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Review

The risks of biomarker-based epidemiology: Associations of circulating calcium levels with age, mortality, and frailty vary substantially across populations

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ABSTRACT

Recent studies have shown contradictory associations between calcium levels and health outcomes. We suspected these conflicting results were the consequence of more general issues with how biomarkers are analyzed in epidemiological studies, particularly in the context of aging. To demonstrate the risks of typical analyses, we used three longitudinal aging cohort studies and their demographic subsets to analyze how calcium levels change with age and predict risk of mortality and frailty. We show that calcium levels either increase or decrease with age depending on the population, and positively or negatively predict frailty depending on the population and analysis; both age and frailty results showed substantial heterogeneity. Mortality analyses revealed few significant associations but were likely underpowered. Variation in population composition (demographics, diseases, diet, etc.) leads to contradictory findings in the literature for calcium and likely for other biomarkers. Epidemiological studies of biomarkers are particularly sensitive to population composition both because biomarkers generally have non-linear and often non-monotonic relationships with other key variables, notably age and health outcomes, and because there is strong interdependence among biomarkers, which are integrated into complex regulatory networks. Consequently, most biomarkers have multiple physiological roles and are implicated in multiple pathologies. We argue that epidemiological studies of aging using biomarkers must account for these factors, and suggest methods to do this.

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1. Introduction

Clinical and epidemiological studies of biomarkers during aging often characterize associations between a single marker and either age or relevant health outcomes (Glei et al., 2011; Liu et al., 2015; Loeffen et al., 2015; Schottker et al., 2015; Schram et al., 2007). However, we and others have previously observed that many standard clinical biomarkers (cholesterol, glucose, blood panel markers, circulating proteins, inflammatory markers, etc.) have surprisingly variable associations with age in different populations (Cohen et al., 2015b; Martin-Ruiz and von Zglinicki, 2014; Sebastiani et al., 2016), and we were worried that many biomarker findings in the literature might be poorly generalizable. We note that our focus here is largely on clinical biomarkers used in epidemiological research of aging, not on single biomarkers of the aging process *per se*. Such single biomarkers have not really been found, though there is some controversy about telomeres (Johnson, 2006).

Individual biomarkers do not necessarily change linearly with, or independently of, other aspects of physiology, but rather are integrated into physiological regulatory networks that can exhibit properties of formally complex systems (Cohen, 2016; Cohen et al., 2012). Accordingly, many biomarkers have multiple physiological determinants and roles and can fluctuate for diverse physiological and pathophysiological reasons (see below for details, taking calcium as an example). Biomarkers are thus rarely a direct window into the underlying physiological processes of interest, but are rather proxies, the efficacy of which can depend heavily on other biomarkers, disease state, and demographics.

Another consequence of complex physiological integration is that changes with age are generally non-linear and usually non-monotonic as well (i.e., showing periods of both increase and decrease). For example, a long-term study of seven biomarkers in the Framingham Heart Study found that six showed non-monotonic changes across the adult life course (Yashin et al., 2010). Thus, since disease prevalence and physiological traits can vary greatly across populations, population composition should have a strong role in determining biomarker-age associations in any given study. For example, if a biomarker happens to be elevated in diabetics, observed changes in the biomarker with age may depend strongly on the prevalence and severity of diabetes in the population, the age structure of diabetes incidence, and impacts of diabetes on other outcomes except aging and mortality, all of which can be quite variable but are unrelated to the fundamental question of how the biomarker may or may not predict aging. Such effects are likely to be present even in longitudinal studies, where biomarkers may evolve differently with age and have different impacts in specific subpopulations.

This article attempts to address this challenge by combining aspects of a review and aspects of an empirical study. We first conduct analyses of how calcium associates with age, mortality, and clinical frailty in three different cohort studies of aging and their demographic subsets. We demonstrate that results are highly heterogeneous across populations. Obviously, calcium is but a single example, so our discussion then goes well beyond our particular findings to consider the theoretical basis of why biomarker studies should generate such unstable results, and how to address the problem. We propose a number of concrete methods to help mitigate the problem.

