Assessment of Valvular Calcification and Inflammation by Positron Emission Tomography in Patients With Aortic Stenosis

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Background—The pathophysiology of aortic stenosis is incompletely understood, and the relative contributions of valvular calcification and inflammation to disease progression are unknown.

Methods and Results—Patients with aortic sclerosis and mild, moderate, and severe stenosis were compared prospectively with age- and sex-matched control subjects. Aortic valve severity was determined by echocardiography. Calcification and inflammation in the aortic valve were assessed by 18F-sodium fluoride (18F-NaF) and 18F-fluorodeoxyglucose (18F-FDG) uptake with the use of positron emission tomography. One hundred twenty-one patients (20 controls; 20 aortic sclerosis; 25 mild, 33 moderate, and 23 severe aortic stenosis) were administered both 18F-NaF and 18F-FDG. Quantification of tracer uptake within the valve demonstrated excellent interobserver repeatability with no fixed or proportional biases and limits of agreement of \( \pm 0.21 \) (18F-NaF) and \( \pm 0.13 \) (18F-FDG) for maximum tissue-to-background ratios. Activity of both tracers was higher in patients with aortic stenosis than in control subjects (18F-NaF: 2.85 ± 0.82 versus 1.55 ± 0.17; 18F-FDG: 1.58 ± 0.21 versus 1.30 ± 0.13; both \( P < 0.001 \)). 18F-NaF uptake displayed a progressive rise with valve severity (\( r^2 = 0.540 \), \( P < 0.001 \)), with a more modest increase observed for 18F-FDG (\( r^2 = 0.218 \), \( P < 0.001 \)). Among patients with aortic stenosis, 91% had increased 18F-NaF uptake (>1.97), and 35% had increased 18F-FDG uptake (>1.63). A weak correlation between the activities of these tracers was observed (\( r^2 = 0.174 \), \( P < 0.001 \)).

Conclusions—Positron emission tomography is a novel, feasible, and repeatable approach to the evaluation of valvular calcification and inflammation in patients with aortic stenosis. The frequency and magnitude of increased tracer activity correlate with disease severity and are strongest for 18F-NaF.

Clinical Trial Registration—http://www.clinicaltrials.gov. Unique identifier: NCT01358513.
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Key Words: aortic stenosis  ■  calcification  ■  inflammation  ■  positron emission tomography

Calcific aortic stenosis is the most common form of valvular heart disease in the Western world and represents a major healthcare burden that is projected to increase with an aging population. However, the underlying pathophysiology remains incompletely defined, and there are currently no effective medical treatments capable of altering its course. Unfortunately, histological studies are limited by the availability of valve tissue from patients with advanced disease and do not lend themselves to the longitudinal study of disease progression. Alternative techniques are therefore required to investigate the pathogenesis and progression of this condition.

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Positron emission tomography (PET) combined with computed tomography (CT) is a noninvasive imaging technique that allows the identification and quantification of specific biochemical processes within small anatomic structures, such as the aortic valve. Furthermore, 2 common PET tracers
target calcification and inflammation, which are believed to
play a key role in the development of the disease. PET/CT
therefore holds considerable promise as a means of investi-
gating the pathophysiology of aortic stenosis.

18F-Fluorodeoxyglucose (18F-FDG) is a glucose analogue
that is taken up into cells by glucose transport proteins and
enters the glycolytic metabolic pathway. After the initial
hexokinase step, 18F-FDG-6-phosphate cannot be metabo-
lized further and becomes trapped within cells that have high
metabolic requirements, such as macrophages. PET imaging
with the use of 18F-FDG has become an established means of
quantifying vascular inflammation in both the aorta and
carotid arteries,3,4 correlating with plaque macrophage bur-
den5 and symptomatic status.4 18F-Sodium fluoride (18F-
NaF) is an alternative PET tracer that is directly incorporated
into exposed bone crystal (hydroxyapatite) via an exchange
mechanism with hydroxyl groups.6 It is therefore thought to
detect areas of novel calcification and regions of calcium
remodeling and is used clinically for the detection of primary
osteoblastic tumors and bone metastases.7 More recently,
studies have described 18F-NaF uptake as a marker of
calcification within atherosclerotic plaque8,9; however, to
date this tracer has not been used to study patients with aortic
stenosis.

In this PET study, we investigated 18F-NaF and 18F-FDG
uptake in the valves of patients with aortic stenosis with 3
major aims: to examine the feasibility of this approach, to
establish its repeatability, and to assess the relative impor-
tance of inflammation and calcification at different stages of
the disease.

Methods

Consecutive patients aged $\geq$50 years with aortic sclerosis and mild,
moderate, and severe aortic stenosis attending the outpatient depart-
ment of the Royal Infirmary of Edinburgh were considered for
participation in this study. Exclusion criteria included insulin-
dependent diabetes mellitus, blood glucose $\geq$200 mg/dL, and inabil-
ity to undergo PET/CT scanning. Patients were not approached if
they fulfilled any of the exclusion criteria or if their clinician believed
that participation was not appropriate. Of the patients approached,
52% agreed to take part in the trial. These patients were then
compared with age- and sex-matched control subjects with a
normal aortic valve and a similar range of comorbidity. The study
was conducted with local research ethics committee approval and the
written informed consent of all patients.

Echocardiography

Valve disease severity was assessed by a single dedicated research
ultrasonographer on a dedicated machine (Phillips Medical Systems,
Best, Netherlands) under standardized conditions and according to a
formal protocol.10,11 Patients were studied with the use of an S51
pure wave transducer (Phillips Medical Systems) for 2-dimensional,
M-mode, and pulsed and continuous wave Doppler studies. Contin-
uous wave Doppler velocities were confirmed with the use of a D2
CWC transducer (Phillips Medical Systems) from the apex, right
sternal edge, and suprasternal notch. Measurements were determined
online and averaged from 3 cardiac cycles or 5 if the patient was in
atrial fibrillation.

Aortic sclerosis was defined as a thickened aortic valve on
echocardiography in the absence of accelerated flow though the
valve (peak jet velocity $<2$ m/s). The severity of aortic stenosis was
graded according to American Heart Association and American
College of Cardiology criteria with the use of peak transvalvular
aortic valve velocity and mean and maximum aortic valve pressure
gradients.12 In our clinical laboratory, we previously demonstrated a
coefficient of reproducibility of 0.32 m/s for the Doppler measure-
ment of peak aortic valve velocity in patients with aortic stenosis.10
Aortic stenosis severity was also assessed with the use of the time
velocity integral, the dimensionless index, and the aortic valve area,
calculated with the continuity equation.

PET and CT

Combined PET/CT scans of the aortic valve were performed on 2
occasions in close succession with the use of a hybrid scanner
(Biograph mCT, Siemens Medical Systems, Erlangen, Germany).
On the first occasion, a target dose of 125 MBq 18F-NaF was
injected intravenously, and patients rested in a quiet environment for
the 60-minute uptake period. An attenuation-correction CT scan
(noenhanced, low dose 120 kV, 50 mAs) was then performed,
followed by PET imaging covering 2 bed positions centered over the
valve in 3-dimensional mode for 10 minutes. On the second
occasion, a target dose of 200 MBq 18F-FDG was injected, and
patients rested in a quiet environment for 90 minutes. Combined
PET/CT imaging was then performed as described for the 18F-NaF
scan but with the use of a 15-minute bed time. Tracer circulation
times were based on previous studies with the use of 18F-FDG and
18F-NaF in atherosclerosis8,9 and aimed to allow for optimal
contrast between the aortic wall, aortic valve, and blood pool. An
ECG-gated breath-hold CT scan (nonenhanced, 40 mAs per rotation
[CareDose], 100 kV) was performed of the aortic valve immediately
after the 18F-NaF PET/CT scan for calculation of the aortic valve
calcium score.

The PET data were reconstructed with the use of the Siemens
Ultra-HD (time of flight+True X) reconstruction algorithm. Correc-
tions were applied for attenuation, dead time, scatter, and random
coincidences. All image analysis was performed on fused PET/CT
data sets.

Dietary Restrictions

Intense uptake of 18F-FDG by the left ventricle leads to difficulties
in discriminating between activity in the aortic valve and the
myocardium. All patients in our cohort were asked to observe a
carbohydrate-free diet for 24 hours before their 18F-FDG scan
because this suppresses myocardial uptake as the heart switches from
glucose to free fatty acid metabolism.13–15 Patients were provided
with a list of food and drink to avoid and reminded of these
restrictions the day before their scan. Dietary diaries were recorded,
and patients were categorized into dietary compliance or noncom-
pliance. Myocardial tracer uptake was assessed by recording the
maximum standardized uptake value (SUV) in the left ventricular
septal myocardium. The SUV is the decay-corrected tissue uptake
divided by the injected dose per body weight and is a semiqualita-
tive dimensionless unit that is a widely used and validated measure
t of tissue 18F-FDG and 18F-NaF uptake.4,8,16 High myocardial
18F-FDG uptake was prespecified as an SUV value $\leq$5.0, whereas
low uptake, indicating successful myocardial suppression, was de-
 fined by measurements $<$5.0.13

Quantification of Tracer Uptake in the
Aortic Valve

PET image quantification is usually performed in the axial, coronal,
or sagittal planes. However, the aortic valve is a complex
3-dimensional structure that does not align perfectly with any of
these orthogonal planes, making accurate identification of the bound-
aries of the valve difficult with the use of standard techniques. To try
to overcome this, the PET and CT images were fused and analyzed
with the use of a workstation (OsirixX version 3.5.1 64-bit; Osirix
Imaging Software, Geneva, Switzerland) that allows for rotation of
the plane of view into the true axis of the valve. This facilitated the
more accurate delineation of regions of interest around the valve, as
described below.
18F-NaF: Short-Axis Method
The fused PET/CT image was rotated in a 3-dimensional multiplanar reconstruction mode to provide a coaxial short-axis view of the aortic valve (Figure 1). Starting superiorly, we drew a circular region of interest around the aortic valve on 3-mm slices guided by anatomic information provided by CT and any obvious valvular calcification (Figure 1C). Further regions of interest were drawn on adjacent slices until the whole valve had been examined. Mean and maximum SUVs were calculated for each slice and then for the valve as a whole after these values were averaged. However, SUV measurements in vascular structures are influenced by variation in 18F-FDG and 18F-NaF activity in the blood pool. Therefore, SUV measurements were divided by an averaged mean SUV value derived from 5 circular regions of interest drawn in the central blood pool of the superior vena cava. This provided mean and maximum tissue-to-background ratios (TBRs).5,17

18F-FDG Analysis
The coaxial short-axis method was performed for 18F-FDG as described above. Whereas the prescan dietary restrictions sought to minimize the difficulties caused by myocardial 18F-FDG uptake, we also explored 2 additional image analysis approaches to define better and to assess more specifically the valvular uptake (Figure 1). In the long-axis technique, images were reoriented as described for 18F-NaF to provide the coaxial short-axis view shown. This patient has intense myocardial 18F-FDG uptake, which appears to spill into the aortic valve (white arrows) and makes appreciation of the less intense activity in the valve difficult. In the short-axis technique, the green region of interest has been drawn to include as much of the valve as possible while avoiding the myocardial activity. E, Long-axis technique. A region of interest has been drawn on the modified coronal images, attempting to avoid the myocardial uptake. F, Center valve technique. In the same patient, the region of interest has been drawn in the center of the valve well away from myocardial uptake around the periphery. Purple borders indicate images taken in the coaxial short-axis view of the valve. Blue borders indicate images taken in the modified coronal view. Yellow borders indicate images taken in the modified sagittal view of the valve.

