^{11}C-METOMIDATE PET/CT IS A USEFUL ADJUNCT FOR LATERALISATION OF PRIMARY ALDOSTERONISM IN ROUTINE CLINICAL PRACTICE

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ABSTRACT

Objective: To describe clinical practice experience of ^{11}C-Metomidate PET/CT as an adjunct to adrenal vein sampling (AVS) in the lateralization of aldosterone producing adenomas (APA) in primary aldosteronism (PA).

Context: Accurate lateralisation of aldosterone producing adenomas in the setting of primary aldosteronism (PA) offers the potential for surgical cure and improved long-term cardiovascular outcomes. Challenges associated with adrenal vein sampling, the current gold-standard lateralization modality, mean that only a small proportion of potentially eligible patients currently make it through to surgery. This has prompted consideration of alternative strategies for lateralisation, including the application of novel molecular PET tracers such as ^{11}C-Metomidate.

Design: Clinical Service Evaluation / Retrospective audit.

Patients: Fifteen individuals with a confirmed diagnosis of PA, undergoing lateralization with ^{11}C Metomidate PET/CT prior to final clinical decision on surgical versus medical management.

Measurements: All patients underwent screening aldosterone renin ratio (ARR), followed by confirmatory testing with the seated saline infusion test, according to Endocrine Society Clinical Practice Guidelines. Adrenal glands were imaged using dedicated adrenal CT. ^{11}C-Metomidate PET/CT was undertaken due to equivocal or failed AVS. Management outcomes were assessed by longitudinal measurement of blood pressure, ARR, number of hypertensive medications following adrenalectomy or institution of medical therapy.

Results: We describe the individual lateralisation and clinical outcomes for 15 patients with PA.

Conclusion: ^{11}C-Metomidate PET/CT in conjunction with adrenal CT and AVS provided useful information which aided clinical decision making for PA within a multidisciplinary hypertension clinic.

INTRODUCTION

Primary Aldosteronism (PA) is the commonest cause of secondary hypertension. It is associated with a higher risk for cardiovascular, cerebrovascular and renal hypertensive complications when compared to blood pressure (BP) matched essential hypertension.[1-3] PA is driven by aldosterone hypersecretion from one or both adrenal glands that bypasses normal renin-angiotensin-aldosterone system (RAAS) regulation. When PA arises from a single adrenal gland, adrenalectomy removes the source of aldosterone excess [4], with resultant biochemical cure and significant improvement or resolution of hypertension.[5-9] Adrenalectomy is also associated with improved cardiovascular outcomes beyond those which can be achieved by medical therapy alone. In contrast, bilateral disease is managed pharmacologically with primacy given to mineralocorticoid receptor antagonists (MRA).[6, 10] Distinguishing between unilateral and bilateral PA, so-called lateralisation, is therefore central to determining whether primary medical therapy or adrenalectomy is recommended.

Lateralisation of PA is challenging. Adrenal vein sampling (AVS), the current gold standard, is technically demanding, resource intensive and only performed to a high standard in a small number of centres. Consensus statements for AVS do not offer uniform advice relating to procedural approach or thresholds for interpretation of the selectivity (SI) or lateralisation (LI) indices.[11, 12] The use of contralateral suppression of aldosterone secretion by the non-lateralising gland can serve as to an indicator of lateralisation where the LI does not reach recommended thresholds. However, this index may also be affected by the use of intra-procedural ACTH-stimulation.[12, 13] Additionally, clinically important APAs which co-secrete cortisol, (suppress DHEA-S and ACTH; lack of dexamethasone suppression) albeit relatively rare highlight the potential for false negative lateralisation using AVS by virtue of yielding a lower aldosterone:cortisol on the side of the lesion. [14] Overall, AVS has been reported to yield unsatisfactory or indeterminate results in 15-30% of all cases [11, 15, 16].

Cross-sectional imaging of the adrenal glands [most commonly with computed tomography (CT)] is a useful imaging modality for lateralisation in the setting of a distinct, unilateral APA >8mm

diameter, with a normal appearing or atrophied contralateral gland.[17-20]. However, the scenario where a unilateral adenoma (>8mm) can be confidently reported to justify equally confident lateralisation using the CT modality alone accounts only for approximately 20% of all PA cases. Lateralisation using CT alone is also of limited value for the detection of smaller adrenal lesions (~5mm) which are commonly encountered in PA, or for individuals who have unilateral disease but bilateral adrenal abnormalities.[21]

Positron emission tomography (PET) using molecular tracers that bind steroidogenic enzymes, such as ^{11}C-Metomidate, represents an additional approach to localising the source of aldosterone excess. Metomidate binds to both CYP11B1 (ACTH-dependent) and CYP11B2 (ACTH-independent). Pre-imaging suppression of *background* adrenal CYP11B1 using low-dose dexamethasone enhances the selectivity of metomidate for CYP11B2 and improves sensitivity and specificity for detection of unilateral APAs.[22] Accordingly, ^{11}C-Metomidate PET/CT has been proposed as an adjunctive or alternative lateralisation modality in individuals for whom AVS and/or adrenal CT has not provided a clear answer in the clinical setting.[22-25])

Since 2014, we have used ^{11}C-Metomidate PET/CT as an adjunct and/or alternative lateralisation modality for PA within the setting of a multidisciplinary hypertension clinic. Herein, we report our experience of using this imaging modality to guide clinical decision-making in routine clinical practice.

