Mega doses of vitamin D for the treatment of autoimmune diseases (update of December, 2016)

Cicero Galli Coimbra, MD, PHD
Internal Medicine and Neurology
Laboratory of Neuropathology & Neuroprotection, head
Associate Professor of Neurology and Neuroscience
Federal University of São Paulo - UNIFESP
Rua Pedro de Toledo 781 - 7th floor
04039-032 - São Paulo - SP - Brazil

Private Office:
Rua Dr. Diogo de Faria 775 - cj 94 - 9th floor
04037-003 - São Paulo - SP – Brazil

The protocol for the treatment of autoimmune diseases with exceptionally high daily doses of vitamin D has been under development since 2002 under the strict observance of the clause #37 of Helsinki declaration (World Medical Association – WMA, 2013) which can be found under the headline "Unproven Interventions in Clinical Practice" – republished for decades with further detail at each revised version (http://www.wma.net/en/30publications/10policies/b3/):

> *"In the treatment of an individual patient, where proven interventions do not exist or other known interventions have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorized representative, may use an unproven intervention if in the physician's judgment it offers hope of saving life, re-establishing health or alleviating suffering. This intervention should subsequently be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information must be recorded and, where appropriate, made publicly available."*

In the long run, therapies with interferon beta have ultimately shown to be unable to prevent accumulation of disabilities.

http://jamanetwork.com/journals/jama/fullarticle/1217239

Likewise biological agents usually loose their effectiveness due to generation of anti-drug antibodies.

http://rheumatology.oxfordjournals.org/content/early/2012/01/18/rheumatology.ker445.short

The treatment with high doses of vitamin D (cholecalciferol) requires individual tailoring of daily doses (usually ranging from 40,000 IU to 320,000 IU per day, most often 1,000 IU per kg per day) according to laboratory results of each patient. Those (occasionally extremely high) daily doses are required to compensate for the individual level of resistance to the biological effects of vitamin D (including its

potent immune modulatory role) that seems to characterize all or nearly all patients with autoimmune disorders. Individual resistance to vitamin D may be due to a myriad of (single or multiple, each of them possibly severe, moderate or mild) genetic polymorphisms (affecting one or both vitamin D hydroxylases, the serum carriers – like DBP – and/or VDR). Other factors like body weigh, body mass index, race and age also play a role.

The effect of enzyme polymorphism on vitamin D metabolism is exemplified in the figure 1 of the following publication showing the enzyme kinetics of three hypothetical polymorphic variants of 25-hydroxyvitamin D-1 α-hydroxylase (CYP27B1), "A," "B" and "C":

http://www.tandfonline.com/doi/full/10.4161/derm.24808

Facing a myriad of possible contributors to individual resistance to vitamin D, we have to use the response of a measurable biological effect of vitamin D to an initial daily dose (an initial testing daily dose, maintained for at least 2-3 months) to do the first adjustment at the second appointment (which usually takes place 4-6 months after the first medical appointment) (Figure 1). The measurable biological effect we have successfully used is the extent of PTH inhibition resulting from an initial testing dose (further explained at: http://www.tandfonline.com/doi/full/10.4161/derm.24808). In other words, we measure serum PTH before and after at least 2-5 months of regular treatment with an initial (testing) daily dose (which may be 1,000 IU per kg of body weight for a patient with normal body mass index – BMI). Using the same clinical laboratory for consecutive measurements of PTH is advisable to facilitate daily dose adjustments (calculation and future recalculations which may be eventually needed as a result of adaptive changes in vitamin D metabolism – like faster catabolism – that may occur in a significant percentage of patients under high-dose vitamin D therapy). Different clinical laboratories may use different kits and reference values, which could compromise comparison of serum PTH levels under different daily doses of vitamin D.

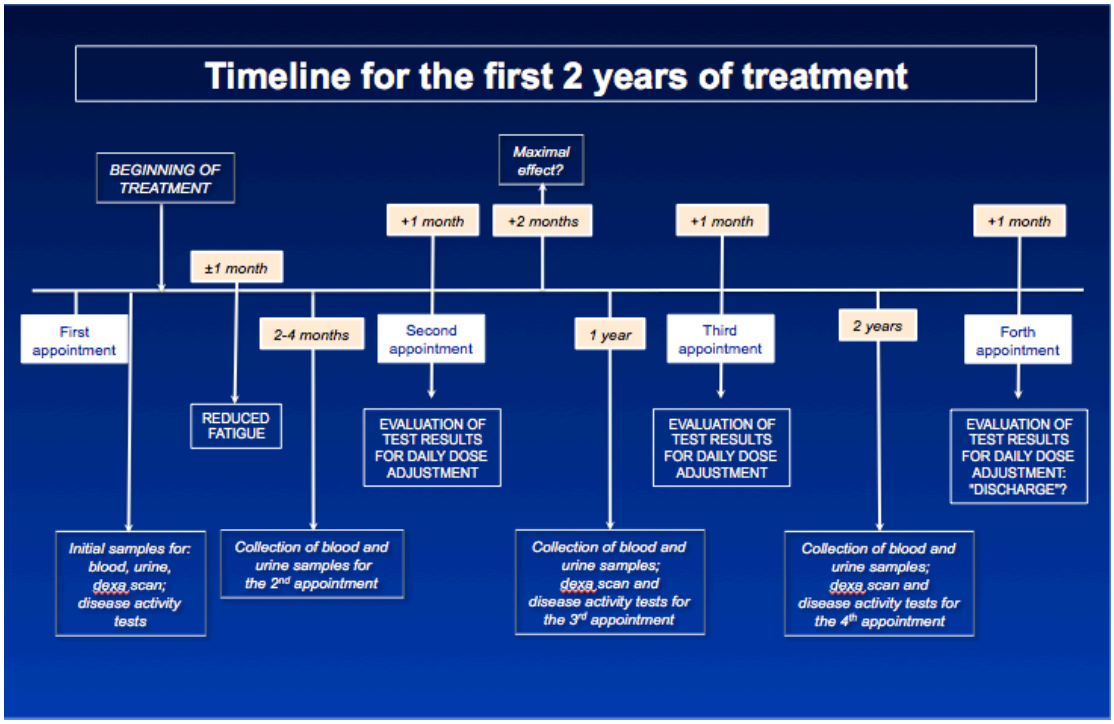

Figure 1 – Suggested timeline for the first 2 years of treatment.

