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Clinical Update

Calcific aortic valve stenosis: hard disease in the heart

A biomolecular approach towards diagnosis and treatment

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Calcific aortic valve stenosis (CAVS) is common in the ageing population and set to become an increasing economic and health burden. Once present, it inevitably progresses and has a poor prognosis in symptomatic patients. No medical therapies are proven to be effective in holding or reducing disease progression. Therefore, aortic valve replacement remains the only available treatment option. Improved knowledge of the mechanisms underlying disease progression has provided us with insights that CAVS is not a passive disease. Rather, CAVS is regulated by numerous mechanisms with a key role for calcification. Aortic valve calcification (AVC) is actively regulated involving cellular and humoral factors that may offer targets for diagnosis and intervention. The discovery that the vitamin K-dependent proteins are involved in the inhibition of AVC has boosted our mechanistic understanding of this process and has opened up novel avenues in disease exploration. This review discusses processes involved in CAVS progression, with an emphasis on recent insights into calcification, methods for imaging calcification activity, and potential therapeutic options.

Keywords Review • Aortic valve stenosis • Calcification

Introduction

Degenerative calcific aortic valve stenosis (CAVS) is the most common type of valvular disease in the Western world, representing a substantial and increasing disease burden in the ageing population.¹ Upon mild valve obstruction, disease progression with increasing haemodynamic severity is inevitable. Once symptomatic severe CAVS has developed, the prognosis without intervention is dismal. Despite growing knowledge, experience, and technological developments, the only treatment for (symptomatic) severe CAVS is surgical or transcatheter aortic valve replacement (AVR), to which not all patients are

suited.² Pharmacological interventions have thus far failed to alter the course of CAVS. Therefore, an unmet clinical need exists to develop new treatment strategies delaying CAVS progression.

We still lack precise molecular insight in to the pathophysiological underlying CAVS, although calcification is well known to play a fundamental role in progressive valvular narrowing. Today calcification is no longer considered a passive consequence of ageing but an active process involving cellular and molecular pathways. The exact processes underlying the initiation and progression of valvular calcification remain unresolved.³ Understanding the biomolecular mechanisms related to the genesis of calcification in CAVS will propel our

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knowledge and open novel avenues for diagnosis and treatment. In this review, we summarize the latest research progress in the pathophysiology of CAVS and offer novel targets holding potential for pharmacological interventions and imaging.

Aortic valve cusp function

Aortic valve cusps (or leaflets) must be both strong and flexible to withstand the considerable mechanical stress and strain associated with valve closure. To maintain cusp function, the specialized cusp microarchitecture is crucial and consists of three layers: fibrosa, spongiosa, and ventricularis⁴ (Figure 1). Valvular endothelial cells (VECs) are located at valvular blood-contacting surfaces, constituting a barrier that regulates valve permeability, the adhesion of inflammatory cells and paracrine signalling. Valvular interstitial cells (VICs), the major cell type, are present throughout all valvular layers. Valvular interstitial cells are key in valve remodelling, regulating both the synthesis and degradation of extracellular matrix components. Physiologically, VICs exist in a quiescent state, with similar characteristics to fibroblasts.⁵ Stimulation of VECs and VICs by molecular and mechanical triggers including high blood pressure, altered shear stress, cytokines, and growth factors contributes to CAVS pathophysiology, altering the local valve environment and making it calcification prone.

Calcific aortic valve stenosis aetiology

Although the most common cause of aortic stenosis in the Western world is degenerative CAVS (referred to as 'CAVS' in this review), rheumatic heart disease remains common in developing countries. Rheumatic aortic stenosis is caused by an abnormal immune response to Group A streptococcal infections. Calcification is again a predominant feature, and although this is believed to relate to chronic inflammation, precise mechanisms remain poorly defined.⁶ Calcific aortic valve stenosis is accelerated in patients with congenitally bicuspid aortic valves (BAVs) with aortic stenosis developing several decades earlier than in patients with trileaflet valves. More than 50% of patients with severe aortic stenosis requiring aortic valve replacement have BAV.⁷

