Vascular calcification in patients with end-stage renal disease

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Abstract
Vascular calcification is the most common type of extra-osseous calcification in end-stage renal disease (ESRD), manifesting as both medial and intimal calcification of large arteries. It is highly prevalent, often progressive and is associated with reduced arterial elasticity and increased mortality. Risk factors for calcification in ESRD include age, duration of dialysis, diabetes mellitus, most probably an elevated calcium–phosphorus product (Ca × P) level, the dose of calcium-containing phosphate binders and the induction of the systemic inflammatory response. Uraemic calcification was thought to be a largely physicochemical process facilitated by elevated Ca × P (i.e. 'metastatic' calcification). It is now well established, however, that vascular smooth muscle cells actively take up phosphate to form bioapatite. This process is associated with a phenotypic transformation of vascular smooth muscle cells during which they express osteoblast markers. In addition to phosphate, various other factors are likely to increase bioapatite formation, e.g. lipids and inflammatory cytokines. There have also been relatively new insights relating to the role of endogenous inhibitors of calcification [i.e. matrix Gla protein and fetuin-A (α2-Heremans–Schmid glycoprotein)], in particular the downregulation of fetuin-A in systemic inflammation. Decreased serum fetuin-A has been shown to be associated with a reduced capacity to inhibit calcium phosphate precipitation in vitro and is predictive of mortality in dialysis patients. These new insights into pathogenesis may lead to better prevention and treatment of calcification (e.g. with calcimimetics, anti-cytokines, etc.). However, the only preventive approach to have been established prospectively to date is the replacement of calcium-containing phosphate binders with sevelamer HCl, a non-calcaemic phosphate binder. Yet, it remains unclear whether sevelamer HCl reduces vascular calcification by preventing episodes of hypercalcaemia and/or by reducing low-density lipoprotein (LDL)-cholesterol levels.

Keywords: calcification; calcimimetics; calcium; end-stage renal disease; phosphorus; sevelamer

Introduction
Calcification is an almost ubiquitous pathological process in patients with end-stage renal disease (ESRD). It can result in a range of pathologies including calcific uraemic arteriolopathy (formerly termed 'calciphylaxis'), extra-osseous soft tissue calcification and solid organ calcification. However, vascular and valvular calcification is the most common and perhaps the most clinically significant manifestation. In this review, the epidemiology, quantification and aetiology of calcification will be discussed with a particular focus on vascular calcification. Potential therapeutic approaches are also addressed.

Vascular calcification has been a concern since the early days of dialysis: radiological skeletal survey examinations in 1976 in patients with severe chronic renal disease showed an incidence of 30% in the 15- to 30-year-old age group and 50% in the 40- to 50-year-old group. The earliest and most common site of calcification was the ankles, followed in frequency by the abdominal aorta, feet, pelvis, and hands and wrists [1]. Autopsy findings in 1969 and 1977 also demonstrated widespread soft tissue calcification, including vascular calcification in 50–80% of haemodialysis (HD) patients [2,3].

Intimal vs medial vascular calcification
Vascular calcification may separately and independently involve particular regions within the vessel wall, namely the intima or the media. Intimal calcification occurs only within atherosclerotic plaques and is seen...
as early as the second decade of life, just after the fatty streak stage. It is a disease of ischaemia-related occlusion in which inflammatory plaques are present in the intima [4]. Risk factors for atherosclerosis are well known in the normal population (systemic hypertension, dyslipidaemia, diabetes mellitus and smoking) [5], but their role in uraemic atherosclerosis is less well established [6]. In mediasclerosis, also known as Mönckeberg’s sclerosis, muscular arteries undergo non-inflammatory medial calcification [7], which is generally asymptomatic. Mediasclerosis does, however, result in the loss of cushioning function in blood vessels, thus inducing pseudohypertension (e.g. systolic hypertension of the elderly), left ventricular hypertrophy and altered coronary perfusion [8,9]. Mediasclerosis commonly occurs in the peripheral arteries of the legs with normal ageing, but is markedly aggravated in uraemia and diabetes mellitus, i.e. two conditions that are associated with accelerated ageing processes of various tissues [7].

In uraemia, the notion that the predominant form of calcification is mediasclerosis, at least in relatively young patients, was confirmed recently by Moe and colleagues, who examined inferior epigastric arteries removed during renal transplantation. They observed moderate calcification in five of the 41 vessels tested and severe calcification in seven vessels [10]. In all cases, calcifications involved the media, and only two arteries exhibited both medial and intimal calcification. In another recent study, patients with predominantly intimal calcifications were older and characterized by the presence of ‘typical’ risk factors (e.g. smoking and dyslipidaemia), whereas patients with medial calcifications were younger and characterized by a longer duration of HD treatment and derangements in their calcium–phosphorus product (Ca × P) balance [11].