BMI is calculated as follows:

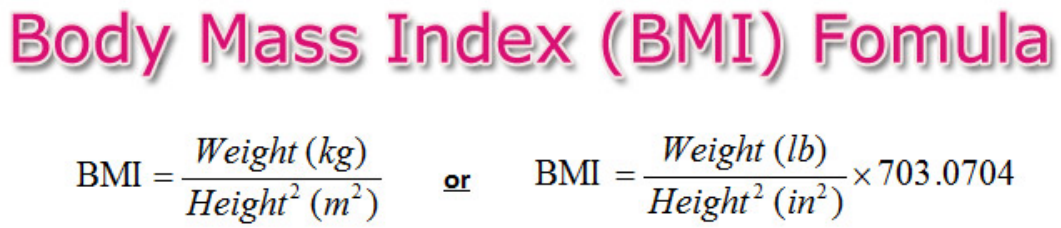

Figure 2 – BMI formula.

There are several BMI calculators available in the Internet that greatly facilitates your work, no matter which units is used in your country (kilograms of pounds, meters or feet). An example follows:

http://halls.md/body-mass-index/av.htm

BMI Categories:
Underweight = <18.5
Normal weight = 18.5–24.9
Overweight = 25–29.9
Obesity = BMI of 30 or greater

To calculate the initial daily dose we are currently adding 25% to the basal daily dose of 1,000 IU per kg of body weight if the patient's body weight is in the category of overweight, or adding 50% to the basal daily dose of 1,000 IU per kg of body weight if the patient's body weight is in the category of obesity).

That initial dose may be even higher for a patient at high risk of becoming permanently and severely disabled – such as a patient with multiple sclerosis or Devic's disease or Behçet disease at risk of becoming blind or paraplegic (such risk may be subjectively estimated according to his / her clinical history). For instance, the quality of life of a patient who is already blind or nearly blind in one eye (either due to optic neuritis or uveitis) would be permanently and severely compromised if the other eye is affected during a unnecessarily delayed process of dose adjustment (consuming several months of consecutive clinical and laboratorial evaluations to achieve the tailored daily dose of cholecalciferol). The same rationale applies to similar circumstances and situations such as to Cogan syndrome: permanent deafness or blindness may occur if the therapy is not assertive enough from the beginning.

The same also applies to a patient with a recently acquired neurologic disability (prior to vitamin D therapy) such as a patient with relapsing-remitting MS (RRMS) who has not fully recovered from a relapse of optic neuritis or paraplegia after 3-5 days of pulse therapy with 1 g of methylprednisolone daily. Remyelination (generation of new myelin sheaths around axons – sometimes documented as progressive fading of demyelinating lesions in consecutive MRIs, particularly at the cervical spinal cord) with consequent recovery from recently acquired (usually not older than 1 year) neurologic disabilities may occur if the preliminary daily doses of vitamin D are sufficiently high from the first medical appointment.

According to the same rationale, MS patients (or patients suffering from other diseases that similarly carry out the risk of permanent disabilities and severe impairment of quality of life) may receive a loading dose (approximately 50% higher than the estimated testing daily dose) per day (for two to five days, according to their BMI, age and skin pigmentation) for earlier achievement of a higher level of 25(OH)D3 and further decreasing the chances of relapses during the first month of treatment: 25(OH)D3 requires 2-3 months to reach a steady serum level when patients are put only on maintenance doses from the beginning of treatment.

The daily dose of 1,000 IU per kg of body weight is currently diminished by 25% if the patient's body weight is in the underweight category and he / she is not at risk of permanent disabilities (for instance, those with psoriasis).

Patients that are not at risk of becoming severely disabled (such, for instance, those with psoriasis) should not receive loading doses and may simply start with a daily dose of cholecalciferol of 1,000 IU per kg of body weight minus 10-20%.

The first adjustment is usually a major one and is made at the second medical appointment. Suppose you are dealing with the MS patient "A". Patient "A" weighs of 60 kg (132.2 pounds) and a height of 1.7 m (5.57 feet), received 100,000 IU of cholecalciferol per day for 4 days), and maintains a daily dose of 60,000 IU until the second appointment. At the second appointment (which usually takes place approximately after 3 months of treatment) suppose that patient A's laboratory results demonstrate that his / her basal serum PTH concentration of 60 pg/mL fell down to 30 pg/mL (reference range: 12-65 pg/mL). Then, aiming at achieving a PTH level of approximately 8 pg/mL, you should increase the daily dose to 110,000 IU (an increase of 66% - you should use a simple "rule of three": the method of finding the fourth

term in a proportion when three terms are given). The initial dose (60.000 IU) reduced PTH by 30 pg/mL and you want to further reduce PTH by additional 26 pg/mL (to achieve the level of 6 pg/mL). The three terms of the "rule of three" would be (where "x" is the fourth term to be calculated):

30 pg/ml (difference between basal and latest PTH levels) ------> 60,000 (daily dose of vitamin D maintained for 2-3 months
26 pg/ml (difference between latest and aimed PTH levels) ------> "x"

Therefore: (26 pg/ml x 60.000 IU) ÷ 30 pg/ml ≅ 50.000 IU. By adding 50.000 IU to the initial (testing dose) one achieves the adjusted daily dose of cholecalciferol (110,000 IU).

By experience we anticipate that the PTH level will probably achieve that aimed level (6 pg/mL, or a level close to that) within a few months and then increases over the first one to two years of treatment. That is probably the result of an adaptive response related to enhanced vitamin D catabolism (possible due to induction of 24-hydroxylase). Suppose that patient "A" comes back to your office after one year of treatment (3rd appointment, second adjustment of the daily dose of cholecalciferol) regularly taking 110.000 IU per day and his/her PTH level has raised from 5 to 20 pg/mL. Then, if you aim at restoring the level of 5 pg/mL, you should consider the initial and latest PTH levels to recalculate the daily dose of cholecalciferol. So, considering 60 pg/mL and 20 pg/mL respectively as the initial (basal) and latest PTH levels under 110.000 IU of cholecalciferol per day, you would increase the daily dose to 150.000 IU.