Calcific aortic valve stenosis pathophysiology

Initiation phase

Calcific aortic valve stenosis can be divided in two distinct phases; the initiation and propagation phase, each dominated by different mechanisms (Figure 2). The initiation phase shows similarities with atherosclerosis, both ignited by endothelial activation/damage and an inflammatory response⁸ and sharing common risk factors including age, male gender, body mass index, smoking, hypertension, and elevated lipid levels including Lp(a).⁹ Moreover, stenotic valves from animals fed a high-fat diet display similar lesions as found in early human atherosclerotic plaques.^{10,11}

Classically, the initiation phase is triggered by mechanical stress in the valve causing endothelial damage and activation. This is perhaps best illustrated by the accelerated development of aortic stenosis in patients with BAV that are characterized by altered flow patterns, increased mechanical stress, and reduced shear stress.¹² The endothelial damage results in lipid infiltration and subsequent oxidation, thereby initiating an inflammatory response within the valvular endothelium involving macrophages, T-lymphocytes, and mast cells.¹¹ Within affected regions, microcalcifications colocalize with lipids. Formation of microcalcifications is mediated by the release of apoptotic bodies and extracellular vesicles, in a similar manner to vesicle-induced calcification in bone and the vasculature.^{8,13} These calcification-prone extracellular vesicles function as nucleating sites for calcium crystal deposition and facilitate formation of hydroxyapatite. Hydroxyapatite crystals in turn set the stage for CAVS progression by (i) expanding quickly (creating more nucleation sites for calcium deposition) and (ii) evoking additional pro-inflammatory responses.^{5,8,14}

Propagation phase: fibrosis and calcification as hallmarks of disease progression

Although the initiation phase is mainly mediated by inflammatory responses, the role of inflammation and lipid deposition is less prominent in the propagation phase (Figure 2). Instead, it is characterized by fibrosis and accelerated calcification, leading to valvular dysfunction

