Imaging intraplaque inflammation in carotid atherosclerosis with $^{11}$C-PK11195 positron emission tomography/computed tomography

Oliver Gaemperli$^{1*}$, Joseph Shalhoub$^{2*}$, David R.J. Owen$^1$, Frederic Lamare$^1$, Saga Johansson$^3$, Naghmeh Fouladi$^3$, Alun H. Davies$^2$, Ornella E. Rimoldi$^{1,4}$, and Paolo G. Camici$^{1,5}$

$^1$Medical Research Council Clinical Sciences Centre and National Heart and Lung Institute, Hammersmith Hospital, Imperial College, London, UK; $^2$Imperial Vascular Unit, Charing Cross Hospital, Imperial College London, London, UK; $^3$GE Healthcare, Medical Diagnostics, Amersham, UK; $^4$CNR, Istituto Bioimmagini Fisiologia Molecolare, Milan, Italy; and $^5$Vita-Salute University and Scientific Institute San Raffaele, Via Olgettina 60, 20132 Milan, Italy

Received 5 April 2011; revised 5 July 2011; accepted 23 August 2011; online publish-ahead-of-print 19 September 2011

This paper was guest edited by Prof. Filippo Crea, Universita Catolica del Santo Cuore, Rome, Italy

See page 1857 for the editorial comment on this article (doi:10.1093/eurheartj/ehr455)

Aims
We sought to determine whether intraplaque inflammation could be measured with positron emission tomography/computed tomography angiography (PET/CTA) using $^{11}$C-PK11195, a selective ligand of the translocator protein (18 kDa) (TSPO) which is highly expressed by activated macrophages.

Methods and results
Patients ($n=32$; mean age $70 \pm 9$ years) with carotid stenoses ($n=36$; 9 symptomatic and 27 asymptomatic) underwent $^{11}$C-PK11195 PET/CTA imaging. $^{11}$C-PK11195 uptake into carotid plaques was measured using target-to-background ratios (TBR). On CTA images, plaque composition was assessed by measuring CT attenuation of the carotid plaque. Eight patients underwent carotid endarterectomy and ultrathin contiguous sections were processed for TSPO and CD68 (using immunohistochemical staining, $^3$H-PK11195 autoradiography, and confocal fluorescence microscopy). Carotid plaques associated with ipsilateral symptoms (stroke or transient ischaemic attack) had higher TBR ($1.06 \pm 0.20$ vs. $0.86 \pm 0.11$, $P=0.001$) and lower CT attenuation [(median, inter-quartile range) $37, 24–40$ vs. $71, 56–125$ HU, $P=0.01$] than those without. On immunohistochemistry and confocal fluorescence microscopy, CD68 and PBR co-localized with $^3$H-PK11195 uptake at autoradiography. There was a significant correlation between $^{11}$C-PK11195 TBR and autoradiographic percentage-specific binding ($r=0.77$, $P=0.025$). Both TBR and CT plaque attenuation had high negative predictive values (91 and 92%, respectively) for detecting symptomatic patients. However, the best positive predictive value (100%) was achieved when TBR and CT attenuation were combined.

Conclusion
Imaging intraplaque inflammation in vivo with $^{11}$C-PK11195 PET/CTA is feasible and can distinguish between recently symptomatic and asymptomatic plaques. Patients with a recent ischaemic event had ipsilateral plaques with lower CT attenuation and increased $^{11}$C-PK11195 uptake.

Keywords
$^{11}$C-PK11195 • Atherosclerosis • Positron emission tomography • CT angiography • Carotid artery

Introduction
Stroke is a leading cause of death and long-term disability in western societies.\(^1\) In two-thirds of ischaemic strokes, the cause is disruption of an atherosclerotic plaque of an extracranial artery which subsequently develops superficial thrombosis and sheds debris into the cerebral vasculature.\(^7\) Although occasionally heralded by transient ischaemic events, the majority of strokes...
occur without prior prodromal symptoms. Up until now, indications for carotid endarterectomy in asymptomatic patients have been based largely on the severity of stenosis. However, in the recent past, the composition and biological activity of plaques have emerged as important determinants of thrombus-mediated ischaemic events alongside the degree of luminal narrowing. Intraplaque inflammation plays an important role in the progression and destabilization of atherosclerotic lesions. On histology, symptomatic carotid plaques [i.e. plaques associated with stroke and transient ischaemic attacks (TIA)] are characterized by a high degree of macrophage infiltration and develop into a more stable non-inflammatory phenotype over a period of 6 months after the ischaemic event. The link between inflammation and ischaemic cerebral events is highlighted by the strong correlation between elevated plasma C-reactive protein, a marker of systemic inflammation, and cerebrovascular events.

