Detection of Atherosclerotic Inflammation by $^{68}$Ga-DOTATATE PET Compared to $[^{18}$F$]$FDG PET Imaging

Jason M. Tarkin, MBBS,a Francis R. Joshi, MBBS, PhD,b Nicholas R. Evans, MBBS,c Mohammed M. Chowdhury, MBBS,b Nichola L. Figg, BSc,c Aarti V. Shah, PhD,d Laksh T. Starks, BSc,a Abel Martín-Garrido, PhD,a Roido Manavaki, PhD,e Emma Yu, MD, PhD,d Rhoda E. Kuc, BSc,f Luigi Grassi, PhD,g Roman Kreuzhuber, MSc,g Myrto A. Kostadima, PhD,g Mattia Frontini, PhD,g Peter J. Kirkpatrick, MD,h Patrick A. Coughlin, PhD,d Tim D. Fryer, PhD,d John R. Buscombe, MD,j Ashley M. Groves, MD,k Willem H. Ouweland, PhD,k, Martin R. Bennett, MD, PhD,a Elizabeth A. Warburton, DM,c Anthony P. Davenport, PhD,d James H.F. Rudd, MD, PhD,a

ABSTRACT

BACKGROUND Inflammation drives atherosclerotic plaque rupture. Although inflammation can be measured using fluorine-18-labeled fluorodeoxyglucose positron emission tomography ($[^{18}$F$]$FDG PET), $[^{18}$F$]$FDG lacks cell specificity, and coronary imaging is unreliable because of myocardial spillover.

OBJECTIVES This study tested the efficacy of gallium-68-labeled DOTATATE ($^{68}$Ga-DOTATATE), a somatostatin receptor subtype-2 (SST2)-binding PET tracer, for imaging atherosclerotic inflammation.

METHODS We confirmed $^{68}$Ga-DOTATATE binding in macrophages and excised carotid plaques. $^{68}$Ga-DOTATATE PET imaging was compared to $[^{18}$F$]$FDG PET imaging in 42 patients with atherosclerosis.

RESULTS Target SST2 gene expression occurred exclusively in “proinflammatory” M1 macrophages, specific $^{68}$Ga-DOTATATE ligand binding to SST2 receptors occurred in CD68-positive macrophage-rich carotid plaque regions, and carotid SST2 mRNA was highly correlated with in vivo $^{68}$Ga-DOTATATE PET signals ($r = 0.89; 95\%$ confidence interval [CI]: 0.28 to 0.99; $p = 0.02$). $^{68}$Ga-DOTATATE mean of maximum tissue-to-blood ratios (mTBRmax) correctly identified culprit versus nonculprit arteries in acute coronary syndrome (median difference: 0.69; interquartile range [IQR]: 0.22 to 1.15; $p = 0.008$) and transient ischemic attack/stroke (median difference: 0.13; IQR: 0.07 to 0.32; $p = 0.003$). $^{68}$Ga-DOTATATE mTBRmax predicted high-risk coronary computed tomography features (receiver operating characteristics area under the curve [ROC AUC]: 0.86; 95\% CI: 0.80 to 0.92; $p < 0.0001$), and correlated with Framingham risk score ($r = 0.52; 95\%$ CI: 0.32 to 0.69; $p < 0.0001$) and $[^{18}$F$]$FDG uptake ($r = 0.73; 95\%$ CI: 0.64 to 0.81; $p < 0.0001$). $[^{18}$F$]$FDG mTBRmax differentiated culprit from nonculprit carotid lesions (median difference: 0.12; IQR: 0.0 to 0.23; $p = 0.008$) and high-risk from lower-risk coronary arteries (ROC AUC: 0.76; 95\% CI: 0.62 to 0.91; $p = 0.002$); however, myocardial $[^{18}$F$]$FDG spillover rendered coronary $[^{18}$F$]$FDG scans uninterpretable in 27 patients (64\%). Coronary $^{68}$Ga-DOTATATE PET scans were readable in all patients.

CONCLUSIONS We validated $^{68}$Ga-DOTATATE PET as a novel marker of atherosclerotic inflammation and confirmed that $^{68}$Ga-DOTATATE offers superior coronary imaging, excellent macrophage specificity, and better power to discriminate high-risk versus low-risk coronary lesions than $[^{18}$F$]$FDG. (Vascular Inflammation Imaging Using Somatostatin Receptor Positron Emission Tomography [VISION]; NCT02021188) (J Am Coll Cardiol 2017;69:1774–91) © 2017 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).
Systemic inflammation triggers culprit pathogenic mechanisms, relating clinical cardiovascular disease (CVD) risk factors to atherosclerotic plaque progression and rupture (1). Quantifying vascular inflammation in atherosclerosis may reveal mechanistic pathways, allow efficacy testing of new drugs, and improve CVD risk prediction.

See Page 1792

Carotid, aortic, and peripheral arterial inflammation can be measured by fluorine-18-labeled fluorodeoxyglucose positron emission tomography/computed tomography ([18F]FDG PET/CT) (2). However, myocardial [18F]FDG signal spillover occurs due to myocardial muscle [18F]FDG uptake, often hampering coronary artery signal quantification (3). Lack of cell specificity and the influence of hypoxia on [18F]FDG uptake within macrophages and other cells (4) are further limitations of [18F]FDG imaging.

