Response to Reviewer no. 00503199

Manuscript Number	25629
Manuscript Title	Vascular calcification: When should we interfere in CKD patients? And How?

	Reviewer Comment	Authors Reply
1.	Provide the actual words of the abbreviations the first time they appear.	It happened once…already fixed (put in red font)
2.	"Hemodialysis (HD) patients have higher calcification scores than either peritoneal dialysis (PD) or CKD G4. More heavily calcified patients were significantly older and mostly male [23]"	No more articles to support So, corrected as you have suggested and put in red font.
3.	The legends of the figures need to be more explanatory	Done
4.	"Increased FGF23 level is associated with increased risk for mortality among incident HD patients, during their first year of treatment [127]. This association was also confirmed in prevalent dialysis patients [128]. Neutralization of FGF23 in CKD rats was found to accelerate V.C. and increases mortality [129]".....In this paragraph you mention two, at first glance, contradictory effects of FGF-23 in VC and mortality. You need to be more specific and explanatory, in order to highlight the different effects.	The initial impression about FGF23 was positive; being a phosphatonin that stimulates urine phosphate excretion. However, my group was the first to report the association of high FGF23 with aortic calcification in incident and prevalent HD patients (10). Together with studies no 127, and 128 and other studies that showed association with vessel calcifications in other sites and left ventricular hypertrophy. This changed the impression about DGF23 from being a friend to a "foe". This paradox stimulated for the search for neutralizing antibodies against FGF23 to protect CKD patients from this foe!!. However, when such antibodies were developed, their use in uremic rats accelerated calcification and mortality of treated rats (129). These results rectified the wrong impression about FGF23. The researchers realized that they were facing a situation simulating hyperinsulinemia in early type 2 DM. In other words, FGF23 is a favorable agent that faces excess resistance to its action in CKD. Failure of FGF23 to perform its favorable effects results in increased calcification, L.V. hypertrophy, and increased mortality beside increased serum concentration. There is comprehensive discussion of this issue in the section of pathogenesis.
5.	We recommend small dose of vitamin D or vitamin D analogues to be given daily as prophylaxis against V.C. in spite of the lack of clinical trials favoring the use of either native or active vitamin D analogues to prevent V.C. progression. The rarity of vitamin D toxicity in general and the privileged survival benefits offered by VDRAs administered in small doses even in cases suffering hyperparathyroidism and/ or increased calcium and phosphorus levels supports this concept."....a)How do you define small doses? b)In which patients? C)Even in those with low iPTH levels? You need to give precise doses and target population as this is your "recommendation". However, I do not agree with such a recommendation as there are no prospective controlled trials that support any survival benefit from any vitamin D compound in CKD patients, especially the VDRAs. Using data from retrospective studies are not enough even for	First, we like to emphasize that the management of vascular calcification is directed to predialysis CKD patients. a)Small doses mean 1000IU of either vitamin D2 or vitamin D3 or 0.25 ug of 1 alpha calcidol, or 0.25 ug of calcitriol or 1ug of paricalcitol every other day. b)This dose Guarantees maintenance of adequate level that can inhibit V.C. in CKD patients . administration should start in the early days of G2. Till patients are indicated for renal replacement therapy. c)the issue of low PTH is concerned with dialysis patients, the recommended management as we mentioned is maily directed to predialysis patients. We like you to revise the following articles that support our view: **Vitamin D receptor agonists increase**

	suggestions. In addition several experimental and prospective clinical data and RCTs (for example from transplant patients) may have harmful effects on patients.	**klotho and osteopontin while decreasing aortic calcification in mice with chronic kidney disease fed a high phosphate diet** *Wei Ling Lau1,5, Elizabeth M. Leaf2,5, Ming Chang Hu3, Marc M. Takeno2, Makoto Kuro-o4, Orson W. Moe4 and Cecilia M. Giachelli2* Kidney International (2012) 82, 1261–1270 **Vitamin D and vascular calcification in chronic kidney disease** *Masahide Mizobuchi a,□, Hiroaki Ogata b, Fumihiko Koiwa c, Eriko Kinugasa b, Tadao Akizawa a* Bone 45 (2009) S26–S29 **Vitamin D and secreted Klotho: a long-awaited panacea for vascular calcification?** *Hirotaka Komaba1 and Masafumi Fukagawa1* Kidney International (2012) 82
6.	Check the title in ref 129	Done, highlighted in red

Finally; accept our great appreciation to your creative criticism

Response to Reviewer no. 00225280

Manuscript Number	25629
Manuscript Title	Vascular calcification: When should we interfere in CKD patients? And How?