Different imaging techniques are currently under evaluation for detecting inflammatory cells in atherosclerotic plaques among which positron emission tomography (PET) has emerged as a promising modality due to its high sensitivity. The C-labelled PET tracer PK11195 is a specific ligand of the translocator protein (18 kDa) (TSPO), formerly known as peripheral benzodiazepine receptor, a protein that is highly expressed in activated cells of the mononuclear phagocyte lineage. Recently, specific in vitro binding of \(^{3}H\)-PK11195 to macrophages has been shown in human carotid endarterectomy samples, and a pilot study in patients with large vessel vasculitis has demonstrated that \(^{11}C\)-PK11195 PET/computed tomography angiography (PET/CTA) can be used to assess vascular inflammation in vivo in humans.

In this proof-of-principle study, we aimed to assess the value of \(^{11}C\)-PK11195 PET/CTA for measuring inflammation in carotid atherosclerotic plaques in vivo, and whether it can be used to discriminate between recently symptomatic and asymptomatic lesions.

**Methods**

**Study population**

Twenty-three asymptomatic and nine symptomatic patients with a carotid stenosis >50% (confirmed by duplex ultrasonography using velocity criteria) were recruited from a vascular tertiary referral centre serving a hyper-acute stroke unit. Asymptomatic subjects were identified through screening carotid ultrasonography of patients with cardiovascular risk factors and/or arterial disease in other territories such as coronary or lower limb. Exclusion criteria were age <40 years or more than 85 years, contraindications to iodinated contrast agents (including known intolerance to contrast agents or a glomerular filtration rate of <60 mL/min/1.73 m\(^2\)), or a positive pregnancy test in women of child-bearing potential. Patients were defined as symptomatic if they had unilateral amaurosis fugax, clinical features consistent with a cortical TIA, or a completed hemispheric stroke in the carotid territory of interest in the preceding 3 months.

The study protocol received Research Ethics Committee approval (09/H0707/2) and all patients gave written informed consent. Radiation exposure was licensed by the UK Administration of Radioactive Substances Advisory Committee (ARSAC; RPC 262-425 (24111)).

**Positron emission tomography/computed tomography scanning protocol**

Imaging of the carotid arteries was performed using a 16-slice PET/CT scanner (Discovery RX, GE Healthcare, Milwaukee, WI, USA) with a 15 cm axial field of view centred on the carotid bifurcations. Radiotrac-ter synthesis of \(^{11}C\)-PK11195 was performed as described previously. After a low-dose CT scan for attenuation correction, a bolus of 475 ± 103 MBq of \(^{11}C\)-PK11195 was injected into an antecubital vein and dynamic PET emission data acquisition was started in list mode for a total duration of 60 min and 30 s. Positron emission tomography data were re-binned into 18 temporal frames (30 s background, 1 × 15, 1 × 5, 1 × 10, 1 × 30 s, 4 × 1, 7 × 5, and 2 × 10 min).

Thereafter, contrast-enhanced CT angiography was performed with the same scan range as the PET scan. Administration of a contrast agent (70 mL Ultravist 370, Schering, Berlin, Germany) was performed through an antecubital vein at 3.5 mL/s. The arrival of contrast in the ascending aorta was synchronized with the CT angiography scan using a bolus tracking technique. The CT angiography acquisition parameters were 120 kV, 180 mA s, 16 × 0.625 mm slice thickness, pitch of 1.0, and 0.5 s rotation time.

The effective dose of CT (including localization, attenuation correction, and CT angiography) was estimated from the product of the dose-length product and an organ weighting factor for the neck (0.0034 mSv/mGy/cm) as proposed by the European Working Group for Guidelines on Quality Criteria in CT.

