



Innate immune cells in the pathophysiology of calcific aortic valve disease: lessons to be learned from atherosclerotic cardiovascular disease?

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Abstract

Calcific aortic valve disease (CAVD) is the most common valvular disease in the developed world with currently no effective pharmacological treatment available. CAVD results from a complex, multifactorial process, in which valvular inflammation and fibro-calcific remodelling lead to valve thickening and cardiac outflow obstruction. The exact underlying pathophysiology of CAVD is still not fully understood, yet the development of CAVD shows many similarities with the pathophysiology of atherosclerotic cardiovascular disease (ASCVD), such as coronary artery disease. Innate immune cells play a crucial role in ASCVD and might also play a pivotal role in the development of CAVD. This review summarizes the current knowledge on the role of innate immune cells, both in the circulation and in the aortic valve, in the development of CAVD and the similarities and differences with ASCVD. Trained immunity and clonal haematopoiesis of indeterminate potential are proposed as novel immunological mechanisms that possibly contribute to the pathophysiology of CAVD and new possible treatment targets are discussed.

Keywords Calcific aortic valve disease · Atherosclerotic cardiovascular disease · Inflammation · Innate immune cells · Trained immunity · Clonal haematopoiesis of indeterminate potential

Introduction

Calcific aortic valve disease (CAVD) is the most common type of valvular heart disease in the Western world and is characterized by valvular inflammation, fibrosis and calcification. It is the leading cause of aortic valve stenosis and, ultimately, it can cause angina, syncope, heart failure and sudden cardiac death [27]. One in four people over 65 years suffer from aortic valve sclerosis of which 10–15%

progresses to aortic valve stenosis [114]. Once symptomatic, untreated patients have a poor prognosis with a 2- and 5-year survival rate of 50% and 25%, respectively [114]. CAVD has a major impact on health care and this is expected to increase in the coming decades due to the ageing population [16]. Currently, no effective pharmacological treatment is available to prevent CAVD or slow down disease progression.

Traditionally, the development of CAVD was seen as a passive, degenerative process, but nowadays it is increasingly recognized as an active, multifactorial process with an important role for activation of the innate immune system. Importantly, this process appears to have many similarities with the pathophysiology of atherosclerosis and the pathophysiological process underlying atherosclerotic cardiovascular disease (ASCVD), such as coronary artery disease (CAD) [19, 82]. However, the exact underlying pathophysiology of CAVD remains incompletely understood, which hampers target-specific development of pharmacotherapy.

In this review, we discuss the role of innate immune cells, and in particular the role of monocytes, in the development

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of CAVD, and its similarities and differences with ASCVD. After a brief comparison of the overall pathophysiology of CAVD and ASCVD, we discuss in detail the current knowledge on valvular and systemic inflammation and innate immune cells in the development and progression of CAVD and the pivotal role of oxidized lipids. For each component, we systematically compare its role in CAVD and ASCVD. Furthermore, we propose two novel immunological mechanisms that might contribute to innate immune system activation in CAVD, namely trained immunity and clonal haematopoiesis of indeterminate potential (CHIP). Finally, we discuss how this knowledge might deliver novel therapeutic targets for the treatment of CAVD.

Summary of the pathophysiology of CAVD

The aortic valve is tricuspid, although 1–2% of individuals have a bicuspid or even a unicuspid or quadricuspid valve. Aortic valve leaflets consist of valvular endothelial cells (VECs), valvular interstitial cells (VICs) and valvular extracellular matrix (VECM) [119]. VECs cover the leaflets and regulate valve permeability and homeostasis. The valvular interstitium is composed of three layers: the laminae fibrosa (aortic side), spongiosa and ventricularis. VICs are found throughout the interstitium and regulate valve remodelling via the synthesis and degradation of VECM components. VICs are quiescent and have characteristics similar to fibroblasts in the homeostatic state [115]. Furthermore, healthy valves contain few resident macrophages, mast cells and dendritic cells as well as a small number of myofibroblast-like cells [40, 113].

The current proposed pathophysiological process of CAVD is divided into an initiation and a propagation phase (Fig. 1) [3]. The initiation phase starts by damage and stimulation of the VECs, which can be initiated by oxidative or mechanical stress [113, 152]. As bicuspid and unicuspid valves are subject to more mechanical stress, they often develop aortic valve stenosis one to two decades earlier [136]. The valvular damage alters the permeability and allows for infiltration of circulating lipoproteins, such as lipoprotein (a) (Lp(a)) and low-density lipoprotein (LDL) and immune cells, including monocytes and T lymphocytes [109, 111, 113]. Oxidized LDL (oxLDL) and Lp(a) stimulate and activate VICs and VECs, creating an inflammatory environment [111, 113] which further propagates the infiltration of immune cells [55]. The inflammatory milieu promotes VECs, VICs and macrophages to secrete extracellular vesicles, and induces apoptosis of macrophages and VICs, which release apoptotic bodies [72, 93]. Both processes cause microcalcifications by dystrophic calcification. Moreover, VICs are stimulated to differentiate into a myofibroblastic phenotype, causing VECM remodelling and fibrosis [25].

Further differentiation of myofibroblasts into an osteoblast-like phenotype results in biomineralization [58].

The propagation phase is characterized by accelerated fibrosis and calcification [102]. Accumulation of calcium and leaflet stiffening creates more mechanical stress and calcium deposition. A self-perpetuating cycle is established which eventually leads to narrowing of the valvular orifice [102, 177]. The valvular obstruction creates left ventricle systolic pressure overload, leading to myocardial hypertrophy, interstitial fibrosis and ultimately results in heart failure [82]. For the purpose of this review, we refer to excellent recent reviews for a more detailed general overview of the pathophysiology of CAVD [57, 72, 82, 115].

CAVD and ASCVD, two sides of the same coin?

The development of CAVD is increasingly considered to be an atherosclerosis-like process, especially in the initiation phase [38]. Both CAVD and ASCVD represent chronic inflammatory disorders, which involve initial endothelial damage and activation, lipid deposition, immune cell recruitment, inflammation, neoangiogenesis and calcification (Fig. 2) [38, 102]. Pathological studies show active remodelling processes regulated by inflammation in both ASCVD and CAVD [27]. Importantly, in atherosclerosis-prone apolipoprotein E-deficient (*ApoE*^{-/-}) mice, atherosclerotic plaques develop first in the aortic valves and aortic root [54]. Furthermore, CAVD and CAD often co-exist [31, 74], are both slowly progressive conditions with precursor lesions that remain asymptomatic for some time and they share important risk factors, including increased age, male sex, cigarette smoking, hypertension, kidney disease, diabetes mellitus, obesity, hyperlipidaemia, elevated Lp(a) levels and shared genetic susceptibility loci [20, 27, 130, 138, 151].

The role of the innate immune system is well established in the pathophysiology of ASCVD. Monocyte-derived macrophages are the principal immune cell type in atherosclerotic plaques and are involved in its initiation, progression and destabilization [95]. Limiting the influx of circulating monocytes into the arterial wall in atherosclerosis-prone mice prevents atherosclerotic plaque formation [17]. In these *ApoE*^{-/-} mice, the lesion size was reduced particularly in the valve leaflet region, where wild-type mice developed the most severe lesions [54]. Furthermore, targeting inflammation can prevent clinical atherosclerotic complications [139]. In addition, accumulating evidence points to the fact that circulating monocytes are characterized by an activated inflammatory phenotype in patients with established ASCVD or risk factors for ASCVD, including elevated LDL-cholesterol and Lp(a). Thus, activation of the innate immune system not only occurs in the inflammatory micro-environment of the