Up-regulation of the G-protein-coupled receptor somatostatin receptor subtype-2 (SST2) occurs on the surface of activated macrophages (5). Pre-clinical (6,7) and retrospective (8-10) studies suggest that gallium-68-labeled [1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid]-n-Phe1, Tyr3-octreotate (DOTATATE), a PET ligand with high-specificity binding affinity for SST2 (11), may be superior to [18F]FDG in marking macrophage activity, particularly in the coronary arteries. However, robust evaluation of 68Ga-DOTATATE in atherosclerosis is lacking.

We present a prospective clinical study evaluating 68Ga-DOTATATE PET for imaging coronary, carotid, and aortic inflammation in patients with CVD.

Methods

RNA SEQUENCING. To determine target specificity of 68Ga-DOTATATE imaging in atherosclerosis, expression of the SSTR1-5 genes within in vitro differentiated macrophage subtypes and other blood-derived cells relevant to atherosclerosis were characterized using population-based “next generation” RNA sequencing data from the European BLUEPRINT (a BLUEPRINT of haematopoietic epigenomes) project, for which all data are publicly available (12). The expression levels of glucose transporter 1 (GLUT1) and glucose transporter 3 (GLUT3) genes were also analyzed from the dataset; these genes encode the main glucose transporters that facilitate uptake of [18F]FDG in atherosclerotic plaques.

Clinical Study. In the VISION (Vascular Inflammation Imaging Using 68Ga-DOTATATE PET; NCT02021188) study, an unselected “real-world” cohort of patients with wide-ranging severity of stable (n = 18) and unstable (n = 24) CVD was prospectively enrolled from Addenbrooke’s Hospital, Cambridge, United Kingdom (Figure 1). “Stable” patients had stable angina or asymptomatic atherosclerosis and at least a 30% stenosis of a major epicardial coronary artery or an internal carotid artery. “Unstable” patients had experienced a clinical event (acute coronary syndrome [ACS] or carotid territory transient ischemic attack [TIA]/stroke) within the 3 months before imaging. Baseline cardiovascular risk factors were noted, including measurement of serum lipids and high-sensitivity C-reactive protein. Patients were

Abbreviations and Acronyms

ACS = acute coronary syndrome
BMI = body mass index
CT = computed tomography
CVD = cardiovascular disease
ECG = electrocardiogram
FDG = fluorodeoxyglucose
ICC = intra-class coefficient
PET = positron emission tomography
SSTR1 = somatostatin receptor subtype-1
SSTR2 = somatostatin receptor subtype-2
TBR = tissue-to-blood ratio
TBRmax = maximum tissue-to-blood ratios
TIA = transient ischemic attack
FIGURE 1 The VISION Study

A

CVD patients assessed for eligibility n=102
(August 2014 - June 2015)

Patients excluded, n=60*

Patients enrolled and consented n=42

Stable CVD n=18

Stable angina n=10

Asymptomatic n=8

Unstable CVD n=24

ACS n=10

TIA or stroke n=14

Carotid endarterectomy n=8

B

Patients with stable and unstable CVD

Clinical imaging n=42

Validation of imaging

Explanted carotid plaques n=8

Cultured macrophages

RNA sequencing data (BLUEPRINT study)

Patient (A) and procedure (B) flowcharts. *Did not meet study criteria, n = 8; other clinical factors, n = 3; declined/cancelled, n = 49. †Coronary artery PET data excluded in ACS patients with ambiguous culprit arteries (n = 2). ‡Carotid artery PET data excluded in patients with prior carotid surgery (n = 2). §[18F]-FDG PET imaging not completed because of timing of surgery (n = 1). ¶Tissue samples excluded owing to insufficient mRNA extracted for quantitative PCR (n = 2); ¶CT scans not completed (calcium scan, n = 1; coronary angiogram, n = 5; carotid angiogram, n = 2). ACS = acute coronary syndrome; CT = computed tomography; CVD = cardiovascular disease; FDG = fluorodeoxyglucose; PCR = polymerase chain reaction; PET = positron emission tomography; TIA = transient ischemic attack; VISION = Vascular Inflammation imaging using Somatostatin receptor positron emission tomography.
older than 40 years of age and provided written, informed consent. The study protocol approved by the local research ethics committee (REC 14/EE/0019) was in accordance with the Declaration of Helsinki.

**PET-CT IMAGING.** Patients underwent $^{68}$Ga-DOTATATE PET-CT and $^{[18F]}$FDG PET-CT imaging, using established methods (13) for vascular PET imaging on a Discovery 690 combined PET-CT system model (GE Healthcare, Little Chalfont, United Kingdom; extended Methods are detailed in the Online Appendix). $^{68}$Ga-DOTATATE had an average radiochemical purity of 99% on quality control testing performed by the manufacturer (Mallinckrodt, St. Louis, Missouri). Patients fasted for 6 h prior to $^{[18F]}$FDG imaging; capillary blood glucose concentration was confirmed as <7.0 mmol/l in nondiabetic patients prior to scanning. Patients with diabetes mellitus were instructed to take their antidiabetic medications as usual prior to $^{[18F]}$FDG scanning but to hold insulin within 4 h of the scan; if glucose level was >11.0 mmol/l, the scan was rescheduled according to our standard clinical practice. Insulin was not administered to any patient prior to $^{[18F]}$FDG PET imaging. The mean injected dose of $^{68}$Ga-DOTATATE was 147.8 ± 31.6 MBq and 248.1 ± 22.3 MBq for $^{[18F]}$FDG. Electrocardiography (ECG)-gated CT coronary angiography plus calcium scanning and carotid angiography were also performed.

