Clinical Chemistry

Accuracy of 6 Routine 25-Hydroxyvitamin D Assays: Influence of Vitamin D Binding Protein Concentration

Bob Barrett: This is the podcast from *'Clinical Chemistry.'* I am Bob Barrett.

Vitamin D is known to play an important role in the prevention of osteoporosis. But recent studies have shown that it has been implicated in prevention of cancer and autoimmune diseases. As a result, clinical laboratories are now receiving numerous requests for measuring 25-hydroxy vitamin D.

Assays for 25-hydroxy vitamin D often involve laborious extraction, reconstitution, and quantification methods. However, several recent studies have sought to automate the measurements of vitamin D.

A major challenge in measuring its concentration is the displacement of 25-hydroxy vitamin D from vitamin D-binding protein. Solvents used to release vitamin D from its binding protein are usually not compatible with most immunoassays or protein-binding assays.

In the March 2012 issue of *'Clinical Chemistry'* Dr. Annemieke Heijboer, a Clinical Chemist and Head of the Endocrine Laboratory of the Department of Clinical Chemistry at the VU University Medical Center in Amsterdam, The Netherlands, and her colleagues, tested the accuracy of the currently available assays by comparing six available routine vitamin D methods to assess the sensitivity to differences in circulating vitamin D-binding protein concentrations.

Dr. Heijboer is our guest in this podcast. Doctor, what makes your comparison study so remarkable?

Dr. Annemieke Heijboer: Well, we made a comparison with all currently available methods for measuring vitamin D, including the second generation assay of Roche which we had the opportunity to use in our comparison before it was commercially available. And I think that's an interesting point about our study but not the most remarkable.

Most method comparisons are made in healthy subject or in a random outpatient population. However, in our comparison we used four groups of different subjects: healthy persons, pregnant women, dialysis patients, and intensive care patients, and I think that's what makes our study so remarkable and interesting.

Bob Barrett: Why did you choose these specific groups for your comparison?

Dr. Annemieke Heijboer: Well, it's widely-known that automated vitamin D assays have some problems, and it's often suggested that one

of the main problems might be vitamin D-binding protein and if you want to measure vitamin D, you first have to get vitamin D off the vitamin D-binding protein.

This can be done effectively using organic solvents, but these can't be used in automated assays. Other options that are compatible with automated assays are less effective, for instance, because of the high turnaround time of the automated assays.

Therefore, it has been suggested that this problem, namely the release of vitamin D from its binding protein, might be a stumbling block in automated assays, but the effect has never been demonstrated and quantified. So to challenge all vitamin D assays we chose these specific groups with increased or decreased concentrations of vitamin D-binding proteins.

Bob Barrett: Were you surprised by the finding that four out of five automated assays were influenced by the concentration of vitamin D-binding protein?

Dr. Annemieke Heijboer: Actually yes. Of course I started the study with this hypothesis, but there are so many interferences that can and will play a role and that influence the data. So after all the work had been done, I was a little bit surprised that the influence of vitamin D-binding protein concentration was so great that a significant influence was seen in four out of five automated assays.

Moreover, in the radioimmunoassay preceded with extraction, there was no effect of vitamin D-binding protein concentration, which was what you would also expect as it's preceded by a thorough extraction.

Bob Barrett: Well, it's great that you quantified this influence of vitamin D-binding protein but what goals do you hope to reach with this study?

Dr. Annemieke Heijboer: Well, I hope and expect that the diagnostic companies realize that this is an important problem of their Vitamin D assays and start working on improving their assays. In the end this will lead to more accurate vitamin D results and to better diagnosis of vitamin D deficiency. With as a consequence that only the vitamin D–deficient persons will be given supplementation and there won't be under-diagnosis. I think that's what's it's all about as it's important to have a sufficient amount of vitamin D.

Bob Barrett: Isn't it possible to diagnose vitamin D deficiency accurately in the general population with the current assays?

Dr. Annemieke Heijboer: Well, we have to look at the percentage of persons that would be sufficient, insufficient, and deficient in every group of persons, and in the healthy persons or what you could call the general population, this differs between assays.

For example depending on the assay used, the percentage of healthy persons being vitamin D-sufficient can range from 30%-60%. These differences are even greater when you look at the pregnant women which you might place under the general population as well.

The percentage of pregnant women being sufficient for vitamin D can range from 25%-65% depending on the assay used.

So to answer your question, no. Even in the general population some of the current assays are not able to diagnose vitamin D deficiency accurately.

Bob Barrett: In your article which is a chemical study, you conclude that authorities should be aware of this issue too. Now will they understand your article, and why should they be aware?

Dr. Annemieke Heijboer: They don't need to understand the analytical part, but I think it is important they are aware of differences between vitamin D assays, differences in quality between assays.

When authorities devise guidelines based on studies, they will have to know where these studies are reliable and from my study it's clear that not all methods are reliable, guidelines should not be based on studies using unreliable methods. And I would like to add that not only authorities, but also doctors should be aware of the method that is used in their hospitals.

Methods might be FDA-approved, but nonetheless inadequate for measuring vitamin D in particular patient groups, for example dialysis patients.

Bob Barrett: Well, finally doctor, what's next? Are you planning more studies about the accuracy of vitamin D assays?

Dr. Annemieke Heijboer: Yes, from the data we got from this study, we saw that more interferences play a role than only the concentration of vitamin D-binding protein. So currently we are studying the influence of other possible interferences and I hope you will be able to read about our new findings soon.

Bob Barrett: Dr. Annemieke Heijboer is a Clinical Chemist and the Head of the Endocrine Laboratory of the Department of Clinical Chemistry at the VU University Medical Center in Amsterdam, The Netherlands. She's been our guest in this podcast from '*Clinical Chemistry.'*

I am Bob Barrett. Thanks for listening!

Total Duration: 7 Minutes