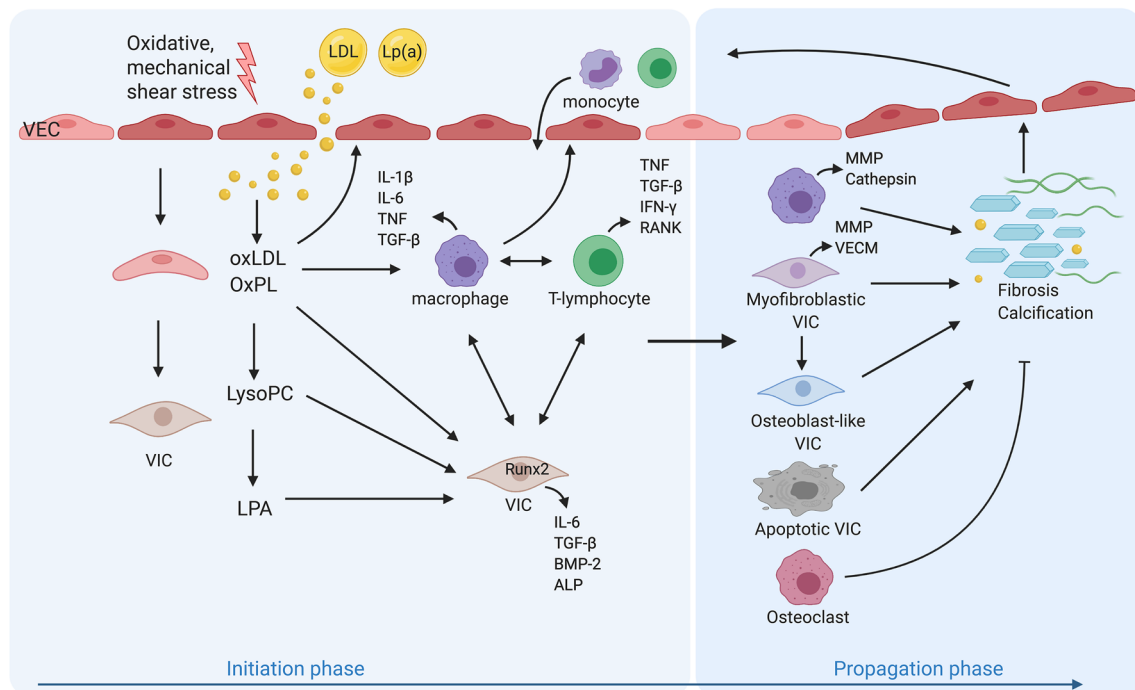


Fig. 1 The pathogenesis of calcific aortic valve disease. In the initiation phase, valvular endothelial cells (VECs) are activated by oxidative, mechanical or shear stress, leading to increased valvular permeability. This results in infiltration of circulating lipids (lipoprotein (a) (Lp(a)) and low-density lipoprotein (LDL)) and immune cells, such as monocytes, neutrophils and lymphocytes. The oxidized LDL (oxLDL) and Lp(a) contain oxidized phospholipids (OxPL), which both activate macrophages and T lymphocytes and stimulate the release of various pro-inflammatory molecules that activate other immune cells, VECs, and valvular interstitial cells (VICs). The oxidized lipids also directly activate VEC and increase the expression of adhesion molecules, prompting the recruitment of more immune cells. OxPL are transformed into lysophosphatidylcholine (LysoPC) by lipoprotein-phospholipase A₂ (Lp-PLA₂), which is subsequently converted into lysophosphatidic acid (LPA) by autotaxin (ATX). LPA then activates VICs, triggering an NF-κB-regulated inflammatory cascade, which results in increased expression of bone morphogenic protein (BMP) 2, IL-6 and Runt-related transcription factor 2 (Runx2) and secretion of alkaline phosphatase (ALP). Additionally,

the OxPL derive LysoPC induces apoptosis in VICs. In the propagation phase, VICs differentiate into a myofibroblastic or osteoblast-like phenotype upon stimulation by the pro-inflammatory molecules and promote fibrosis and calcification, respectively. The activated macrophages and myofibroblastic VICs secrete matrix remodelling proteins and valvular extracellular matrix (VECM) components. The continuous redeposition and destruction of VECM creates valvular stiffness. The chronic inflammation stimulates apoptosis of macrophages and VICs and the release of extracellular vesicles, including apoptotic bodies, which both promote the continuous deposition of microcalcifications and crystals. Osteoblast-like VICs induce biomineralization in a way akin to osteogenesis. T lymphocytes stimulate proinflammatory polarization of macrophages and the osteogenic differentiation of VICs. IFN-γ, produced by T lymphocytes, inhibits the function of macrophage-derived osteoclasts. Together, these processes create accumulation of calcium and leaflet stiffening, creating more mechanical stress and thereby prompting more calcium deposition, establishing a self-perpetuating cycle which eventually leads to valvular outflow obstruction

plaque, but also in circulating monocytes [13, 14, 134, 163]. Moreover, recent studies have pointed out that the activation of innate immune cells in ASCVD also occur at the level of the myeloid progenitors in the bone marrow compartment [107, 164].

There are also important differences between CAVD and ASCVD, particularly in the advanced stages of the diseases. Firstly, there are patients with severe CAVD who do not suffer from advanced ASCVD and vice versa [62]. Secondly, statins do not prevent cardiovascular events in CAVD as opposed to their beneficial effect in patients with ASCVD, suggesting different pathophysiological processes [23, 48, 126]. Moreover, advanced lesions display histological differences, such as the fibrous cap and

necrotic cores rich in foam cells in atheromas, which are not present in CAVD [89], and the limited presence of foam cells in calcified valves [71]. Lastly, adverse events in atherosclerosis are often related to plaque ruptures leading to acute coronary syndrome, while in CAVD, they are mostly caused by slowly progressive valve narrowing driven by progressive calcification [38, 90]. Despite the differences between CAVD and ASCVD, the profound commonalities in risk factors and similarities in (early) pathological features suggest overlap in pathophysiology, including a key role for inflammation and activation of the innate immune system. A systematic overview of the similarities and differences between CAVD and ASCD is given in Table 1.

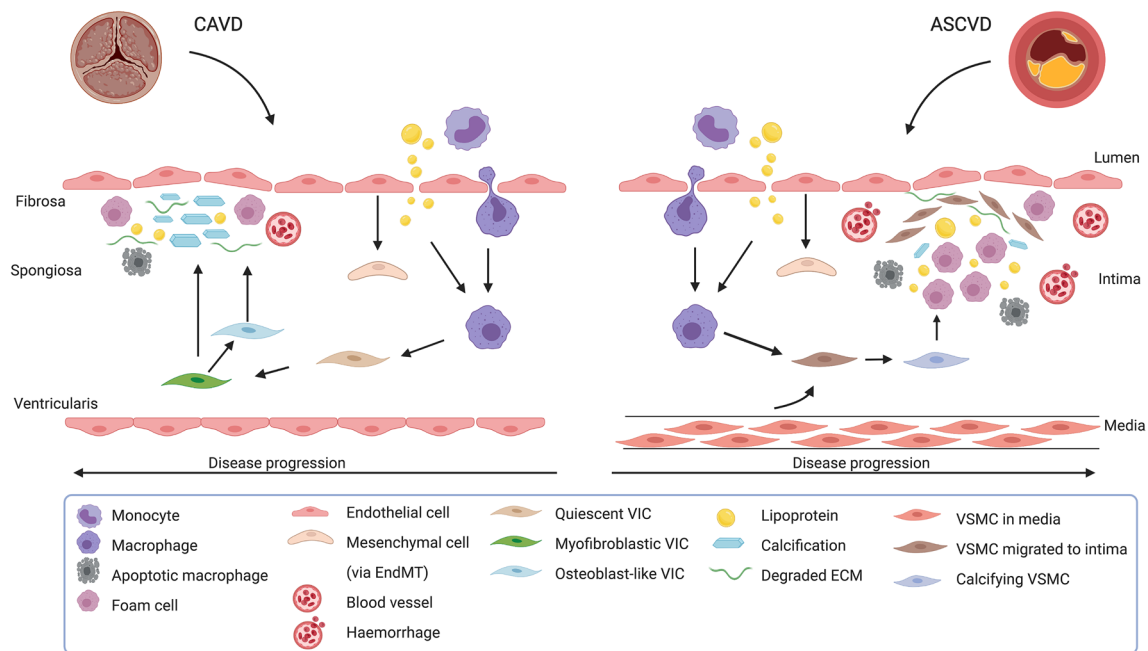


Fig. 2 Schematic overview of innate immune cells in the pathophysiology of calcific aortic valve disease and atherosclerotic cardiovascular disease. The underlying pathophysiology of calcific aortic valve disease (CAVD) and atherosclerotic cardiovascular disease (ASCVD) shows many similarities in the initiation phase. In both CAVD as ASCVD, endothelial cells are damaged and activated, leading to lipoprotein infiltration and immune cell recruitment. The macrophages take up lipoproteins, leading to activation with subsequent secretion of proinflammatory cytokines and proteolytic enzymes and foam cell formation. Activated endothelial cells differentiate into mesenchymal cells (endothelial to mesenchymal transition) and transmigrate to the valvular interstitium or intima of the vessel wall. When the CAVD and ASCVD progress, the lesions start to show more differ-