Repeatability Studies
Twenty-five patients with a range of aortic valve disease were selected at random from the cohort. After establishment of the aortic valve image analysis methodology, all scans from these patients were analyzed independently by 2 trained observers (M.R.D., C.J.). For each technique, this provided measures of interobserver repeat-
ability for mean and maximum SUV and TBR values. To assess intraobserver variation, both observers repeated the analyses at least 2 weeks later to minimize recall bias.

### Statistical Methods

Continuous variables were expressed as mean±SD and compared with the unpaired Student t test or 1-way ANOVA when appropriate. All continuous variables were tested for normal distribution with the Shapiro-Wilk test. In cases in which data were not normally distributed, they were presented as the median (interquartile range). Categorical variables were expressed as percentages and analyzed with the χ² test. Correlations between normally distributed data were performed with Pearson correlation and presented as r² values. Spearman correlation was used for nonparametric data. The 95% normal range for differences between sets of SUV and TBR measurements (the limits of agreement) were estimated by multiplying the SD of the mean of the differences by 1.96."19 Intraclass correlation coefficients with 95% confidence intervals were calculated for intraobserver and interobserver variation. Statistical analysis was performed with the use of SPSS version 18 (SPSS Inc, Chicago, IL). A 2-sided P<0.05 was regarded as statistically significant.

### Results

#### Patient Population
A total of 121 patients were recruited (aged 72±8 years; 69% male; peak aortic valve velocity 2.8±1.2 m/s) and had both 18F-NaF (66±7 minutes after 124±10 MBq) and 18F-FDG (94±7 minutes after 197±14 MBq) scans of their aortic valve <1 month apart. This cohort comprised 20 control subjects, 20 patients with aortic sclerosis, and 25 patients with mild, 33 with moderate, and 23 with severe aortic stenosis. Patients were well matched for age, sex, and comorbidities (Table 1).

#### Dietary Restrictions and Blood Pool Uptake
Average myocardial SUV across the entire cohort was 4.6±3.6, and dietary restrictions effectively suppressed 18F-FDG myocardial uptake (SUV <5) in 67% of patients, similar to that observed in previous studies."20 On the basis of dietary diaries, 61% of patients complied with the dietary

### Table 1. Baseline Subject Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Aortic Sclerosis</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>20</td>
<td>20</td>
<td>25</td>
<td>33</td>
<td>23</td>
<td>...</td>
</tr>
<tr>
<td>Age, y</td>
<td>70±8</td>
<td>71±9</td>
<td>73±8</td>
<td>72±7</td>
<td>73±11</td>
<td>0.726</td>
</tr>
<tr>
<td>Male sex, %</td>
<td>65</td>
<td>75</td>
<td>60</td>
<td>76</td>
<td>65</td>
<td>0.687</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26±3</td>
<td>29±6</td>
<td>27±3</td>
<td>29±5</td>
<td>28±4</td>
<td>0.051</td>
</tr>
<tr>
<td>Ischemic heart disease, %</td>
<td>35</td>
<td>40</td>
<td>48</td>
<td>36</td>
<td>22</td>
<td>0.445</td>
</tr>
<tr>
<td>Cardiovascular disease, %</td>
<td>35</td>
<td>45</td>
<td>48</td>
<td>39</td>
<td>22</td>
<td>0.373</td>
</tr>
<tr>
<td>Smoking, %</td>
<td>50</td>
<td>35</td>
<td>48</td>
<td>52</td>
<td>61</td>
<td>0.566</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>10</td>
<td>15</td>
<td>20</td>
<td>13</td>
<td>17</td>
<td>0.886</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>40</td>
<td>55</td>
<td>64</td>
<td>73</td>
<td>61</td>
<td>0.203</td>
</tr>
<tr>
<td>Chronic kidney disease stage ≥3, %</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>24</td>
<td>30</td>
<td>0.927</td>
</tr>
<tr>
<td>Creatinine, mg/dL</td>
<td>0.91±0.20</td>
<td>0.99±0.26</td>
<td>0.97±0.32</td>
<td>1.05±0.26</td>
<td>1.09±0.41</td>
<td>0.314</td>
</tr>
<tr>
<td>Urea (BUN), mg/dL</td>
<td>20.2±5.1</td>
<td>19.0±6.8</td>
<td>20.5±10.4</td>
<td>21.0±4.6</td>
<td>22.3±7.8</td>
<td>0.651</td>
</tr>
<tr>
<td>Calcium, mg/dL</td>
<td>9.2±0.2</td>
<td>9.2±0.7</td>
<td>9.2±0.5</td>
<td>9.3±0.4</td>
<td>9.5±0.9</td>
<td>0.228</td>
</tr>
<tr>
<td>Phosphate, mg/dL</td>
<td>3.7±0.5</td>
<td>3.6±0.6</td>
<td>3.6±0.5</td>
<td>3.6±1.5</td>
<td>3.6±0.4</td>
<td>0.973</td>
</tr>
<tr>
<td>Alkaline phosphatase, U/L</td>
<td>75±19</td>
<td>85±30</td>
<td>79±21</td>
<td>82±22</td>
<td>102±89</td>
<td>0.314</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>191±42</td>
<td>194±53</td>
<td>210±59</td>
<td>171±41</td>
<td>203±52</td>
<td>0.040*</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td>98±44</td>
<td>101±41</td>
<td>121±47</td>
<td>89±37</td>
<td>119±48</td>
<td>0.036*</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>58±15</td>
<td>60±35</td>
<td>57±19</td>
<td>49±11</td>
<td>49±13</td>
<td>0.165</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>63±35</td>
<td>72±33</td>
<td>69±41</td>
<td>77±52</td>
<td>82±45</td>
<td>0.613</td>
</tr>
<tr>
<td>Statin therapy, %</td>
<td>35</td>
<td>50</td>
<td>52</td>
<td>67</td>
<td>57</td>
<td>0.262</td>
</tr>
<tr>
<td>ACE inhibitor therapy, %</td>
<td>35</td>
<td>40</td>
<td>52</td>
<td>36</td>
<td>30</td>
<td>0.604</td>
</tr>
<tr>
<td>Peak aortic jet velocity, m/s</td>
<td>1.3±0.2</td>
<td>1.7±0.2</td>
<td>2.5±0.2</td>
<td>3.4±0.3</td>
<td>4.6±0.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Peak aortic valve gradient, mm Hg</td>
<td>7.1±2.2</td>
<td>11.1±2.6</td>
<td>25.5±6.6</td>
<td>46.2±7.7</td>
<td>84.3±24.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean aortic valve gradient, mm Hg</td>
<td>3.7±1.0</td>
<td>5.9±1.4</td>
<td>13.2±2.7</td>
<td>24.9±4.4</td>
<td>48.8±15.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time-velocity integral, m</td>
<td>0.29±0.05</td>
<td>0.38±0.05</td>
<td>0.58±0.07</td>
<td>0.81±0.10</td>
<td>1.13±0.19</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Aortic valve area, cm²</td>
<td>2.70±0.67</td>
<td>2.37±0.54</td>
<td>1.49±0.37</td>
<td>1.19±0.31</td>
<td>0.81±0.28</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Dimensionless index</td>
<td>0.73±0.11</td>
<td>0.62±0.11</td>
<td>0.44±0.99</td>
<td>0.31±0.06</td>
<td>0.24±0.07</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Agatston aortic valve calcium score, AU</td>
<td>1.6±3.8</td>
<td>343±377</td>
<td>702±485</td>
<td>2084±1324</td>
<td>3956±2300</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are mean±SD unless indicated otherwise. BUN indicates blood urea nitrogen; LDL, low-density lipoprotein; HDL, high-density lipoprotein; ACE, angiotensin-converting enzyme; and AU, Agatston unit.

*There was no correlation between peak aortic valve velocity and either serum total cholesterol (Pearson correlation, r²=0.000, P=0.976) or LDL cholesterol (r²=0.007, P=0.36) concentrations.

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Reproducibility statistics reflected this and demonstrated that short- and long-axis techniques, particularly for the latter. Avoiding myocardial uptake was difficult with the use of the 18F-Fluorodeoxyglucose and intraobserver repeatability were all excellent agreement (Table 3). Limits of agreement for intraobserver measurements were fell within narrow limits of agreement: no fixed or proportional bias with narrow limits of agreement. The majority of data for the mean and maximum SUV and TBR values. There were no fixed or proportional biases, and the data fell within narrow limits of agreement: ±0.20 for mean and ±0.21 for maximum TBR measurements (Table 2 and Figure 2). Limits of agreement for intraobserver measurements were similarly good, and intraclass coefficients for interobserver and intraobserver repeatability were all >0.95, indicating excellent agreement (Table 3).

### 18F-Fluorodeoxyglucose

Avoiding myocardial uptake was difficult with the use of the short- and long-axis techniques, particularly for the latter. Reproducibility statistics reflected this and demonstrated that the variability was much greater than for 18F-NaF. The interobserver limits of agreement for the short-axis technique were ±0.28 and ±0.72 for the mean and maximum TBR values, respectively, and were ±0.78 and ±1.18 with the long-axis approach (Table 2 and Figure 2). The intraclass coefficients for the short-axis technique were 0.76 and 0.59 for the mean and maximum TBR values, respectively, and were 0.39 and 0.52 with the long-axis approach (Table 3). Intraobserver repeatability measures were similarly poor.

The center valve analysis was more reproducible. There were no fixed or proportional biases in the differences between interobserver measurements, and the data fell within narrow limits of agreement: ±0.11 for mean and ±0.13 for maximum TBR values (Table 2 and Figure 2). Intraobserver repeatability was similarly good, and intraclass coefficients were all >0.90, indicating excellent agreement (Tables 2 and 3).

There were concerns that the center valve technique might underestimate 18F-FDG activity in the valve by excluding the valve ring. However, there was no difference between mean uptake values calculated by the center valve technique and the short-axis method (center valve TBR: 1.43±0.17; short

### Table 2. Interobserver and Intraobserver Repeatability Statistics for 18F-NaF and 18F-FDG Quantification in the Aortic Valve

