Vitamin D Toxicity: A 16-Year Retrospective Study at an Academic Medical Center

John P. Lee, DO,[1] Michael Tansey, MD,[2] Jennifer G. Jetton, MD,[3] Matthew D. Krasowski, MD, PhD[1]*

Laboratory Medicine 49:2:123–129

DOI: 10.1093/labmed/lmx077

ABSTRACT

Background: Interest in vitamin D has increased during the past 2 decades, with a corresponding increase in laboratory testing of 25-hydroxyvitamin D [25(OH)D]. The vast majority of specimens tested display normal or deficient levels of 25(OH)D; concentrations rarely fall in the potentially toxic range.

Methods: We performed a retrospective investigation of elevated 25(OH)D levels during a 16-year period at the University of Iowa Hospitals and Clinics (UIHC), a 734-bed tertiary-/quaternary-care academic medical center in the midwestern United States. Detailed medical-record review was performed for patients with serum/plasma 25(OH)D concentrations higher than 120 ng per mL.

Results: A total of 127,932 serum/plasma 25(OH)D measurements were performed on 73,779 unique patients. Of these patients, 780 (1.05%) had results that exceeded 80 ng per mL and 89 patients (0.12%) had results that exceeded 120 ng per mL. Only 4 patients showed symptoms of vitamin D toxicity. Three of these cases involved inadvertent misdosing of liquid formulations.

Conclusions: Symptomatic vitamin D toxicity is uncommon, and elevated levels of 25(OH)D do not strongly correlate with clinical symptoms or total serum/plasma calcium levels. Our study highlights the potential risks of the liquid formulation of vitamin D.

Keywords: vitamin D, vitamin D toxicity, hypercalcemia, drug overdose, toxicology, pharmaceutical solutions

During the past 2 decades, interest in vitamin D has significantly increased, partly due to newly hypothesized connections of vitamin D to the immune system, cardiovascular health, and even cancer prevention.[1] Also, widespread recognition of vitamin D deficiency has prompted increased supplementation, to prevent important adverse health consequences such as osteoporosis.[2] We note that supplementation, in many cases, may be patient driven, rather than prescribed by physicians. Although evidence for the benefits of vitamin D supplementation is still emerging, the rate of its use has increased, along with a concomitant rise in vitamin D testing.[3] Despite that most test results are from patients with normal or deficient levels, the incidence of vitamin D toxicity has also risen.[4]

Vitamin D toxicity is poorly understood and not well defined in the literature.[5] Multiple commercial reference laboratories have varying cut-off values for the upper limit of normal for 25-hydroxyvitamin D [25(OH)D] levels. Further, potentially toxic levels of 25(OH)D have been shown to correlate poorly with hypercalcemia and symptomatic presentation. Vitamin D toxicity has been reported in multiple age groups and from multiple causes, including manufacturing errors, errors in milk fortification, incorrect dosing from liquid preparations, and intentional (although with no intent to harm) ingestion of megadoses of vitamin D supplements.[6-10] Among these causes, the most harmful appears to be sustained ingestion of megadoses (eg 50,000 IU) and incorrect dosing of supplements in children. We performed a medical-record review in patients with elevated 25(OH)D levels during a 16-year period at an academic medical center, in

Abbreviations

25(OH)D, 25-hydroxyvitamin D; UIHC, University of Iowa Hospitals and Clinics; RWB, Reporting Workbench; LC/MS, liquid chromatography–mass spectrometry; MVI, multivitamin

[1]Department of Pathology, University of Iowa Hospitals and Clinics, Iowa City, IA, Divisions of [2]Pediatric Endocrinology and [3]Pediatric Nephrology, Dialysis, and Transplantation, University of Iowa Stead Family Children's Hospital, Iowa City, IA

*To whom correspondence should be addressed.
John-p-lee@uiowa.edu

an effort to describe the causes of hypervitaminosis D and the extent to which vitamin D levels correlate with serum calcium levels and clinical symptoms.

