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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.
Mitral 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.

Hybrid positron emission and computed tomography (PET-CT) allows for the simultaneous noninvasive evaluation of disease activity and heart valve anatomy. CT provides a detailed assessment of calcium burden, and PET can measure the activity of specific disease processes dependent on the availability of suitable tracers. 18F-Sodium fluoride (18F-fluoride) is a marker of vascular and valvular calcification activity that has been used to investigate vascular atherosclerosis and aortic stenosis. 18F-Fluorodeoxyglucose (18F-FDG) has been used to measure vascular inflammation because of its accumulation within tissue macrophages.

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 $^{18}$F-fluoride 125 MBq and 90 minutes after $^{18}$F-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 $^{18}$F-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
Mitral 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. MAC on (CT-MAC) was defined as calcium score ≥0 Agatston units (AU) in the mitral annulus.

Image Analysis: PET
Mitral annular $^{18}$F-fluoride and $^{18}$F-FDG PET activity were quantified according to a standardized protocol using OsiriX. Regions of interest were drawn around maximal areas of $^{18}$F-fluoride and $^{18}$F-FDG activity to obtain the maximum standardized uptake values ($SUV_{max}$), which were divided by blood pool uptake values in the right atrium (2 cm² area) to obtain $TBR_{max}$ values. Given the difficulty in determining the exact borders of the mitral annulus, $SUV_{mean}$ and $TBR_{mean}$ values were not quantified.

Uptake of $^{18}$F-fluoride and $^{18}$F-FDG in the aortic valve, aorta, and coronary arteries was measured as previously reported (Data Supplement). BMD and $^{18}$F-fluoride bone uptake were measured in 4 thoracic vertebrae as detailed previously. $^{18}$F-fluoride SUV values were quantified in the same regions of interest. Myocardial $^{18}$F-FDG uptake was assessed by recording the maximum SUV in the left ventricular septum. A diffuse pattern of myocardial $^{18}$F-FDG uptake accompanied by SUV $\geq$5.0 indicated failed myocardial suppression. Patients with failed suppression were excluded from the analysis of FDG data, but not from analysis of $^{18}$F-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 transmitral continuous-wave Doppler envelopes were traced to obtain an average diastolic transmural gradient. Mitral 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. Mitral annular disease progression was assessed using the annualized change in CT calcium score and transmirtal 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 $\chi^2$ or Fisher’s exact test. Correlations were calculated using Spearman correlation coefficients. Data are presented by presence or absence of CT-MAC or mitral annular $^{18}$F-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 $^{18}$F-fluoride activity. Logarithmic transformation of $^{18}$F-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, $^{18}$F-FDG TBR$_{mean}$ was added to the model to identify FDG as a predictor of $^{18}$F-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 transmirtal 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-
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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).

Mitral annular 18F-FDG TBRmax uptake was negatively correlated with total cholesterol and low-density lipoprotein (\( r = -0.30 ; P = 0.014 \)) and was higher in women (1.33 [1.16–1.45]) than men (1.19 [1.12–1.32]; \( P = 0.037 \)); there was no correlation with other serum biomarkers nor 18F-FDG activity measured in remote areas (aortic valve, \( r = -0.05 ; P = 0.658 \); aorta, \( r = -0.23 ; P = 0.060 \); 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/1.73m²²</td>
<td>74.5±17.8</td>
<td>63.5±18.9</td>
<td>0.004</td>
<td>70.3±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±8.8</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 mean±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.
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 1.36 [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

### Table 2. Imaging Characteristics by Presence of Mitral Annular Calcification (Prevalence) and Mitral Annular 18F-Fluoride Uptake (Disease Activity)