<table>
<thead>
<tr>
<th>Mean Difference Between Aortic Valve SUV and TBR Measurements</th>
<th>Interobserver</th>
<th>M.R.D.</th>
<th>C.J.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean SUV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18F-NaF short axis</td>
<td>0.04 (−0.12 to 0.20)</td>
<td>0.02 (−0.10 to 0.14)</td>
<td>0.05 (−0.14 to 0.24)</td>
</tr>
<tr>
<td>18F-FDG short axis</td>
<td>0.02 (−0.18 to 0.20)</td>
<td>−0.01 (−0.10 to 0.12)</td>
<td>−0.02 (−0.19 to 0.15)</td>
</tr>
<tr>
<td>18F-FDG long axis</td>
<td>0.05 (−0.57 to 0.67)</td>
<td>0.05 (−0.37 to 0.47)</td>
<td>0.00 (−0.14 to 0.14)</td>
</tr>
<tr>
<td>18F-FDG center valve</td>
<td>0.01 (−0.05 to 0.07)</td>
<td>0.01 (−0.05 to 0.07)</td>
<td>−0.02 (−0.16 to 0.12)</td>
</tr>
<tr>
<td>Maximum SUV</td>
<td>0.02 (−0.16 to 0.20)</td>
<td>0.04 (−0.11 to 0.19)</td>
<td>0.00 (−0.19 to 0.19)</td>
</tr>
<tr>
<td>18F-NaF short axis</td>
<td>0.02 (−0.80 to 0.84)</td>
<td>−0.11 (−0.64 to 0.42)</td>
<td>−0.09 (−0.89 to 0.72)</td>
</tr>
<tr>
<td>18F-FDG long axis</td>
<td>−0.03 (−1.06 to 1.00)</td>
<td>0.12 (−0.44 to 0.68)</td>
<td>0.10 (−0.41 to 0.61)</td>
</tr>
<tr>
<td>18F-FDG center valve</td>
<td>0.03 (−0.07 to 0.13)</td>
<td>0.02 (−0.13 to 0.17)</td>
<td>−0.06 (−0.26 to 0.14)</td>
</tr>
<tr>
<td>Mean TBR</td>
<td>0.07 (−0.13 to 0.27)</td>
<td>−0.01 (−0.15 to 0.17)</td>
<td>0.05 (−0.17 to 0.27)</td>
</tr>
<tr>
<td>18F-NaF short axis</td>
<td>0.06 (−0.22 to 0.34)</td>
<td>0.00 (−0.15 to 0.15)</td>
<td>0.01 (−0.12 to 0.14)</td>
</tr>
<tr>
<td>18F-FDG long axis</td>
<td>0.06 (−0.72 to 0.84)</td>
<td>0.07 (−0.50 to 0.64)</td>
<td>0.02 (−0.22 to 0.26)</td>
</tr>
<tr>
<td>18F-FDG center valve</td>
<td>−0.01 (−0.12 to 0.10)</td>
<td>0.02 (−0.06 to 0.10)</td>
<td>−0.01 (−0.07 to 0.05)</td>
</tr>
<tr>
<td>Maximum TBR</td>
<td>0.02 (−0.19 to 0.23)</td>
<td>−0.04 (−0.16 to 0.24)</td>
<td>0.00 (−0.21 to 0.21)</td>
</tr>
<tr>
<td>18F-NaF short axis</td>
<td>0.06 (−0.66 to 0.78)</td>
<td>−0.09 (−0.39 to 0.57)</td>
<td>0.07 (−0.06 to 0.20)</td>
</tr>
<tr>
<td>18F-FDG long axis</td>
<td>0.01 (−1.17 to 1.19)</td>
<td>0.12 (−0.50 to 0.74)</td>
<td>0.06 (−0.33 to 0.39)</td>
</tr>
<tr>
<td>18F-FDG center valve</td>
<td>−0.02 (−0.15 to 0.11)</td>
<td>0.02 (−0.11 to 0.15)</td>
<td>−0.05 (−0.14 to 0.04)</td>
</tr>
</tbody>
</table>

Mean difference between standard uptake value (SUV) and tissue-to-background ratio (TBR) measurements for 18F-sodium fluoride (18F-NaF) valve uptake with the use of the short-axis technique and 18F-fluorodeoxyglucose (18F-FDG) uptake with the use of the short-axis, center valve, and long-axis techniques is shown (95% confidence intervals in parentheses). The short-axis technique for 18F-NaF and the center valve technique for 18F-FDG display no fixed proportional bias with narrow limits of agreement.

Restrictions and had lower myocardial 18F-FDG uptake than noncompliers (SUV 3.2±2.3 versus 6.7±4.2; P<0.001). Across the cohort, blood pool uptake in the SVC was 0.99±0.18 for 18F-NaF and 1.26±0.20 for 18F-FDG.
Maximum TBR values were lower with the use of the former approach (center valve: 1.60 ± 0.20; short axis: 1.80 ± 0.45; \( P = 0.041 \)). However, this difference was no longer apparent when patients with high myocardial uptake (n = 11) were excluded from the analysis (center valve: 1.56 ± 0.18; short axis: 1.64 ± 0.20; \( P = 0.245 \)), reflecting the wide limits of agreement (±1.05) for the short-axis technique when myocardial suppression was poor. By contrast, limits of agreement for the center valve technique were equally good in patients with low and high myocardial uptake (±0.13 versus ±0.14, respectively; \( P = 0.919 \)). Given this and other advantages, subsequent analysis of the entire cohort was performed with the center valve method for 18F-FDG.

### Aortic Valve Uptake

#### 18F-Sodium Fluoride

Focal 18F-NaF uptake was observed in the valves of patients with calcific aortic valve disease in areas overlying, adjacent to, and remote from existing calcification. Areas of established calcium were also observed frequently in the absence of increased 18F-NaF activity (Figure 3). Compared with control subjects, valvular 18F-NaF uptake was higher in patients with both aortic sclerosis (maximum TBR: 1.55 ± 0.17 versus 1.92 ± 0.31; \( P < 0.001 \)) and aortic stenosis (maximum TBR: 1.55 ± 0.17 versus 2.87 ± 0.82; \( P < 0.001 \)). The highest maximum TBR value in the control group was 1.97, which was used to divide patients with aortic valve disease into those with increased 18F-NaF uptake (>1.97).
and those without ($\leq 1.97$). Overall, 45% of patients with aortic sclerosis and 91% of those with aortic stenosis had increased uptake. The proportion of patients with increased activity rose sharply with increasing disease severity such that 100% of patients with severe disease had increased uptake (Table 4).

All measures of 18F-NaF uptake displayed a progressive rise with increasing aortic jet velocity (maximum TBR: $r^2=0.540$, $P<0.001$; Table 4 and Figure 4), the aortic valve calcium score ($r^2=0.641$, $P<0.001$), and other echocardiographic measures of aortic stenosis severity (time-velocity integral: $r^2=0.546$, $P<0.001$; aortic valve area: $r^2=0.387$, $P<0.001$; dimensionless index: $r^2=0.527$, $P<0.001$).

18F-Fluorodeoxyglucose
18F-FDG showed a more diffuse pattern of activity within the valve (Figure 3), and compared with control subjects, uptake was increased in patients with aortic sclerosis (maximum TBR: $1.30 \pm 0.13$ versus $1.47 \pm 0.15$; $P<0.001$) and aortic stenosis (maximum TBR: $1.30 \pm 0.13$ versus $1.58 \pm 0.21$; $P<0.001$). The highest maximum TBR value in the control group was 1.63, which was used to divide patients with aortic valve disease into those with increased 18F-FDG uptake (>1.63) and those without ($\leq 1.63$). Overall, 20% of patients with aortic sclerosis and 35% of patients with aortic stenosis had increased uptake. The proportion of patients with increased activity in the valve again rose with increasing aortic valve disease; however, this rise was more gradual than for 18F-NaF, with only 52% of patients with severe disease demonstrating increased activity (Table 4).

All measures of 18F-FDG activity displayed a progressive rise with increasing aortic jet velocity (maximum TBR: $r^2=0.218$, $P<0.001$; Table 4 and Figure 4), the aortic valve Agatston score ($r^2=0.138$, $P<0.001$), and other echocardiographic measures of aortic stenosis (time-velocity integral: $r^2=0.246$, $P<0.001$; aortic valve area: $r^2=0.184$, $P<0.001$; dimensionless index: $r^2=0.229$, $P<0.001$). These correlations were weaker and more modest than for 18F-NaF. A modest correlation was also observed between valvular 18F-NaF and 18F-FDG activities (maximum TBR: $r^2=0.174$, $P<0.001$).

**Discussion**
In this PET study, we have established the feasibility of evaluating 18F-NaF and 18F-FDG activity in patients with aortic stenosis. Moreover, we have demonstrated excellent repeatability for the quantification of these tracers in the valve as measures of calcification and inflammation, respectively. 18F-NaF and 18F-FDG activity was increased in patients with both aortic sclerosis and stenosis, displaying a

<table>
<thead>
<tr>
<th>Table 3. Intraclass Coefficient Values for 18F-NaF and 18F-FDG Quantification in the Aortic Valve</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intraclass Coefficient Values Comparing Aortic Valve SUV and TBR Measurements</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Mean SUV</td>
</tr>
<tr>
<td>18F-NaF short axis</td>
</tr>
<tr>
<td>18F-FDG short axis</td>
</tr>
<tr>
<td>18F-FDG long axis</td>
</tr>
<tr>
<td>18F-FDG center valve</td>
</tr>
<tr>
<td>Maximum SUV</td>
</tr>
<tr>
<td>18F-NaF short axis</td>
</tr>
<tr>
<td>18F-FDG short axis</td>
</tr>
<tr>
<td>18F-FDG long axis</td>
</tr>
<tr>
<td>18F-FDG center valve</td>
</tr>
<tr>
<td>Mean TBR</td>
</tr>
<tr>
<td>18F-NaF short axis</td>
</tr>
<tr>
<td>18F-FDG short axis</td>
</tr>
<tr>
<td>18F-FDG long axis</td>
</tr>
<tr>
<td>18F-FDG center valve</td>
</tr>
<tr>
<td>Maximum TBR</td>
</tr>
<tr>
<td>18F-NaF short axis</td>
</tr>
<tr>
<td>18F-FDG short axis</td>
</tr>
<tr>
<td>18F-FDG long axis</td>
</tr>
<tr>
<td>18F-FDG center valve</td>
</tr>
</tbody>
</table>

Intraobserver M.R.D. C.J.

**Intraobserver correlation coefficient (ICC) values (95% confidence intervals in parentheses) for the short-axis 18F-sodium fluoride (18F-NaF) technique and the center valve 18F-fluorodeoxyglucose (18F-FDG) method are shown. SUV indicates standard uptake value; TBR, tissue-to-background ratio.**
progressive rise in uptake with increasing disease severity. However, calcification rather than inflammation appears to be the predominant process affecting the valve, particularly in the latter stages of the disease, in which a more marked progression in 18F-NaF activity was observed that was disproportionate to 18F-FDG.

Valve calcification plays a key role in the development of aortic stenosis. Hydroxyapatite becomes deposited on a bonelike matrix containing collagen, osteopontin, and other bone matrix proteins to form nodules that progress until, by the end stage of the disease, lamellar bone, microfractures, and hemopoietic tissue can all be identified. This appears to occur as part of a highly regulated process, coordinated by increased osteoblast activity and the local production of osteopontin, osteocalcin, bone sialoproteins, and bone morphogenetic protein-2, all of which are more commonly associated with skeletal bone formation. Established aortic valve calcium can be measured accurately with the use of CT, but measurement of 18F-NaF uptake offers, for the first time, the possibility of detecting areas of developing calcification within the valve. In this study, 18F-NaF uptake was observed in regions adjacent to and remote from existing calcium, suggesting expansion of the calcific process to new areas of the valve. In addition, uptake was observed in regions adjacent to and remote from existing calcium, suggesting expansion of the calcific process to new areas of the valve. In addition, uptake was observed in regions
overlapping with that of established calcium, and, in these areas, activity is likely to represent calcium remodeling and maturation of the calcific process. 18F-NaF activity was increased in the valves of patients with aortic sclerosis and stenosis compared with control subjects and demonstrated a marked progressive rise with increasing disease severity accounting for \( \frac{1}{2} \) of the variance associated with valve stenosis. Moreover, increased valvular 18F-NaF activity was observed in 45% of patients with aortic sclerosis, 91% of patients with aortic stenosis, and all patients with severe stenosis. Calcium accumulation is the predominant mechanism by which valve cusp rigidity increases and aortic stenosis advances. As such, this technique offers considerable promise as a biomarker of disease activity and as a means of predicting disease progression. Longitudinal studies are now required to determine whether calcification activity quantified by 18F-NaF uptake is an accurate predictor of disease progression and superior to baseline measures of valve severity and calcium scores. If confirmed, these studies would pave the way for mechanistic studies of medical interventions to interrupt progressive calcific disease with the use of 18F-NaF activity as a surrogate biomarker and end point.