**IMAGE ANALYSIS.** Static PET images were reconstructed using 3-dimensional (3D) iterative time-of-flight ordered-subset expectation maximization with point spread function modeling to reduce partial volume error. ECG-gated coronary PET images were reconstructed in diastole (50% to 75% of the R-R interval). PET-CT images were coregistered and analyzed by experienced observers masked to the clinical details, using OsiriX imaging software (version 7.0; Pixmeo, Bernex, Switzerland). CT angiography was used as the anatomical reference standard; 2D regions of interest were drawn on consecutive, fused PET-CT slices to quantify the maximum arterial radioactivity concentration, normalized by mean blood pool activity in the superior vena cava (maximum tissue-to-blood ratio [TBR$_{\text{max}}$]). Mean (m) and most diseased segment (mds) TBR$_{\text{max}}$ values were measured for each coronary segment, carotid artery, and thoracic aorta. Reproducibility of $^{68}$Ga-DOTATATE TBR measurements were tested by 2 independent observers using 10% of the coronary and carotid scans (n = 4 for both) selected at random, with 1 week between intraobserver readings. Coronary artery PET data were deemed uninterpretable if the maximum myocardial standardized uptake value was >5.0.

Coronary lesions were classified according to established CT criteria for plaque composition (calcified, noncalcified, or mixed plaque) and the presence of high-risk morphological features (spotty calcification [<3.0 mm], low attenuation [<30 HU], and positive remodeling [cross-sectional area >10% of a reference segment]) (14).

“Culprit” lesions were defined in patients with ACS or TIA/stroke by the attending cardiologist or stroke physician before PET imaging, with no involvement of the VISION study team. Assignment of culprit artery status took clinical data into consideration (e.g., ECG, angiographic and echocardiographic findings, site of any neurological deficit at time of clinical presentation, and carotid artery or brain imaging). Arteries targeted for intervention (with percutaneous coronary intervention or carotid endarterectomy surgery) were presumed to be culprit arteries. In patients who were managed medically, if the culprit lesion was uncertain, the relevant data were excluded from this part of the analysis.

**QUANTITATIVE POLYMERASE CHAIN REACTION.** The pattern of SSTR2 gene expression observed using population-based RNA-sequenced data was confirmed in lipopolysaccharide-stimulated macrophages from subjects in our imaging cohort by using quantitative real-time polymerase chain reaction assay results and compared to those of age- and sex-matched healthy volunteers (n = 3 for both). SSTR2 and CD68 mRNA levels were measured in excised carotid plaques, and compared with $^{68}$Ga-DOTATATE signals in PET images obtained prior to surgery.

**AUTORADIOGRAPHY AND HISTOLOGY.** To confirm specific ligand binding in atherosclerotic plaques, $^{68}$Ga-DOTATATE autoradiography was performed in carotid tissue sections adjacent to those used for quantitative polymerase chain reaction. After the radioactivity decayed, sections were stained with antibodies for SSTR$_2$, the panmacrophage marker CD68, and Movat’s pentachrome stain for anatomic characterization. Autoradiography and immunostaining were similarly tested in cultured macrophages. Colocalization of SSTR$_2$ and CD68 staining in macrophages within carotid plaque sections were assessed by immunofluorescence, with isotype and concentration-matched immunoglobulin G (IgG) as the negative control. The retention, storage, and use of tissue sections and blood samples were compliant with the UK Human Tissue Act of 2004.

**STATISTICAL ANALYSIS.** The primary outcome was comparison of culprit versus nonculprit coronary and carotid artery $^{68}$Ga-DOTATATE mTBR$_{\text{max}}$ in
patients with ACS or TIA/stroke. Pre-specified secondary outcomes included comparisons of vascular 
$^{68}$Ga-DOTATATE mTBR$_{\text{max}}$ values versus clinical CVD risk factors, CT plaque morphology, $^{[18F]}$FDG mTBR$_{\text{max}}$, and SSTR2/CD68 gene expression levels in excised carotid plaques. Primary and secondary outcome data expressed as medians (interquartile range [IQR]) were compared using Wilcoxon signed rank test or Mann-Whitney U test, as appropriate, with differences of medians derived for paired data. For comparisons between more than 2 groups, the Kruskal-Wallis test was used.

Based on $^{68}$Ga-DOTATATE TBR values from our pilot work and previously published data (9), our sample size ($n = 42$) was chosen to detect differences in mTBR$_{\text{max}}$ of $\geq 1.13$ between high- and low-risk arteries, with 80% power and a 2-sided p value of $< 0.05$. Patients with stable and unstable CVD were not formally matched as our primary comparison used “within patient” data (culprit versus nonculprit artery) rather than stable versus unstable patients. We anticipated that if one-third of patients had TIA/stroke, this would yield a comparable number of explanted carotid specimens to similar PET validation work performed by our group (15).

Spearman’s correlation and simple linear regression were used to identify statistically significant clinical and biochemical predictors of $^{68}$Ga-DOTATATE mTBR$_{\text{max}}$ that were then evaluated together using multivariate analysis. In the regression analysis, mean arterial values were used to mitigate the problem of multiple observations, as each patient contributed an equal number of arteries. Two-sided p values of $< 0.05$ were considered significant. Statistical analysis was performed using Prism version 6.0 software (GraphPad Software, Redwood, California) and Stata version 14.1 software (StataCorp, Cary, North Carolina).

