Disease Activity in Mitral Annular Calcification - a Multimodality Study

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**ORIGINAL ARTICLE**

**Disease Activity in Mitral Annular Calcification**
**A Multimodality Study**

**BACKGROUND:** Mitral annular calcification (MAC) is associated with cardiovascular events and mitral valve dysfunction. However, the underlying pathophysiology remains incompletely understood. In this prospective longitudinal study, we used a multimodality approach including positron emission tomography, computed tomography, and echocardiography to investigate the pathophysiology of MAC and assess factors associated with disease activity and progression.

**METHODS:** A total of 104 patients (age 72±8 years, 30% women) with calcific aortic valve disease, therefore predisposed to MAC, underwent 18F-sodium fluoride (calcification activity) and 18F-Fluorodeoxyglucose (inflammation activity) positron emission tomography, computed tomography calcium scoring, and echocardiography. Sixty patients underwent repeat computed tomography and echocardiography after 2 years.

**RESULTS:** MAC (mitral annular calcium score >0) was present in 35 (33.7%) patients who had increased 18F-fluoride (tissue-to-background ratio, 2.32 [95% CI, 1.81–3.27] versus 1.30 [1.22–1.49]; P<0.001) and 18F-Fluorodeoxyglucose activity (tissue-to-background ratio, 1.44 [1.37–1.58] versus 1.17 [1.12–1.24]; P<0.001) compared with patients without MAC. MAC activity (18F-fluoride uptake) was closely associated with the local calcium score and 18F-Fluorodeoxyglucose uptake, as well as female sex and renal function. Similarly, MAC progression was closely associated with local factors, in particular, baseline MAC. Traditional cardiovascular risk factors and calcification activity in bone or remote atherosclerotic areas were not associated with disease activity nor progression.

**CONCLUSIONS:** MAC is characterized by increased local calcification activity and inflammation. Baseline MAC burden was associated with disease activity and the rate of subsequent progression. This suggests a self-perpetuating cycle of calcification and inflammation that may be the target of future therapeutic interventions.
Mitrail annular calcification (MAC) is a common finding on cardiovascular imaging studies with an estimated prevalence ranging from 8% to 42% depending on age of the population studied and analysis method. Often associated with aortic, coronary artery, and aortic valve calcification (AVC), MAC has been linked to increased atherosclerotic burden, incident stroke, and cardiovascular mortality. Although MAC is associated with endothelial damage, lipid infiltration, and progressive valve calcification, the pathophysiology of MAC remains incompletely understood and medical therapies to halt its progression are lacking. MAC also has functional consequences, helping to drive progressive mitral stenosis and mitral regurgitation, the severe stages of which can only be remedied through surgical or, potentially, percutaneous intervention.

Several epidemiological studies have investigated risk factors for MAC, finding similar determinants as for calcific aortic valve disease, including age, obesity, smoking, and serum phosphate. Important differences have also been observed, with MAC showing female predominance and a stronger association with chronic kidney disease and dysregulated mineral metabolism. An association with low bone mineral density (BMD) has been suggested but remains unproven. Despite the various investigations into risk factors for MAC incidence and prevalence, no studies to date have evaluated risk factors governing disease activity.

In this clinical imaging study, our aim was to use a state-of-the-art multimodality imaging approach to investigate activity and inflammation to elucidate factors associated with disease prevalence, activity, and progression.
PET-CT Imaging
PET-CT scans of the heart and aorta were performed with a hybrid scanner (Biograph mCT, Siemens Medical Systems, Erlangen, Germany). Two scans were performed at least 24 hours apart, 60 minutes after administration of 

18F-fluoride 125 MBq and 90 minutes after 

18F-FDG 200 MBq. ECG-gating was not used, and all counts were used for analysis. All patients were asked to adhere to a carbohydrate-free diet for 24 hours preceding their 

18F-FDG scan to suppress myocardial uptake, as previously described. Patients were given a list of foods (high in fat and low in carbohydrate) to eat and also those to avoid. An ECG-gated breath-hold CT scan (noncontrast-enhanced, 40 mA/rot [CareDose], 100 kV) of the heart was performed for calcium scoring.

