REVIEW

Vascular and valvar calcification: recent advances

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The last few years have seen a surge of interest in the measurement of coronary artery calcification to predict and monitor the presence of coronary atherosclerosis. In addition, the rising clinical burden of valvar calcification in the aging population has highlighted the importance of understanding the processes of calcification within vascular tissues.

Vascular calcification occurs in both the intima and the media of arteries, and there is evidence that these two sites of calcification are distinct entities (table 1). Intimal calcification only occurs within atherosclerotic plaques and is seen as early as the second decade of life, just after the fatty streak stage.¹ In contrast, medial calcification occurs independently of intimal calcification and atherosclerosis. It commonly occurs in the peripheral arteries of the lower limbs in otherwise healthy elderly patients (Monckeberg's sclerosis), where it is seen as "rail tracking" on plain radiographs. However, it also occurs in younger patients with diabetes and chronic renal failure. In diabetic patients, medial calcification appears to be a strong independent predictor of cardiovascular mortality and occurs particularly in those with neuropathy.² Its presence can make palpating pulses and hearing Korotkoff sounds difficult and can lead to false elevation of cuff systolic blood pressure measurements. It also causes problems with the surgical management of vascular shunts.

More importantly, arterial wall stiffness has been shown to be correlated independently with aortic calcification.³ In theory this increased arterial stiffness from calcification may lead to an increase in cardiac work. In addition the reduction in aortic compliance may result in a decrease in diastolic coronary perfusion, as this is dependent on the recoil of the aorta which has been stretched during systole.⁴ Furthermore, increased arterial stiffness leads to an increase in pulse pressure, which is a highly significant predictor of myocardial infarction

and cardiovascular death.⁵ It has therefore been suggested that the high ischaemic heart disease mortality rates in patients with renal failure may be partly attributable to increased vascular calcification.⁶ Severe medial calcification is also seen in calcific uraemic arteriolopathy (calciphylaxis)—a relatively rare and often fatal syndrome of ischaemic necrosis of skin, muscles, and subcutaneous fat occurring almost exclusively in uraemic patients.

Imaging of coronary calcification

Medial calcification is unusual in the coronary arteries and therefore any detectable coronary calcification is taken to reflect calcium within intimal atherosclerotic lesions. Intravascular ultrasound (IVUS) detects calcification as hyperechoic areas within plaques. Although IVUS is highly sensitive and specific for calcification, it is invasive, non-quantitative, and only visualises a limited portion of the coronary tree.⁷ Electron beam computed tomography (EBCT) is the most accurate non-invasive way of measuring coronary calcium and generates a "calcium score". Within the coronary circulation, the calcium score correlates closely with plaque apatite content. The absence of a moving x ray source in EBCT allows for very rapid scanning times, and recent evidence suggests that similar data can be obtained using less sophisticated spiral and conventional systems. Radiation dosimetry for a single screening EBCT scan is two- to threefold less than during angiography.⁷

EBCT IN THE DIAGNOSIS OF CORONARY DISEASE AND RISK STRATIFICATION

Necropsy studies have shown that the amount of intimal calcium in the coronary arteries is related closely to the amount of plaque. Furthermore, a direct relation has been demonstrated between coronary artery calcium score, as measured by EBCT, and histological measures of plaque burden.⁸ In other words, where there is calcification there is usually plaque, and where there is plaque there is usually calcification. In vitro studies of explanted coronary vessels from hearts obtained at necropsy⁹ have shown that EBCT sensitively detects significant luminal disease as assessed histologically. It also predicts the presence of angiographically significant disease in patients with symptomatic coronary disease with high sensitivity but low specificity. Therefore, although EBCT can detect the presence of

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Table 1 Characteristics of intimal versus medial calcification

	Intimal	Medial
Morphology	Patchy scattered deposits	Continuous linear deposits
Histological association	Inflammatory cells Lipid Vascular smooth muscle cells	Vascular smooth muscle cells Elastin
Disease association	Atherosclerosis	Diabetes Chronic renal failure Monckeberg's sclerosis

atherosclerosis, the calcium score does not have a direct correlation with degree of luminal narrowing and provides little advantage over traditional methods for investigating patients with probable or definite symptomatic coronary disease.

A positive correlation has been shown between coronary calcium score and subsequent clinical events in patients with known coronary artery disease.¹⁰ However, the extent to which this predicts coronary events independently of traditional risk factors—particularly in asymptomatic patients—needs further study.^{11–15} The National Institutes of Health funded multiethnic study of atherosclerosis (MESA) will provide some of these data over the next decade.