METHODOLOGY

Study Design & Patients

A clinical service evaluation was performed of patients attending a resistant/young hypertension clinic at Galway University Hospital between 2014 and 2018, who met the following criteria: (i) diagnosis of PA, (ii) had undergone lateralisation with AVS, ^{11}C Metomidate PET/CT or both, (iii) had outcome data for >6 months following adrenalectomy or commencement of MRA (for bilateral disease or those awaiting adrenalectomy). Each patient (n=15) had been referred for work-up and management of hypertension from primary or secondary care for the following reasons: (i) young age of hypertension onset (<40 years), (ii) hypertension complicated by cardiovascular disease or hypokalaemia, (iii) difficulty in controlling hypertension on three or more anti-hypertensive medications. Approval was received from the Research Ethics Committee at Galway University Hospital (GUH) and data were used with their informed consent.

Screening and Diagnosis of Primary Aldosteronism

All patients underwent standardised screening and diagnostic approach for endocrine hypertension. PA was diagnosed according to Endocrine Society Guidelines.[26] Screening used a mid-morning aldosterone renin ratio (ARR) following two hours of ambulation with each patient seated for 15 minutes prior to venesection. Washout of interfering medications was performed on all patients. Confirmatory testing was performed using the seated saline infusion test (SSIT).[27] BP was controlled during screening and diagnosis using a combination of doxazosin and a calcium channel antagonist. Potassium was corrected where necessary in advance of all blood sampling. Threshold ARR and aldosterone values are reported according to locally determined laboratory reference ranges: suppressed plasma renin activity (PRA) was accepted at values < 1.0 ng/mL/h, and direct renin concentrations (DRC) < 9.0 mIU/L; raised aldosterone renin ratio (ARR) was accepted at values >800 pmol/L:ng/mL/h when calculated using the PRA and >35 pmol:mIU when using the DRC.[28]

Lateralisation

Upon diagnosis of PA, each patient underwent 3mm slice CT scan of the adrenal glands (pre-contrast and venous phases). Lateralisation was assessed using adrenal vein sampling (AVS) and/or ^{11}C-Metomidate PET/CT. Adrenal vein sampling is the lateralisation modality of choice and is

considered for all patients. ^{11}C-Metomidate PET/CT was chosen as an adjunct or alternative lateralisation modality under the following circumstances: (i) prior failed AVS; (ii) unable to stop interfering medications for diagnosis or lateralisation; (iii) patient refusal of AVS; (iv) equivocal lateralisation using AVS (defined as a LI between 2 and 3.9 using ACTH-unstimulated AVS); (iv) discordance between CT appearance of the adrenals and the LI on AVS.

Adrenal Vein Sampling

AVS was performed at GUH by a single operator (GJOS). Patients were maintained overnight in the supine position. AVS was performed without ACTH stimulation. Selective, sequential catheterization under ultrasound guidance, was performed of the right, followed by the left adrenal vein, using a 5F catheter optimised for acquisition of sufficient blood volumes. Duplicate blood samples were collected for cortisol and aldosterone from each adrenal vein, coupled with a peripheral sample from the antecubital fossa and an inferior vena cava sample.

Selectivity indices (SI) were assessed using the cortisol ratios between the adrenal and peripheral veins; the lateralisation index (LI) was determined using the left to right adrenal vein aldosterone ratios adjusted for cortisol.[12] Successful catheterisation for each adrenal vein was demonstrated by a threshold SI $\geq$2. A threshold LI $\geq$4 indicated unilateral PA, with values between 2 and 4 considered equivocal. AVS results were correlated with images from CT adrenals.[11, 12] The contralateral suppression index was calculated for each patient by dividing the aldosterone: cortisol ratio on the non-dominant side by the peripheral aldosterone: cortisol ratio. Values <1.0 were interpreted as indicative of contralateral suppression.[12, 13]

^{11}C-Metomidate PET/CT

^{11}C-Metomidate PET/CT was performed at Addenbrooke's Hospital Cambridge. All patients were pretreated with 0.5mg dexamethasone 6 hourly for 72 hours prior to scanning.[22] ^{11}C-Metomidate was manufactured on site in compliance with good manufacturing practice using a GE Medical Systems PETtrace cyclotron (Milwaukee, WI), as previously described.[22]

PET-CT was performed on a GE Discovery 690 PET-CT scanner (GE Medical Systems). Noncontrast CT images were acquired over the adrenals (140 kV, 64 mA, slice width 3.75 mm). After an IV injection of ^{11}C-metomidate (150–500 MBq), dynamic PET images were acquired for 45 min. Attenuation and decay-corrected images were converted to standardised uptake value maps

through division by injected activity per patient weight. The maximum standardised uptake values (SUV_{max}) over regions of interest were determined for 10-min static images starting 35 min after the injection. Images were analysed and reported in line with previously published standards.[22] Lateralisation of ^{11}C Metomidate PET/CT was interpreted as an SUV_{max} ratio of 1.25 to the lateralising side [22], or focal uptake of metomidate within a lateralising nodule.