Suppose you are dealing with the MS patient "B". Patient "B" also weighs 60 kg (132.2 lbs) and a height of 1.7 m (5.57 feet), and (after a loading dose of 100,000 IU of cholecalciferol per day for 4 days) also maintains a daily dose of 60,000 IU until the second appointment. At the second appointment (which may take place after 3-6 months of treatment) suppose that B's laboratory results demonstrate that a basal serum PTH concentration of 60 pg/mL fell to 20 pg/mL (reference range: 12-65 pg/mL). The initial dose (60.000 IU) reduced PTH by 40 pg/mL and you want to further reduce PTH by 14 pg/mL (to achieve the level of 6 pg/mL). Then, by using the same simple "rule of three", you increase the daily dose to 80,000 IU (a 33% increase). Due to an adaptive response related to enhanced vitamin D, suppose that patient "B" comes back to your office after one year of treatment taking 80,000 IU per day (regularly) since the second appointment and his/her PTH level is again 20 pg/mL (rather than 6 pg/mL as expected, which may have been achieved a few months after the second appointment). Then, if you aim at restoring the 6 pg/mL level, you should consider the initial and latest PTH levels to recalculate the daily dose of cholecalciferol. So, considering 60 ng/mL and 20 ng/mL respectively as the initial (basal) and latest PTH levels under 80.000 IU of cholecalciferol per day, you would increase patient B's daily dose to 110.000 IU at one year of treatment.

Suppose you are dealing with the MS patient "C". Patient "C" weighs 95 kg (209.4 pounds) and also a height of 1.7 m (5.57 feet) (BMI = 32.7 → obesity), and maintains a daily dose of 140,000 IU until the second appointment; if necessary – according to the risk of developing or turning recently acquired disabilities (only partially diminished after pulse therapy with IV methylprednisolone) into permanent

disabilities that could significantly decrease quality of life, you may prescribe a loading dose of 200,000 IU per day for 2-4 days. At the second appointment (which usually takes place after 3 months of treatment) suppose that the laboratory results of patient C demonstrate that his / her basal serum PTH concentration of 60 pg/mL fell to 30 pg/mL (reference range: 12-65 pg/mL). Then, by using the same simple "rule of three" (aiming at a level of PTH of 6 pg/mL), you increase the daily dose to 250,000 IU (a 78% increase). Months later you will probably see that patient C's serum PTH concentration decreased to 6 pg/mL (your aimed level). Again (after an initial decrease down to the aimed level), PTH may increase until the end of first year of treatment due to an adaptive response related to enhanced vitamin D catabolism. Suppose that patient "C" comes back to your office after one year of treatment taking 250,000 IU per day (regularly) and his/her PTH level is now 16 pg/mL (rather than 6 pg/mL as expected). Then, if you aim at restoring the level of 6 pg/mL (that may have been achieved at some point after adjusting the initial dose), you should consider the initial (basal) and latest PTH levels to recalculate the daily dose of cholecalciferol. So, considering 60 ng/mL and 16 ng/mL as the initial and latest PTH levels under 250.000 IU of cholecalciferol per day, you would increase the daily dose of patient “C" to about 300.000 IU.

The treatment requires (1) a low calcium diet (basically free of milk and dairy products) to prevent excessive absorption of calcium from intestinal contents, and (2) minimal daily hydration of 2,5 L of liquids (water, juices, etc.) to provide urinary calcium dilution. Patients should not drink or eat calcium-enriched foods like soya "milk", or (if also calcium-enriched) other vegetal "milks" like rice "milk"; oat "milk"; they should not eat nuts or similar foods like peanuts, almonds, chestnuts, cashew, granola, pistachio, hazelnuts, etc., and dry fruits containing seeds. Also avoid typical food of the Lebanese diet containing sesame paste (tahini) such as hummus (hummus) and babaganoush. Sardines and anchovies with bones, as well as the so-called Green juice (with blended dark-green vegetables like kale) should be also avoided. In some countries mineral water has a large concentration of calcium and the physician should be able to advice his / her patients about the brands of mineral water with the lowest content of calcium for adequate hydration or preferentially use fruit juices. These are possible causes of increased urinary calcium output. As stated above, PTH should not be fully suppressed to avoid hypercalcemia from enhanced osteoclastic activity. Recorded serum levels of 25(OH)D typically reach 300 to 4,000 ng/mL 2 months after adjustment of the daily dose, but 25(OH)D levels are not used for adjustments. The most important laboratory measurements are PTH and 24-hour urinary calcium (with recorded urinary volume to estimate compliance to required daily hydration volume). Other important measurements would be serum total and ionized calcium, urea (or BUN) and creatinine, 24(OH)D3 and 1,24(OH)2D3, serum phosphorus and 24-hour urinary phosphorus. Vitamin B12 deficiency (if present) should be corrected: it is needed for myelin synthesis. If the patient does not follow the advised data, its calcium level can occasionally reach levels capable of suppressing PTH synthesis, thereby compromising the use of PTH for the adjustment of the daily dose of vitamin D.

Therefore, for optimizing the protective effect of vitamin D against autoimmune aggression we basically aim at reducing PTH to a level that is lower than its lowest normal limit (but higher than zero pg/mL) and maintaining that level with further adjustments (nearly invariably elevations) of daily doses of vitamin D as needed

(there might be some adaptive response as time goes by, with increased ability to catabolize vitamin D). PTH may ultimately reach a level that is lower than its lowest normal limit but should not be undetectable to avoid a major vitamin D-induced stimulation of osteoclastic activity (hypercalcemia due to large amounts of calcium released from the bone tissue).

However, sometimes a patient under mega-dose vitamin D therapy may show a laboratory analysis that shows a PTH level that its lower than its lowest normal limit (but higher than zero pg/mL) and, subsequently (without any change in the daily dose of vitamin D), PTH becomes undetectable. That may occur, for instance, because the latest level was collected in the summertime or by the end of the summertime, while the previous o ne vas collected in the wintertime or by the beginning of spring (when less or no skin synthesis of vitamin D occurs from exposure of skin to sunlight). In that case, provided that serum calcium is within the normal range, there is nothing to worry about and the daily dose of vitamin D should not be reduced or stopped (PTH will almost invariably raise again).