ences. In CAVD, the valvular interstitial cells (VIC) are stimulated to differentiate to myofibroblasts or osteoblast-like cells and promote fibrosis and calcification, respectively. There are few foam cells and there is only little neovascularization. Apoptotic macrophages, VICs and foam cells contribute to the calcification. In ASCVD, foam cells are abundant and found across the intima and there is intraplaque haemorrhage due to leaky neovessels. Vascular smooth muscle cells (VSMCs) migrate from the media to the intima and form a fibrous cap. The activated macrophages stimulate osteoblastic differentiation of VSMCs subsequently. Macrophages, foam cells and VSMCs can die in advanced lesions by apoptosis, generating a necrotic core. Calcification is caused by osteoblast-like cells and the deposition of microcalcifications, which are generated by apoptotic cells

CAVD, a chronic inflammatory disease

Inflammation in CAVD occurs on several levels. Besides local inflammation in the aortic valves, inflammation can be observed in the circulation, by activated immune cells and increased inflammatory proteins. In this section, we will describe the various components of the inflammatory process in CAVD, from local to systemic inflammation, and compare this to the situation in ASCVD.

Local valvular inflammation

CAVD develops by an active inflammatory process driven by infiltrated lipoproteins and immune cells. Histopathological examination of human calcified aortic valves shows subendothelial thickening with lipid deposition, immune cell infiltration (predominantly macrophages and T lymphocytes) and mineralization in early CAVD lesions and more

advanced lesions in further progressed CAVD [29, 89, 94, 111, 113]. The inflammatory infiltrates are associated with valvular remodelling, neovascularization and osseous metaplasia [29]. Moreover, calcified aortic valves show an upregulated expression of multiple proinflammatory cytokines in total valve tissue, including interleukin (IL)-1 β , IL-6, tumour necrosis factor (TNF), anti-inflammatory cytokines, as IL-10, and transforming growth factor (TGF)- β , as well as chemokines such as chemokine C-X-C ligand (CXCL) 5, CXCL9, chemokine C-C ligand (CCL) 19 and CCL 21 as summarized in Raddatz et al. [118]. The expression of the anti-inflammatory cytokine IL-37 is downregulated in calcified aortic valves [172]. The cells contributing to this valvular inflammation will be discussed below.

Valvular endothelial damage

Early in CAVD development, altered haemodynamic forces on the valve affect the phenotype of VECs, leading to endothelial dysfunction. These altered forces result for

Table 1 Similarities and differences between calcific aortic valve disease and atherosclerotic cardiovascular disease

	CAVD	ASCVD
Risk factors	<ul style="list-style-type: none"> ✓ Older age predisposition ✓ Male sex ✗ Bicuspid aortic valve ✗ Endocarditis 	<ul style="list-style-type: none"> ✓ Diabetes mellitus ✓ Renal failure ✓ Obesity ✓ Hypertension ✓ Genetic ✓ Smoking ✓ Dyslipidaemia ✓ Radiotherapy of chest
Pathology:		
- Endothelial cells	<ul style="list-style-type: none"> ✓ Activation by altered stress, leading to infiltration of lipids and immune cells ✓ EndMT 	
- Interstitial cells	<ul style="list-style-type: none"> ✗ VICs differentiation into myofibroblasts and osteoblast-like cells 	<ul style="list-style-type: none"> ✗ VSMCs migrate to the intima ✗ Fibrous cap surrounding necrotic core
- Lipids	<ul style="list-style-type: none"> ✓ Subendothelial lipid accumulation ✓ Inflammation driven by oxidized lipids ✓ Induce apoptosis 	
- Calcification	<ul style="list-style-type: none"> ✓ Macro- and microcalcifications ✓ Calcification by apoptotic cells 	
	<ul style="list-style-type: none"> ✗ Calcification by osteoblast-like VICs 	<ul style="list-style-type: none"> ✗ Calcification by osteochondrogenic VSMCs
- Blood vessels	<ul style="list-style-type: none"> ✗ Little haemorrhages and neovascularisation 	<ul style="list-style-type: none"> ✗ Frequent neovascularisation ✗ Frequent intraplaque haemorrhages
Innate immune cells		
- Macrophages	<ul style="list-style-type: none"> ✓ Macrophage infiltration ✓ Production of pro-inflammatory markers and MMPs, contributing to local inflammation, ECM degradation and calcification. 	
	<ul style="list-style-type: none"> ✗ Few foam cells, restricted to their inflow or outflow surfaces 	<ul style="list-style-type: none"> ✗ Many foam cells, abundant throughout the entire neointima ✗ Contribute to the necrotic core
- Monocytes	<ul style="list-style-type: none"> ± Possible altered (pro-)inflammatory phenotype of circulating monocytes 	<ul style="list-style-type: none"> ± Pro-inflammatory circulating monocytes with increased cytokine production capacity.
- Neutrophil	<ul style="list-style-type: none"> ± N/L ratio is associated with the presence, severity and prognosis of CAVD ± Exact role still unclear 	<ul style="list-style-type: none"> ± Circulating granulocyte count and N/L ratio is associated with ASCVD. ± Promote vascular inflammation.
Systemic inflammation	<ul style="list-style-type: none"> ± Exact relationship with circulating pro-inflammatory markers is still unknown 	<ul style="list-style-type: none"> ± Elevated levels of pro-inflammatory markers are associated with MACE.
Adverse events	<ul style="list-style-type: none"> ✗ Valve narrowing leading to ventricular hypertrophy and heart failure 	<ul style="list-style-type: none"> ✗ Blood flow disruption and occlusion of blood vessel after erosion, rupture or thrombosis, leading to ischemia.
Therapy	<ul style="list-style-type: none"> ✗ Lipid lowering is not effective in preventing cardiovascular events in CAVD ± Anti-inflammatory agents are not yet investigated in CAVD 	<ul style="list-style-type: none"> ✗ Lipid lowering is effective in preventing cardiovascular events in ASCVD ± Anti-inflammatory agents are beneficial in preventing MACE.

✓ Similar in CAVD and ASCVD, ✗ different in CAVD and ASCVD, ± unclear.

ASCVD, atherosclerotic cardiovascular disease; CAVD, calcific aortic valve disease; EndMT, endothelial to mesenchymal transition; MACE, major adverse cardiovascular event; MMP, matrix metalloproteinases; N/L ratio, Neutrophil-to-lymphocyte ratio; VIC, valvular interstitial cell; VSMC, vascular smooth muscle cell.

example from hypertension [83], by stiffening of the valvular tissue due to ageing [143, 165], or from increased oxidative stress. In addition, other risk factors, such as diabetes or dyslipidaemia could precipitate endothelial dysfunction. The relevance of VEC injury is demonstrated by histopathological studies showing lipoprotein accumulation mainly in

regions of low shear stress [109]. In the aortic valve, the damaged VECs subsequently express adhesion molecules, stimulating the recruitment of immune cells [55]. These immune cells produce cytokines leading to further stimulation of VECs and the transition of VECs into VICs by endothelial to mesenchymal transition [84]. Furthermore,

VECs express endothelial nitric oxide synthase (eNOS), which regulates the production of nitric oxide. Calcified valves express reduced levels of eNOS, leading to increased oxidative stress which contributes to valvular inflammation by increasing lipoprotein oxidation [120, 153]. By these very processes, VECs promote inflammation, fibrosis and calcification.

