criteria of eligibility for authorship: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; (c) final approval of the published article; and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved. Nobody who qualifies for authorship has been omitted from the list.

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Commentary on Understanding Elevated Vitamin D Measurements to Uncover Hypercalcemia Etiology

Etienne Cavalier [a,*] and Jean-Claude Souberbielle[b]

Sajid et al. perfectly illustrate in this report the challenges associated with immunoassays for 25(OH)D and/or $1,25(OH)_2D$ quantification. Their report showcases the exploration of severe hypercalcemia associated with acute kidney injury, suppressed PTH, and markedly elevated 25(OH)D and $1,25(OH)_2D$ concentrations measured via immunoassay. When the diagnosis of vitamin D intoxication was initially made due to the elevated 25(OH)D concentration, it did not fit with the elevated $1,25(OH)_2D$ level. The authors thus explored the possibility of granulomatous disease (usually characterized by increased $1,25(OH)_2D$ but normal or high-normal 25(OH)D) or inactivating variants in the CYP24A1 gene (usually characterized by high-normal or slightly increased 25(OH)D and $1,25(OH)_2D$ and a decreased $24,25(OH)_2D$/25(OH)D ratio) without success. They also treated the patient with ketoconazole, which might have been detrimental. As a final option, they opted for a multiplex LC-MS/MS approach for the measurement of vitamin D metabolites, which showed elevated 25(OH)D and $24,25(OH)_2D$ concentrations, as expected in vitamin D intoxication, but normal $1,25(OH)_2D$ concentrations, which is also expected in vitamin D intoxication but contradicted the results of the immunoassay. Notably, they also found an elevated $1,24,25(OH)_3D$ concentration. Based on that pattern, vitamin D intoxication was retained as the final diagnosis and the diagnostic confusion explained by an interference in the $1,25(OH)_2D$ immunoassay, attributed to a

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probable cross-reactivity with $1,24,25(OH)_3D$ (this information is not displayed in the manufacturer's instructions for use). This report confirms the superiority of LC-MS/MS over immunoassays for the measurement of vitamin D metabolites and emphasizes the role of $1,24,25(OH)_3D$ in vitamin D intoxication. Indeed, this metabolite can also actively bind the vitamin D receptor with approximately 50% of the affinity of $1,25(OH)_2D$ and can cause hypercalcemia (1). Finally, this report raises concerns about the reliability of "unregulated" vitamin D supplements, which potentially do not contain the claimed vitamin D content, advocating for the use of pharmaceutical-grade vitamin D supplements (2).

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Commentary on Understanding Elevated Vitamin D Measurements to Uncover Hypercalcemia Etiology

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This very interesting case provides excellent learning opportunities in relation to vitamin D metabolism and clinical decision-making. It highlights the enormous amount of investigational data that diagnosticians can access in modern healthcare and the fact that sometimes making a working diagnosis is simpler when less information is at hand.

The patient presented with symptomatic hypercalcemia, not driven by parathyroid hormone excess. In the absence of hyperthyroidism or biochemical evidence of a plasma cell dyscrasia, the raised 25-hydroxyvitamin D in a person taking a vitamin D supplement suggested the correct diagnosis: hypervitaminosis D. The complicating factor here related to the unexpected finding of a significantly raised 1,25-dihydroxyvitamin D concentration on immunoassay. This measurand is typically found to be low, normal, or only slightly elevated in hypervitaminosis D (1).

Finding a significantly abnormal and unexpected result triggered further testing, and, in this instance, the patient was investigated with plain radiography, computed tomography imaging, and positron emission tomography along with specialized biochemical and immunological testing. Eventually, LC-MS/MS testing revealed that the highly abnormal 1,25-dihydroxyvitamin D result was spurious, probably relating to assay cross-reactivity with other vitamin D metabolites.

In the complex modern world of medical diagnostics, it is often refreshing to go back to basics and the idea of Occam's razor, which, loosely speaking, dictates that a simple explanation for a particular situation is often the most satisfactory explanation. In instances where detailed investigations are required, there is a tendency among requestors of laboratory tests to view the output from laboratories as infallible. In such instances, when laboratory results deviate significantly from what is expected, requestors and laboratorians should have a high index of suspicion

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