In the early stages of aortic stenosis, endothelial damage secondary to mechanical stress and lipid deposition triggers an inflammatory response within the valve. This is characterized by increased macrophage and T-cell activity within the valve leaflets and the expression of a range of proinflammatory cytokines including transforming growth factor-\( \beta \), tumor necrosis factor-\( \alpha \), and interleukin-1\( \beta \). The inflammatory response is thought to trigger the fibrotic and calcific processes that subsequently drive valve orifice narrowing. Thus, identifying and quantifying valvular inflammation with 18F-FDG have the potential to be critical in the evaluation of aortic stenosis. In the present study, 18F-FDG uptake was higher in patients with aortic sclerosis and stenosis compared with control subjects, and activity again rose with increasing valve severity. However, this association was weaker and the increase in activity was more modest than for 18F-NaF. Indeed, increased valvular 18F-FDG activity was only observed in 20% of patients with aortic sclerosis, 35% of patients with aortic stenosis, and 52% of patients with severe stenosis. Although this may reflect the high cutoff used for increased activity or an insensitivity of 18F-FDG in detecting inflammation, these data suggest that calcification is the predominant pathogenic process in aortic stenosis and a better target for novel therapeutic strategies. It might also explain the disappointing results of statin therapy in this condition, which has consistently failed to modify vascular calcification even in the coronary circulation despite reducing systemic markers of inflammation.

### Table 4. Correlation Between Aortic Stenosis Severity and Radiotracer Uptake

<table>
<thead>
<tr>
<th>Correlation With Peak Aortic Jet Velocity</th>
<th>Aortic Stenosis</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>( r^2 )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Aortic Sclerosis</td>
<td>Aortic Sclerosis</td>
<td>Aortic Sclerosis</td>
<td>Aortic Sclerosis</td>
<td>Aortic Sclerosis</td>
<td>Aortic Sclerosis</td>
</tr>
<tr>
<td>18F-NaF</td>
<td>Variance</td>
<td>1.20 (1.10–1.55)</td>
<td>1.35 (1.24–1.59)</td>
<td>1.59 (1.38–1.73)</td>
<td>1.82 (1.67–2.05)</td>
<td>2.10 (1.78–2.51)</td>
</tr>
<tr>
<td>Mean SUV</td>
<td>Variance</td>
<td>1.23 (1.20–1.36)</td>
<td>1.53 (1.34–1.59)</td>
<td>1.73 (1.45–1.92)</td>
<td>2.03 (1.71–2.28)</td>
<td>2.17 (1.82–2.36)</td>
</tr>
<tr>
<td>Maximum SUV</td>
<td>Variance</td>
<td>1.54 (1.33–1.86)</td>
<td>1.77 (1.58–2.09)</td>
<td>2.21 (1.84–2.45)</td>
<td>2.57 (2.27–2.99)</td>
<td>3.25 (2.47–4.42)</td>
</tr>
<tr>
<td>Maximum TBR</td>
<td>Variance</td>
<td>1.56 (1.41–1.64)</td>
<td>1.96 (1.63–2.11)</td>
<td>2.45 (1.94–2.71)</td>
<td>2.89 (2.31–3.24)</td>
<td>3.25 (2.62–3.63)</td>
</tr>
<tr>
<td>Patients with increased uptake, %</td>
<td>Variance</td>
<td>0</td>
<td>45</td>
<td>76</td>
<td>95</td>
<td>100</td>
</tr>
<tr>
<td>18F-FDG</td>
<td>Variance</td>
<td>1.49 (1.33–1.56)</td>
<td>1.73 (1.46–1.88)</td>
<td>1.66 (1.53–1.88)</td>
<td>1.71 (1.61–1.91)</td>
<td>1.76 (1.61–2.18)</td>
</tr>
<tr>
<td>Mean SUV</td>
<td>Variance</td>
<td>1.18 (1.09–1.26)</td>
<td>1.35 (1.19–1.44)</td>
<td>1.29 (1.21–1.45)</td>
<td>1.33 (1.26–1.47)</td>
<td>1.42 (1.36–1.62)</td>
</tr>
<tr>
<td>Maximum SUV</td>
<td>Variance</td>
<td>1.62 (1.47–1.68)</td>
<td>1.91 (1.64–2.07)</td>
<td>1.85 (1.72–2.07)</td>
<td>1.95 (1.81–2.18)</td>
<td>2.07 (1.88–2.25)</td>
</tr>
<tr>
<td>Maximum TBR</td>
<td>Variance</td>
<td>1.27 (1.21–1.40)</td>
<td>1.47 (1.31–1.61)</td>
<td>1.44 (1.37–1.63)</td>
<td>1.58 (1.41–1.65)</td>
<td>1.65 (1.55–1.85)</td>
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<tr>
<td>Patients with increased uptake, %</td>
<td>Variance</td>
<td>0</td>
<td>20</td>
<td>24</td>
<td>30</td>
<td>52</td>
</tr>
</tbody>
</table>

Values are median±interquartile range, with Pearson correlation values. 18F-NaF indicates 18F-sodium fluoride; 18F-FDG, 18F-fluorodeoxyglucose; SUV, standard uptake value; and TBR, tissue-to-background ratio.

Figure 4. Uptake of 18F-fluorodeoxyglucose (18F-FDG) and 18F-sodium fluoride (18F-NaF) according to the severity of aortic stenosis. Box plots show the median and interquartile ranges of the tissue-to-background ratios (TBR) for 18F-NaF (white boxes) and 18F-FDG (gray boxes) with whiskers to 1.5×interquartile range.
A recent retrospective study described increased 18F-FDG uptake in the valves of 42 patients with cancer who were coincidentally found to have aortic stenosis.\(^\text{18}\) However, in contrast to our study, a reduction in activity was observed in patients with severe compared with moderate disease. This difference is likely to reflect the small number of subjects in the severe subgroup (n = 8), the retrospective nature of the study analysis, and the confounding effects of coexistent malignancy. In contrast, we have prospectively recruited a larger, well-defined cohort of patients with aortic stenosis who are more likely to be representative of those seen in cardiology practice. Moreover, we studied patients following dietary restrictions to minimize the effects of myocardial uptake and spillover into the valve. Our data are in agreement in those with advanced disease.

**Study Limitations**

With exclusion of part of the valve, it is possible that the center valve technique employed for 18F-FDG analysis may underestimate valvular inflammation. However, there were no differences in mean uptake values compared with the short-axis technique. This reflects the diffuse nature of 18F-FDG activity in stenotic valves and the equal distribution of lesions between the base of the valve leaflets (54%) and the mid portion and tips (46%).\(^\text{33}\)

This study has not validated 18F-FDG and 18F-NaF activity against histological samples. Although the mechanism of uptake for both tracers has been investigated in other tissues, further work is required in the valve to address this issue (see the online-only Data Supplement).

**Conclusion**

The evaluation of aortic stenosis with the use of PET is feasible and highly reproducible, with 18F-FDG and particularly 18F-NaF holding considerable promise as novel biomarkers of disease activity. Both calcification and inflammation are increased in patients with aortic valve disease compared with control subjects, and the activity of both rises steadily with increasing disease severity. However, calcification appears to be the predominant pathological process, particularly in the latter stages of the disease, and would therefore appear to be a better target for future potential medical therapies.

**Acknowledgments**

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**Disclosures**

None.

**References**


**CLINICAL PERSPECTIVE**

Aortic stenosis is the most common form of valvular heart disease in the Western world and represents a major healthcare burden that is projected to increase with an aging population. However, there are currently no effective medical treatments or biomarkers of disease activity. The pathogenesis of aortic stenosis is incompletely understood, and defining the various stages of this process will be highly important to develop the therapies and biomarkers that are required. Positron emission tomography combined with computed tomography is a noninvasive imaging technique that allows the identification and quantification of specific pathological processes within small anatomic structures, such as the aortic valve. In this study, we sought to test the feasibility, repeatability, and validity of this technique in the evaluation of aortic valve stenosis. Positron emission tomography/computed tomography imaging of the aortic valve was performed to assess inflammation (18F-fluorodeoxyglucose) and active calcification (18F-sodium fluoride) of the valve leaflets. The positron emission tomography/computed tomography findings were compared in 121 patients with a full spectrum of disease severity. Our data have clearly established that this technique is both feasible and repeatable, indicating that these tracers may prove to be useful biomarkers of disease activity. Furthermore, we have demonstrated that 18F-fluorodeoxyglucose and 18F-sodium fluoride activity increase with progressive disease severity. However, uptake of 18F-sodium fluoride appears to predominate in the early and latter stages of this disease. This may explain the disappointing effects of statin therapy in this condition and indicates that calcification might represent a better target for novel therapeutic interventions.
Assessment of Valvular Calcification and Inflammation by Positron Emission Tomography in Patients With Aortic Stenosis
Marc Richard Dweck, Charlotte Jones, Nikhil V. Joshi, Alison M. Fletcher, Hamish Richardson, Audrey White, Mark Marsden, Renzo Pessotto, John C. Clark, William A. Wallace, Donald M. Salter, Graham McKillop, Edwin J.R. van Beek, Nicholas A. Boon, James H.F. Rudd and David E. Newby

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Supplemental Material

Supplemental Methods

One patient underwent an aortic valve replacement 3 months after their PET scans. The valve was harvested and incubated with 18F-FDG, Dulbecco’s modified eagle medium (Invitrogen, Paisley, UK) and 10% fetal calf serum for 90 min before PET/CT imaging using the same protocol as the in vivo clinical scans. The next day, PET/CT imaging was repeated after incubation with 18F-NaF for 60 min. Finally the valve was fixed in formalin and decalcified using ethylene diamine tetracetic acid before immunohistochemistry was performed to examine for macrophage accumulation (CD68) and active calcification (osteocalcin).

Supplemental Results

18F-NaF uptake was consistent on both the in vivo and ex vivo PET/CT scans. Furthermore uptake co-localized with the distribution of osteocalcin staining on histology, extending beyond the boundaries of existing macroscopic calcification (Supplemental Figure 1). The pattern of valvular 18F-FDG uptake was again consistent between in-vivo and ex-vivo PET/CT scans. Furthermore activity mapped closely to areas of increased macrophage density on immunohistochemistry (Supplementary Figure 2).
Supplemental Figure Legends

Supplemental Figure 1. 18F-NaF studies on excised aortic valve.

A Excised portion of a stenotic bicuspid aortic valve removed at the time of an aortic valve and root replacement. The aortic side of one of the valve leaflets is shown.

B Immunohistochemistry of the valve for osteocalcin in a region of the valve adjacent to a calcific nodule. Osteocalcin is incorporated into the bone matrix where it binds to hydroxyapatite during active mineralization. Osteocalcin immunoreactivity can be seen at the periphery of the existing calcified nodule. Inset (black border) shows cytoplasmic staining of cells adjacent to this.

C. Immunohistochemistry in a region remote from existing calcification with no staining present. This focal distribution of staining matches the pattern of 18F-NaF activity in the valve on both the in vivo and ex vivo scans described below.

D. In vivo 18F-NaF PET/CT scan performed 3 months prior to the operation. Note the focal areas of uptake overlying the area of calcification at the bottom left of the valve and adjacent to the smaller area of calcification in the top right. This closely matches the pattern of uptake observed on the ex vivo PET/CT scan performed on the excised valve (E).
**Supplemental Figure 2. 18F-NaF studies on excised aortic valve.**

**A.** Excised portion of the same aortic valve as in Figure 1.

**B.** Immunohistochemistry for CD 68 in a region of the valve adjacent to a calcific nodule demonstrating the presence of macrophages around the lesion in the fibrosa of the valve leaflet. **C.** Immunohistochemistry in a region remote from existing calcification, which again displays staining for macrophages. **D.** *In vivo* 18F-FDG PET/CT of the valve shows a more diffuse pattern of uptake than for 18F-NaF matching the distribution of macrophages as well as the pattern of uptake observed in the *ex vivo* PET/CT scan of the excised valve tissue (E).
Supplemental Figures

Supplemental Figure 1
대동맥판협착증의 병태생리는 아직 완전하게 밝혀져 있지 않으며, 특히 판막 석회화 및 염증이 협착증의 진행에 미치는 영향에 대해서는 알려진 바 없다.

