DATA SUPPLEMENT

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

Short Title ^{11}C–Metomidate PET-CT in Primary Aldosteronism

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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 3·5mmol/L. To confirm the diagnosis of PA, patients were required to have either plasma aldosterone concentration (PAC) >140pmol/L after intravenous saline-loading test; or spontaneous hypokalemia with undetectable plasma renin activity (PRA) and PAC >550pmol/L.[1] 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 0·6-3·0ng/ml/hr, and 580pmol/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 3·75mm) 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[2], in three of four patients, bilateral simultaneous adrenal vein cannulation without cosyntropin infusion (non-stimulated AVS) was done by an experienced interventional radiologist[3]. 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[4]. After non-stimulated samples were taken, cosyntropin bolus of 250mcg was administered, followed by a continuous infusion at 50mcg/hour, and repeat stimulated samples were taken 15 minutes later.

Histological analysis

All adrenal glands were paraffin-embedded, and cut into 4um 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.[5]

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.[6] 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).[6] 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[7] in line with previously-published assessments.[8,9] 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 = $4/3\pi r^3$, 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.

First AVS – sequential under continuous cosyntropin stimulation			
	Right Adrenal Vein	Peripheral Vein	Left Adrenal Vein
Aldosterone, pmol/l	290100	1758	46840
Cortisol, nmol/L	27920	865	22460
Second AVS – bilateral simultaneous unstimulated			
	Right Adrenal Vein	Peripheral Vein	Left Adrenal Vein
Aldosterone, pmol/l	1385	443	9169
Cortisol, nmol/L	853	163	2180
Metanephrine, nmol/L	14.69	0.05	8.58
Aldosterone-to-Cortisol Ratio	1.62	2.72	4.21

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

ID	Treat-ment	Final Subtype	Lateralization on PET and/or AVS	Tumor SUVmax	Contra–lateral SUVmax	PET SUVmax Ratio	AVS Lateral–isation Ratio	CT nodule	PASO Biochemical Outcome	PASO Clinical Outcome	IHC by HISTALDO / diameter (mm)	APM
10	Surgery	Left	Both	37·0	16·6	2·23	202·5	Left	Complete Success	Partial Success	Single APA / 16	2
7	Surgery	Left	Both	34·1	16·1	2·12	40·3	Left	Complete Success	Partial Success	Single APA / 12	2
8	Surgery	Left	Both	50·5	26·6	1·90	77·4	Left	Complete Success	Partial Success	Single APA / 10	0
20	Surgery	Left	Both	26·8	16·2	1·65	11·6	Left	Complete Success	Partial Success	Single APA /20	1
21	Surgery	Right	Both	34·6	24·1	1·44	32·3	Right	Complete Success	Absent Success [d]	Single APA / 10	2
12	Surgery	Right	Both	42·3	29·7	1·42	15·9	Right	Complete Success	Absent Success [d]	Single APA / 19	NA[e]
13	Surgery	Right	Both	22·9	16·1	1·42	350·9	Right	Complete Success	Partial Success	Single APA / 16	1
3	Surgery	Right	Both	24·5	17·5	1·40	69·9	Right	Complete Success	Absent Success	Single APN / 9	0
19	Surgery	Right	Both	24·6 [b]	17·9	1·37	22·9	Nil	Complete Success	Absent Success	Single APN / 5	0
17	Surgery	Right	Both	13·2 [b]	10·1	1·31	14·4	Nil	Complete Success	Partial Success	Single APN / 5	3
1	Surgery	Right	Both	29·5	24·4	1·21	10·5	Right	Complete Success	Complete Success	Single APA / 14	2

22	Surgery	Right	Both	24·0	22·0	1·09	5·5	Right	Complete Success	Partial Success	Non–classical (Two CYP11B2–negative APA) / 7	3
24	Surgery	Left	PET	46·7	22·8	2·05	2·8	Left	Complete Success	Absent Success	Single APN / 7	2
23	Surgery	Right	PET	47·3	24·2	1·95	3·1	Right	Complete Success	Complete Success	Single APA / 11	1
15	Surgery	Right	PET	32·2	20·1	1·60	1·0 [a]	Right	Complete Success	Complete Success	Single APA / 15	0
18	Surgery	Right	PET	30·5 [b]	24·9	1·22	3·4 [a]	Nil	Complete Success	Partial Success	Single APN / 5	3
16	Surgery	Right	AVS	35·6 [c]	33·4	1·07 [c]	9·6	Nil	Complete Success	Complete Success	Single APA / 11	1
4	Surgery	Left	AVS	34·3	37·0	0·93	22·6	Bilateral	Complete Success	Partial Success	Non–classical (APN+hyperplasia) / 8	1
2	Surgery	Right	AVS	39·2	60·0	0·65	7·8	Right	Complete Success	Partial Success	Single APA / 11	1
6	Surgery	Right	Neither [a]	11·6	16·7	0·69	3·4 [a]	Right	Complete Success	Complete Success	Single APA / 23	2
14	Medication	Left	PET	41·7 [c]	28·2	1·48 [c]	3·5	Right	NA	NA	NA	NA
25	Medication	Left	PET	28·5	25·0	1·14	3·4	Left	NA	NA	NA	NA
9	Medication	Indeter–minate	PET–left AVS–right	37·2	15·0	2·48	5·0	Left	NA	NA	NA	NA
5	Medication	Bilateral	None	26·9 [c]	24·3	1·11 [c]	2·3	Nil	NA	NA	NA	NA

11	Medication	Bilateral	None	21·1	25·9	0·81	1·3	Left	NA	NA	NA	NA

[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;

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