**Image reconstruction**

All PET emission scans were normalized and corrected for randoms, dead time, scatter, and attenuation. Correction for radiotracer decay was deferred to the post-processing of the images. The 18 frames of the dynamic emission scans were reconstructed using the three-dimensional re-projection algorithm (with the ramp and Colsher filters set to Nyquist frequency) and using an ordered subset expectation maximization (OSEM) algorithm with 2 iterations and 21 subsets. The matrix size was 256 × 256 × 47 for both reconstructions.

Reconstruction parameters for CT angiography were 0.625 mm slice thickness, 0.625 mm increment, 30 cm wide reconstruction field-of-view, window width of 300 HU, and window level of 30 HU.

Images were transferred to a dedicated workstation (Advantage Workstation 4.2, GE Healthcare) for visual assessment. In the case of misalignment between the CT and the PET data sets, images were manually re-aligned using the vertebrae and the sternum as anatomical landmarks. A 25 min static frame was created by addition of OSEM PET dynamic frames 5 min after tracer injection. Computed tomographic images were reduced to a matrix size of 256 × 256 × 47 to match the size of the reconstructed PET images and superimposed to the PET images to help defining the regions of interest (ROIs).

**Measurement of \(^{11}C\)-PK11195 uptake**

Quantitative measurement of \(^{11}C\)-PK11195 uptake was performed using dedicated software (MATLAB, The MathWorks Inc.). After PET and CT images were resliced to obtain magnified cross-sections of the carotid artery, ROIs were placed on the CT images encompassing the carotid plaque and superimposed on the PET images. To quantify plaque tracer uptake, standardized uptake values (SUVs) were calculated as the average radioactivity concentration in each volume of interest (in Bq/mL) divided by the total injected activity per body weight (in Bq/g). A background SUV was obtained in a venous structure (superior vena cava, subclavian, or internal jugular vein) and
plaque target-to-background ratios (TBR) were calculated as the ratio of plaque SUV over venous blood SUV.\textsuperscript{19}

**Computed tomographic analysis of plaque composition and stenosis severity**

Computed tomographic analysis was performed from axial source images and multiplanar reformations. The amount of plaque calcification (PC) was semi-quantitatively determined from carotid cross-sections according to a previously reported scale:\textsuperscript{20} a score of 0 was assigned when PC was absent, 1 was assigned for small PCs covering <10% of the vessel circumference, 2 was assigned if the PC involved 10–25% of the vessel circumference, 3 if 26–50%, and 4 if >50% of the vessel circumference were involved.

Additionally, all plaque cross-sections were analysed to identify areas of low attenuation at fixed window settings (width 700 HU, level 200 HU). In at least five planes, a circular or elliptical ROI (≥ 2 mm\textsuperscript{2}) was placed within the plaque, at sufficient distance from calcifications or contaminations with contrast material to avoid beam-hardening artefacts. Computed tomographic attenuation was measured as the mean CT density (in HU) in the ROI with the lowest attenuation. The severity of stenosis was determined according to the NASCET criteria:\textsuperscript{21} in brief, on cross-sections strictly perpendicular to the axis of the vessel, the minimal luminal diameter (MLD) was measured using an electronic calliper and compared with the reference diameter (RD) in a more distally located non-diseased segment. The degree of stenosis was calculated as (RD – MLD)/RD.

**Ex vivo plaque processing**

Eight patients (five asymptomatic and three symptomatic) underwent carotid endarterectomy within 9 ± 11 days of the PET/CT study. The diseased intimal arterial segments were immediately snap frozen in liquid nitrogen and stored at −80 °C for batch analysis. Frozen samples were embedded in optimum cutting temperature compound (TissueTek, Qiagen) and adjacent 12 μm cryosections were used for autoradiography, immunohistochemistry, and immunofluorescence.

