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25-hydroxyvitamin D Levels and Coronary Heart Disease Risk Reclassification in Hypertension – Is it worth the “Hype”?

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Atherosclerotic cardiovascular disease (CVD) [i.e. coronary heart disease (CHD) or stroke], remains the leading cause of morbidity and mortality in developed countries worldwide. All cardiovascular prevention guidelines begin with a screening recommendation that assesses global risk, which is calculated using traditional CVD risk factors to estimate one's 10-year risk of CHD or CVD events. Estimation of risk takes place via calculators such as the Framingham Risk Score (FRS) or more recently the AHA/ACC Pooled Cohort Equations. However these risk scoring systems have been criticized for both underestimating(1) and over-estimating risk(2), warranting consideration of additional factors not included in those models for refinement of individualized risk assessment. The goal for improving classification of risk is to further identify high-risk individuals that might benefit from intensified lifestyle changes to promote cardiovascular health or warrant additional treatments to modify their risk. Identification of a low vitamin D state might be one of these novel risk markers that can help guide personalized risk assessment.

There is strong observational epidemiologic evidence that suggests that low levels of serum 25-hydroxyvitamin D [25(OH)D] are a novel risk factor for all-cause mortality(3), cardiovascular mortality(4), CHD events(5, 6), stroke(7), and heart failure(8). Suboptimal vitamin D is thought to influence CVD risk by acting on established risk factors such as hypertension, diabetes, and inflammation(9).

In this issue of *Atherosclerosis*, Dr. Nargesi and colleagues advance our understanding of 25(OH)D as a novel marker of CHD risk in several ways. First, they evaluate this question in a specific subgroup of patients – those with essential hypertension. Since activated vitamin D is a negative inhibitor of the renin-angiotensin-aldosterone system(10) and associated with incident hypertension(11), hypertension has been noted as a possible mediator in the causal pathway between low 25(OH)D and incident CHD. However, the authors report that even among those who already have hypertension at baseline, low 25(OH)D is still predictive of future CHD risk independent of multiple traditional CVD risk factors.

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Their study population included 1586 hypertensive patients from Tehran that were enriched with diabetics by study design (68%) and followed for an average of 8.5 years. This high risk population indeed had low 25(OH)D levels with half of the population with clinical deficiency (<20 ng/ml) and only 5% had optimal levels >30 ng/ml. Compared to the highest quartile of 25(OH)D (>23.6 ng/ml), those in the lowest quartile (<16.1 ng/ml) had a nearly 3-fold higher risk of incident CHD. Findings from this study – which was exclusively limited to hypertensive patients – are concordant with a previous study from the Framingham Offspring Study cohort which found that the risk of CVD conferred by low vitamin D was actually stronger among those with hypertension compared to those without(12).

However, to determine the clinical usefulness of a marker for upgrading or downgrading risk, it is not sufficient for a biomarker to simply be associated with an outcome independent of traditional CVD risk factors, but rather a novel marker should show improvement in discrimination, calibration, and net reclassification of risk. Most previous papers evaluating the association of 25(OH)D with CVD outcomes have simply considered 25(OH)D in a multivariable hazard or relative risk model. Nargesi and colleagues are to be congratulated for their careful and thorough examination of this issue through their comprehensive use of statistical methods including Akaike and Bayesian information criteria (tests of model fitness), Gronnesby and Borgan χ^2 (a test of calibration), Harrell's concordance index (a marker of discrimination), integrated discriminant improvement (IDI), and both continuous and categorical net reclassification improvement (NRI) [tests to assess reclassification]. NRI may be particularly useful for evaluating whether new markers change risk strata and potentially alter treatment decisions(13). The authors found that adding 25(OH)D to the FRS improved NRI for incident CHD by successfully reclassifying 11.2% of hypertensive patients according to CHD risk categories of 5%, 6–10%, 11–20% and >20%. The continuous NRI was 33. The authors should be commended for their comprehensive statistical modeling; while it is reassuring that the various tests resulted in the same conclusion – that inclusion of 25(OH)D added additional information beyond that of established CVD risk factors - it is important to note that all of these statistical methods including NRI have various limitations and imperfections(14).

Also, as fully acknowledged by the authors, their study has some important limitations that should be considered when placing these findings into clinical context. First, a single measure of 25(OH)D may not reflect lifetime vitamin D status or even annual-averaged vitamin D levels. For areas significantly above or below the equator, there is substantial variation in the peak and trough of 25(OH)D levels by season(15), but unfortunately the authors were unable to account for seasonal variation in their analysis. Therefore there may be misclassification of risk without considering seasonal changes in 25(OH)D levels and estimates of annual-average 25(OH)D levels may be more appropriate.

