Pediatr Nephrol (2003) 18:969–975
DOI 10.1007/s00467-003-1276-0

EDITORIAL COMMENTARY

**Sharon M. Moe · Neal X. Chen**

# Calciphylaxis and vascular calcification: a continuum of extra-skeletal osteogenesis

Received: 24 April 2003 / Revised: 4 July 2003 / Accepted: 8 July 2003 / Published online: 21 August 2003


**Abstract** Calciphylaxis, more appropriately termed calcific uremic arteriolopathy, is a clinical syndrome of skin necrosis due to medial calcification of the small arterioles. While this disease has been described as a distinct entity, we believe it is but a single manifestation of a complicated systemic process that leads to vascular calcification in both the intimal and medial layers of all forms of arteries in patients with chronic kidney disease. The pathophysiology of vascular calcification includes injury leading to vascular smooth muscle cell de-differentiation to osteoblast-like cells, a nidus "formation" of matrix proteins with and without apoptotic bodies, initiation and extension of mineralization, and a balance of pro-calcification factors and inhibitory calcification factors. The clinical manifestations depend upon the location of the affected artery. The rationale behind this hypothesis is discussed in this review.

**Keywords** Calciphylaxis · Calcific uremic arteriolopathy · Vascular calcification · Dialysis · Chronic kidney disease · Core-binding factor α-1

## Introduction

The first case of calciphylaxis was described in 1898 [1]. The term "calciphylaxis" was coined by Hans Selye in a book of his lifetime work published in 1962. He described an animal model in which he was able to induce calcification of tissues with a sensitizer [vitamin D, parathyroid hormone (PTH), diet high in calcium and phosphorus, renal failure], followed by a "critical time" interval before a challenger (iron salts, steroids, trauma, egg albumin). While this extra-skeletal calcification model was generated by many of the clinical and mineral metabolism risk factors identified in dialysis patients with extra-skeletal calcification, the pathological lesions were calcification of the dermis, not of the arterioles [2]. In patients with chronic kidney disease (CKD), calciphylaxis lesions are usually located in two distinct patterns: distal, with lesions on the lower extremities, or proximal, with lesions on the abdomen, inner thighs, and buttocks [3]. Calciphylaxis is characterized by lesions that present as painful red nodules, often with livedo reticularis that progress to ulcers with necrotic centers and violaceous borders. Histologically, there is vascular calcification of small arterioles (usually 40–50 μm) in the medial layer with resultant ischemic epidermolysis. In general, the lesions are not associated with an inflammatory cell infiltrate, and only rarely is there thrombosis and intimal layer changes. The mineral that is deposited is composed of hydroxyapatite (calcium phosphate) [4]. Thus, the calcification is in arterioles, not dermis, in contrast to the calcification model of Selye. As a result of these pathological differences, Coates et al. [4] (and we concur) recommended using the term calcific uremic arteriolopathy (CUA) to describe the clinical disease observed in dialysis patients. Recently, there has been an increase in the number of publications about CUA, but whether this represents an increased incidence or renewed interest is unknown.

Unfortunately, most of the literature on CUA consists of case reports or case series. The differential diagnosis of CUA includes calcinosis cutis (calcification in skin, not vessels, truly Selye's calciphylaxis), panniculits, often associated with pancreatitis, subcutaneous fat necrosis of the newborn, pseudoxanthoma elasticum, scleroderma, and porphyria cutanea tarda [5]. It is important to note that dialysis is not a prerequisite, nor is renal failure, as there have been isolated case reports in patients with a functioning renal allograft [3, 6] and in primary hyperparathyroidism [7]. Several early case series identified elevated parathyroid hormone as a key risk factor, leading to the recommendation that patients with

S. M. Moe (✉) · N. X. Chen
Indiana University School of Medicine,
1001 W. 10th Street; OPW 526, Indianapolis, IN 46202, USA
e-mail: smoe@iupui.edu
Tel.: +1-317-2782868, Fax: +1-317-2782860

S. M. Moe
Roudebush Veterans Affairs Medical Center,
Indianapolis, Indiana, USA

CUA undergo a parathyroidectomy [3, 6, 8]. In particular, Hafner et al. [8] in a large review of the literature reported that 38 of 58 patients that underwent parathyroidectomy survived, whereas only 13 of 37 without parathyroidectomy survived ($P<0.01$). This same paper reported that the survival was 75% for those patients with the distal form of the disease and only 26% for those with the proximal form of the disease [8]. Another review of the literature noted that hyperparathyroidism was found in 82% of cases compared with hyperphosphatemia in 68%, hypercalcemia in 20%, and elevated calcium×phosphorus product greater than 70 $mg^2/dl^2$ in 33% [9]. However, Mawad et al. [10] recently reported 7 cases from 100 dialysis patients; 5 of these 7 patients had biopsy-proven adynamic bone disease, clearly demonstrating that elevated PTH is not required. Several medications have been identified as potential culprits in the etiology of CUA, including Coumadin (sodium warfarin) [11], insulin injections [12], calcium-based phosphate binders [13, 14], and intravenous iron dextran [15].