RESULTS

POPULATION-BASED VALIDATION OF SSTR2 GENE EXPRESSION IN MACROPHAGES. Prior to clinical PET imaging, we tested the target expression of SSTR2 in blood-derived macrophages compared to other relevant cell types by using data from a large-scale population study. High levels of SSTR2 mRNA were detected exclusively in proinflammatory M1 macrophages and no other macrophage phenotype. This pattern and degree of expression was not seen for any other SST receptor subtype or cell line (Figure 2). SSTR3 was expressed by CD4$^+$ T lymphocytes to a lesser extent, as is known to occur (16).

Very low levels of SSTR2 mRNA were detected in unstimulated M0 macrophages and alternatively activated M2 macrophages, but SSTR2 was not expressed by any of the following cells: monocytes, T or B lymphocytes, natural killer cells, platelets, neutrophils, and endothelial cells. GLUT1 and GLUT3 were highly expressed by all cell types, demonstrating that SSTR2 offers greater cell specificity as an inflammation imaging target than glucose metabolism.

CLINICAL STUDY. Baseline clinical data are summarized in Table 1. The median time interval between ACS and PET imaging was 35 days (IQR: 21 to 66 days) and 18 days (IQR: 11 to 25 days) for TIA/stroke.

The reproducibility of $^{68}$Ga-DOTATATE TBR$_{\text{max}}$ measurements was excellent for both intraobserver observations (coronary artery intraclass coefficient value [ICC]: 0.90; 95% confidence interval [CI]: 0.85 to 0.94; carotid artery ICC: 0.96; 95% CI: 0.95 to 0.97) and interobserver observations (coronary artery ICC: 0.96; 95% CI: 0.94 to 0.97; carotid artery ICC: 0.91; 95% CI: 0.88 to 0.94).

$^{68}$Ga-DOTATATE IDENTIFIES CULPRIT ACS LESIONS IN CORONARY ARTERIES. Myocardial binding of $^{68}$Ga-DOTATATE was sufficiently low in all patients to allow unimpeded coronary artery PET signal measurement (Central Illustration, Online Figure 1). In patients with ACS, culprit $^{68}$Ga-DOTATATE uptake was consistently greater than the highest nonculprit coronary segment within the same individual (median difference mTBR$_{\text{max}}$: 0.69; IQR: 0.22 to 1.15; $p = 0.008$; median difference mdsTBR$_{\text{max}}$: 1.17; IQR: 0.45 to 1.70; $p = 0.02$), regardless of whether the lesion had been stented prior to imaging (culprit stented mTBR$_{\text{max}}$: 2.91; IQR: 2.66 to 4.63 vs. stable stented mTBR$_{\text{max}}$: 2.00; IQR: 1.51 to 2.70; $p = 0.006$) (Online Figure 2).

Using receiver operator characteristic (ROC) analysis, coronary $^{68}$Ga-DOTATATE mTBR$_{\text{max}}$ values $> 2.66$ had 87.5% (95% CI: 47.4 to 99.7) sensitivity and 78.4% (95% CI: 72.4 to 83.6) specificity to detect a culprit coronary segment (ROC area under the curve [AUC]: 0.86; 95% CI: 0.78 to 0.93; $p = 0.0006$).

$^{68}$Ga-DOTATATE IDENTIFIES HIGH-RISK STABLE LESIONS IN CORONARY ARTERIES. Data from 6 ± 2 coronary segments were analyzed from each patient. Increased $^{68}$Ga-DOTATATE signals were often observed in nonculprit (bystander) lesions in ACS patients, particularly in low-attenuation plaques defined by CT (Figure 3). $^{68}$Ga-DOTATATE mTBR$_{\text{max}}$ values were higher in nonculprit coronary segments in patients with both stable and unstable CVD, with
either noncalcified/mixed plaque morphology or with high-risk CT features (spotty calcification, low attenuation, or positive remodeling) versus heavily calcified or normal arteries with no high-risk features (p < 0.0001) (Figure 4).

Coronary \(^{68}\text{Ga-DOTATATE}\) mTBR\(_{\text{max}}\) > 2.12 had 83.3% (95% CI: 67.2% to 93.6%) sensitivity and 71.7% (95% CI: 64.6% to 78.0%) specificity (ROC AUC: 0.86; 95% CI: 0.80 to 0.92; p < 0.0001) to detect a segment with at least 1 high-risk CT feature.

\(^{68}\text{Ga-DOTATATE IDENTIFIES CULPRIT TIA/STROKE LESIONS IN CAROTID ARTERIES.}\) In patients with TIA or stroke, increased \(^{68}\text{Ga-DOTATATE}\) inflammatory signals reliably differentiated between culprit carotid plaques and contralateral nonculprit carotid arteries (median difference mTBR\(_{\text{max}}\): 0.13; IQR: 0.07 to 0.32; p = 0.003; median difference mdsTBR\(_{\text{max}}\): 0.34; IQR: −0.01 to 0.53; p = 0.005) (Figure 5). Contralateral carotid \(^{68}\text{Ga-DOTATATE}\) mdsTBR\(_{\text{max}}\) in patients with TIA/stroke was also greater than in diseased
TABLE 1 Baseline Clinical Factors