<table>
<thead>
<tr>
<th>Imaging 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>Control, n (%)</td>
<td>3 (4.4)</td>
<td>2 (5.7)</td>
<td>0.034</td>
<td>4 (6.1)</td>
<td>1 (2.8)</td>
<td>0.012</td>
</tr>
<tr>
<td>Sclerosis, n (%)</td>
<td>16 (23.3)</td>
<td>2 (5.7)</td>
<td></td>
<td>17 (25.8)</td>
<td>1 (2.8)</td>
<td></td>
</tr>
<tr>
<td>Mild stenosis, n (%)</td>
<td>20 (29.0)</td>
<td>5 (14.3)</td>
<td></td>
<td>17 (25.8)</td>
<td>7 (19.4)</td>
<td></td>
</tr>
<tr>
<td>Moderate stenosis, n (%)</td>
<td>18 (26.1)</td>
<td>15 (42.9)</td>
<td></td>
<td>18 (27.3)</td>
<td>15 (41.7)</td>
<td></td>
</tr>
<tr>
<td>Severe stenosis, n (%)</td>
<td>12 (17.4)</td>
<td>11 (31.4)</td>
<td></td>
<td>10 (15.2)</td>
<td>12 (33.3)</td>
<td></td>
</tr>
<tr>
<td>AVC calcium score, AU</td>
<td>801 (298–2174)</td>
<td>1501 (600–3314)</td>
<td>0.030</td>
<td>771 (309–2076)</td>
<td>1598 (1007–3230)</td>
<td>0.003</td>
</tr>
<tr>
<td>MAC calcium score, AU</td>
<td>0</td>
<td>837 (300–2129)</td>
<td>...</td>
<td>834 (139–2107)</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Aorta calcium score, AU</td>
<td>894 (190–2548)</td>
<td>1733 (396–7984)</td>
<td>0.058</td>
<td>997 (144–3181)</td>
<td>1378 (374–4036)</td>
<td>0.170</td>
</tr>
<tr>
<td>Aortic valve 18F-fluoride TBRmax</td>
<td>2.44 (1.91–2.99)</td>
<td>2.58 (2.21–3.14)</td>
<td>0.192</td>
<td>2.34 (1.96–2.91)</td>
<td>2.74 (2.38–3.18)</td>
<td>0.028</td>
</tr>
<tr>
<td>Mitral annulus 18F-fluoride TBRmax</td>
<td>1.30 (1.22–1.49)</td>
<td>2.32 (1.81–3.27)</td>
<td>&lt;0.001</td>
<td>1.29 (1.22–1.41)</td>
<td>2.30 (1.84–3.07)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Coronary artery 18F-fluoride TBRmax</td>
<td>1.50 (1.33–1.75)</td>
<td>1.60 (1.35–2.09)</td>
<td>0.274</td>
<td>1.50 (1.35–1.76)</td>
<td>1.59 (1.35–2.02)</td>
<td>0.590</td>
</tr>
<tr>
<td>18F-fluoride TBRmax, in aorta</td>
<td>2.06 (1.82–2.28)</td>
<td>2.14 (1.19–2.38)</td>
<td>0.081</td>
<td>2.05 (1.84–2.26)</td>
<td>2.20 (1.91–2.50)</td>
<td>0.060</td>
</tr>
<tr>
<td>Aortic valve 18F-FDG TBRmax</td>
<td>1.52 (1.44–1.63)</td>
<td>1.39 (1.33–1.63)</td>
<td>0.072</td>
<td>1.51 (1.40–1.63)</td>
<td>1.46 (1.35–1.68)</td>
<td>0.83</td>
</tr>
<tr>
<td>Mitral annulus 18F-FDG TBRmax</td>
<td>1.17 (1.12–1.24)</td>
<td>1.44 (1.37–1.58)</td>
<td>&lt;0.001</td>
<td>1.17 (1.12–1.26)</td>
<td>1.38 (1.24–1.56)</td>
<td>0.002</td>
</tr>
<tr>
<td>Aorta 18F-FDG TBRmax</td>
<td>1.84 (1.69–1.94)</td>
<td>1.68 (1.50–1.78)</td>
<td>0.002</td>
<td>1.83 (1.68–1.92)</td>
<td>1.69 (1.61–1.83)</td>
<td>0.116</td>
</tr>
</tbody>
</table>

Continuous variables are presented as median (IQR). AVC indicates aortic valve calcification; 18F-FDG, 18F-Fluorodeoxyglucose; MAC, mitral annular calcification; and TBRmax, tissue-to-background ratio.

* n=33 patient with failed myocardial FDG suppression were excluded.
chronic kidney disease \( (P=0.933) \). There were no associations with MAC progression for low-density lipoprotein \( (r=-0.10; P=0.444) \), 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 (median progression rate 199 [63–480] AU/y). Eight (21.1\%) of the 38 patients without baseline CT-MAC (CT−) developed 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 calcium score \( (\beta=0.048\text{ per 100\ AU}; P=0.013) \) was an independent predictor of log-transformed MAC progression after adjustment for age \( (\beta=0.008\text{ per year}; P=0.847) \), sex \( (\beta=-0.580; P=0.368) \) and eGFR \( (\beta=-0.063\text{ per 10}\ \text{mL/min}; P=0.718) \).

