Editorial

Markus Herrmann

Towards a personalized assessment of vitamin D status

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The growing interest in vitamin D has stimulated intensive research activities aiming to address unresolved analytical, clinical and physiological aspects of vitamin D [1–4]. This work has led to an increasing awareness that our knowledge about vitamin D metabolism and its assessment in clinical practice harbours substantial limitations. For example, Blacks have a markedly lower average 25(OH)D concentration than Whites [2, 5, 6], but exhibit higher bone mineral density (BMD) and a lower risk of fragility fracture [7–9]. Also, the relationship between 25(OH)D and parathyroid hormone (PTH) seems to differ between races [2]. These findings have led researchers to look for other markers that are capable of providing more accurate information about the adequacy of patients' vitamin D supply. Several studies suggested that free and bioavailable 25(OH)D reflect vitamin D metabolism better than 25(OH)D [2, 10, 11]. However, both markers require the measurement of vitamin D binding protein (VDBP). Early studies quantified VDBP with either monoclonal or polyclonal immunoassays. However, later studies that employed LC-MS/MS based methods have demonstrated that these immunoassay are strongly biased due to common genetic polymorphisms [4]. The limited number of laboratories that offer VDBP measurement by LC-MS/MS and the lack of a reference measurement procedure hamper a wider use of free and bioavailable 25(OH)D in clinical studies. Another potential surrogate marker of vitamin D metabolism is 24,25$(OH)_2$D, the major product of 25(OH)D catabolism. The circulating concentrations of both metabolites are strongly correlated [12] and can reliably be measured by LC-MS/MS [13–16]. The simultaneous quantitation of 24,25$(OH)_2$D and 25(OH)D has been proposed as a dynamic measure of vitamin D metabolism that allows distinguishing CYP24A1 deficiency from vitamin D intoxication and granulomatous disease. However, the interpretation of 25(OH)D and 24,25$(OH)_2$D results is still a matter of intensive debate. Previous studies have established reference intervals [17, 18] and clinical cut-offs [19–22]. However, the close relationship between 25(OH)D and 24,25$(OH)_2$D implies that a meaningful interpretation is only possible when both metabolites are considered together. This has led to the idea of a ratio between 24,25$(OH)_2$D and 25(OH)D, also known as vitamin D metabolite ratio (VMR) [23]. Theoretically, a higher VMR indicates better supply with vitamin D so that excessive 25(OH)D is catabolized to 24,25$(OH)_2$D. Several studies have investigated the clinical utility of VMR, but results are inconclusive [3, 24–26]. In addition, the VMR cannot be calculated when 24,25$(OH)_2$D is below the limit of quantitation. When one measurand has a much lower concentration than the other, calculating the ratio between the two enhances the intrinsic measurement uncertainty. In this issue of *Clinical Chemistry and Laboratory Medicine* (*CCLM*) a study by Cavalier et al. has analyzed 24,25$(OH)_2$D and 25(OH)D simultaneously in 1200 samples from children, adolescents and young adults [27]. Instead of calculating the VMR the authors propose to compare the 24,25$(OH)_2$D and 25(OH)D concentrations of patients with those of healthy subjects classified according to their 25(OH)D concentration. They assume that a low or undetectable 24,25$(OH)_2$D concentration has a different meaning in the context of high or low 25(OH)D. Theoretically, a vitamin D-deficient patient cannot afford to waste 25(OH)D and CYP24A1 is down regulated. Consequently, little or no 24,25$(OH)_2$D is produced. According to Cavalier et al., with lower 25(OH)D concentrations undetectable 24,25$(OH)_2$D concentrations are increasingly likely and most probably indicate functional vitamin D deficiency. In turn, when 25(OH)D is high, the organism aims to protect itself against hypercalcemia by eliminating excessive amounts of 25(OH)D through 24-hydroxylation. As a result, undetectable 24,25$(OH)_2$D concentrations are highly unlikely in this context and would rather suggest an enzyme defect than vitamin D deficiency. Cavalier et al. suggest that in clinical practice the concentrations of 24,25$(OH)_2$D and 25(OH)D should be reported together with the probability that this constellation occurs in healthy subjects. This information would help physicians judging their patients' metabolic status in a more dynamic fashion and leave the historical concept of vitamin D deficiency on the basis of a universal 25(OH)D cut-off [19–22]. With the established 25(OH)D cut-offs a large portion of the population has vitamin D deficiency or at least insufficiency,

which, in many cases, would trigger vitamin D supplementation even in the absence of risk factors for metabolic bone disease or manifest osteoporosis [20, 28, 29]. Although robust evidence is lacking, some clinicians and researchers believe that every person has an individual set point above which vitamin D supplementation has no beneficial effects. In many persons this set point is probably below the commonly used cut-off of 50 nmol/L for sufficiency [28]. The interpretation of $24,25(OH)_2D$ and 25(OH)D proposed by Cavalier et al. allows an individual evaluation of patients' metabolic situation.

The data presented by Cavalier show that amongst individuals with a 25(OH)D concentration above 52 nmol/L over 99% exhibit detectable amounts of $24,25(OH)_2D$ and thus are probably vitamin D sufficient. This finding supports the 50 nmol/L cut-off recommended by the IOF [30]. Finally, this study represents a valuable data set that provides a robust overview about the vitamin D status in Belgian infants, children, adolescents and adults. Of note, more than 50% of these individuals have 25(OH)D concentrations <50 nmol/L. This finding is critical as vitamin D deficiency at this age may interfere with bone growth and mineralization. A particular strength of this study is that analyses have been performed with a VDSP certified LC-MS/MS method.

In summary, the study by Cavalier et al. is a nice example of personalized medicine and may trigger similar approaches for other analytes, such as B-vitamins and homocysteine. It also highlights the valuable contribution that laboratory doctors can provide for patient care.

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Prof. Dr. med. habil. FRCPA Markus Herrmann, Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz, Auenbruggerplatz 15/1, 8036 Graz, Austria, Phone: +43 (0) 316 385 131 45, Fax: +43 (0) 316 385 134 30, E-mail: markus.herrmann@medunigraz.at