DATA SUPPLEMENT

**11C–Metomidate PET–CT versus Adrenal vein sampling to subtype primary aldosteronism: a prospective clinical trial**

**Short Title** 11C–Metomidate PET-CT in Primary Aldosteronism

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* Dr Weekes passed away during the study
Extended Methods

**Diagnostic work-up and Hormonal tests**
Prior to hormonal and subtype tests, antihypertensive medications that interfered with renin-angiotensin-aldosterone system were discontinued at least two weeks in most patients, and potassium-sparing diuretics stopped at least eight weeks in all patients. Patients were prescribed potassium supplementation if hypokalemia was present, aiming for serum potassium of at least 3.5 mmol/L. To confirm the diagnosis of PA, patients were required to have either plasma aldosterone concentration (PAC) > 140 pmol/L after intravenous saline-loading test; or spontaneous hypokalemia with undetectable plasma renin activity (PRA) and PAC > 550 pmol/L. PAC and PRA were analyzed by Mayo Clinic Laboratories, Rochester, Minnesota, USA using high performance liquid chromatography tandem mass spectrometry, and the reference ranges were 0.6-3.0 ng/ml/hr, and 580 pmol/L or less, respectively.

**Production of $^{11}$C-Metomidate**
$^{11}$C-metomidate was manufactured in the Clinical Imaging Research Center using a GE Medical Systems PETtrace 860 cyclotron in compliance with good manufacturing practice (appendix). $^{11}$C-methyl iodide was reacted with a solution of (R)-methyl 1-(1-phenylethyl)-1H-imidazole-5-carboxylic before purification and re-formulation. Patients received an injected dose of 150–300 MBq of $^{11}$C-metomidate with PET acquisition on a Siemens PET-CT scanner. Non-contrast CT images were acquired over the adrenal (140 kV, 64 mA, slice width 3.75 mm) for anatomical correlation. Attenuation and decay-corrected images were converted to standardized uptake value (SUV) maps through division by (injected activity per patient weight). The maximum SUV (SUVmax) over regions of interest were determined for 10-min static images starting 35 min after the injection.

**Repeat AVS**
For the repeat AVS, since cosyntropin stimulation may affect lateralization ratio, in three of four patients, bilateral simultaneous adrenal vein cannulation without cosyntropin infusion (non-stimulated AVS) was done by an experienced interventional radiologist. Non-stimulated AVS was deemed successful if cortisol levels in both adrenal veins were three times or greater than peripheral vein, and lateralization ratios above two were consistent with unilateral PA. After non-stimulated samples were taken, cosyntropin bolus of 250 mcg was administered, followed by a continuous infusion at 50 mcg/hour, and repeat stimulated samples were taken 15 minutes later.

**Histological analysis**
All adrenal glands were paraffin-embedded, and cut into 4 μm thick slides and stained with H&E. Immunohistochemistry was performed on formalin-fixed, paraffin-embedded adrenal sections (4 μm) using an automated immuno-stainer with cover tile technology (Bond-III system, Leica Biosystems). Custom-made antibodies – mouse monoclonal anti-human CYP11B2 (1:5000 dilution), rat monoclonal anti-human CYP11B1 (1:100 dilution), and mouse monoclonal anti-human CYP17A1 (1:800 dilution) were used as primary antibodies.
The diagnosis of classical (single aldosterone producing adenoma, APA) and non-classical (multiple APAs or hyperplasia) PA was made in accordance with the histology of primary aldosteronism (HISTALDO) consensus. Cellular composition of the adenoma was determined by the percentage of cells that were either zona fasciculata-like (clear and lipid rich) or zona glomerulosa-like (compact and eosinophilic). Tissue histology and staining was scored blindly by an experienced histopathologist (M.T).

Immunoreactivity for CYP11B2 was assessed semi-quantitatively by a modified McCarty H-score in line with previously-published assessments. In each field, the percentage of immunopositive cells was assessed and multiplied by a factor (0, 1 or 2) according to the intensity of the immunopositivity (0=no positivity; 1=weak, 2=strong). We assumed that the tumor shape was a sphere and estimated the tumor volume using the formula volume = \( \frac{4}{3}\pi r^3 \), where ‘r’ represents the radius of the nodule.
Supplementary Table S1. Patient with discordant adrenal vein sampling (AVS) and 11C-Metomidate PET-CT (PET) findings (Figure 2F). First AVS showed lateralisation to the right, with increased PET uptake on the left. Second AVS (unstimulated) showed lateralisation to the left.

<table>
<thead>
<tr>
<th></th>
<th>First AVS – sequential under continuous cosyntropin stimulation</th>
<th></th>
<th>Second AVS – bilateral simultaneous unstimulated</th>
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<td></td>
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<td>Peripheral Vein</td>
<td>Left Adrenal Vein</td>
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<td>Metanephrine, nmol/L</td>
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AVS, adrenal vein sampling
### Supplementary Table 2. Individual data of all 25 patients recruited into study, who underwent $^{11}$C–Metomidate PET–CT (PET) and adrenal vein sampling (AVS), and their final subtype diagnosis, treatment and post–treatment outcome

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<th>ID</th>
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<th>Lateralization on PET and/or AVS</th>
<th>Tumor SUVmax</th>
<th>Contra–lateral SUVmax</th>
<th>PET SUVmax Ratio</th>
<th>AVS Lateralisation Ratio</th>
<th>CT nodule</th>
<th>PASO Biochemical Outcome</th>
<th>PASO Clinical Outcome</th>
<th>IHC by HISTALDO / diameter (mm)</th>
<th>APM</th>
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</table>

a Initial AVS was successful but did not show lateralization. Repeat AVS subsequently showed lateralization
b small visible tumor seen on PET–CT, but not initially seen on CT
c no obvious tumor, and Tumor SUVmax taken from side with higher uptake, and SUVmax ratio taken from higher to lower side
d absent clinical success by PASO consensus, although decrease in DDD of medications, but rise in blood pressure
e minimal normal adrenal tissue to assess

APA, aldosterone–producing adenoma; APN, aldosterone–producing nodule; APM, aldosterone–producing micronodule; CT, computed tomography; IHC, immunohistochemistry; HISTALDO, histopathology of primary aldosteronism consensus; NA, not applicable; PASO, Primary Aldosteronism Surgery Outcome; SUVmax, maximal standardized uptake value;
References