**Quantification of calcification**

Various non-invasive methods have been developed to detect and measure vascular and valvular calcifications. The two most valuable methods are electron beam computed tomography (EBCT) and multislice spiral CT [12] (Figure 1). Both techniques are gated to the electrocardiograph (ECG) so that an image can be taken at a specific point of the RR interval. Both of these techniques offer good spatial resolution, low motion artefacts and a low signal-to-noise ratio. Furthermore, both techniques can be used to calculate a semi-quantitative Agatston score (the product of the area of the plaque and a density coefficient) [13]. Historically, most studies assessing vascular calcification in uraemics have been performed using EBCT [14–16], whereas multislice spiral CT, which is now more widely available, has only recently been introduced for this purpose [17,18]. Important shortcomings of both CT-based imaging methods are that they cannot distinguish between calcifications of the vascular intima and media and that they cannot be employed in patients with atrial fibrillation. Other less frequently used methods include ultrasound, echocardiography, optical coherence tomography, digital subtraction angiography and magnetic resonance tomography.

Overall, these new, sensitive, non-invasive techniques have increased our ability to quantify calcification and have thus facilitated research in patients with ESRD. Their improved ability to detect and quantify calcifications may have also contributed to the impression that vascular calcification is a new epidemic, a notion that remains difficult, if not impossible, to substantiate given the lack of comparable studies before the 1990s.

**Clinical consequences of vascular calcification**

The haemodynamic and cardiac consequences of vascular calcification have been widely studied in ESRD patients. Guerin *et al*. [19], for example, showed that an increasing semi-quantitative calcification score was associated with a small increase in systolic blood pressure and a parallel decrease in diastolic blood pressure, i.e. higher pulse pressure. They related these effects to aortic pulse wave velocity, which increased from 9 m/s in the group with the lowest calcification scores to 13 m/s in the group with the most calcification. Calcified arteries become stiffer, causing increased pulse wave velocity. Waves therefore return more quickly from the periphery, causing the observed changes in blood pressure (Figure 2). By increasing left ventricular afterload, a high aortic pulse wave velocity is also associated with increased left ventricular mass [20], thereby providing a link between vascular calcification and left ventricular hypertrophy [19].

Vascular calcification is also linked with poor outcomes in ESRD patients. Baseline data from a study by Raggi *et al*. [14] show that previous myocardial infarction, angina and known coronary artery disease...
(CAD) are all more common in patients with higher calcification scores. Moreover, in a 5–6 year follow-up study, Blacher et al. [21] noted a strong association between the presence and extent of vascular calcifications and cardiovascular and all-cause mortality in ESRD patients.

Vascular calcification—risk factors

Early histological studies in the early 1970s, discussed above, had already shown that calcification is more prevalent in dialysis patients than in subjects with normal renal function and that it increases with age. Braun et al. [16] were the first to use EBCT to assess calcification in 49 HD patients and 102 non-HD patients, who were then stratified according to the presence of CAD. In those with normal renal function, there was some vascular calcification of the coronary arteries, but this was largely restricted to patients with CAD. This was, however, not the case in HD patients, who displayed a high degree of calcification even in the absence of clinical CAD.

Braun et al. [16] also demonstrated an age-related increase in calcification scores of coronary arteries in both groups, with an exaggerated rise seen in the HD group. Furthermore, the calcification score of aortic valves in the HD group increased markedly after only 1 year of follow-up, particularly in the 60- to 69-year-old age group. These results subsequently were extended to patients with childhood-onset chronic renal failure presently treated by dialysis or renal transplantation [15,22]. Both of these later studies showed a very high prevalence of coronary calcifications in HD patients aged >20 years, while levels of calcification were very low in almost all control subjects [15].

In addition to the duration of dialysis and age, a number of other risk factors for calcification have been identified, which are summarized in Table 1. These include diabetes mellitus, which is not unexpected given that mediasclerosis is typically associated with diabetes even in the absence of uraemia. Furthermore, several studies noted that the risk of calcification increases in the presence of hypercalcaemia, hyperphosphataemia or elevated Ca×P and/or the use of calcium-containing phosphate binders [14,15,22,23]. Others, in contrast, found no such association between the risk of calcification and the presence of hypercalcaemia, hyperphosphataemia or elevated Ca×P [16,19]. This may be related to sample sizes, in particular to the difficulty of relating a very long-term event such as calcification to parameters such as serum calcium or phosphorus, which may change rapidly and are rarely constant in a dialysis patient. Similarly, only a few studies on vascular or valvular calcification [22,23], but not others dealing with vascular calcification [15,16,19], were able to detect an association between calcification and intact parathyroid hormone (iPTH) levels. The importance of PTH for vascular calcification is also unclear in view of the fact that pronounced vascular calcification or even uraemic calcific arteriolopathy (‘calciphylaxis’) has been reported in the absence of hyperparathyroidism [24,25].