Patients with increased mitral annular \(^{18}\)F-fluoride PET uptake demonstrated faster progression than patients without (CT-MAC progression: PET+ 200 [47–480] AU vs. PET− 0 [0–3] AU/y; \( P<0.001) \). In multinominal 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 median: \( OR, 1.95\text{ per 0.1 increment in TBR}_{\text{max}}; 95\%\ CI 1.38–2.75, P<0.001\) ; 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 progression (median MAC progression, 0 [0–0] AU/y, n=32), while MAC progression was highest in PET+CT+ patients (270 [68–493] AU/y, n=18). Intermediate progression was observed in PET+CT− (47 [0–95] AU/y, n=18). When considering PET and CT data together, PET−CT− patients did not demonstrate MAC progression (median MAC progression, 0 [0–0] AU/y, n=32), while MAC progression was highest in PET+CT+ patients (270 [68–493] AU/y, n=18). Intermediate progression was observed in PET+CT− (47 [0–95] AU/y, n=18). When considering PET and CT data together, PET−CT− patients did not demonstrate MAC progression (median MAC progression, 0 [0–0] AU/y, n=32), while MAC progression was highest in PET+CT+ patients (270 [68–493] AU/y, n=18). Intermediate progression was observed in PET+CT− (47 [0–95] AU/y, n=18). When considering PET and CT data together, PET−CT− patients did not demonstrate MAC progression (median MAC progression, 0 [0–0] AU/y, n=32), while MAC progression was highest in PET+CT+ patients (270 [68–493] AU/y, n=18). Intermediate progression was observed in PET+CT− (47 [0–95] AU/y, n=18). When considering PET and CT data together, PET−CT− patients did not demonstrate MAC progression (median MAC progression, 0 [0–0] AU/y, n=32), while MAC progression was highest in PET+CT+ patients (270 [68–493] AU/y, n=18). Intermediate progression was observed in PET+CT− (47 [0–95] AU/y, n=18). When considering PET and CT data together, PET−CT− patients did not demonstrate MAC progression (median MAC progression, 0 [0–0] AU/y, n=32), while MAC progression was highest in PET+CT+ patients (270 [68–493] AU/y, n=18). Intermediate progression was observed in PET+CT− (47 [0–95] AU/y, n=18). When considering PET and CT data together, PET−CT− patients did not demonstrate MAC progression (median MAC progression, 0 [0–0] AU/y, n=32), while MAC progression was highest in PET+CT+ patients (270 [68–493] AU/y, n=18). Intermediate progression was observed in PET+CT− (47 [0–95] AU/y, n=18).

**DISCUSSION**

We used state-of-the-art multimodality imaging to investigate MAC, providing novel insights into the pathophysiology of this common condition and factors associated with its prevalence, disease activity, and progression. We confirmed that MAC is characterized by both calcification and inflammatory activity that increases proportionally 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 already present within the valve annulus. Similar observations 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 characterized 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 focus on breaking this vicious calcification cycle.

Despite its high prevalence, contribution to mitral valve dysfunction and adverse prognosis, the pathobiology of MAC remains incompletely understood. Moreover, therapeutic options are limited since effective medical therapy is lacking and surgical intervention is made complicated by its presence. There is, there-

}&
\text{Local factors} \\
& \text{Mitrail annulus CT calcium score} \\
& 0.78 <0.001 0.50 <0.001 \\
& \text{Mitrail annulus }^{18}\text{F-fluoride} \\
& ... ... 0.54 <0.001 \\
& \text{Mitrail annulus }^{18}\text{F-FDG} \\
& 0.54 <0.001 ... ... \\
\hline
\text{Remote factors} \\
& \text{Aortic valve CT calcium score} \\
& 0.24 0.017 0.15 0.218 \\
& \text{Aortic valve }^{18}\text{F-fluoride} \\
& 0.19 0.053 −0.02 0.848 \\
& \text{Aortic valve }^{18}\text{F-FDG} \\
& −0.02 0.895 −0.05 0.658 \\
& \text{Coronary artery CT calcium} \\
& 0.03 0.789 0.12 0.327 \\
& \text{Coronary artery }^{18}\text{F-fluoride} \\
& 0.14 0.159 0.08 0.518 \\
& \text{Aorta CT calcium score} \\
& 0.20 0.049 0.14 0.262 \\
& \text{Aorta }^{18}\text{F-fluoride} \\
& 0.23 0.025 −0.02 0.884 \\
& \text{Aorta }^{18}\text{F-FDG} \\
& −0.16 0.127 −0.23 0.060 \\
& \text{Bone mineral density} \\
& −0.19 0.065 −0.15 0.247 \\
& \text{Bone }^{18}\text{F-fluoride} \\
& 0.02 0.861 0.01 0.989 \\
\hline
\text{Serum biomarkers} \\
& \text{Calcium} \\
& 0.15 0.126 0.04 0.774 \\
& \text{Phosphate} \\
& −0.02 0.828 0.14 0.260 \\
& \text{Alkaline phosphatase} \\
& 0.11 0.264 −0.02 0.887 \\
& \text{Creatinine} \\
& 0.07 0.494 −0.02 0.848 \\
& \text{LDL cholesterol} \\
& −0.03 0.746 −0.30 0.014 \\
& \text{HDL cholesterol} \\
& −0.04 0.677 −0.01 0.919 \\
& \text{Total cholesterol} \\
& −0.07 0.500 −0.24 0.500 \\
& \text{Triglycerides} \\
& −0.07 0.484 0.00 0.992 \\
& \text{Lipoprotein(a)} \\
& 0.11 0.286 0.08 0.507 \\
\end{tabular}