**Autoradiography**

Translocator proteins were labelled with \(^{3}\text{H}-\text{PK11195}\) (specific activity 86.4 Ci/mmol; 1 mCi/mL, PerkinElmer Inc.) for autoradiography. Non-specific binding was determined in the presence of excess non-radioactive \(^{1}\text{H}-\text{PK11195}\) (1 μmol). Slides were apposed to a \(^{3}\text{H}-\text{sensitive film for 5 months. Calibration standards were included in the cassette (}^{3}\text{H-microscales 0–16 and 3–110 nCi/mg; GE Lifesciences, UK). Films were developed using an automatic film processor (Amersham Hyperfilm, GE Lifesciences) under safelight conditions. Quantitative analysis was performed using a computerized image analysis system (MCID, Interfocus, Cambridge, UK). Optical density readings, corrected for background, were made and values standardized against the linear portion of a curve generated using \(^{3}\text{H-microscale standards and expressed as nCi/mg. Specific binding values were determined by subtracting non-specific binding values from total binding values.}\)**

**Immunohistochemistry**

Tissue sections processed for immunohistochemistry were fixed in ice-cold 70% ethanol for 10 min, followed by 10 min incubation in 0.3% H\textsubscript{2}O\textsubscript{2} and 30 min incubation in 10% normal goat serum to block non-specific labelling. Adjacent tissue sections were thereafter incubated with primary antibodies raised against either TSPO (rabbit anti-TSPO, RnD Systems, 1:100) or CD68 (mouse anti-CD68, Dako, 1:100) overnight at 4 °C. Secondary labelling was performed using species-specific kits with HRP-conjugated secondary antibodies (Envision) and incubated for 30 min at room temperature. The substrate of 3,3′-diaminobenzidine (0.7 mg/mL, Sigma) was used as a chromogen. Plaque sections were counterstained in haematoxylin, dehydrated, and mounted in DPX mounting media (Merck). The slides were rinsed with phosphate-buffered saline between each step during the process. Images of TSPO- and CD68-labelled tissue sections were captured using a Motic camera and software connected to a Nikon microscope.

**Confocal fluorescence microscopy**

Selected sections were double-labelled for TSPO and CD68 to evaluate co-expression. Following incubation with primary antibodies overnight at 4 °C, the sections were incubated with fluorescence secondary antibodies (ALEXA546 anti-rabbit and ALEXA488 anti-mouse, Invitrogen, 1:1000) for 1 h at room temperature. Finally, the tissue sections were mounted in Vectashield mounting media with DAPI (Vector Labs). The fluorescent slides were imaged using a Nikon/Perkin-Elmer spinning disc confocal microscopy system.

**Statistical analysis**

Statistical analysis was performed with SPSS 16.0.1 (SPSS, Inc., Chicago, IL, USA). Continuous variables are expressed as mean ± standard deviation (SD) or median [with inter-quartile range (IQR)] where appropriate. Normal distribution was assessed from Q–Q plots and confirmed using the Shapiro–Wilks test. Categorical variables are expressed as frequencies (percentages). The Mann–Whitney U-test was used for comparison of continuous values and the χ\textsuperscript{2} or Fisher’s exact test for comparison of categorical data (all tests two-sided). As four patients had bilateral stenoses, a mixed-model analysis was used, given the dependence of multiple segments per patient, assuming an unstructured covariance. Least square means of the difference between the asymptomatic and symptomatic groups, with 95% confidence intervals (CI) were estimated, and \(P\)-values for the comparison between groups were calculated. An agreement between CTA and Duplex ultrasound for the measurement of stenosis severity was determined with linear correlation and the Bland–Altman limits of agreement.\textsuperscript{22} Receiver-operating characteristics analysis was used to determine optimal cut-offs for CTA- and PET-derived parameters to predict ischaemic events and their respective diagnostic accuracies. Pearson’s or Spearman’s method was used (where appropriate) to assess the correlation between \(^{1}\text{C-PK11195 PET TBR, CT plaque attenuation or PC score, and }^{3}\text{H-PK11195 plaque autoradiography. A }P\text{-value of } <0.05\text{ was considered statistically significant.}\)**