Recently, there have been four case-control series that have helped further clarify these risk factors. The first, by Bleyer et al. [16] examined 9 cases of pathologically proven proximal CUA over 6 years (excluding 1 case of distal CUA), and compared these patients with their remaining dialysis patient population of 347 patients. We performed a similar case-control study examining 10 cases of pathologically proven CUA from a dermatopathology database over 5 years (both proximal and distal lesions), and compared these patients with our dialysis patient population of 235 patients [17]. Mazhar et al. [18] identified 19 cases of calciphylaxis, 16 of which had pathologically proven disease. One patient had a functioning transplant; the others were undergoing hemodialysis. This study compared the CUA cases with controls (3 per case) from their hemodialysis center, matched for the date of initiation of hemodialysis [18]. Lastly, Fine and Zacharias [19] reported 36 new cases of CUA in 7 years, the majority of which were early, nonulcerating lesions, noting an increasing incidence rate of 4.5/1,000 patient-years in the years 1998–2001. They compared these "plaque only" cases with 2 controls per case, controlled for dialysis modality, duration of dialysis, and gender [20]. The results of these four case-control studies [16, 17, 18, 19] demonstrate female gender, Caucasian race, and obesity as clear risk factors. In contrast, diabetes was a risk factor in two of the four series, and Coumadin was not significantly associated with CUA, although case-control studies do have limitations. The mortality ranged from 45% to 65%. Analysis of the biochemical abnormalities in these case-control studies demonstrates that hyperphosphatemia and hypoalbuminemia are associated risk factors, whereas hyperparathyroidism and hypercalcemia are not associated with CUA (Fig. 1).

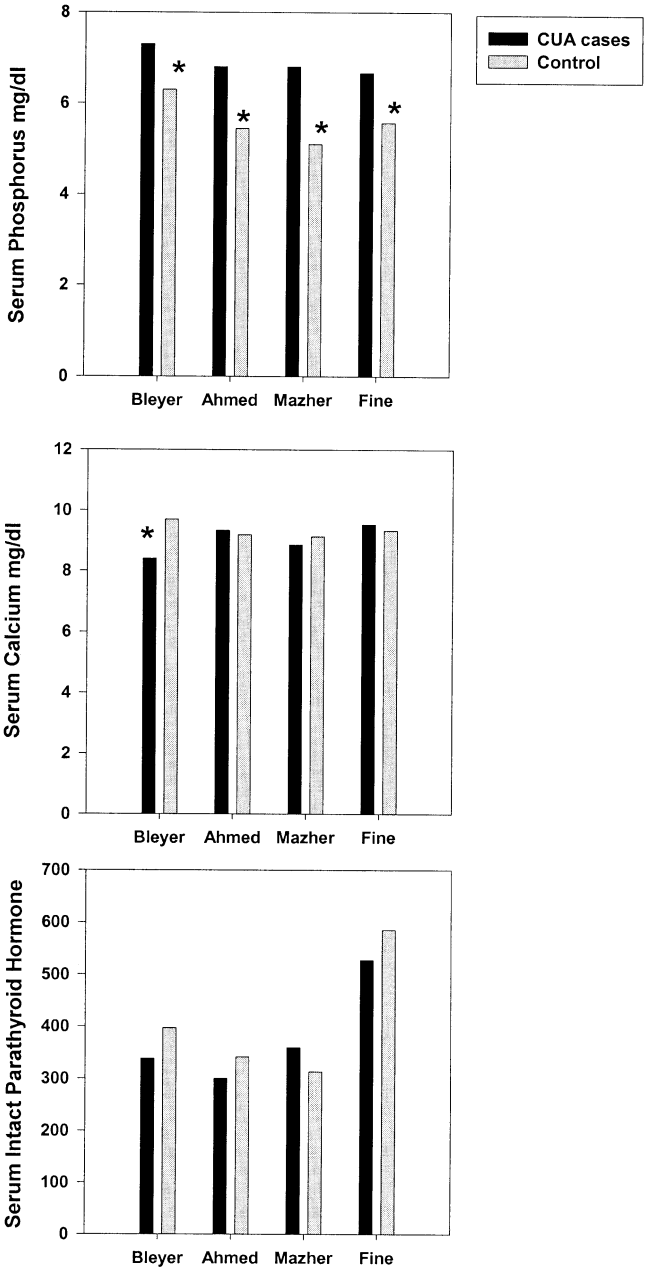


**Fig. 1** Role of mineral metabolism in the pathogenesis of calcific uremic arteriolopathy (CUA, calciphylaxis). Data from four case-control studies [16, 17, 18, 20] are presented with results for serum levels of phosphorus (*top panel*), calcium (*middle panel*), and parathyroid hormone (*lower panel*). Serum phosphorus levels were higher in CUA patients compared with control patients in all four studies. In contrast, the serum levels of calcium were lower in CUA than control patients only in the study by Bleyer et al. [16]. Similarly, there was no difference in the intact parathyroid hormone levels in the CUA patients and control patients in any of the studies. (*$P<0.05$ comparing CUA patients with control patients within a given study)

