Molecular imaging of calcific aortic valve disease

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Received Nov 21, 2017; accepted Nov 27, 2017
doi:10.1007/s12350-017-1158-7

Calcific aortic valve disease (CAVD) can progress to symptomatic aortic stenosis in a subset of patients. The severity of aortic stenosis and the extent of valvular calcification can be evaluated readily by echocardiography, CT, and MRI using well-established imaging protocols. However, these techniques fail to address optimally other important aspects of CAVD, including the propensity for disease progression, risk of complications in asymptomatic patients, and the effect of therapeutic interventions on valvular biology. These gaps may be addressed by molecular imaging targeted at key biological processes such as inflammation, remodeling, and calcification that mediate the development and progression of CAVD. In this review, recent advances in valvular molecular imaging, including ^{18}F-fluorodeoxyglucose (FDG) and ^{18}F-sodium fluoride (NaF) PET, and matrix metalloproteinase-targeted SPECT imaging in the preclinical and clinical settings are presented and discussed.

Key Words: Aortic stenosis • aortic valve • molecular imaging • matrix metalloproteinases • FDG • sodium fluoride

Calcific aortic valve disease (CAVD) is the most common cause of aortic stenosis (AS), which according to recent US statistics affects ~ 0.4% of the general population.[1] The prevalence of AS increases with age with moderate to severe AS affecting nearly 3% of patients ≥ 75 years old.[1] In addition, 17,000 deaths and 55,000 hospitalizations are annually attributable to aortic valve disease (not just AS). The Natural history of CAVD involves a long asymptomatic period during which the initial fibrotic thickening of aortic valve leaflets with limited calcification (aortic sclerosis) progresses to more extensive valvular calcification and ultimately, hemodynamically significant aortic stenosis. The resulting pressure overload promotes left ventricular hypertrophy, an adaptive response that underlies the typically long period of asymptomatic disease present despite gradual worsening of aortic stenosis. The rise in wall stress may lead to impairment of coronary perfusion and development of sub-endocardial ischemia, which in turn triggers cardiomyocyte apoptosis and myocardial fibrosis. It is postulated that eventually, ventricular decompensation triggers angina, heart failure, syncope, or sudden death. While symptomatic AS is indeed the hallmark of severe CAVD, not all patients with moderate AS progress to symptomatic disease. Even in patients with severe AS (peak systolic velocity ≥ 4 m/s by Doppler echocardiography), ~ 30% do not develop symptoms over a 5-year period.[2] On the

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s12350-017-1158-7) contains supplementary material, which is available to authorized users.

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Funding This work was supported by grants from NIH (R01-HL114703, R01-HL138567), and Department of Veterans Affairs (I0-BX001750).

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J Nucl Cardiol 2018;25:1148–55.
1071-3581/$34.00

other hand, a non-negligible minority of such asymptomatic patients die of sudden cardiac death,[2] highlighting the complex nature of the relation between aortic valve structure and physiology (including the severity of AS), ventricular response, and symptoms.

In addition to age, the number of leaflets is another major risk factor for the development of CAVD. Indeed, while bicuspid aortic valve (BAV) is found in ~ 1.4% of live births, BAV accounts for a large fraction of aortic valve surgeries.[3-5] Reflecting the similarity between CAVD and atherosclerosis, male sex, smoking, hypertension, diabetes, hypercholesterolemia, and elevated Lp(a) are additional risk factors for the development of CAVD.[4] Many of the factors that increase the risk of CAVD development are also risk factors for CAVD progression. In particular, disease progression is accelerated with the severity of stenosis, higher extent of calcification, older age, and presence of BAV.[4] Moreover, aortic valve area and left ventricular hypertrophy are independent predictors of the development of symptoms.[2] Importantly, the presence of CAVD, even in the absence of severe disease or symptoms, portends a higher risk of all-cause mortality.[2,6,7] The predictors of all-cause mortality in aortic stenosis include age, chronic renal failure, inactivity, aortic valve velocity,[2] left ventricle mass,[8] and left ventricle wall stress.[9]

