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Short communication

The prevalence of vitamin deficiency in clinical practice is assay-dependent

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SUMMARY

Background & aim: Vitamin D deficiency is an important concern in clinical settings and recently, international agencies have recognised the importance of 25-OHD assays in defining vitamin D status. Thus, our aim was to assess the consistency of different vitamin D assays in clinical practice. Methods: 25-OH-vitamin D was measured in 332 patients by ultra-fast liquid chromatography (UHPLC) and two immunoassays (Liaison Total 25(OH) and ADVIA Centaur Vitamin D Total Assay). Samples from the Vitamin D External Quality Survey (DEQAS) and the Standard Reference Material SRM 972 were used for analytical quality control. Results: All methods displayed an acceptable performance with DEQAS samples but immunoassays showed a significant bias against certified materials. Compared to UHPLC, differences were significant for both immunoassays in the deficiency interval but the systematic bias was higher for the ADVIA assay throughout the whole range of concentrations. Conclusion: The prevalence of vitamin D deficiency in clinical practice is assay-dependent and physicians should be aware of the uncertainty associated with vitamin D assessment.

1. Introduction

Vitamin D is a fat-soluble vitamin that is essential for maintaining normal calcium metabolism, and it can be found in two forms (D_3 (cholecalciferol) and D_2 (ergocalciferol)). Vitamin D itself is biologically inactive and, in the liver, both forms (D_3 and D_2) are rapidly hydroxylated to form 25-hydroxy-vitamin D_3 and D_2, respectively. This metabolite is further hydroxylated to the biologically active form, 1,25-dihydroxy-vitamin D_3 ($1,25(OH)_2D_3$) or 1,25-dihydroxyvitamin D_2 ($1,25(OH)_2D_2$) mainly in the kidney but also in other tissues. 25-hydroxy-vitamin D constitutes the major circulating form of vitamin D in serum and it is widely considered the best indicator of vitamin D nutritional status.[1,2]

Vitamin D deficiency is an important concern in clinical settings as hypovitaminosis D has been associated with important short- and long-term health effects, including rickets, osteomalacia and the risk of osteoporosis. Additionally, vitamin D status has been associated with common chronic diseases such as diabetes, cardiovascular conditions, cancer and autoinmune disorders, although for these conditions the evidence is still inconsistent, inconclusive as to causality, and insufficient to inform nutritional requirements.[3] However, at present, there is no consensus on the optimum reference intervals for 25-OH-vitamin D to classify patients with moderate to severe vitamin D deficiency.[4,5]

Recently, the UK Food Standards Agency and the US NIH Office of Dietary Supplements have recognised the importance of 25-OHD assays in defining vitamin D status.[6,7] Due to the exponential demand for this analysis, novel immunoassays suitable for integrated auto-analyzers in high-volume workload clinical labs have been developed, although little is known about its performance in the clinical routine. In this study, we approach the measurement of 25-OH-vitamin D using simultaneously three methods to evaluate their analytical performance and their adequacy for the management of patients in clinical practice.

2. Materials and methods

2.1. Serum samples

We assessed the status of 25-OH-vitamin D in 332 patients (mostly outpatients) living in the Health Area VI of Madrid (Spain) during the period March–April 2011. Samples were not selected for any criteria and were processed according to routine pre-analytical procedures in the hospital. To avoid chromatographic interferences,

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blood samples are drawn into plain tubes with no anticoagulants or separating gel, centrifuged and the serum frozen until analysis (<1 week).

2.2. Methods of analysis

25-OH-vitamin D was simultaneously determined by two immunoassays (Diasorin Liaison 25(OH) Vitamin D Total Assay and ADVIA Centaur Vitamin D Total Assay (Siemens Healthcare Diagnostics)) and an ultra-fast chromatographic method (UPLC, with UV–VIS detection).[8] Both immunoassays provide values for total 25-OH-vitamin D (the sum of 25-OH-vitamin D_3 and 25-OH-vitamin D_2; reactivity >95% for 25-OH-vitamin D_3 and D_2 but null cross-reactivity for the C-3 epimer (C3-epi-25-OH-vitamin D_3), as stated in the corresponding inserts). Under the chromatographic conditions used, 25-OH-vitamin D_3 and D_2 may be resolved although the C3-epi-25-vitamin D_3 coelutes with 25-OH-vitamin D_3. The methods displayed an adequate imprecision (CV < 10%) and a good linearity over the physiological range (<4–150 ng/ml) as specified by the manufacturer and published elsewhere.[8]

2.3. Quality control study

The performance of the methods was tested using samples from an international quality control programme (i.e. Vitamin D External Quality Assessment Scheme -DEQAS, January 2011 distribution cycle; Charing Cross Hospital, London, UK) and certified materials (Standard Reference Material SRM 972 (NIST, USA)). This material provides four vials with certified concentrations of 25-OH-vitamin D_3, 25-OH-vitamin D_2 and C3 epi-25-OH-vitamin D_3. Samples from DEQAS and SRM 972 were transferred to "blinded" vials and distributed for analysis on the same day. Additionally, serum pools were prepared to test the imprecision over time (six consecutive days).