<table>
<thead>
<tr>
<th></th>
<th>Stable CVD (n = 18)</th>
<th>Unstable CVD* (n = 24)</th>
<th>All (n = 42)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yrs</td>
<td>67 ± 10</td>
<td>71 ± 7</td>
<td>69 ± 9</td>
</tr>
<tr>
<td>Male</td>
<td>14 (78)</td>
<td>20 (83)</td>
<td>34 (81)</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>29 ± 5</td>
<td>27 ± 4</td>
<td>28 ± 5</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>57 ± 9</td>
<td>58 ± 6</td>
<td>57 ± 8</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>141 ± 22</td>
<td>144 ± 24</td>
<td>143 ± 21</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>74 ± 9</td>
<td>76 ± 10</td>
<td>75 ± 9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Occurrences of previous cardiovascular history</th>
</tr>
</thead>
<tbody>
<tr>
<td>Occurrences of previous cardiovascular history</td>
</tr>
<tr>
<td>Hypertension</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
</tr>
<tr>
<td>Noninsulin dependent diabetes</td>
</tr>
<tr>
<td>Smoking habit (ex or current)</td>
</tr>
<tr>
<td>Family history of coronary heart disease†</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Occurrences of cardiovascular risk factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angina</td>
</tr>
<tr>
<td>Myocardial infarction</td>
</tr>
<tr>
<td>Coronary stenting</td>
</tr>
<tr>
<td>Coronary artery bypass surgery</td>
</tr>
<tr>
<td>Transient ischemia attack or stroke</td>
</tr>
<tr>
<td>Carotid endarterectomy surgery</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Occurrences of cardiovascular medications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirin</td>
</tr>
<tr>
<td>Clodigore</td>
</tr>
<tr>
<td>Statin</td>
</tr>
<tr>
<td>β-Adrenergic receptor blocker</td>
</tr>
<tr>
<td>Angiotensin converting enzyme inhibitor/receptor blocker</td>
</tr>
<tr>
<td>Calcium-channel blocker</td>
</tr>
<tr>
<td>Other antithrombotic</td>
</tr>
<tr>
<td>Oral nitrates</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Random lipid profile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol, mmol/l</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/l</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/l</td>
</tr>
<tr>
<td>Triglycerides, mmol/l</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/l</td>
</tr>
<tr>
<td>Median high-sensitivity CRP, mg/l</td>
</tr>
<tr>
<td>Median peak serum troponin-I concentration, ng/l</td>
</tr>
<tr>
<td>Median %10-year Framingham risk score</td>
</tr>
<tr>
<td>Median coronary artery calcium score, Agatston units</td>
</tr>
</tbody>
</table>

Values are mean ± SD, n (%), or mean (interquartile range). *Unstable CVD = ACS or TIA/stroke within the previous 3 months. †≤56 years of age. ACS patients. ACS = acute coronary syndrome; CRP = C-reactive protein; CVD = cardiovascular disease; HDL = high-density lipoprotein; LDL = low-density lipoprotein; TIA = transient ischemic attack.

(p = 0.01) or normal (p = 0.0001) carotids from patients with stable CVD (i.e., those without TIA/stroke or ACS). Nonculprit carotid 68Ga-DOTATATE mTBRmax was also higher in patients with unstable CVD (either TIA/stroke or ACS) versus stable CVD (p = 0.02).

AORTIC 68Ga-DOTATATE SIGNALS ARE RELATED TO CORONARY 68Ga-DOTATATE SIGNALS. 68Ga-DOTATATE mTBRmax values in the coronary arteries and neighboring aorta showed a moderate correlation (r = 0.43; 95% CI: 0.11 to 0.66; p = 0.008). Aortic 68Ga-DOTATATE mTBRmax was negatively correlated with coronary calcium scores in patients with a total score of <400 (r = −0.66; 95% CI: −0.87 to 0.26; p = 0.003). VASCULAR 68Ga-DOTATATE SIGNALS ARE RELATED TO CLINICAL CVD RISK FACTORS. Relationships between vascular 68Ga-DOTATATE signals and clinical CVD risk factors were evaluated to explore possible mechanistic links between 68Ga-DOTATATE and underlying disease pathology. Age (r = 0.44; 95% CI: 0.20 to 0.62; p = 0.0004), total cholesterol (r = 0.51; 95% CI: 0.30 to 0.68; p < 0.0001), and Framingham risk score (r = 0.53; 95% CI: 0.32 to 0.69; p < 0.0001) showed significant correlations with carotid 68Ga-DOTATATE mTBRmax (Figure 6). Carotid 68Ga-DOTATATE mTBRmax also differed significantly across patients grouped according to Framingham risk score (p < 0.0001). Body mass index (BMI) was positively correlated with aortic 68Ga-DOTATATE mTBRmax (r = 0.38; 95% CI: 0.06 to 0.64; p = 0.017). When age, total cholesterol, and BMI were evaluated with other relevant clinical factors using multivariate linear regression, they remained significant predictors of 68Ga-DOTATATE mTBRmax (Online Table 1).

Carotid 68Ga-DOTATATE TBRmax values also varied significantly in patients without TIA/stroke who were taking statins, with lower values seen in patients taking high-intensity statins compared to those taking moderate or low dosages (p = 0.004) (Figure 7).

In the 1.6 ± 0.2 years following PET imaging, 2 patients attended the emergency department with nonanginal chest pain, and there were 2 out-of-hospital deaths; our study was not powered to assess the ability of PET imaging to predict clinical events.