Materials and Methods

The study was conducted at the University of Iowa Hospitals and Clinics (UIHC), a 734-bed tertiary-/quaternary-care academic medical center located in Iowa City, Iowa. The data in the study were collected as part of a retrospective study approved by the University of Iowa Institutional Review Board (protocol #201612810), covering the time period from January 1, 2000, through December 31, 2016. This study was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki).

As described in a previous study coauthored by one of us, Epic Reporting Workbench (RWB) was used to retrieve past laboratory results and medication administration records.[11] In the retrospective timeframe, all serum/plasma 25(OH)D levels derived from testing performed for clinical purposes were retrieved from the electronic medical record. We did an additional search using RWB for patients in the retrospective time period with clinical encounter diagnostic codes related to "vitamin D poisoning" (ICD-9: 278.4; ICD-10, E67.3), "vitamin D overdose" or "vitamin D poisoning" (ICD-9: 963.5, E858.1, E962.0, E969, E980.4; ICD-10, T45.2X), and "vitamin D toxicity" (ICD-9: 278.4; ICD-10, T45.2X).

Multiple 25(OH)D assays were used during this time period, although none simultaneously. From January 2000 through mid-July 2005, the Nichols ADVANTAGE 25-OH Vitamin D immunoassay (Nichols Institute Diagnostics, Inc) was performed in-house. From late July 2005 through January 2012, specimens were tested at ARUP Laboratories using the DiaSorin immunoassay (DiaSorin). An in-house assay, the Abbott ARCHITECT 25-OH Vitamin D (Abbott Laboratories, Inc.) was used from mid-January 2012 through mid-October 2012. Starting in mid-October 2012, the laboratory moved to the Roche Elecsys Vitamin D assay (Roche Diagnostics for all products mentioned in this paragraph) on the in-house Modular E platform. Ultimately, in October of 2013, the laboratory switched to Roche cobas e602 analyzers. Total calcium assays were first performed on Roche Modular P analyzers. In October 2013, the laboratory switched to Roche cobas c702 analyzers. Both testing platforms used colorimetric methodologies.

Elevated 25(OH)D levels were defined as levels higher than 80 ng per mL based on a case report of a patient showing toxicity with a 25(OH)D serum/plasma concentration of only 80 ng per mL.[12] However, the published literature has shown that toxicity is unlikely unless 25(OH)D concentrations exceed 120 ng per mL.[12-14] Thus, our detailed medical record review focused on patients whose 25(OH)D exceeded 120 ng per mL. The medical record review included assessment for presence or absence of symptoms, total calcium levels (if performed), and type and concentration of vitamin D supplementation. *Patients having symptoms* were defined as those having 1 or more of the following symptoms in the absence of any other diagnosable cause: polydipsia, polyuria, decreased appetite, vomiting, constipation, abdominal pain, renal failure, nephrocalcinosis, and/or failure to thrive. These symptoms were required to be present at the time of the blood draw to test 25(OH)D levels. *Normal total serum/plasma calcium concentrations* are defined as 8.5 to 10.5 mg per dL.

Results

During the 16-year study period, there were 127,932 measurements of 25(OH)D performed on specimens from 73,779 unique patients. We identified 1068 samples from 780 unique patients with 25(OH)D concentrations greater than 80 ng per mL. Thus, specimens with 25(OH)D levels greater than 80 ng per mL comprised 0.8% of total 25(OH)D measurements and 1.1% of patients tested.

The age range of those affected was 0.3 to 100.6 years, with a mean age of 49.0 years. Of these patients, 86 were younger than 18 years (11.0%). A total of 776 25(OH)D measurements were performed on specimens from 559 unique female patients (71.7% of unique patients tested), with a range from 81 to 480 ng per mL. A total of 290 25(OH)D measurements were performed in 221 unique male individuals (**Table 1**), with a range of 81 to 805 ng per mL. Among the patients with elevated levels, 89 (0.1% of unique patients tested) had 25(OH)D values greater than 120 ng per mL (**Figure 1**). Based on reported history, 17 patients were taking 50,000-IU tablets, 4 were taking 20,000-IU tablets, 6

