapy are summarized in Table 1.

Frequency and spectrum of supplementary medication

Overall, 72.6% reported having taken over-the-counter vitamins or dietary supplements currently or in the past (Figure 1(a)). The most frequently used was vitamin D, by 60.0% of all patients (Figure 1(b)). Of these, 24 patients provided full information on both dose and frequency of vitamin D intake, with 3 patients taking more than 5000 IE/day. One patient reported a current high-dose use of vitamin D of 20,000 IE/day and in the past up to 50,000 IE/day. Of 177 patients who completed the questions regarding taking biotin supplements, 26 patients (14.7%) answered yes. Of the patients answering no (151/177), another 6 (3.4% of total) nevertheless reported intake of mixed dietary supplements containing low doses of biotin (Figure 1 (c)). Of those patients deliberately taking biotin, 15 did not indicate a dose. No patient reported a high-dose intake (100–300 mg/day).

In the hierarchy of vitamins and dietary supplements, vitamin D and biotin were followed by vitamin B12 (11.8%), magnesium (11.3%), vitamin B combination products (10.8%), folic acid (6.5%), vitamin C (5.9%), zinc (5.4%) and selenium (2.7%) (Figure 1(d)).

Analysis of subgroups revealed that female patients tended to use vitamins and dietary supplements more frequently overall (female: 75.6% vs male: 66.1%), and specifically vitamin D (63.8% vs 51.7%) and biotin (20.0% vs 14.0%), none of which were statistically significant using Fisher's exact test (Figure 2(a)). The use of supplementary medication was not dependent on age when dividing our cohort into two approximately equal subgroups born before or after 1 January 1980. Total intake of vitamins and dietary supplements was more frequent in patients with progressive forms of MS than with relapsing–remitting MS (85.0% vs 71.1%) although this was not statistically significant using the chi-square and Fisher's exact test. Patients suffering from primary progressive MS tended to use biotin more frequently (30.0%) (Figure 2(b)).

No interference of biotin with sNfL measurement

Next, five healthy volunteers were recruited to take biotin in two different dosages of 10 mg per day, corresponding to standard doses for hair and nail care, and 300 mg per day, corresponding to high-dose treatment from MS study protocols, for a week each. The mean baseline serum concentration of biotin was 0.282 µg/l (median 0.262 µg/l). After a week's intake of biotin in doses of 10 mg per day and 300 mg per day, serum concentration of biotin rose to a mean of 103.3 µg/l (median 90.1 µg/l) and a mean of 1577.3 µg/l (median 1170.3 µg/l), respectively (Figure 3(a)). In the healthy controls, the low baseline concentration of sNfL did not change with biotin intake as measured by Simoa (Figure 3(b)).

As biotin interacts directly with laboratory assays, its interference potential can be determined by subsequent addition to a sample of interest. To this end, CSF from three different patients with MS was used to analyse NfL concentration *in vitro* using Simoa without biotin and with three different doses of biotin, namely 500 µg/l, 1000 µg/l and 1500 µg/l. These doses were chosen based on the concentration of biotin measured in healthy controls after intake of biotin 10 mg per day and 300 mg per day as mentioned above. No decrease in NfL concentration was observed after addition of biotin (Figure 4).

Discussion

Supplementary medication continues to be a highly topical issue among patients with MS. Treating

Table 1. Patient characteristics.

	n	%
All patients	186	100.0
Sex		
Female	127	68.3
Male	59	31.7
Age		
Born before 1 January 1980	92	49.5
Born on or after 1 January 1980	94	50.5
Form of MS		
RRMS	166	89.2
PPMS	11	5.9
SPMS	9	4.8
Disease-modifying treatment		
None	25	13.4
Glatirameracetate	8	4.3
Interferon beta	16	8.6
Teriflunomide	8	4.3
Dimethyl fumarate	58	31.2
Fingolimod	23	12.4
Natalizumab	23	12.4
Cladribine	5	2.7
Ocrelizumab	11	5.9
Rituximab	4	2.2
Alemtuzumab	4	2.2
Methotrexate	1	0.5
Intake of vitamins or dietary supplements in the present or past		
Yes	135	72.6
No	51	27.4
Intake of vitamin D		
Number of patients answering the question	185	
thereof yes	111	60.0
thereof no	74	40.0
Intake of biotin (vitamin B7/H)		
Number of patients answering the question	177	
thereof yes, self-reported	26	14.7
thereof intake in mixed dietary supplements	6	3.4
thereof no	145	81.9

MS: multiple sclerosis; RRMS: relapsing-remitting multiple sclerosis; PPMS: primary progressive multiple sclerosis; SPMS: secondary progressive multiple sclerosis.

physicians are often only aware of prescribed medication, although vitamins and dietary supplements available over-the-counter also bear risks of side effects or interaction. Our study showed that the majority of MS patients in our cohort use vitamins and dietary supplements in addition to their disease-modifying treatment.