COMPARISON OF 68Ga-DOTATATE VERSUS [18F] FDG-DEFINED INFLAMMATION. The time interval between 68Ga-DOTATATE and [18F]FDG imaging was a median of 2 days (IQR: 1 to 7 days). Coronary, carotid, and aortic 68Ga-DOTATATE and [18F]FDG mTBRmax values were strongly correlated with each other (r = 0.73; 95% CI: 0.64 to 0.81; p < 0.0001), although coronary artery [18F]FDG data were uninterpretable in 27 (64%) patients because of high myocardial spillover. Of 2 ACS patients with interpretable coronary [18F]FDG data, culprit mTBRmax values were numerically higher than the highest nonculprit segment in 1 patient.

[18F]FDG mTBRmax but not mdsTBRmax differentiates culprit from contralateral carotids (median
Comparisons between coronary $^{18}$F-FDG mTBRmax values and CT morphology are shown in Figure 4 and clinical risk factors in Figure 6. Coronary $^{68}$Ga-DOTATATE PET (F, H, I) clearly detected intense inflammation in this high-risk atherosclerotic plaque/distal portion of the stented culprit lesion (arrow) and recently infarcted myocardium (*). In contrast, using $^{18}$F-FDG PET (G, J), myocardial spillover completely obscures the coronary arteries. CT = computed tomography; $^{18}$F-FDG = fluorine-18-labeled fluorodeoxyglucose; $^{68}$Ga-DOTATATE = gallium-68-labeled DOTATATE; PET = positron emission tomography.

TARGET VALIDATION IN MACROPHAGES FROM CVD PATIENTS. In CVD patients, macrophage SSTR2
mRNA was increased a median 91-fold (IQR: 56 to 104) above baseline versus 13-fold (IQR: 4.0 to 25) in age- and sex-matched healthy volunteers ($p = 0.01$), after stimulation with lipopolysaccharide. Presence of SST$_2$ receptors was confirmed by immunostaining and specific binding of $^{68}$Ga-DOTATATE to SST$_2$ in cultured macrophages shown by autoradiography.
AUTORADIOGRAPHIC AND HISTOLOGICAL TARGET VALIDATION IN CAROTID PLAQUES. Following PET-CT imaging, 8 patients underwent carotid endarterectomy. The PET scan-to-surgery time interval was a median of 9 (IQR: 3 to 35) days. Ex vivo 68Ga-DOTATATE carotid autoradiography showed high levels of specific ligand binding to SST2 receptors in all specimens (n = 8). A small degree of nonspecific binding was seen in relation to freshly cut calcium and as a result of edge artifact, which occurs when tissue edges curl causing the ligand to remain trapped during the experiment.

(A) X-ray and (D) CT coronary angiograms of a 67-year-old man with stable angina (hatched oval) with spotty calcification ([*] calcium scan) and calcified plaque in the LAD artery. Although 68Ga-DOTATATE PET (B, E) allows unimpeded interpretation of inflammation in the LCx lesion ([arrow]), and lack of signal in the LAD, coronary [18F]FDG imaging is obscured by patchy myocardial tracer uptake (C). Graphs compare 68Ga-DOTATATE (F) with [18F]FDG (G) coronary TBRmax, values by CT plaque morphology in coronary segments. 68Ga-DOTATATE: NCP or MP, n = 86; normal, n = 45; spotty calcium, n = 30; large calcium, n = 72; LA or PR, n = 11; no high-risk CT, n = 186; [18F]FDG: NCP or MP, n = 43; normal, n = 13; spotty calcium, n = 15; large calcium n = 14; LA or PR, n = 4; no high-risk CT, n = 66), and ROC analysis demonstrating good diagnostic accuracy for high-risk coronary lesions. LA = low attenuation; LAD = left anterior descending; LCx = left circumflex; NCP = noncalcified plaque; MP = mixed plaque; PR = positive remodeling; other abbreviations in Figures 1 to 3.
68Ga-DOTATATE binding within carotid plaques occurred mainly in the necrotic cores and shoulder regions, where there was strong colocalization of CD68 and SST2 staining (Figure 8, Online Figure 3). Neither 68Ga-DOTATATE binding nor SST2 staining was observed within thick fibrous cap regions consisting mainly of vascular smooth muscle cells.
CAROTID SSTR2/CD68 mRNA VERSUS 68GA-DOTATATE ACTIVITY. SSTR2 and CD68 mRNA levels were highly correlated within carotid plaque ($r = 0.93$; 95% CI: 0.49 to 0.99; $p = 0.007$) (Figure 9). Carotid SSTR2 and CD68 mRNA levels also showed strong correlation with in vivo 68Ga-DOTATATE TBR$_{\text{max}}$ values measured at the corresponding level in clinical PET images, orientated at the bifurcation ($SSTR2 r = 0.89$; 95% CI: 0.28 to 0.99; $p = 0.02$; $CD68 r = 0.84$; 95% CI: 0.09 to 0.98; $p = 0.04$). Moreover, immunofluorescence demonstrated high cell specificity of colocalized of SST2 and CD68 staining in carotid plaque macrophages. These data provided both histological and molecular validation of 68Ga-DOTATATE as a specific marker of atherosclerotic inflammation.

**DISCUSSION**

There have been previous reports of 68Ga-DOTATATE imaging in atherosclerosis, but they have been preclinical or retrospective studies, with the exception of 1 report limited to the carotid arteries. We provide the first definitive prospective validation of 68Ga-DOTATATE imaging as a marker of atherosclerotic inflammation.