Laboratory assays

ARR was calculated from renin and aldosterone measured using 2 assay systems, the Immunodiagnostics Systems (IDS/iSYS®) chemiluminescent immunoassay (CLIA) and the (RADIM/MAIA®, Adaltis, Bologna, Italy), radioimmunoassay (RIA). DRC and aldosterone were measured in ethylenediaminetetraacetic acid (EDTA) plasma using the IDS/iSYS® platform. DRC was measured using a sandwich immunoassay employing two monoclonal antibodies. The assay is calibrated to the WHO International Standard 68/356. The inter-assay precision expressed as coefficient of variation (CV_A%), at a mean DRC of 14mIU/L, 100.3mIU/L and 390.2mIU/L was 7.7%, 8.4% and 4.9% respectively. Aldosterone was measured by competitive immunoassay traceable to liquid chromatography-tandem mass spectrometry. The between-run CV_A% at a mean aldosterone of 238 pmol/L, 442pmol/L and 1648pmol/L was 9.71%, 9.37% and 3.83% respectively. PRA and aldosterone were also measured using RADIM/MAIA® RIA, in EDTA and serum respectively. Between-assay precision for PRA gave CV_A% of <10% at PRA of 0.69ng/mL/h and <5% at PRA of 2.96 - 6.33 ng/mL/h. For aldosterone the inter-assay precision gave a CV_A% of <5% at concentrations of between 244 – 3805pmol/L. Screening ARR was calculated using either of the two assay systems, based on the time of sampling. Analysis of aldosterone from samples drawn during AVS was performed using the Immunodiagnostics Systems (IDS/iSYS®) chemiluminescent immunoassay (CLIA).

Results from each assay system were used to calculate the aldosterone renin ratio (ARR) to provide the following decision thresholds: for IDS-iSYS® assay, the decision threshold was >35pmol:mIU; for RADIM/MAIA® RIA assay, the decision threshold was >800 pmol/L:ng/mL/h.

For AVS, dexamethasone suppression tests and ACTH-stimulation tests, serum cortisol was measured using Roche Diagnostics Cortisol II electrochemiluminescence immunoassay (ECLIA) on the Cobas® 8000 analyser. The lower and upper limits of measurements were 0.5 and 1750 nmol/L.

The between-run CV_A% at a mean cortisol of 112 nmol/L, 485 nmol/L and 1080 nmol/L was 3.4%, 2.5% and 3.2% respectively.

All assays were performed within a medical testing laboratory accredited to ISO15189: 2012 standards.

Management and Follow-up

Primary aldosteronism was managed surgically by unilateral adrenalectomy or medically with MRA (spironolactone or eplerenone) or amiloride. The latter was used only when MRA therapy was not tolerated or in sexually active women of reproductive age, not using contraception.

Unilateral adrenalectomy was performed using a posterior retroperitoneoscopic approach. Post-operatively, each patient was followed up with measurement of BP, serum potassium, and plasma renin and aldosterone within 24 hours, and at three monthly intervals thereafter for one year.

For medical management, either spironolactone, eplerenone or amiloride were used as primary therapy, with doses up-titrated to maintain: (i) renin and potassium within the normal reference range, (ii) optimised BP control and (iii) <20% decrease in estimated GFR.[6, 10, 16] Where potassium sparing diuretic monotherapy did not produce adequate BP control, the second line agent of choice was a dihydropyridine calcium channel antagonist.

RESULTS

Patient Characteristics

In total, 15 individuals with PA underwent ^{11}C Metomidate PET/CT scan (Table 1 & 2). AVS was performed in 9 of these cases, and successful cannulation of both adrenal veins was achieved in 8 of 9 (94%). An additional two patients (Cases 1 & 3) had unsuccessful AVS performed elsewhere, prior to referral to our service. In total, 42 AVS procedures have been carried out at our centre since 2014 with a success rate for cannulation of both adrenal veins (SI > 2.0) in 41/42 (97%).

The reasons for performing ^{11}C Metomidate PET/CT were as follows: (i) Three patients underwent unsucccessful AVS procedures (Table 1; Cases 1, 3 & 8); (ii) two patients were deemed unsuitable for AVS due to onset of severe hypertension or hypokalaemia upon stopping interfering medications (Table 1 Cases 10 & 13); (iii) two patients refused AVS (Case 4 & 12); (iv) four patients had LI between 2.0 and 4.0 (Table 1 Cases 5, 6, 11 & 14); (v) four patients had LI <2.0 but multidisciplinary discussion recommended further investigation based on visible unilateral adrenal nodules, difficult to control hypertension and/or hypokalaemia (Table 1 Cases 2, 7, 9 & 15).