Image Analysis: CT
Mitrail annulus, aortic valve, coronary artery, and aortic CT calcium scores were determined using dedicated analysis software (VScore, Vital Images, Minnetonka, and OsiriX Lite version 8.5.1, OsiriX Imaging Software, Geneva, Switzerland). Agatston scores were calculated using a threshold of 130 Hounsfield units.21 MAC on (CT-MAC) was defined as calcium score >0 Agatson units (AU) in the mitral annulus.

Image Analysis: PET
Mitrail annular 

18F-fluoride and 

18F-FDG PET activity were quantified according to a standardized protocol using OsiriX. Regions of interest were drawn around maximal areas of 

18F-fluoride and 

18F-FDG activity to obtain the maximum standardized uptake values (SUVmax), which were divided by blood pool uptake values in the right atrium (2 cm² area) to obtain 

TBRmax values. Given the difficulty in determining the exact borders of the mitral annulus, SUVmean values and TBRmean values were not quantified.

Uptake of 

18F-fluoride and 

18F-FDG in the aortic valve, aorta, and coronary arteries was measured as previously reported (Data Supplement).15 BMD and 

18F-fluoride bone uptake were measured in 4 thoracic vertebrae as detailed previously.18 Briefly, 0.5 cm² regions of interest were drawn within the cancellous bone. The average Hounsfield unit density within those regions was used as a relative measure of BMD.22 Maximum 

18F-fluoride SUV values were quantified in the same regions of interest. Myocardial 

18F-FDG uptake was assessed by recording the maximum SUV in the left ventricular septum. A diffuse pattern of myocardial 

18F-FDG uptake accompanied by SUV ≥5.0 indicated failed myocardial suppression.15 Patients with failed suppression were excluded from the analysis of FDG data, but not from analysis of 

18F-fluoride data.

Repeatability Studies
All CT and PET quantifications were independently performed in a blinded fashion by 2 trained observers (M.G. Trivieri and D. Massera). Disagreements were resolved by consensus with involvement of a third observer (R. Abgral).

Image Analysis: Echocardiography
Examination of the mitral valve apparatus was performed in a blinded fashion by one cardiologist (J. Andrews). At least 3 diastolic transmural continuous-wave Doppler envelopes were traced to obtain an average diastolic transmural gradient. Mitrail regurgitation severity was assessed according to the American Society of Echocardiography guidelines. No adjustment for heart rate was performed because 87% of patients had a heart rate of <80 bpm.

Disease Progression Studies
A subset of study participants underwent repeat CT and echocardiography using the same protocol and equipment 2 years after initial imaging. Mitrail annular disease progression was assessed using the annualized change in CT calcium score and transmural pressure gradient.

Statistical Analysis
Continuous variables are reported as mean±SD or median (interquartile range [IQR]) and were compared with the unpaired Student t test, Wilcoxon rank-sum or Kruskal-Wallis tests, as appropriate. Categorical variables are reported as proportions and analyzed with the χ² or Fishers exact test. Correlations were calculated using Spearman correlation coefficients. Data are presented by presence or absence of CT-MAC or mitral annular 

18F-fluoride activity or were dichotomized at the median CT-MAC calcium score. Bland-Altman mean differences and limits of agreement were obtained. Intraclass correlation coefficients were calculated with 2-way mixed-effects models. Multivariable linear and logistic regression models were used to identify predictors of MAC prevalence and 

18F-fluoride activity. Logarithmic transformation of 

18F-fluoride uptake was performed to achieve a normal distribution. Initially, all variables with P<0.2 in bivariate comparisons were included in the model, as well as important cardiovascular risk factors (age, sex, hypertension, diabetes mellitus, smoking, low-density lipoprotein cholesterol, and prior cardiovascular disease). Subsequently, a backward stepwise selection process was used with age and sex forced into the model. Separately, 

18F-FDG TBRmean was added to the model to identify FDG as a predictor of 

18F-fluoride uptake. Multiple linear and multinomial logistic regression models were used to identify predictors of MAC progression. All analyses were performed with STATA 14.2 (StataCorp LP, College Station, TX). A 2-tailed P<0.05 was used to define statistical significance.

