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Case Report

Serum Vitamin D Measurement May Not Reflect What You Give to Your Patients

Etienne Cavalier,[1] A Michael Wallace,[2] Susan Knox,[2] Virginie I Mistretta,[1] Catherine Cormier,[3] and Jean-Claude Souberbielle[4]

The recognized index of vitamin D (VTD) status is the measurement of circulating concentrations of 25-OH VTD (25VTD). A concentration of 30 ng/ml 25VTD (75 nM) is considered by many experts as the minimum optimal concentration.[1] There is currently a growing interest in VTD far beyond bone and calcium metabolism,[2] including cancer, immunology, and hypertension, which has caused a recent upsurge in requests for 25VTD evaluation,[3] necessitating the need for accurate measurement.

We report here the case of a 60-yr-old woman diagnosed as having VTD deficiency (serum 25VTD measured with the automated Roche Elecsys method at 12 ng/ml). She was given a single 600,000U VTD_2 oral dose. Because serum 25VTD measured with the same assay 2 wk later was still low (11 ng/ml), she was referred to our unit for extensive laboratory testing. All biochemical parameters were normal, including 25VTD (50 ng/ml), but this time the Diasorin radioimmunoassay (RIA) was used to quantify 25VTD. To study the cause for these discrepant results further, we conducted measurements of 25VTD by a specific liquid chromatography-mass spectrometry (LC/MS/MS) method. The LC/MS/MS method separates and quantifies 25-hydroxylated metabolites of both VTD_2 and VTD_3, which are summed to get the total 25VTD concentration. The LC/MS/MS is considered by many as the candidate reference method for 25(OH)D measurement,[4] although drawbacks because of the recognition of other compounds such as epimers have been highlighted, especially in pediatric subjects.[5,6] In addition to the index case, all three methods were used to measure 25VTD in serum collected from 11 healthy subjects (5 men and 6 women; age, 21–62 yr) before (D0) and 7 and 28 days after a single 600,000U VTD_2 dose to mimic the above-mentioned case. Pooling the results from the three time-points, we found that the LC/MS/MS results were highly correlated with the RIA values (Spearman's $\rho = 0.94$; $p < 0.0001$) but not with the Elecsys values ($\rho = 0.16$; not significant).

On day 0, the mean concentration [SD] was similar with the three assays (Diasorin RIA: 29.3 [6.8] ng/ml; Roche Elecsys: 30.2 [6.0] ng/ml; LC/MS/MS: 27.8 [6.0] ng/liter). At day 7, 25VTD increased similarly when measured by the Diasorin RIA and LC/MS/MS assays (77.5 [22.2] and 78.4 [22.8] ng/ml, respectively) but decreased (to 27.4 [5.4] ng/ml) with the Roche Elecsys assay. All subjects had a 25VTD concentration >30 ng/ml with LC/MS/MS and the Diasorin RIA, whereas this was the case in only two of them with the Elecsys. At day 28, 25VTD remained >30 ng/ml in all subjects when measurements were conducted by Diasorin RIA (52.0 [20.3] ng/ml) and LC/MS/MS (52.8 [8.5] ng/ml), whereas it was <30 ng/ml (21.4 [4.9] ng/ml) in all subjects with the Elecsys assay (Fig. 1). The LC/MS/MS data confirmed that the increases observed were solely caused by an increase in the $25VTD_2$ metabolite.

The supplementation with 600,000 IU of VTD_2 did not produce a significant rise in calcium and phosphorus levels (2.35, 2.35, and 2.39 mM, respectively, for day 0, 7, and 28 median calcium levels and 1.07, 1.05, and 1.06 mM, respectively, for phosphorus concentrations at the same times). We did not observe any significant variation in parathormone levels (41 versus 44 pg/ml before and after 28 days, respectively).