**Results**

**Patients**

Thirty-four patients were enrolled in the study and 32 successfully underwent the PET/CTA scan (9 symptomatic and 23 asymptomatic patients). In two patients, the scans were unsuccessful due to failed tracer production (\(n = 1\)) or technical problems (\(n = 1\)). The patient baseline characteristics are shown in Table 1. In the symptomatic group, the index cerebrovascular events were amaurosis fugax (\(n = 4\)), hemispheric TIA (\(n = 4\)), and hemispheric stroke (\(n = 1\)). The interval between the index event and PET/CTA scan was 20 ± 21 days (range 5–75 days). Effective radiation dose was 3.2 ± 1.2 and 2.1 ± 0.5 mSv for CTA and PET, respectively, and 5.3 ± 1.7 mSv for the combined PET/CTA study.
Table 1  Patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>All patients (n = 32)</th>
<th>Symptomatic (n = 9)</th>
<th>Asymptomatic (n = 23)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years (median, IQR)</td>
<td>69, 63–77</td>
<td>67, 65–68</td>
<td>72, 64–78</td>
<td>0.57</td>
</tr>
<tr>
<td>Female gender, n (%)</td>
<td>11 (34)</td>
<td>4 (44)</td>
<td>7 (30)</td>
<td>0.68</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.1 ± 4.5</td>
<td>24.1 ± 3.7</td>
<td>26.8 ± 4.6</td>
<td>0.22</td>
</tr>
<tr>
<td>History of stroke/TIA, n (%)</td>
<td>17 (53)</td>
<td>9 (100)</td>
<td>8 (35)¹</td>
<td>0.001</td>
</tr>
<tr>
<td>S/p CEA, n (%)</td>
<td>7 (22)</td>
<td>1 (11)</td>
<td>6 (26)</td>
<td>0.64</td>
</tr>
<tr>
<td>CvRF, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td>21 (66)</td>
<td>6 (67)</td>
<td>15 (65)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Arterial hypertension</td>
<td>22 (69)</td>
<td>5 (56)</td>
<td>17 (74)</td>
<td>0.41</td>
</tr>
<tr>
<td>Dyslipidaemia</td>
<td>23 (72)</td>
<td>7 (78)</td>
<td>16 (70)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>7 (22)</td>
<td>2 (22)</td>
<td>5 (22)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Positive FHx</td>
<td>11 (34)</td>
<td>2 (22)</td>
<td>9 (39)</td>
<td>0.44</td>
</tr>
<tr>
<td>Medical treatment, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>25 (78)</td>
<td>7 (78)</td>
<td>18 (78)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Statin</td>
<td>31 (97)</td>
<td>8 (89)</td>
<td>23 (100)</td>
<td>0.28</td>
</tr>
<tr>
<td>ACE-inhibitors/ARB</td>
<td>14 (44)</td>
<td>4 (44)</td>
<td>10 (43)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>β-Receptor antagonists</td>
<td>8 (25)</td>
<td>2 (22)</td>
<td>6 (26)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>CCB</td>
<td>11 (34)</td>
<td>2 (22)</td>
<td>10 (43)</td>
<td>0.11</td>
</tr>
</tbody>
</table>

Data are given as mean ± SD unless otherwise indicated. BMI, body mass index; TIA, transient ischaemic attack; CEA, carotid endarterectomy; CvRF, cardiovascular risk factors; FHx, family history; ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; CCB, calcium channel blocker.
¹Stroke/TIA in asymptomatic patients occurred 7 months to 12 years prior to PET/CT.

Table 2  Imaging results

<table>
<thead>
<tr>
<th>Stenosis severity (CT) (%)</th>
<th>All stenoses (n = 36)</th>
<th>Symptomatic stenoses (n = 9)</th>
<th>Asymptomatic stenoses (n = 27)</th>
<th>Mean difference* (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>70 ± 17</td>
<td>77 ± 16</td>
<td>68 ± 16</td>
<td>−9 (−23, 4)</td>
<td>0.18</td>
</tr>
<tr>
<td>Plaque SUV for ¹¹C-PK11195</td>
<td>0.62 ± 0.10</td>
<td>0.69 ± 0.08</td>
<td>0.60 ± 0.10</td>
<td>−0.08 (−0.16, −0.01)</td>
<td>0.03</td>
</tr>
<tr>
<td>Plaque TBR for ¹¹C-K11195</td>
<td>0.91 ± 0.16</td>
<td>1.06 ± 0.20</td>
<td>0.86 ± 0.11</td>
<td>−0.19 (−0.30, −0.08)</td>
<td>0.001</td>
</tr>
<tr>
<td>Venous blood SUV</td>
<td>0.68 ± 0.09</td>
<td>0.66 ± 0.10</td>
<td>0.69 ± 0.09</td>
<td>0.04 (−0.04, 0.11)</td>
<td>0.33</td>
</tr>
<tr>
<td>CT plaque attenuation (median, IQR)</td>
<td>65, 38–117</td>
<td>37, 24–40</td>
<td>71, 56–125</td>
<td>43 (11, 76)</td>
<td>0.01</td>
</tr>
<tr>
<td>PC score</td>
<td>2.4 ± 1.3</td>
<td>2.6 ± 1.7</td>
<td>2.4 ± 1.2</td>
<td>−0.1 (−1.2, 1.0)</td>
<td>0.82</td>
</tr>
</tbody>
</table>