Thank you very much for your prompt response,

	Reviewer Comment	Authors Reply
1.	The title of the review implies that the review will focus on the clinical aspects of the vascular calcification (**who and when to treat**). The main text is disproportionally divided; half of the pages are focused eg on pathogenesis. I would prefer a more extended reference to the clinical aspects of VC.	The title is **"When should we interfere in CKD patients?** And How? In this review we are trying to change the concept, from waiting till calcification appears to start treatment..to an aggressive prophylactic approach. The pathogenesis section is serving to support this strategy. We really do not understand what do you mean with "clinical aspects of VC". We already discussed the clinical relevance of V.C. and the clinical consequences of such pathology.
2.	Regarding the clinical implications of VC, I believe that the authors should insist more on analyzing the current debate about a) VC as a marker and prognostic factor of the kidney disease but not as a relevant etiological factor in the arterial disease	We would like the reviewer to revise the introduction and the clinical relevance sections beside our comment on the discrepancy of the effect of sevelamer in the different stages of CKD (last paragraph of page17 and 1st paragraph of page 18" to realize that we were fighting all through this review to confirm that VC is a real risk factor rather than a risk marker. Arrest of VC in the INDEPENDENT trial resulted in a significant decrease in Mortality. Failure to get similar effect in DCOR is simply due to the very late interference. We would like to refer to the review article **"Vascular Calcification: The Killer of Patients with Chronic Kidney Disease"** by *Masahide Mizobuchi, Dwight Towler,† and Eduardo Slatopolsky* J Am Soc Nephrol 20: 1453–1464, 2009, and to the clinical trial done by Block GA, et al., published in Kidney International, Mar;71(5):438-41, 2007. In this trial; decreased rate of calcification progression was associated with decreased mortality of borderline significance. On the other hand, we mentioned the criticism of Zoccali C, and London G., is based on trials of non-calcium based phosphate binder and the calcium receptor antagonist, cinacalcet, in prevalent hemodialysis patients. Dialysis patients usually have advanced V.C. rendering their arteries permanently and irreversibly damaged. All the studies that tried to tackle different traditional (4D trial, AURORA trial, SHARP trial and Alberta Kidney Disease Network) and non traditional (DCOR

trial, ADVANCE trial and EVOLVE trial) risk factors failed to show a significant impact on cardiovascular morbidity or mortality among such patients. We would like to refer to the review article written by New SE and Aikawa E. in circulation research 2011 May 27;108(11):1381-91 who affirmed that vascular calcification becomes irreversible once established and detected by radiology. On the other hand, when the same intervention tools were applied to CKD patients at earlier stages of the disease before the calcification is established such interventions succeeded to significantly decrease cardiovascular events and or mortality (SHARP trial, Alberta Kidney Disease Network. and INDEPENDENT study). We would like to emphasize that predialysis patients are also affected with vascular calcification, but less frequently and with significantly lower score that makes them able to respond in comparison to dialysis patients.

In addition, we mentioned under the topic **CLINICAL RELEVANCE OF VASCULAR CALCIFICATION:** CKD patients sill need prospective clinical trials evaluating the prognostic impact of aortic, coronary and carotid calcification in different CKD stages

2. b)the necessity of screening for VC or not

We referred for the debate about screening for VC under the topic: **CLINICAL RELEVANCE OF VASCULAR CALCIFICATION:** and we wrote: The European Renal Best Practice (ERBP) work group recommends screening of incident dialysis patients [169], whereas some national guidelines dictated the screening of any CKD patient[170]. And under the topic: **IMAGING OF VASCULAR CALCIFICATION:** we wrote: In 2009, the Kidney Disease Outcomes Quality Initiative (KDOQI) guidelines did not recommend the routine screening of V.C. as long as there is no clear clinical utility [177]. However, in some cases, imaging of V.C. might help to guide the treatment plan [178].
However, New SE and Aikawa E. considered that the traditional imaging modalities such as computed tomography, although perfectly adept at identifying and quantifying advanced calcification, cannot detect the early stages of this disorder and offer limited insight into the

2. c) lack of trials based on VC as a clinical end point.(Zoccali C and London G, Nephrol Dial Transplant, 2015 and Zoccali C et al, Hypetension, 2015;66:3-9).

mechanisms of mineral dysregulation.

All the following studies are presented in the review and all of them got use of VC as one of the endpoints:

223.**Block GA,** Wheeler DC, Persky MS, et al., Effects of phosphate binders in moderate CKD. J Am Soc Nephrol. Aug;23(8):1407-15, 2012.

226.**Di Iorio B**, Bellasi A, Russo D; INDEPENDENT Study Investigators. Mortality in kidney disease patients treated with phosphate binders: a randomized study. Clin J Am Soc Nephrol. Mar;7(3):487-93, 2012.