Vascular calcification is not a new phenomenon in patients with CKD. In 1979 Ibels et al. [21] demonstrated that patients undergoing a renal transplant had increased intimal thickness and calcification, and increased medial thickness and calcification, of internal iliac and renal arteries compared with transplant donors and autopsy subjects. Furthermore, the magnitude of calcification quantified by biochemical techniques correlated with duration

of dialysis [21]. In 2000, Schwarz et al. [22] demonstrated increased calcification of atherosclerotic plaques in dialysis patients at autopsy compared with non-dialysis patients, but the volume of plaque was not different. In addition, the medial layer was markedly thickened compared with patients not on dialysis. New imaging techniques, such as electron beam computed tomographic scan (EBCT), have demonstrated markedly increased coronary artery calcification in dialysis patients compared with non-uremic patients with angiographically proven coronary artery disease [23]. This process also occurs in children and young adults [24]. In 2002, we demonstrated medial calcification in the inferior epigastric artery of many asymptomatic patients undergoing a renal transplant in association with expression of bone-specific factors [25, 26]. Thus, calcification of both intimal/atherosclerotic lesions and the medial layer of arteries are increased in patients with CKD.

## Is CUA simply another manifestation of vascular calcification?

There are case reports of CUA, defined as medial calcification of small arterioles, leading to gastrointestinal hemorrhage [4, 27, 28] and penile gangrene [29]. Pathologically, the arteries involved in these unusual clinical sequelae of CUA have medial calcification indistinguishable from skin lesions of CUA and from the medial calcification we observed in the inferior epigastric artery [25]. Furthermore, this medial calcification is also pathologically the same as that in distal peripheral arteries, the so called Mönckeberg calcification seen in individuals with diabetes, which is associated with increased cardiovascular mortality [30]. Even calcification of small arteries in the breasts of individuals without renal failure correlates with cardiovascular mortality [31, 32]. Clearly, calcification of arteries, whether arterioles of the skin, small arteries in the abdomen or breast, medium arteries in the peripheral extremities, the aorta, or coronary arteries is pathological, and is a marker for disease. The precise clinical manifestation may be due to the location of the abnormal artery and the tissue it perfuses. In addition, there is likely some sort of systemic process or disease that leads some individuals to calcify their arteries and others not. However, in CUA the lesions appear more rapidly progressive and may cause greater mortality than in many other forms of vascular calcification. This may be because small arterioles are the final point of blood supply and therefore collateral blood flow is not an alternative, or there is a systemic process that rapidly depletes the body of naturally occurring inhibitors of mineralization.

Although arterial calcification has been recognized for more than 150 years [33], the basic pathogenesis of this disorder is still not completely understood. The work of Stary et al. [34] in mammals and in human autopsy studies demonstrated that calcification in atherosclerotic (intimal) disease appears to be a late process, occurring well after the intimal plaque. However, in medial calcification, the calcification is the first obvious sign of disease, although medial wall thickening may precede the mineral deposition. So how can intimal/atherosclerotic calcification and medial calcification processes be related? Until recently, the actual calcification of the vascular tissue had been regarded as a passive process associated with atherosclerosis or normal aging, resulting from a non-specific response to tissue injury or necrosis. Now there is strong evidence that vascular calcification is an active and regulated process that shares features with normal embryonic bone formation and bone repair [17, 25, 35, 36, 37, 38, 39, 40, 41, 42]. Bones form through endochondral ossification with a cartilage intermediate, or by direct intra-membranous ossification from condensations of mesenchymal cells. Osteoblasts differentiate from a pluripotent mesenchymal stem cell to a mature osteoblast with different morphology, and gene and matrix protein production in each phase of differentiation, bone formation, and mineralization. The latter is felt to occur through blebs from osteoblasts called matrix vesicles that contain hydroxyapatite, which are guided to areas by collagen and non-collagenous matrix proteins [43].

These same mesenchymal stem cells form vascular smooth muscle cells (VSMC). Work in the last decade has demonstrated that VSMC and/or vascular pericytes are capable of producing bone-like proteins in cell culture [44, 45]. Additional experiments in both human and bovine VSMC have demonstrated that phosphorus, in the form of β-glycerophosphate (which is cleaved by alkaline phosphatase to form free phosphate), can induce calcification similar to that observed in osteoblast cultures [46, 47]. To examine the pathophysiology of vascular calcification observed in dialysis patients, we examined arteries histologically [17, 25]. We found expression of osteopontin at the base of the calcium spicules in skin arterioles with calcification from patients with CUA, but no expression of osteopontin in arterioles without calcification in the same section [17]. We also found evidence of other bone proteins, including osteonectin and bone sialoprotein (unpublished observation), and evidence for matrix vesicles in CUA specimens [17]. We then prospectively evaluated inferior epigastric arteries from patients with CKD stage 5 undergoing renal transplantation [25]. Many of these arteries demonstrated calcification in the form of medial calcinosis in association with the expression of the "bone" matrix proteins osteopontin, alkaline phosphatase, type I collagen, and bone sialoprotein. Positive immunostaining for these bone proteins was found more frequently than was overt calcification, which suggests that the deposition of these proteins precedes calcification [25]. These findings suggest that the initial changes that occur in the vessels of dialysis patients are the deposition of these bone matrix proteins into the medial layer, followed by calcification. These results confirm a cell-mediated process in vascular calcification in patients with end-stage renal disease (ESRD). This is similar to findings in vessels of non-dialysis pa-

