Practical Laboratory Medicine 2 (2015) 1–14

Contents lists available at ScienceDirect

Practical Laboratory Medicine

journal homepage: www.elsevier.com/locate/plabm

Measurement of circulating 25-hydroxyvitamin D: A historical review

C. Le Goff [a], E. Cavalier [a], J.-C. Souberbielle [b], A. González-Antuña [a], E. Delvin [c,*]

[a] Service de Chimie Clinique, CHU de Liège, Belgium
[b] Service des Explorations Fonctionnelles Hôpital Necker-Enfants Malades, Assistance Publique-Hôpitaux de Paris (AP-HP), Paris, France
[c] Centre de Recherche, CHU Sainte-Justine, 3175 Côte Sainte-Catherine, Montréal, Québec, Canada H3T 1C5

ARTICLE INFO

Article history:
Received 22 January 2015
Received in revised form
24 March 2015
Accepted 16 April 2015
Available online 12 May 2015

Keywords:
Vitamin D
25-Hydroxycholecalciferol
25-Hydroxyergocalciferol
HPLC
Mass spectrometry
Immunoassays

ABSTRACT

The constantly increasing requests for the measurement of serum 25-hydroxyvitamin D over the last years has led reagent manufacturers to market different automated and semi-automated methods, that being unfortunately not fully harmonized, yield different results. Liquid chromatography coupled to tandem mass spectrometry (LC/MS^2) has more recently been introduced. This approach allows the distinction between the two forms of 25-hydroxyvitamin D and to measure other metabolites. This approach also requires harmonization to curtail the differences between the different analytical methods. To meet this requirement, the American National Institutes of Health (NIH), the Centre for Disease Control and Prevention (CDC) in Atlanta, the National Institute of Standards and Technology (NIST) and the vitamin D Reference laboratory of Ghent University have pooled their expertise to develop a standardization program.

This article reviews the main elements and the difficulties of the automated and semi-automated methods for 25-hydroxyvitamin D, from sample preparation to the analytical phase, as well as those related to mass spectrometry. It also emphasizes the need for standardization to better define the clinical decision thresholds of vitamin D nutritional status.

Contents

* Corresponding author. Tel.: +1 514 345 4931x6268.
E-mail address: delvine@sympatico.ca (E. Delvin).

http://dx.doi.org/10.1016/j.plabm.2015.04.001

1. Introduction

The role of cholecalciferol or vitamin D_3 in growth and bone metabolism is well established [1]. Its effects in the prevention and treatment of diseases as varied as diabetes, multiple sclerosis and cancer have also been reported, but are still matter of debate [2–6]. Both the Institute of Medicine (IoM) [7] and the Agency for Healthcare Research and Quality (AHRQ) [8] have published extensive documents dampening the optimism aroused by these reports. The AHRQ report [8] makes the case that studies (observational, randomised controlled interventions) and systematic reviews or meta-analyses based on those, involved different types of assays that, except for the most recently published, did not use appropriate reference material. It also shows, as a series of bubble plots, that there was an important variation in responses to vitamin D supplementation (Fig. 1). This apparent variation is multifactorial. The individual response to sun exposure and the formulation of the vitamin D supplement are parts of the equation. However, inter-laboratory variations also contribute to this observation as they hinder comparison between results. Indeed, the inter-laboratory differences between the mean serum 25-hydroxyvitamin D (25OHD) values, that reached almost 32%, according to a Vitamin **D** **E**xternal **Q**uality **A**ssessment **S**cheme (DEQAS) survey in 1994, could have lead to misclassification of patients in terms of vitamin D nutritional status. Since then, the standardization process has improved, and in 2009, the inter-laboratory imprecision had dramatically decreased [9], and thus if similar experiments were conducted today, the vitamin D dose-response relationship should be stricter. However at the present time, the observed wide-spread difference in circulating 25OHD concentrations restrain the conclusions of past epidemiological studies on the circulating 25OHD concentrations required for optimal health status, and confuse the efforts in developing international evidence-based public health guidelines. To solve this challenge, the NIH Office of Dietary Supplements (ODS), jointly with the Center for Disease Control (CDC) National Center for Environmental Health (NCEH), the National Institute of Standards and Technology (NIST) and Ghent University, established in 2010 the Vitamin D Standardization Program (VDSP) with the main goal of promoting consistency in the methods for the measurement of 25OHD [10]. This consortium is thus advocating, based on the recommendations of Stöckl et al. [11], an imprecision (CV) of $\leq 10\%$ and a bias $\leq 5\%$ as current goals for the analytical performance of vitamin D assays in routine clinical laboratories [12]. This initiative has resolved the imprecision issue. However, the trueness or accuracy although improved, remains a work in progress.