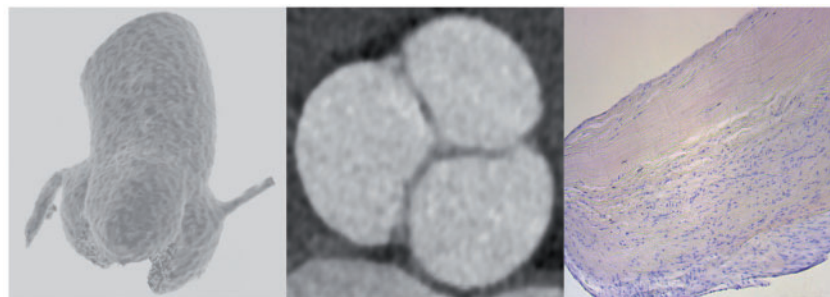
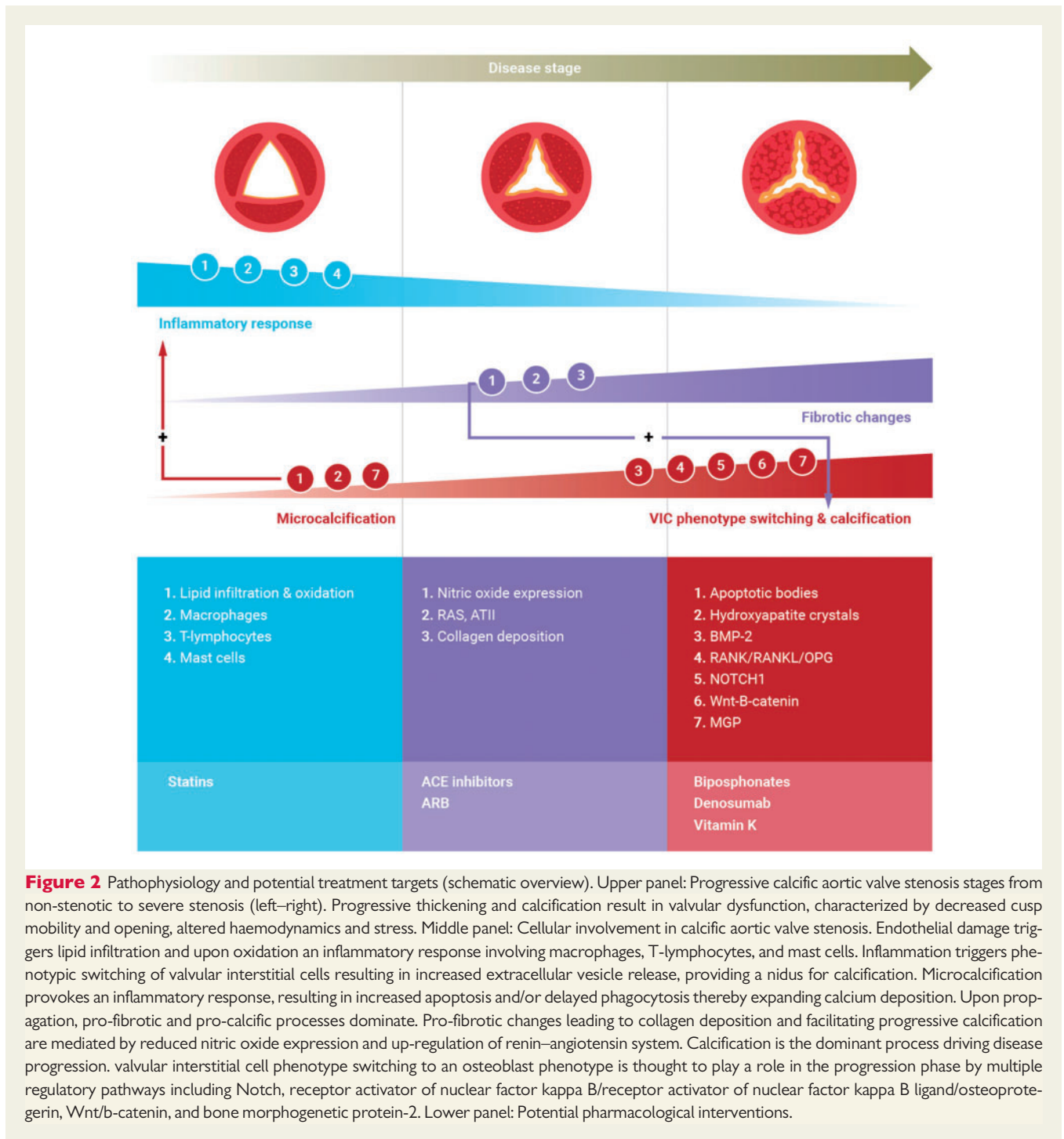


Figure 1 Aortic valve. Left panel: 3D reconstruction (from bottom to top): aortic valve with three cusps and proximal ascending aorta. Middle panel: 2D view. Right panel: valvular histology (bottom to top): ventricularis, spongiosa, and fibrosa.



and changes in mechanical stress and flow, thereby creating self-sustaining mechanisms underlying CAVS progression. Pro-fibrotic processes are mediated by (i) reduced nitric oxide expression following endothelial injury¹⁵ and (ii) up-regulation of the renin–angiotensin system (RAS), and formation of angiotensin II (ANGII). Down-regulation of expression of the ANGI type 2 receptor has been shown to result in a predominant pro-fibrotic profile, resulting in collagen deposition and the facilitation of progressive calcification.¹⁶ Calcific aortic valve stenosis is viewed as a fibrocalcific disease; however, once

calcification becomes abundant, pro-osteogenic mechanisms become overwhelming, ultimately leading to severe calcification and valvular dysfunction. The phenotypic switching of VICs into an osteoblast-like phenotype is thought to be the fundamental step in accelerating valvular calcification, initiated at least in part by inflammation. In the propagation phase, disease progression is driven by calcific regulatory pathways including Notch, receptor activator of nuclear factor kappa B (RANK)/receptor activator of nuclear factor kappa B ligand (RANKL)/osteoprotegerin (OPG), Wnt/b-catenin, and bone