tients with both calcified atherosclerotic coronary arteries [35, 36, 48, 49, 50] and medial calcinosis in small distal vessels [38]. The presence of bone proteins in all forms of vascular calcification was also recently confirmed by Canfield et al. [51] who found osteopontin and cartilage oligomeric matrix protein in arteries from patients with CUA, atherosclerosis, and chronic vascular rejection in renal allografts, but no evidence for staining in normal arteries.

## What makes vascular calcification so prominent in dialysis patients?

Dialysis patients are known to have many vascular risk factors, such as a history of cardiac disease, diabetes, elevated levels of homocysteine and oxidized lipids, and hypertension. In addition to these traditional vascular risk factors, there is increasing evidence that elevated serum phosphorus, serum calcium×phosphorus product, and/or calcium load in the form of calcium-containing phosphate binders are associated with various vascular end-points including CUA [17], coronary artery calcification by EBCT [24], carotid and aortic calcification [40, 52], and hemodynamic abnormalities [53]. These studies suggest a relationship between these laboratory values and positive calcium and phosphorus balance with vascular disease. Chertow et al. [54] recently demonstrated in a prospective randomized trial that the non-calcium phosphate binder sevelamer can arrest coronary artery and aorta vascular calcification in ESRD patients, whereas calcium-based phosphate binders increased calcification in both coronary arteries and aorta. We and others have also demonstrated a healing of CUA lesions in patients with removal of calcium-containing phosphate binders [14, 55]. Jono et al. [56] demonstrated that phosphorus-induced calcification was dependent on the sodium-phosphate (Na/Pi) co-transporter. Furthermore, exogenous phosphate added to human VSMC culture upregulated core-binding factor α-1 (Cbfa1) expression [56], a transcription factor critical for osteoblast differentiation and the expression of the bone matrix proteins osteopontin, osteocalcin, and type I collagen [57]. Cbfa1 knock-out mice fail to form mineralized bone, proving that Cbfa1 is critical for the initial differentiation of osteoblasts [58], thus indicating that VSMC de-differentiate to osteoblast-like cells. In addition, arteries from the matrix gla protein knock-out mice lose smooth muscle markers and gain expression of Cbfa1 as they progressively mineralize their arteries [59]. Thus, the in vivo data confirm a role for abnormal mineral metabolism in vascular calcification in ESRD patients, and in vitro data in VSMC indicate that phosphorus can lead to calcification, and that phosphorus can induce Cbfa1 and the expression of bone matrix proteins.

In order to further understand the mechanism by which kidney disease or the uremic state can induce vascular calcification, we incubated bovine VSMC in the presence of pooled normal human serum versus pooled human serum from patients on hemodialysis for at least 2 years (to eliminate residual renal function) [60]. Using these pooled sera in vitro, we demonstrated that uremic serum led to increased and accelerated calcification of VSMC. Furthermore, the uremic serum upregulated the expression of osteopontin in VSMC compared with normal serum. Unlike the exogenous addition of phosphorus in the form of β-glycerophosphate, the uremic serum-induced osteopontin was only partially dependent on both alkaline phosphatase and Na/Pi co-transport [60]. Of importance, the final media concentration of phosphorus was similar in the VSMC cultures with 10% normal and those with 10% uremic serum (~0.5 mM) [60], well below levels known to induce calcification in the study of Jono et al. (2.0 mM) [56]. We also found that uremic serum, compared with control human serum, induced the expression of Cbfa1 in bovine VSMC in a time-dependent, non-phosphorus-mediated mechanism. Furthermore, we have found ex vivo evidence of the expression of Cbfa1 in both medial and intimal VSMC in calcified inferior epigastric arteries obtained at the time of kidney transplant [26]. These results suggest that Cbfa1 may be a key regulatory factor in vascular calcification observed in dialysis patients. However, expression of Cbfa1 was also simultaneously observed in calcification of atherosclerotic plaques from patients without CKD [61]. These results suggest that multiple factors may lead to Cbfa1 expression, and that this expression is associated with calcification. Whether Cbfa1 is a marker of VSMC differentiation or a cause remains to be determined.