The most commonly used supplementary vitamin is vitamin D, which is the only vitamin sometimes specifically prescribed to MS patients but, at least in lower dosages, is also obtainable without prescription. Conflicting results have been reported for the impact of vitamin D on pro- and anti-inflammatory cytokines in MS patients.[20,21] Nevertheless, there is an epidemiologic correlation between low vitamin D levels in neonates and risk of MS, and higher vitamin D levels are associated with lower relapse risk.[2,22] These findings provide a rationale to screen for decreased levels in serum, although a
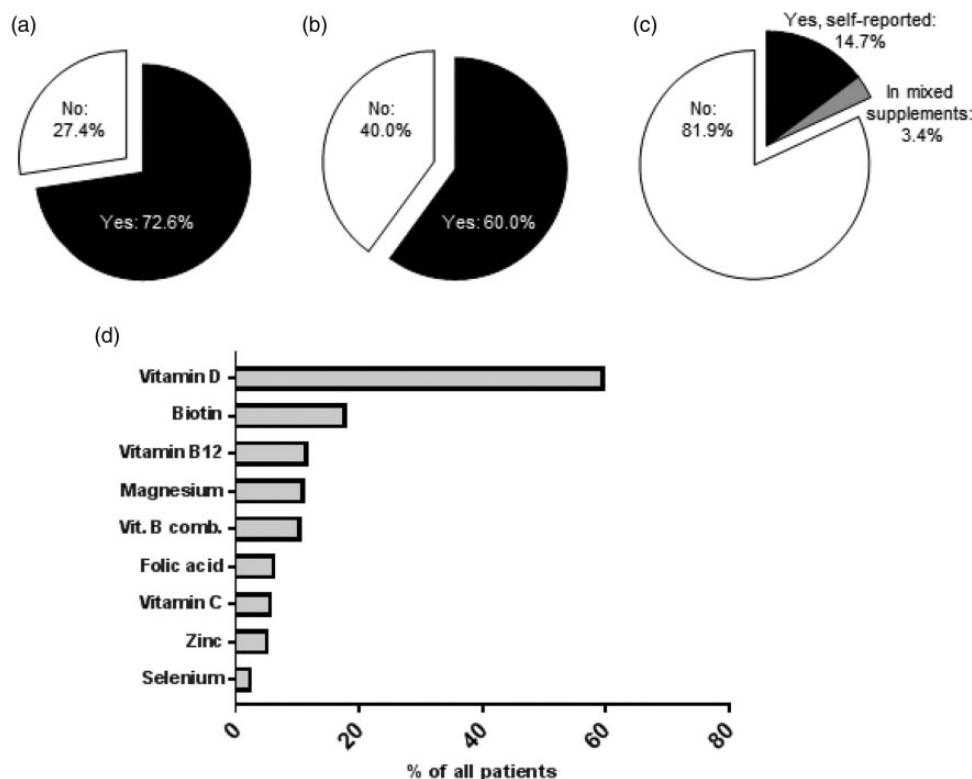
Figure 1. Use of vitamins and dietary supplements: (a) overall intake of over-the-counter vitamins or dietary supplements currently or in the past, (b) intake of vitamin D, (c) intake of biotin and (d) hierarchy of vitamins and dietary supplements.

recent Cochrane review showed no benefit of vitamin D for patient-important outcomes.[23] Notably, one patient in our cohort reported a high-dose intake of vitamin D. Although the frequency of high-dose intake is expected to be low, the risks of high dosages should be discussed with MS patients in order to avoid potentially life-threatening side effects.

Remarkably, 18.1% of the patients in our cohort were currently or had taken biotin or biotin-containing compounds in the past. Although the subgroup of patients with primary progressive MS was small and no statistically significant conclusions could be drawn from this cohort, biotin seems to have been used more frequently among these patients, since these were the study groups in clinical biotin trials.[6–9] An information guide on the use of vitamin D and biotin with evidence in favour of and against their supplementation is provided in Box 1.

Limitations of this study include its one-centre, retrospective design based on self-reported data. In addition, patients may underreport self-medication[24] as they may expect disapproval of their behaviour by

the treating physician. Patients opposed to standard disease-modifying drugs and relying completely on therapeutic approaches that are not (or not yet) evidence-based, such as high-dose biotin, may not be referred to a university medical centre and would therefore be missed by our study. Furthermore, our study population consists mostly of patients having relapsing–remitting MS and only a small number of individuals with progressive MS, which is the targeted population for biotin therapy.