morphogenetic proteins (BMPs).¹⁶ Notch-1 is essential in the development of the aortic valve during embryology and a mutation in Notch-1 is associated with development of BAV (but multiple genetic factors associated with BAV and CAVS have been described¹⁷). Also, Notch-1 is associated with early valve calcification by stimulating BMP-2.¹⁸ Bone morphogenetic protein-2 is up-regulated through binding of RANKL to RANK. Activation of the RANK/RANKL pathway results in the formation of proteins involved in calcification such as alkaline phosphatase and osteocalcin¹⁶ and is involved in CAVS (Figure 2).

Belonging to the multifunctional TGF- β superfamily, BMP-2 is an important osteogenic differentiation factor. Bone morphogenetic protein-2 is a key protein in phenotypic switching of VICs and hence in the development of aortic valve calcification/calcium (AVC).¹⁶ Physiologically, BMP is inhibited by matrix Gla-protein (MGP).¹⁹ The vital role of MGP to inhibit vascular calcification was demonstrated in MGP-deficient mice, showing lethal rupture of severely calcified arteries >2 months after birth.²⁰ Although the inhibitory function of MGP on BMP-2 and subsequent VIC differentiation in CAVS seems evident, MGP also exerts its effect via a second mechanism. Matrix Gla-protein interacts directly with hydroxyapatite, inhibiting the growth of hydroxyapatite crystals in vascular tissue.¹⁹ Since we hypothesize that hydroxyapatite crystals are involved in the early phase of CAVS, MGP is a potential target to inhibit microcalcification. Matrix Gla-protein is a vitamin K-dependent protein and is present in two distinct forms: uncarboxylated inactive (ucMGP) and carboxylated active (cMGP). Like all vitamin K-dependent proteins, MGP requires vitamin K-induced carboxylation to exert its function¹⁹ (Figure 3). Vitamin K antagonist (VKA) inhibits recycling of vitamin K, thereby inducing inactive vitamin K-dependent proteins. Although VKA is important for prophylaxis of thrombo-embolic events in certain patient populations, calcification should be acknowledged as a side effect. In animal models, warfarin treatment increased vascular and valvular calcification, similar to the MGP knock-out mouse.²¹ The detrimental effect of warfarin was also identified in humans, where patients using VKA demonstrated more vascular and valvular calcification.^{22,23} With our expanding knowledge of CAVS pathophysiology, possible treatment targets for pharmacological interventions become evident.

Pharmacological treatment targets in calcific aortic valve stenosis

Current guidelines do not recommend pharmacological interventions to halt CAVS progression. However, the importance and need to reduce or even reverse progression of CAVS is evident. Therefore, multiple observational studies and randomized controlled trials (RCTs) have attempted to repurpose commonly used pharmacological interventions to slow CAVS progression.

Angiotensin-converting enzyme inhibitors and angiotensin receptor blockers

Hypertension affects the stenotic aortic valve and increases afterload, thereby accelerating left ventricular (LV) hypertrophy. Both LV hypertrophy and high valvuloarterial impedance are associated with