Because of immunoassays quantified total 25-OH-vitamin D (i.e. 25-OH-D_3 plus 25-OH-vitamin D_2), comparisons with UPLC data were made using the sum of both vitamers (including the C-3 epimer) except otherwise stated (Table 1, footnote). Also, since the assigned value in the DEQAS may be somehow biased to the mean of the methods most frequently used,[5] performance of the UPLC was also compared with the "method mean" of the HPLC users (as provided by DEQAS). For comparisons, in the present study, the "reference" method was selected based on the overall performance of each assay against the Standard Reference Material SRM 972, DEQAS samples and in-house control.

2.4. 25-OH-vitamin D cut-offs

Definition of deficiency based on 25-OH-vitamin D concentrations is controversial. To assess the (mis)classification of the patients according to each method, we followed the categories used by the Institute of Medicine[9] to define the vitamin D status; <12 ng/mL (<25 nmol/l) (risk of deficiency relative to bone health; 12–20 ng/mL (25–50 nmol/l), some but not all persons at risk of deficiency; >20 ng/mL (>50 nmol/l), all persons as sufficient; >50 ng/mL (>125 nmol/l), some level of concern regarding safety). Additionally, considering the uncertainty associated with the measurement,[10] we also assessed the vitamin D status by correcting the sufficiency cut-off (>20 ng/mL) for the maximum analytical bias allowed to meet the proficiency target in the DEQAS (at present, ±25% from the Average Trimmed Laboratory Mean (ATLM)). Therefore, this cut-off was re-calculated [serum levels < 15 ng/ml (20 ng/ml − 25%) (37.5 nmol/l)] and interpreted in terms of practical "discriminant" concentrations below which subjects could be classified as "not sufficient"(insufficiency) with the least analytical uncertainty.

2.5. Statistics

We performed the statistical analysis excluding the most discordant results, i.e. excluding 5% of the samples corresponding to the extreme differences observed between each pair of methods ($n = 14$). Thus, a total of 318 samples were finally included in the statistical analysis. To compare the methods, paired T test for all samples and according to the categories of vitamin D status, and Pearson correlation coefficient were calculated. For clinical

Table 1
Performance of the methods with quality control samples, certified materials and in-house controls.

DEQAS[a]	UPLC		ADVIA	LIAISON	"Assigned Value" (ng/mL)	
	Bias from ATLM	Bias from method mean-HPLC	Bias from ATLM	Bias from ATLM	ATLM[a]	Method mean-HPLC[b]
Sample 386	+6%	+5%	+23%	−33%	12.5	12.8
Sample 387	+8%	0%	−2%	−8%	23.3	25.3
Sample 388	+15%	−1%	−5%	−12%	29.7	34.7
Sample 389	+23%	+23%	+13%	+2%	20.5	20.4
Standard reference material (SRM 972)[c]					Certified concentrations (ng/mL)	
Level 1 (ng/mL)	25-OH-D_3 → 25.8 (+4%)		18.8 (−20%)	20.5 (−12%)	25-OH-D_3 → 23.9 ± 0.8	
Level 2 (ng/mL)	25-OH-D_3 → 11.4 (−3%)[d]		17.3 (+18%)	16.5 (+12%)	25-OH-D_2 → 1.71 ± 0.08 25-OH-D_3 → 12.3 ± 0.6	
Level 3 (ng/mL)	25-OH-D_2 → 20.0 (−18%) 25-OH-D_3 → 18.7 (0%)		42.2 (0%)	30.7 (−26%)	25-OH-D_2 → 26.4 ± 2.0 25-OH-D_3 → 18.5 ± 1.1	
Level 4 (ng/mL)	25-OH-D_3 → 70.3 (0%) [d,e]		28.9 (−17%)	26.0 (−26%)	25-OH-D_2 → 2.40 ± 0.21 25-OH-D_3 → 33.0 ± 0.8 C3-epi-25-OH-D_3 → 37.7 ± 1.2	
Imprecision (ng/mL)[f] (mean, SD (var. coef.))	19.0 ± 1.3 (6.9%)		19.7 ± 2.0 (10.2%)	20.2 ± 3.7 (17.8)	–	

Values expressed in ng/mL (×2.5 = nmol/L). Number in italics means Out of performance target (± 25% ATLM).

[a] Vitamin D external quality assurance survey (DEQAS). ATLM refers to the average trimmed laboratory Mean. All values correspond to total 25-OH-vitamin D, except otherwise stated. Serum 390 was excluded from the final performance analysis (DEQAS).

[b] Method mean for all labs using chromatographic methods (LC-UV, including UPLC).

[c] Values between brackets refer to the estimated bias from the certified range for 25-OH-vitamin D_3 or total 25-OH-vitamin D (for immunological assays). For level 4, bias of immunological assays was estimated excluding the value for C3-epi-25-OH-vitamin D_3 (assuming a null reactivity for this compound).

[d] 25-OH-D_2 below the limit of quantitation (<3 ng/ml).

[e] Under the UPLC conditions used, 25-OH-D_3 and C3-epi-vitamin D_3 coelute and thus, values correspond to the sum of both compounds.

[f] Serum pool assayed on 6 consecutive days.