Table 1. Demographics of Patients With Elevated Levels of 25(OH)D

25(OH)D(ng/mL)	No. of Patients	Male	Female	Male Age, Average (y)	Female Age, Average (y)
>80	780	221	559	46.7	49.8
>120	89	29	60	47.3	50.1

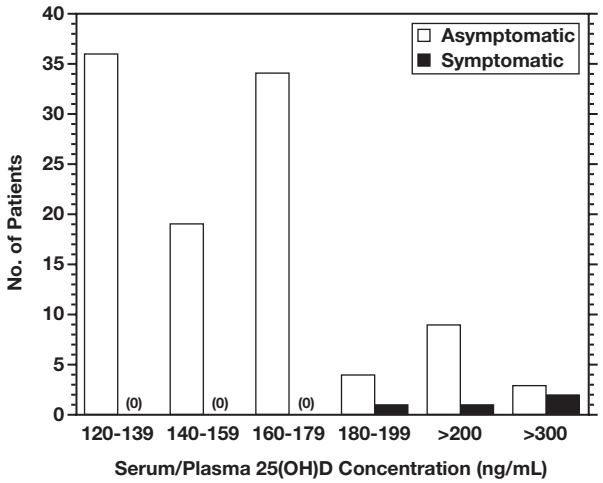

Figure 1

Distribution of patients with and without symptoms at various vitamin D concentrations.

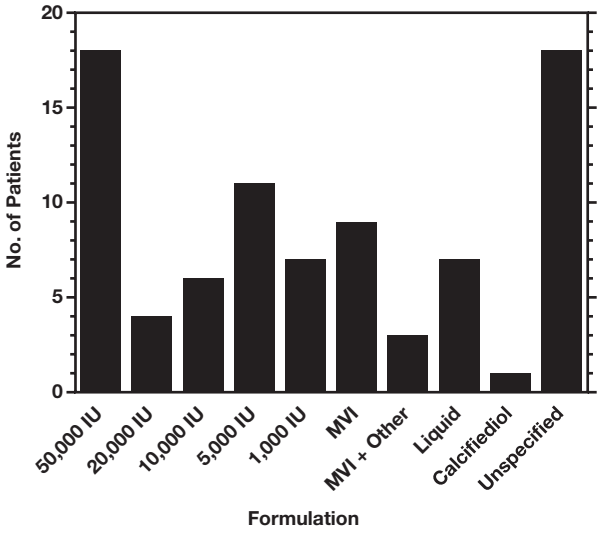

Figure 2

Varying vitamin D supplementation concentrations and their frequency among patients with elevated 25(OH)D levels. MVI indicates multivitamin.

were taking 10,000-IU tablets, 11 were taking 5000-IU tablets, and 6 were taking 1000-IU tablets (Figure 2). Also, 7 were taking liquid formulations with varying concentrations. The remaining patients were reportedly taking a combination of multivitamin and "other" supplements (Table 2). Eighteen patients had no vitamin D supplementation recorded in their medical record.

A total of 53 patients had concomitant serum/plasma total calcium drawn at the time of 25(OH)D testing. Only 7 of these patients had total calcium levels higher than the upper limit of the reference range (3 of whom were experiencing symptoms). In these 7 patients, total calcium values ranged from 10.8 to 19.8 mg per dL. The median was 13.3 mg per dL, and the mean was 13.8 mg per dL. For the remaining 47 patients, the total calcium concentrations ranged from 7.3 to 10.5 mg per dL (normal range, 8.5 to 10.5 mg/dL), with a median of 9.4 mg per dL. Based on linear regression statistical analysis, the correlation between vitamin D concentrations and total serum/plasma calcium concentrations was weak, with an r^2 value of 0.10 (Figure 3).