Thus, we hypothesize that vascular calcification in the course of dialysis may be a four-step process (Table 1). First, VSMC are stimulated by injury or uremic toxins [26], including phosphorus [56], oxidized low-density lipoproteins [62], and perhaps ischemic injury, to transform into osteoblast-like cells via upregulation of Cbfa1. In CUA, this injury may be ischemic due to altered blood flow in the pannus of obese women or injection sites. In intimal disease this injury may represent the diverse factors that lead to plaque formation. These cells then lay down a bone matrix of type I collagen and non-collagenous proteins. Some of the cells may also undergo apoptosis [63]. In step 2, this framework of matrix proteins with and without apoptotic bodies may act as a nidus for mineralization, similar to the recent observations in kidney stone formers [64]. In step 3, extension of the mineralization of this nidus/matrix occurs, in part through a physiochemical process due to supersaturation of serum and in part through a process "guided" by the matrix proteins and osteoblast-like cells. This step is likely to be accelerated in the presence of elevated calcium×phosphorus product in the serum, as well as positive calcium balance due to calcium-containing phosphate binders [24, 40, 54]. In addition, a lack of naturally occurring inhibitors likely plays a role. This includes decreased matrix gla protein, which is decreased in animals on Coumadin [65, 66], and fetuin-A, which is decreased in ESRD patients with acute inflammation, and in pa-

**Table 1** Proposed pathogenesis of vascular calcification in chronic kidney disease (*VSMC* vascular smooth muscle cells, *Cbfa1* core-binding factor α-1, *LDL* low-density lipoprotein, *CUA* calcific uremic arteriolopathy)

| Stage | Cause | Current therapeutic approach |
|---|---|---|
| 1. Injury leading to VSMC cellular de-differentiation to osteoblast-like cells via upregulation of Cbfa1 | Elevated phosphorus | Lower phosphorus |
| | Uremic toxins | Optimize dialysis, consider daily or nocturnal dialysis |
| risk factors | Oxidized LDL | Minimize or eliminate traditional vascular |
| | ?Ischemia | Antioxidant therapy [70] |
| | ?Hypertension | |
| 2. Nidus formation | Production of matrix proteins by de-differentiated VSMC | Unknown |
| | Production of atherosclerotic plaque | |
| | Apoptosis of VSMC | |
| 3. Mineralization initiation and extension | Balance of | Remove all calcium intake, including vitamin D |
| | Excessive Ca×P, increased phosphorus, calcium load from binders | Decrease inflammatory response |
| | Lack of naturally occurring inhibitors (fetuin, matrix gla protein) | Stop Coumadin |
| | Abnormal regulation of mineralization by matrix proteins | ?Bisphosphonates [69] |
| 4. Clinical manifestations | Ischemia of tissue from poor perfusion due to calcified vessel | Wound care for CUA |
| | | Antibiotics if infected |
| | | Surgery |
| | | Cardiovascular procedures |

tients with CUA [67, 68]. Thus, the mineralization step is a balance of the pro-mineralization and anti-mineralization forces at the cellular level. The fourth stage is the resultant ischemia of the tissue perfused by the calcified artery. In the case of CUA, the damage is skin necrosis due to calcification of the skin arterioles. In the case of coronary artery disease, the damage is an acute myocardial infarction with occlusion of the coronary artery or sudden death when the calcified distal vessels fail to supply extra oxygen needed during stress. We believe these are all manifestations of vascular calcification, a continuum of extra-skeletal osteogenesis exacerbated by injury and aggravated by the uremic milieu.

Unfortunately, definitive therapies for these manifestations of vascular calcification, including CUA, are lacking. At present, prevention appears to be the only hope, as no studies have demonstrated an ability to modulate, or remove, the mineral once it is deposited. Unfortunately, therapy at present is limited (Table 1), but should include (1) reducing traditional and non-traditional vascular disease risk factors, (2) minimizing mineral overload by aggressive control of serum phosphate with non-calcium binders or increased dialysis frequency and decreasing vitamin D use, and (3) aggressive wound care. In addition, optimizing naturally occurring inhibitors such as fetuin-A by decreasing inflammation and bisphosphonate therapy (at least in animal models [69]), and stopping Coumadin therapy to increase matrix gla protein remain theoretical at present. Nevertheless, while we strive to understand the pathophysiology of this complicated process, we owe it to our patients to minimize cardiovascular risk factors that may lead to initial injury and avoid positive calcium and phosphorus balance that add further insult to injury.