방법 및 결과
대동맥판경화증(aortic sclerosis) 환자와 경증, 중등도, 중증 대동맥판협착증 환자를 연령과 성별을 맞춘 대조군과 전향적으로 비교하였다. 협착증의 중증도는 심초음파 검사로 평가하였다. 협착증의 중증도는 심초음파 검사로 평가하였다. 18F-sodium fluoride(18F-NaF)와 18F-fluorodeoxyglucose(18F-FDG)를 이용한 PET 영상으로 판막의 석회화 및 염증을 평가하였다. 총 121명(대조군 20명; 대동맥판협착증 20명; 경증 25명; 중등도 33명; 중증 대동맥판협착증 23명)에서 18F-NaF와 18F-FDG를 모두 투여하였다. 판막 내 추적자 섭취(tracer uptake)를 정량화하였을 때 아주 좋은 관찰자 간 일치도를 보였으며, 최대 조직-배경 비(tissue-to-background ratio)의 일치한도Limits of agreement)는 각각 ±0.21(18F-NaF), ±0.13(18F-FDG)였다. 두 추적자 모두 대동맥판협착증에서 대조군에 비해 섭취율이 높았다(18F-NaF: 2.87±0.82 vs. 1.55±0.17; 18F-FDG: 1.58±0.21 vs 1.30±0.13; both P<0.001). 18F-NaF 섭취는 판막협착증이 심할수록 높게 나타났으며(r^2=0.540, P<0.001), 18F-FDG의 증가는 다소 낮은 편이었다(r^2=0.218, P<0.001). 대동맥판협착증 환자의 91%에서 18F-NaF 섭취가 증가되었으며(19.7), 35%에서 18F-FDG 섭취가 증가되었다(r=1.63). 두 추적자의 섭취량 간에 약한 상관관계가 관찰되었다(r^2=0.174, P<0.001).

결론
PET 영상은 대동맥판협착증 환자에서 판막의 석회화 및 염증을 평가할 수 있는 새로운, 쉽게 적용가능하고, 반복적으로 시행할 수 있는 유용한 검사법이다. 추적자의 섭취증가가 판막병변의 중증도와 좋은 상관관계를 보였으며, 특히 18F-NaF에서 강한 상관관계를 보였다.
대동맥판협착증은 북미, 유럽국가에서는 가장 흔한 판막질환이며, 우리나라에서도 고령화 및 생활습관의 변화에 따라 급격하게 증가하고 있다. 하지만 현재까지 협착증이 진행을 늦출 수 있는 약물치료나 질병 상태를 반영하는 biomarker는 없는 형편이다. 대동맥판협착증의 병태생리는 아직 완전히 밝혀져 있지 않은데, 이 과정을 좀 더 명확히 밝히는 것이 효과적인 약물치료나 biomarker의 개발을 위해 중요하다.

PET-CT 영상은 대동맥판막과 같이 작은 구조물에서 특정 병리학적 과정을 발견하고 정량화할 수 있는 비

**Figure 1.** 18F-NaF(A–F)와 18F-FDG(G–I)의 PET–CT 영상(정상대조군: A, G; 대동맥판경화증: B; 경증: C, H; 중등도: D; 중증 협착증: E, F, I)
침습적인 방법이다. 본 연구에서는 대동맥판막의 석회화(18F-NaF) 및 염증(18F-FDG)을 평가하는 데 있어 PET-CT 영상의 실현 가능성이, 정확도 등을 평가하였다. 그 결과 매우 좋은 관찰자가 일치도를 얻었으며, 추적자 직 석회화가 협착증의 중증도와 좋은 상관관계를 보여 협착증의 활성도를 반영하는 biomarker로서의 가능성을 제시하였다. 특히, 18F-FDG에 비해 18F-NaF에서 높은 상관관계를 보여 염증반응보다는 석회화가 협착증의 진행에 있어 중요한 타깃임을 시사하였다. 이는 스타틴 연구에서 스타틴 치료가 협착증의 진행을 막지 못하고 실패한 이유를 설명해주주는 증거라고 하겠다.

판막내 염증 및 석회화의 정량화는 몇 가지 측면에서 큰 의미가 있다. 첫째, 대동맥판협착증에서 biomarker로서의 가능성이 있다. 스타틴을 비롯한 몇몇 약물치료가 대동맥판협착증 환자에서 실망스러운 결과를 보인 이유 중 하나는 그 증가점이 사망이나 판막수술과 같은 중대한 임상사건이었기 때문이다. 즉, 중대한 심혈관계 임상사건들은 대동맥판협착증뿐 아니라 동반된 질환에 의해 크게 영향을 받는데, 특히 대동맥판협착증 환자들이 고령이라는 점을 감안하면 이런 부분이 특히 중요하다. 따라서 정확한 biomarker가 있어서 약물치료에 의한 판막변성의 진행 정도만을 정확히 평가할 수 있다면, 임상시험에서 약물치료의 유효성을 평가하는 데 매우 유리할 것이다. 또한, biomarker의 특성상 연구에 소요되는 시간과 비용을 크게 줄여 임상연구를 확대할 수 있는 장점이 있다. 둘째, 대동맥판협착증의 병태생리에 의해하는 데 유용하다. 이는 새로운 양식치료를 개발하고 새로운 치료대상을 발굴하는 데 매우 유용할 것으로 기대된다. 특히, 염증반응이 중요한 역할을 하는 대동맥판막염증 초기에 효과적인 양식치료가 기대된다.

본 연구에서는 사용된 추적자인 18F-NaF 와 18F-FDG는 여러 조직에서 그 유용성이 입증되었기는 하지만, 이 연구에서 환자들의 조직에는 직접 검증되지 못한 점은 아쉬운 점이라고 하겠다. 또한, 각 추적자들의 석회화 협착증의 중증도에 따라 증가되었기는 하지만, 중증도를 나타내는 이상의 의미가 있는지에 대한 추가연구가 필요할 것으로 생각된다. 즉, 같은 중증도를 가진 환자에서 추적자의 석회화에 따라 협착증의 진행경과가 달라진다면, 이는 임상적으로 매우 유용한 정보가 될 것이다. 따라서 추적자의 석회화와 실제 조직변화를 비교하여 validation 연구와 추적자의 석회화로 평가한 염증 및 석회화와 협착증의 진행 경과 간의 관계를 평가하는 longitudinal 연구가 꼭 필요할 것으로 생각된다.
Assessment of Valvular Calcification and Inflammation by Positron Emission Tomography in Patients With Aortic Stenosis

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Background—The pathophysiology of aortic stenosis is incompletely understood, and the relative contributions of valvular calcification and inflammation to disease progression are unknown.

Methods and Results—Patients with aortic sclerosis and mild, moderate, and severe stenosis were compared prospectively with age- and sex-matched control subjects. Aortic valve severity was determined by echocardiography. Calcification and inflammation in the aortic valve were assessed by 18F-sodium fluoride (18F-NaF) and 18F-fluorodeoxyglucose (18F-FDG) uptake with the use of positron emission tomography. One hundred twenty-one subjects (20 controls; 20 aortic sclerosis; 25 mild, 33 moderate, and 23 severe aortic stenosis) were administered both 18F-NaF and 18F-FDG. Quantification of tracer uptake within the valve demonstrated excellent interobserver repeatability with no fixed or proportional biases and limits of agreement of \(\pm 0.21\) (18F-NaF) and \(\pm 0.13\) (18F-FDG) for maximum tissue-to-background ratios. Activity of both tracers was higher in patients with aortic stenosis than in control subjects (18F-NaF: \(2.87 \pm 0.82\) versus \(1.55 \pm 0.17\); 18F-FDG: \(1.58 \pm 0.21\) versus \(1.30 \pm 0.13\); both \(P<0.001\)). 18F-NaF uptake displayed a progressive rise with valve severity \((r^2=0.540, P<0.001)\), with a more modest increase observed for 18F-FDG \((r^2=0.218, P<0.001)\). Among patients with aortic stenosis, 91% had increased 18F-NaF uptake \((>1.97)\), and 35% had increased 18F-FDG uptake \((>1.63)\). A weak correlation between the activities of these tracers was observed \((r^2=0.174, P<0.001)\).

Conclusions—Positron emission tomography is a novel, feasible, and repeatable approach to the evaluation of valvular calcification and inflammation in patients with aortic stenosis. The frequency and magnitude of increased tracer activity correlate with disease severity and are strongest for 18F-NaF.

Clinical Trial Registration—http://www.clinicaltrials.gov. Unique identifier: NCT01358513.

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Key Words: aortic stenosis ■ calcification ■ inflammation ■ positron emission tomography

Calcific aortic stenosis is the most common form of valvular heart disease in the Western world and represents a major healthcare burden that is projected to increase with an aging population. However, the underlying pathophysiology remains incompletely defined, and there are currently no effective medical treatments capable of altering its course. Unfortunately, histological studies are limited by the availability of valve tissue from patients with advanced disease and do not lend themselves to the longitudinal study of disease progression. Alternative techniques are therefore required to investigate the pathogenesis and progression of this condition.

Clinical Perspective on p 130

Positron emission tomography (PET) combined with computed tomography (CT) is a noninvasive imaging technique that allows the identification and quantification of specific biochemical processes within small anatomic structures, such as the aortic valve. Furthermore, 2 common PET tracers
target calcification and inflammation, which are believed to play a key role in the development of the disease. PET/CT therefore holds considerable promise as a means of investigating the pathophysiology of aortic stenosis.

18F-Fluorodeoxyglucose (18F-FDG) is a glucose analogue that is taken up into cells by glucose transport proteins and enters the glycolytic metabolic pathway. After the initial hexokinase step, 18F-FDG-6-phosphate cannot be metabolized further and becomes trapped within cells that have high metabolic requirements, such as macrophages. PET imaging with the use of 18F-FDG has become an established means of quantifying vascular inflammation in both the aorta and carotid arteries, correlating with plaque macrophage burden and symptomatic status. 18F-Sodium fluoride (18F-NaF) is an alternative PET tracer that is directly incorporated into exposed bone crystal (hydroxyapatite) via an exchange mechanism with hydroxyl groups. It is therefore thought to detect areas of novel calcification and regions of calcium remodeling and is used clinically for the detection of primary osteoblastic tumors and bone metastases. More recently, studies have described 18F-NaF uptake as a marker of calcification within atherosclerotic plaque; however, to date this tracer has not been used to study patients with aortic stenosis.

In this PET study, we investigated 18F-NaF and 18F-FDG uptake in the valves of patients with aortic stenosis with 3 major aims: to examine the feasibility of this approach, to establish its repeatability, and to assess the relative importance of inflammation and calcification at different stages of the disease.

**Methods**
Consecutive patients aged >50 years with aortic sclerosis and mild, moderate, and severe aortic stenosis attending the outpatient department of the Royal Infirmary of Edinburgh were considered for participation in this study. Exclusion criteria included insulin-dependent diabetes mellitus, blood glucose >200 mg/dL, and inability to undergo PET/CT scanning. Patients were not approached if they fulfilled any of the exclusion criteria or if their clinician believed that participation was not appropriate. Of the patients approached, 52% agreed to take part in the trial. These patients were then compared with age- and sex-matched control subjects with a normal aortic valve and a similar range of comorbidity. The study was conducted with local research ethics committee approval and the written informed consent of all patients.