RESULTS
Patient Population
The study cohort comprised 104 patients (mean age 72±8 years, 30% women; baseline characteristics are presented in Tables 1 and 2). The median transmural mean diastolic pressure gradient was 1.4 (IQR, 1.0–2.1) mm Hg (Data Supplement). In addition, a control cohort of 17 subjects without heart valve calcification was included (68±8 years; Data Supplement). The effective radiation dose per patient was 9.7±1.2 mSv (CT conversion factor 0.014 mSv/mGy/cm). Interobserver reproducibility for MAC-CT calcium scoring (intraclass coefficient, 1.00 [95% CI, 0.99–1.00]) and PET quantifi-
Massera et al; Mitral Annular Calcification PET-CT

Factors Associated with MAC Prevalence

The median baseline MAC-CT calcium score was 0 (IQR, 0–316) AU and was higher in women (283 [0–1082] AU) compared with men (0 [0–0] AU; P = 0.001). Overall, 35 (33.7%) patients had MAC on CT (CT+; 837 [300–2129] AU), who were older, twice as likely to be female, had more AVC, lower BMD, and reduced estimated glomerular filtration rate (eGFR) compared with patients without MAC (CT−). Both groups had extensive cardiovascular disease risk factor burden (Table 1). In a multiple logistic regression model, female sex and AVC calcium score were statistically significantly associated with MAC prevalence (Table 3).

Mitral Annular Inflammatory Activity (18F-FDG PET)

Thirty-three patients (32%) met criteria for failed myocardial suppression of physiological 18F-FDG uptake and were excluded from further analysis of FDG data only. In the remaining patients, median mitral annular 18F-FDG TBRmax was 1.21 (IQR, 1.14–1.39), higher in patients with CT-MAC (CT+ 1.44 [1.37–1.58]) compared with those without (CT− 1.17 [1.12–1.24]; P<0.001) or with controls (1.06 [1.04–1.17]; P<0.001). A moderate correlation was observed between mitral annular 18F-FDG TBRmax and CT-MAC scores (r = 0.50, P<0.001; Table 4).

MAC Activity (18F-Fluoride PET)

Median mitral annular 18F-Fluoride TBRmax uptake in the entire study cohort (104 patients) was 1.44 (IQR, 1.27–1.89). Patients with CT-MAC had higher 18F-Fluoride uptake (CT+ 2.32 [1.81–3.27]) than those without (CT− 1.30 [1.22–1.49]; P<0.001). Mitral annular 18F-Fluoride activity appeared most closely related to local markers of disease burden. A strong correlation was observed

Table 1. Baseline Characteristics by Presence of Mitral Annular Calcification (Prevalence) and Mitral Annular 18F-Fluoride Uptake (Disease Activity)