## References


1. Bryant JH, White WH (1898) A case of calcification of the arteries and obliterative endarteritis, associated with hydronephrosis, in a child aged six months. Guys Hospital Rep 55:17–20
2. Selye H, Gabbiani G, Strebel R (1962) Sensitization to calciphylaxis by endogenous parathyroid hormone. University of Chicago Press, Chicago, pp 554–558
3. Chan YL, Mahony JF, Turner JJ, Posen S (1983) The vascular lesions associated with skin necrosis in renal disease. Br J Dermatol 109:85–95
4. Coates T, Kirkland GS, Dymock RB, Murphy BF, Brealey JK, Mathew TH, Disney AP (1998) Cutaneous necrosis from calcific uremic arteriolopathy. Am J Kidney Dis 32:384–391
5. Walsh JS, Fairley JA (1995) Calcifying disorders of the skin. J Am Acad Dermatol 33:693–706
6. Gipstein RM, Coburn JW, Adams DA, Lee DB, Parsa KP, Sellers A, Suki WN, Massry SG (1976) Calciphylaxis in man. A syndrome of tissue necrosis and vascular calcification in 11 patients with chronic renal failure. Arch Intern Med 136: 1273–1280
7. Anderson DC, Stewart WK, Piercy DM (1968) Calcifying panniculitis with fat and skin necrosis in a case of uraemia with autonomous hyperparathyroidism. Lancet II:323–325
8. Hafner J, Keusch G, Wahl C, Sauter B, Hurlimann A, Weizsacker F von, Krayenbuhl M, Biedermann K, Brunner U, Helfenstein U (1995) Uremic small-artery disease with medial calcification and intimal hyperplasia (so-called calciphylaxis): a complication of chronic renal failure and benefit from parathyroidectomy. J Am Acad Dermatol 33:954–962
9. Budisavljevic MN, Cheek D, Ploth DW (1996) Calciphylaxis in chronic renal failure. J Am Soc Nephrol 7:978–982
10. Mawad HW, Sawaya BP, Sarin R, Malluche HH (1999) Calcific uremic arteriolopathy in association with low turnover uremic bone disease. Clin Nephrol 52:160–166
11. Rudwaleit M, Schwarz A, Trautmann C, Offermann G, Distler A (1996) Severe calciphylaxis in a renal patient on long-term oral anticoagulant therapy. Am J Nephrol 16:344–348
12. Ruggian JC, Maesaka JK, Fishbane S (1996) Proximal calciphylaxis in four insulin-requiring diabetic hemodialysis patients. Am J Kidney Dis 28:409–414