The following additional observations may be useful when you have to decide about the initial maintenance daily dose of vitamin D or calculate the adjustment of daily doses:

1) Young adults, adolescents and children frequently have PTH basal serum concentrations of 20-25 pg/mL or lower even when 25(OH)D is deficient and they have a concomitant inherited resistance to vitamin D. Still the same "rule of three" should be applied: the difference between the basal and second (achieved under the testing dose) PTH level is used as one of the terms of the "rule of three". For instance, PTH may decrease from a basal level of 22 pg/mL to 15 pg/mL in a young adult or adolescent in response to a daily dose of 60,000 IU of vitamin D sustained for 2-3 months. That indicates that the daily dose should be increased to 120,000 IU to reach a PTH level of 8 pg/mL.
2) Major changes in body weight (> 10 kg) during treatment may influence laboratory results and daily doses of vitamin D.
3) Slight seasonal variations of PTH may occur during the treatment as a consequence of solar exposure during summertime compared to lack of solar exposure during wintertime.
4) Fair skinned patients may have less severe inherited resistance to vitamin D as a result of natural selection that has occurred through generations as human populations moved to higher latitudes.
5) Patients with multiple autoimmune disorders are among those with most severe inherited resistance as compared to patients with only one autoimmune disease.
6) It seems advisable (as a general rule that should accept exceptions) to avoid more than doubling the previous dose at each adjustment of the daily dose of vitamin D.
7) We have not used more than 320,000 IU of vitamin D daily (so far).
8) If required (due to imminent irreversibility of a recent disability capable of

severely affecting quality of life – like significant visual loss or significant impairment of walking) an initial maintenance daily dose of 140,000 IU may be used in a patient with average weight and height, provided that the recommended diet and hydration are strictly followed.

9) Poor compliance with the recommended diet may disturb calculation of the adjusted daily dose by intermittently increasing serum calcium to levels close to the upper limit of the normal range; that is because increased serum calcium directly inhibits PTH synthesis independently on the effect of changes in serum 25OHD3 concentration on PTH production. Alert your patients about that.

10) Conversely, a diet excessively restricted in calcium may also disturb calculation of the adjusted daily dose by decreasing serum calcium to levels close to the lower limit of the normal range, consequently increasing PTH level independently on the effect of changes in serum 25OHD3 concentration on PTH synthesis.

11) Whenever (rarely) PTH increases (and the diet is not excessively restricted in calcium) compared to the previous measurement we cannot use the "rule of three" and we simply double the daily dose of vitamin D.

12) The most important laboratory tests are PTH (which will tell you whether you should increase the daily dose) and 24-hour calciuria (which will tell whether you can increase the daily dose; it also provides reliable information on compliance to the recommended diet and hydration).

13) Patients should be asked to use the same laboratory for measuring PTH to avoid different reference values that would make calculations more difficult.

14) The serum concentration of 25(OH)D3 may reach thousands of ng/mL during the treatment. Chemiluminescence technique usually provides a result like "higher than 160 ng/mL". You may ask the laboratory to dilute blood samples to measure higher levels, but most laboratories would refuse doing that because the achieved level would not be reliable or accurate. HPLC technique would not cause that problem but laboratories rarely use it because it is more expensive.

15) The serum concentration of 1,25(OH)2D3 remains within the normal range even if the patient is intoxicated with vitamin D (with hypercalciuria or hypercalcemia, with or without elevated creatinine) or deficient in vitamin D; at very high levels of 25(OH)D3, the chemiluminescence technique artifactually shows elevated serum concentrations of 1,25(OH)2D3 (false high levels).

16) At the beginning of each subsequent medical appointment we start by asking about the consumption of each of items of the diet that should be avoided, as well as level of hydration, source of vitamin D and emotional status (imaginary scale of stress – where level zero is not possible, 5 is the average level or common people who have no disease and level 10 is the maximal imaginable level of stress is usually presented for self-evaluation). That preliminary information would be essential for analyzing laboratory results and clinical response.

Poor absorption of vitamin B2 (riboflavin) is an inherited disorder that may affect 10-15% of the world population:
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1918332/pdf/ajhg00044-0124.pdf

This may lead to low availability of its active forms (FNM and FAD). During vitamin D activation (hydroxylation), the heme group of hydroxylases gets oxidized, and before they can convert another molecule of vitamin D into the hydroxylated form, the enzyme must turn into the reduced state. This chemical process (enzyme reduction) is carried out by another enzyme called cytochrome P-450 reductase that works for the whole family cytochrome P-450. This reduction process requires the presence of both active forms of vitamin B2 (Figure 2).

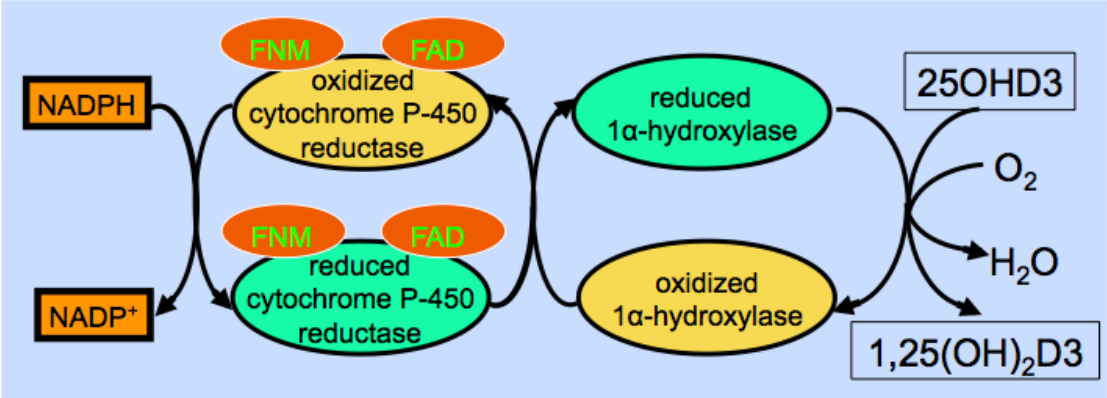

Figure 2 – Both FMN and FAD are prosthetic groups of the reductase of cytochrome P-450 enzymes (a family of enzymes to which all hydroxylases of vitamin D belong). Therefore both FNM and FAD are indirectly essential for the activities of (a) 25-hydroxylase of cholecalciferol, (b) 1α-hydroxylase of 25(OH)D3 (depicted), and (c) 24-hydroxylase of all metabolites of vitamin D.