In patients with new presentation of chest pain, an EBCT calcium score of zero has a negative predictive value of about 95% for significant stenosis in any major vessel.¹⁶ In two recent studies, a negative EBCT scan was used to predict which patients could safely be discharged (with a predictive value of 100%).^{17,18} Thus because EBCT can be performed rapidly it may have a role to play in excluding coronary disease in patients presenting to the emergency department with atypical chest pain and a low probability of coronary disease.⁷

Similarly EBCT may have a role to play in the assessment of coronary artery disease in heart transplant recipients, many of whom currently undergo frequent cardiac catheterisations.^{19–22} EBCT has recently been shown to have a sensitivity of 94%, a specificity of 79%, and a negative predictive value of 99% for detecting significant stenosis in the transplanted heart.²²

EBCT may also have a role in monitoring disease progression in patients undergoing treatment. Studies in animal models of atherosclerosis have shown that statins reduce the amount of calcium in atherosclerotic plaques.²³ In a recent clinical study it was found that statin treatment also causes a reduction in coronary calcium score in man,²⁴ raising the possibility that EBCT might be used to monitor plaque regression.

Calcification and coronary intervention

Angioplasty to highly calcified lesions carries an increased risk, even in the stent and glycoprotein IIb/IIIa era, because of higher failure and complication rates. Calcified lesions are stiff and resistant to adequate dilatation, leading to increased risk of vessel closure from dissections originating at the junction of the calcified and non-calcified regions of the plaque.²⁵ In addition, the high inflation pressures required increase the risk of balloon rupture and even vessel perforation.²⁵ Rotational atherectomy has been shown to increase success and reduce the complication rate in calcified lesions; however, the restenosis rate remains unchanged.²⁵

Stenting of highly calcified lesions is also problematic because of lack of full stent expansion and difficulties in delivery to the target site. Several studies have now shown improved

results by using rotational atherectomy to debulk the calcium before stent placement.^{26,27} These studies showed appropriate stent expansion in most cases and a reduced restenosis rate compared with rotational atherectomy followed by angioplasty alone.

Valvar calcification

NATIVE VALVE CALCIFICATION

Calcific aortic stenosis is the most common valvar lesion in the USA and a major cause of aortic valve replacement.²⁸ It is associated with an increase of approximately 50% in the risk of death from cardiovascular causes, even in the absence of haemodynamically significant obstruction.²⁹

Calcification of the mitral annulus is one of the most common cardiac abnormalities found at necropsy, and when severe is a major cause of mitral regurgitation.²⁸ When subvalvar or intravalvar extension is extensive, mitral stenosis may result.²⁸ Furthermore, mitral valve calcification is an indicator of poor prognosis during balloon valvuloplasty, leading to an inadequate increase in valve area and reduced long term survival.

The risk factors for development of calcific aortic stenosis and mitral annular calcification overlap with those for coronary artery disease.²⁸ However, accelerated forms of these calcifications are seen, particularly in patients with uraemia, where they should always be suspected when new or changing murmurs are detected.

BIOPROSTHETIC VALVE CALCIFICATION

Bioprosthetic valves made from either glutaraldehyde cross linked porcine aortic valves or bovine pericardium have superior haemodynamic and thromboresistant properties compared with mechanical valves. However, calcification of the cusps often causes the clinical failure of these devices. By 10 years approximately 30% of such valves require replacement and the process of calcification is accelerated in chronic renal failure and in children.

Pathophysiology of vascular calcification

Skeletal bone formation is the result of tightly regulated mineralisation of a specialised extracellular matrix resulting in calcium apatite deposition. This requires initiation of apatite accumulation (nucleation) and growth, elaboration of a permissive matrix, and production of regulatory proteins. Vascular calcification appears to be a regulated process very similar to that of developing bone, with apatite crystal nucleation, growth, and possibly degradation in association with an extracellular matrix that regulates tissue mineralisation.