Data are given as mean ± SD unless otherwise indicated.  
*Mean differences between asymptomatic and symptomatic patients. CI, confidence intervals; CT, computed tomography; PC, plaque calcification; SUV, standardized uptake value; TBR, target-to-background ratio.

Positron emission tomography/computed tomography angiography imaging results

A total of 36 carotid stenoses were available for image analysis (4 asymptomatic subjects had bilateral stenoses). There was no significant difference in the severity of carotid stenoses on CT angiography between symptomatic and asymptomatic patients (77 ± 16 vs. 68 ± 16%, P = 0.18; Table 2). An excellent agreement was found between CTA- and ultrasound-derived stenosis severity (r = 0.93, P < 0.001; Bland–Altman limits of agreements −15 to 10%) with only a slight underestimation with CTA by 3% compared with ultrasound.

¹¹C-PK11195 SUV and TBR were higher in the carotid plaques of symptomatic compared with asymptomatic patients (Table 2) (Figures 1–3). On CTA, the mean PC score was comparable in both patient groups (Table 2). However, carotid plaques of symptomatic patients had areas of lower attenuation compared with asymptomatic plaques [(median, IQR) 37, 24–40 vs. 71, 56–125 HU, P = 0.01]. There was no significant correlation between ¹¹C-PK11195 TBR and either CT plaque attenuation (r = −0.22, P = 0.23) or PC score (r = −0.01, P = 0.97). Figure 2 shows the distribution of ¹¹C-PK11195 TBR and CT plaque attenuation among symptomatic and asymptomatic patients.

ROC analysis revealed an optimal cut-off to discriminate between symptomatic and asymptomatic patients at 0.92 for...
Target-to-background ratios on $^{11}$C-PK11195 PET significantly correlated with specific binding by $^3$H-PK11195 autoradiography ($r = 0.77$, $P = 0.025$).

**Discussion**

In the present study, we demonstrate that $^{11}$C-PK11195 PET allows the non-invasive detection and quantification of intraplaque inflammation in patients with carotid stenoses. Moreover, the combination of $^{11}$C-PK11195 PET with contrast-enhanced CTA provides an integrated assessment of plaque structure, composition, and biological activity and allows the distinction between recently symptomatic vulnerable plaques and asymptomatic plaques with a high positive predictive value. Focal $^{11}$C-PK11195 uptake did not correlate with either the presence of low attenuation plaques or the PC score. This is in line with prior reports obtained with $^{18}$F-fluorodeoxyglucose (FDG) PET/CT and indicates that information obtained with these two modalities is complementary and enhances the diagnostic performance of the hybrid technique to detect potentially vulnerable plaques. The absence of

$^{11}$C-PK11195 TBR and at 44 HU for CT plaque attenuation. Sensitivity, specificity, and negative and positive predictive values for TBR, CT plaque attenuation, one feature positive, and two features positive plaques are shown in Figure 4.

**Autoradiography, immunohistochemistry, and immunofluorescence**

The mean $^3$H-PK11195-specific binding on autoradiography was $60.6 \pm 16.0\%$, with no significant difference between plaque sections from symptomatic ($n = 3$) or asymptomatic ($n = 5$) patients.