<table>
<thead>
<tr>
<th>Baseline Characteristics</th>
<th>MAC−, (n=69)</th>
<th>MAC+, (n=35)</th>
<th>P Value</th>
<th>18F-Fluoride−, (n=66)</th>
<th>18F-Fluoride+, (n=36)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>70.6±7.9</td>
<td>75.1±8.2</td>
<td>0.011</td>
<td>70.8±7.9</td>
<td>74.8±8.7</td>
<td>0.026</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>14 (20.3)</td>
<td>17 (48.6)</td>
<td>0.003</td>
<td>12 (18.2)</td>
<td>19 (52.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>27.6±4.2</td>
<td>28.7±4.9</td>
<td>0.276</td>
<td>27.4±3.9</td>
<td>28.9±5.0</td>
<td>0.093</td>
</tr>
<tr>
<td>Ischemic heart disease, n (%)</td>
<td>27 (39.1)</td>
<td>11 (31.4)</td>
<td>0.441</td>
<td>28 (42.4)</td>
<td>9 (25.0)</td>
<td>0.080</td>
</tr>
<tr>
<td>Cardiovascular disease, n (%)</td>
<td>31 (44.9)</td>
<td>11 (31.4)</td>
<td>0.185</td>
<td>32 (48.9)</td>
<td>9 (25.0)</td>
<td>0.021</td>
</tr>
<tr>
<td>Current smoking, n (%)</td>
<td>8 (11.6)</td>
<td>4 (11.4)</td>
<td>0.980</td>
<td>7 (10.6)</td>
<td>4 (11.1)</td>
<td>0.937</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>9 (13.2)</td>
<td>6 (17.1)</td>
<td>0.594</td>
<td>9 (13.9)</td>
<td>6 (16.7)</td>
<td>0.703</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>41 (59.4)</td>
<td>23 (65.7)</td>
<td>0.533</td>
<td>39 (59.1)</td>
<td>23 (63.9)</td>
<td>0.635</td>
</tr>
<tr>
<td>Osteoporosis, n (%)</td>
<td>2 (2.9)</td>
<td>0 (0)</td>
<td>0.309</td>
<td>2 (3.0)</td>
<td>0 (0)</td>
<td>0.539</td>
</tr>
<tr>
<td>Bone mineral density (mean, HU)</td>
<td>160.6±43.2</td>
<td>142.1±38.5</td>
<td>0.035</td>
<td>159.7±41.0</td>
<td>144.8±43.6</td>
<td>0.096</td>
</tr>
<tr>
<td>eGFR, ml/min/1.73m²</td>
<td>74.5±17.8</td>
<td>63.5±18.9</td>
<td>0.004</td>
<td>73.0±17.6</td>
<td>67.0±20.1</td>
<td>0.121</td>
</tr>
<tr>
<td>Urea, mg/dL</td>
<td>20.0±7.1</td>
<td>22.3±7.7</td>
<td>0.159</td>
<td>20.0±5.5</td>
<td>22.4±9.8</td>
<td>0.187</td>
</tr>
<tr>
<td>Calcium, mg/dL</td>
<td>9.3±0.7</td>
<td>9.4±0.3</td>
<td>0.119</td>
<td>9.2±0.5</td>
<td>9.5±0.7</td>
<td>0.047</td>
</tr>
<tr>
<td>Phosphate, mg/dL</td>
<td>3.6±1.1</td>
<td>3.5±0.5</td>
<td>0.606</td>
<td>3.5±0.5</td>
<td>3.7±1.4</td>
<td>0.411</td>
</tr>
<tr>
<td>Alkaline phosphatase, U/dL</td>
<td>78.7±20.2</td>
<td>99.1±74.9</td>
<td>0.133</td>
<td>80.2±22.9</td>
<td>95.6±74.3</td>
<td>0.255</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>195.6±52.6</td>
<td>183.8±51.5</td>
<td>0.280</td>
<td>190.2±50.1</td>
<td>193.2±56.2</td>
<td>0.781</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td>107.4±44.4</td>
<td>101.0±46.2</td>
<td>0.511</td>
<td>101.1±41.2</td>
<td>110.6±49.2</td>
<td>0.307</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>55.4±23.2</td>
<td>50.4±12.0</td>
<td>0.146</td>
<td>55.8±23.4</td>
<td>50.5±12.4</td>
<td>0.133</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>75.7±47.2</td>
<td>70.7±37.1</td>
<td>0.554</td>
<td>76.0±46.6</td>
<td>71.5±39.6</td>
<td>0.621</td>
</tr>
<tr>
<td>Lipoprotein(a), mg/dL</td>
<td>18.6 (8.9–62.9)</td>
<td>18.1 (9.0–54.9)</td>
<td>0.845</td>
<td>17.6 (8.3–67.4)</td>
<td>20.5 (9.0–55.6)</td>
<td>0.660</td>
</tr>
<tr>
<td>Statin therapy, n (%)</td>
<td>39 (56.5)</td>
<td>52 (60.0)</td>
<td>0.734</td>
<td>40 (60.6)</td>
<td>18 (50.0)</td>
<td>0.301</td>
</tr>
<tr>
<td>ACE inhibitor therapy, n (%)</td>
<td>27 (39.1)</td>
<td>14 (40.0)</td>
<td>0.932</td>
<td>26 (39.4)</td>
<td>14 (38.9)</td>
<td>0.960</td>
</tr>
</tbody>
</table>