In the context of the potential aetiology of vascular calcification, several other risk factors associated with vascular calcification are noteworthy (Table 1). A number of studies noted an association of calcifications with the malnutrition, inflammation and
Atherosclerosis (MIA) syndrome or with serum parameters related to this, such as increased fibrinogen, C-reactive protein or hypoalbuminaemia [15,19, 21–23,26]. Dyslipidaemia was associated with more rapid progression of uraemic vascular/valvular calcification in some studies [27,28], but not in others [14,23].

Aetiology of vascular calcification

Vascular calcification, intimal and/or medial, can occur in the presence of normal renal function, for example in the course of atherosclerosis or diabetes mellitus. However, even a moderate decrease of the glomerular filtration rate (GFR) to 50 ml/min dramatically augments the prevalence and extent of vascular and valvular calcification [29]. Thus, it is clear that vascular calcification is multifactorial. However, a number of specific factors related to uraemia or dialysis may aggravate the problem, as highlighted below.

The role of phosphate

Phosphate appears to play an important role in the calcification process. For example, when normal human aortic smooth muscle cells were cultured for 9 days in a solution containing a normal clinical level of phosphate (1.4 mmol/l), no calcification was present. However, when the same cell line was exposed to a 2.0 mmol/l phosphate solution for the same period, bioapatite, a form of calcium–phosphate precipitation, was formed in association with the extracellular matrix [30]. The bioapatite formation by vascular smooth muscle cells can be completely inhibited by phosphonoformic acid, an antagonist of their neutral sodium–phosphate co-transporter [30,31] (Figure 3). This observation is probably the most convincing evidence available that calcium and phosphate precipitation is not just a passive physico-chemical ('metastatic') phenomenon, but an active cellular process involving increases in intracellular phosphate.

Increased intracellular phosphate appears to induce the formation of matrix vesicles via as yet unknown pathways [31]. Matrix vesicles, in turn, are known to be important in osteogenesis [32]. In addition, high intracellular phosphate downregulates typical smooth muscle cell genes and stimulates the production of core binding factor 1 (Cbfa-1), which is a central transcription factor in osteogenic differentiation [30]. Increased intracellular phosphate levels also contribute to various other osteoblast-like phenotypic changes of vascular smooth muscle cells, including the expression of alkaline phosphatase on their surface, production of calcium-binding proteins, such as osteocalcin and osteopontin, and the laying down of a collagen-rich extracellular matrix [31,33]. Moe et al. [10,34] subsequently have demonstrated in vivo that Cbfa-1, alkaline phosphatase and osteopontin are present in calcified arteries, but are absent from the vessel wall of non-calcified arteries.
However, in addition to high phosphate in uraemia, other factors that are present in uraemic serum apparently also affect the phenotypic transformation of vascular smooth muscle cells. Moe et al. [34] found in vitro that uraemic serum upregulated the expression of Cbfa-1 in vascular smooth muscle cells independently of phosphate levels or blockade of the sodium–phosphate co-transporter. This suggests the presence of, as yet, unidentified modulators of calcification, which are not present in normal serum.

Other modulators of calcification

Blood lipids have been suggested as possible modulators of calcification. In vitro, minimally oxidized, low-density lipoprotein (LDL) and several other lipid oxidation products increased vascular smooth muscle cell calcification [35], while high-density lipoprotein (HDL) inhibited spontaneous and IL-1/IL-6-induced calcification [36]. In vivo, the reduction of total cholesterol slowed the progression of coronary artery calcification in non-ESRD patients [37]. However, in vivo evidence for a role for lipids in uraemic calcification is less clear. Low levels of HDL-cholesterol and high levels of LDL-cholesterol have been linked with arterial stiffening [20]. Nevertheless, as mentioned above, epidemiological studies on the association of dyslipidaemia with uraemic calcifications are contradictory [14,23,27], and low cholesterol even predicts cardiovascular mortality in ESRD patients, a phenomenon termed ‘reverse epidemiology’ [6,38].

In contrast to lipids, the concept of inflammation as a protagonist of calcification in ESRD is reasonably well supported by epidemiological studies (Table 1) and in vitro observations. In vitro, monocyte- and macrophage-derived tumour necrosis factor (TNF)-α and oncostatin-M have been demonstrated to induce alkaline phosphatase production and calcification in vascular smooth muscle cells [39,40]. Furthermore, the effect of inflammation in contributing to both osteoporosis and vascular calcification may explain the correlation between increased calcification scores and decreased bone mineral densities observed in dialysis patients [16].