This is comparable to the initiation phase in atherogenesis, where endothelial cell (EC) activation occurs in areas of disturbed shear stress, such as near arterial bifurcations, which permits circulating ApoB-containing lipoprotein and immune cells to enter the intimal space [81]. Stimulated ECs can also undergo endothelial to mesenchymal transition and migrate to the intima, where they can contribute to inflammation and intimal thickening [81]. Endothelial to mesenchymal transition is associated with atherosclerotic plaque instability [42].

Valvular interstitial cells contribute to valvular inflammation

VICs are the most abundant cells in the valvular tissue and transiently transition into myofibroblasts during normal hemodynamic stress on the valvular tissue to remodel the ECM. During CAVD progression, the transition into myofibroblasts is more persistent, leading to pathological fibrosis. Ultimately, the myofibroblasts can differentiate into an osteoblast-like phenotype, which promotes calcium depositions. Although the exact underlying process driving valvular fibrosis and calcification remains uncertain [77], it is clear that proinflammatory communication between VICs and immune cells plays an important role. Firstly, Toll-like receptors (TLRs), especially TLR-2 and -4, are upregulated in VICs in calcified aortic valves. Several pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) promote inflammation and osteogenesis by activating the nuclear factor- κ B (NF- κ B) pathway in VICs via TLR stimulation. This leads to the production of proinflammatory and pro-calcifying molecules [52]. Recently, it was demonstrated that the upregulation of TLR2 in VICs is enhanced through paracrine signalling of TNF by activated monocytes [174]. IL-37, which shows a lower expression in calcified valves, suppresses the production of proinflammatory mediators by human VICs after TLR stimulation [172, 173]. Secondly, the differentiation of VICs into myofibroblasts or osteoblast-like cells is stimulated by cytokines, including TGF- β 1 [76] or TNF, IFN- γ , IL-6 and receptor activator of NF- κ B ligand (RANKL) [51, 57, 65]. In addition, myofibroblasts upregulate the expression of leptin-like oxidized LDL receptor-1 and scavenger CD36 receptors, leading to uptake of oxidized lipids and production of inflammatory molecules, resembling the foam cell-forming potential of macrophages in atherosclerotic

lesions [147]. Lastly, VICs promote calcification via apoptosis and osteogenesis [77].

The processes of valvular fibrosis and calcification by VICs differ substantially from the extracellular matrix remodelling that occurs in atherosclerotic plaques, in which local inflammation stimulates smooth muscle cells to form a fibrous cap that shields to growing necrotic core, and stimulates vascular calcification which is summarized in detail in other reviews [37, 81].

Macrophages

Macrophages are present in healthy valves, although histopathologic examination of explanted calcified valves demonstrates a higher abundance with CAVD progression [79, 113]. The majority of macrophages are located close to calcium deposits and areas of vascularization [99] and the inflammatory infiltrates are associated with active VECM remodelling, the severity of the stenosis and haemodynamic progression [29].

Macrophages are presumed essential in the initiation phase of CAVD (Fig. 1). After infiltration of circulating monocytes and differentiation into macrophages, the cells take up modified lipids via their scavenger receptors and can become foam cells [71, 111]. Calcified aortic valves contain only few foam cells, although fatty streaks are prominently found at the inflow and outflow surface of the valves [71]. In addition, macrophages are activated by cytokines and oxidized lipoproteins via pattern recognition receptors (PRRs) resulting in activation of the NF- κ B pathway. Activated macrophages secrete multiple proinflammatory molecules, including IL-1 β , IL-6, TNF, TGF- β , cathepsins, osteopontin and matrix metalloproteinases (MMPs) [47, 72]. The predominant macrophage subset found in human explanted calcified aortic valves consists of proinflammatory CD11c-positive macrophages [79] with an increased mRNA expression of iNOS, monocyte chemoattractant protein 1 (MCP-1), TNF, IL-6 and IL-12 [78]. In addition, the number of anti-inflammatory macrophages (CD206+) is lower compared to healthy valves [79]. Chronic inflammation arises as the macrophages stimulate VECs, VICs and other immune cells, thereby promoting further immune cell recruitment, apoptosis, myofibroblastic and osteogenic differentiation of VICs and the differentiation of VECs into VICs via endothelial to mesenchymal transition [57, 96].

In the propagation phase, macrophages contribute to accelerated valvular fibrosis and calcification [57, 72]. Fibrous VECM remodelling is dependent on fibrosis and proteolysis. Activated macrophages produce TGF- β 1, which in turn induces myofibroblastic differentiation of VICs [76] and secrete matrix remodelling proteins, such as MMPs, which promotes proteolysis.

Valvular calcification relies on two distinct processes; dystrophic calcification and biomineralization. Dystrophic calcification is defined by continuous deposition of microcalcifications and hydroxyapatite by apoptotic macrophages and VICs and extracellular vesicles, and is responsible for most of the calcification [72, 93]. Biomineralization is induced by osteoblast-like VICs, resembling osteogenesis. Inflammatory communication might play an important role in the calcification potential of these VICs [58, 79]. Conditioned medium of proinflammatory macrophages deactivates myofibroblasts and stimulates their proliferation, which is attributed to TNF and IL-1 β [58]. TNF and IL-6, also secreted by proinflammatory macrophages, stimulate osteogenic differentiation of VICs and upregulate the expression of osteogenic markers by VICs [58, 79]. Macrophages can also contribute to clearance of mineralization through osteoclastogenesis after stimulation by RANKL and macrophage colony-stimulating factor (M-CSF), produced by osteoblast-like cells and T lymphocytes [18, 33]. These osteoclast-like cells can be found in calcified aortic valves and express proteins involved in mineral uptake and bone resorption [93, 97]. However, interferon (IFN)- γ produced by activated T lymphocytes impairs this osteoclastic activity. As a result, the osteoclast-like cells cannot counterbalance the osteoblastic activity from the VICs [98]. These processes, orchestrated by macrophages, progressively increase valvular fibrosis and calcification. The role of the adaptive immune system in the development of CAVD is discussed in recent excellent reviews [10, 118].

The role of macrophages in ASCVD is well established and shows many similarities with the pathogenesis of CAVD (Table 1). Monocyte-derived macrophages are the main immune cell type found in the atherosclerotic plaque and play a central role in all stages of atherogenesis [95]. Proinflammatory stimuli within the atherosclerotic plaque stimulate the macrophages to produce multiple proinflammatory chemokines and cytokines, creating an inflammatory milieu [24, 145]. However, contrary to in CAVD, foam cells are abundant in the atherosclerotic plaque and are distributed randomly across the neointima [71]. During progression of the atherosclerotic plaque, the death of foam cells and macrophages and subsequent impaired clearance of apoptotic cells by phagocytic cells (efferocytosis), contribute to the formation of a lipid and necrotic core [69, 80]. Moreover, macrophages contribute to destabilization of the atherosclerotic plaque by producing proteases [103].

Oxidized lipids: central regulators of inflammation in CAVD

Lipoproteins take centre stage in the development and progression of CAVD by orchestrating the underlying

inflammatory process. Observational studies and Mendelian randomization studies indicate that elevated LDL-cholesterol and Lp(a) levels are risk factors for CAVD [5, 7, 91, 137, 144]. Elevated Lp(a) levels are also associated with accelerated disease progression [7, 20, 176]. A single nucleotide polymorphism in the Lp(a) locus (rs1045872) is associated with elevated Lp(a) levels [7] and with aortic valve calcification [150]. Patients with mild to moderate CAVD with elevated Lp(a) levels suffer from a faster CAVD progression [21]. Lipids and lipid loaded macrophages localize predominantly in the subendothelial region of the fibrosa side of the valve [71, 111]. Valves containing higher amounts of oxLDL have denser inflammatory infiltrates, increased valvular tissue remodelling and higher expression of TNF [94]. The crucial role of lipoproteins is further demonstrated in the Reserva mouse model in which rapid normalisation of circulating cholesterol after a period of hyperlipidemia leads to a normalization of valvular oxidative stress, suppression of pro-osteogenic signalling, and a prevention of disease progression [91].