To compensate for a possible inherited poor absorption of vitamin B2 we have added the oral administration of 50-100 mg of riboflavin QID (four times a day: at meals + bed time) of riboflavin to the protocol. The reason why we administer vitamin B2 QID is as follows. Probably the same (inherited) defective mechanism responsible for impaired intestinal absorption should also carry out the reabsorption of riboflavin from glomerular filtrate at proximal renal tubules – implying in increased urinary loss and decreased half-life of riboflavin.

Magnesium plays a critical role in the synthesis and metabolism of PTH and vitamin D. The activities of three major enzymes determining 25(OH)D level [25-hydroxylase of cholecalciferol, 1α-hydroxylase of 25(OH)D3, and 24-hydroxylase of all vitamin D metabolites] and vitamin D binding protein are magnesium-dependent:

https://bmcmedicine.biomedcentral.com/articles/10.1186/1741-7015-11-187

Magnesium deficiency is more prevalent than usually suspected (probably the most under diagnosed electrolyte deficiency):

http://www.sciencedirect.com/science/article/pii/000293438790129X

http://www.sciencedirect.com/science/article/pii/0002914989902130

Causes of magnesium deficiency include not only the chronic intake of some drugs (diuretics, digitalis, proton-pump inhibitors):

http://njmonline.nl/getpdf.php?id=788

Causes also include stress (ubiquitous among patients with autoimmunity):

https://www.researchgate.net/publication/12555615_Alterations_in_magnesium_and_oxidative_status_during_chronic_emotional_stress

To avoid interference of magnesium deficiency on the adjustment of daily dose of cholecalciferol (through impaired PTH response to vitamin D and impaired vitamin D hydroxylation and serum transportation) we have administered 120 mg of elemental magnesium QID (with riboflavin).

All patients should have periodic (at least annually) dexa scans. All those who remain sedentary will slowly loose bone mass under mega doses of vitamin D. If they maintain a daily aerobic exercise (like 30 minutes of brisk walking) they will steadily gain bone density. Those high doses of vitamin D stimulate both osteoblastic and osteoclastic activities simultaneously as demonstrated by measuring P1NP and CTX respectively. Aerobic exercise (initiated after the second medical appointment) will induce production of calcitonin and efficiently inhibit osteoclastic activity. Those disabled patients (due to neurologic disabilities or joint damage like in rheumatoid arthritis) who are therefore not capable of doing aerobic exercise should receive bisphosphonates. We have used alendronate 70 mg per week (sometimes we have to increase to 70 mg every 5 days. Vitamin K2 has been useless under the doses of vitamin D we have employed. Aerobic exercise should start from the second medical appointment (when most fatigue is already gone).

Disabling fatigue (fatigue at rest, not under physical activity; when asking about this symptom make sure that your patient is not mistaken fatigue for leg weakness: they usually do) and intolerance to environmental heat affects around 80% of MS patients. We always record the percentage of improvement of fatigue (the feeling of lack of energy even at rest; some patients describe as sensation of fever without increased body temperature) and intolerance to heat as clinical indexes of reduced disease activity (autoimmune aggression) in response to treatment. From the second visit percentage of recovery from fatigue is subjectively evaluated by patients from 0% to 100% where 0% is equivalent to no improvement and 100% improvement (always compared to the first appointment) is equivalent to resumption of normal state of physical and mental energy. Patients usually start noticing reduction of fatigue around 1 month of treatment. Some patients who are in the first few months or years of disease activity will only recognize they had fatigue in the second appointment. When asking about intolerance to environmental heat in response to treatment you may also use percentage of improvement (0% to 100%) but make sure that your patient understands that 100% is equivalent to the same level of discomfort that a normal person would experience when subjected to the same level of environmental heat.

Also try to quantify the extent of (sensory and motor) disabilities as well as urinary dysfunction. Recording the answers to quantitative questions [like “How many meters / blocks can you maximally walk continuously with and without unilateral / bilateral

support of crutch(es) / cane(s)?" or "For how long can you stand?"] are really useful to evaluate response to therapy at each medical appointment. Similar questions may be asked to quantify the strength of the upper limbs such as "Can you open a PET bottle?" Urinary dysfunction can also be evaluated by asking proper questions. For spastic bladder: "For how long you think you could maximally hold in urine from the time the need for passing urine begins?" For retention type of neurogenic bladder: "How long do you have to wait to start passing urine (in how many out of 10 urinations)?" Is there a feeling of sensation of incomplete emptying of bladder or slow urinary flow (in how many out of 10 urinations)?

A quantified neurological examination is similarly helpful. For instance, you may record how long the patient takes to walk 5 meters straight forward and another 5 meters straight back to the starting point, firstly in normal and then in forced speed, with and without unilateral / bilateral support of crutch(es) / cane(s). Also, in a patient lying on dorsal decubitus and keeping his / her knee slightly flexed with his / her heel on the stretcher, you may record whether you manage incompletely or fully extending the patient's partially flexed knee (counteracting the patient's strength with his / her heel supported on the stretcher) using one, two, three, or four of your fingers, or one hand or two of your hands. Or whether you cannot move the patient's knee at all. Or whether the patient's knee extends spontaneously as a consequence of spasticity.

Patients with spastic bladder should not restrict hydration to avoid frequent use of restrooms, but rather advised to urinate preventively to avoid incontinence. In addition to dilute urinary calcium (thereby helping to prevent nephrocalcinosis), increased hydration contributes to prevent urinary tract infection (UTI).