Data presented in study cohort patients \( (n=104) \). In \(^{18}\)F-FDG analyses, patients with failed myocardial suppression were excluded \( (n=33) \). CT indicates computed tomography; \(^{18}\)F-FDG, \(^{18}\)F-fluorodeoxyglucose; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PET, positron emission tomography; and \( TBR_{\text{max}} \), tissue-to-background ratio.
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 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

Table 5. Factors Associated With Disease Activity in MAC

<table>
<thead>
<tr>
<th></th>
<th>Model 1 (n=98)</th>
<th></th>
<th>Model 2 (n=68)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β Value</td>
<td>95% CI</td>
<td>P Value</td>
<td>β Value</td>
</tr>
<tr>
<td>Age (per 10 y)</td>
<td>−0.002</td>
<td>−0.070 to 0.066</td>
<td>0.953</td>
<td>0.065</td>
</tr>
<tr>
<td>Male sex</td>
<td>−0.172</td>
<td>−0.289 to −0.054</td>
<td>0.005</td>
<td>−0.082</td>
</tr>
<tr>
<td>AVC (per 100 AU)</td>
<td>0.003</td>
<td>−0.000 to 0.005</td>
<td>0.052</td>
<td>0.002</td>
</tr>
<tr>
<td>eGFR (per 10 mL/min)</td>
<td>−0.032</td>
<td>−0.061 to −0.003</td>
<td>0.030</td>
<td>−0.001</td>
</tr>
<tr>
<td>MAC (per 100 AU)</td>
<td>0.014</td>
<td>0.011 to 0.018</td>
<td>&lt;0.001</td>
<td>0.010</td>
</tr>
<tr>
<td>¹⁸F-FDG TBRₘ₃₃ (per 0.1)</td>
<td>…</td>
<td>…</td>
<td>…</td>
<td>…</td>
</tr>
</tbody>
</table>

Predictors of log-transformed ¹⁸F-fluoride TBRₘ₃₃ 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 TBRₘ₃₃ in addition to variables in model 1. AVC indicates aortic valve calcification; ¹⁸F-FDG, ¹⁸F-Fluorodeoxyglucose; MAC, mitral annular calcification; and TBRₘ₃₃, 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 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.

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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Correspondence

Marc R. Dweck, MD, PhD, British Heart Foundation Centre for Cardiovascular Sciences, University of Edinburgh, 49 Little France Crescent, Edinburgh, EH164SB, United Kingdom. Email marc.dweck@ed.ac.uk

Affiliations

Leon H. Charney Division of Cardiology, New York University School of Medicine, New York, New York (D.M.); Department of Cardiology, Icahn School of Medicine at Mount Sinai, New York, NY (M.G.T., S.S.); British Heart Foundation Centre for Cardiovascular Science, University of Edinburgh, United Kingdom (J.P.M.A., A.R.C., W.S.A.J., A.T.V., T.A.P., D.E.N., M.R.D.); Department of Nuclear Medicine, University Hospital of Brest, France (R.A.); Department of Vascular Medicine, Academic Medical Center, Amsterdam, The Netherlands (K.H.Z.); and Cardiology Section, San Francisco Veterans Affairs Health Care System and Department of Epidemiology and Biostatistics, University of California, San Francisco, CA (J.R.K.).

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