Lp(a) and oxLDL are powerful stimuli that drive valvular inflammation and calcification by oxidized phospholipids (OxPL) (Fig. 1). Lp(a) is the major carrier of OxPL in the circulation [163]. Antigen-presentation of oxLDL and apoB can activate T lymphocytes that subsequently stimulate VICs [92]. Oxidized lipids directly stimulate VECs leading to increased expression of bone morphogenic protein (BMP) 2 and adhesion molecules, thereby promoting calcification and the recruitment of immune cells [55, 109, 146]. OxLDL can augment the osteogenic response of human VICs through modulation of the NF- κ B pathway and NOTCH1 activation [171]. Furthermore, OxPLs are transformed into lysophosphatidylcholine (LysoPC) and subsequently into lysophosphatidic acid (LPA) by Lp-PLA₂ (lipoprotein-phospholipase A₂), leading to apoptosis in VICs. LPA triggers an NF- κ B-regulated inflammatory cascade in VICs and leads to increased expression of BMP2, IL-6 and Runt-related transcription factor 2 (Runx2) and secretion of alkaline phosphatase, stimulating valvular calcification [108, 130]. Additionally, LysoPC induces apoptosis in VICs [85]. In addition to Lp(a) and oxLDL, also triglyceride-rich lipoproteins are associated with an increased CAVD risk. Triglyceride-rich lipoproteins likely contribute to the lipid deposition and local inflammation by the release of monoacylglycerols and free fatty acids [66].

OxPL play a similar role in the development of ASCVD. OxLDL induces endothelial dysfunction and activation, triggers the recruitment of circulating immune cells and foam cell formation, and stimulates the VSMC migration and proliferation in the atherosclerotic plaque. Furthermore, oxLDL contributes to the destabilization of the

atherosclerotic plaque by inducing apoptosis and the release of MMPs [116]. The OxPL on Lp(a) cause a proinflammatory monocyte response and inflammation of the arterial wall in humans [163].

Beyond the valve: systemic inflammation and circulating immune cell activation

There is accumulating evidence that CAVD, just like ASCVD, is characterized not only by local valvular inflammation, but also by low-grade systemic inflammation, although this is still controversial. In ASCVD, it is well established that elevated circulating levels of proinflammatory markers, e.g. IL-6 and high-sensitive C-reactive protein (hsCRP), are associated with major adverse cardiovascular events, which is independent of other traditional risk factors [68, 121, 124, 169]. Some studies also suggest an association between circulating hsCRP and the presence, severity and progression of CAVD [50, 88, 129], mostly in patients with advanced CAVD. Other studies, however, did not find a relationship between elevated hsCRP, the presence of aortic sclerosis or CAVD and disease progression [59, 106]. Also, no correlation was found between hsCRP and valvular inflammatory infiltrates [88]. The exact relationship between hsCRP levels and CAVD therefore remains uncertain. In a small observational study, patients with severe CAVD had increased levels of circulating TNF, but it is still not known whether this is causally related to CAVD pathophysiology or results from the haemodynamic consequences of CAVD [67]. More evidence points to activation of circulating innate immune cells in CAVD pathophysiology, which is highlighted below.

Monocyte activation in CAVD

Human monocytes can be divided in three subsets based on the surface expression of CD14 and CD16: classical monocytes are CD14⁺⁺CD16⁻, intermediate monocytes CD14⁺⁺CD16⁺ and non-classical monocytes are CD14⁺CD16⁺⁺ [141, 178]. In addition, other surface markers can be used to characterize subsets with specific functions. For example, classical monocytes are known for their high C-C chemokine receptor type 2 (CCR2) expression, whereas intermediate monocytes have high CCR5 expression [46, 141]. In general, circulating CD16⁺ monocytes, especially the intermediate monocytes, are associated with atherosclerotic disease [125, 155].

Few studies have characterized monocyte subsets in the setting of CAVD. Most of these studies are cross-sectional and observational in small patient groups, hence results need to be interpreted with caution. Shimoni et al. showed increased levels of CD14⁺ monocytes in patients with severe

CAVD compared to controls which was inversely correlated with the aortic valve area surface [133]. Hewing et al. showed that patients with severe CAVD have higher levels of proinflammatory intermediate monocytes [59]. The level of intermediate monocytes has been reported to drop after aortic valve replacement (AVR) [60, 101], although the effect of the surgical intervention itself was not evaluated with a control group. The relationship between disease severity and monocyte subtypes is still unclear [59, 101]. It remains speculative whether the increased levels of circulating (intermediate) monocytes play a causal role in the pathophysiology of CAVD or, are rather a consequence of the disease through haemodynamic changes or valvular inflammation. Although these studies suggest that the phenotype of circulating monocytes is altered in patients with CAVD, more in-depth exploration of monocyte function and phenotype has not yet been performed.

Changes in monocyte phenotype have been described in the setting of ASCVD and also for several risk factors for CAVD. In patients with established CAD, circulating monocytes are characterized by an augmented cytokine production capacity [14, 134]. In addition, patients with elevated levels of Lp(a) show an increased level of intermediate monocytes, which correlates with OxPL/apoB, independent of circulating CRP and IL-6 [73]. Also, monocytes from patients with increased levels of LDL-cholesterol as well as Lp(a) show a hyperresponsive state, with an enhanced cytokine production capacity and increased transendothelial migration [13, 131, 163], which is associated with increased arterial wall inflammation in high Lp(a) conditions [163]. Circulating monocytes stem from bone marrow hematopoietic stem and progenitor cells. In patients with CAD, the bone marrow myeloid progenitor cells are programmed towards a proinflammatory phenotype [107]. This has never been investigated in the context of CAVD.

Neutrophils: important players in CAVD?

The role of neutrophils in cardiovascular inflammation and their possible contribution to the pathogenesis of CAVD only recently gained attention. Patients with severe CAVD have a higher absolute circulating neutrophil count compared to healthy controls [140]. An increased neutrophil-to-lymphocyte ratio is associated with the presence, severity and prognosis of CAVD [9, 26, 140]. In addition, Kopytek et al. demonstrated that calcified aortic valves exhibit significantly more neutrophil extracellular trap (NET) formation compared to healthy valves and that the amount of valvular NETs correlates with disease severity, suggesting a role for neutrophils in the progression of CAVD [70]. In contrast, by using electron microscopy, Kostyunin et al. did not find neutrophils to be present in severely calcified aortic valves [71]. More research is needed to identify the exact role of

neutrophils in the fibro-calcific remodelling of the aortic valve.

The role of neutrophils and NETs in ASCVD is more established. A recent prospective epidemiological study demonstrated that circulating granulocyte count is strongly associated with the future occurrence of ASCVD [43]. Furthermore, an increased granulocyte-to-lymphocyte ratio is a risk factor for ASCVD [43]. Neutrophil activation and recruitment is promoted by chemokines, such as CC-chemokine ligand 5, during atherogenesis [135]. Activated neutrophils then secrete granule proteins, including Cathepsin G, at the luminal side, which can activate chemokines resulting in further myeloid cell recruitment. The secretion of ROS and myeloperoxidase, which mediates LDL oxidation and subsequently promotes foam cell formation, further promotes atherosclerotic disease progression [135]. Besides,

neutrophils can promote vascular wall inflammation by the secretion of proinflammatory microvesicles [56] and the formation of NETs [35].