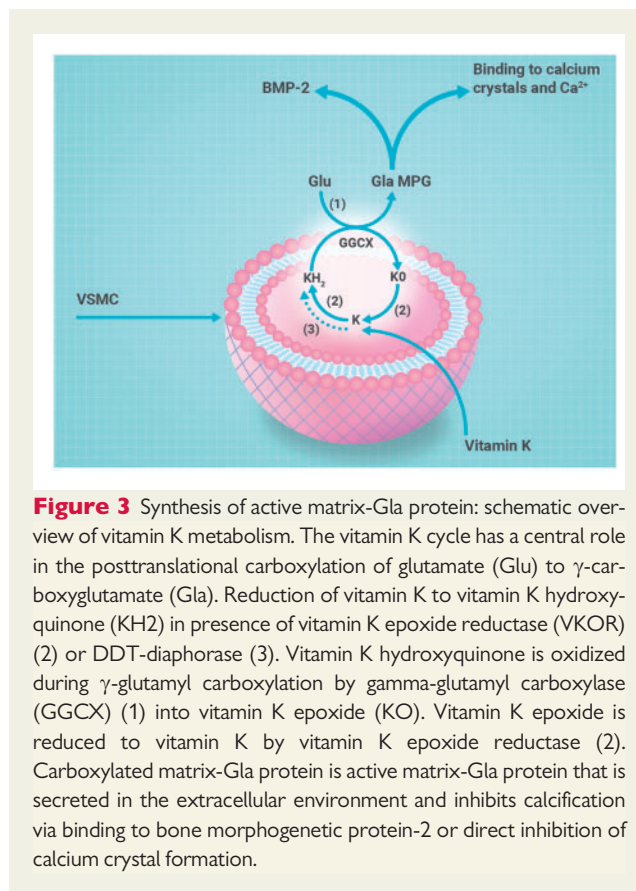


Figure 3 Synthesis of active matrix-Gla protein: schematic overview of vitamin K metabolism. The vitamin K cycle has a central role in the posttranslational carboxylation of glutamate (Glu) to γ -carboxyglutamate (Gla). Reduction of vitamin K to vitamin K hydroxyquinone (KH₂) in presence of vitamin K epoxide reductase (VKOR) (2) or DDT-diphosphorase (3). Vitamin K hydroxyquinone is oxidized during γ -glutamyl carboxylation by gamma-glutamyl carboxylase (GGCX) (1) into vitamin K epoxide (KO). Vitamin K epoxide is reduced to vitamin K by vitamin K epoxide reductase (2). Carboxylated matrix-Gla protein is active matrix-Gla protein that is secreted in the extracellular environment and inhibits calcification via binding to bone morphogenetic protein-2 or direct inhibition of calcium crystal formation.

adverse events in patients with CAVS.^{24,25} Therefore, current guidelines recommend treatment of concomitant hypertension.²

Renin-angiotensin system (RAS) is an important player in cardiovascular disease, being involved in pathological processes in both the valve and myocardium in CAVS. Angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs) are well-known attenuators of RAS effects. However, observational retrospective studies investigating the ACE inhibitor effects on CAVS progression provided conflicting results. Treatment with ACE inhibitors was associated with less AVC²⁶ but did not appear to slow haemodynamic progression.²⁷ In principle, ARBs might have superior effects on both valve fibrosis and calcification,²⁸ but prospective RCTs are lacking. With respect to the LV hypertrophic response, the RIAS RCT showed a modest but significant reduction of myocardial hypertrophy in patients with CAVS treated with ramipril.²⁹ Finally, clinical observational studies have suggested that ACE inhibitors and ARBs are associated with favourable effects on symptoms (dyspnoea and exercise tolerance) and improved survival in patients with CAVS.³⁰ Again RCT data are lacking.

Statins

Statins are widely used for lipid lowering in atherosclerosis and inflammation, being a specific inhibitor of hydroxymethylglutaryl-coenzyme A-reductase (HMG-CoA-reductase). Although retrospective studies suggested that statins might also be of benefit in CAVS, subsequent RCTs demonstrated that statins in fact have no effect on CAVS progression or clinical outcomes. This conclusion was

confirmed by a subsequent meta-analysis.³¹ The most plausible explanation for this failure is that whilst statins might intervene with inflammation and lipid deposition in the initiation phase, they have little effect once the propagation phase has become established when fibrosis and calcification are the dominant pathological processes.

