Look More Closely at the Valve: Imaging Calcific Aortic Valve Disease

Running title: Aikawa et al.; Calcific valve disease

Elena Aikawa, MD, PhD¹; Catherine M. Otto, MD²

¹Brigham and Women's Hospital, Harvard Medical School, Boston, MA; ²Division of Cardiology, University of Washington, Seattle, WA

Address for Correspondence:
Catherine M. Otto, MD
Division of Cardiology
University of Washington
Box 356422
1959 NE Pacific Street, AA510
Seattle, WA 98195
Phone: 206-685-1397
Fax: 206-616-4847
Email: cotto@u.washington.edu


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Outcome measures in patients with aortic stenosis

Clinical trials of medical therapy to prevent progression in calcific aortic valve disease (CAVD) are hampered by the lack of sensitive measures of disease activity in the valve leaflets. Although the long-term goal of therapy is to prevent adverse cardiovascular outcomes, including mortality and aortic valve replacement, these endpoints have several limitations. First, the “hard” endpoint of mortality is frequently impacted by comorbid conditions. Patients with CAVD typically are elderly with a high prevalence of coronary and noncardiac disease so that isolating the effect of valve obstruction on survival is problematic. Second, the endpoint of valve replacement for symptom onset reflects considerable physician variation in the threshold for intervention, as well as patient preferences. In addition, some patients are not promptly referred for valve replacement at symptom onset, sometimes inappropriately but often due to concerns about surgical risk or to patient factors such as extreme age, dementia, or a severely reduced life expectancy due to coexisting conditions. Inclusion of these endpoints in clinical trials might obscure any benefit of medical therapy. Finally, clinical outcomes provide limited information about the mechanism of benefit; for example, medical therapy might improve outcomes by effects on coronary disease or ventricular function, rather than affecting the disease process in the valve leaflets.

Hemodynamic measures of aortic stenosis (AS) severity provide some insight into the disease process. Noninvasive Doppler velocity, gradient and valve area measurements have provided the foundation of our knowledge about CAVD progression and are useful measures for evaluating the effects of therapy on valve obstruction in clinical trials. Yet even hemodynamic assessment is not the ideal measure of disease because stenosis occurs late in the clinical course, may not be
a sensitive marker of changes in the leaflets once significant disease is present, and is not useful for studying early disease. Similarly, looking at the left ventricular response to AS reflects late and longstanding disease, not the early disease process. Even though newer imaging approaches allow detection of diastolic dysfunction and myocardial fibrosis before overt ventricular hypertrophy is evident, these changes still reflect disease severe enough to cause hemodynamic effects. We now understand that calcific AS is the end-stage of a disease that progresses from microscopic early changes to aortic sclerosis and then, in a subset of patients, to severe AS. In order to impact this disease process, we need to be able to “see” the earliest stages of disease so that we can measure the effects of targeted therapy on the microscopic processes in the leaflets themselves. Progress in the understanding, diagnosis, and treatment of CAVD has been hindered by our inability to spatially resolve and quantify in vivo the dynamic molecular events associated with early calcific changes in valves.

**Current experimental and clinical approaches to valve imaging**

Recent studies have shown that innovative optical molecular imaging employing near-infrared fluorescence imaging agents enables simultaneous in vivo visualization of early mineralization, allowing measurement of inflammation and osteogenic activity associated with early CAVD. These studies extended a paradigm of inflammation-dependent mechanisms of calcification, as opposed to the long-held belief that CAVD is an inevitable consequence of aging. Molecular imaging visualizes three stages of CAVD, and demonstrates that inflammation precedes calcification (Figure). During inflammation (the “initiation” phase), activated macrophages produce pro-osteogenic factors that induce the transition of valvular interstitial myofibroblasts into osteoblast-like cells. Severe pro-inflammatory conditions further enhance inflammation and
the formation of microcalcifications, contributing to more abundant calcium deposition and leaflet stiffening (the “propagation” phase). During these early stages, when calcification is accompanied closely by inflammation, medical therapies targeting pro-inflammatory and pro-osteogenic pathways might retard osteogenic changes. Sensitive diagnostic imaging tools are needed to accomplish this goal. Late-stage advanced calcification, in contrast, is readily detected by conventional imaging modalities but is difficult to treat. Recent clinical trials support these observations, showing that statins cannot prevent CAVD due to late implementation, after calcification has progressed to the irreversible stage.