Continuous variables are presented as means±SD or median (IQR). eGFR indicates estimated glomerular filtration rate (CKD-EPI); HDL, high-density lipoprotein; LDL, low-density lipoprotein; and MAC, mitral annular calcification.

Table 4. Correlation of Mitral Annular 18F-FDG TBRmax and Baseline Characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mitral Annular 18F-FDG TBRmax</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>0.139</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>-0.261</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>-0.180</td>
</tr>
<tr>
<td>Ischemic heart disease, n (%)</td>
<td>0.241</td>
</tr>
<tr>
<td>Cardiovascular disease, n (%)</td>
<td>0.314</td>
</tr>
<tr>
<td>Current smoking, n (%)</td>
<td>0.194</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>0.214</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>0.224</td>
</tr>
<tr>
<td>Osteoporosis, n (%)</td>
<td>0.184</td>
</tr>
<tr>
<td>Bone mineral density (mean, HU)</td>
<td>0.154</td>
</tr>
<tr>
<td>eGFR, ml/min/1.73m²</td>
<td>0.174</td>
</tr>
<tr>
<td>Urea, mg/dL</td>
<td>0.164</td>
</tr>
<tr>
<td>Calcium, mg/dL</td>
<td>0.154</td>
</tr>
<tr>
<td>Phosphate, mg/dL</td>
<td>0.144</td>
</tr>
<tr>
<td>Alkaline phosphatase, U/dL</td>
<td>0.134</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>0.124</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td>0.114</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>0.104</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>0.094</td>
</tr>
<tr>
<td>Lipoprotein(a), mg/dL</td>
<td>0.084</td>
</tr>
<tr>
<td>Statin therapy, n (%)</td>
<td>0.074</td>
</tr>
<tr>
<td>ACE inhibitor therapy, n (%)</td>
<td>0.064</td>
</tr>
</tbody>
</table>

The remaining patients, median mitral annular 18F-Fluoride TBRmax was 0.87 [0.82–0.91]) was good (Data Supplement).

33.7% patients had MAC on CT (CT+; 837 [300–2129] AU), who were older, twice as likely to be female, had more AVC, lower BMD, and reduced estimated glomerular filtration rate (eGFR) compared with patients without MAC (CT−). Both groups had extensive cardiovascular disease risk factor burden (Table 1). In a multiple logistic regression model, female sex and AVC calcium score were statistically significantly associated with MAC prevalence (Table 3).
between mitral annular 18F-fluoride activity and baseline CT-MAC score (r=0.79, P<0.001; Figure 1A) while a moderate correlation was observed with 18F-FDG uptake (r=0.32, P=0.001; Figure 1B). By comparison, modest or no correlations were observed between mitral annular 18F-fluoride uptake and uptake in other areas (aorta, r=0.23, P=0.025; aortic valve, r=0.19, P=0.053; coronary arteries, r=0.14, P=0.159; and bone, r=0.02, P=0.861) or serum biomarkers including calcium, alkaline phosphatase, and lipid markers (Table 3). Mitral annular 18F-fluoride uptake was higher in women compared with men (2.01 [1.31–2.69] versus 1.26 [1.26–1.63]; P=0.002), and in patients with impaired eGFR (<60 mL/min/1.73m²) compared with preserved renal function (1.39 [1.10–1.61] versus 1.26 [1.04–1.36]; P=0.046).