Currently, there is no known treatment to slow down the progression of CAVD, and surgical or transcatheter aortic valve replacement remains the only effective therapeutic options for advanced, symptomatic disease. Despite considerable overlap between the risk factors for CAVD and atherosclerosis and promising results of observational studies,[10] randomized clinical trials of statins have failed to demonstrate benefit in slowing down the progression of aortic valve calcification or stenosis.[11,12] Inadequate duration of statin therapy and late stage of the disease, as well as the procalcific effects of statins, may have contributed to this failure. In addition to statins, several other approaches are currently under investigation to prevent CAVD progression.[13] Examples include Niacin [NCT02109614, to reduce Lp(a) levels], denosumab or alendronic acid (NCT02132026, to target bone metabolism), tadalafil (NCT01275339, to inhibit phosphodiesterase Type 5), and ataciguat (NCT02481258, to promote guanylate cyclase activity). Irrespective of the drug tested, considerable heterogeneity of CAVD progression makes it difficult to demonstrate therapeutic effectiveness in clinical trials. For instance in the Simvastatin and Ezetimibe in Aortic Stenosis (SEAS) trial, the mean (± SD) change in peak aortic-jet velocity during a median follow-up of 52.2 months was 0.62 ± 0.61 $m \cdot s^{-1}$ in the placebo group.[12] It is reasonable to assume that by targeting therapeutic interventions to those patients who are at the highest risk for progression, it is easier to establish any potential therapeutic efficacy.

The initial diagnosis and evaluation of CAVD and AS is typically based on clinical findings and echocardiography. In addition, a subset of patients (e.g., those with inconclusive echocardiography results) may require invasive evaluation of the valve. However, classical non-invasive imaging techniques, echocardiography, CT, and MRI, which focus on aortic valve anatomy and physiology (e.g., calcification, area, flow rates and transvalvular gradient) and associated aortic and myocardial abnormalities (focal and diffuse myocardial fibrosis, global and basilar longitudinal strain) inadequately inform of the patient progression risk and prognosis. This risk is directly related to valvular (and myocardial) pathobiology, which can be appraised by molecular imaging. As such, in vivo assessment of valvular biology by molecular imaging can potentially help clinical decision making regarding the need for aortic valve replacement in patients with moderate AS who are to undergo coronary artery bypass grafting, and possibly in patients with severe asymptomatic AS, who might be at increased risk for sudden cardiac death. Selection of patients who might benefit from emerging medical therapies and assessment of the response to therapeutic interventions within a relatively short period of time are additional applications of such techniques for drug development. Last, but certainly not least, molecular imaging can help address important gaps in CAVD pathophysiology through in vivo assessment of aortic valve biology and serial imaging in the same subject.

PATHOBIOLOGY

CAVD was once presumed to be a degenerative disease associated with passive calcium deposition in leaflets. Over the past two decades, advances in our understanding of aortic valve pathobiology have led to a paradigm shift of our view of the disease to an active process of valvular thickening and calcification starting with asymptomatic, hemodynamically insignificant aortic sclerosis that progresses to aortic stenosis.[14]

Aortic valve leaflets are covered by valvular endothelial cells (VECs) and are composed of three layers, namely the collagen-rich fibrosa on the aortic side, the proteoglycan-rich spongiosa, and the elastin-rich ventricularis.[15] The valve-specific, fibroblast-like valvular interstitial cells (VICs) are interspersed within these layers and play a central role in fibrocalcific remodeling of the leaflets.[16] Environmental factors, biomechanical perturbations, and genetic predisposition contribute to the leaflet remodeling process, which

culminates in the development of hemodynamically significant aortic stenosis.

VEC activation induced by changes in shear stress or other stimuli, e.g., atherosclerotic risk factors, initiates the development of CAVD by promoting subendothelial lipid retention and oxidation, and recruitment of inflammatory cells to the leaflet.[17,18] Inflammation, VIC transformation, angiogenesis, extracellular matrix remodeling, and calcification are pathologic hallmarks of CAVD. Infiltrated macrophages and T cells may trigger tissue remodeling by promoting oxidative stress, release of proteases, e.g., matrix metalloproteinases and cathepsins, and differentiation of quiescent VICs into myofibroblasts. This promotes fibrotic remodeling of the valve. In parallel, these cells may undergo osteogenic differentiation, resulting in the formation of calcific nodules, a process which starts at the base of the fibrosa layer.[19-22] Ultimately, the thickening, fibrosis, and calcification of the leaflets lead to hemodynamically significant aortic stenosis in a subset of patients. Of note, while the immune-inflammatory (oxidative stress-driven) pathway underlies most cases of CAVD, an alternative hyperphosphatemia-driven pathway of calcification appears to dominate in patients with chronic kidney disease. These issues are discussed in detail elsewhere.[14,23]