Secondly, despite the prospective study design, reverse causation and residual confounding are a concern in observational epidemiology studies of 25(OH)D. Low 25(OH)D levels may simply be a marker of a poorer health state. Less healthy people may have reduced outdoor physical activity and thus less sunlight exposure which in turn confers low vitamin D status. Unfortunately the authors did not have measures of physical activity to adjust for this factor.

Thirdly, the authors used an enzyme linked immunosorbent assay (ELISA) to measure 25(OH)D; however there is substantial between-method variability in the validity and reliability of 25(OH)D assays(16), and liquid chromatography-tandem mass spectrometry (LC-MS/MS) is now considered the gold standard. Although the use of an ELISA to measure 25(OH)D likely resulted in misclassification, one would expect the degree of misclassification to be similar between those who ultimately developed incident CHD and those who did not. As such, the misclassification would be non-differential, and the study findings would likely be biased toward the null. Unfortunately, LC-MS/MS assays involve expensive equipment; thus alternate assays are commonly used to quantify 25(OH)D status. To improve comparability of vitamin D measurements both across and within assay methods, in 2010, the Center for Disease Control and the National Institutes of Health's Office of Dietary Supplements initiated a Vitamin D Standardization Program (VDSP). The over-arching goal of the VDSP is to assure accurate and reliable clinical vitamin D measurements to improve the diagnosis, treatment, and prevention of diseases conferred by low vitamin D states(17). In thinking about 25(OH)D as a risk marker and when comparing results across studies, it is important to be cognizant of these methodology issues.

Fourth, only incident CHD was considered here, but assessment of low vitamin D states may help refine risk assessment for a number of health conditions including other types of CVD, such as stroke and heart failure.

Fifth, it is unclear whether 25(OH)D is the optimal biomarker for defining vitamin D status, and evaluating the relation of vitamin D to CVD risk. Although circulating 25(OH)D, a prohormone, has been the biomarker universally measured for assessing vitamin D status, 1,25(OH)₂D [i.e. calcitriol] is the active hormonal form. Furthermore, approximately 85–90% of circulating 25(OH)D is tightly bound to vitamin D binding protein and is generally believed to be inactive. Of the remaining circulating 25(OH)D, 10–15% is bound to albumin and <1% is free. The free D is readily accessible for use by cells. 25(OH)D is bound to albumin loosely, and thus may also be available for use by tissues(18). The sum of free plus albumin-bound 25(OH)D is referred to as bioavailable D. Existing research has focused on total 25(OH)D; however, as highlighted in a 2015 U.S. Preventive Services Task Force (USPSTF) Recommendation Statement(19), it is possible that free or bioavailable D are the constructs of 25(OH)D most relevant to human health. Bioavailable D may correlate better with markers of mineral metabolism such as parathyroid hormone compared to total 25(OH)D(20). Also, bioavailable and free D have been shown to be better correlated with bone mineral density than 25(OH)D in some(21) but not all studies(22).

Finally, and most importantly of all, even if we have firmly established that 25(OH)D adds incremental CHD risk prediction and can re-classify patients, the next clinically relevant question then becomes whether such reclassification and application of targeted treatment strategies can alter clinical outcomes. Targeted treatment strategies could involve traditional CVD risk prevention approaches (e.g. improve lipid profile and treat diabetes and hypertension) or, if justified by ongoing clinical trials, could involve treating low vitamin D states with supplementation and/or modest sunlight exposure to reduce CHD risk. Notably, clinical trials to date have failed to show any CVD benefit for vitamin D supplementation, but there have been limitations with previous trials including low supplementation doses

used. Thus, we eagerly await the results of on-going clinical trials to answer this question, such as the VITamin D and Omega-3 Trial [VITAL](23).

Of critical note, since most of the hazard associated with low CHD risk across studies is confined to those with very low 25(OH)D levels (<15 ng/ml), there may be a treatment threshold where supplementation only benefits those with marked deficiency below this threshold but not the general population. Clinical trials targeting supplementation to the general population unselected for deficiency may indeed show no benefit. While the Institute of Medicine (IOM) report indicated that levels greater than 20 ng/ml should cover the nutritional requirements of at least 97.5% of the population, the IOM also noted that levels of 16 ng/ml should meet the requirements for approximately half of the population; as such not everyone with levels <20 ng/ml are truly deficient.

The impact of vitamin D supplementation on CVD risk reduction remains inconclusive and is a subject of much investigation and debate(24). The findings by Dr. Nargesi and colleagues are intriguing; however they require replication in independent populations. At this time, without concrete guidance of how to best clinically manage these individuals identified to be at higher CHD risk by virtue of their low vitamin D status in order to improve CVD outcomes, it may not be worth the “hype” to measure 25(OH)D for CHD risk re-classification among patients with hypertension.

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