Article:
Deborah French
The (Sun)Light and Dark of 25-Hydroxyvitamin D Testing.
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Guest: Dr. French is the assistant director of chemistry and director of mass spectrometry at UCSF Health Clinical Laboratories. She is also an associate clinical professor in the Department Laboratory Medicine at the University of California, San Francisco.

Randye Kaye: Hello, and welcome to this edition of "JALM Talk," from *The Journal of Applied Laboratory Medicine*, a publication of the American Association for Clinical Chemistry. I'm your host, Randye Kaye.

The potential benefits of maintaining sufficient vitamin D levels has led to a steady increase in testing of vitamin D status in the general population. This increase has impacted laboratories in a variety of ways, as organizations adapt to growing demand while striving for accuracy and test standardization.

"The (Sun)Light and Dark of 25-Hydroxyvitamin D Testing" was published in the November 2018 issue of *the Journal of Applied Laboratory Medicine*. The Review details some of the analytical issues surrounding vitamin D in laboratory testing. The author is Dr. Deborah French. Dr. French is the assistant director of chemistry and director of mass spectrometry at UCSF Health Clinical Laboratories. She is also an associate clinical professor in the Department Laboratory Medicine at the University of California, San Francisco.

Welcome Dr. French. You mention in your Review that vitamin D has become a hot topic in the past few years. Can you tell us the function of vitamin D?

Dr. French: So, vitamin D plays important roles in the body in terms of promoting calcium absorption from your guts, and this is necessary to maintain adequate serum calcium and serum phosphate concentration. And we need vitamin D in order to maintain normal bone remodeling and bone growth, and if you don't have enough of it, your bones can become brittle or misshapen, and in children is going to lead to rickets and in adults also osteomalacia.

Vitamin D also protects you against osteoporosis, which is obviously important. It is postulated to have other roles in the body, such as modulation of cell growth, neuromuscular and immune functions, and also it's thought to play role in

reducing inflammation. I think the main reason that the profile of vitamin D was raised in the last couple of years in the lay press is because it was implicated or has been implicated in many pathological conditions, such as cancer, multiple sclerosis, and diabetes. However, the evidence is kind of self-conflicting in the literature, as to whether these associations actually exist.

Randye Kaye: So, you need to measure the vitamin D in a patient obviously, but what form of vitamin D should be measured to determine a patient's vitamin D status?

Dr. French: So, we usually measure a patient's 25-hydroxyvitamin D concentration as the first line of testing and that's what we recommend to our physicians. And so, 25-hydroxyvitamin D is the form of vitamin D that circulates in your body. It has a half life of a couple of weeks and it's found in nanogram per ml concentrations in your blood. Contrary to that, the active form of the hormone is 1,25-hydroxyvitamin D and the concentrations of the active form are much lower than 25-hydroxyvitamin D, because they're found in picogram per ml concentrations.

It also has the very short circulating half life of a few hours. And so, if you measure the concentrations of 1,25-hydroxyvitamin D, you're kind of just measuring what's going on in the body of the patient at that particular point in time, and not really getting a good overview of what their overall vitamin D status is. And so, we usually only test for the active form of the hormone in patients that have a normal 25-hydroxyvitamin D but they're showing signs of deficiency, and that can occur in patients with kidney disease, because they can't actually make the active form of the hormone, because in the kidneys, that's where the 25-hydroxyvitamin D is converted to the active form 1,25.

Randye Kaye: That makes sense. What are some of the challenges of developing an accurate immunoassay to measure 25-hydroxyvitamin D?

Dr. French: Yes. There's actually a number of challenges and one of them is that there's two forms of 25-hydroxyvitamin D. So, there's D2 and D3, and really you would like for an immunoassay to detect both forms with a 100% cross reactivity. And if you read the Review, that's not the case. There's also a number of other forms and these include three epi-25-hydroxyvitamin D as well as 24, 25-dihydroxyvitamin D. And these often cross-react with immunoassays and they can give falsely elevated 25-hydroxyvitamin D concentrations.

If you're using electrochromatography tandem mass spectrometry method to measure 25-hydroxyvitamin D, it's

very important that you chromatographically separate 25-hydroxyvitamin D from three epi-25-hydroxyvitamin D before they enter the mass spectrometer, and the reason for that is they're isomers of each other. And so, the mass spectrometer is going to see them as the same thing, and so you will quantify them together and again, the measurement will be inaccurate.

And so, one of the major issues of developing an assay to measure 25-hydroxyvitamin D accurately is that 25-hydroxyvitamin D actually circulates in the body binds of vitamin D, binding protein and in order to accurately measure the concentrations, you need to disrupt this binding.

So, you need to disrupt the binding between 25-hydroxyvitamin D and vitamin D binding protein. With some immunoassays, when you just put the sample straight on the instrument, they do have reagents onboard, the instrument that do take care of that, but it's not always complete, it's not always a 100%.

And then finally, as with any assay, differences in calibration between assays can cause differences in the accurate quantification of 25-hydroxyvitamin D. So, the good news is there is a program available that enables both assay manufacturers or people developing methods to measure 25-hydroxyvitamin D to align the calibration of their assay to a reference measurement procedure, or there's also a standard reference material available, so you can use that to check the calibration of your assay.

So, while a little progress has been made in trying to harmonize methods to measure vitamin D, it's not been fully accomplished yet. And the hard part for clinicians is that when you're trying to determine if your patient is sufficient in vitamin D, there's defined cut-off concentrations and if assay's measure different amounts of vitamin D, which they do currently, then it's hard for the clinician to know if the patient is sufficient or deficient in vitamin D based on the clinical cut-off.

Randye Kaye: Okay. So, that's is a lot of challenges and a lot of things to look out for. So, that's wonderful information. If I could ask you, what is the take home message that you have for the listeners, what would you answer be to that?

Dr. French: Good question, so I think the main point is that all 25-hydroxyvitamin D assays were not created equal, right? So, you need to be aware of the issues that your immunoassay, has I guess. If you are being monitored as a patient, then I would suggest getting your 25-hydroxyvitamin D concentration measured by the same assay each time just

so you can follow. Normally, we're trying to increase our vitamin D levels and so, it's good to follow the increase using the same assay. And also, if you're being monitored for your 25-hydroxyvitamin D level, I wouldn't allow your doctor to order any more than once every six months to a year, because even if you are taking supplements that really doesn't change that fast. I've been taking supplements for maybe four years and my level hasn't changed any in the last two years.

Randye Kaye: Interesting. I take them as well, so that's good to know. All right, awesome. Thank you so much. Thank you for joining us today.

Dr. French: Sure.

Randye Kaye: That was Dr. Deborah French, Associate Clinical Professor in the Department of Laboratory Medicine at the University of California, San Francisco, talking about "The (Sun)Light and Dark of 25-Hydroxyvitamin D Testing" from the November 2018 issue of JALM.

Thanks for tuning in for "JALM Talk." See you next time and don't forget to submit something for us to talk about.