Reviewer's report

Title: 25-Hydroxyvitamin D level is Inversely Associated with Serum MMP-9 in a cross-sectional study of African American ESRD Patients

Version: 2 Date: 23 September 2010

Reviewer: Barbara J J Boucher

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Review of MS by Wasse et al. “25-hydroxyvitamin D level is inversely associated with serum MMP-9 in a cross-sectional study of African American ESRD patients.”

This elegant and well presented report provides data showing vitamin D status in end stage renal dialysis patients, as assessed by serum 25-hydroxyvitamin D concentration, to be inversely related to circulating markers of inflammation including matrix metalloproteinase-9 [known to contribute to inflammatory atherosclerosis] and LDL-cholesterol [a major risk factor for cardiovascular disease]. Vitamin D status was also directly correlated with the anti-inflammatory factor IL-10. The data for MMP-9 and CRP confirms observations made earlier in South Asians and the other findings support various reports in the literature. The study design is clear and the findings well presented and easy to follow. The only major concern is that there is an absence of information on whether or not any patients were taking vitamin D, calcitriol or its analogues [see 1. Below].

‘Major’ Points that need to be addressed.

1. The patients studied had end stage renal disease and were having renal dialysis. Many such patients would be on treatment with either calcitriol or a calcitriol analogue such as 1-alpha-calcidol. Since modest supplementation with vitamin D reduced plasma MMP-9 by a mean of nearly 70% in ‘healthy’ South Asians, [ref 18 in this MS], it is important that the reader is told whether any of these patients were taking such supplements. If they were we should be told whether the supplementation contributed to serum 25(OH)D. This is unlikely since the assay systems used for 25(OH)D would not have detected them. However, this point should be clarified and the use of any such medication reported and the effects of their use on the associations examined would need to be examined and reported. If patients on these supplements were not included in this study we should be told if this was an exclusion factor?

2. There is in fact considerable earlier work showing vitamin D to act upon MMP9 in bones, in joint tissues and in inflammatory joint problems, e.g. by Tetlow et al. some years ago, that should be mentioned in the first Para on page 5.

3. How long were the intervals between blood sampling for vitamin D status and for the routine biochemical testing that provided the other biochemical data including lipid profiles? If there is more than a few weeks between these samples vitamin D status is likely to have changed considerably [with season] and any
associations that might exist might well be progressively weakened as this gap enlarges, thus these intervals should be given and they may need to be included as a likely confounder of the associations examined since adjustment for them could either strengthen or weaken the associations reported with vitamin D status, depending on the rates of clearance from the blood of the different factors.

4. MMP9 measurement in plasma reflects tissue production, but, if serum is used the MMP9 concentrations increase greatly due to its release from white cells during clotting. It must be made crystal clear, therefore, whether plasma or serum was used for these analyses.

‘Minor’ points that it would be helpful to deal with.

Circulating factors measured in blood, plasma or serum are measured as concentrations rather than levels. This paper uses the term concentrations correctly but in some places where ‘level’ is used, concentration would be more correct; this could usefully be tidied up with the aid of the search/find facility.

Page 8, last sentence of Para 1, Is the description of the hs-CRP kit as ‘BNll, correct?

Page 10, line 13, it would be useful to add ‘correlation’ before (Pearson) analysis

Were AVGs, associated with higher MMP9 values etc, recent? The more recent they are, the more likely that increases in plasma MMP-9 would be associated with their use rather than vitamin D?

It is a pity, if no vitamin D treatment was being used in this cohort, that there is no follow-up data on the effects of vitamin D administration as there was in ref 18 where MMP-9, and hsCRP fell with supplementation, since falls in LDL-C and increases in IL-10 could well be expected with supplementation from other published data. Such findings would add to the evidence for causality. If the authors have this data and are planning a second paper on it, I suggest it would be better to include it in this MS, or as a twin paper, in order to emphasize which of these associations may indeed be causal.

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests.