In the early 1900s, cholesterol deposition in atherosclerosis was considered a passive, degenerative, inevitable and end-stage process of ageing. After decades of research, it is now recognized as an active, regulated, treatable and preventable disorder related to deposition and oxidation of lipoprotein components. Similarly, in the past few decades, vascular calcification also has been considered a passive, degenerative, inevitable, and end-stage process of ageing. But, after recent clinical and laboratory findings, there is increasing recognition that vascular calcification is an active, regulated process related to oxidized lipids that may be treatable and preventable.

The fact that complete bone tissue forms within the atherosclerotic artery wall has been known since at least the 1800s. In 1863, Virchow observed that vascular calcium deposits were not mere calcification, but ossification.1 In 1908, investigators reported red marrow elements in bone tissue within atherosclerotic plaque.2,3 Experimental models of atherosclerosis also have cartilage and marrow within plaque.4

As an overview, vascular calcification in general, and coronary calcification in particular, increase with ageing, are present in almost all subjects over age 65, are more frequent in diabetics, less common in African-Americans, and extremely common in end-stage renal disease. Current studies of coronary calcification utilize electron beam computed tomographic scanning (EBCT). This method has been described as an ‘inaccurate’ predictor of stenosis severity. While this is not incorrect, it may leave the wrong impression since EBCT is accurate when used to predict the presence of significant coronary artery disease, and the degree of plaque burden.5

Mechanism of vascular calcification

The mechanism of vascular calcification is under investigation. There may be more than one mechanism, since atherosclerosis itself occurs by several different mechanisms, and also because atherosclerotic calcification, which primarily involves the intimal layer of the arteries, appears to be in a different category than medial calcification (also known as Monckeberg’s medial calcinosi), which is particularly common in diabetic patients.6,7

Such a determination is difficult, though, because EBCT does not identify the layer of the artery affected.

Investigators in the 1980s recognized bone-like features of vascular calcification including the mineral hydroxyapatite and matrix vesicles. Similarities between artery and bone at the molecular level were identified by Giachelli et al.8 who discovered a bone matrix protein, osteopontin, expressed in immature vascular cells. Schor et al. demonstrated that microvascular pericytes were capable of producing mineralization in vitro.9 The possibility that atherosclerotic calcification occurs by the same molecular mechanism as embryonic bone formation was proposed by Bostrom et al.10 who demonstrated expression of the potent embryonic bone differentiation factor, BMP-2, in human calcified plaque.10 A variety of bone proteins were then found in atherosclerotic lesions.11–16

Vascular calcification can be studied in tissue culture models. A subpopulation of cells from the aortic medial layer spontaneously produce bone mineral (hydroxyapatite) in tissue culture.10 These calcifying vascular cells (CVC) recapitulate the sequence of molecular events defining osteoblastic differentiation including co-ordinate expression of alkaline phosphatase, collagen I, osteopontin, osteonectin, and osteocalcin.17,18 Key mechanisms of vascular calcification include genetic determination, inflammatory mediators, apoptosis, matrix components, and homeobox genes.19–24

Clinical significance of vascular calcification

Measurement of vascular calcification

Aortic calcification is often measured by visual indexes of simple lateral roentgenography, though it is more accurately measured by quantitative computed tomographic (CT) scanning, since apparent density depends on penetration and technique. Coronary calcification is most often measured by EBCT, which differs from ordinary CT scanning in that images are acquired rapidly enough to minimize heart motion artefacts. Some groups are now attempting to validate routine CT scanning coupled with ECG gating to substitute for the more expensive EBCT. Coronary calcification is also measured by intravascular ultrasound, which detects mineral by sound reflection at the inner edge of the mineral deposit.
Coronary events

The degree of coronary calcification by EBCT is a sensitive and specific predictor for future cardiac events. The earliest evidence for this was limited by the need to include ‘soft’ endpoints to achieve sufficient numbers of events,\textsuperscript{25} such as interventional or surgical treatment, which could be influenced by the results of the EBCT scan. Other early studies suggested that there was not a relationship with hard endpoints at early follow-up.\textsuperscript{26} As follow-up time has increased, the correlation between EBCT results and the hard endpoints, myocardial infarction and coronary death, has remained positive.\textsuperscript{27} However, patients with the least circumferential extent of coronary calcification, measured by intravascular ultrasound, have more occasions of acute coronary syndrome.\textsuperscript{28} These findings are difficult to reconcile with those of EBCT, suggesting that the circumferential extent of calcification is not related to calcium score by EBCT, and that the circumferential versus longitudinal distribution have different implications.

Plaque rupture

Cardiac events are often the result of plaque rupture or ulceration.\textsuperscript{29} Plaque disruption may be prevented or promoted by calcium deposits because they may strengthen the plaque against circumferential mechanical stress,\textsuperscript{30} but they also introduce solid shear stress concentration where the nondistensible mineral interfaces with distensible tissue. Under mechanical stress induced by balloon angioplasty, calcified plaque is more likely to rupture than non-calcified plaque,\textsuperscript{31} and the rupture occurs along the interface between the calcium deposit and soft tissue.\textsuperscript{32} Thus, the ratio of surface area to volume in calcium deposits may determine whether they are harmful or protective. The presence of calcium deposits also correlates with adverse outcomes\textsuperscript{33} and restenosis\textsuperscript{34} in coronary interventional procedures.

Loss of the Windkessel effect in aortic calcification

Mineralization of the aorta may have greater significance than of the coronary. The normal aorta, with its multiple layers of elastin, is highly resilient. This resilience serves a pump function, known as the Windkessel effect. During systole, the aorta distends which reduces the work of the heart by reducing afterload. During diastole, the aorta recoils, with an energy that propels blood throughout the vasculature, particularly into the coronary tree, which depends on this diastolic aortic recoil for most of its perfusion. When the aorta calcifies and becomes rigid, it loses its Windkessel function,\textsuperscript{35} the work of the heart increases,\textsuperscript{36} and coronary flow is reduced\textsuperscript{37} leading to left ventricular hypertrophy, congestive heart failure and coronary insufficiency in patients with coronary disease.\textsuperscript{38–41} Congestive heart failure and myocardial infarction are major health problems in the over 65 age group. Aortic calcification, present in the vast majority of these individuals,\textsuperscript{42} is considered a factor in both.\textsuperscript{43–46}

Cardiac valve calcification

Calcific valvular stenosis is responsible for significant cardiovascular morbidity and mortality. For decades, valvular stenosis was considered independent of atherosclerosis and its risk factors. However, it is now known that cardiac valvular calcification shares risk factors with atherosclerosis\textsuperscript{47} and it has many features of bone.\textsuperscript{48–50} In addition, its progression is reduced in response to lipid lowering therapy.\textsuperscript{51}

Accelerated vascular calcification in dialysis patients

In haemodialysis patients, vascular calcification develops early and progresses rapidly,\textsuperscript{52} paralleling their high rate of premature cardiovascular disease.\textsuperscript{53} These patients often receive treatment with vitamin D and warfarin. At high doses, vitamin D promotes vascular calcification,\textsuperscript{39,54} and warfarin, a widely-used anticoagulant, blocks vitamin K dependent carboxylation\textsuperscript{55} which is critical for the function of some proteins in the clotting cascade and two involved in mineralizing tissue, osteocalcin and matrix GLA protein (MGP). Matrix GLA protein is expressed in the artery wall, and it regulates in vitro vascular calcification,\textsuperscript{56} and mice deficient in MGP develop complete ossification of the aorta and all its branches. There is now evidence that MGP binds and inhibits bone morphogenetic protein (BMP-2),\textsuperscript{57,58} the level of which determines the lineage to be taken by mesenchymal progenitor cells. Hence, in the MGP null mouse, where BMP-2 activity would be unopposed, the high level of activity would be expected to direct medial cells along an osteoblastic rather than smooth muscle lineage, thus accounting for the phenotype. Since MGP function depends on gamma-carboxylation, warfarin treatment may interfere with its protective function in the vasculature. This raises the question of whether warfarin treatment contributes to the accelerated vascular calcification in dialysis patients.\textsuperscript{59}

Vascular calcification and osteoporosis

Osteoporosis treatment efficacy is often assessed by bone densitometry of the lumbar vertebrae. In this technique, an X-ray beam is projected through the abdominal wall and lumbar spine. Since the amount of beam attenuation corresponds with the amount of calcium mineral in the beam path, the density of mineral in the vertebrae can be calculated.\textsuperscript{60} However, it is often unappreciated that this beam path includes the abdominal aorta, a prominent and early site of vascular calcification. Thus, a treatment that increased only aortic calcification would also increase lumbar densitometric beam attenuation, potentially leading to its incorrect identification as successful osteoporosis treatment. In general, postmenopausal women are advised to take calcium supplements to prevent or treat osteoporosis, implying that bone loss is due to insufficient dietary calcium. Yet, in many patients with osteoporosis, loss of bone tissue from the skeleton occurs at the same time as formation of bone in the artery wall. This paradox suggests that dietary calcium is not the limiting factor. The association of osteoporosis with vascular calcification has been reported widely,\textsuperscript{61–66} and it may may not\textsuperscript{68–70} be explained by their mutual correlation with ageing. In rodents, vascular calcification and osteoporosis co-exist under at least three conditions: deficiency of osteoprotegerin, an osteoclast inhibitory factor,\textsuperscript{71} deficiency of dietary essential fatty acids\textsuperscript{72} and hyperlipidaemia.
Lipids and biomineralization