Lateralisation

Table 1 demonstrates lateralisation data for AVS and/or ^{11}C-Metomidate PET/CT for all 15 patients. Lateralisation to one adrenal gland was demonstrated in 5/15 individuals (Table 1, Figure 1). Bilateral disease was present in ten cases (Table 1, Figure 1).

AVS, adrenal CT and metomidate were concordant in 6 of 8 (75%) individuals who underwent both lateralisation modalities (Table 1, Figure 1). For cases 2 and 15, AVS and metomidate were not fully concordant. In case 2, AVS was consistent with bilateral disease with a LI of 1.5 to the left and without contralateral suppression of aldosterone. However, a single lesion was identified on CT in the body of the left adrenal gland, and this lesion demonstrated the highest level of metomidate uptake (Figure 1C). While metomidate uptake didn't meet previously published criteria for definitive lateralisation,[22] the patient was intolerant of MRA therapy and elected to proceed to left adrenalectomy. Aldosterone at 24h post-operatively was undetectable and the patient has controlled hypertension on fewer agents without hypokalaemia. Subsequent follow-up ARR were performed while the patient was taking a beta-blocker for left ventricular hypertrophy (LVH) that may have affected renin (Table 2).[29, 30] For Case 15, AVS was also consistent with bilateral

disease, showing a LI 1.4 in favour of the left, without contralateral aldosterone suppression. Adrenal CT demonstrated a right-sided nodule (9mm diameter) without nodularity on the left. ^{11}C-Metomidate PET/CT demonstrated a clear focus of increased tracer uptake within the visualised right adrenal nodule (Figure 2D) and therefore considered to have lateralised to the right. This patient has moved to another jurisdiction and awaits adrenalectomy.

For those who were unsuitable for, declined AVS or for whom AVS was unsuccessful three of seven individuals lateralised using ^{11}C-Metomidate PET/CT (Table 1: Cases 1, 3 & 13). Two of these have proceeded to surgery. The third, case 13 requires the presence of a bariatric and adrenal surgeon for adrenalectomy and is undergoing weight optimisation. Of note, for case 13, ^{11}C-Metomidate PET/CT aided diagnosis and lateralisation (Table 1, Case 13, Figure 1E).

For adrenal CT, lateralisation criteria as described by the SPARTACUS investigators (i.e. a unilateral nodule $\geq$8mm diameter with no nodularity of the contralateral gland) [18] were present in 7 patients and these findings were not in agreement with AVS (which showed bilateral disease in 6 of 7). Following PET/CT, four of seven patients satisfied ^{11}CMettomidate PET/CT criteria for unilateral disease (Cases 1, 2, 13 & 15) demonstrating focal metomidate uptake in the visible nodule. Of these cases 13 and 15 await surgical outcome. The remaining three individuals with visible nodules on CT were classified as bilateral disease (Cases 7, 11 & 12).

Treatment and Outcomes

Surgical Management

In total, ^{11}C-Metomidate PET/CT informed the decision to proceed to adrenalectomy in four patients who would not otherwise have been offered surgery.

For the three (Cases 1-3) lateralised cases, two satisfied criteria for complete postoperative cure (biochemistry fully normalised, BP within target and off antihypertensive agents) at follow-up beyond six months (Table 2: Cases 1 & 3).

Two of 4 patients that underwent surgery demonstrated partial cure only (Table 2: Cases 2 & 4). Case 2 is described in detail above. Case 4 (Table 1 & 2; Figures 1F) demonstrated focal uptake in multiple nodules on ^{11}C-Metomidate PET/CT, but with left sided dominance. The patient experienced clinically significant hypokalaemia and grade 3 hypertension upon MRA withdrawal. A decision to proceed to left adrenalectomy was taken with the aim of achieving improved BP and K

control in advance of fertility therapy. Surgery resulted in partial cure with improved BP on fewer agents (from six preoperatively to 2 post-operatively) and maintained at >2 years (Table 2). Biochemistry remains suggestive of persistent PA, albeit confounded by beta-blocker therapy. [24]

No complications relating to surgical procedure were observed for any individual. ACTH stimulation tests were performed on all surgical patients on the first post-operative morning (8-10am). Two of four patients had low morning cortisol and/or a suboptimal response to 250mcg tetracosactrin (serum cortisol <450 nmol/L) in the immediate post-operative period, which resolved within 3 months.[12] For each surgical patient at least one adrenal adenoma, with zona fasciculata-like cells, >5mm was described (Supplementary Table).

Medical Management

Within the medically managed cohort, two individuals lateralised and await adrenalectomy for reasons already discussed (Table 1 & 2; Cases 13 & 15). Each has target BP control with improving renin concentrations with MRA therapy.

Of the remaining cases, nine of eleven had bilateral disease. Case 12 is of reproductive age, declined AVS and elected not to take MRA therapy. Her BP control has improved since commencing a progesterone only pill in combination with dihydropyridine CCB.