UTI is a major concern of ours as it may trigger acute hypercalcemia and increased creatinine levels in patients who have been on the same daily mega-dose vitamin D and maintaining diet and hydration as recommended for months or years. If UTI progresses to acute pyelonephritis (high fever is usually present in this case) it may increase local conversion of 25(OH)D3 to 1,25(OH)2D3 (as a result of increased macrophagic 1-alpha-hydroxylase activity, rather than of enhanced activity of 1-alpha-hydroxylase activity in proximal tubules; nevertheless, the enhanced activity of 1-alpha-hydroxylase activity ultimately increases tubular reabsorption of calcium from the glomerular filtrate and hypercalcemia (Tsao YT et al. Severe hypercalcemia in nonobstructive pyelonephritis with acute renal failure: hit or miss? Am J Emerg Med. Am J Emerg Med 2012 Oct 17;30:1665.e5-7); such effect of pyelonephritis is expected to be exceedingly increased in the presence of high circulating concentrations of 25(OH)D3. Pyelonephritis may also impair glomerular filtration rate, which contributes to restrict calcium excretion; the resulting hypercalcemia may further impair renal function and calcium excretion. Those patients with chronic urinary retention with or without need for multiple bladder catheterizations are especially prone to develop pyelonephritis and may particularly resist increasing hydration to avoid more frequent bladder catheterizations. If needed, bladder catheterizations should be performed with maximal hygiene as possible, but as frequent as required to avoid post-void residual urine, which favors urinary infection. Bacteria from the intestinal flora cause most UTIs. Therefore, the risk urinary infections may be efficiently reduced in patients with neurogenic bladder if they wash the perianal area with antibacterial soap after each bowel movement. An ointment containing the association of bacitracin (250-500 mg) and neomycin (5mg),

easily available in several countries marketed under diverse trade names, should be applied on the perianal area after washing with the antibacterial soap. This is important because flatus (gas passed out of the rectum) carries intestinal secretion containing bacteria that may recolonize the perianal area; local moisture and heat facilitates bacterial growth and spread to the adjacent perineal skin reaching the external urethral orifice. Such ointment – not cream – should be reapplied after each bath (shower). Those who require bladder catheterizations should also apply the same ointment on the anterior part of vulva or glans penis after bath. Daily (once or twice daily, one hour before meals) administration of acid-resistant probiotic strains like Lactobacillus and Bifidobacteria (with higher survivability in the upper gastrointestinal tract) may contribute to a healthier colonic microbioma. Acid-resisted (coated) capsules may be helpful in delivering probiotics to colonic environment (in addition, administration with empty stomach reduces the exposure of capsules to gastric acid). Trademarks that provide expiration date (expiry date) declared on the label are more reliable since probiotics are living organisms. Always ask your patients about past history of recurrent or chronic UTI (even subclinical), independently on the presence of neurogenic bladder; if confirmed, treat (whenever active) UTI and prescribe the preventive measures described.

Uncontrolled hyperthyroidism precludes the use of high doses of vitamin D and Graves' disease may be associated to other autoimmune disorders like MS. Patients with associated Graves' disease should have their thyroid hormone levels normalized (with methimazole or a similar drug) before starting the treatment with high doses of vitamin D. High thyroid hormone levels increase osteoclastic activity, thereby triggering hypercalcemia when associated with the range of high doses of vitamin D proposed here. Very rarely, Graves or other causes of hyperthyroidism may appear in the course of (in spite of) high-dose vitamin D therapy (we have had 3 cases of Graves' disease among thousands of MS patients, contrasting with much higher comorbidity reports, where Graves' disease is expected to affect about 5% of MS patients on "standard" therapies). Periodic measurement of TSH levels has been included in our protocol. Methimazole may be withdrawn when the levels of thyroid stimulating hormone receptor antibody become normalized as a result of megadose vitamin D therapy itself.

On the other hand, concomitant use of high doses of biotin may produce false laboratory results indicating abnormal thyroid function tests as well as elevated antibodies (thyroid peroxidase antibody, thyroglobulin antibody and thyroid stimulating hormone receptor antibody) suggesting acute thyroiditis; this condition is recognized by the lack of clinical and echographic findings compatible with acute thyroiditis in a patient taking high doses of biotin.

High doses of vitamin C may also trigger hypercalcemia and affect renal function in patients under high-dose vitamin D therapy. We believe the reason is systemic oxalosis – a condition associated to high levels of oxalate derived from vitamin C. In systemic oxalosis, oxalate deposits are found in bone and kidney tissues, with increased osteoclastic activity leading to increased calcium released from bone. We had one patient who was taking 70 g o vitamin C per day (on her own) and developed symptoms of vitamin D intoxication plus symptoms of oxalosis.

Any physician who uses high-dose vitamin D therapy for autoimmune disorders

should be prepared to face and efficiently treat vitamin D intoxication – no matter how rare it occurs when this protocol is strictly followed. Intoxication (hypercalcemia and hypercalciuria, with or without increase in serum creatinine) may occur (in spite of strict observance of calcium-restricted diet) as a consequence of uncontrolled hyperthyroidism, pyelonephritis (underlying mechanism described elsewhere), concomitant intake of high doses of vitamin C (leading to systemic oxalosis), or when daily doses far exceed those daily doses minimally required to fully suppress serum PTH (for instance, when an unreliable compounding pharmacy prepares cholecalciferol oil solution in concentration much higher than prescribed). We have suggested a list of compounding pharmacies that are ruled by responsible pharmacists who we know to be particularly skilled in supplying cholecalciferol in oil solution. Alternatively, patients may use industrialized soft gels containing 10,000 IU of cholecalciferol diluted in oil which are commonly marketed by US websites.

In vitamin D intoxication 1,25(OH)2D is low or within the reference range due to inhibition of renal 1-alpha-hydroxylase by calcium; at high serum concentrations, 25(OH)D becomes the main agonist of VDR. If serum concentration of 25(OH)D far exceed the level necessary to compensate for individually inherited vitamin D resistance (which should not occur when the calculation rules are followed) bone calcium becomes the main source of hypercalcemia and hypercalciuria. In addition to correction of the specific triggering factor and stopping vitamin D administration, the most important therapeutic measure is inhibiting osteoclast activity and inducing osteoclast apoptosis by administering bisphosphonates like alendronate. In addition, increased hydration and furosemide administration (+ potassium supplementation) are used up to normalization of creatinine, serum and urinary calcium and resumption of PTH secretion (when serum PTH concentration resumes detectable levels). Thereafter, alendronate 70 mg per week is to be maintained for additional 2-3 months, when treatment (with high-dose vitamin D) is then resumed, initially administering half the daily dose that the patient was taking at the time of intoxication.