**Echocardiography**
Valve disease severity was assessed by a single dedicated research ultrasonographer on a dedicated machine (Phillips Medical Systems, Best, Netherlands) under standardized conditions and according to a formal protocol. Patients were studied with the use of an S51 pure wave transducer (Phillips Medical Systems) for 2-dimensional, M-mode, and pulsed and continuous wave Doppler studies. Continuous wave Doppler velocities were confirmed with the use of a D2 CW transducer (Phillips Medical Systems) from the apex, right sternal edge, and suprasternal notch. Measurements were determined online and averaged from 3 cardiac cycles or 5 if the patient was in atrial fibrillation.

Aortic sclerosis was defined as a thickened aortic valve on echocardiography in the absence of accelerated flow though the valve (peak jet velocity <2 m/s). The severity of aortic stenosis was graded according to American Heart Association and American College of Cardiology criteria with the use of peak transvalvular aortic valve velocity and mean and maximum aortic valve pressure gradients. In our clinical laboratory, we previously demonstrated a coefficient of reproducibility of 0.32 m/s for the Doppler measurement of peak aortic valve velocity in patients with aortic stenosis.

Aortic stenosis severity was also assessed with the use of the time velocity integral, the dimensionless index, and the aortic valve area, calculated with the continuity equation.

**PET and CT**
Combined PET/CT scans of the aortic valve were performed on 2 occasions in close succession with the use of a hybrid scanner (Biograph mCT, Siemens Medical Systems, Erlangen, Germany). On the first occasion, a target dose of 125 MBq 18F-NaF was injected intravenously, and patients rested in a quiet environment for the 60-minute uptake period. An attenuation-correction CT scan (nonenhanced, low dose 120 kV, 50 mAs) was then performed, followed by PET imaging covering 2 bed positions centered over the valve in 3-dimensional mode for 10 minutes. On the second occasion, a target dose of 200 MBq 18F-FDG was injected, and patients rested in a quiet environment for 90 minutes. Combined PET/CT imaging was then performed as described for the 18F-NaF scan but with the use of a 15-minute bed time. Trajectory and circulation times were based on previous studies with the use of 18F-FDG and 18F-NaF in atherosclerosis and aimed to allow for optimal contrast between the aortic wall, aortic valve, and blood pool. An ECG-gated breath-hold CT scan (nonenhanced, 40 mAs per rotation [True Dose], 100 kV) was performed of the aortic valve immediately after the 18F-NaF PET/CT scan for calculation of the aortic valve calcium score.

The PET data were reconstructed with the use of the Siemens Ultra-HD (time of flight + True X) reconstruction algorithm. Corrections were applied for attenuation, dead time, scatter, and random coincidences. All image analysis was performed on fused PET/CT data sets.

**Dietary Restrictions**
Intense uptake of 18F-FDG by the left ventricle leads to difficulties in discriminating between activity in the aortic valve and the myocardium. All patients in our cohort were asked to observe a carbohydrate-free diet for 24 hours before their 18F-FDG scan because this suppresses myocardial uptake as the heart switches from glucose to free fatty acid metabolism. Patients were provided with a list of food and drink to avoid and reminded of these restrictions the day before their scan. Dietary diaries were recorded, and patients were categorized into dietary compliance or noncompliance. Myocardial tracer uptake was assessed by recording the maximum standardized uptake value (SUV) in the left ventricular septal myocardium. The SUV is the decay-corrected tissue uptake divided by the injected dose per body weight and is a semiquantitative dimensionless unit that is widely used and validated measure of tissue 18F-FDG and 18F-NaF uptake. High myocardial 18F-FDG uptake was prespecified as an SUV value ≥5.0, whereas low uptake, indicating successful myocardial suppression, was defined by measurements <5.0.

**Quantification of Tracer Uptake in the Aortic Valve**
PET image quantification is usually performed in the axial, coronal, or sagittal planes. However, the aortic valve is a complex 3-dimensional structure that does not align perfectly with any of these orthogonal planes, making accurate identification of the boundaries of the valve difficult with the use of standard techniques. To try to overcome this, the PET and CT images were fused and analyzed with the use of a workstation (Osirix version 3.5.1 64-bit; Osirix Imaging Software, Geneva, Switzerland) that allows for rotation of the plane of view into the true axis of the valve. This facilitated the more accurate delineation of regions of interest around the valve, as described above.
18F-NaF: Short-Axis Method

The fused PET/CT image was rotated in a 3-dimensional multiplanar reconstruction mode to provide a coaxial short-axis view of the aortic valve (Figure 1). Starting superiorly, we drew a circular region of interest around the aortic valve on 3-mm slices guided by anatomic information provided by CT and any obvious valvular calcification (Figure 1C). Further regions of interest were drawn on adjacent slices until the whole valve had been examined. Mean and maximum SUVs were calculated for each slice and then for the valve as a whole after these values were averaged. However, SUV measurements in vascular structures are influenced by variation in 18F-FDG and 18F-NaF activity in the blood pool. Therefore, SUV measurements were divided by an averaged mean SUV value derived from 5 circular regions of interest drawn in the central blood pool of the superior vena cava. This provided mean and maximum tissue-to-background ratios (TBRs).5,17

18F-FDG Analysis

The coaxial short-axis method was performed for 18F-FDG as described above. Whereas the prescan dietary restrictions sought to minimize the difficulties cause by myocardial 18F-FDG uptake, we also explored 2 additional image analysis approaches to define better and to assess more specifically the valvular uptake (Figure 1). In the long-axis technique, images were reoriented into a modified coronal view, from which it was hoped the boundaries of myocardial uptake would be more clearly observed and therefore avoided. In the center valve technique, regions of interest were again drawn in the center of the valve well away from myocardial uptake around the periphery. Purple borders indicate images taken in the coaxial short-axis view of the valve. Blue borders indicate images taken in the modified coronal view. Yellow borders indicate images taken in the modified sagittal view of the valve.

Repeatability Studies

Twenty-five patients with a range of aortic valve disease were selected at random from the cohort. After establishment of the aortic valve image analysis methodology, all scans from these patients were analyzed independently by 2 trained observers (M.R.D., C.J.). For each technique, this provided measures of interobserver repeat-
ability for mean and maximum SUV and TBR values. To assess intraobserver variation, both observers repeated the analyses at least 2 weeks later to minimize recall bias.

Statistical Methods

Continuous variables were expressed as mean±SD and compared with the unpaired Student t test or 1-way ANOVA when appropriate. All continuous variables were tested for normal distribution with the Shapiro-Wilk test. In cases in which data were not normally distributed, they were presented as the median (interquartile range). Categorical variables were expressed as percentages and analyzed with the χ² test. Correlations between normally distributed data were performed with Pearson correlation and presented as r values. Spearman correlation was used for nonparametric data. The 95% normal range for differences between sets of SUV and TBR measurements (the limits of agreement) were estimated by multiplying the SD of the mean of the differences by 1.96.19 Intraclass correlation coefficients with 95% confidence intervals were calculated for intraobserver and interobserver variation. Statistical analysis was performed with the use of SPSS version 18 (SPSS Inc, Chicago, IL). A 2-sided P<0.05 was regarded as statistically significant.

Table 1. Baseline Subject Characteristics

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<th></th>
<th>Control</th>
<th>Aortic Sclerosis</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>P</th>
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<td>20</td>
<td>25</td>
<td>33</td>
<td>23</td>
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<td>Age, y</td>
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<td>71±9</td>
<td>73±8</td>
<td>72±7</td>
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<td>Male sex, %</td>
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<td>75</td>
<td>60</td>
<td>76</td>
<td>65</td>
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<td>Body mass index, kg/m²</td>
<td>26±3</td>
<td>29±6</td>
<td>27±3</td>
<td>29±5</td>
<td>28±4</td>
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<tr>
<td>Ischemic heart disease, %</td>
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<td>48</td>
<td>36</td>
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<td>Cardiovascular disease, %</td>
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<td>45</td>
<td>48</td>
<td>39</td>
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<td>Smoking, %</td>
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<td>48</td>
<td>52</td>
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<td>Diabetes mellitus, %</td>
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<td>20</td>
<td>13</td>
<td>17</td>
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<td>Hypertension, %</td>
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<td>55</td>
<td>64</td>
<td>73</td>
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<td>Chronic kidney disease stage ≥3, %</td>
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<td>20</td>
<td>20</td>
<td>24</td>
<td>30</td>
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<td>Creatinine, mg/dL</td>
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<td>0.97±0.32</td>
<td>1.05±0.26</td>
<td>1.09±0.41</td>
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<td>21.0±4.6</td>
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<td>Calcium, mg/dL</td>
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<td>9.2±0.5</td>
<td>9.3±0.4</td>
<td>9.5±0.9</td>
<td>0.228</td>
</tr>
<tr>
<td>Phosphate, mg/dL</td>
<td>3.7±0.5</td>
<td>3.6±0.6</td>
<td>3.6±0.5</td>
<td>3.6±1.5</td>
<td>3.6±0.4</td>
<td>0.973</td>
</tr>
<tr>
<td>Alkaline phosphatase, U/L</td>
<td>75±19</td>
<td>85±30</td>
<td>79±21</td>
<td>82±22</td>
<td>102±89</td>
<td>0.314</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>191±42</td>
<td>194±53</td>
<td>210±59</td>
<td>171±41</td>
<td>203±52</td>
<td>0.040*</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>98±44</td>
<td>101±41</td>
<td>121±47</td>
<td>89±37</td>
<td>119±48</td>
<td>0.036*</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td>58±15</td>
<td>60±35</td>
<td>57±19</td>
<td>49±11</td>
<td>49±13</td>
<td>0.165</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>63±35</td>
<td>72±33</td>
<td>69±41</td>
<td>77±52</td>
<td>82±45</td>
<td>0.613</td>
</tr>
<tr>
<td>Statin therapy, %</td>
<td>35</td>
<td>50</td>
<td>52</td>
<td>67</td>
<td>57</td>
<td>0.262</td>
</tr>
<tr>
<td>ACE inhibitor therapy, %</td>
<td>35</td>
<td>40</td>
<td>52</td>
<td>36</td>
<td>30</td>
<td>0.604</td>
</tr>
<tr>
<td>Peak aortic jet velocity, m/s</td>
<td>1.3±0.2</td>
<td>1.7±0.2</td>
<td>2.5±0.2</td>
<td>3.4±0.3</td>
<td>4.6±0.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Peak aortic valve gradient, mm Hg</td>
<td>7.1±2.2</td>
<td>11.1±2.6</td>
<td>25.5±4.6</td>
<td>46.2±7.7</td>
<td>84.3±24.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean aortic valve gradient, mm Hg</td>
<td>3.7±1.0</td>
<td>5.9±1.4</td>
<td>13.2±2.7</td>
<td>24.9±4.4</td>
<td>48.8±15.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time-velocity integral, m</td>
<td>0.29±0.05</td>
<td>0.38±0.05</td>
<td>0.58±0.07</td>
<td>0.81±0.10</td>
<td>1.13±0.19</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Aortic valve area, cm²</td>
<td>2.70±0.67</td>
<td>2.37±0.54</td>
<td>1.49±0.37</td>
<td>1.19±0.31</td>
<td>0.81±0.28</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Dimensionless index</td>
<td>0.73±0.11</td>
<td>0.62±0.11</td>
<td>0.44±0.99</td>
<td>0.31±0.06</td>
<td>0.24±0.07</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Agatston aortic valve calcium score, AU</td>
<td>1.6±0.38</td>
<td>343±377</td>
<td>702±485</td>
<td>2084±1324</td>
<td>3956±2300</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are mean±SD unless indicated otherwise. BUN indicates blood urea nitrogen; LDL, low-density lipoprotein; HDL, high-density lipoprotein; ACE, angiotensin-converting enzyme; and AU, Agatston unit.