APATITE ACCUMULATION

The process of tissue matrix mineralisation is remarkably poorly understood, even in bone. In fact mammalian extracellular fluids are oversaturated with respect to the precipitation of hydroxyapatite and yet widespread calcification does not usually occur. This is thought to reflect the complexing of calcium by organic molecules and the fact that energy is required

Table 2 Some proteins with potential roles in the regulation of vascular calcification

Protein	Possible function
Collagen type I	May act as a nucleator ^{55 56}
Elastin	May act as a nucleator ⁴⁹
Bone sialoprotein	May act as a nucleator ³⁷
Osteopontin	Adhesion molecule binding cells to apatite and inhibiting crystal growth by binding to crystal surfaces ³⁶ Inhibitor of hydroxyapatite nucleation in vitro ³⁶
Osteonectin	High affinity for apatite and collagen ⁵⁷
Matrix Gla protein	Found in association with areas of vascular calcification ⁴³ Gla residues capable of binding hydroxyapatite ³⁸ Inhibition of Gla formation by warfarin leads to medial and valvar calcification in rats ⁴⁵ Possible inhibitor of calcification as mouse knockout has medial vascular calcification ³⁸
Osteocalcin	Gla residues capable of binding hydroxyapatite ⁵⁸
BMP-2	Osteogenic differentiation factor expressed in areas of vascular calcification ⁴⁷
Osteoprotegerin	Osteoclastogenesis inhibitory factor ⁵⁹ Soluble member of the tumour necrosis factor (TNF) receptor family ⁵⁹ Possible inhibitor of calcification as mouse knockout has medial vascular calcification ⁴⁰
Klotho	Possible inhibitor of calcification as mouse knockout has medial vascular calcification ³⁹
Carbonic anhydrase	Possible inhibitor of calcification as mouse knockout has medial vascular calcification ⁴¹
Fibrillin-I	Possible inhibitor of calcification as mouse knockout has medial vascular calcification ⁴²

to achieve the phase transformation from solution to solid crystal. The energy required can be decreased by the presence of nuclei of solid particles, which serve as a substrate for heterogeneous nucleation. Therefore, although apatite crystallisation does not occur in isolated serum, the addition of preformed apatite results in a rapid proliferation of apatite. In tissues, this phase change—or nucleation—is thought to occur on an organic component of the matrix which, in bone, may be represented by collagen fibrils, certain bone regulatory proteins, and matrix vesicles. Matrix vesicles are small subcellular remnants that are thought to be crucially involved in bone mineralisation.

Nucleation sites such as matrix vesicles, collagen fibrils, mitochondria, lipids, and certain bone regulatory proteins exist in the vessel wall. For example, intimal calcification has been described in association with matrix vesicles, cholesterol crystals, and mitochondria-like structures in intimal vascular smooth muscle cells (VSMCs).^{1 30}

Structures similar to matrix vesicles, arising from VSMCs³¹ and dead macrophage foam cells,¹ have been identified in the vessel wall. There is growing evidence that VSMC apoptosis in the media, and possibly macrophage apoptosis in the intima, may lead to matrix vesicle formation and apatite nucleation.³² In support of this notion, Kim showed calcification of matrix vesicles derived from VSMCs within the arterial media.³¹ In addition, we have shown that apoptotic bodies derived from human VSMCs—like matrix vesicles from bone—accumulate calcium and might therefore initiate calcification.³³ From these data we

have speculated that apoptosis (of either VSMCs or macrophages) is a crucial initiating event in vascular calcification, and that once an initial nucleation site has been established, apatite crystal growth can occur.

CALCIFICATION REGULATING PROTEINS

Human VSMCs, both in vivo and in vitro, express many of the calcification regulating proteins commonly found in bone^{34 35} (table 2). Several of these proteins have calcium and apatite binding properties and are concentrated in areas of vascular calcification, where they may serve a variety of functions, including regulation of apatite crystal nucleation and growth. For example, osteopontin appears to bind to the apatite crystal surface and inhibits its growth,³⁶ whereas bone sialoprotein can act as a nucleator of crystal formation.³⁷

Evidence that some of the proteins are potent natural inhibitors of calcification comes from the development of extensive vascular calcification in mice lacking specific genes.^{38–42} The most impressive of these is the matrix Gla protein (MGP) knockout mouse which develops such extensive medial vascular calcification that it dies of arterial rupture within two months of birth.³⁸ This suggests that MGP is a potent inhibitor of vascular calcification. MGP contains several residues of an uncommon amino acid— γ -carboxyglutamic acid (Gla)—formed by a vitamin K dependent modification of specific glutamic acid residues. The Gla residues appear to confer calcium binding properties to these proteins.⁴³ In addition there are data from both humans and rats that inhibition of the vitamin K dependent process of Gla residue formation by warfarin may lead to an increase in calcification.⁴⁴ In culture, warfarin increases calcification in VSMC nodules.³⁴ Similarly warfarin feeding of rats leads to accelerated medial and valvar calcification.⁴⁵ Finally, warfarin use has been associated with the occurrence of accelerated medial calcification in calcific uraemic arteriolopathy.⁴⁶