Among the patients with elevated 25(OH)D levels greater than 120 ng per mL, only 4 showed symptoms of vitamin D toxicity (Table 3). The first patient was a 4-month old boy with a 25(OH)D concentration of 496 ng per mL and a concomitant calcium value of 19.8 mg per dL. His symptoms included failure to thrive, constipation, weight loss, and nephrocalcinosis. He was receiving an incorrect dose of liquid vitamin D from a dropper, consisting of approximately 100,000 to 150,000 IU per day. The second patient was a 3-year-old girl with a 25(OH)D concentration of 480 ng per mL and total calcium level of 13.3 mg per dL at the time of diagnosis. Her symptoms included severe gastroenteritis, abdominal pain, vomiting, weight loss, polydipsia, polyuria, and nephrocalcinosis. In similar circumstances to the first patient, patient 2 was receiving an incorrect dose of liquid vitamin D from a dropper, of approximately 40,000 to 80,000 IU per day.

The third patient was a 62-year-old non-Hispanic white woman with a 25(OH)D concentration of 247 ng per mL. She

DOI: 10.1093/labmed/lmx077

Table 2. Varying 25(OH)D Levels and Corresponding Presence or Absence of Symptoms and/or Hypercalcemia

25(OH)D (ng/mL)	No. of Patients	Age, Average (y)	Age, Range (y)	Male	Female	Hypercalcemia[a]	With Symptoms
>300	5	26.4	0.3–66.6	4	1	2	2
>200	10	48.4	4.3–62.5	3	7	0	1
180–199	5	53.2	1.9–77.2	1	4	1	1
160–179	14	53.9	1.5–90.2	6	8	0	0
140–159	19	51.5	17.2–90.2	4	15	1	0
120–139	36	49	3.2–90.9	11	25	2	0

[a]*Defined as serum/plasma concentration >10.5 mg/dL.*

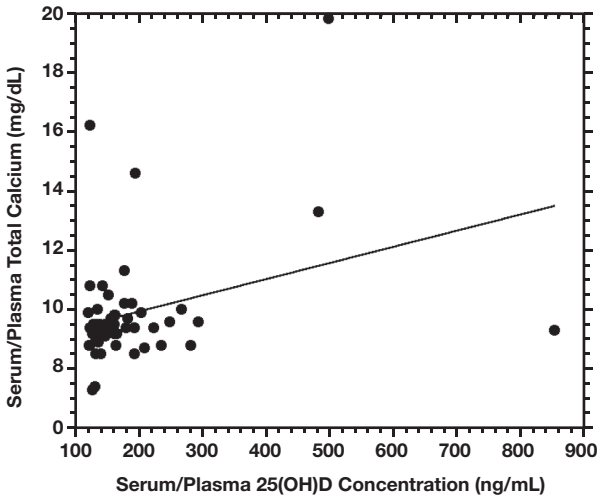

Figure 3

Correlation between 25(OH)D levels and serum/plasma calcium concentrations. Linear regression statistics (95% confidence interval in parentheses): slope, 19.1 (3.5–34.7), *y* intercept, –0.14 (–157.1 to 156.9), r^2 = 0.10.

had a normal total serum/plasma calcium concentration, at 9.6 mg per dL, but had abdominal pain, severe constipation, and nausea. She was taking 50,000 units of vitamin D per day, also in liquid form. Patient 4 was a 70-year-old non-Hispanic white woman with a 25(OH)D concentration of 194 ng per mL. She had an elevated calcium concentration of 11.4 mg per dL and had experienced renal failure, weakness, confusion, low mood, poor balance, and slurred speech. Her vitamin D supplementation reportedly consisted of only a single 1000 IU tablet per day; however, the reported history may be inaccurate.

The 2 patients with the most severe symptoms were pediatric patients receiving liquid vitamin D via "droppers." In both instances, the incorrect dosage was administered due to confusion between the words "dropperful" and "drop." As a result, the patients received an entire dropperful of liquid vitamin D and, on occasion, 2 to 3 entire droppersful, instead of the correct 1 to 3 drops.

The healthcare professionals treating those patients were able to obtain the original bottles of vitamin D and to determine that a dropper contained approximately 50 drops. There were 1000 IU per drop. Thus, patient 1 was receiving 100,000–150,000 IU per day. Also, based on the amount of vitamin D left in the bottle, it was estimated that he had received approximately 1.6 to 1.7 million units during an 8-week period. The parents of the second patient misinterpreted the instructions and also administered 1 dropperful instead of 1 drop. In this case, each drop contained 2000 IU. It was estimated that patient 2 received 3.5 to 5.5 million IU during a 6-month period, based on the amount of supplement left in the bottle.