An important pitfall in treatment with biotin is its interference with laboratory assays. Many assays include streptavidin binding of biotinylated antibodies directed against the molecule of interest. Here, free biotin may interfere leading to falsely negative or positive results for the antibody-bound molecule, depending on the assay used. This is of particular importance for parameters required in emergency settings such as cardiac troponin. Some troponin assays may already be affected by an intake of 10 mg biotin per day according to the susceptibility thresholds observed in a recent study.[14] If biotin supplementation is known, pre-treatment of biotin-containing sera with streptavidin-coated magnetic
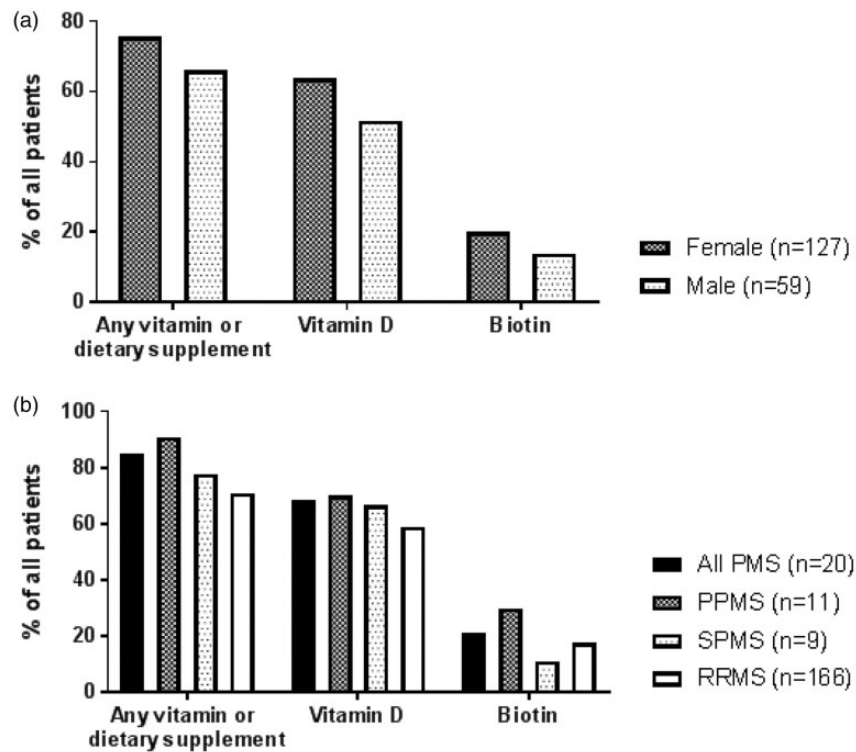
Figure 2. Patient subgroups show different use of supplementary medication: (a) female patients (darker pattern) use supplements more frequently than male patients (lighter pattern) and (b) intake of vitamins and dietary supplements is more frequent in patients with progressive forms of MS (black) than with relapsing-remitting MS (white). Patients with primary progressive MS (darker pattern) have the highest percentage of biotin intake. Numbers of patients in the respective subgroup are given in brackets. All PMS: all patients with a progressive form of multiple sclerosis; PPMS: primary progressive multiple sclerosis; SPMS: secondary progressive multiple sclerosis; RRMS: relapsing–remitting multiple sclerosis.
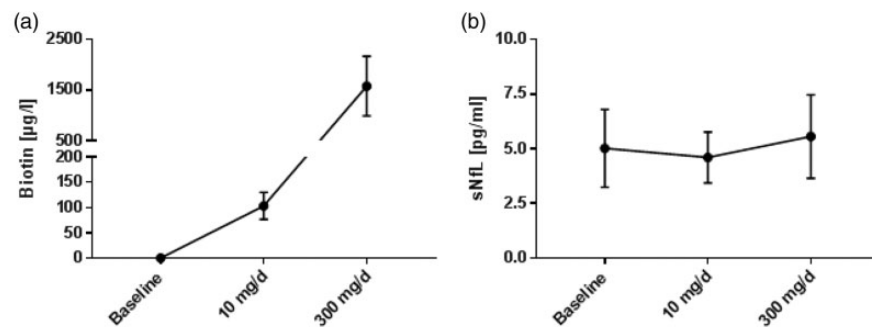

