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LETTER TO THE EDITOR

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Letter to Editor in response to the article "Vitamin D insufficiency as a potential culprit in critical COVID-19 patients"

To The Editor,

We carefully read the meta-analysis article on the association between serum vitamin D status and outcome of COVID-19 by Munshi et al.[1] and would like to make a technical critique about the pooled estimation of serum vitamin D level. Munshi et al.[1] have enrolled six articles that are heterogeneous in vitamin D measurement methods as follows.

Article reference **No.8:** Chemiluminescence Immunoassay (CLIA); article reference **No.16:** Electrochemiluminescence (ECL); article reference **No.19:** Liquid Chromatography Tandem Mass-Spectrometry (LC-MS/MS); article reference **No.20:** is an author's reply to a letter to the editor and have no experimental data; article reference **No.21:** is a letter to editor and has no any information regarding the method of vitamin D assay; article reference **No.22:** Enzyme-Linked Fluorescent Assay (ELFA).[1] In this regard, we believe that all the aforementioned articles are not eligible for estimation of pooled serum vitamin D level.

Of note, two major metabolites of vitamin D, that is, 25OH-Vit D3 and D2 are being considered as markers for evaluating vitamin D status in serum.[2] To measure these metabolites in clinical settings, various methods are in use, most notably immunoassay-based methods such as Enzyme-Linked Immunosorbent Assay, ELFA,

TABLE 1 Advantages and disadvantages of different 25 OH Vitamin D measurement methods

	Advantages	Disadvantages
CPBA	✓ Inexpensive ✓ Can be performed on a small sample size ✓ Co-specific for 25OH-Vit D2 and D3.[3]	– Underestimates 25OH-Vit D3 at low levels and overestimates it at high levels. – Poor reproducibility. – Sensitive to nonspecific interfering substances. – Instability of the binding proteins.[3,4]
RIA	✓ Inexpensive ✓ Less susceptible to nonspecific interference ✓ Accurate.[3]	– Requires the use of radionuclides. – Some RIAs are not able to detect both 25OH-Vit D2 and D3 equally.[4]
ELISA and ELFA	✓ Inexpensive ✓ Acceptable precision, accuracy, sensitivity, and specificity.[5]	– Some ELISA/ELFA kits are not able to detect both 25OH-Vit D2 and D3 equally and may underestimate the 25OH-Vit D2. – Interferences due to matrix effect.[5]
CLIA and ECL	✓ It is a sensitive and specific method.[6]	– In some cases, it has acceptable performance in healthy individuals and in vitamin D3-supplemented patients, but the performance is unacceptable in patients who receive vitamin D2 -supplements. – In some cases it overestimates the circulating 25OH-Vit D3 concentrations.[6]
HPLC	✓ Can detect 25OH-Vit D2 and D3 separately. ✓ Much evidence for the precision and accuracy of the test. [4,7]	– Time consuming and low throughput. – Needs an expert technician. – Sometimes assay is subject to interference.
LC-MS/MS	✓ Can detect 25OH-Vit D2 and D3 separately. ✓ Minimizes the interferences and matrix effects. ✓ It is considered as a gold-standard method. ✓ High sensitivity, specificity and repeatability.[3,7,8]	– Time-consuming and low throughput.[3,9] – A common problem with LC-MS/MS is its relative inability to discriminate between 25OH-Vit D3 and its inactive isomer 3-epi-25OH-Vit D3 which causes overestimation of total concentration of vitamin D. [3,7,9]

Abbreviations: CLIA, chemiluminescence immunoassay; CPBA, competitive protein binding assay; ECL, electrochemiluminescence; ELFA, enzyme-linked fluorescent assay; ELISA, enzyme-linked immunosorbent assay; HPLC, high-performance liquid chromatography; LC-MS/MS, liquid chromatography tandem mass-spectrometry; RIA, radioimmunoassay.

radioimmunoassay, CLIA, and ECL along with Competitive Protein Binding Assay, High-Performance Liquid Chromatography, and LC-MS/MS.[3] The advantages and disadvantages of these methods are reviewed in Table 1. Considering the differences in principle of measurement and extraction method, inconsistency between the results is expectable[3] which leads to remarkable difficulties in interpretation of the clinical data and decision making. Therefore, it can be inferred that various vitamin D measurement methods may affect the result outcomes for investigation in both clinical practice and basic research.

Obviously, choosing an inappropriate method for vitamin D measurement can lead to misclassification of the disease status and advising a wrong treatment strategy. As a consequence, clinicians should be aware of the limitations and possible differences in result interpretation between different methods before ordering the vitamin D test.

One suggestion for overcoming the vitamin D measurement challenges is to monitor vitamin D status in different stages of the disease in the same laboratory. Moreover, the variations in the results of vitamin D assays have been decreased using the vitamin D standardization-certification program (VDSCP), under the authority of the Centers for Disease Control and Prevention (CDC) that evaluates the accuracy and reliability of vitamin D tests using well-established methods. This program certifies those methods that have bias and CV equal to or less than 5% and 10%, respectively. Thus, it is recommended to use commercially available vitamin D assay kits that meet CDC's analytical performance criteria. The list of VDSCP certified participants is available from https://www.cdc.gov/labstandards/vdscp_participants.html.

In conclusion, there are some significant differences in the vitamin D measurement results obtained by various methods. It is suggested that Munshi et al.[1] should consider the advantages and disadvantages of each method and re-evaluate the interpretation of the results based on such differences.

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