The highest-resolution non-invasive clinical imaging modality currently has a resolution of approximately 1 mm, which is insufficient to visualize cells and early calcification. Micro-optical coherence tomography (μOCT) — a novel form of optical imaging technology with 1-μm resolution — was developed recently. Microcalcifications and other cellular-level features, such as cholesterol crystals, macrophages, and structural proteins can be visualized by μOCT. Because μOCT data are three-dimensional and acquired from intact specimens, it provides histologic level resolution with the speed and reliability of imaging in vivo. This method is superior to near-infrared molecular imaging because it does not require injection of molecular imaging agents, and therefore may be translated to human patients more easily. The major disadvantages of near-infrared molecular imaging and μOCT are limited tissue penetration and the potential need of an invasive approach.

More established non-invasive diagnostic imaging modalities currently allow identification of significant AS and risk stratification of patients with suspected CAVD. Echocardiography has
long been a useful clinical tool for evaluation of leaflet motion and hemodynamic obstruction, but it does not have the resolution for quantifying and monitoring aortic valve calcium at early stages, even with newer 3D approaches. Computed tomography (CT) is a useful tool for the assessment of valve anatomy, particularly during planning for transcatheter aortic valve implantation and is an accurate technology for quantification of established, but not early, valve calcification. Cardiac magnetic resonance imaging (MRI) provides accurate, reproducible, and non-invasive data on left ventricle volumes and function and has the potential for quantitation of valve obstruction, but offers little insight into the disease process in the valve leaflets with current instrumentation.

**Positron emission tomography (PET) imaging as a measure of aortic valve inflammation and calcification**

During the last decade, it has become clear that CAVD is an actively regulated disease process rather than mere degeneration, and that inflammation is critical in its initiation. No clinical data, however, have directly linked inflammation and calcification during the progression of CAVD. A primary reason for the lack of such studies is that a reliable technology to non-invasively measure aortic valve inflammation and calcium burden remains unavailable in a clinical setting. Emerging evidence indicate that PET imaging may non-invasively assess inflammation in the aortic valves of patients with AS.

In this issue of *Circulation*, Dweck et al. used PET imaging combined with CT, and two common PET tracers that target inflammation and calcification, to investigate the feasibility of this approach. In addition, they measured the relative contributions of inflammation and
calcification at different stages of CAVD by administration of both tracers to the same patients. The glucose analogue 18F-fluorodeoxyglucose (18F-FDG), an imaging agent approved for clinical use, is taken up by cells through glucose transport proteins. Following phosphorylation, 18F-FDG becomes trapped within macrophages, pro-inflammatory phagocytes with high metabolic requirements. 18F-sodium fluoride (18F-NaF) is directly incorporated into hydroxyapatite via an exchange mechanism with hydroxyl groups and thus is a potential marker of active tissue calcification. In a series of 121 adults over age 50 years, spanning the range of CAVD from normal valves to aortic sclerosis to severe stenosis, the authors demonstrated excellent repeatability for the quantification of 18F-FDG and 18F-NaF tracers in the valve leaflets. In the early stages of CAVD, both tracers were increased, reflecting both inflammation and active calcification, and both tracers showed a progressive stepwise increase in uptake with more severe CAVD. Calcification appeared to be a more predominant process than inflammation, particularly in the later stages of CAVD, supporting our current understanding of the pathogenesis of this disease. The study provides the first demonstration that 18F NaF can detect early calcification in patients with CAVD. Importantly, 18F-NaF uptake was observed in regions remote from advanced calcification, indirectly suggesting that 18F-NaF identifies microcalcifications, which has not been possible previously using non-invasive imaging in humans. Overall, increased 18F-NaF activity was observed in 45% of patients with aortic sclerosis, in 91% of patients with mild or moderate AS, and in 100% of patients with severe AS. The next steps in this line of study will be to examine more quantitatively, in a larger study population, whether the magnitude of 18F-NaF signal associates positively with CAVD progression, and to validate this tracer against histological or other sensitive imaging methods. If
confirmed, perhaps 18F-NaF activity could serve as a marker of disease progression in medical intervention trials.

