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A Few Follow-up Questions to a Recent Calcitriol and Sepsis Study

To the Editor:

Vitamin D and critical illness is an area of growing interest and research, with a suggested association between vitamin D deficiency and intensive care unit mortality (1).

In their recent publication, Dr. Leaf and colleagues (2) did not find an appreciable difference in clinical outcomes in septic patients receiving calcitriol versus placebo. However, in the intervention group of the trial (patients who received 2 μg calcitriol), the data show that some patients had an increase in plasma 1,25-dihydroxyvitamin D levels at 6 hours, whereas others who were also receiving the therapy did not have as large of a response. Our question is whether this division merits a subgroup analysis within the control group, involving comparing patients who received calcitriol and had a significant increase in plasma levels (responders) with patients who received calcitriol but did not have a significant increase in plasma levels (nonresponders), and assessing the difference in clinical outcomes.

Further, the dose used in the trial, 2 μg intravenous calcitriol, was based on dosages used in patients with chronic kidney disease. Although the authors indicate this dose was chosen in part to avoid adverse effects, are there intentions for further trials with higher doses?

What remains unknown is predicting which patients will be responders versus nonresponders and, of course, whether this is clinically significant. We believe further research is needed in the area of vitamin D deficiency and severe illness, both in understanding its role in sepsis and potential interventions. ■

Author disclosures are available with the text of this letter at www.atsjournals.org.

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Reply: Active and Native Vitamin D in Critical Illness

From the Authors:

We appreciate the comments by Schnedl and colleagues in response to our trial (1) and wish to respond in detail to provide further clarification. They raise several points. First, drawing an analogy to insulin, antibiotics, and beta blockers, they question whether the treatment group was vitamin D deficient. However, the analogy may not be relevant to our trial, which used calcitriol and not vitamin D_3. Moreover, vitamin D deficiency/insufficiency is nearly universal in critically ill patients (99–100% in some studies) (2, 3). In our study, 93% of patients at enrollment had 25-hydroxyvitamin D [25(OH)D] levels below 30 ng/ml, and the other 7% had 25(OH)D levels between 30 and 40 ng/ml.

Schnedl and colleagues suggest analyzing whether patients with enrollment 25(OH)D levels below versus above the median had a more potent immunologic response to calcitriol. We did not present subgroup analyses in the original manuscript because of the modest sample size. However, the suggested analysis is shown in Figure 1. Fold elevation in leukocyte human cathelicidin antimicrobial peptide 18 messenger RNA expression was similar in calcitriol-treated versus placebo-treated patients with enrollment 25(OH)D levels below versus above the median level (17 ng/ml).

Second, Schnedl and colleagues comment that the interassay coefficients of variation (CVs) for 25(OH)D and 1,25-dihydroxyvitamin D [1,25(OH)D] are "surprisingly high." We note that our study assessed interassay CV using blinded split sample replicates from intensive care unit patients, rather than manufacturer-reported CVs or CVs from standard reference materials. We further note that our CVs are within the range reported by the National Institutes of Standards and Technology for 25(OH)D measurement (4). Finally, all vitamin D metabolites were measured using immunoaffinity enrichment and liquid chromatography–tandem mass spectrometry, which is widely regarded to be the gold standard method of measurement (5, 6).

Third, Schnedl and colleagues comment that a different drug (vitamin D_3 instead of calcitriol), dosing regimen, and patient population should have been studied. Each of these items merits careful consideration.

Drug

There has been an explosion of observational studies on 25(OH)D, in contrast to the limited studies of the active metabolite, 1,25(OH)D. The argument in favor of using vitamin D_3 as an immunomodulatory

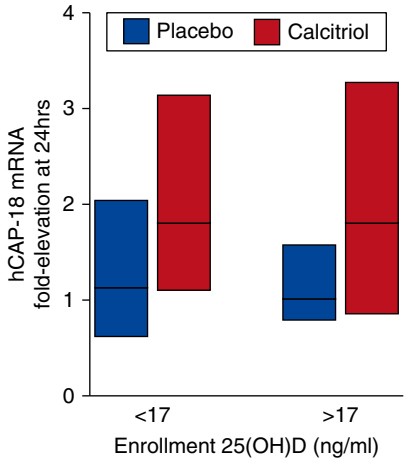

Figure 1. Leukocyte hCAP-18 mRNA expression in patients with enrollment 25-hydroxyvitamin D [25(OH)D] levels below (n = 29) versus above (n = 29) the median level of 17 ng/ml. Bars represent median (25th–75th interquartile range). hCAP-18 = human cathelicidin antimicrobial peptide 18. A 1.0-fold elevation is equivalent to no change.

agent is certainly reasonable: by raising local 25(OH)D levels, endogenous 1,25(OH)D production in monocytes and other cells may be enhanced. We note that nearly all preclinical studies (performed both *in vitro* and in animals) demonstrating immunomodulatory effects of vitamin D metabolites used 1,25(OH)D, rather than precursors such as vitamin D_3. Furthermore, the increase in plasma 1,25(OH)D levels induced by high-dose vitamin D_3 supplementation in critically ill patients (7) was lower than what we observed with direct calcitriol administration (1). We recognize, however, that plasma 1,25(OH)D levels may not be the best measure of local or intracellular levels, which could conceivably be more effectively increased by strategies focused on circulating 25(OH)D. Which drug is more effective would require a head-to-head comparison, which would only be relevant if both were shown in separate randomized controlled trials to be beneficial.