*There was no correlation between peak aortic valve velocity and either serum total cholesterol (Pearson correlation, r²=0.000, P=0.976) or LDL cholesterol (r²=0.007, P=0.36) concentrations.

Results

Patient Population

A total of 121 patients were recruited (aged 72±8 years; 69% male; peak aortic valve velocity 2.8±1.2 m/s) and had both 18F-NaF (66±7 minutes after 124±10 MBq) and 18F-FDG (94±7 minutes after 197±14 MBq) scans of their aortic valve <1 month apart. This cohort comprised 20 control subjects, 20 patients with aortic sclerosis, and 25 patients with mild, 33 with moderate, and 23 with severe aortic stenosis. Patients were well matched for age, sex, and comorbidity (Table 1).

Dietary Restrictions and Blood Pool Uptake

Average myocardial SUV across the entire cohort was 4.6±3.6, and dietary restrictions effectively suppressed 18F-FDG myocardial uptake (SUV <5) in 67% of patients, similar to that observed in previous studies.20 On the basis of dietary diaries, 61% of patients complied with the dietary...
Reproducibility statistics reflected this and demonstrated that short- and long-axis techniques, particularly for the latter. Avoiding myocardial uptake was difficult with the use of the short-axis, center valve, and long-axis techniques is shown (95% confidence intervals in parentheses). The short-axis technique for 18F-NaF and the center valve technique for 18F-FDG display no fixed proportional bias with narrow limits of agreement.

### Repeatability Studies

#### 18F-Sodium Fluoride

Among the 25 patients selected (aged 74±10 years; 64% male; aortic valve peak velocity 3.8±1.1 m/s), aortic valve 18F-NaF uptake showed excellent interobserver repeatability for the mean and maximum SUV and TBR values. There were no fixed or proportional biases, and the majority of data fell within narrow limits of agreement: ±0.20 for mean and ±0.21 for maximum TBR measurements (Table 2 and Figure 2). Limits of agreement for intraobserver measurements were similarly good, and intraclass coefficients for interobserver and intraobserver repeatability were all >0.95, indicating excellent agreement (Table 3).

#### 18F-Fluorodeoxyglucose

Avoiding myocardial uptake was difficult with the use of the short- and long-axis techniques, particularly for the latter. Reproducibility statistics reflected this and demonstrated that the variability was much greater than for 18F-NaF. The interobserver limits of agreement for the short-axis technique were ±0.28 and ±0.72 for the mean and maximum TBR values, respectively, and were ±0.78 and ±1.18 with the long-axis approach (Table 2 and Figure 2). The intraclass coefficients for the short-axis technique were 0.76 and 0.59 for the mean and maximum TBR values, respectively, and were 0.39 and 0.52 with the long-axis approach (Table 3). Intraobserver repeatability measures were similarly poor.

The center valve analysis was more reproducible. There were no fixed or proportional biases in the differences between interobserver measurements, and the data fell within narrow limits of agreement: ±0.11 for mean and ±0.13 for maximum TBR values (Table 2 and Figure 2). Intraobserver repeatability was similarly good, and intraclass coefficients were all >0.90, indicating excellent agreement (Tables 2 and 3).

There were concerns that the center valve technique might underestimate 18F-FDG activity in the valve by excluding the valve ring. However, there was no difference between mean uptake values calculated by the center valve technique and the short-axis method (center valve TBR: 1.43±0.17; short

### Table 2. Interobserver and Intraobserver Repeatability Statistics for 18F-NaF and 18F-FDG Quantification in the Aortic Valve

<table>
<thead>
<tr>
<th></th>
<th>Mean Difference Between Aortic Valve SUV and TBR Measurements</th>
<th>Intraobserver</th>
<th>M.R.D.</th>
<th>C.J.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean SUV</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18F-NaF short axis</td>
<td>0.04 (−0.12 to 0.20)</td>
<td>0.02 (−0.10 to 0.14)</td>
<td>0.05 (−0.14 to 0.24)</td>
<td></td>
</tr>
<tr>
<td>18F-FDG short axis</td>
<td>0.02 (−0.18 to 0.20)</td>
<td>−0.01 (−0.10 to 0.12)</td>
<td>−0.02 (−0.19 to 0.15)</td>
<td></td>
</tr>
<tr>
<td>18F-FDG long axis</td>
<td>0.05 (−0.57 to 0.67)</td>
<td>0.05 (−0.37 to 0.47)</td>
<td>0.00 (−0.14 to 0.14)</td>
<td></td>
</tr>
<tr>
<td>18F-FDG center valve</td>
<td>0.01 (−0.05 to 0.07)</td>
<td>0.01 (−0.05 to 0.07)</td>
<td>−0.02 (−0.16 to 0.12)</td>
<td></td>
</tr>
<tr>
<td><strong>Maximum SUV</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18F-NaF short axis</td>
<td>0.02 (−0.16 to 0.20)</td>
<td>0.04 (−0.11 to 0.19)</td>
<td>0.00 (−0.19 to 0.19)</td>
<td></td>
</tr>
<tr>
<td>18F-FDG short axis</td>
<td>0.02 (−0.80 to 0.84)</td>
<td>−0.11 (−0.64 to 0.42)</td>
<td>−0.09 (−0.89 to 0.72)</td>
<td></td>
</tr>
<tr>
<td>18F-FDG long axis</td>
<td>−0.03 (−1.06 to 1.00)</td>
<td>0.12 (−0.44 to 0.68)</td>
<td>0.10 (−0.41 to 0.61)</td>
<td></td>
</tr>
<tr>
<td>18F-FDG center valve</td>
<td>0.03 (−0.07 to 0.13)</td>
<td>0.02 (−0.13 to 0.17)</td>
<td>−0.06 (−0.26 to 0.14)</td>
<td></td>
</tr>
<tr>
<td><strong>Mean TBR</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18F-NaF short axis</td>
<td>0.07 (−0.13 to 0.27)</td>
<td>−0.01 (−0.15 to 0.17)</td>
<td>0.05 (−0.17 to 0.27)</td>
<td></td>
</tr>
<tr>
<td>18F-FDG short axis</td>
<td>0.06 (−0.22 to 0.34)</td>
<td>0.00 (−0.15 to 0.15)</td>
<td>0.01 (−0.12 to 0.14)</td>
<td></td>
</tr>
<tr>
<td>18F-FDG long axis</td>
<td>0.06 (−0.72 to 0.84)</td>
<td>0.07 (−0.50 to 0.64)</td>
<td>0.02 (−0.22 to 0.26)</td>
<td></td>
</tr>
<tr>
<td>18F-FDG center valve</td>
<td>−0.01 (−0.12 to 0.10)</td>
<td>0.02 (−0.06 to 0.10)</td>
<td>−0.01 (−0.07 to 0.05)</td>
<td></td>
</tr>
<tr>
<td><strong>Maximum TBR</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18F-NaF short axis</td>
<td>0.02 (−0.19 to 0.23)</td>
<td>−0.04 (−0.16 to 0.24)</td>
<td>0.00 (−0.21 to 0.21)</td>
<td></td>
</tr>
<tr>
<td>18F-FDG short axis</td>
<td>0.06 (−0.66 to 0.78)</td>
<td>−0.09 (−0.39 to 0.57)</td>
<td>0.07 (−0.06 to 0.20)</td>
<td></td>
</tr>
<tr>
<td>18F-FDG long axis</td>
<td>−0.01 (−1.17 to 1.19)</td>
<td>0.12 (−0.50 to 0.74)</td>
<td>0.06 (−0.33 to 0.39)</td>
<td></td>
</tr>
<tr>
<td>18F-FDG center valve</td>
<td>−0.02 (−0.15 to 0.11)</td>
<td>0.02 (−0.11 to 0.15)</td>
<td>−0.05 (−0.14 to 0.04)</td>
<td></td>
</tr>
</tbody>
</table>

Mean difference between standard uptake value (SUV) and tissue-to-background ratio (TBR) measurements for 18F-sodium fluoride (18F-NaF) valve uptake with the use of the short-axis technique and 18F-fluorodeoxyglucose (18F-FDG) uptake with the use of the short-axis, center valve, and long-axis techniques is shown (95% confidence intervals in parentheses). The short-axis technique for 18F-NaF and the center valve technique for 18F-FDG display no fixed proportional bias with narrow limits of agreement.
axis: 1.47±0.45; \(P=0.473\)). Maximum TBR values were lower with the use of the former approach (center valve: 1.60±0.20; short axis: 1.80±0.45; \(P=0.041\)). However, this difference was no longer apparent when patients with high myocardial uptake (n=11) were excluded from the analysis (center valve: 1.56±0.18; short axis: 1.64±0.20; \(P=0.245\)), reflecting the wide limits of agreement (±1.05) for the short-axis technique when myocardial suppression was poor. By contrast, limits of agreement for the center valve technique were equally good in patients with low and high myocardial uptake (±0.13 versus ±0.14, respectively; \(P=0.919\)). Given this and other advantages, subsequent analysis of the entire cohort was performed with the center valve method for 18F-FDG.

**Aortic Valve Uptake**

**18F-Sodium Fluoride**

Focal 18F-NaF uptake was observed in the valves of patients with calcific aortic valve disease in areas overlying, adjacent to, and remote from existing calcification. Areas of established calcium were also observed frequently in the absence of increased 18F-NaF activity (Figure 3). Compared with control subjects, valvular 18F-NaF uptake was higher in patients with both aortic sclerosis (maximum TBR: 1.55±0.17 versus 1.92±0.31; \(P<0.001\)) and aortic stenosis (maximum TBR: 1.55±0.17 versus 2.87±0.82; \(P<0.001\)). The highest maximum TBR value in the control group was 1.97, which was used to divide patients with aortic valve disease into those with increased 18F-NaF uptake (>1.97).

**Figure 2.** Bland-Altman plots of interobserver variability. A, Maximum 18F-sodium fluoride (18F-NaF) tissue-to-background ratio (TBR) values with the use of the short-axis technique. The dark central line is the mean difference between the 2 analyses, which does not show any fixed or proportional biases. The light outer lines show the limits of agreement (mean of the differences ±1.96×SD), which are narrow. Maximum 18F-fluorodeoxyglucose (18F-FDG) values with the use of the short-axis (B), long-axis (C), and center valve techniques (D) are shown. Note the wide limits of agreement for the short-axis and long-axis 18F-FDG techniques but the excellent measures of repeatability for the center valve technique. Also note that there is a single outlier not plotted on the long-axis graph (C); the mean difference for this patient is given in parentheses.
and those without (≤1.97). Overall, 45% of patients with aortic sclerosis and 91% of those with aortic stenosis had increased uptake. The proportion of patients with increased activity rose sharply with increasing disease severity such that 100% of patients with severe disease had increased uptake (Table 4).

All measures of 18F-NaF uptake displayed a progressive rise with increasing aortic jet velocity (maximum TBR: $r^2=0.540$, $P<0.001$; Table 4 and Figure 4), the aortic valve calcium score ($r^2=0.641$, $P<0.001$), and other echocardiographic measures of aortic stenosis severity (time-velocity integral: $r^2=0.546$, $P<0.001$; aortic valve area: $r^2=0.387$, $P<0.001$; dimensionless index: $r^2=0.527$, $P<0.001$).