While this study successfully identified early calcification, the authors also found a progressive increase of valvular 18F-FDG, an agent currently used as a tracer for inflammation. Increased 18F-FDG activity was observed in 20% of patients with aortic sclerosis, in 35% of patients with mild or moderate AS, and in 52% of patients with severe AS, supporting the hypothesis that CAVD is an inflammatory disease.¹ The authors also detected somewhat elevated uptake of 18F-FDG in patients with severe AS; in contrast to previous imaging studies, which suggested that inflammation is reduced in late-stage CAVD.⁶ This controversial finding could be explained by the recent study by Folco et al. that evaluated the effects of inflammation and hypoxia on FDG activity, and found that macrophages exhibited increased glucose uptake in response to hypoxia, but not to inflammatory cytokines.⁸ Because aortic valves are largely avascular structures, and valve thickness is significantly increased due to fibrosis and calcification, we can reasonably hypothesize that aortic valve tissue suffers from a hypoxic environment during CAVD progression. This could explain the increased FDG tracer in patients with severe AS.

Previous studies also found that results from FDG PET in vivo imaging studies of atherosclerosis are often inconsistent, probably due to variations in lesion composition, presence of calcification, disease severity, and degrees of hypoxia. Furthermore, FDG uptake by metabolically active cardiac myocytes surrounding aortic valve tissue produce substantial background signal, which may interfere with the FDG read-out. Because FDG uptake appears to depend on multiple factors, we should interpret the findings from this study carefully.
Imaging in clinical trials – understanding mechanisms versus clinical endpoints

Medical therapy to prevent initiation and progression of CAVD will soon be within reach as ongoing research fills the gaps in our understanding of the specific disease pathways involved at the tissue level. In translating these advances from the basic science laboratory to the clinical research trial, we need to ensure that we do not lose sight of the mechanisms of therapeutic benefit in our eagerness to impact overall clinical outcomes. Of course, making patients live longer and feel better is our goal regardless of the mechanism of benefit, but we also need to understand how a specific intervention works in order to target our therapies more effectively.

Compared to outcome trials, mechanistic studies also offer the opportunity to perform initial clinical studies with a smaller patient population, over a shorter time interval and at a lower cost.

The study by Dweck et al. has set the stage for the use of PET valve imaging as a potential marker for evaluating drug efficacy in clinical trials. Further studies are needed to establish how early PET imaging can detect CAVD, and to evaluate the utility of PET for prediction of disease progression and adverse cardiovascular outcome. Although current clinical imaging approaches, such as echocardiography, remain essential for clinical management, and for following CAVD progression once valve obstruction is present, PET may be the method of choice for detection of early calcification and inflammation in clinical trials. In addition, because histopathological examination is not possible during longitudinal non-invasive imaging studies (e.g., echocardiography, CT, PET), validated high-resolution invasive imaging modalities (e.g., molecular imaging, μOCT) might be required to understand the mechanisms at various stages of CAVD. Large, prospective, event-driven studies of non-invasive imaging techniques will be required to determine the place of these modalities in future clinical practice. As we evaluate
potential medical therapies for CAVD, it is not enough to know if it works, we need to know why.

**Conflict of Interest Disclosures:** None.

**References:**


Figure Legend:

Figure 1: Inflammation-dependent mechanism of calcification. Mice were co-injected with near-infrared fluorescence nanoparticles to visualize macrophages (green), and a spectrally distinct imaging agent to detect calcification (red). Molecular imaging detects three stages of calcification in CAVD: *initiation, propagation,* and *late-stage calcification.*
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