As it has often been mentioned, the number requests for the measurement of circulating 25OHD, the accepted biomarker for the vitamin D nutritional status [13,14], has constantly increased over the last 3 decades, imposing structural and financial burdens on laboratory facilities and public funding. The Ontario Health Technology Advisory Committee (OHTAC) has reported that, the volume of laboratory vitamin D tests had increased from approximately 30,000 in 2004 to over 730,000 in 2009 [15]. Similar observations were made worldwide. This increased request load has lead most of the clinical laboratories to abandon manual binding-protein assays and radio-immunoassays (RIAs), the methods mostly utilized clinical laboratories in the 1980s and early 1990s, in favor of automated competitive binding-protein assays (CBPA), enzyme-linked immunoassays (ELISAs) or chemiluminescent immunoassays (CLIA). Techniques based on high-performance liquid chromatography (HPLC), coupled or not tandem mass spectrometry (LCMS-MS), while more exact,

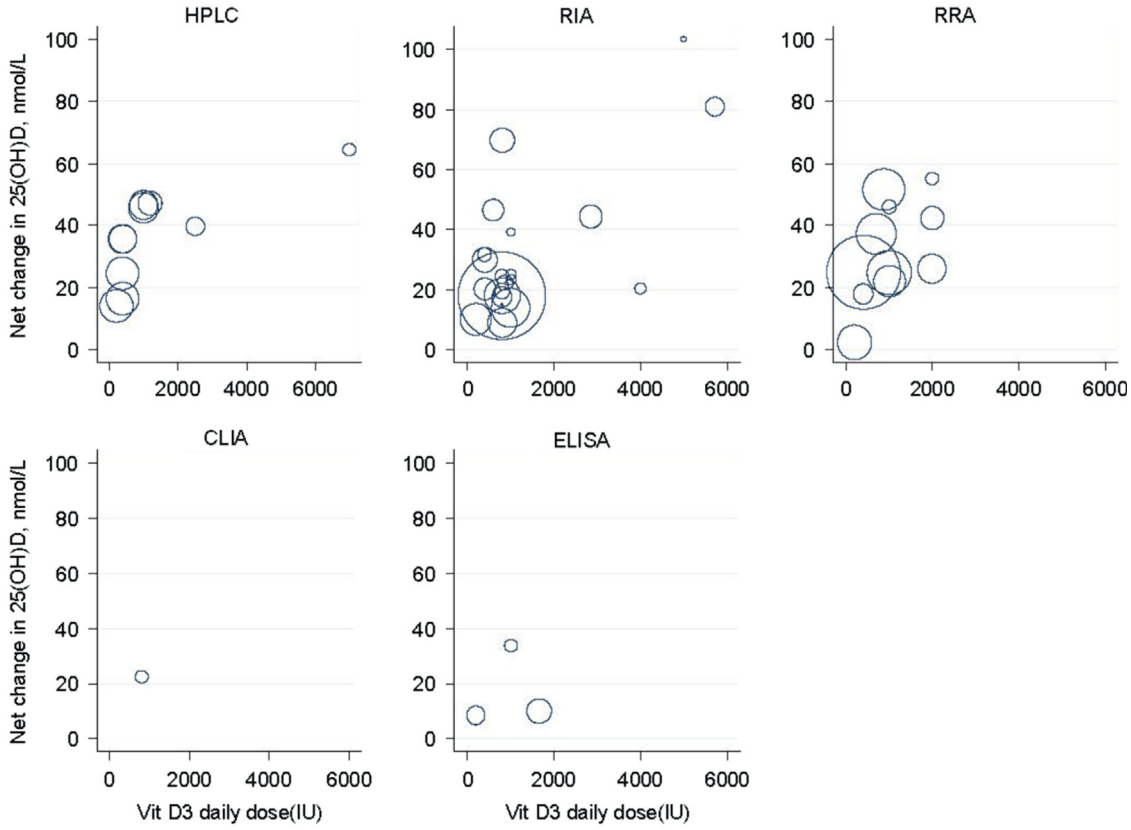

Fig. 1. Relationship between doses of vitamin D_3 supplementation and net changes in serum 25OHD concentrations in RCTs by assay type. *Legends*: Each empty circle represents one study. The area of the circle is proportional to the inverse of the within-study variances. The larger the bubble is, the larger the sample size and the smaller the standard error of the changes in 25OHD.
Reprinted with permission from Newberry et al. [8].