TSPO$^+$ and CD68$^+$ cells were observed in all tissue specimens, and both markers were seen in corresponding areas on adjacent plaque sections (Figure 5C–F). Translocator protein expression by macrophages was confirmed by double immunofluorescence and confocal microscopy (Figure 5G–J). There was no significant difference in the presence of TSPO$^+$ CD68$^+$ cells between the symptomatic and asymptomatic groups. TSPO$^+$ and CD68$^+$ cells were located in areas of high $^3$H-PK11195-specific binding on autoradiography (Figure 5A–F).
either increased $^{11}$C-PK11195 TBR $>$0.92 or low CT attenuation areas $<$44 HU was associated with asymptomatic plaques, whereas all plaques positive for both features were found in patients with an ipsilateral event.

Translocator proteins were discovered in the 1970’s as benzodiazepine-binding sites outside the central nervous system.24 Subsequent studies have shown a high TSPO density in circulating human phagocyte populations, particularly in monocytes and polymorphonuclear neutrophils, with up to 750,000 binding sites per cell.25 Translocator protein expression is higher in mature monocytic cell lines compared with promonocytic or promyelocytic lines and its density increases by a factor of 2–3 after in vitro monocyte activation with interferon-γ or phorbol 12-myristate 13-acetate. Stimulated human monocytes can

---

**Figure 3** Computed tomography angiography and co-registered positron emission tomography/computed tomography cross-sections at 3 mm intervals illustrate differences in plaque composition and $^{11}$C-PK11195 uptake on positron emission tomography/computed tomography among symptomatic (Patient A) and asymptomatic (Patient B) patients. In Patient A, areas of low attenuation (23.5 HU) can be seen (arrows, first column) in close vicinity with foci of increased $^{11}$C-PK11195 uptake (block arrows, second column). In contrast, in Patient B, plaque computed tomography attenuation was higher (76.2) indicating a predominance of fibrotic tissue (Column 3), and no $^{11}$C-PK11195 uptake was noted (Column 4).

---

**Figure 4** Sensitivity (Sens), specificity (Spec), and negative (NPV) and positive predictive values (PPV) to identify patients with cerebrovascular events.
express more than 2,000,000 binding sites for PK11195 and this increase is paralleled by an enhanced expression in CD11a and CD11b surface antigens and augmented production of interleukin-1, -8, and tumour necrosis factor, indicating that TSPO over-expression is a marker of activated phagocytes. PK11195 is a selective TSPO ligand which binds specifically to macrophages in specimens of human carotid atherosclerotic plaque. In a previous publication from this group, we have demonstrated that \(^{15}\)C-PK11195 PET/CTA allows in vivo detection and quantification of vascular inflammation in patients with large vessel vasculitides and can distinguish symptomatic from asymptomatic patients. The present study builds on these existing data and further extends the use of \(^{15}\)C-PK11195 PET/CTA imaging onto patients with atherosclerotic disease. Our results, if
confirmed by larger prospective studies, would suggest that \(^{11}\text{C}-\text{PK11195}\) PET/CTA may help to improve risk stratification in patients with asymptomatic carotid stenoses and identify those at risk for ischaemic cerebrovascular events.

The PET tracer FDG, a biological glucose analogue, has been extensively evaluated for measuring intraplaque inflammation in atherosclerotic lesions.\(^{27,28}\) However, since FDG is taken up by any metabolically active tissue, concerns have been raised about the specificity of this tracer for imaging inflammatory cells. Indeed, micro-autoradiography studies in aortic sections of ApoE\(^{-/-}\) mice have shown that \(^{11}\text{C}-\text{FDG}\) uptake correlates poorly with fat content and selective macrophage staining with anti-CD68.\(^{29}\) Davies et al. reported that in vivo FDG microPET SUVs in atherosclerotic lesions of rabbit aorta were not correlated with macrophage density \((r = 0.16, P = 0.57)\) and there was no significant difference in FDG uptake seen between rabbits with highly inflamed aortic walls, those with low levels of inflammation, or controls.\(^{30}\) Conversely, a high degree of specific binding to macrophages of atherosclerotic plaques has been observed with \(^{3}\text{H}-\text{PK11195}\) in autoradiographic studies which is confirmed by the ex vivo results of the present study.\(^{31}\) However, no definite conclusion on the relative specificity of the aforementioned tracers can be drawn until a direct head-to-head comparison is performed.