Proposed new mechanisms for circulating immune cell activation in CAVD

Until now, it is unclear how activation of innate immune cells might contribute to CAVD pathophysiology. Trained immunity and CHIP are recently described immunological mechanism that could potentially contribute to long-term activation of innate immune cells and we propose that these mechanisms could contribute to the development of ASCVD and CAVD (Fig. 3).

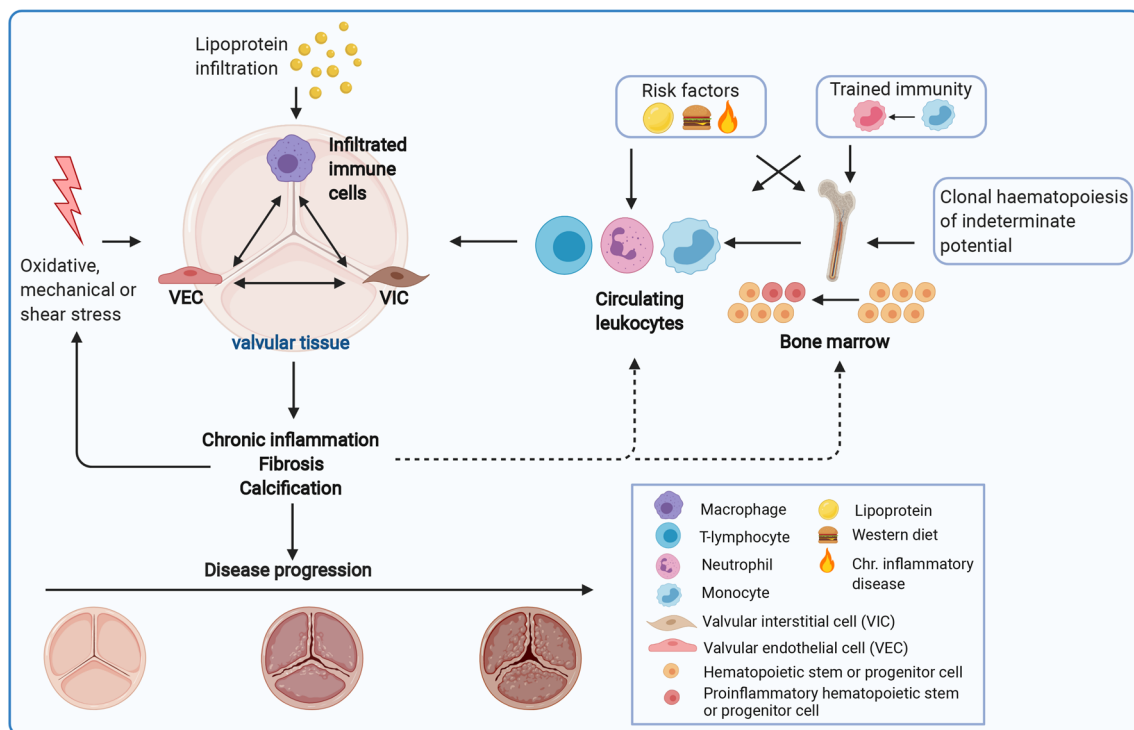


Fig. 3 A schematic illustration of how systemic immune cell reprogramming can contribute to CAVD pathophysiology. Oxidative, mechanical or shear stress damages and activates valvular endothelial cells (VECs), altering endothelial permeability. This causes lipoproteins and immune cells to infiltrate the valvular tissue, creating an inflammatory environment. Local migrated immune cells and activated VECs and valvular interstitial cells (VICs) continue to stimulate each other, thereby causing chronic inflammation, fibrosis and calcification. This leads to valve leaflet stiffening and thickening, which increases mechanical stress, establishing a self-perpetuating cycle. Activation of innate immune cells, such as monocytes, macrophages and neutrophils, contributes to the initiation and develop-

ment of CAVD. Risk factors for CAVD, such as hyperlipidaemia, elevated Lp(a) levels and a Western diet, activate hematopoietic stem and progenitor cells (HSPCs) and circulating immune cells. Trained immunity can lead to a persistent pro-inflammatory phenotype of circulating innate immune cells and myeloid progenitor cells. Clonal haematopoiesis of indeterminate potential (CHIP) results in a pro-inflammatory phenotype of HSPCs. The proinflammatory leukocytes infiltrate the valvular tissue and contribute to the development of CAVD by creating an inflammatory environment. The chronic inflammation that arises might in turn impact on HSPCs and circulating leukocytes

Trained immunity

Trained immunity describes the phenomenon that innate immune cells, including monocytes and macrophages, are able to adapt their function after a first encounter with a DAMP or PAMP, leading to a long-term hyperresponsive phenotype [11]. Although this mechanism is beneficial in the context of recurrent infections, it might be detrimental in chronic inflammatory diseases in which these innate immune cells themselves contribute to pathophysiology and tissue damage, such as atherosclerosis [45]. Trained immunity is dependent on intracellular metabolic and epigenetic reprogramming, resulting in a persistent proinflammatory phenotype, characterized by an increased cytokine production capacity [100]. In vitro, brief exposure of isolated human monocytes to oxLDL, Lp(a), uric acid or adrenaline/noradrenaline induces a trained macrophage phenotype [12, 161, 163].

Recent studies translated the concept of trained immunity to patients with established ASCVD or risk factors for ASCVD. In patients with CAD, the augmented cytokine production of circulating monocytes was associated with increased glycolysis and enrichment of epigenetic histone markers, characteristic of trained immunity [14]. In addition, monocytes from treatment-naïve patients with familial hypercholesterolaemia have an increased cytokine production capacity and enrichment of activating histone modifications on the promoters of these cytokine genes, which persisted for three months after cholesterol lowering with statins [13]. A similar hyperresponsive monocyte phenotype was observed in patients with elevated Lp(a) levels [163].

The prolonged presence of monocytes with a trained hyperresponsive phenotype is explained by the fact that training occurs at the level of myeloid precursors in the bone marrow [100]. In this regard, it was recently shown that isolated bone marrow mononuclear cells of patients with severe CAD demonstrate an increased cytokine production capacity and a higher metabolic rate than individuals without CAD. The bone marrow composition of the CAD patients showed skewing towards myelopoiesis and the hematopoietic stem and progenitor cells demonstrated enriched monocyte and neutrophil related pathways [107]. Moreover, several risk factors for both CAVD and ASCVD are described to reprogram myeloid progenitor cells in mouse models [28, 132, 167]. A Western type diet in *Ldlr*^{-/-} mice induces long-term epigenetic and transcriptional reprogramming of myeloid progenitor cells, leading to increased myelopoiesis and augmented innate immune responses, which persist despite switching back to a chow diet [28]. Furthermore, bone marrow transplantation from Western type diet fed *Ldlr*^{-/-} mice into *Ldlr*^{-/-} mice on a chow diet was associated with an increased number of

circulating inflammatory leukocytes and an increased aortic root plaque sizes compared to chow fed donor bone marrow [167]. Similarly, transplantation of bone marrow progenitors from diabetic mice into normoglycemic atherosclerosis-prone mice accelerated atherosclerosis by trained immunity [41].

Clonal haematopoiesis and CAVD

Recently, clonal haematopoiesis of indeterminate potential (CHIP) has been identified as an important mechanism of innate immune cell activation [49, 64]. Clonal haematopoiesis (CH) describes the disproportionate clonal growth of leukocytes arising from a single progenitor cell harbouring a somatic mutation, without the presence of haematologic malignancy [142]. CH is rare in young patients, but the prevalence increases with age, affecting > 10% of individuals older than 65 years [53, 170]. CH-driver mutations (CHDM) provide a survival advantage to the mutated cells and allow progressive clonal expansion, leading to accumulation of circulating mutant leukocytes [142]. CHDM occur mainly in genes encoding for epigenetic regulators, such as ten–eleven translocation 2 (TET2) and DNA methyltransferase 3A (DNMT3A). CH is associated with an augmented all-cause mortality risk [53, 63] and an increased risk of atherosclerotic CVD [49, 64]. At least for TET2 driver mutations, this association appears to be driven by a hyper-inflammatory phenotype of clonal monocytes. This is mediated, at least partly, due to the fact that TET2-deficient macrophages exhibit an increased NLRP3 inflammasome-dependent secretion of IL-1 β [49], which is key to the development of atherosclerosis [123]. DNMT3A deficiency has been associated with diminished immunosuppressive function of myeloid-derived suppressor cells, proinflammatory activation of mast cells and an increased production of IFN- γ by T lymphocytes. However, a direct pathophysiological connection between DNMT3A loss-of-function and atherosclerosis has not yet been established [6]. Interestingly, it was demonstrated that increased haematological stem cell proliferation, driven by atherosclerosis itself, can accelerate CH, creating a vicious cycle [61].

