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In Focus

FGF23 and vascular calcifications: another piece of the puzzle?

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At the time of its early discovery and characterization, fibroblast growth factor 23 (FGF23) was identified as a hormonal substance produced by bone cells (osteoblast/osteocytes) mainly devoted to controlling phosphorus balance by directly reducing phosphate reabsorption in the proximal renal tubule and indirectly decreasing phosphorus intestinal absorption, through a reduced bioavailability of the most active vitamin D metabolite (calcitriol) [1–3]. These characteristics formerly made this new hormone the object of interest mainly in the endocrinological field encompassing some acquired or genetically transmitted hypophosphatemic syndromes, such as tumor-induced (TIO), X-linked, autosomal dominant and recessive hypophosphatemic rickets/osteomalacia (XLHR/ADHR/ARHR) syndromes which have been demonstrated to be directly or indirectly linked to some disturbances associated with FGF23 and other strictly related phosphaturic substances (PHEX, DMP-1, MEPE). However, many subsequent experimental and observational studies reported on increased FGF23 concentrations from the very early stages of chronic kidney disease (CKD) and provided data suggesting that high FGF23 levels might represent an independent risk factor for both global and/or cardiovascular morbidity/mortality [4–6], not only in CKD patients but also in the general population [7].

However, the presence of many potential confounders and contradictions soon became evident, preventing a clear interpretation of the results of this bulk of published studies, with many questions still remaining unanswered.

An important unanswered question concerns whether the increased circulating levels of FGF23 (the hormonal component) or some disturbance in its tissue expression and/or signaling (the autocrine/paracrine or 'local' component) can be implicated in the putative pathogenic link with cardiovascular morbidity.

Anyway, for both these putative conditions a series of critical points arise.

The increase of FGF23 levels in CKD probably represents a homeostatic response directed to maintain a neutral phosphorus balance, given the trend toward a reduced renal excretion, in spite of an almost unchanged intestinal phosphorus absorption in the course of CKD. It is worth underlining that the trend to phosphorus retention in CKD is secondary not only to the reduced glomerular filtration volume, but probably also to the tubular resistance to the phosphaturic action of FGF23, secondary to the progressive reduction of α-Klotho, which plays a basic role in increasing the affinity of FGF23 receptors.

Since both phosphorus retention and α-Klotho reduction may independently play a pathogenic role in CV morbidity, it is not easy to understand how much of this putative pathogenic role should be ascribed to the FGF23 *per se* or to the above quoted primary conditions causative for the FGF23 increase [8]. In particular, the reduced tissue α-Klotho availability has recently gained much credit as a primary CV pathogenic factor [9, 10].

On the other hand, it cannot be excluded that extremely high FGF23 levels may cease to represent a compensatory phenomenon, displaying a direct pathogenic 'off-target' effect, possibly through a non-specific occupation of FGF receptors even in the absence of Klotho, given the exceedingly high concentrations occurring at the latest stages of CKD [6, 11, 12].

However, even if this is the case, uncertainties would still remain on what the FGF23 level threshold is above which the pathogenic role starts to be evident. In fact, looking at the results of the study by Faul *et al.* [6], the FGF23 levels associated with a pathogenic effect on cardiac mass in the experimental models were manifoldly higher than those found in the clinical observational section of the same study.

Overall, when the effects of FGF23 have been blocked with monoclonal anti-FGF23 antibodies in an experimental animal model of CKD, even if hyperparathyroidism was better controlled, the net result was a net increase in animal mortality [13], these data cast some doubt on the putative direct pathogenic effect of FGF23.

However, a basic limitation in making clear any message on the role of the circulating levels of FGF23 is represented by the lack of a widely accepted gold-standard method for dosing this hormone. In fact, circulating FGF23 is present as both intact-

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FGF23 and C-terminal fragment(s). There are available assays for measuring all these forms, however most of the published studies have limited themselves to use only one of these methods. Just citing the most relevant limitations related to this point, it is worth stressing that it is well accepted that the full activity of FGF23 is held by the intact molecule, while conflicting results have been reported on the possible activity of the C-terminal fragment(s), since some authors reported on a possible maintained activity [14] while others suggested a potential antagonist effect of C-terminal fragment(s) on the main activity of the full moiety [15]. Second, since there are multiple available assays for the measurement of the intact molecule, it has been clearly shown that there is limited comparability among the different available assays measuring either the intact or the C-terminal molecule [16, 17]. Furthermore, critical preanalytical problems have been reported, with a trend to an increase and a reduction of the C-terminal and intact molecule, respectively, when the sample is maintained at room temperature for >30 min in the absence of protease inhibitors [18].

To complicate the interpretation of FGF23/Klotho system, recent studies suggest that quite similar analytical problems might be encountered for the assessment of circulating levels of Klotho too [19, 20].

It is well recognized that vascular calcification in CKD patients is an active and cell-regulated process driven by a great number of putative causal factors inducing local phenotypic transdifferentiation of vascular smooth muscle cells towards an osteochondrogenic phenotype [21].