18F-Fluorodeoxyglucose

18F-FDG showed a more diffuse pattern of activity within the valve (Figure 3), and compared with control subjects, uptake was increased in patients with aortic sclerosis (maximum TBR: $r^2=0.218$, $P<0.001$; Table 4 and Figure 4), the aortic valve Agatston score ($r^2=0.138$, $P<0.001$), and other echocardiographic measures of aortic stenosis severity (time-velocity integral: $r^2=0.246$, $P<0.001$; aortic valve area: $r^2=0.184$, $P<0.001$; dimensionless index: $r^2=0.229$, $P<0.001$). These correlations were weaker and more modest than for 18F-NaF. A modest correlation was also observed between valvular 18F-NaF and 18F-FDG activities (maximum TBR: $r^2=0.174$, $P<0.001$).

Discussion

In this PET study, we have established the feasibility of evaluating 18F-NaF and 18F-FDG activity in patients with aortic stenosis. Moreover, we have demonstrated excellent repeatability for the quantification of these tracers in the valve as measures of calcification and inflammation, respectively. 18F-NaF and 18F-FDG activity was increased in patients with both aortic sclerosis and stenosis, displaying a
progressive rise in uptake with increasing disease severity. However, calcification rather than inflammation appears to be the predominant process affecting the valve, particularly in the latter stages of the disease, in which a more marked progression in 18F-NaF activity was observed that was disproportionate to 18F-FDG.

Valve calcification plays a key role in the development of aortic stenosis. Hydroxyapatite becomes deposited on a bonelike matrix containing collagen, osteopontin, and other bone matrix proteins to form nodules that progress until, by the end stage of the disease, lamellar bone, microfractures, and hemopoietic tissue can all be identified. This appears to occur as part of a highly regulated process, coordinated by increased osteoblast activity and the local production of osteopontin, osteocalcin, bone sialoproteins, and bone morphogenetic protein-2, all of which are more commonly associated with skeletal bone formation. Established aortic valve calcium can be measured accurately with the use of CT, but measurement of 18F-NaF uptake offers, for the first time, the possibility of detecting areas of developing calcification within the valve. In this study, 18F-NaF uptake was observed in regions adjacent to and remote from existing calcium, suggesting expansion of the calcific process to new areas of the valve. In addition, uptake was observed in regions

Figure 3. 18F-Sodium fluoride (18F-NaF) and 18F-fluorodeoxyglucose (18F-FDG) uptake in patients with aortic stenosis. 18F-NaF: Fused positron emission tomography/computed tomography scans demonstrating uptake of 18F-NaF on coaxial short-axis views of the aortic valve in patients with a normal aortic valve (A), aortic sclerosis (B), and mild (C), moderate (D), and severe aortic stenosis (E and F). White areas show regions of existing calcium, and yellow and red areas show areas of 18F-NaF uptake. Focal areas of uptake are observed in regions overlying existing calcium as well as in areas remote from it. Furthermore, areas of existing calcification are observed in the absence of overlying 18F-NaF uptake. Note the increased activity with increasing severity of valve disease. Regions of interest have been drawn around the periphery of the valve (white lines) with the use of the short-axis technique. 18F-FDG: Fused positron emission tomography/computed tomography scans demonstrating uptake of 18F-FDG on coaxial short-axis views of the aortic valve in patients with a normal aortic valve (G), mild aortic stenosis (H), and severe aortic stenosis (I). Patients all have excellent myocardial suppression, allowing uptake to be visualized in the patients with aortic valve disease. Regions of interest have been drawn with the use of both the short-axis and center valve techniques (green lines).
18F-NaF activity was increased in the valves of patients with aortic sclerosis and stenosis compared with control subjects and demonstrated a marked progressive rise with increasing disease severity accounting for $\sim 50\%$ of the variance associated with valve stenosis. Moreover, increased valvular 18F-NaF activity was observed in 45% of patients with aortic sclerosis, 91% of patients with aortic stenosis, and all patients with severe stenosis. Calcium accumulation is the predominant mechanism by which valve cusp rigidity increases and aortic stenosis advances. As such, this technique offers considerable promise as a biomarker of disease activity and as a means of predicting disease progression. Longitudinal studies are now required to determine whether calcification activity quantified by 18F-NaF uptake is an accurate predictor of disease progression and superior to baseline measures of valve severity and calcium scores. If confirmed, these studies would pave the way for mechanistic studies of medical interventions to interrupt progressive calcific disease with the use of 18F-NaF activity as a surrogate biomarker and end point.

In the early stages of aortic stenosis, endothelial damage secondary to mechanical stress and lipid deposition triggers an inflammatory response within the valve. This is characterized by increased macrophage$^{27}$ and T-cell activity within the valve leaflets and the expression of a range of proinflammatory cytokines including transforming growth factor-$\beta$, tumor necrosis factor-$\alpha$, and interleukin-1$\beta$. The inflammatory response is thought to trigger the fibrotic and calcific processes that subsequently drive valve orifice narrowing. Thus, identifying and quantifying valvular inflammation with 18F-FDG have the potential to be critical in the evaluation of aortic stenosis. In the present study, 18F-FDG uptake was higher in patients with aortic sclerosis and stenosis compared with control subjects, and activity again rose with increasing valve severity. However, this association was weaker and the increase in activity was more modest than for 18F-NaF. Indeed, increased valvular 18F-FDG activity was only observed in 20% of patients with aortic sclerosis, 35% of patients with aortic stenosis, and 52% of patients with severe stenosis. Although this may reflect the high cutoff used for increased activity or an insensitivity of 18F-FDG in detecting inflammation, these data suggest that calcification is the predominant pathogenic process in aortic stenosis and a better target for novel therapeutic strategies. It might also explain the disappointing results of statin therapy in this condition, which has consistently failed to modify vascular calcification even in the coronary circulation despite reducing systemic markers of inflammation.$^{11,30–32}$

### Table 4. Correlation Between Aortic Stenosis Severity and Radiotracer Uptake

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Aortic Sclerosis</th>
<th>Aortic Stenosis</th>
<th>Correlation With Peak Aortic Jet Velocity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mild</td>
<td>Moderate</td>
</tr>
<tr>
<td><strong>18F-NaF</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean SUV</td>
<td>1.20 (1.10–1.55)</td>
<td>1.35 (1.24–1.59)</td>
<td>1.59 (1.38–1.73)</td>
<td>1.82 (1.67–2.05)</td>
</tr>
<tr>
<td>Mean TBR</td>
<td>1.23 (1.20–1.36)</td>
<td>1.53 (1.34–1.59)</td>
<td>1.73 (1.45–1.92)</td>
<td>2.03 (1.71–2.28)</td>
</tr>
<tr>
<td>Maximum SUV</td>
<td>1.54 (1.33–1.86)</td>
<td>1.77 (1.58–2.09)</td>
<td>2.21 (1.84–2.45)</td>
<td>2.57 (2.27–2.99)</td>
</tr>
<tr>
<td>Maximum TBR</td>
<td>1.56 (1.41–1.64)</td>
<td>1.96 (1.63–2.11)</td>
<td>2.45 (1.94–2.71)</td>
<td>2.89 (2.31–3.24)</td>
</tr>
<tr>
<td>Patients with increased uptake, %</td>
<td>0</td>
<td>45</td>
<td>76</td>
<td>95</td>
</tr>
<tr>
<td><strong>18F-FDG</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean SUV</td>
<td>1.49 (1.33–1.56)</td>
<td>1.73 (1.46–1.88)</td>
<td>1.66 (1.53–1.88)</td>
<td>1.71 (1.61–1.91)</td>
</tr>
<tr>
<td>Mean TBR</td>
<td>1.18 (1.09–1.26)</td>
<td>1.35 (1.19–1.44)</td>
<td>1.29 (1.21–1.45)</td>
<td>1.33 (1.26–1.47)</td>
</tr>
<tr>
<td>Maximum SUV</td>
<td>1.62 (1.47–1.68)</td>
<td>1.91 (1.64–2.07)</td>
<td>1.85 (1.72–2.07)</td>
<td>1.95 (1.81–2.18)</td>
</tr>
<tr>
<td>Maximum TBR</td>
<td>1.27 (1.21–1.40)</td>
<td>1.47 (1.31–1.61)</td>
<td>1.44 (1.37–1.63)</td>
<td>1.58 (1.41–1.65)</td>
</tr>
<tr>
<td>Patients with increased uptake, %</td>
<td>0</td>
<td>20</td>
<td>24</td>
<td>30</td>
</tr>
</tbody>
</table>

Values are median ± interquartile range, with Pearson correlation values. 18F-NaF indicates 18F-sodium fluoride; 18F-FDG, 18F-fluorodeoxyglucose; SUV, standard uptake value; and TBR, tissue-to-background ratio.
A recent retrospective study described increased 18F-FDG uptake in the valves of 42 patients with cancer who were coincidentally found to have aortic stenosis. However, in contrast to our study, a reduction in activity was observed in patients with severe compared with moderate disease. This difference is likely to reflect the small number of subjects in the severe subgroup (n=8), the retrospective nature of the study analysis, and the confounding effects of coexistent malignancy. In contrast, we have prospectively recruited a larger, well-defined cohort of patients with aortic stenosis who are more likely to be representative of those seen in cardiology practice. Moreover, we studied patients following dietary restrictions to minimize the effects of myocardial uptake and spillover into the valve. Our data are in agreement with a modest yet sustained and progressive increase in inflammation even in those with advanced disease.

Study Limitations

With exclusion of part of the valve, it is possible that the center valve technique employed for 18F-FDG analysis may underestimate valvular inflammation. However, there were no differences in mean uptake values compared with the short-axis technique. This reflects the diffuse nature of 18F-FDG activity in stenotic valves and the equal distribution of lesions between the base of the valve leaflets (54%) and the mid portion and tips (46%). This study has not validated 18F-FDG and 18F-NaF activity against histological samples. Although the mechanism of uptake for both tracers has been investigated in other tissues, further work is required in the valve to address this issue (see the online-only Data Supplement).

Conclusion

The evaluation of aortic stenosis with the use of PET is feasible and highly reproducible, with 18F-FDG and particularly 18F-NaF holding considerable promise as novel biomarkers of disease activity. Both calcification and inflammation are increased in patients with aortic valve disease compared with control subjects, and the activity of both rises steadily with increasing disease severity. However, calcification appears to be the predominant pathological process, particularly in the latter stages of the disease, and would therefore appear to be a better target for future potential medical therapies.

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Disclosures

None.

References


**CLINICAL PERSPECTIVE**

Aortic stenosis is the most common form of valvular heart disease in the Western world and represents a major healthcare burden that is projected to increase with an aging population. However, there are currently no effective medical treatments or biomarkers of disease activity. The pathogenesis of aortic stenosis is incompletely understood, and defining the various stages of this process will be highly important to develop the therapies and biomarkers that are required. Positron emission tomography combined with computed tomography is a noninvasive imaging technique that allows the identification and quantification of specific pathological processes within small anatomic structures, such as the aortic valve. In this study, we sought to test the feasibility, repeatability, and validity of this technique in the evaluation of aortic valve stenosis. Positron emission tomography/computed tomography imaging of the aortic valve was performed to assess inflammation (18F-fluorodeoxyglucose) and active calcification (18F-sodium fluoride) of the valve leaflets. The positron emission tomography/computed tomography findings were compared in 121 patients with a full spectrum of disease severity. Our data have clearly established that this technique is both feasible and repeatable, indicating that these tracers may prove to be useful biomarkers of disease activity. Furthermore, we have demonstrated that 18F-fluorodeoxyglucose and 18F-sodium fluoride activity increase with progressive disease severity. However, uptake of 18F-sodium fluoride appears to predominate in both the early and latter stages of the disease. This may explain the disappointing effects of statin therapy in this condition and indicates that calcification might represent a better target for novel therapeutic interventions.