Among the control cohort, the highest 18F-fluoride TBRmax value was 1.64. This cutoff was used to categorize patients in the study cohort as having increased 18F-fluoride uptake (>1.64, PET+) or not (≤1.64, PET−). Overall, 36 (35.6%) patients had increased 18F-fluoride uptake (median TBRmax, 2.30 [1.84–3.07]). PET+ patients had a median CT-MAC calcium score of 834 (139–2107), while PET− patients had no MAC (Figure 1C). Compared with PET− patients, PET+ patients were older, more likely to be female, had more AVC, lower BMD, and eGFR (Table 1). In a multiple linear regression model, CT-MAC and AVC calcium scores, female sex, and eGFR demonstrated a statistically significant association with MAC disease activity. When 18F-FDG TBRmax was added to the model, significant predictors of MAC 18F-fluoride activity were baseline CT-MAC and 18F-FDG TBRmax in the subset of patients with successful myocardial suppression (Table 5).

### Disease Progression in Mitral Annular Calcification

Sixty patients in the study cohort underwent repeat echocardiography and CT after a median of 741 (IQR, 726–751) days (Figure 2 includes examples of 3 patients). The annual progression rate of CT-MAC calcium score was 2 (0–166) AU per year. The strongest associations of MAC progression were observed with baseline CT-MAC (r=0.82, P<0.001; Figure 3A), 18F-fluoride (r=0.75, P<0.001; Figure 3B) and 18F-FDG activity (r=0.48; P<0.002). Women tended to have a higher rate of MAC progression (34 [0–409] AU/y) than men (0 [0–68] AU/y; P=0.083). There was no association between baseline eGFR and MAC progression (r=−0.13; P=0.308) nor differences in the rate of MAC progression between those with and without advanced disease.
chronic kidney disease ($P=0.933$). There were no asso-
ciations with MAC progression for low-density lipopro-
tein ($r=−0.10; P=0.444$), HDL (high-density lipoprotein; $r=−0.08, P=0.524$) or lipoprotein(a) ($r=0.07, P=0.629$).

All 22 (36.7%) patients with baseline CT-MAC (CT+) demonstrated progression in their CT-MAC scores (me-
dian progression rate 199 [63–480] AU/y). Eight (21.1%) of the 38 patients without baseline CT-MAC (CT−) de-
veloped new MAC (CT-MAC score at second exam 135 [40–291] AU). MAC regression was not observed. In a
multiple linear regression model, baseline CT-MAC cal-
cium score ($β=0.048$ per 100 AU; $P=0.013$) was an in-
dependent predictor of log-transformed MAC progression
after adjustment for age ($β=0.008$ per year; $P=0.847$),
sex ($β=−0.580; P=0.368$) and eGFR ($β=−0.063$ per 10
mL/min; $P=0.718$).

Patients with increased mitral annular $^{18}$F-fluoride PET uptake demonstrated faster progression than patients
without (CT-MAC progression: PET+ 200 [47–480] ver-
sus PET− 0 [0–3] AU/y; $P<0.001$). In multinomial logistic
regression models adjusted for age and sex, there was a
stronger association of positive $^{18}$F-fluoride PET uptake
(PET+) with a MAC progression rate above median (OR,
100.03; 95% CI 10.88–919.62; $P<0.001$), than below
median (OR, 17.25; 95% CI 2.76–107.92; $P=0.002$). Similar results were obtained with $^{18}$F-fluoride uptake as continuous variable (MAC progression above me-
dian: OR, 1.95 per 0.1 increment in TBRmax; 95% CI
1.38–2.75, $P<0.001$; MAC progression below median: OR,
1.71; 95% CI 1.23–2.37; $P=0.001$).

When considering PET and CT data together,
PET−CT− patients did not demonstrate MAC progres-
sion (median MAC progression, 0 [0–0] AU/y, n=32),
while MAC progression was highest in PET+CT+ pa-
patients (270 [68–493] AU/y, n=18). Intermediate pro-
gression was observed in PET+CT− (47 [0–95] AU/y,
n=5) and PET−CT+ patients (102 [39–166] AU/y, n=4;
Figure 3C).