Recent studies suggested that FGF23 could also play some role in the CV disease of CKD patients, showing that FGF23 can be expressed both in cardiomyocytes and in the calcified aorta wall, suggesting that in addition, and probably independently of the circulating levels of FGF23, the tissue expression of this protein might play a potential direct pathogenic role [22, 23]. In this issue of the Nephrology, Dialysis and Transplantation journal, van Venrooij *et al.* [24] report on the expression of FGF23 in the coronary arteries of the explanted hearts in more than half of the 50 patients who received a heart transplantation. The renal function was lower in patients whose coronary arteries expressed FGF23 than in patients with no FGF23 coronary expression, though part of the positive patients had completely normal renal function. Another important finding of this study was that FGF23 protein was co-expressed with its main receptors (FGFR-1 and FGFR3) and its co-receptor Klotho. Furthermore, the degree of FGF23 expression was positively correlated with coronary calcium score and with the expression of a specific osteocyte protein (DMP1). Interestingly, the expression of FGF23 was mainly evident in infiltrating macrophage cells. Other authors had recently reported on an association between circulating FGF23 levels and markers of inflammation in CKD patients [25], the potential links between FGF23 and inflammation being manifold (Figure 1). These hypothetical links are mainly pertinent to systemic inflammatory conditions. However, at variance with these previous studies, the results from van Venrooij *et al.* seem to suggest that FGF23 expression in the coronary wall is mainly associated with local more than systemic inflammation, given that the authors did not find any difference in systemic inflammatory markers between patients with and without FGF23 expression in the coronary arteries. These results fit well with those from a recent study which demonstrated that the accumulation of macrophage foam cells in established atherosclerotic lesions is mainly derived from 'in loco' macrophage proliferation within the lesion and not from monocyte recruitment from circulation [26].

However, these results raise new questions which are far from being answered. The first point to be clarified regards whether the increased, either circulating or local, levels of FGF32 are a primary or secondary event in relationship to the vascular inflammation and/or to the calcification process. The second point to be demonstrated is whether increased FGF23 plays a promoting or a protective role in the vascular disease. The third point to be clarified is whether the FGF23, secreted by the infiltrating macrophages, actively contributes to these

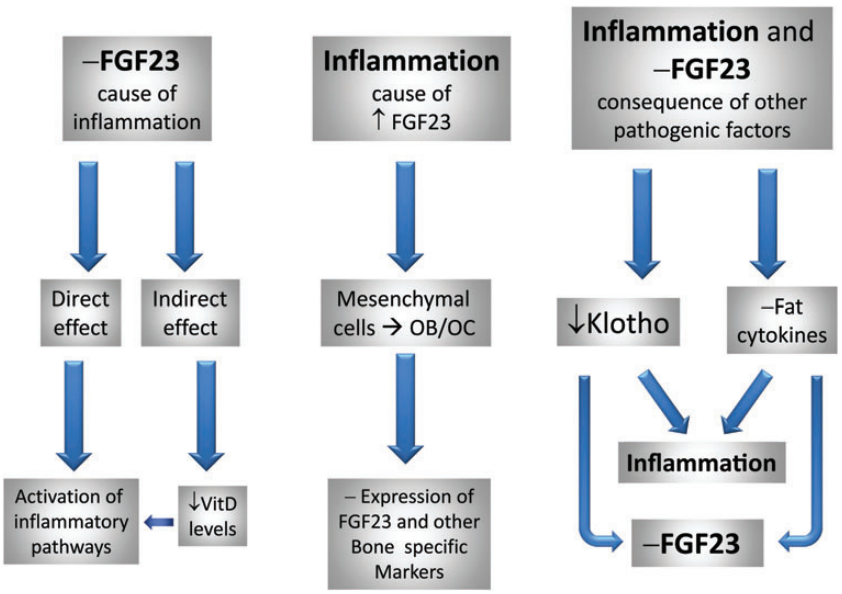

FIGURE 1: Potential relationships between FGF23 and inflammation: OB, osteoblast; OC, osteocyte.

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roles or if it simply represents a marker of a trans-differentiation process of macrophage itself toward an osteochondrogenic cell. Furthermore, a point to be addressed concerns whether this extra-skeletal production of FGF23 can contribute to its increased circulating levels in the same way of other pathological conditions (e.g. tumor-induced osteomalacic syndromes). Finally, it is not completely clear whether the potential positive or negative effects of FGF23 on the vascular wall are mediated or not by Klotho.

Overall, in addition to all the uncertainties listed in the previous paragraphs, it still remains to be ascertained which of the cardiovascular pathological conditions are mainly associated with higher FGF23 levels. In fact, while some studies reported an independent association between circulating FGF23 concentration and the severity and extent of coronary artery stenosis or with the progression of coronary calcifications [27, 28], other recent data seem to suggest a prevalent association of FGF23 levels with left ventricular mass and heart failure but not with ischaemic heart disease [29, 30].

In conclusion, the study from van Venrooij *et al.* sheds further light on this ever more complicated issue, though at the same time it also raises new unanswered questions.

As for any experimental study, we look forward to seeing replication of these unexpected and exciting results in further studies, and, if confirmed, future research could further clarify this increasingly complex issue.

Any new piece to be added in this tangled puzzle will be welcome.

CONFLICT OF INTEREST STATEMENT

The results presented in this paper have not been published previously in whole or part.

(See related article by van Venrooij *et al.* FGF23 protein expression in coronary arteries is associated with impaired kidney function. *Nephrol Dial Transplant* 2014; 29: 1525–1532.)

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