Three of nine patients (Table 2; Cases 5, 6, & 7) are treated with single-agent MRA only and demonstrate normal renin concentrations. A further three of these nine patients (Table 2; Cases 8, 10 & 11) similarly have normal renin concentrations and BP within the target range, but are taking one or more anti-hypertensives in addition to MRA. Finally, two patients (Table 2: Cases 9 & 14) continue to show a suppressed renin level and BP above target despite MRA therapy. Both patients admit to variable compliance with MRA therapy.

All patients treated with MRA are taking fewer anti-hypertensives with better BP control when compared to the period prior to addition of MRA.

DISCUSSION

We demonstrate that molecular imaging with ^{11}C-Metomidate PET/CT is a useful adjunct to lateralisation and guiding treatment decisions in PA, within a centre with high quality AVS (success rate 97%).

Within our centre and in keeping with previously published series (1-3,5,9), approximately 50% of cases demonstrate unilateral disease (data not shown). The majority of these cases are readily identified using AVS. The cases represented herein demonstrated equivocal lateralisation results using AVS and CT, had an AVS procedure that was unsuccessful or did not undergo AVS for reasons described above. Four of these individuals underwent unilateral adrenalectomy, two of whom demonstrated complete cure and two demonstrated partial cure despite many years of poorly controlled hypertension. Two further individuals have lateralised definitively and await adrenalectomy. Overall, six of fifteen individuals (40%) were offered surgery with greater diagnostic certainty than would have been otherwise available. Our findings therefore suggest that molecular (PET) imaging can complement AVS and adrenal CT in identifying patients with unilateral PA, and aid surgical referral where there is equivocation. Within this small series we demonstrate 75% concordance between AVS and ^{11}C Metomidate PET/CT. For the discordant cases, there was CT evidence of a unilateral nodule on the lateralising side in agreement with ^{11}C Metomidate PET/CT.

The importance of accurate lateralisation of PA is supported by outcome-based clinical studies. Retrospective datasets from multiple centres have demonstrated consistent association between PA and cardiovascular disease, which is higher than that of matched essential hypertension.[9, 10, 16] Within several series, adrenalectomy provided improved longterm cardiovascular outcomes when compared to MRA therapy.[6, 7, 9, 10] A recent observational study has suggested that biochemical control of PA in addition to BP control is essential, even amongst medically treated cohorts. Specifically, in patients receiving MRA, better cardiovascular outcomes were achieved only in those with normalised renin.[16, 31] Within the presented cohort, amongst those who lateralised and underwent adrenalectomy, 2 of 3 met criteria for complete cure.(5) Of those without complete cure, all readily achieved target BP on fewer anti-hypertensives following surgery. Eight of 10 individuals treated with MRA achieved normal renin, which was sustained for >6 months after initiating therapy.

It is well recognised that AVS presents significant challenges which limit its wider application, including: (i) difficulty in successfully cannulating both adrenal veins and, (ii) lack of consensus in relation to procedural methodology i.e. ACTH-stimulated versus unstimulated, sequential versus simultaneous cannulation. As such, in spite of its '*gold standard'* status, there is variability of technique and considerable subjectivity of interpretation of the SI and LI from centre-to-centre.[11, 12, 15, 32] Sequential, unstimulated AVS has been performed by a single operator (GJO'S) at our centre since late 2014, producing success rates for cannulation of both adrenal veins which are above 97%, when applying a SI of 2.0. We consider definitive lateralisation as ≥4.0 in line with international consensus [12] and equivocal lateralisation at LI values between 2.0 and 4.0. In this regard 11C Metomidate PET/CT confirmed bilateral disease in 3 individuals and demonstrated unilateral disease in 1, which directly influenced our clinical recommendation. In addition, these data have assisted us in defining local threshold LI cut-offs within our relatively young service. It is noteworthy that lateralisation within our clinical service takes a median duration of 2.3 years from presentation to lateralisation, and involves 1.7 episodes where medication changes were made to facilitate interpretable investigation. This highlights the complexity of the preparation necessary for AVS and speaks to the need for simpler lateralisation modalities, less susceptible to medication interference.

Historical studies suggest that lateralisation of an APA using CT alone is not sufficient to justify adrenalectomy, given poor sensitivity and specificity, with the exception of patients aged less than 35 years.[33] A recent prospective study from the SPARTACUS investigators challenged these findings and renewed debate relating to the utility of CT.[18] In their prospective study, CT localisation of APAs demonstrated equivalent sensitivity and specificity to AVS. However, study participants had visible adrenal lesions >7mm and a large proportion had hypokalaemia (or "*low normal" serum* potassium) at the time of presentation.[21, 34] When applied to the broader characteristics of patients diagnosed with PA, the sensitivity of CT remains low when adhering to criteria based on the patient characteristics within the SPARTACUS study.