*In vitro* and *in vivo* studies show that oxidized lipids not only promote mineralization of vascular cells but they also inhibit mineralization of bone cells. Low density lipoprotein (LDL) levels correlate with both coronary and aortic valve calcification progression, and LDL proteins accumulate in calcified aortic valves. Hyperlipidaemia is associated with rapid progression of coronary calcification, and lipid-lowering therapy reduces progression of both coronary and valvular calcification. Oxidized lipids induce osteoblastic differentiation in vascular cells *in vitro*, and hyperlipidaemia reduces bone mineral density *in vivo*.

The paradox of simultaneous osteolysis and ectopic ossification

One possible unifying theme explaining this paradox is that accumulation and oxidation of lipid deposits in tissue may mimic chronic infection and stimulate immune responses that promote hardening of soft tissue and the softening of hard tissue. The bacterial cell wall contains lipids, and they are modified by oxidizing factors released by phagocytic cells, such as superoxide radical and nitric oxide from macrophages. Thus, oxidized lipids in general may trigger the immune system to respond as it does to persistent bacterial infection. It is well known that the immune response to longstanding infection or inflammation in bone is osteolysis, which would dissolve a substrate for bacterial infectious growth. It is also well known that the immune response to longstanding infection or inflammation in soft tissue is heterotopic bone formation around the site, which would wall off any infectious organism. Tuberculous granulomata result from this process. Thus, lipid accumulation and oxidation may lead to a reversal of the normal regional control of biomineralization, promoting calcification of soft tissue and osteolysis of bone, accounting for the paradox of bone formation in the arteries of patients who are losing bone from their skeletons.

Additional epidemiological considerations

In a case-control study from Thailand, serum biomarkers of osteoporosis and coronary heart disease were compared. No statistically significant difference was found in bone turnover rates between cases compared to control subjects. Since a reduction in osteogenesis is not always accompanied by changes in turnover, however, such measures may not detect an association between coronary disease and reduced bone formation. If accurate markers of bone differentiation/formation are developed in the future, this type of study, comparing degree of vascular calcification with serum markers of bone differentiation or formation may help determine whether such a relation exists.

From a mechanical standpoint, whether calcium deposits in arteries are circumferential versus longitudinal may influence stability. These assessments are difficult to make by EBCT because of resolution limitations, and they are difficult by intravascular ultrasound because calcium deposits reflect the echoes allowing assessment only of the edge of the deposit closest to the transducer. Beckman *et al.* compared the extent of circumferential calcification (not longitudinal), and found that patients with acute coronary syndromes had less circumferential extent than those with stable angina. Although there are potential confounding effects in this study, it raises the possibility that circumferential calcification has a stabilizing effect.

Although long-term warfarin use in atrial fibrillation, by reducing function of MGP, would be expected to promote arterial calcification, clinical events are generally reduced in treated patients. The likely reason, of course, is warfarin’s direct effect on blood coagulation and its contribution to thrombosis, the major event in atrial fibrillation. It also remains possible that calcification could stabilize plaque as well as reduce clot formation.

It remains controversial whether coronary heart disease risk factor profiles (e.g. the Framingham score) have a greater predictive value if the extent of arterial calcification is included. Arad *et al.* determined the areas under the receiver-operator characteristics curves as 0.84 and 0.86 for predicting non-fatal myocardial infarctions and deaths from coronary calcification score. Overall, the view is that calcification scores make a small improvement in predictive value.

Coronary calcification and osteoporosis have been associated with presence of infectious agents, such as *Chlamydia pneumoniae* and *Helicobacter pylori*, as well as markers of chronic infection, such as C-reactive protein. While this most likely suggests that arterial calcification is a chronic inflammatory process, it remains possible that inflammatory processes in bone alter the serum bone regulatory factor levels, resulting in indirect effects on vascular calcification.

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