**WHICH CELLS EXPRESS SSTR2 IN ATHEROSCLEROSIS?** We confirmed that high target SSTR2 gene expression occurs exclusively among activated proinflammatory M1 macrophages in atherosclerosis and demonstrated the presence of SST$_2$ receptors in macrophages from patients with CVD. As a glucose analog, [18F]FDG lacks cell specificity, but there is some evidence that [18F]FDG accumulates more in M1 macrophages than in other macrophage subtypes because of differing glycolytic activity between these cells (17).

**BINDING OF 68GA-DOTATATE WITHIN ATHEROSCLEROTIC PLAQUES.** We observed specific 68Ga-DOTATATE ligand binding to SST$_2$ receptors within CD68$^+$ macrophage-rich carotid plaque regions and strong correlations between carotid SSTR2 mRNA and in vivo 68Ga-DOTATATE activity. Although low levels

---

**FIGURE 6 Vascular Inflammation Versus Clinical Risk Factors**

Graphs show correlations of vascular inflammation detected by 68Ga-DOTATATE (A to C) and [18F]FDG (D) versus clinical cardiovascular disease risk factors. (Carotid arteries $n = 62$; aortas, $n = 38$; note data from patients not taking statins [$n = 4$] were excluded to control for this variable). Abbreviations as in Figure 1.
of SST2 expression have been previously reported in vascular smooth muscle cells, we did not observe 68Ga-DOTATATE binding nor SST2 staining within the thick fibrous cap regions where these cells are abundant, suggesting that the synthetic atherosclerotic vascular smooth muscle cell phenotype is unlikely to express SST2 to a degree that would be detectable by clinical imaging. These laboratory-based findings provide robust histological and molecular validation of 68Ga-DOTATATE as a specific marker of atherosclerotic inflammation.

**CULPRIT AND HIGH-RISK PLAQUE INFLAMMATION.**
In clinical imaging, we found that 68Ga-DOTATATE PET correctly identified culprit coronary and carotid arteries in individuals with ACS or TIA/stroke. The median difference between culprit and nonculprit carotid arteries was less pronounced than in coronary arteries, but these 2 regions are not necessarily directly comparable because of the high prevalence of asymptomatic contralateral carotid disease, differing imaging time points affecting tracer kinetics, and local factors determining tracer delivery and clearance. 68Ga-DOTATATE demonstrated reliable diagnostic accuracy to detect stable yet inflamed coronary lesions with high-risk CT morphological features.

**SYSTEMIC INFLAMMATION.** The ability of 68Ga-DOTATATE to detect generalized vascular
FIGURE 8 **68Ga-DOTATATE Ligand Binding to Macrophage SST$_2$ in Carotid Plaque**

In vivo CT angiography views of culprit carotid artery (hatched oval = internal carotid artery) in axial (A) and sagittal (E) views, with corresponding fused $^{68}$Ga-DOTATATE PET-CT (B). Ex vivo views of macrographic images of the explanted carotid specimen (I, hatched line signifies location of carotid section); phosphor autoradiographic image shows the total binding of $^{68}$Ga-DOTATATE to SST$_2$ receptors in macrophages within a transverse carotid section (C) corresponding to the level shown in clinical images. Adjacent section was incubated with $^{68}$Ga-DOTATATE and cold competing ligand (D) showing very low levels of nonspecific binding. Brightfield photomicrographs show brown immunoreactive SST$_2$ staining (G, J, M) of macrophages identified with the panmacrophage marker CD68 (H, K, N), colocalized SST$_2$ (brown), and CD68 (blue) staining in the same section (L); Movat’s pentachrome stain (F).
inflammation was shown by the close relationship between PET signals in neighboring coronary and aortic vasculature and increased inflammatory signals arising from nonculprit carotids in patients with unstable CVD. Both of these features have been previously demonstrated using $^{[18F]}$FDG (2). Moreover, significant correlations were observed between clinical CVD risk factors and generalized vascular $^{68}$Ga-DOTATATE inflammatory signals, which were overall lower in patients receiving high-intensity statins and with increasing coronary calcium scores up to 400. The inverse relationship between statin dosages and signal intensity provide anecdotal evidence that $^{68}$Ga-DOTATATE PET may provide a useful imaging platform for monitoring the anti-inflammatory effects of atherosclerosis drugs.

$^{68}$Ga-DOTATATE OUTPERFORMS $^{[18F]}$FDG. Although $^{68}$Ga-DOTATATE signals were strongly correlated with $^{[18F]}$FDG-defined inflammation in multiple vascular territories, disparity between these 2 tracers reflects the fact that $^{68}$Ga-DOTATATE is a specific macrophage marker in atherosclerosis, whereas $^{[18F]}$FDG provides a nonspecific measurement of glucose metabolism within plaque cells. Superiority of $^{68}$Ga-DOTATATE compared with $^{[18F]}$FDG was
shown by better power to discriminate high-risk versus low-risk coronary atherosclerotic lesions, higher signal-to-blood ratios, and consistently lower myocardial activity, affording clear coronary signal interpretation.

[18F]FDG imaging is notoriously unreliable in coronaries; in contrast, myocardial 68 Ga-DOTATATE binding was sufficiently low to allow coronary artery inflammation imaging in all patients. 68 Ga-DOTATATE inflammatory signals differentiated culprit from contralateral carotids, using both “mean of the whole artery” and “most-diseased segment” methods, but [18F]FDG only detected a difference in mean carotid uptake, hinting that 68 Ga-DOTATATE may offer a more focal approach. 68 Ga-DOTATATE signals also appeared more discretely localized than [18F]FDG signals in clinical images (Figures 3 and 5). Given the higher cost of 68 Ga-DOTATATE than [18F]FDG, its use for non-coronary vascular imaging may not be justified, although in the context of research, increased macrophage specificity of 68 Ga-DOTATATE potentially holds significant advantage for detection of subtle changes in vascular biology that may not be as clearly appreciated using a blunter imaging tool such as [18F]FDG.