227.**Floege J**, and Ketteler M. Vascular calcification in patients with end-stage renal disease. Nephrol Dial Transplant 19(Suppl 5): V59–V66, 2004.

228.**London GM,** Marchais SJ, GuerinAP et al. Association of bone activity, calcium load, aortic stiffness, and calcifications in ESRD. J Am Soc Nephrol 2008.

230.**Chertow GM,** Burke SK, and Raggi P; Treat to Goal Working Group. Sevelamer attenuates the progression of coronary and aortic calcification in hemodialysis patients. Kidney Int. Jul;62(1):245-52, 2002.

231.**Block GA,** Spiegel DM, Ehrlich J et al. Effects of sevelamer and calcium on coronary artery calcification in patients new to hemodialysis. Kidney Int 68: 1815–1824, 2005.

232.**Asmus, H.,** Braun, J., Krause, R., et al. Two year comparison of sevelamer and calcium carbonate effects on cardiovascular calcification and bone density. Nephrol Dial Transplant 20: 1653–1661, 2005.

233.**Braun, J.,** Asmus, H., Holzer, H., et al. Long-term comparison of a calcium-free phosphate binder and calcium carbonate–phosphorus metabolism and cardiovascular calcification. Clin Nephrol 62: 104–115, 2004.

234.**Kakuta, T.,** Tanaka, R., Hyodo, T., et al. Effect of sevelamer and calcium-based phosphate binders on coronary artery calcification and accumulation of circulating advanced glycation end products in hemodialysis patients. Am J Kidney Dis 57: 422–431, 2011.

235.Shantouf, R., Ahmadi, N., Flores, F., et al. Impact of phosphate binder type on coronary artery calcification in hemodialysis patients. Clin Nephrol 74: 12–18, 2010.

237.**Russo, D.,** Miranda, I., Ruocco, C., et al. The progression of coronary artery calcification in predialysis patients on calcium carbonate or sevelamer. Kidney Int 72: 1255–1261, 2007.

239.**Block GA,** Raggi P, Bellasi A, et al., Mortality effect of coronary calcification and phosphate binder choice in incident hemodialysis patients. Kidney Int. Mar;71(5):438-41, 2007.

160.**García-Canton C,** Bosch E, Ramírez A, et al., Vascular calcification and 25-hydroxyvitamin D levels in non-dialysis patients with chronic kidney

	disease stages 4 and 5. Nephrol Dial Transplant. Jul;26(7):2250-6, 2011. 251.**Toussaint ND,** Lau KK, Polkinghorne KR, and Kerr PG: Attenuation of aortic calcification with lanthanum carbonate versus calcium-based phosphate binders in haemodialysis: A pilot randomized controlled trial. Nephrology (Carlton), 16:290–298, 2011.
3. Moreover all the related clinical trials which the authors refer to, should be critically reviewed (pitfalls, end points and surrogate end points, discrepancies etc)	We did our best to discuss the results of the different trials, and critical appraisal was done whenever needed. The volume of the review does not allow more appraisals or analyses.
4. The pathogenesis section should be re-written in a more concise and precise way. For example FGF23-klotho section is too long with too many references. On the contrary, there is no clear description of the sequence of the mechanisms involved from the early to late stages eg inflammation-associated osteogenesis, cytokines, transcription factors, conversion of VSM cells,micro RNAs etc. I suggest reference to excellent reviews like Neil J. Paloian and Cecilia M. Giachelli. A current understanding of vascular calcification in CKD Am J Physiol Renal Physiol 307: F891–F900, 2014	By the time we started writing this review, most of the data on FGF23 – klotho axis were novel. Nonetheless, we already made it more concise. Moreover, we would like to express our surprise. In the first point of your comment, you mentioned that the pathogenesis is disproportionately large. Again in this point your comment is that the section about FGF23- klotho is lengthy. On the other hand you want us to add to the pathogenesis description of inflammation, cytokines, transcription factors, conversion of VSMCs , Micro-RNA etc. This suggestion would add around 3 more pages of data that, contrary to klotho, do not help in the target of the article, namely, when and how to manage. These items might be of value in the future when new therapeutic modalities involving such factors are added to the existing management.
5. Conclusion section should be shorter with clear suggestions	Done
6. Too many pointless references. For example, the authors illustrate the high CVD mortality of CKD patients (a common knowledge) and cite 5 references.	The five references about high CV mortality in CKD are concerned with the association of CV mortalty to VC or FGF23 in different situations. Needless to mention we are not fond of citation collection. **12.Rodriguez Garcia M**, Naves Diaz M, and Cannata Andia JB. Bone metabolism, vascula calcifications and mortality: association beyond mere coincidence. J Nephrol,18:458 463, 2005. Review. [PMID: 16245255] 36.**Blacher J**, Safar ME, Guerin AP, Pannier E Marchais SJ, London GM. Aortic pulse wav velocity index and mortality in end-stage rena disease. Kidney Int. 63:1852–1860, 2003