Schnedl and colleagues also argue in favor of vitamin D_3 on the basis of the longer half-life of 25(OH)D (2–3 wk) compared with that of 1,25(OH)D (several hours). However, whether the immunomodulatory effects of oral vitamin D_3 are superior to intravenous calcitriol cannot be assessed on the basis of a comparison of their half-lives. Moreover, patients with critical illness often have impaired gastrointestinal absorption and/or difficulty taking oral medications. Even in the absence of impaired absorption, the faster onset of intravenous calcitriol would arguably be preferable to the slower effects of oral vitamin D_3 in a critically ill population: plasma 1,25(OH)D levels peak within minutes of administration of the former (8), whereas plasma 25(OH)D levels require several days (9), if not weeks (10), to peak after administration of the latter.

Dosing Regimen

We tested a single dose of calcitriol because of the physiologic nature of the study. The concentrations of 1,25(OH)D used in most *in vitro* studies, which ranged between 1 and 100 nmol/L, are much higher than physiologic levels in humans and may be higher than can be safely achieved without the development of hypercalcemia. However, immune responses *in vitro* have also been observed with 1,25(OH)D concentrations as low as 0.1 nmol/L (11–13)—levels that were achieved in the current study [median plasma 1,25(OH)D levels at 6 h were 0.2 (interquartile range [IQR], 0.1–0.3) nmol/L]. In addition, others have evaluated the pharmacokinetics of higher doses of intravenous calcitriol: after a single 10-μg intravenous dose of calcitriol among patients with advanced solid tumors, mean plasma 1,25(OH)D levels peaked at 1.1 (standard deviation, 0.5) nmol/L and were well tolerated (8). Thus, the possibility remains that with higher and repeated dosing of intravenous calcitriol, greater immunomodulatory effects may be observed. Such a strategy may indeed be appropriate for future studies of calcitriol in critically ill patients, as alluded to in the letter by Chapman and Alhajhusain.

Patient Population

Schnedl and colleagues make the argument that we excluded the only patient group likely to benefit from calcitriol: patients with end-stage renal disease. However, the studies we cited on the impaired conversion of 25(OH)D to 1,25(OH)D in the presence of fibroblast growth factor-23 (FGF23) included patients with end-stage renal disease as well as healthy volunteers (14). It is quite likely that conversion of 25(OH)D to 1,25(OH)D is impaired in critically ill patients. We (2) and others (15, 16) have documented elevated plasma levels of FGF23, a potent phosphaturic hormone release from bone, among critically ill patients with and without acute kidney injury. FGF23 inhibits 25-hydroxyvitamin D-1α-hydroxylase and also stimulates 25-hydroxyvitamin D-24-hydroxylase, thereby simultaneously inhibiting the activation and enhancing the catabolism of 25(OH)D, respectively (17, 18). Until recently, these effects were thought to primarily involve the kidneys, but recent data indicate that FGF23 also inhibits extrarenal 25-hydroxyvitamin D-1α-hydroxylase in monocytes (14). Even if conversion of 25(OH)D to 1,25(OH)D is not universally impaired in all patients with critical illness, additional pharmacologic augmentation of 1,25(OH)D may prove to be clinically beneficial. Only additional randomized trials will provide the answer.

Chapman and Alhajhusain also question whether clinical outcomes were different in "responders" versus "nonresponders," as defined by changes in plasma 1,25(OH)D levels from 0 to 6 hours. Every patient who received calcitriol had an increase in plasma 1,25(OH)D levels at 6 hours (range, 14–225 pg/ml on an absolute basis and 1.6- to 84-fold on a relative basis). We found a graded relation between fold elevation in 1,25(OH)D levels (0–6 h) and fold elevation in human cathelicidin antimicrobial peptide 18 and interleukin 10 leukocyte mRNA expression (0–24 h) (Figures 4B and E1A from Reference 1). Whether clinical outcomes differ between patients who demonstrate a greater versus lesser increase in plasma 1,25(OH)D levels in response to calcitriol is an interesting question, but our study was not powered to evaluate clinical outcomes, particularly among subgroups. Nonetheless, we analyzed hospital mortality and length of stay among patients who received calcitriol and had an absolute increase in 1,25(OH)D levels above versus below the median. We found similar rates of hospital mortality (18 and 22%; $P > 0.99$) and length of stay (median, 20 [IQR, 16–28] and

21 [IQR, 13–38] d; $P = 0.95$), which were also similar to rates in the placebo group. ■

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Proper Reading of Pulmonary Artery Vascular Pressure Tracings

To the Editor:

Kovacs and colleagues (1) address an important subject: accurate measurements of pulmonary artery pressures. The historical discussion on choices of the appropriate level for fluid-filled systems is especially well covered. Unfortunately, their concluding recommendation that pulmonary vascular pressures should be averaged over several respiratory cycles in spontaneously breathing patients returns to an error made by Sharpey-Schafer in his discussion on heart–lung interactions in the 1965 *Handbook of Physiology* (2).

Vascular pressures are primarily elastic forces with much smaller kinetic components. As such, the force is caused by the difference in pressure inside versus outside the elastic structures, which is referred to as transmural pressure. The pressure outside intrathoracic structures, including both sides of the heart and pulmonary vessels, is pleural pressure, which is not the same as atmospheric pressure. However, pleural pressure generally is not accessible, so transducers have to be zeroed relative to atmospheric pressure, which introduces an error in the measurement of intrathoracic pressures, although not for systemic pressures or flow gradients into and out of the thorax. Pulmonary artery occlusion pressure falls relative to atmosphere during spontaneous inspiration, which would seem to indicate that left heart volume decreases during this phase. Sharpey-Schafer accordingly waxed eloquently about why left heart volumes fall during inspiration. However, pleural pressure most often falls more than left atrial pressure, which means left heart transmural pressure increases, and Sharpey-Schafer needed to explain why the left heart gets bigger, not smaller (3–5). The reason for this is that some blood is squeezed out of the lungs during lung inflation, except when left heart filling pressures are very low (6).