For early detection of vitamin D intoxication, patients are routinely alerted (in the first medical appointment) about symptoms like exceptional sustained thirst (despite increased hydration) and persistent nausea as possibly related to intoxication. The caring physician should consider persisting nausea particularly alarming, especially if bowel movements have stopped or are remarkably decreased. Whenever those symptoms appear they are instructed to measure urinary calcium using extra laboratory prescriptions they receive in the first medical appointment. If urinary calcium concentration is higher than 250 mg / L they are have to stop vitamin D ingestion and communicate with the caring physician. Nearly invariably (when the advised diet is strictly followed) “dry mouth” (which disappears by simply taking a sip of water) is frequently mistaken for real thirst (which is releived by drinking a couple of full glasses of water). “Dry mouth” is not related to hypercalcemia or hypercalciuria.

Sometimes a patient may show fully suppressed PTH (undetectable serum PTH) in a subsequent laboratory analysis without having increased the daily dose of vitamin D. This may due (as stated elsewhere) to occasional (intentional or unintentional) consumption of high-calcium containing foods close to the date of blood and urinary sampling. It may also due to seasonal differences in blood and urinary sampling dates; for instance, PTH may be 6 pg/mL (reference range: 10-65 pg/mL) when measured in

winter, and (while keeping the same dose of vitamin D taken orally) may become undetectable in summertime due to the addition of the natural skin synthesis of vitamin D due to solar exposure in summer. So, when PTH becomes transiently undetectable in absence of hypercalcemia, while oral vitamin D daily dose has not been changed for months, the caring physician should not reduce or stop the treatment. Reducing or stopping the treatment in a circumstance like that (where serum calcium is within the normal range in spite of transiently undetectable PTH) would put an MS patient at risk of having a new relapse or a new active lesion detectable on MRI without increasing safety.

Previous chronic renal failure is a challenging situation whenever high dose vitamin D is considered as a potential therapy for autoimmunity. On one side, patients with recent autoimmune aggression to their kidneys may resume normal renal function. On the other side, measurements of urinary calcium concentration become useless and the risk of intoxication (hypercalcemia) increases as the kidney ability to excrete calcium decreases. We have not had enough experience to provide solid advice on how to deal with that situation. For the moment we have used weekly measurements of serum calcium, urinary calcium, creatinine, urea (BUN) and monthly measurements of PTH, while the daily dose of cholecalciferol is cautiously increased.

We had tens of cases of pregnancy during the treatment with high doses of vitamin D, nearly all of then in MS patients who gave birth to normal babies showing precocious neuropsychological development.

From the very first appointment, emotional behavior should be inquired about. Patients should be emphatically advised to avoid emotional stress (stressful life events precede 85% of MS relapses in RRMS; it is acknowledged as by far the most important triggering factor of relapses). Unnecessary exposure to potentially stressful situations (like pointless family quarrels) should be avoided. In addition, chronically sustained emotional instability (depression, irritability, chronic fear) may partially or totally compromise the benefits of any treatment for autoimmune disorders. The detrimental effect of sustained emotional instability should be re-emphasized at each appointment, and patients presenting those features should be vigorously treated (including with antidepressants, magnesium supplements, acupuncture, tai chi, meditation, mindfulness in the context of cognitive-behavioral therapy, etc.) and encouraged to change their emotional reactivity to ordinary problems and to disease-related disabilities and limitations. We have advise them to regard their problems in a healthier positive way: as an opportunity to achieve a higher level of maturity as human beings as they learn to peacefully and patiently do their best to achieve feasible solutions, while serenely accepting whatever problems (economic and family issues, etc.) that are beyond their power to resolve. The following links can be used to emphasize the importance of emotional issues:

http://www.recentscientific.com/auto-immune-diseases-and-their-psychosocial-risk-factors-review

http://onlinelibrary.wiley.com/doi/10.1111/imm.12341/full

http://journals.lww.com/psychosomaticmedicine/Abstract/2002/11000/Stressful_Life_Events_Precede_Exacerbations_of.9.aspx

http://jnnp.bmj.com/content/52/1/8.full.pdf

http://www.sciencedirect.com/science/article/pii/S0006322302013598
http://onlinelibrary.wiley.com/doi/10.1111/imm.12341/full

https://www.researchgate.net/profile/Lisa_Goehler/publication/51448988_Depressions_multiple_comorbidities_explained_by_(neuro)inflammatory_and_oxidative__nitrosative_stress_pathways/links/0912f505b414700e84000000.pdf

http://download.springer.com/static/pdf/790/art%253A10.1186%252F1741-7015-11-200.pdf?originUrl=http%3A%2F%2Fbmcmedicine.biomedcentral.com%2Farticle%2F10.1186%2F1741-7015-11-200&token2=exp=1480304393~acl=%2Fstatic%2Fpdf%2F790%2Fart%25253A10.1186%25252F1741-7015-11-200.pdf*~hmac=c02dfb8445cb273be2b761749b05f29909fa9b5fdbca88d2967ee9150865df66

This is particularly important in patients with primary progressive or secondary progressive MS: we have found that progressive types of MS are consistently associated with *sustained* irritability (including irritability due to – or worsened by – loss of motor skills) and/or *sustained* fear (like fear of MS progression) and/or *sustained* depression (like depression related to poor quality of life due to potentially irreversible disabilities already accumulated prior to beginning of mega-dose vitamin D therapy). *Either primarily or secondarily progressive types of MS most likely will not respond to any treatment (including the expected therapy with megadoses of vitamin D as expected) if these emotional issues are not resolved or at least markedly diminished by employing all possible therapeutic resources. At each appointment the caring physician should maximally emphasize the importance of developing the ability to emotionally cope with ongoing daily life common frustrations and disappointments and, as much as possible, also with long-lasting consequences of major issues such as unemployment, financial crisis, affective ruptures, divorce, miscarriage, death of a beloved one, etc.*

Literature data indicates that chronically sustained emotional distress leads to release of inflammatory cytokines in the nervous tissue thereby maintaining diffuse inflammatory phenomena affecting the whole central nervous system (http://www.sciencedirect.com/science/article/pii/S1568997213000438). During long-term or indefinitely sustained stress, we usually observe a pattern of gradual neurological worsening in the clinical course of either primarily or secondarily progressive types of MS despite administering daily mega-doses of vitamin D. Such patients consistently accumulate disabilities in the following usual sequence: (1) first, affecting the motor function of one or both lower limbs (with progressive spasticity and impairment of gait) associated or not with worsening of neurogenic bladder; (2) second, deterioration of motor function one or both upper limbs. Commonly, under mega-doses of vitamin D no new demyelinating lesions nor contrast enhancement of demyelinating lesions appear on consecutive MRIs, but we may see progressive thinning of spinal cord, as well as progressive enlargement of lateral ventricles and cortical sulci, suggesting progressive atrophy of the central nervous system.