MOLECULAR IMAGING

Preclinical Studies

The small size of aortic valve and motion are challenges to in vivo imaging. Accordingly, a number of studies have relied on ex vivo imaging modalities [near-infrared fluorescence (NIRF) imaging, and magnetic resonance imaging and spectroscopy (MRI/MRS)] to image various aspects of valvular pathobiology in rodent models of CAVD, including endothelial cell activation, proteolytic activity, phagocytic activity, osteogenic activity and angiogenesis.[24-26] While valuable as complement to conventional molecular and valvular biology techniques, the inherent limitations of these techniques preclude their application in longitudinal studies and ultimately, clinical implementation. This is in sharp contrast with emerging nuclear imaging-based approaches, which can be readily applied to non-invasive imaging in humans.

MMPs play a key role in extracellular matrix remodeling. Several members of MMP family, MMP-1, -9, and -12, are amongst the most highly up-regulated genes in CAVD.[27] Inflammatory cells are major sources of MMP production and activity. Accordingly, MMP activation and inflammation are closely intertwined in CAVD. The feasibility of MMP-targeted imaging was recently shown in apolipoprotein $E^{-/-}$ mice fed a Western diet for up to 9 months to induce CAVD. In this model, a subset of animals develops hemodynamically significant aortic stenosis. In vivo microSPECT-CT imaging using RP805, a ^{99m}Tc-labeled tracer that binds to several activated MMPs, demonstrated specific in vivo aortic valve uptake of the tracer (Figure 1).[28] Interestingly, the MMP signal peaked at 6 months of Western diet, while key features of CAVD (leaflet thickening, valvular calcification, and aortic stenosis) were most pronounced after 9 months. This suggests that MMPs are implicated in CAVD progression and MMP imaging may serve as a predictive tool in CAVD. A significant correlation between valvular macrophage staining (CD68) and MMP signal in vivo suggested that this technique may be used for detection of valvular inflammation and remodeling. Whether this technique can be used for imaging CAVD in the absence of atherosclerotic lesions (which co-exist in the aforementioned model) or tracking the response to therapeutic interventions remains to be determined.

Clinical Studies

FDG PET. Despite some concern regarding its specificity, ^{18}F-FDG PET has been extensively used for imaging vascular inflammation and has served as a non-invasive tool for early assessment of the effect of novel therapeutic interventions in clinical trials. Indeed, several studies have shown a close relationship between the ^{18}F-FDG signal and macrophage content of atherosclerotic plaques.[29-33] The potential role of ^{18}F-FDG PET in aortic valve disease was first investigated in a retrospective analysis of whole body ^{18}F-FDG PET/CT images acquired for oncological staging.[34] In this study, FDG uptake [expressed as target-to-blood ratio (TBR)] was significantly higher in patients with aortic stenosis ($n = 42$) than in the matched control group (Figure 2). When categorized based on severity of stenosis and calcification, ^{18}F-FDG uptake was significantly increased in patients with mild and moderate disease, but not in those with severe disease.

Another retrospective study evaluated the relation between aortic valve ^{18}F-FDG signal in 111 patients without active cancer or aortic stenosis who underwent at least 2 ^{18}F-FDG PET/CT studies within a period of 1-5 years.[35] When categorized as non-progressors or progressors based on aortic valve calcium score determined at baseline and follow-up time points, the ^{18}F-FDG signal (expressed as SUVmax) in aortic valve was found to be higher in the progressor group compared to non-progressor group. A similar difference was seen in the subset of subjects without aortic valve calcification at baseline. Interestingly, in this study the ^{18}F-FDG