Although we did not perform a detailed medical record review for patients with 25(OH)D less than 120 ng per mL, we did a search in the electronic medical records for patients with diagnostic codes related to "vitamin D poisoning," "vitamin D overdose," "vitamin D poisoning," or "vitamin D toxicity," to determine whether any hypervitaminosis D cases were missed. The results of this search identified many of the patients with serum/plasma 25(OH)D concentrations greater than 120 ng per mL whose cases were already reviewed in detail and also identified 7 patients assigned these diagnostic codes who did not have serum/plasma 25(OH)D concentrations exceeding 120 ng per mL. All 7 of these patients had at least a single 25(OH)D concentration greater than 80 ng per mL but none had values exceeding 120 ng per mL. None of these 7 case individuals had any symptoms suggestive of vitamin D toxicity. Further, 6 of the 7 patients had serum/plasma total calcium measurement(s) concurrent with the elevated 25(OH)D levels. For

Table 3. Patients Experiencing Symptoms Who Have Corresponding Calcium Levels, and Type and Concentration of Vitamin D Supplementation

Patient	Age (y)	25(OH)D (ng/mL)	Calcium (mg/dL)	Vitamin D Supplement Type
1	0.3	496	19.8	Liquid (1000 IU)
2	3.0	480	13.3	Liquid (2000 IU)
3	62.0	247	9.6	Liquid (50,000 IU)
4	70.0	194	11.4	Tablet (1000 IU)

these 6 patients, total calcium concentrations were all within the reference range; the remaining patient did not have total calcium analysis performed.

Discussion

Consistent with the findings of previous studies,[3,15,16] we found that the vast majority of 25(OH)D test results at our academic medical center have shown concentrations below 80 ng per mL. Only 1.1% of patients tested in a 16-year period revealed 25(OH)D levels higher than 80 ng per mL, and only 0.1% had levels exceeding 120 ng per mL. However, 25(OH)D toxicity levels are not well defined.[12] Major clinical laboratories have varying cut-off points for excess levels.[13] A previous study[14] reported that a value of 125 ng per mL can be used as an upper limit of normal. In another study, toxicity was not observed until values exceeded 200 ng per mL. However, toxicity was mentioned in 1 patient report of a 25(OH)D serum/plasma concentration of only 80 ng per mL.[12]

We did not encounter such outcomes in patients until a 25(OH)D concentration of 194 ng per mL was achieved. Of the remaining patients experiencing symptoms, one had a level higher than 200 ng per mL, whereas the others had levels higher than 400 ng per mL. Further, similar to results reported by Dudenkov et al,[17] we discovered that potentially "toxic" levels of vitamin D did not strongly correlate with hypercalcemia. We limited our detailed medical-record review to cases in which the level of 25(OH)D exceeded 120 ng per mL. It is possible that some patients showed toxicity at lower 25(OH)D levels; however, our results align with those of other studies, such that these events appear to be rare.

The results of previous studies[3,12,15] have shown that most cases of potentially toxic 25(OH)D levels occurred due to use of high-dose vitamin D supplementation. Patients taking high-dose concentrations and liquid supplementation were at increased risk for higher 25(OH)D levels. Doses containing 50,000 IU were common in patients with high 25(OH)D levels in our study. This finding highlights the risk of someone overdosing or taking the incorrect amount with such concentrated doses.

We observed a higher rate of adverse outcomes associated with liquid vitamin D formulations. Specifically, we found 2 pediatric overdose cases in which an incorrect amount of supplement was dispensed from a dropper. Instead of receiving a drop of vitamin D, both patients received 1 entire dropperful due to confusion between the terms "drop" and "dropperful." Both misunderstandings resulted in these children receiving hundreds of thousands of IU or more of vitamin D during the course of a few months. Both patients showed symptoms and had elevated calcium levels and required hospital stays and treatment. Other case reports have also reported vitamin D toxicity resulting from liquid formulations.[8,18,19] In each case, the infant received dropperful instead of drops. In one case, a dropperful had been the correct dose on a previous formulation; however, the new supplement only required 1 drop. Our experience and the documented case reports highlight the danger associated with liquid formulations and the lack of standardized dosing in this area. Perhaps it is time for standardization of supplement formulation and simplification of administration methods, or other such measures, to enhance safety.