13. Campistol JM, Almirall J, Martin E, Torras A, Revert L (1989) Calcium-carbonate-induced calciphylaxis. Nephron 51:549–550
14. Russell R, Brookshire MA, Zekonis M, Moe SM (2002) Distal calcific uremic arteriolopathy in a hemodialysis patient responds to lowering of Ca x P product and aggressive wound care. Clin Nephrol 58:238–243
15. Braden G, Goerdt P, Pekow P, O'Shea M, Mulhern J, Sweet S, Slater J, Madden R, Lipkowitz G, Germain M (1997) Calciphylaxis (C) in hemodialysis (HD) patients: patient profiles and temporal association with IV iron dextran (abstract). Abstracts of the American Society of Nephrology Annual Meeting
16. Bleyer AJ, Choi M, Igwemezie B, Torre E de la, White WL (1998) A case control study of proximal calciphylaxis. Am J Kidney Dis 32:376–383
17. Ahmed S, O'Neill KD, Hood AF, Evan AP, Moe SM (2001) Calciphylaxis is associated with hyperphosphatemia and increased osteopontin expression by vascular smooth muscle cells. Am J Kidney Dis 37:1267–1276
18. Mazhar AR, Johnson RJ, Gillen D, Stivelman JC, Ryan MJ, Davis CL, Stehman-Breen CO (2001) Risk factors and mortality associated with calciphylaxis in end-stage renal disease. Kidney Int 60:324–332
19. Fine A, Zacharias J (2002) Calciphylaxis is usually non-ulcerating: risk factors, outcome and therapy. Kidney Int 61:2210–2217
20. Fine A (2002) Relevance of C-reactive protein levels in peritoneal dialysis patients. Kidney Int 61:615–620
21. Ibels LS, Alfrey AC, Huffer WE, Craswell PW, Anderson JT, Weil Rd (1979) Arterial calcification and pathology in uremic patients undergoing dialysis. Am J Med 66:790–796
22. Schwarz U, Buzello M, Ritz E, Stein G, Raabe G, Wiest G, Mall G, Amann K (2000) Morphology of coronary atherosclerotic lesions in patients with end-stage renal failure. Nephrol Dial Transplant 15:218–223
23. Braun J, Oldendorf M, Moshage W, Heidler R, Zeitler E, Luft FC (1996) Electron beam computed tomography in the evaluation of cardiac calcification in chronic dialysis patients. Am J Kidney Dis 27:394–401
24. Goodman WG, Goldin J, Kuizon BD, Yoon C, Gales B, Sider D, Wang Y, Chung J, Emerick A, Greaser L, Elashoff RM, Salusky IB (2000) Coronary-artery calcification in young adults with end-stage renal disease who are undergoing dialysis. N Engl J Med 342:1478–1483
25. Moe SM, O'Neill KD, Duan D, Ahmed S, Chen NX, Leapman SB, Fineberg N, Kopecky K (2002) Medial artery calcification in ESRD patients is associated with deposition of bone matrix proteins. Kidney Int 61:638–647
26. Moe SM, Duan D, Doehle BP, O'Neill KD, Chen NX (2003) Uremia induces the osteoblast differentiation factor Cbfa1 in human blood vessels. Kidney Int 63:1003–1011
27. Brown DF, Denney CF, Burns DK (1998) Systemic calciphylaxis associated with massive gastrointestinal hemorrhage. Arch Pathol Lab Med 122:656–659
28. Rivera-Nieves J, Bamias G, Alfert J, Bickston SJ, Moskaluk CA, Cominelli F (2002) Intestinal ischemia and peripheral gangrene in a patient with chronic renal failure. Gastroenterology 122:495–499
29. Jenkins MA, Pirani JF, Conner WT (2001) Calciphylaxis presenting as penile gangrene. Infect Urol 14:105–109
30. Lehto S, Niskanen L, Suhonen M, Ronnemaa T, Laakso M (1996) Medial artery calcification. A neglected harbinger of cardiovascular complications in non-insulin-dependent diabetes mellitus. Arterioscler Thromb Vasc Biol 16:978–983
31. Noord PA van, Beijerinck D, Kemmeren JM, Graaf Y van der (1996) Mammograms may convey more than breast cancer risk: breast arterial calcification and arterio-sclerotic related diseases in women of the DOM cohort. Eur J Cancer Prev 5:483–487
32. Crystal P, Crystal E, Leor J, Friger M, Katzinovitch G, Strano S (2000) Breast artery calcium on routine mammography as a potential marker for increased risk of cardiovascular disease. Am J Cardiol 86:216–217
33. Virchow R (1971) Sclerosis and ossification of arteries. In: Cellular pathology: as bases upon physiological and pathological histology. Dorer, New York, pp 404–408
34. Stary HC (2000) Natural history of calcium deposits in atherosclerosis progression and regression. Z Kardiol 89 [Suppl 2]:28–35
35. Bostrom K, Watson KE, Horn S, Wortham C, Herman IM, Demer LL (1993) Bone morphogenetic protein expression in human atherosclerotic lesions. J Clin Invest 91:1800–1809
36. Fitzpatrick LA, Severson A, Edwards WD, Ingram RT (1994) Diffuse calcification in human coronary arteries. Association of osteopontin with atherosclerosis. J Clin Invest 94:1597–1604
37. Mori K, Shioi A, Jono S, Nishizawa Y, Morii H (1999) Dexamethasone enhances in vitro vascular calcification by promoting osteoblastic differentiation of vascular smooth muscle cells. Arterioscler Thromb Vasc Biol 19:2112–2118
38. Shanahan CM, Cary NR, Salisbury JR, Proudfoot D, Weissberg PL, Edmonds ME (1999) Medial localization of mineralization-regulating proteins in association with Monckeberg's sclerosis: evidence for smooth muscle cell-mediated vascular calcification. Circulation 100:2168–2176
39. Shanahan CM, Proudfoot D, Tyson KL, Cary NR, Edmonds M, Weissberg PL (2000) Expression of mineralisation-regulating proteins in association with human vascular calcification. Z Kardiol 89 [Suppl 2]:63–68
40. Guerin AP, London GM, Marchais SJ, Metivier F (2000) Arterial stiffening and vascular calcifications in end-stage renal disease. Nephrol Dial Transplant 15:1014–1021
41. Reslerova M, Moe SM (2003) Vascular calcification in dialysis patients: pathogenesis and consequences. Am J Kidney Dis 41:S96–S99
42. Moe SM, Chen NX (2002) Vascular calcification in end stage renal disease. Clin Calcium 12:51–56
43. Anderson HC (1995) Molecular biology of matrix vesicles. Clin Orthop:266–280
44. Doherty MJ, Canfield AE (1999) Gene expression during vascular pericyte differentiation. Crit Rev Eukaryot Gene Expr 9:1–17
45. Bostrom KI (2000) Cell differentiation in vascular calcification. Z Kardiol 89 [Suppl 2]:69–74
46. Shioi A, Nishizawa Y, Jono S, Koyama H, Hosoi M, Morii H (1995) Beta-glycerophosphate accelerates calcification in cultured bovine vascular smooth muscle cells. Arterioscler Thromb Vasc Biol 15:2003–2009
47. Bellows CG, Aubin JE, Heersche JN (1991) Initiation and progression of mineralization of bone nodules formed in vitro: the role of alkaline phosphatase and organic phosphate. Bone Miner 14:27–40
48. Shanahan CM, Cary NR, Metcalfe JC, Weissberg PL (1994) High expression of genes for calcification-regulating proteins in human atherosclerotic plaques. J Clin Invest 93:2393–2402
49. Nakaoka T, Gonda K, Ogita T, Otawara-Hamamoto Y, Okabe F, Kira Y, Harii K, Miyazono K, Takuwa Y, Fujita T (1997) Inhibition of rat vascular smooth muscle proliferation in vitro and in vivo by bone morphogenetic protein-2. J Clin Invest 100:2824–2832
50. Proudfoot D, Skepper JN, Shanahan CM, Weissberg PL (1998) Calcification of human vascular cells in vitro is correlated with high levels of matrix Gla protein and low levels of osteopontin expression. Arterioscler Thromb Vasc Biol 18:379–388
51. Canfield AE, Farrington C, Dziobon MD, Boot-Handford RP, Heagerty AM, Kumar SN, Roberts IS (2002) The involvement of matrix glycoproteins in vascular calcification and fibrosis: an immunohistochemical study. J Pathol 196:228–234
52. Kimura K, Saika Y, Otani H, Fujii R, Mune M, Yukawa S (1999) Factors associated with calcification of the abdominal aorta in hemodialysis patients. Kidney Int [Suppl] 71:S238–S241
53. Marchais SJ, Metivier F, Guerin AP, London GM (1999) Association of hyperphosphataemia with haemodynamic disturbances in end-stage renal disease. Nephrol Dial Transplant 14:2178–2183