Within our small series, CT did not demonstrate adequate specificity as a lateralisation modality, even when using the criteria described by the SPARTACUS investigators, to justify referral for surgery when AVS was equivocal or not performed. However, the presence of a nodule on CT informed referral for ^{11}C Metomidate PET/CT in seven individuals, four of whom lateralised. The numbers presented in this manuscript are not sufficient to draw definitive conclusions in relation to

the performance of CT as a sole lateralising modality. However, it is not our usual clinical practice to justify referral for adrenalectomy in cases of PA on the basis of adrenal CT alone. In this regard however, ^{11}C Metomidate PET/CT has demonstrated its greatest clinical usefulness in lateralising an APA in the presence of a unilateral adrenal nodule on CT. Future data from larger studies, such as the ongoing MATCH study will inform the true utility of metomidate PET/CT as an adjunct to CT adrenals, equivocal AVS or both.

Metomidate is a high affinity ligand for both CYP11B1 and CYP11B2.[22] This presents a concern that sensitivity for CYP11B2 expressing lesions may be subject to interference from non-specific binding of metomidate to CYP11B1.[35] It also raises the possibility that specificity for CYP11B2-binding within APAs may be imprecise due to tracer sink. These concerns are relevant when using imaging such as single-photon emission computed tomography (SPECT), but are largely alleviated when using PET/CT preceded by dexamethasone pre-treatment to suppress pituitary ACTH secretion, and background adrenocortical expression of CYP11B1.[22] Ongoing research in several centres is directed towards identifying CYP11B2-selective tracers, as well as chemokine-tracers which may offer improved specificity for APAs. However, it is noteworthy that less specific tracer binding to steroidogenic CYP11B1 and CYP11B2 may offer benefits in identifying and localising adenomas which co-secrete glucocorticoids and mineralocorticoids. A recent study of urinary steroid metabolomics data described excess glucocorticoid secretion from APAs, within individuals who have appropriate cortisol suppression following dexamethasone (ascribed *"Connshing's Syndrome").*[14]. In this study, post-operative partial adrenocortical insufficiency was also described in up to 30% of individuals with PA who underwent unilateral adrenalectomy. Within our cohort, partial adrenocortical insufficiency occurred in 2 of four adrenalectomised patients (Supplementary Table), and within our total cohort, we see partial adrenocortical insufficiency in 40% following adrenalectomy for APA.

Our data highlight several 'real world' clinical practice dilemmas encountered during the investigation and management of PA, and provide insights in to potential additional benefits of ^{11}C-Metomidate PET/CT. We describe a single case (Case 4) in whom the decision to proceed to unilateral adrenalectomy, with improved clinical outcome, was taken in the context of investigations that suggested non-lateralising disease.(26) Clearly further prospective studies will be required to determine whether PET/CT alone, or in combination with AVS, has the potential to guide unilateral intervention to reduce disease burden even when bilateral disease is suspected.

While we describe the utility of ^{11}C-Metomidate PET/CT, as a less complicated functional imaging modality for lateralisation of PA, it is not without its drawbacks. For ^{11}C-Metomidate PET/CT imaging in PA, an SUV_{Max} of 1:1.25 previously demonstrated a specificity of 87% to lateralise APAs. .[22] While we have employed these thresholds, we acknowledge that the study by Burton *et al* was carried out in a small cohort of 39 individuals and that the performance of this imaging modality awaits validation within a larger PA cohort. The major limitation of metomidate PET/CT relates to the short half-life of C11 tracers and the need for imaging to be carried out close to the tracer manufacturing site. Currently, only a handful of centres worldwide routinely manufacture ^{11}C-Metomidate and access to this imaging modality is limited by the long distances some patients may have to travel to a provider centre. Given this limitation, the widespread availability of ^{11}C-Metomidate is unlikely to occur. However, several more stable novel ^{18}F tracers are under development which may permit wider availability to lateralisation of APAs using PET/CT imaging in the future. The pursuit of molecular imaging in this context is important and worthwhile.[35-37]

In summary, we report our clinical experience of combining cross-sectional imaging, adrenal vein sampling and molecular imaging with ^{11}C-Metomidate PET/CT for lateralisation of PA in patients attending a hypertension clinical service. We highlight the potential utility of functional imaging as an adjunct to lateralisation with conventional CT and AVS. We describe how ^{11}C-Metomidate PET/CT aided clinical decision making which allowed more patients to be considered for adrenalectomy by increasing diagnostic confidence, especially when AVS has been unsuccessful or when there was a visible unilateral nodule on adrenal CT. Given the high prevalence of PA in patients with hypertension and the inherent challenges of AVS, our findings suggest that efforts to increase the availability of molecular tracers that can reliably identify the source of aldosterone excess in PA is an important and worthwhile pursuit.

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Figure 1

Merged CT and PET images which demonstrate functioning aldosterone producing lesions for Cases 2, 3, 4, 10, 13, 15 . Each case is also represented in Tables 1 & 2. Yellow arrows mark the aldosterone producing lesions, as identified using ^{11}CMetomidate PET/CT.