[PMID: 12675863 doi:10.1046/j.1523 1755.2003.00932.x]

44.Russo D, Morrone L, and Russo L Coronary artery calcification an cardiovascular mortality in predialysis patients Kidney Int 79: 258, 2011. [PMID 21191391doi: 10.1038/ki.2010.405]

127.Gutierrez OM, Mannstadt M, Isakova T Rauh-Hain JA, Tamez H, Shah A, Smith K, Lee H, Thadhani R, Jüppner H, Wolf M. Fibroblast growth factor 23 and mortality among patients undergoing hemodialysis. N Engl J Med 359: 584–592, 2008 [PMID: 18687639 doi: 10.1056/NEJMoa0706130]

128.**Jean G**, Terrat JC, Vanel T, Hurot JM, Lorriaux C, Mayor B, Chazot C. High levels o serum fibroblast growth factor (FGF)-23 are associated with increased mortality in long haemodialysis patients. Nephrol Dial Transplant. Sep;24(9):2792-6, 2009. [PMID: 19395730 doi: 10.1093/ndt/gfp191]

129Shalhoub V, Shatzen EM, Ward SC, Davi J, Stevens J, Bi V, Renshaw L, Hawkins N, Wang W, Chen C, Tsai MM, Cattley RC, Wronski TJ, Xia X, Li X, Henley C, Eschenberg M, Richards WG. FGF23 neutralization improves chronic kidney disease-associated hyperparathyroidism yet increases mortality. J Clin Invest 122:2543-2553, 2012. [PMID: 22728934doi: 10.1172/JCI61405]

162. Pilz S, Iodice S, Zittermann A, Grant WB, Gandini S. Vitamin D status and mortality risk in CKD: a meta-analysis of prospective studies. Am J Kidney Dis 58: 374-382, 2011. [PMID: 21636193 doi: 10.1053/j.ajkd.2011.03.020]

166.London GM, Guérin AP, Marchais SJ, Métivier F, Pannier B, Adda H. Arterial media calcification in end-stage renal disease: Impact on all-cause and cardiovascular mortality. Nephrol Dial Transplant 18 : 1731– 1740, 2003. [PMID: 12937218 doi:10.1093/ndt/gfg414]

173.Jamal SA, Vandermeer B, Raggi P,

		Mendelssohn DC, Chatterley T, Dorgan M, Lok CE, Fitchett D, Tsuyuki RT. Effect of calcium-based versus non-calcium-based phosphate binders on mortality in patients with chronic kidney disease: an updated systematic review and meta-analysis. Lancet; 382 1268–1277, 2013. [PMID: 23870817 doi: 10.1016/S0140-6736(13)60897-1] **192.Kestenbaum B**, Sampson JN, Rudser KD Patterson DJ, Seliger SL, Young B, Sherrard DJ, Andress DL. Serum phosphate levels and mortality risk among people with chronic kidney disease. J Am Soc Nephrol 16: 520–528,2005. Epub 2004 Dec 22 [PMID: 15615819 doi:10.1681/ASN. 2004070602] **208.Agarwal R.** Blood pressure and mortality among hemodialysis patients. Hypertension. 55:762–768, 2010. [PMID: 20083728 doi: 10.1161/HYPERTENSIONAHA.109.144899]
	They describe FGF-klotho association with VC and cite 50 references (this is not a review for klotho!)	Discovery of FGF23 axis and its role in VC pathogenesis is behind the changed strategy of VC management. In fact, this is the core of this review article. You mentioned that the search for VC in pubmed release more than 1600 article. If you do a similar search for either klotho or FGF23 you will get more than 1300 articles!!. We super selected the most relevant articles to write this review.
7.	Minor comments 1. Plain definition of VC in the beginning of the manuscript.	Done
	2. In the introduction section, is the definition of ESAD valid? (where and when used?reference?)	Is our suggestion. Upon revising the article by New SE and Aikawa E. in circulation research 2011 May 27;108(11):1381-91 who affirmed that vascular calcification becomes irreversible once established and detected by radiology. Patients on prevalent hemodialysis get accelerated progression of VC renering them far beyond what New SE and Aikawa E. have described.
	3. Put the ideal strategy…The verb develop is better.	Done (red font)
	4. Large instead of big vessels.	Done (red font)
	5. I would prefer explanatory	Done

tables about eg treatment strategies instead of the images