2. The example of calcium

Within the aging context, calcium has not particularly been proposed as a single biomarker of aging, and is generally analyzed rather as one among many markers in a panel (Martin-Ruiz et al., 2011; Szewieczek et al., 2015). Nevertheless, calcium levels are increasingly believed to be associated with risk of mortality and morbidity. Normal calcium reference ranges are 8.9–10.1 mg/dl in adults. While many studies with middle-aged adults report associations between high calcium levels and increased risk of mortality (Larsson et al., 2010; Reid et al., 2016), findings in oldest adults tend to show reduced adverse outcomes for higher calcium levels, perhaps reflecting the concomitant higher albumin levels within this group (Szewieczek et al., 2015). Non-linear associations have also been reported (Larsson et al., 2010), notably U- or J-shaped curves for risk (Durup et al., 2012; Lu et al., 2016).

Calcium is involved in many crucial physiological functions, notably neuronal transmission, immune cell stimulation, apoptosis, bone health maintenance, muscle contraction – including the heart – and blood coagulation (Shaker and Deftos, 2000). In the blood, its ionized form is believed to be the more closely regulated one, and accounts for approximately 50% of total serum calcium, the rest being bound to serum proteins, mainly albumin (Brown, 2001). Abnormalities in albumin and globulins may affect total serum calcium levels, but the ionized level should remain unchanged for a given pH (Payne et al., 1973). Hypercalcaemia has various causes, notably cancer and hyperparathyroidism, whereas hypocalcaemia is generally due to hypoparathyroidism or chronic renal failure (Parfitt, 1979). Many undetected pathological conditions related to age may influence calcium homeostasis and as such remain undiagnosed for long-time.

The relationship between calcium and generalized risks of adverse outcomes during aging thus appears to be a complex one. First, both high and low levels might have detrimental effects on physiological homeostasis. Thus, population composition, most notably age, might strongly affect possible associations between calcium and risk of health outcomes. For instance, while high calcium levels at mid-life may be linked to adverse outcomes, the inverse relationship might take place at older ages. To demonstrate this idea, we looked at age-related changes in calcium, and its potential association with frailty and mortality, across three longitudinal cohort studies of aging. Analyses were repeated in different cohort subsets (sex, race, and age) to assess the effect of population composition on potential associations. Our objective was to demonstrate the limits of such single-biomarker epidemiology in the context of the complex process of aging.

Detailed Methods are provided in the online Appendix; here we note principally that we have taken care to reproduce the methods most common in the literature. For example, it is common to use measures of calcium adjusted for albumin levels, and we thus used both a raw and albumin-adjusted version. There are two principal exceptions where we opted for methods more sophisticated than are standard. First, many analyses use tertiles or other quantiles, and we disfavour this approach because it causes a loss of statistical power and can cause important biases and inflation of Type-I error without any important justification (Barnwell-Ménard et al., 2015). We thus always consider calcium as a continuous variable. Second, age was sometimes controlled using a cubic spline, a more sophisticated approach than age categories or age as a linear variable.

3. Findings on calcium

3.1. Association of calcium with age

First, we looked at the association between calcium and age through correlation analyses in three longitudinal cohort studies of community dwelling older adults (Table 1, see online Appendix for details), as might be obtained in many cross-sectional studies. As shown in Table 2, calcium is consistently negatively correlated with age, except for WHAS I (nearly significant) and WHAS II; however *albumin-adjusted calcium (alb-adj ca)* shows unstable results (see Table A.1 for other versions). *Alb-adj ca* is clearly positively correlated with age in the InCHIANTI cohort, but not in the other two data sets. Calcium is correlated with albumin in all three cohorts ($0.33 \le r \le 0.44$, all $p < 0.001$). Calcium and *alb-adj ca* also correlate strongly ($0.78 \le r \le 0.87$, all $p < 0.001$). Surprisingly, *alb-adj ca* is not much more correlated with ionized calcium than the unadjusted measure ($r = 0.45$ compared to $r = 0.43$) and thus might not serve as a good proxy for ionized calcium (Fig. A.1).

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