A small number of previous studies have investigated SST2 PET imaging in CVD. Two studies demonstrated autoradiographic 68 Ga-DOTATATE binding within macrophage-rich aortic atherosclerotic plaques in mice (6,7). Five retrospective analyses of PET scans from patients who underwent imaging for oncological indications reported significant statistical relationships between vascular SST2 signals and clinical CVD factors, including older age, male sex, hypercholesterolemia, presence of calcified coronary plaque, prior CVD events, and Framingham risk score calculated using BMI (8-10,18,19). In 1 study, a strong correlation was observed between 68 Ga-DOTATATE and [18F]FDG vascular TBR values, although signals from the 2 tracers did not colocalize at the sites of highest tracer uptake (9). In another study of 11 patients who underwent 3 serial 68 Ga-DOTATATE scans following peptide receptor radionuclide therapy with lutetium-177-labeled DOTATATE, good interscan reproducibility of 68 Ga-DOTATATE TBR measurements prior to radionuclide therapy was observed, as well as significant signal reduction 1 month after, which was most pronounced in relation to noncalcified plaques (10). These studies, although retrospective and without CT angiography or ECG-gating, are consistent with our findings.

Our finding that SST2 PET can differentiate culprit from contralateral carotid arteries is supported by another study of 64 Cu-DOTATATE PET cardiac magnetic resonance in 10 patients with carotid TIA/stroke (20). However, in that study, correlation between carotid copper-64-labeled DOTATATE signals and gene expression of the monocyte/macrophage marker CD63 was observed using a multivariate regression model, leading the authors to conclude that this tracer reports on alternatively activated M2 macrophages. As hemoglobin-haptoglobin scavenging by CD63 in the setting of intraplaque hemorrhage directs monocyte differentiation toward an atheroprotective M2 phenotype (21), the finding of increased CD63 mRNA within advanced ruptured plaques is unsurprising. However, there is no current evidence to indicate that significant SST2 expression occurs in M2 macrophages. Our findings agree with those of previous work indicating that 68 Ga-DOTATATE signals in atherosclerosis occur because of intracellular tracer accumulation following cell surface binding and receptor internalization among dense clusters of classically activated M1 macrophages (22).

Next steps involve testing in a larger, longitudinal study with clinical outcomes, similar to the ongoing BiImage (NCT00738725) and PESA (Progression of Early Subclinical Atherosclerosis; NCT01410318) [18F] FDG studies.

**STUDY LIMITATIONS.** Limitations of our study include inherent technical challenges of vascular PET imaging, namely low spatial resolution (~5 mm) and image artifacts created by cardiorespiratory motion that are confounded by the high positron energy of 68 Ga (E\text{\textsubscript{max}}: 1.9 MeV; average positron range: 2.4 mm). To overcome these difficulties, we used CT angiography for precise anatomical PET signal localization (spatial resolution: 0.5 to 0.6 mm), ECG-gated PET reconstruction to reduce the impact of motion, and iterative time-of-flight reconstruction with point spread function modeling to provide resolution recovery and reduce partial volume error. Coronary signal-to-noise ratio could potentially be improved further by motion correction methods in active development (23).

We did not attempt myocardial suppression of [18F]FDG using dietary manipulation or prolonged fasting, because in our experience, these methods are ineffective in ~50% of cases (3) and are inconvenient for patients. Nevertheless, others have reported success using these methods.

Most of the ACS patients underwent stenting prior to PET imaging and persistence of procedure-related inflammation could have, in theory, augmented inflammatory signals detected in culprit coronary lesions. Clinical identification of culprit arteries can be challenging, particularly in the coronary arteries; although intravascular imaging can be used to
confirm plaque rupture, this investigation was not performed in any of the patients in this study. Last, although the novel finding of increased SST2 expression in LPS-stimulated macrophages from patients with CVD versus healthy volunteers is intriguing, further testing in a larger patient cohort is needed.

CONCLUSIONS

We provide gene-, cell-, plaque-, and patient-level data demonstrating that SST2 PET imaging using 68Ga-DOTATATE provides a quantifiable, cell-specific marker of atherosclerotic inflammation that outperforms [18F]FDG in the coronary arteries. Further work is needed to confirm these findings in a larger patient population and to compare imaging with clinical outcomes. 68Ga-DOTATATE PET offers measurement of both generalized atherosclerotic disease activity and detailed information about local plaque functional phenotype to complement multimodal assessments of anatomic, morphologic, and hemodynamic disease severity. This approach, in selected patient populations, has the potential to improve CVD risk prediction, allowing personalized tailoring of therapies aimed to improve clinical outcomes.

REFERENCES

19. Malmberg C, Ripa RS, Johnbeck CB, et al. 64Cu-DOTATATE for noninvasive assessment of atherosclerosis in large arteries and its correlation with risk factors: head-to-head comparison with...


KEY WORDS
atherosclerosis, inflammation, macrophages, molecular imaging, positron emission tomography, somatostatin receptor

APPENDIX
For an expanded Methods section and supplemental figures and a table, please see the online version of this article.