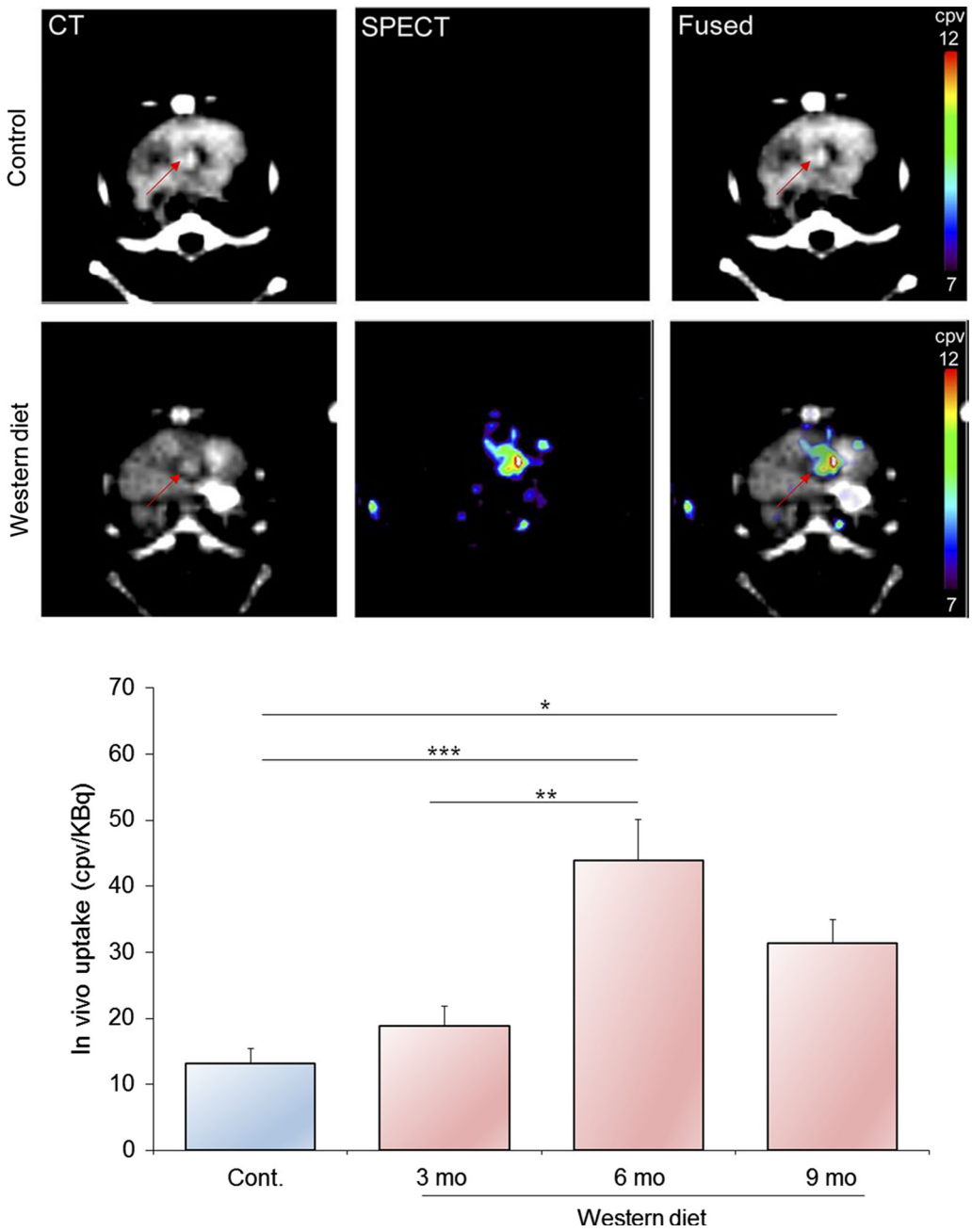

Figure 1. In vivo matrix metalloproteinase-targeted imaging in apolipoprotein $E^{-/-}$ mouse fed on Western diet for up to 9 months to induce CAVD. *Top panels* Examples of transverse contrast-enhanced CT, ^{99m}Tc-RP805 (matrix metalloproteinase-targeted) SPECT, and fused images in a control, wild-type mouse, and an apolipoprotein $E^{-/-}$ mouse fed on Western diet for 6 months. *Bottom panel* Background-corrected quantification of aortic valve ^{99m}Tc-RP805 uptake in control animals and apolipoprotein $E^{-/-}$ mice fed on Western diet for up to 9 months. *Arrows* point to aortic valve area. *cpv*, counts per voxel. $*P < .05$, $**P < .01$, $***P < .0001$, n = 7-13 in each group. This research was originally published in Journal of Nuclear Medicine (Jung et al.[28] © by the Society of Nuclear Medicine and Molecular Imaging, Inc.).