Inhibitory proteins such as MGP are expressed constitutively by normal medial VSMCs, where they may serve to prevent apatite accumulation in the event of physiological or pathological cell death. However, once calcification is established VSMCs have been found to express proteins that are normally expressed in developing and mature bone.³⁵ In so doing, VSMCs appear to adopt an osteogenic phenotype. The molecular signals that regulate this phenotypic change are not yet fully understood, but VSMCs and osteoblasts share a common embryonic mesenchymal derivation, and several of the bone morphogenetic proteins and their receptors are expressed in the vascular wall.⁴⁷ It is unclear whether this change to an osteogenic phenotype leads to the calcification process or whether it is secondary to it. It is possible that these osteogenic cells migrate into the calcified vessel wall from elsewhere. However, they are most probably derived from VSMCs (or a subset) already resident in the vessel wall. This is because VSMCs show a large degree of plasticity and can, for example, spontaneously

Figure 1 A model for the initiation of calcification by apoptosis, and potential regulatory influences.

adopt an osteogenic phenotype forming calcified nodules in culture.³⁴

On the basis of the above data we would suggest that vascular calcification occurs when there is either physiological or pathological cell death and a failure of clearance of the resulting apoptotic bodies, which go on to nucleate apatite (fig 1). A relative lack of function of constitutive inhibitory proteins, such as MGP, would lead to crystal growth and a concomitant transition of VSMCs to an osteogenic phenotype either as a prerequisite for calcification or as a consequence of it.

Pathophysiology of valvar calcification

NATIVE VALVE CALCIFICATION

Cell death with subsequent failure of clearance of apoptotic bodies which can act as nucleators is probably the basic mechanism of calcification within native valves. It is associated with matrix vesicles, collagen, and lipid which are thought to act as nucleation sites. There is evidence that alterations in calcification regulatory proteins and change to an osteogenic phenotype occur in an analogous fashion to vascular calcification.⁴⁸ Indeed native valve fibroblasts in culture spontaneously form nodules which calcify in a fashion analogous to VSMCs.⁴⁸

The importance of calcification regulatory proteins has been demonstrated in a rat model in which warfarin inhibition of vitamin K dependent Gla formation results in significant valvar calcification.⁴⁵

The role of cell death has been highlighted by Kim, who showed that when cultured canine valve fibroblasts were injured by anoxia and freeze-thawing, the cells calcified, whereas the uninjured control cells did not.⁴⁹

The strong clinical association between calcific aortic stenosis and hypercholesterolaemia is consistent with a role for lipids as

nucleators of aortic valve calcification. Moreover, the pronounced regression of aortic stenosis in a patient with homozygous familial hypercholesterolaemia who was treated with plasmapheresis suggests an exciting possibility for potential therapeutic intervention in calcific aortic stenosis by use of lipid lowering treatment.⁵⁰

BIOPROSTHETIC VALVE CALCIFICATION

In both vascular and native valve calcification the nucleation sites are thought to be generated by cells. However, glutaraldehyde treated bioprosthetic valves are devoid of live cells. It appears that cell death again plays a key role in this process. Glutaraldehyde fixation seems to induce an immediate, sustained, and massive rise in intracellular calcium in porcine aortic valve fibroblasts in culture.⁵¹ Furthermore cellular blebs have been observed following glutaraldehyde treatment which seem to isolate the overloaded calcium and act to nucleate apatite.⁵¹ These structures may be analogous to matrix vesicles. In fact matrix vesicle like structures have been observed in both fresh and fixed bioprosthetic tissue valves at sites of mineralisation.⁵²

The role of lipids (including lipid in matrix vesicle membranes) has been further highlighted by the demonstration that ethanol pretreatment (which removes lipids) of porcine bioprosthetic aortic valves before glutaraldehyde fixation inhibits calcification when these valves are placed in sheep hearts.⁵³

Interestingly, the calcification of bioprosthetic valves in vivo takes place after a period of years, in contrast to the calcification of many in vitro models which occurs immediately. This may indicate that in vivo factors are present which act to inhibit calcification in these valves. One such factor is osteopontin. For example, when glutaraldehyde treated porcine valves were implanted into mice lacking osteopontin they accumulated four to five times as much mineral as those implanted in wild type mice.⁵⁴

Conclusions

Vascular and valvar calcifications are associated with significant morbidity and mortality. Recent studies have shown that these calcifications are regulated processes with many similarities to developing bone, and that they may therefore be amenable to therapeutic modification. Measurement of coronary calcification by electron beam computed tomography provides a tool for confidently excluding coronary disease in low risk individuals and a possible means of monitoring plaque modifying treatment non-invasively in patients with established disease.

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