## DISCUSSION

We used state-of-the-art multimodality imaging to inves-
tigate MAC, providing novel insights into the pathophys-
ilogy of this common condition and factors associated
with its prevalence, disease activity, and progression. We
confirmed that MAC is characterized by both calcifica-
 tion and inflammatory activity that increases propor-
tionally to the baseline MAC burden. Importantly, while
female sex, renal dysfunction, and local inflammatory
activity were associated with MAC disease activity, the
strongest correlate was the local burden of calcium al-
ready present within the valve annulus. Similar observa-
tions were made with respect to progression, with the
fastest progression observed in patients with the largest
baseline burden of MAC. We, therefore, suggest that
once established, MAC activity and progression are char-
acterized by a vicious cycle of established calcium, injury,
and inflammation within the valve that prompts further
calcification activity. These findings support the concept
that therapeutic strategies targeting MAC will need fo-
cus on breaking this vicious calcification cycle.

Despite its high prevalence, contribution to mitral
valve dysfunction and adverse prognosis,$^4$ the patho-
biology of MAC remains incompletely understood.
Moreover, therapeutic options are limited since effec-
tive medical therapy is lacking and surgical intervention
is made complicated by its presence.$^24$ There is, there-
fore, an urgent need to illuminate the pathophysiology underlying MAC and to identify novel therapeutic strategies to prevent its clinical sequelae. We describe a new multimodality imaging approach to help address this need. First, we have applied CT calcium scoring to define the presence of MAC and to quantify disease prevalence, burden, and progression. Second, we used 18F-FDG to measure inflammatory activity. Although 18F-FDG was only interpretable in two-thirds of patients, our data clearly demonstrate that MAC is an inflammatory condition with the 18F-FDG PET signal increasing in proportion to baseline disease severity. Finally, we used 18F-fluoride PET as marker of calcification activity demonstrating a close association with subsequent progression and building upon a growing body of literature using 18F-fluoride to image developing cardiovascular microcalcification. The use of a cohort of patients with calcific aortic valve disease provided a patient population at high risk of developing MAC, as evidenced by the particularly high prevalence. This gave us the opportunity to assess disease activity and progression in patients with established MAC, but also in patients who subsequently developed MAC during follow-up. It also provided insights into why certain patients with aortic stenosis develop MAC, while others do not, with female sex, renal impairment, and advanced AVC appearing to be of particular importance in this population.

Factors Associated With Disease Activity in MAC

Using 18F-fluoride PET, we demonstrated that calcification activity in the mitral annulus is closely related to the local inflammatory signal provided by 18F-FDG imaging. This is consistent with histological studies of excised mitral valves demonstrating increased expression of pro-calcific cells and mediators adjacent to T-lymphocytic infiltrates and suggests that calcium deposition is closely related to inflammatory activity. However, MAC activity was, in fact, most closely associated with the baseline CT-MAC calcium score. Similar results were observed for progression: patients with rapid disease progression and highest disease activity were those with the highest baseline CT calcium scores. Indeed, baseline MAC was the strongest predictor of MAC progression, here replicating the findings from the Multiethnic Study of Atherosclerosis.

We believe our concordant data on MAC disease activity and progression have important therapeutic implications. The findings are remarkably similar to observations made in aortic stenosis, where it has been suggested that calcium within the valve increases mechanical stress and injury leading to inflammation and increased calcification activity. A similar self-perpetuating cycle of calcification inducing further calcification

![Figure 1. Relationship of mitral annular calcification (MAC) 18F-fluoride activity, MAC calcium score, and 18F-FDG activity.](http://ahajournals.org)