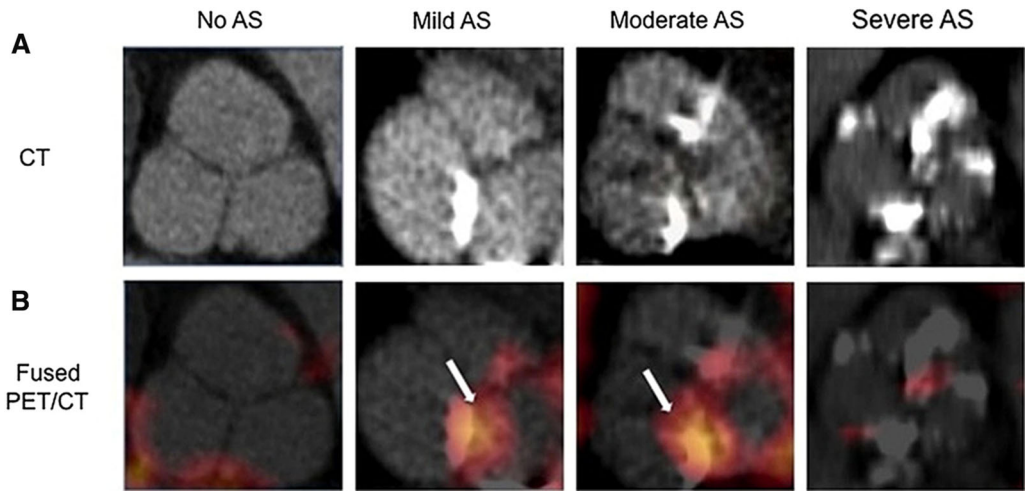

Figure 2. ^{18}F-FDG imaging of CAVD. Examples of CT images (**A**) and fused ^{18}F-FDG-PET/CT images (**B**) of aortic valve in patients with different stages of CAVD. *Arrows* point to ^{18}F-FDG uptake in aortic valve. *AS*, Aortic stenosis. Adapted with permission from Elsevier and Copyright Clearance Center, Journal of the American College of Cardiology,[34] © 2011.

signal could independently predict subsequent calcification after adjusting for cardiovascular risk factors.

In a prospective study of ^{18}F-FDG PET (and ^{18}F-NaF) imaging involving 121 subjects (including 20 controls), the aortic valve ^{18}F-FDG signal (maximum TBR) was significantly higher in patients with aortic stenosis than in controls and the uptake modestly increased with the severity of stenosis.[36] A subset of the subjects of the original cohort (n = 30) was followed for 1 year, during which 12 subjects underwent aortic valve replacement (AVR) for symptomatic AS. In the remaining subjects without severe AS, there was no correlation between the baseline ^{18}F-FDG signal and 1-year change in calcium score.[37] In those subjects who underwent valve replacement, there was no correlation between the ^{18}F-FDG signal in vivo and CD68 staining of the surgical specimens. A follow-up evaluation of the full cohort of the subjects (including the controls) at 2 years showed weak correlations between the baseline ^{18}F-FDG signal and aortic calcification (CT) and stenosis (echocardiography). In addition, the baseline ^{18}F-FDG signal was able to predict clinical outcomes (composite of cardiovascular death and aortic valve replacement) independently of age and sex.[38]

While the non-linear relation between ^{18}F-FDG signal in aortic valve and the severity of aortic stenosis is intriguing, existing data raise many questions about the biological and clinical significance of the ^{18}F-FDG signal in the valve. In addition, uptake in the myocardium is a practical barrier to broader implementation of aortic valve ^{18}F-FDG PET imaging, indicating that alternative, more robust techniques are needed for evaluation of CAVD in humans.