Figure 3. Levels of biotin and neurofilament light chain in serum: (a) serum levels of biotin in five healthy volunteers at baseline, after intake of 10 mg per day for one week and after intake of 300 mg per day for one week and (b) corresponding serum levels of neurofilament light chain (sNfL).

microparticles could deplete biotin from samples before carrying out the assay.[25]

Nevertheless, not all laboratory assays based on biotin–streptavidin interaction are affected by biotin intake, as we showed in our analysis of sNfL. This is due to differences in the assay set-up. Measurement of NfL by enzyme-linked immunosorbent assay (ELISA) or Simoa involves binding the molecule of interest to paramagnetic beads with NfL attachment sites.[26] Subsequently, the beads are washed and only afterwards incubated with a biotinylated detection antibody and then with β-galactosidase-labelled streptavidin. In this way, excess biotin is washed out

Box 1. Information guide on the use of vitamin D and biotin in multiple sclerosis.

Information guide on the use of vitamin D and biotin in Multiple Sclerosis

Vitamin D (cholecalciferol):

Pro:

- There is an epidemiologic correlation between low vitamin D levels in neonates and risk for MS (Nielsen et al., Neurology 2017).

- Higher vitamin D levels are associated with lower relapse risk (Simpson et al., Ann Neurol 2010).

- Patients with low baseline vitamin D levels may benefit from supplementation (Berezowska et al., Int J Mol Sci 2019).

Contra:

- Cochrane Review provides no evidence for benefit in patient-important outcomes (Jagannath et al., Cochrane Database Syst Rev 2018).

- High-dose supplementation of vitamin D may lead to severe hypercalcemia and acute renal failure (Fragoso et al., J Neurol Sci 2014).

- In EAE, an animal model of MS, high dose vitamin D worsens disease symptoms due to overactivation of T cells (Häusler et al., Brain 2019).

Biotin (vitamin B7 / H):

Pro:

- There are still limited treatment options for patients with chronic progressive MS.

- High-dose biotin (100-300 mg per day) was reported to improve disability and progression in some studies (Sedel et al., Mult Scler Relat Disord 2015; Tourbah et al., Mult Scler 2016).

Contra:

- Two relatively large prospective studies reported no benefit of high-dose biotin on disability in progressive MS (Birnbaum et al., Mult Scler Relat Disord 2017 Couloume et al., Mult Scler 2019). Failure to meet primary and secondary endpoints was preliminarily reported from a large phase III trial in progressive MS (NCT02936037, press release from MedDay, March 10, 2020).

- Interference with a large number of common laboratory assays, such as thyroid hormones, TSH, hepatitis B and C viral serologies, and cardiac troponin (Barbesino, Thyroid 2016; Pourcher et al., Mult Scler 2018; Frame et al, Am J Clin Pathol 2019). This has led to safety communications by the FDA and the European Medicines Agency.

- High-dose biotin may cause myopathy as side effect (Maillart et al., Neurology 2019).
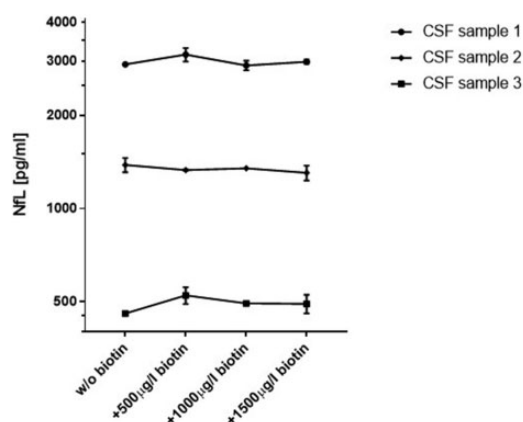

Figure 4. Biotin does not interfere with neurofilament light chain (NfL) measurement by Simoa in cerebrospinal fluid (CSF) of MS patients.

before it is able to bind to streptavidin and thereby have an impact on the result. This example demonstrates that knowledge of both the patient's medication and the laboratory assays used is needed to interpret aberrant values.

In this context, it is important to note that six patients in our cohort reported no intake of biotin, but were currently or had taken mixed dietary supplements containing low doses of biotin in the past. Thus, biotin intake is not only missed by the treating physicians, but the patients themselves are not always aware of it.

In conclusion, self-medication with over-the-counter compounds is an underestimated feature in the treatment of MS patients. Our data provided evidence of a high real-world frequency of the use of vitamins and dietary supplements. When applied in high dosages, these supplements may cause side effects and blur vital diagnostics. Thus, treating physicians should be aware of the frequency and pitfalls of supplementary treatment, and educate their patients accordingly.

Acknowledgements

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Supplemental material

Supplemental material for this article is available online.

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