**Table 5. Factors Associated With Disease Activity in MAC**

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Model 1 (n=98)</th>
<th>Model 2 (n=68)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (per 10 y)</td>
<td>β Value</td>
<td>95% CI</td>
</tr>
<tr>
<td>Male sex</td>
<td>−0.002</td>
<td>−0.070 to 0.066</td>
</tr>
<tr>
<td>AVC (per 100 AU)</td>
<td>−0.172</td>
<td>−0.289 to −0.054</td>
</tr>
<tr>
<td>eGFR (per 10 mL/min)</td>
<td>0.003</td>
<td>−0.000 to 0.005</td>
</tr>
<tr>
<td>MAC (per 100 AU)</td>
<td>0.032</td>
<td>−0.061 to −0.003</td>
</tr>
<tr>
<td>18F-FDG TBRₘₐₓ (per 0.1)</td>
<td>0.014</td>
<td>0.011 to 0.018</td>
</tr>
</tbody>
</table>

Predictors of log-transformed 18F-fluoride TBRₘₐₓ in multiple linear regression model. Model 1 includes age, sex, hypertension, diabetes mellitus, smoking, LDL, prior cardiovascular disease, and variables with P>0.2 in bivariate comparisons, followed by backwards stepwise elimination process. Model 2 includes 18F-FDG TBRₘₐₓ in addition to variables in model 1. AVC indicates aortic valve calcification; 18F-FDG, 18F-Fluorodeoxyglucose; MAC, mitral annular calcification; and TBRₘₐₓ tissue-to-background ratio.
might also underlie MAC. The development of effective medical therapy in both conditions is, therefore, likely to require strategies that interrupt this cycle without impacting bone health. Studies are currently underway testing such therapies in patients with aortic stenosis (SALTIRE2, NCT02132026) providing an opportunity to investigate their impact on bystander MAC.

Study Limitations

Our study cohort comprised participants with calcific aortic valve disease. Although this ensured high proportions of prevalent and incident MAC, our results may not directly apply to patients with isolated mitral valve disease or other conditions known to be associated with MAC. Moreover, our sample size was modest, precluding more detailed examination of determinants and consequences of microcalcification and inflammation. In addition, one-third of patients met criteria for failed myocardial FDG suppression and were excluded from the analysis of FDG data. Further studies exploring the role of PET-CT in larger samples and different patient populations are warranted. Such studies may benefit from the use of contrast CT to better investigate the spatial distribution of PET uptake within the mitral annulus and to improve interobserver reproducibility. In addition, advanced imaging processing technologies such as adaptive thresholding may improve uptake delineation, and ECG-gating of the PET acquisition may reduce image blurring because of cardiac motion.

Figure 2. Baseline computed tomography mitral annular calcification (CT-MAC), 18F-fluoride positron emission tomography (PET) activity, and 2-year progression in 3 patients. 

First row, Mild MAC at baseline (A), associated with mild mitral annular 18F-fluoride uptake (B) and modest progression after 2 y (change in CT-MAC 69 AU (C)).

Second row, Moderate MAC at baseline (A), moderate 18F-fluoride uptake (B), and intermediate progression after 2 y (change in CT-MAC 2404 AU (C)).

Third row, Severe MAC at baseline (A), bifocal high-intensity 18F-fluoride uptake (B), and rapid progression (change in CT-MAC 9446 AU (C). Note de novo areas of MAC that developed at the site of intense 18F-fluoride uptake in the lateral annulus.
Conclusions
In this cohort, although female sex, renal dysfunction, and local inflammatory activity emerged as important determinants of disease activity in MAC, the strongest determinant was the baseline CT-MAC calcium score. Moreover, the higher the baseline burden of MAC, the higher the disease activity and the faster the rate of progression. This may reflect a vicious cycle of established calcium begetting further calcification within the mitral annulus that may be a suitable target of future therapies.

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Figure 3. Relationship of mitral annular calcification (MAC) progression with baseline MAC calcium score and 18F-fluoride activity. MAC progression (AU/y) increased with the burden of baseline MAC (box plots by categories of baseline CT-MAC calcium score: zero/ below median [≥837 AU] above median [≥837 AU]) (A) and was virtually absent in patients without 18F-fluoride activity (B). A steady increase in MAC progression was observed on moving from 18F-fluoride PET−CT− to PET−CT+, to PET+CT−, and finally to PET+CT+ patients (C). CT indicates computed tomography; and PET, positron emission tomography.