NaF PET. ^{18}F-NaF is retained at the sites of calcification through binding to hydroxyapatite via an exchange mechanism with hydroxyl groups.[39] However, the biological basis of ^{18}F-NaF uptake in aortic valve is not well defined. Studies in human atherosclerotic lesions have linked the NaF signal in atherosclerosis to the sites of calcification. As such, in carotid endarterectomy specimens, NaF uptake ex vivo detected by autoradiography corresponded to the areas of calcification (Alizarin red staining), but not CD68 (macrophages), CD31 (endothelial cells) or alpha-smooth muscle actin (smooth muscle cells) expression.[40] The higher ^{18}F-NaF retention at the sites of microcalcification as opposed to larger masses of calcified material has been attributed to the relatively larger surface area of the former available for ^{18}F-NaF binding.

The potential of ^{18}F-NaF PET for imaging valvular biology in CAVD was brought up by retrospective analysis of aortic valve signal in ^{18}F-NaF PET-CT studies of a small group of subjects (n = 5) with cancer or rheumatological disease and concomitant aortic stenosis (and control subjects without aortic valve calcification), which showed significantly higher ^{18}F-NaF (both maximal SUVs and TBRs) in those with aortic stenosis.[41] The aforementioned prospective study of 121 subjects with aortic sclerosis or stenosis of different severity or control subjects without apparent aortic valve disease who underwent both ^{18}F-FDG and ^{18}F-NaF PET/CT shortly followed this initial observation and demonstrated higher ^{18}F-NaF signal in aortic valve of patients with CAVD than controls with the intensity of the signal increasing with the severity of the aortic stenosis.[36] Of note, while 91% of patients with

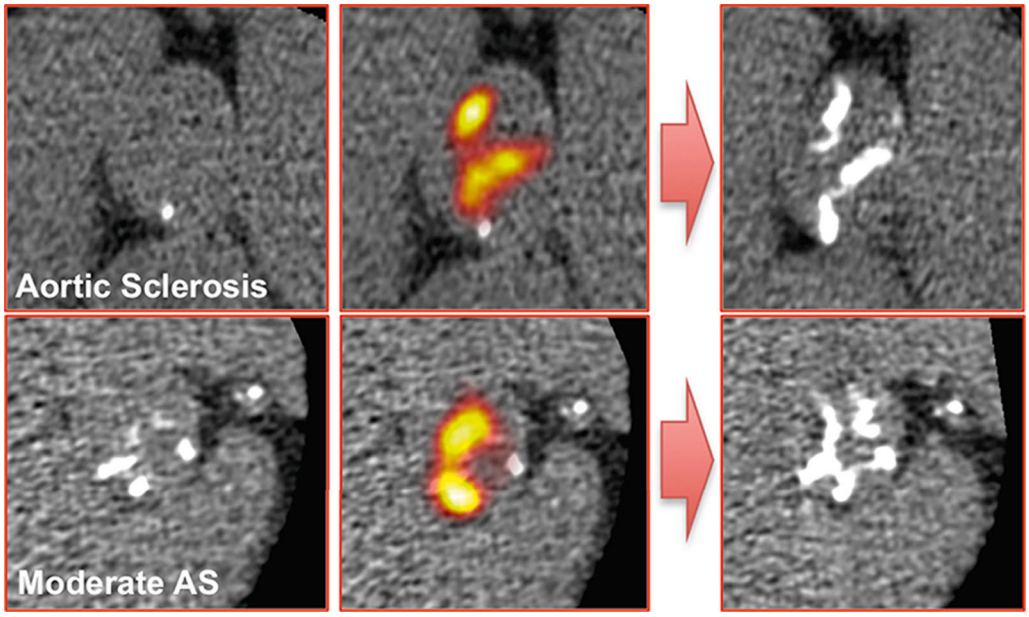

Figure 3. ^{18}F-NaF imaging and progression of valvular calcification in CAVD. Baseline CT images (*left*) and fused ^{18}F-NaF-PET/CT images (*middle*), and 2-year follow-up CT images (*right*) in two patients with CAVD. Adapted with permission from Elsevier and Copyright Clearance Center, Journal of the American College of Cardiology,[38] © 2015.