Because inflammation impairs the production of neurons from stem cells in the adult brain (adult neurogenesis) and increases neurodegeneration (https://www.researchgate.net/profile/Lisa_Goehler/publication/51448988_Depressions_multiple_comorbidities_explained_by_(neuro)inflammatory_and_oxidative__nitrosative_stress_pathways/links/0912f505b414700e84000000.pdf; https://www.researchgate.net/publication/5402574_Inflammation_is_detrimental_for_neurogenesis_in_adult_brain), sustained emotional distress would also impair recovery from previously disabilities acquired, even during the treatment with daily megadoses of vitamin D.

So, "Happiness and healthiness go hand in hand" (http://onlinelibrary.wiley.com/doi/10.1111/imm.12341/full), implying that "unhappiness and disease go hand in hand".

There is a potentially fundamental vicious cycle involving occasional of sustained emotional stress, magnesium and vitamin D:

1) emotional stress reduces magnesium levels (http://europepmc.org/abstract/med/10761188);
2) low magnesium levels reduces the activity of vitamin D hydroxylases (https://bmcmedicine.biomedcentral.com/articles/10.1186/1741-7015-11-187), thereby compromising the immune regulatory effect of vitamin D in mega-dose vitamin D therapy;
3) the resulting reduced vitamin D levels further increases emotional instability (http://link.springer.com/article/10.1007/s002130050517) and may thereby further reduce magnesium levels.

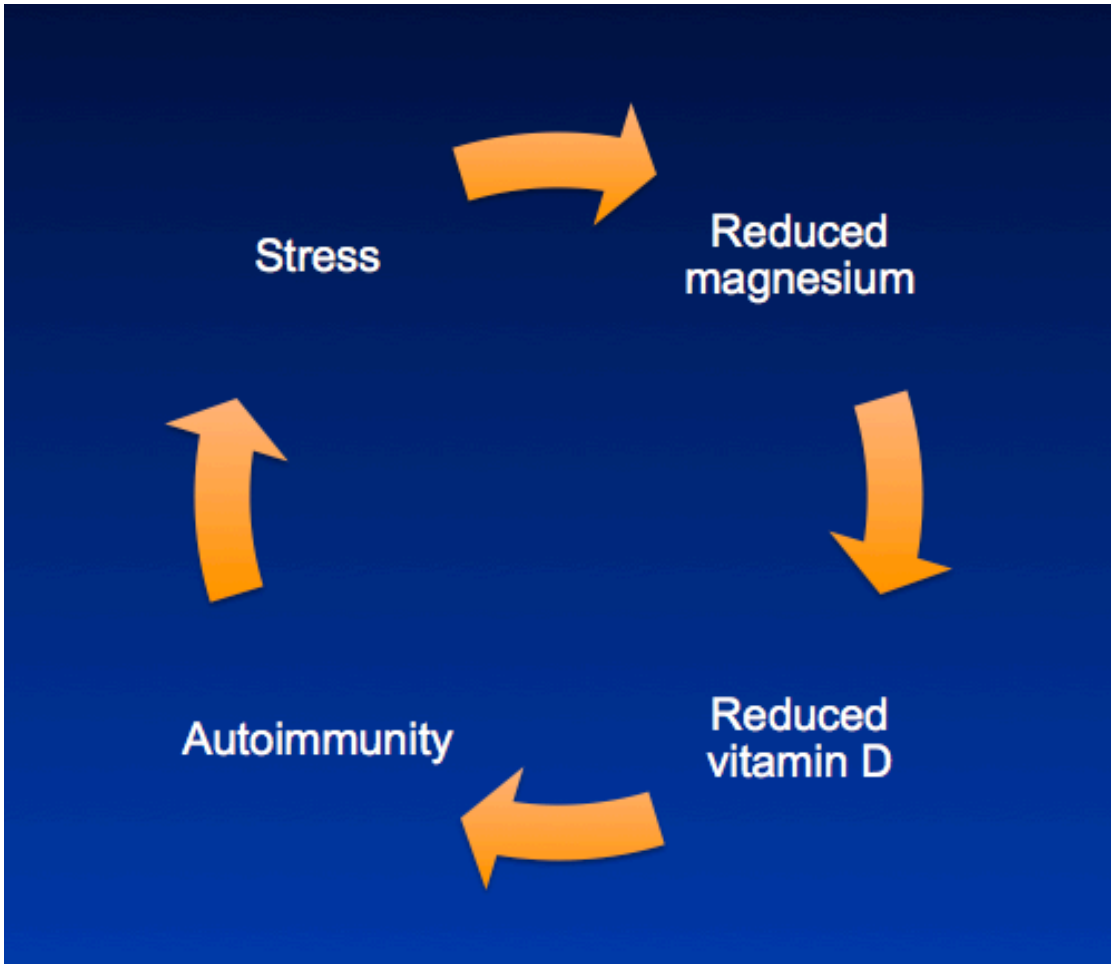

Figure 3: Interplay among emotion, magnesium, vitamin D and immune system.

Patients with autoimmune disorders should avoid, prevent or promptly fight other factors that may also impair the results of high-dose vitamin D therapy. Those include excessively hot baths (usually a habit since childhood – especially found in women) and sauna, frequent alcohol drinking (alcohol inhibits vitamin D hydroxylases), smoking, and repeated urinary (preventive measures described elsewhere) or respiratory tract infections.

One may expect normal life for emotionally stabilized MS patients who have not accumulated significant disabilities years earlier by the time they begin their treatment with high doses of vitamin D. Particularly, disabilities acquired during the latest 12 months before the beginning of treatment may completely disappear as months or years under daily mega doses of vitamin D go by.

The link below will lead to a video with English subtitles.

https://www.youtube.com/watch?v=hOfO29rL-gI

Another video (Portuguese only):
https://www.youtube.com/watch?v=2vdJbowhTxY

I think this initial guidance and video will advance and cover most of the basic

issues.

All the best,
Cicero Galli Coimbra