Lipoprotein(a)

Lipoprotein(a), the preferential plasma carrier of oxidized phospholipids, is an LDL-like particle, containing additional apolipoprotein(a) and apolipoprotein-B100. A causal relationship between AVC and a single nucleotide polymorphism in the LPA locus was suggested.³² Although the precise mechanisms of action of Lp(a) require further elucidation, there is considerable interest in investigating whether Lp(a) is a modifiable target in CAVS. Statins are ineffective in reducing Lp(a);³³ however, several other therapeutic agents are currently in different stages of investigation. IONIS-APO(a)_{Rx} and IONIS-APO(a)-L_{Rx} ([Ligand-conjugated] antisense oligonucleotides targeting hepatic apolipoprotein(a) mRNA) have been investigated in Phase 1 and 2 trials, demonstrating an ability to reduce Lp(a) concentrations.³⁴ Other promising Lp(a) lowering alternatives are proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors and Niacin.³⁵ The effects of Niacin/PCSK9 on aortic stenosis are currently being investigated ('EAVaLL'. Clinicaltrials.gov identifier: NCT02109614 and 'PCSK9 inhibitors in the progression of aortic stenosis', Clinicaltrials.gov identifier: NCT03051360).

Bisphosphonates and denosumab

The calcification paradox implies that treatments for bone diseases (f.i. bisphosphonates or denosumab) might have a beneficial effect on vascular and valvular calcification while maintaining bone health.³⁶ Bisphosphonates inhibit osteoclast-mediated bone resorption, resulting in decreased bone loss.³⁷ The inhibitory effect of bisphosphonates on vascular calcification was demonstrated in animals.³⁸ Retrospectively, a delay in CAVS progression was confirmed,³⁹ whereas a more recent study failed to show a positive effect on haemodynamic CAVS progression or survival.⁴⁰ These data are, however, confounded by the disease accelerating effects of osteoporosis. The ongoing SALTIRE 2 (Clinicaltrials.gov identifier: NCT02132026) RCT will help determine the true impact of bisphosphonates. Denosumab, a human monoclonal antibody targeting RANKL, has been investigated in pre-clinical models. Its binding prevents the interaction between RANK and RANKL, resulting in inhibition of vascular calcification in mice.⁴¹ It is being investigated as part of SALTIRE 2.

Vitamin K

Vitamin K is a fat-soluble vitamin consisting of two forms, namely phyloquinone (vitamin K1, VK1) present in green leafy vegetables and menaquinones (vitamin K2, VK2) present in fermented food. Long-chain menaquinones (i.e. MK7) are transported more efficiently to extrahepatic tissues.⁴² However, dietary intake of vitamin K is not sufficient to ensure full activation of MGP.¹⁹ Vitamin K supplementation is an attractive option to replenish vascular vitamin K stores to ensure optimal calcification inhibition. Vitamin K supplementation in rats showed regression of warfarin-induced vascular calcification.⁴³ The prospective Rotterdam study was the first to report that dietary intake of VK2 showed an inverse relation with vascular calcification and mortality.⁴⁴ Furthermore, low vitamin K status was shown to be

associated with increased ucMGP levels and coronary artery calcification.¹⁹ Although promising, these studies were limited by the short-term follow-up, precluding measurable effects on clinical endpoints. Recently, the first in-man RCT demonstrated that vitamin K supplementation decelerated valvular calcification on computed tomography (CT) in a small group of patients with CAVS.⁴⁵ The effectiveness of vitamin K supplementation to reduce or hold calcification progression is currently subject of investigation in multiple trials ('iPACK-HD'. Clinicaltrials.gov identifier: NCT01528800, 'VitaVasK'. ClinicalTrials.gov identifier: NCT01742273, 'VitaK-CAC trial'. ClinicalTrials.gov identifier: NCT01002157, 'BASIK2'. ClinicalTrials.gov identifier: NCT02917525).

Imaging in calcific aortic valve stenosis: from assessment of haemodynamics to disease progression

The development of potential CAVS therapies creates a need for novel imaging techniques to assess their efficacy in Phase 2 clinical trials. These would select the most promising agents to proceed to larger and more expensive Phase 3 trials incorporating clinical endpoints. Several of such imaging approaches are discussed below and are listed in *Figure 4*.