Table 1: Summary of Lateralisation Data for Patients with Confirmed Primary Aldosteronism

		Adrenal Vein Sampling			CT Abdomen	11C Metomidate PET/CT					
Treatment	**Lateralisation Modality**	**Lateralisation Index**	**Selectivity Index**	**C/L Suppressibility Index**	**Lateralising Nodule >8mm (Left/Right)**	**SUVMax Background**		**Peak SUVMax Nodule**	**SUVMax Lateralising Ratio**	**Reason for Metomidate**	**Decision**
						Right	**Left**				
Surgery											
1	Both	-	1.04 (R); 46.4 (L)	NA	Yes (R)	15.4	13.3	23 (R)	1.72 (R)	(i)	Unilateral R
2	Both	1.5 L	29.6 (R) 61.2 (L)	1.64	Yes (L)	13.5	11	16 (L)	1.23 (L)	(v)	Unilateral L
3	Both	-	0.79 (R) 1.88 (L)	NA	No	16.9	16.7	25.4 (L)	1.52 (L)	(i)	Unilateral L
4	Met	-	-	-	No	15.5	15.2	26.5 (L) 21.7 (R)	1.22 (L)	(iii)	Bilateral L>R
Medical											
5	Both	2.0 R	42.9 (R) 81.3 (L)	0.8	No	11.5	12.1	NA	1.05 (L)	(iv)	Bilateral
6	Both	2.8 L	5.85 (R) 35.1 (L)	1.48	No	13.5	14.8	NA	1.09 (L)	(iv)	Bilateral
7	Both	1.0	14.3 (R) 21.5 (L)	NA	Yes (L)	11.3	9.2	10.8 (L)	1.22 (L)	(v)	Bilateral
8	Both	-	1.5 (R) 67.9 (L)	NA	No	16.8	16.0	NA	1.05 (R)	(i)	Bilateral
9	Both	1.3 R	50 (R) 41.5 (L)	1.53	No	19.2	21.3	NA	1.10 (L)	(v)	Bilateral
10	Met	-	-	-	No	22	21	29 (R); 28 (L)	1.03 (R)	(ii)	Bilateral
11	Both	3.5 L	10.3 (R) 40.9 (L)	1.34	Yes (L)	20	20	20	1.0	(iv)	Bilateral
12	Met	-	-	-	Yes (L)	22.1	22.4	Undetectable	1.01	(iii)	Bilateral
13	Met	-	-	-	Yes (L)	16	16	36 (L)	2.25 (L)	(ii)	Unilateral L
14	Both	2.3 R	98 (R) 42 (L)	1.35	No	17.7	19.9	NA	1.12 (L)	(iv)	Bilateral
15	Both	1.4 L	6.8 (R) 18.4 (L)	1.75	Yes (R)	25	21	38 (R)	1.80 (R)	(v)	Unilateral R

Adrenal vein sampling: Sequential, unstimulated sampling.
Lateralisation Index (LI): Aldosterone:Cortisol Ratio in left versus right adrenal vein. Threshold for lateralisation ≥4.0 to the lateralising side. Equivocal LI: 2.0-3.9. Non-lateralising <2.0.
Selectivity Index: Adrenal Vein:Peripheral Vein Cortisol Ratio. Threshold for successful adrenal vein cannulation (≥2.0).
Contralateral Suppression: Aldosterone:Cortisol Ratio in non-lateralising adrenal vein (where relevant) versus peripheral vein.

R: Right; L: Left

SUVMax Time of Flight measured at 10 minutes.

Reason for Metomidate: (i) prior failed AVS; (ii) unable to stop interfering medications for diagnosis or lateralisation; (iii) refused AVS, (iv) demonstrated equivocal lateralisation using AVS (defined as a LI between 2 and 4 using ACTH-unstimulated AVS); (iv) discordance between CT appearance of the adrenals and the LI on AVS.

Table 2: Summary of Outcomes Following therapy for Primary Aldosteronism

	Pre-Treatment								Post-Treatment						
		Blood Pressure (mmHg)							Blood Pressure (mmHg)						
	Lateralisation Modality	Systolic	Diastolic	#AntiHTN	Aldosterone (pmol/L)	DRC *PRA	ARR DRC *ARR PRA	Potassium (mmol/L)	Systolic	Diastolic	#AntiHTN	Aldosterone (pmol/L)	DRC *PRA	ARR DRC *ARR PRA	Potassium (mmol/L)
Surgery															
1	Met	136	96	3	723	7	103	3.9	121	86	0	278	40.1	7	4.6
2	Both	188	103	6	556*	0.3*	1853*	2.9	136	86	3 (BB)	559	15	37	4.4
3	Met	195	105	3	586*	0.2*	2930*	3.1	143	85	0	242	79.7	3	5.1
4	Met	150	85	6	1449*	0.2*	7245*	2.9	122	87	2 (BB)	883	3.8	232	4.5
Medical															
5	Both	158	92	3	416*	0.2*	2020*	3.5	141	88	MRA	250	9.5	26	4.3
6	Both	115	70	3	222	4.8	46	3.3	135	85	MRA	712	29.8	24	4.2
7	Both	149	69	4	524	0.3*	1747*	3.8	130	73	MRA	1063	51	21	4.6
8	Both	129	80	3	471*	0.2*	5.3	3.5	134	86	MRA + 1	673	29.8	23	4.4
9	Both	146	98	4	274	<1.8	152	3.7	144	104	MRA + 2	932	6.9	135	4.4
10	Met	168	92	4	678	0.6*	1130*	3	117	82	MRA + 2	626	26	24	4.9
11	Both	149	86	4	912*	0.4*	2280*	3.9	122	86	MRA + 1	1477	14.7	100	4.9
12	Met	186	102	4	275	2.6	106	3.4	128	72	1 + POP	531	5.1	104	4.6
13	Met	188	110	6	1474	2.9	508	2.6	142	78	MRA +2	1750	17.4	101	3.9
14	Both	190	115	5	874	1.8	485	3.5	146	88	MRA + 1	2120	6.1	348	4.0
15	Both	143	70	4	341	3.6	95	3.9	135	85	MRA	2419	90.8	27	4.2