Finally, links have been made in vitro with a number of other potential modulators of calcification in ESRD. For example, increased calcification has been associated with calcitriol levels [41], diabetes, advanced glycation end-products [42], leptin [43] and with some genetic factors [44–46]. Other factors that have been found to decrease rates of calcification include PTH and PTH-related peptide (PTHrP) [47], osteopontin [48], osteoprotegerin [49] and bone morphogenic protein (BMP)-7 [50]. The relevance of these factors for uraemic calcifications in vivo currently is unknown.

Inhibitors of calcification

When calcium and phosphate are mixed at concentrations that are equivalent to high serum levels, they form an insoluble precipitate—calcium phosphate. Clearly, this does not happen in vivo, suggesting the presence of natural inhibitors of calcification.

Matrix Gla protein (MGP), a vitamin K-dependent protein, was the first inhibitor of vascular calcification to be discovered. In a study of MGP-knockout mice, which developed severe medial calcification and died of aortic rupture [51], staining for mineralization using alizarin red showed that their aortas were entirely calcified. In humans, MGP has been found in
atherosclerotic plaques [52], with the highest concentrations being observed in areas surrounding calcifications. At least in the aorta, MGP may therefore represent a major local inhibitor of calcification, its role being to limit excessive plaque calcification.

A major systemic inhibitor of calcification has also been recently identified called fetuin-A or α2-Heremans–Schmid glycoprotein (AHSG). In vitro, fetuin-A has been shown to be a highly potent inhibitor of hydroxyapatite formation and to reduce crystal formation in solutions containing calcium and phosphate [53]. Furthermore, when fetuin-A-knockout mice were generated, all mouse tissues became severely calcified [54].

In humans, serum fetuin-A levels were found to be significantly lower in both long- and short-term HD patients, compared with controls who had normal renal function. Moreover, serum from dialysis patients was less efficient at inhibiting calcium phosphate crystal formation than normal serum, an effect that was reversed by the addition of fetuin-A [18]. Finally, fetuin-A was identified as an independent predictor of all-cause and cardiovascular mortality, with low fetuin-A levels being associated with reduced survival [18]. Fetuin-A and its regulation may therefore be of interest as a novel therapeutic target (Figure 4).

**Therapeutic approaches**

Existing treatment and prevention options for calcification include modifying calcium load and the use of phosphate binders. Sevelamer HCl, a phosphate-binding polymer, has been demonstrated to be more effective than calcium-containing phosphate binders at slowing the progression of calcification in coronary arteries and the aorta [55]. A randomized study comparing the effects of sevelamer with calcium-based phosphate binder showed that although there were no significant differences between phosphorus levels in both groups and only a small difference in calcium levels, the group treated with the calcium-containing phosphate binder had significantly more episodes of hypercalcaemia [55]. It should be noted, however, that in addition to its phosphate-binding effects, sevelamer is a bile acid sequestrant. Indeed, in the above study, patients assigned to sevelamer had lower LDL-cholesterol than controls. Thus, it remains to be determined whether the effects of sevelamer on calcification relate to its prevention of hyperphosphataemia/hypercalcaemia or to its lipid-lowering properties. Intervention studies to distinguish between the role of phosphorus and lipids in uraemic vascular calcification are ongoing.

The possible aetiological mechanisms described above may lead to the development of novel approaches to treat and prevent calcification in dialysis patients. Calcimimetics, vitamin D analogues and bisphosphonates may provide alternative ways to modify calcium and phosphorus levels, i.e. lower Ca × P, while the control of lipid levels with statins or bile acid sequestrants may also have beneficial effects. Possible anti-inflammatory strategies could include the use of pyrogen-free dialysate and therapies to reduce

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**Fig. 4.** Relative fetuin-A deficiency: a novel mechanism contributing to uraemic extra-osseous calcification. Schematic graph of the regulation and effects of fetuin-A deficiency in dialysis patients. Besides inflammation-dependent fetuin-A downregulation, other putative factors including uraemic toxins, mode of dialysis treatment and genetic disposition may be involved, but will require further studies. Potential therapeutic modifiers and additional biological properties of fetuin-A as depicted in the graph were reviewed recently in Ketteler et al. [56].
cytokine levels. Finally, the synthesis of endogenous inhibitors of calcification, such as fetuin-A, could also offer an insight into novel therapeutic agents.

Conflict of interest statement. None declared.

References


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