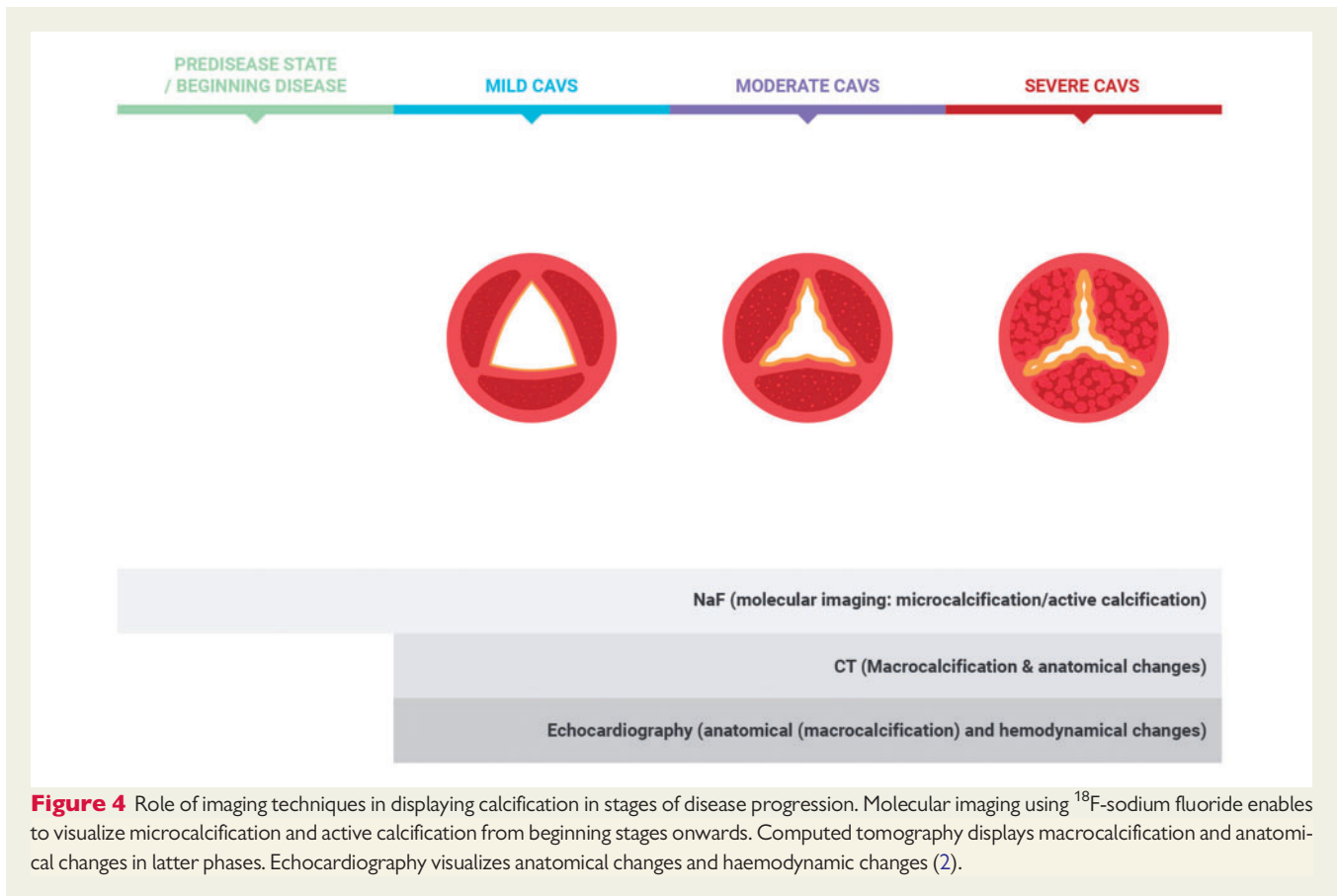
Echocardiography

Echocardiography is the most commonly used imaging technique to assess patients with aortic stenosis, providing detailed information on aortic stenosis severity, LV wall thickness, and function.² Despite slow annual rate of CAVS progression and relatively high scan-rescan variability, echocardiography is the most commonly used method for assessing aortic stenosis progression.