Recently, in patients with severe CAVD undergoing transcatheter AVR, a higher prevalence of DNMT3A and TET2 mutations was found in circulating monocytes compared to other cohorts of healthy subjects or to subjects with CAD. Patients with CHDM had a markedly increased all-cause mortality during the first eight months after a successful transcatheter AVR. Compared to non-CHDM carriers, patients with TET2 mutations had elevated levels of proinflammatory non-classical monocytes and patients with DNMT3A mutations showed proinflammatory T lymphocyte polarization [87]. Moreover, another

study demonstrated that monocytes of patients with severe degenerative aortic valve stenosis or chronic postischemic heart failure, who harbour DNMT3A or TET2 CHDM, appeared to be primed for excessive inflammatory responses by assessing the transcriptome of circulating peripheral monocytes of CAVD by single-cell RNA sequencing [2].

Imaging of valvular inflammation and calcification

Currently, echocardiography is used to clinically assess the severity of the aortic valve stenosis and computed tomography (CT) is used to quantify valvular macrocalcification and disease severity and progression [36]. However, it is important that we can properly measure inflammation, both systemically and locally, to obtain a better understanding of how inflammation contributes to CAVD pathophysiology. Active arterial wall inflammation can be visualized by ^{18}F -fluorodeoxyglucose (^{18}F -FDG) positron-emission tomography (PET) combined with CT [127]. In atherosclerotic plaques, FDG-uptake correlates with plaque macrophage burden [149]. Marincheva-Sancheva et al. were the first to demonstrate higher valvular ^{18}F -FDG uptake in patients with mild and moderate CAVD, but not in patients with severe CAVD, compared to controls. This suggests that inflammation plays a more important role in the early phases of CAVD than in advanced disease. Also, patients with higher baseline ^{18}F -FDG uptake showed an increased disease progression [86]. In addition, ^{18}F -sodium fluoride (^{18}F -NaF) PET-CT can be used, to detect recent calcification activity and calcium remodeling. Dweck et al. demonstrated that both valvular ^{18}F -FDG and ^{18}F -NaF uptake were higher in CAVD patients compared to controls. However, the ^{18}F -NaF uptake displayed a more progressive rise with disease severity than the more modest increased uptake of ^{18}F -FDG. Moreover, uptake of ^{18}F -NaF and not ^{18}F -FDG strongly correlates with disease severity [39]. A follow-up study demonstrated that both ^{18}F -FDG and ^{18}F -NaF uptake independently predict disease progression and adverse outcomes. ^{18}F -NaF uptake correlates strongly with CT calcium score progression and novel calcium depositions develop in the same distribution as baseline ^{18}F -NaF uptake. Interestingly, Abdelbacky et al. demonstrated that valvular ^{18}F -FDG uptake independently predicted subsequent calcification in patients without CAVD at baseline and thus indicated that inflammation precedes calcification [1]. Together, these studies support that valvular inflammation plays an important role in early CAVD and precedes calcification, which predominantly drives disease progression in later stages. These findings correspond to recent findings in patients

with atherosclerosis, showing mainly ^{18}F -FDG uptake in arterial segments without advanced plaques, suggesting an arterial inflammatory state at early stages of atherosclerosis [44].

Finally, another emerging imaging technique in the imaging of CAVD patients is the use of PET combined with magnetic resonance imaging (MRI). In addition to the potential to detect valvular inflammation with tracers, the PET/MRI has particular added value in assessing prognostic factors, including characterization of the myocardial tissue for remodelling, fibrosis and hypertrophy [156].

Pharmacological treatment to prevent severe CAVD

Although CAVD can be diagnosed at an early stage, there is currently no effective medical treatment available and ‘watchful waiting’ is the only option until endovascular or surgical intervention is needed. Elucidation of the pathophysiology of CAVD will hopefully reveal potential pharmacological targets for prevention or treatment. Given the pivotal roles of (oxidized) lipoproteins, various trials have been performed with lipid-lowering drugs (Table 2). In addition, targeting systemic inflammation and the immune system might be an effective strategy to prevent or treat CAVD. For other examined agents in the search for a pharmacological treatment for CAVD, we refer to the recent review of Donato et al. [34].

Lipid-lowering therapy

The pivotal role of oxidized lipoproteins in CAVD suggest lipid-lowering as possible treatment. However, four double-blind randomized controlled trials (RCTs) showed that statins do not halt or slow down CAVD progression, in contrast to strong beneficial effects on ASCVD (Table 2) [23, 30, 32, 126]. This might be explained by the fact that these patients already had established CAVD with a self-perpetuating calcification process. Another explanation is that statins do not lower Lp(a), and can even increase Lp(a) levels [154]. Additionally, statins are suggested to increase vascular calcifications, which might increase plaque stability and reduce the number of cardiovascular events in the context of atherosclerosis [117, 122], but further drives calcification and subsequent disease progression in CAVD. Interestingly, a post hoc meta-analysis of three studies investigating the effect of 80 mg atorvastatin mg per day in patients with stable CVD without CAVD, did not show a reduced risk for developing CAVD [8]. However, two of the three included trials compared low-dose statin therapy as control; treatment naïve patients as control group may have resulted in different results.

Table 2 Major published clinical trials investigating lipid-lowering therapy in calcific aortic valve disease

Trial	Patients	Study design	Intervention	Primary+echocardiographic outcomes	Inflammatory outcome	Refs.
SALTI ^{RE}	155 patients with CAVD and aortic jet velocity ≥ 2.5 m/s	Double-blind, placebo-controlled RCT FU: 25 months (median, range 7–36)	Atorvastatin 80 mg per day versus placebo	- Aortic jet velocity change/year Atorvastatin: 0.199 ± 0.210 m/s Placebo: 0.203 ± 0.208 m/s ($P = 0.95$) - Calcification progression/year Atorvastatin: $22.3 \pm 21.0\%$ Placebo: $21.7 \pm 19.8\%$ ($P = 0.93$)	None mentioned	[30]
TASS	47 patients with asymptomatic CAVD with mean systolic gradient ≥ 15 mmHg and peak velocity ≥ 2.0 m/s	Placebo-controlled RCT FU: 2.3 ± 1.2 years (median, SD)	Atorvastatin 20 mg per day versus placebo	- HR 0.78 (95% CI 0.32–1.87; $P = 0.569$) for MACE - CAVD progression: Mean gradient at last FU (mmHg): Atorvastatin: 31.3 ± 12.3 Placebo: 29.9 ± 14.8 ($P = NS$) Aortic valve calcification at last FU (Agatston score): Atorvastatin: 2979 ± 1228 Placebo: 2749 ± 1376 ($P = NS$)	CRP level (mg/dl) 0.14 ± 0.66 in atorvastatin group versus 0.33 ± 1.39 in control group ($P < 0.05$) after 12 month FU. $P = NS$ after 24 months FU	[32]
SEAS	1873 patients with mild to moderate, asymptomatic CAVD	Multicentre, double-blind, placebo-controlled RCT FU: 52.2 months (median)	Simvastatin 40 mg + ezetimibe 10 mg per day versus placebo	- HR 0.96 (95% CI 0.83 to 1.12; $P = 0.59$) for MACE - Increase in peak velocity (m/s): Simvastatin + ezetimibe: 0.61 ± 0.59 Placebo: 0.62 ± 0.61 ($P = 0.83$) - Increase mean gradient (mmHg/year): Simvastatin + ezetimibe: 2.7 ± 0.1 Placebo: 2.8 ± 0.1 - Decrease in AVA (cm ² /year): -0.03 ± 0.01 in both groups	None mentioned	[126]
ASTRON-OMER	269 patients with mild or moderate CAVD	Multicentre, double-blind, placebo-controlled RCT FU: 3.5 years (median, IQR 2.1–4.5)	Rosuvastatin 40 mg per day versus placebo	- Annualized peak AS gradient increase (mmHg): Rosuvastatin: 6.3 ± 6.9 Placebo: 6.1 ± 8.2 ($P = 0.83$) - Increase in mean gradient (mmHg): Rosuvastatin: 10.7 Placebo: 9.6 ($P = 0.49$) - Decrease in AVA (cm ²): Rosuvastatin: -0.19 Placebo: -0.16 ($P = 0.79$)	CRP reduction of 0.33 mg/L in rosuvastatin group compared to an increase of 0.095 mg/L in the placebo group ($P = 0.002$)	[23]