As a result of its retrospective nature, our study has certain limitations. First, the medical record review focused on common, recognizable symptoms of vitamin D toxicity. It is possible that more subtle toxicity was not evident on medical record review.

Second, we did not perform a detailed medical record review on patients who had vitamin D levels less than 120 ng per mL.

It is possible that a patient with a 25(OH)D level lower than 120 ng per mL had symptoms. However, this possibility seems unlikely, based on the results of previous studies and the 25(OH)D levels at which we observed symptoms. Also, we did a search for patients with diagnosis codes related to vitamin D intoxication, overdose, poisoning, or toxicity. The results identified 7 patients with 1 or more of these diagnosis codes and who had 25(OH)D levels between 80 and 120 ng per mL. None of these patients had any symptoms consistent with vitamin D toxicity.

Third, a large cohort of patients did not have a calcium measurement performed at the time of vitamin D concentration. The study would have better power in terms of correlation between vitamin D and calcium levels with more concomitant testing. Also, vitamin D concentration and route were not listed in some of the medical records.

Last, a global challenge with 25(OH)D measurements has been the variation between assays and challenges with harmonization.[20-23] Although the criterion standard method of liquid chromatography–mass spectrometry (LC/MS) is increasingly being used for 25(OH)D measurement, immunoassays are still widely used. 25(OH)D immunoassays can vary from one another in cross-reactivity with 25(OH)D2 and 25(OH)D3, analytical measurement range, dilution protocols, and accuracy/precision. Variability between immunoassays may be especially large at very high 25(OH)D concentrations, thus making it challenging to compare patient results over time if different assays were used. This finding may be a factor in the present study, in which our institution changed 25(OH)D assays 3 times during the retrospective time period. Thus, we grouped 25(OH)D concentrations into broad ranges (eg, >80 ng/mL and >120 ng/mL) to minimize variations in the 25(OH) concentration interpretation. We hope that ongoing efforts to standardize and harmonize 25(OH)D assays will provide more consistency with measurement of potentially toxic 25(OD)D concentrations.[20-23]

Conclusion

We report that symptomatic vitamin D toxicity is quite rare. We investigated a large cohort of patients with vitamin D levels characterized as elevated by current guidelines; however, most of these patients did not show untoward effects from the elevated levels. Further, we did not observe a strong correlation between elevated 25(OH)D levels and elevated total calcium levels. Irrespective of what constitutes vitamin D toxicity, we observed that certain formulations were common in patients with high 25(OH)D levels, including high-concentration doses (eg, 50,000 IU) and liquid preparations. We advocate for standardized droppers for pediatric vitamin D supplementation. Further study is required regarding vitamin D supplementation guidelines and vitamin D toxicity definitions. **LM**

Acknowledgments

The authors thank the Department of Pathology (Nitin Karandikar, MD, PhD, Department Executive Officer) for providing research funding for this study.