Another application of echocardiography is quantification of valve calcification, using a semi quantitative four-point scale. Although echo-assessed calcification is an independent predictor of events (death or AVR) and disease progression,⁴⁶ it is not widely used largely because of poor reproducibility and repeatability.

Computed tomography

Non-contrast multislice CT provides a more detailed and reproducible calcification scoring system. Computed tomography aortic valve calcification/calcium (CT-AVC) scoring enables quantification of mass, density, and volume of macroscopic valvular calcification, expressed in Agatston units (AU), similar to the approach developed for the coronary arteries. Computed tomography aortic valve calcification/calcium correlates well with haemodynamic parameters on echocardiography.⁴⁷ Interestingly, women require less calcification to develop severe CAVS than men, resulting in gender-specific CT-AVC thresholds for severe CAVS (1275AU/2065AU for females/males), with additional prediction of subsequent disease progression and clinical events.⁴⁸ Furthermore CT-AVC demonstrates relatively large annualized changes and specific calcification patterns provide additional insight for surgical and TAVI planning.⁴⁹ Computed tomography aortic valve calcification/calcium is therefore appealing as an alternative method to assess disease severity and progression and



was recommended in the recent ESC guidelines for this purpose.² Although CT-AVC provides excellent quantification of the established valve calcific burden, it does not inform about disease activity or the biological mechanisms underlying CAVS.

Positron emission tomography

In contrast to echocardiography and CT, positron emission tomography (PET) is an imaging technique that informs about the activity of specific biological processes. Inflammation and calcification can both be targeted using the PET tracers ^{18}F -fluorodeoxyglucose (^{18}F -FDG) and ^{18}F -sodium fluoride (^{18}F -NaF), respectively. ^{18}F -fluorodeoxyglucose has been applied to quantify vascular inflammation in the carotid arteries, correlating with macrophage infiltration.⁵⁰ Increased valvular ^{18}F -FDG uptake was demonstrated recently in CAVS and associated with faster subsequent disease progression.^{51–53} However, assessment of valvular ^{18}F -FDG activity is frequently obscured by uptake in the adjacent myocardium⁵⁴ and may reflect glucose utilization by a range of different cells or stimulating mechanisms.⁵⁵ ^{18}F -sodium fluoride has been used for many decades for the detection of bone metastases and primary osteoblastic tumours.⁵⁶ In the vasculature, it has been used to image developing microcalcification in carotid, coronary, and aortic atheroma^{57,58} and in CAVS,⁵⁹ providing complementary information to CT-AVC. Indeed, a striking mismatch has consistently been observed between the localization of the macroscopic calcium deposits on CT and the developing microcalcification identified by ^{18}F -NaF. ^{18}F -sodium

fluoride preferentially adsorbs to the available surface area of active hydroxyapatite crystal growth in areas of microcalcification, while uptake is low in regions with established areas of macroscopic calcification.⁶⁰ Histological validation of ^{18}F -NaF uptake in the valve in CAVS has been provided, demonstrating a close correlation with proteins involved in active calcification.⁵⁹

Prospective longitudinal studies have demonstrated that areas of microcalcification on ^{18}F -NaF PET develop with time into novel areas of macroscopic calcification. Thus ^{18}F -NaF PET acts as a good predictor of early disease progression in CAVS.^{52,59} On this basis, ^{18}F -NaF serves as a marker of calcification activity in CAVS and holds major potential as a surrogate endpoint to test the efficacy of novel pharmacological interventions.

Conclusion and future perspectives

Calcific aortic valve stenosis represents an increasing health care burden, leading to either adverse events or the requirement for major heart surgery. The pathophysiological mechanisms involved in CAVS initiation and progression are being rapidly elucidated and include inflammation, fibrosis, and calcification. With this advancing knowledge, we have identified novel therapeutic targets like vitamin K and new imaging techniques such as ^{18}F -NaF PET that can be used to test the efficacy of novel agents and further inform our pathophysiological

understanding. Indeed, several potential pharmacological treatments are under current investigation to achieve the ultimate goal, i.e. the inhibition of disease progression in CAVS.

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