Whereas skin exposure to UVB produces VTD_3 and the food sources of VTD are mainly VTD_3, supplementation is still often made with VTD_2, especially in the United States. Several experts recommend exclusive use of VTD_3,[7] because it has been reported that VTD_3 maintains an adequate 25VTD concentration for a longer period than VTD_2.[8] This recommendation has been recently challenged,[9] and the choice of the best vitamin D supplement requires further study. Thus, as long as VTD_2 is available (and prescribed), it is mandatory to measure 25VTD with a method that recognizes both $25VTD_2$ and $25VTD_3$. This is the situation if LC/MS/MS or the Diasorin RIA is used, whereas the Roche Elecsys assay exclusively measures $25VTD_3$. The case briefly described above shows that measuring 25VTD with an assay exclusively specific for $25VTD_3$, such as the Roche Elecsys assay, underestimates VTD status in patients supplemented with VTD_2. This can potentially cause overtreatment, leading to further expensive and stressful studies.

The authors state that they have no conflicts of interest.

[1]Department of Clinical Chemistry, University Hospital of Liege, University of Liege, Liege, Belgium; [2]Department of Clinical Biochemistry, Glasgow Royal Infirmary, Glasgow, United Kingdom; [3]Service of Rheumatology, Hopital Cochin, Paris, France; [4]Université Paris Descartes, Inserm U845, and, Hôpital Necker, Service d'Explorations Fonctionnelles, AP-HP, Paris, France.

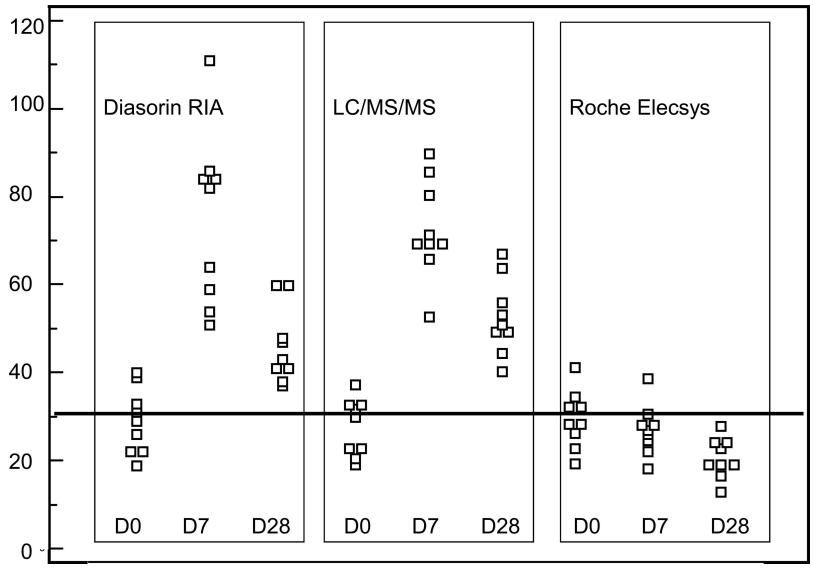

FIG. 1. 25VTD results obtained with the three methods (Diasorin RIA, LC/MS/MS, and Roche Elecsys) largely used worldwide in 11 healthy volunteers before and after a single oral dose of 600,000 IU of VTD_2. Before taking VTD_2 (day 0), the subjects were classified similarly with the three methods with regard to the 30 ng/ml (75 nM) cut-off concentration, below which vitamin D insufficiency is diagnosed (horizontal line). By contrast, after 7 and 28 days, the rise in 25-OH VTD was only observed with the Diasorin RIA method, because of nonrecognition of 25-OH VTD_2 by the Roche Elecsys method (confirmed by specific LC/MS/MS analysis). The consequence was that most subjects were considered vitamin D insufficient with the Roche Elecsys assay, whereas they clearly had a normal concentration when measured by the Diasorin RIA.

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Address reprint requests to:
E Cavalier, PhD
Department of Clinical Chemistry
University of Liege
Liege 4000, Belgium
E-mail: etienne.cavalier@chu.ulg.ac.be

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