References

1. Holick MF. Vitamin D status: measurement, interpretation, and clinical application. *Ann Epidemiol*. 2009;19(2):73–78.
2. Beastall G, Rainbow S. Vitamin D reinvented: implications for clinical chemistry. *Clin Chem*. 2008;54(4):630–632.
3. Genzen JR, Gosselin JT, Wilson TC, Racila E, Krasowski MD. Analysis of vitamin D status at two academic medical centers and a national reference laboratory: result patterns vary by age, gender, season, and patient location. *BMC Endocr Disord*. 2013;13:52.
4. Khan AH, Majid H, Iqbal R. Shifting of vitamin D deficiency to hypervitaminosis and toxicity. *J Coll Physicians Surg Pak*. 2014;24(7):536.
5. Massart C, Souberbielle JC. Serum 25-hydroxyvitamin D immunoassays: recommendations for correct clinical interpretation. *Clin Chem*. 2009;55(6):1247–1248.
6. Anık A, Çatlı G, Abacı A, Dizdarer C, Böber E. Acute vitamin D intoxication possibly due to faulty production of a multivitamin preparation. *J Clin Res Pediatr Endocrinol*. 2013;5(2):136–139.
7. Bansal RK, Tyagi P, Sharma P, et al. Iatrogenic hypervitaminosis D as an unusual cause of persistent vomiting: a case report. *J Med Case Rep*. 2014;8:74.
8. Cantrell L, Hogen E. Vitamin D overdosage in an infant from nonprescription vitamin D drops. *Am J Health Syst Pharm*. 2015;72(15):1262–1263.
9. Jacobsen RB, Hronek BW, Schmidt GA, Schilling ML. Hypervitaminosis D associated with a vitamin D dispensing error. *Ann Pharmacother*. 2011;45(10):e52.
10. Singh P, Trivedi N. Tanning beds and hypervitaminosis D: a case report. *Ann Intern Med*. 2014;160(11):810–811.
11. Krasowski MD, Chudzik D, Dolezal A, et al. Promoting improved utilization of laboratory testing through changes in an electronic medical record: experience at an academic medical center. *BMC Med Inform Decis Mak*. 2015;15:11.
12. Vieth R. Vitamin D supplementation, 25-hydroxyvitamin D concentrations, and safety. *Am J Clin Nutr*. 1999;69(5):842–856.
13. Krasowski MD. Pathology consultation on vitamin D testing. *Am J Clin Pathol*. 2011;136(4):507–514.
14. Adams JS, Lee G. Gains in bone mineral density with resolution of vitamin D intoxication. *Ann Intern Med*. 1997;127(3):203–206.
15. Shea RL, Berg JD. Self-administration of vitamin D supplements in the general public may be associated with high 25-hydroxyvitamin D concentrations. *Ann Clin Biochem*. 2017;54(3):355–361.

16. Pérez-Barrios C, Hernández-Álvarez E, Blanco-Navarro I, Pérez-Sacristán B, Granado-Lorencio F. Prevalence of hypercalcemia related to hypervitaminosis D in clinical practice. *Clin Nutr*. 2016;35(6):1354–1358.

17. Dudenkov DV, Yawn BP, Oberhelman SS, et al. Changing incidence of serum 25-hydroxyvitamin D values above 50 ng/mL: a 10-year population-based study. *Mayo Clin Proc*. 2015;90(5):577–586.

18. Rajakumar K, Reis EC, Holick MF. Dosing error with over-the-counter vitamin D supplement: a risk for vitamin D toxicity in infants. *Clin Pediatr (Phila)*. 2013;52(1):82–85.

19. Ketha H, Wadams H, Lteif A, Singh RJ. Iatrogenic vitamin D toxicity in an infant–a case report and review of literature. *J Steroid Biochem Mol Biol*. 2015;148:14–18.

20. Binkley N, Dawson-Hughes B, Durazo-Arvizu R, et al. Vitamin D measurement standardization: the way out of the chaos. *J Steroid Biochem Mol Biol*. 2017;173:117–121.

21. Elsenberg E, Ten Boekel E, Huijgen H, Heijboer AC. Standardization of automated 25-hydroxyvitamin D assays: how successful is it? *Clin Biochem*. 2017; doi: 10.1016/j.clinbiochem.2017.06.011.

22. Wyness SP, Straseski JA. Performance characteristics of six automated 25-hydroxyvitamin D assays: mind your 3s and 2s. *Clin Biochem*. 2015;48(16-17):1089–1096.

23. Black LJ, Anderson D, Clarke MW, Ponsonby AL, Lucas RM; Ausimmune Investigator Group. Analytical bias in the measurement of serum 25-hydroxyvitamin D concentrations impairs assessment of vitamin D status in clinical and research settings. *PLoS One*. 2015;10(8):e0135478.