54. Chertow GM, Burke SK, Raggi P (2002) Sevelamer attenuates the progression of coronary and aortic calcification in hemodialysis patients. Kidney Int 62:245–252
55. Kleinpeter MA (2000) Spectrum of complications related to secondary hyperparathyroidism in a peritoneal dialysis patient. Adv Perit Dial 16:286–290
56. Jono S, Peinado C, Giachelli CM (2000) Phosphorylation of osteopontin is required for inhibition of vascular smooth muscle cell calcification. J Biol Chem 275:20197–20203
57. Ducy P, Zhang R, Geoffroy V, Ridall AL, Karsenty G (1997) Osf2/Cbfa1: a transcriptional activator of osteoblast differentiation. Cell 89:747–754
58. Komori T, Yagi H, Nomura S, Yamaguchi A, Sasaki K, Deguchi K, Shimizu Y, Bronson RT, Gao YH, Inada M, Sato M, Okamoto R, Kitamura Y, Yoshiki S, Kishimoto T (1997) Targeted disruption of Cbfa1 results in a complete lack of bone formation owing to maturational arrest of osteoblasts. Cell 89:755–764
59. Steitz SA, Speer MY, Curinga G, Yang HY, Haynes P, Aebersold R, Schinke T, Karsenty G, Giachelli CM (2001) Smooth muscle cell phenotypic transition associated with calcification: upregulation of Cbfa1 and downregulation of smooth muscle lineage markers. Circ Res 89:1147–1154
60. Chen NX, O'Neill KD, Duan D, Moe SM (2002) Phosphorus and uremic serum up-regulate osteopontin expression in vascular smooth muscle cells. Kidney Int 62:1724–1731
61. Tyson KL, Reynolds JL, McNair R, Zhang Q, Weissberg PL, Shanahan CM (2003) Osteo/chondrocytic transcription factors and their target genes exhibit distinct patterns of expression in human arterial calcification. Arterioscler Thromb Vasc Biol 23:489–494
62. Parhami F, Morrow AD, Balucan J, Leitinger N, Watson AD, Tintut Y, Berliner JA, Demer LL (1997) Lipid oxidation products have opposite effects on calcifying vascular cell and bone cell differentiation. A possible explanation for the paradox of arterial calcification in osteoporotic patients. Arterioscler Thromb Vasc Biol 17:680–687
63. Proudfoot D, Skepper JN, Hegyi L, Bennett MR, Shanahan CM, Weissberg PL (2000) Apoptosis regulates human vascular calcification in vitro: evidence for initiation of vascular calcification by apoptotic bodies. Circ Res 87:1055–1062
64. Evan AP, Lingeman JE, Coe FL, Parks JH, Bledsoe SB, Shao Y, Sommer AJ, Paterson RF, Kuo RL, Grynpas M (2003) Randall's plaque of patients with nephrolithiasis begins in basement membranes of thin loops of Henle. J Clin Invest 111: 607–616
65. Howe AM, Webster WS (2000) Warfarin exposure and calcification of the arterial system in the rat. Int J Exp Pathol 81:51–56
66. Price PA, Faus SA, Williamson MK (2000) Warfarin-induced artery calcification is accelerated by growth and vitamin D. Arterioscler Thromb Vasc Biol 20:317–327
67. Westenfeld R, Heiss A, Ketteler M, Floege J, Schwarz A, Jahnen-Dechent W (2002) Calciphylaxis is linked to systemic deficiency of the calcification inhibitor aghs/fetuin. American Society of Nephrology
68. Ketteler M, Bongartz P, Westenfeld R, Wildberger JE, Mahnken AH, Bohm R, Metzger T, Wanner C, Jahnen-Dechent W, Floege J (2003) Association of low fetuin-A (AHSG) concentrations in serum with cardiovascular mortality in patients on dialysis: a cross-sectional study. Lancet 361:827–833
69. Price PA, Faus SA, Williamson MK (2001) Bisphosphonates alendronate and ibandronate inhibit artery calcification at doses comparable to those that inhibit bone resorption. Arterioscler Thromb Vasc Biol 21:817–824
70. Tepel M, Giet M van der, Statz M, Jankowski J, Zidek W (2003) The antioxidant acetylcysteine reduces cardiovascular events in patients with end-stage renal failure: a randomized, controlled trial. Circulation 107:992–995