Clinical Chemistry Trainee Council
Pearls of Laboratory Medicine
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Title: Vitamin D

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Slide 1: Title Slide

Slide 2: Outline

This presentation will cover the basics of vitamin D testing. It will start with vitamin D structure and function and a discussion of the clinical utility of vitamin D. Next, we'll focus on vitamin D testing and the issues with measurement. Finally, we'll cover vitamin D supplementation, toxicity, deficiency, and clinical targets.

Slide 3: Vitamin D Structure

Vitamin D broadly refers to a number of different fat soluble compounds known as secosteroids. These include vitamin D proper, which occurs in two forms, vitamin D2 on the left and vitamin D3, 2nd from the left. Vitamin D2 and D3 differ by a double bond between carbons 22 and 23 and a methyl group on carbon 24. These vitamins are biologically inactive. They are enzymatically converted to an intermediate compound, 25-hydroxy vitamin D, which in turn serves as a pool for the biologically active form of vitamin, called 1,25 hydroxy vitamin D. The key differences between these forms are highlighted with the blue circles.

Slide 4: Table of Vitamin D forms

This table shows the concentrations of vitamin D, the intermediate compound 25 hydroxy vitamin D, and the bioactive compound 1,25 hydroxy vitamin D in circulation. Half-lives, control, and the % free are also indicated. 25 hydroxy vitamin D has a relatively long half life, higher relative concentration, and is unregulated, making it the best compound to determine the patient's overall vitamin D status. This point is worth emphasizing, as labs often see orders for other forms of vitamin D even when the goal is to assess vitamin D stores. Many labs end up reviewing or restricting orders for 1,25OH vitamin D, as it has narrow, but valid clinical utility.

Slide 5: Vitamin D Synthesis

Vitamin D synthesis occurs through many steps. It begins with conversion of a cholesterol-derivative, through sunlight or irradiation yielding vitamin D. Both D2 and D3 forms of vitamin D are converted to 25-hydroxy vitamin D in the liver by the enzyme 25 hydroxylase. 25-hydroxy vitamin D is in turn converted to 1,25 hydroxy vitamin D, the active compound, by 1-alpha hydroxylase in the kidney. The 1-alpha hydroxylase enzyme is positively regulated, among other things, by parathyroid hormone. From the synthesis diagram, you can infer that either liver or kidney damage will affect the availability of vitamin D. Likewise, inadequate exposure to sunlight will also cause deficiency in the absence of supplementation. Supplements containing both vitamin D2 and D3 are available.

Slide 6: Vitamin D Function

Vitamin D has a number of functions in the body. The best known function of vitamin D is the control of circulating calcium and phosphate. Produced primarily by the kidney, 1,25OH vitamin D directly increases intestinal absorption of both calcium and phosphorus, increasing their concentrations in circulation. 1,25OH vitamin D is also involved in bone formation, resorption, and mineralization. The mechanisms of bone remodeling are quite complex and beyond the scope of this short vignette. It is clear that in patients with osteoporosis, the risk of bone fracture is inhibited by vitamin D supplementation along with calcium.

Vitamin D is also known to play an important role in immune regulation and has an emerging role in neuromuscular and cardiovascular function, as well as cell proliferation in soft tissues. In fact, most cells in the body express vitamin D receptors and many express converting enzyme such as 1-alpha hydroxylase. You can be certain to hear more about this pleiotropic hormone in the years to come.

Slide 7: Vitamin D Measurement

25-hydroxy vitamin D is the best molecule to measure for assessment of overall vitamin D status. It is decreased with low exposure to sunlight, inadequate dietary intake, malabsorption, and liver disease. It is increased with toxicity, which occurs exclusively through dietary intake such as over supplementation; prolonged exposure to sunlight does not cause toxicity as the excess is converted into inactive compounds such as 24,25 vitamin D. As far as test utilization, it is important to consider when to test for vitamin D deficiency. While supplementation has a clear role in several disorders, such as bone disease, to my knowledge, there is little evidence demonstrating that measuring vitamin D actually improves clinical outcomes. This is a topic of active research. Not to be entirely discounted, 1,25 hydroxy vitamin D does have some specific clinical utility. It is useful for detecting granulomatous diseases, such as sarcoidosis, differentiation of different types of rickets, and for some types of hyperparathyroidism.

Slide 8: Prevalence of Vitamin D Deficiency

There are many hundreds of studies which have assessed vitamin D deficiency in various populations, disease categories, and ethnicities around the world. Shown here are a few examples reviewed by Holick in the Mayo Clinic Proceedings. While some define deficiency as <20 ng/mL or <50 nmol/L, it is worth recognizing that these definitions differ between studies as do methods for measurement, making comparisons a bit challenging. These issues are discussed later on in the presentation. Nevertheless, there appears to be a very high prevalence of 25OH vitamin D deficiency globally, with estimates of 1 billion people worldwide. There are a number of factors which contribute to deficiency. For example,

darker skinned individuals with low exposure to sunlight living at high latitudes who have low dietary intake of vitamin D are very likely to have low levels. This audience will appreciate that medical residents are at risk for vitamin D deficiency due to the lifestyle afforded them by the demands of their work.