aortic stenosis had increased ^{18}F-NaF uptake, only 35% showed increased ^{18}F-FDG uptake. The poor correlation between aortic valve signal from these tracers indicated that they target different aspects of aortic valve biology. The 1-year follow-up study of a subset of the original cohort (n = 30, including 12 subjects who had undergone surgical AVR and 18 with asymptomatic disease) reported a statistically significant correlation between the baseline ^{18}F-NaF signal and changes in calcium score. A similar correlation was found between the baseline and subsequent changes in calcium score.[37] Histological analysis of the aortic valve from those subjects who underwent AVR during this 1-year period showed an association (albeit modest) between the baseline ^{18}F-NaF signal and markers of calcification (alkaline phosphatase and osteocalcin), but not inflammation (CD68). Importantly, areas of osteocalcin expression in the leaflet extended beyond the area of Von Kossa staining (established calcium) and corresponded to the area with maximal ex vivo ^{18}F-NaF uptake on autoradiography. The 2-year follow-up evaluation of this cohort reported the development of new sites of macrocalcification corresponding to the sites of baseline ^{18}F-NaF signal, and a good correlation ($r^2 = 0.64$) between the baseline ^{18}F-NaF signal and subsequent changes in calcium score (Figure 3). However, the relationship with echocardiographic measures of hemodynamic progression was much weaker, highlighting the role of other factors (fibrosis, distribution of calcification) in aortic stenosis.[38] Consistent with the observations at 1-year follow-up, ^{18}F-NaF PET-CT was not an independent predictor of clinical outcomes at 2 years after correction for baseline CT-based calcium scores.

PERSPECTIVE AND CONCLUSIONS

The utility of ^{18}F-NaF PET as a marker of disease activity in CAVD is currently under evaluation in several clinical trials [NCT02132026 (SALTIRE2), NCT02740088, NCT03095313]. While potentially useful as a tool to track the effect of therapeutic interventions on valvular calcification, ^{18}F-NaF PET imaging probably misses the fibrotic component of the disease and additional molecular imaging tools are necessary to fully evaluate different aspects of this pathology. The size of aortic valve and its continuous motion are major hurdles to imaging quantitative assessment of valvular biology by molecular imaging. In the case of ^{18}F-FDG, despite the emergence of several protocols aimed at suppressing myocardial uptake of the tracer, consistent, complete suppression remains an additional challenge. Recent advances in image acquisition and quantification methodology have improved the image quality and reproducibility of aortic valve ^{18}F-NaF PET images, and more progress is expected to lead to more reliable data for clinical studies.[42,43] While PET is a powerful tool for clinical imaging, the spatial

resolution of microPET is a challenge to imaging in the mouse and microSPECT is more suitable in this setting. Preclinical studies of CAVD are also hampered by the paucity of relevant animal models, and in this regard, emergence of non-atherosclerotic models of the disease with leaflet calcification (similar to human disease) would be of great value. Furthermore, despite many similarities between valvular and vascular calcification, there are also key differences between the two processes that should not be overlooked.[44] Nevertheless, a number of new tracers that are under evaluation for imaging atherosclerosis may be of value in CAVD.[45] The limitations of ^{18}F-FDG and ^{18}F-NaF PET imaging highlight the need for such new approaches to detect key aspects of CAVD pathophysiology.

In conclusion, molecular imaging may address some of the existing gaps in CAVD management. Accordingly, there is a great need for non-invasive, highly specific molecular imaging tools with real potential for clinical translation. As only a subset of patients with aortic sclerosis progress to advanced CAVD, detection of early molecular and functional abnormalities in aortic valve disease that predict the future risk for disease progression can help identify subjects who would most benefit from novel therapeutic approaches to prevent aortic valve stenosis. In addition to its clinical relevance, the development of an imaging approach for tracking valvular biology in CAVD will help advance our understanding of pathophysiology and potentially lead to novel medical therapies for this prevalent disease.

Disclosure

Jae-Joon Jung and Farid Jadbabaie have nothing to disclose. Mehran M. Sadeghi is a consultant for Bracco Research, USA.

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