One of the strengths of our study compared with prior PET/CT reports\(^{31,32}\) was the use of contrast-enhanced CTA with a high-end CT device. This facilitated delineation of the atherosclerotic plaque thereby improving co-registration with the PET signal and allowed concurrent evaluation of plaque composition. Measurement of CT attenuation within carotid plaques has been shown to identify lipid-rich cores, fibrous tissue, and areas of calcifications on histology.\(^{33}\) The presence of low attenuation plaques has previously been identified as an important predictor for plaque rupture and thrombotic events in the coronary vasculature.\(^{34}\) In our study, a cut-off of 44 HU was identified to provide the best statistical discrimination between symptomatic and asymptomatic plaques, indicating that the presence of lipid-rich areas was a significant determinant of plaque vulnerability. A similar cut-off (30 HU) has been found to be a good discriminator between rupture-prone and stable plaques in the coronary circulation.\(^{34}\) Plaque calcifications were not associated with ischaemic events in our study, which is contrary to prior reports where calcified plaques were up to 21 times less likely to be symptomatic.\(^{35}\)

**Limitations**

The present manuscript was conceived as a proof-of-principle study, and therefore, its results cannot be extrapolated to a general population with carotid artery disease. Moreover, we acknowledge a relatively small number of patients. This precluded the use of multivariate models to test for independent characteristics associated with ischaemic events and warrants larger prospective trials to confirm our findings. Additionally, we did not obtain blood samples to measure serum markers of subclinical inflammation and correlate them with PET/CT plaque findings.

The proximity of the blood pool and limited thickness of the arterial wall can result in spillover and partial volume effects. However, this should affect asymptomatic and symptomatic patients to the same extent and is unlikely to account for any of the differences observed between the two groups. Additionally, care was taken to restrict the ROIs to the plaque and avoid the residual vessel lumen. Correction for spillover and the quantification of receptor kinetics should overcome these potentially confounding factors and further quantitative studies are indicated.

The short physical half-life of \(^{11}\text{C}\)-labelled compounds mandates an onsite cyclotron facility, thus limiting its clinical applicability. However, the introduction of new \(^{18}\text{F}\)-labelled TSPO ligands, which are currently under pre-clinical investigation and have shown high affinity across species in the brain, may overcome some of these limitations.\(^{36}\)

Finally, the added radiation exposure from PET and CT remains an important concern, particularly if repeated studies are performed to assess inflammatory activity before and after treatment. The total effective radiation dose in our patients was well below 10 mSv, which is comparable to a standard cardiac FDG scan.\(^{37}\)

**Conclusions**

The present study provides proof of the concept that imaging intraplaque inflammation in vivo with \(^{11}\text{C}-\text{PK11195}\) and PET combined with contrast-enhanced CT angiography is feasible and can distinguish between recently symptomatic and asymptomatic plaques. Plaques with low CT attenuation and increased \(^{11}\text{C}-\text{PK11195}\) uptake were associated with prior ipsilateral ischaemic events. The clinical value of integrated \(^{11}\text{C}-\text{PK11195}\) PET/CTA plaque assessment needs to be investigated in larger prospective studies.

**Acknowledgements**

We are thankful to Hammersmith Imanet radiographers Andrew Blyth, Hope McDevitt, and Andrea Williams and to our radiochemists Shaun Creasey and Safiye Osman for their excellent technical support. We are thankful to Matthew Morrison, GE Healthcare, Medical Diagnostics, Amersham, UK, for his valuable contribution to study design and revision of the manuscript. We are thankful to Dr Giliola Calori, Division of Metabolic and Cardiovascular Science, Vita-Salute University and Scientific Institute San Raffaele, Milan, Italy, for her statistical support.

**Funding**

O.G. was financially supported by a Swiss National Science Foundation (SNF) research grant. J.S. is supported by the Circulation Foundation Mary Davies Research Fellowship and the Royal College of Surgeons of England/Rosetrees Trust Research Fellowship.

**Conflict of interest:** none declared.

**References**