Slide 9: Seasonality of Vitamin D

This figure shows seasonal variation in vitamin D levels for individuals in the Northern Hemisphere. Given the mechanism of vitamin D synthesis, it is not surprising that levels drop in the winter months, when there is lower UV radiation in Northern climates. In fact, without dietary intake, it is impossible to convert the cholesterol-derivative into 25OH vitamin D for many months of the year. This is because photolysis of 7-dehydrocholesterol to 25OH vitamin in the skin requires radiation in the UVB range 290-315 nm. At latitudes north or south of 37°, there is insufficient radiation for this conversion in the winter. Sunburn remains possible, but not vitamin D conversion. In Boston, home to the Editor of *Clinical Chemistry*, there is a period between November through February where conversion of vitamin D in the skin will not occur. Further north (or south), to 52°N, for example in Edmonton, Canada or parts of the UK, this ineffective winter period runs from October through March.

Slide 10: What to Measure

One question many laboratorians will be asked is: which form of vitamin D should be measured? Assuming that we want to measure someone's overall vitamin D status, it is important to consider whether we want to measure vitamin D2, vitamin D3, or both. Again, these are the 25 hydroxy forms to which I'm referring. The answer to this question really depends on your patient population; if you have patients who are taking vitamin D2 supplements, principally the prescription form, it is going to be important to identify vitamin D2 to detect rare cases of toxicity. However, management of overall vitamin D status is based on total vitamin D levels so it's certainly important to report at least the total. Another consideration is whether clinicians will be able to appreciate the subtleties of the differences between reporting vitamin D2 and D3. It is useful to provide interpretive comments on reports to help test users understand the results. Others have advocated for providing a total OH vitamin D level and only differentiating the forms in the context of total excess.

Slide 11: Measurement Methods

Once it is understood what to measure, the next step is to choose how to measure it. Available methods for vitamin D measurement include: radioimmunoassays, enzyme-linked immunosorbant assays, enhanced chemiluminescence, and protein-binding assays. Also available are chromatography based methods including HPLC, GC-MS, and LC-MS/MS.

Slide 12: Method Limitations

There are issues with measurement by any of these methods. Immunoassays are not capable of detecting both vitamin D2 and D3 with equal efficacy. In fact, some methods are incapable of detecting vitamin D2 at all. There is also a lack of traceability and generally poor agreement between various platforms. Chromatography-based methods are not immune to problems either. They suffer from lack of standardization, and some are subject to interference by the C3-epimer; the C3-epimer is a compound of unknown clinical significance which is a structural mirror image of the 25-OH form that is the desired target for measurement.

Slide 13: Reference Interval Problems

This figure illustrates the effect of differential detection of vitamin D between methods. Beginning with the red line, the proportion of people deficient for vitamin D at 50 ng/L is much higher than it would be with a different assay. Although these differences are exaggerated to emphasize the point, it is important to consider the appropriate reference interval for your assay. These differences also highlight challenges in defining a global definition of deficiency and in comparing research between different methods. The National Health and Nutrition Examination Survey (NHANES) was famously affected by an assay reformulation, which made it appear that there was a dramatic decline in vitamin D levels over time. Vitamin D testing is fraught with challenges.

Slide 14: Vitamin D Supplements

Most vitamin D supplements on the market are of the D3 variety. Many concentrations are available depending on the brand and source. Laboratorians do need to be aware that D2 is also available. Largely, this is as a prescription for 50,000 IU which is only available in the US. By only available in the US, that means they can also be obtained over the internet in other countries around the world. As for the amount that should be consumed, there are as many different guidelines beyond the scope of this presentation. Sufficed to state that patients with bone disease are generally accepted to have higher requirements, and that vitamin D taken with calcium has more potent effects on fracture risk reduction. As far as toxicity, there are no reports of adverse effects for daily doses at or below 10,000 IU/d (D2 or D3). Doses of 4000 IU/d for 3 months and 50,000 IU/wk for 2 months have also been administered without toxicity.

Another question about supplementation is whether the D2 or D3 form is more potent. A paper by Holick et al in 2007 showed equivalence using doses of 1000 IU/d. A more recent study in JCEM by Heaney et al in 2011 shows greater potency of vitamin D3. Potency here is defined as higher measurable 25OH in circulation. The difference between the studies was that the more recent one used higher doses of Vitamin than the Holick study. The more recent study was based on giving 50,000 IU of vitamin per week. D2 was given at 50,000 IU once a week, and D3 was given 5 times per week at a dose of 10,000 IU. Another study of children with nutritional rickets, published in the Journal of Bone and Mineral research (2010), suggests that the increase in serum 25OH vitamin is prolonged with D3 supplementation as opposed to D2 supplementation. However, control children did not demonstrate a difference in 25OH vitamin D levels between supplements. Collectively, the evidence points to greater efficacy of D3 supplementation at certain doses and clinical contexts.

Slide 15: Deficiency, Sufficiency, and Toxicity

If you assume that we can agree upon which method to use to measure vitamin D, the clinical targets remain the subject of debate. It is reported that a serum vitamin D level of less than 20 ng/mL or 50 nmol/L indicates deficiency. Concentrations between 30 and 60 ng/ml are considered to indicate sufficiency based on data from immunoassays. Finally, the toxic concentration is considered above 150 ng/mL. Regardless of the vitamin D level, it should be interpreted in the context of another test such as calcium, phosphate, and parathyroid hormone. Ongoing research is likely to further define and refine these intervals.

Slide 16: Summary

In summary, vitamin D has an emerging role in several disease states. People with vitamin D deficiency are at risk for a number of different diseases beyond just osteoporosis. To assess overall vitamin D status, 25-hydroxy vitamin D should be measured. As a laboratorian, it is important to know which test you are using and what it measures, to be aware of issues of standardization, and to employ an appropriate reference interval for your test. Likewise, an awareness of the vitamin D forms and supplements is necessary for test selection and result interpretation.