Table 2 (continued)

Trial	Patients	Study design	Intervention	Primary + echocardiographic outcomes	Inflammatory outcome	Refs.
FOURIER (post hoc analysis)	27,564 patients with stable atherosclerotic disease receiving statin therapy	Multicentre, double-blind, placebo-controlled RCT FU: 26 months (median, IQR 22–30)	Subc. evolocumab injection (140 mg biweekly or 420 mg monthly) versus placebo	- Post hoc analysis: HR 0.48 (95% CI, 0.25–0.93) for overall CAVD events after first year of evolocumab	None mentioned	[15]

AVA aortic valve area, CAVD calcific aortic valve disease, CI confidential interval, CRP C-reactive protein, FU follow-up; HR, hazard ratio, IQR interquartile range, LDL low-density lipoprotein, Lp(a) lipoprotein (a), MACE major adverse cardiovascular events, RCT randomized controlled trial, subc. subcutaneous

Convertase subtilisin/kexin type 9 (PCSK9) inhibitors are monoclonal antibodies that bind to circulating PCSK9 and inhibit PCSK9-mediated LDL-receptor degradation. This results in a powerful LDL-cholesterol lowering. In addition, PCSK9 inhibitors also lower Lp(a) concentration by 20–30% respectively [110, 128]. The FOURIER trial examined the effect of the PCSK9-inhibitor evolocumab in patients with stable atherosclerotic disease receiving statin therapy and demonstrated a reduced cardiovascular event risk [128]. Interestingly, a post hoc analysis of this trial displayed that treatment with evolocumab might also reduce CAVD related events [15], which is in line with the findings that patients with a PCSK9 loss-of-function mutation have a reduced CAVD risk [75]. These findings suggest that Lp(a) lowering might be able to prevent or slow down the progression of CAVD. These preliminary findings need further validation with RCTs and currently, the effects of PCSK9 inhibitors in CAVD are being investigated (Table 3) [157]. In addition, the effect of Lp(a) lowering on CAVD progression by niacin is being explored (Table 3) [158].

Another promising therapy targeting Lp(a) is antisense oligonucleotide therapy. These synthetic oligonucleotides bind to apoB or apo(a) mRNA in hepatocytes, resulting in a decreased production of apoB-containing lipoproteins and Lp(a). This leads to a significant reduction in circulating OxPL and Lp(a) [168]. Currently, the HORIZON trial, a large phase 3 multicentre RCT is recruiting patients to assess the impact of the antisense oligonucleotide TQJ230 on major cardiovascular events in patients with CVD (Table 3) [160]. This therapy might also be beneficial in patients with CAVD.

Anti-inflammatory agents

To the best of our knowledge, there are no clinical trials investigating the effect of anti-inflammatory drugs on CAVD. In the setting of ASCVD, however, recently several RCTs have reported effectiveness of the anti-inflammatory drugs colchicine and canakinumab in the prevention of CVD, following the publication of many neutral trials with other anti-inflammatory drugs [175]. Given the overlap in inflammatory components in the pathophysiology of atherosclerotic CVD and CAVD, these drugs might also have beneficial effects in the context of CAVD.

The low-dose colchicine for secondary prevention of cardiovascular disease (LoDoCo) trial demonstrated that colchicine, a broad anti-inflammatory agent, reduces the risk of cardiovascular events in patients with stable CAD [104]. This beneficial effect was confirmed in two large RCTs in patients after recent myocardial infarction [148], or with chronic coronary disease [105]. In addition to its known inhibitory effect on inflammasome activation, colchicine appears to attenuate neutrophil activation [112]. Given these actions, colchicine might be an attractive candidate to limit

Table 3 Clinical trials investigating lipid-lowering therapies of interest for calcific aortic valve disease

Trial	Patients	Study design	Intervention	Primary outcome	Clinicaltrials.gov identifier	Refs.
PCSK9 inhibitors in the progression of aortic stenosis	140 patients with mild to moderate CAVD	Phase 2 multicentre randomized, double-blind, placebo-controlled clinical trial Follow-up 2 years	Subcutaneous injection of EPC biweekly versus placebo	Calcium score progression measured by cardiac CT (Agatston score) and by NaF PET-CT	NCT03051360	[157]
EAVaLL	238 patients with aortic sclerosis or mild CAVD and elevated Lp(a)	Phase 2, randomized, placebo-controlled clinical trial Follow-up 2 years	Niacin 1500–2000 mg versus placebo	Calcium score progression measured by cardiac CT	NCT02109614	[158]
Lp(a) HORIZON	7680 patients with established CVD and elevated Lp(a)	Phase 3 multicentre, randomized, double-blind, placebo-controlled clinical trial Follow-up 4 years	Subcutaneous injection of TQQJ230 80 mg monthly versus placebo	Time to first occurrence of MACE	NCT04023552	[160]

CAVD calcific aortic valve disease, CI confidential interval, CT computed tomography, CVD cardiovascular disease, HR hazard ratio, IQR interquartile range, LDL low-density lipoprotein, Lp(a) lipoprotein (a), MACE major adverse cardiovascular events, NaF sodium fluoride, PET positron emission tomography

CAVD progression and this hypothesis will soon be tested in a new randomized controlled clinical trial [159].

The Canakinumab Anti-inflammatory Thrombosis Outcome Study (CANTOS) demonstrated that inhibition with the human anti-IL-1 β antibody canakinumab decreases cardiovascular event rates in patients with a recent myocardial infarction [123]. This trial did not evaluate the effects on CAVD. Nonetheless, IL-1 β production by macrophages induces calcification by vascular mesenchymal cells [22], which makes IL-1 β also an interesting candidate for CAVD treatment. Furthermore, the CHIP-associated aberrant inflammation, such as the IL-1 β overexpression by TET2 deficient macrophages, further strengthens the possible role for IL-1 β as therapeutic target in CAVD [49]. As a consequence of the promising results of the CANTOS trial, attention has moved upstream of the IL-1 β pathway to target inflammasomes and downstream IL-6. Animal studies using NLRP3 inflammasome inhibitors and IL-6 receptor antagonists have shown promising results in targeting atherosclerosis [4, 162, 166], but larger trials are needed to investigate their clinical relevance.

Conclusion

In conclusion, evidence is gradually accumulating that the complex pathophysiology of CAVD is an inflammatory process in which various immune cells plays a prominent role. Considering the central role of innate immune cells in the pathophysiology of ASCVD and the similarities between ASCVD and CAVD, it is rational to hypothesize that activation of the innate immune system also contributes to the

initiation and progression of CAVD. Further elucidation of the driving processes of innate immune cell activation in CAVD, including trained immunity and CHIP, might identify novel therapeutic targets that can be used for prevention and treatment of CAVD. The recent exciting evidence that anti-inflammatory strategies potentially limits atherosclerotic CVD further underscores the importance of this scientific field.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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