Met: 11C Metomidate PET/CT; Both: Adrenal Vein Sampling + 11C Metomidate PET/CT

*Aldosterone measured using radioimmunoassay.
*PRA: Plasma Renin Activity
MRA: Mineralocorticoid antagonist
POP: Progesterone Only Pill
BB: Beta Blocker
+n represents number of medications in addition to MRA in medically treated patients.

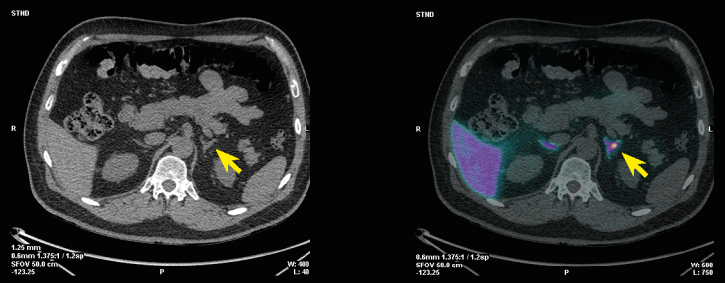
ARR DRC Threshold $\geq$ 37; ARR PRA Threshold $\geq$ 800
DRC Reference Range: 6.8-86.6 mIU/L
PRA Reference Range: Erect: 0.98 - 4.18 ng/mL/h

Supplementary Table: Post-operative ACTH-stimulation tests and adrenal histology.

		Cortisol (nmol/L)		
	PA Cure Category	**Baseline (Morning)**	**30 Min Post ACTH**	**Histology**
Surgery				
1	Complete	79	260	1.5cm adenoma; Fasciculata-like cells Normal Adjacent Gland
3	Partial	41	298	8mm adrenal adenoma Fasciculata-like cells Normal Adjacent Gland
4	Complete	121	446	4mm adrenal adenoma Fasciulata-like Cells Normal Adjacent Cortex
5	Partial	181	683	Two adrenal adenomas 8mm and 6mm respectively Fasciculata-like cells

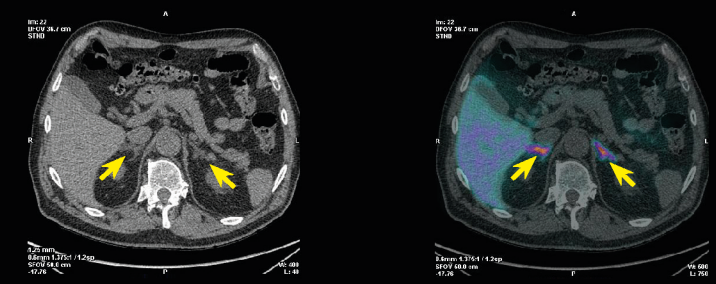
Figure 1

A



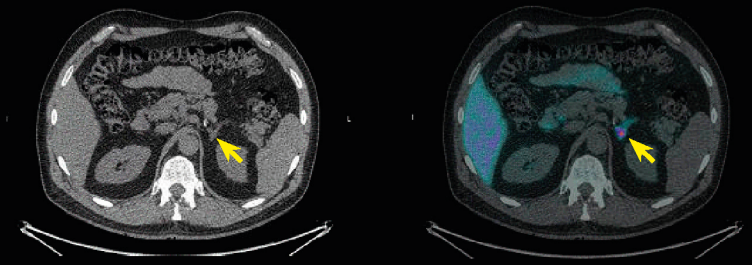
Unilateral Aldosterone Producing Adenoma
Case 3

B



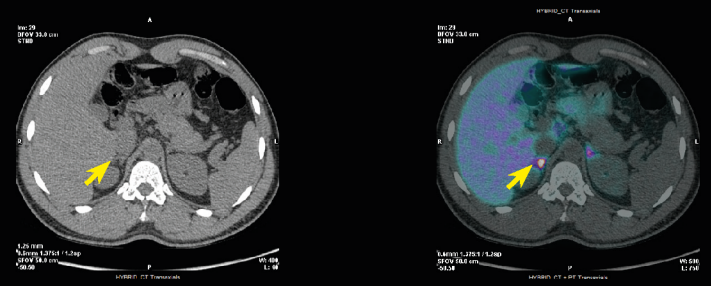
Bilateral Aldosterone Production in Primary Aldosteronism
Case 10

C



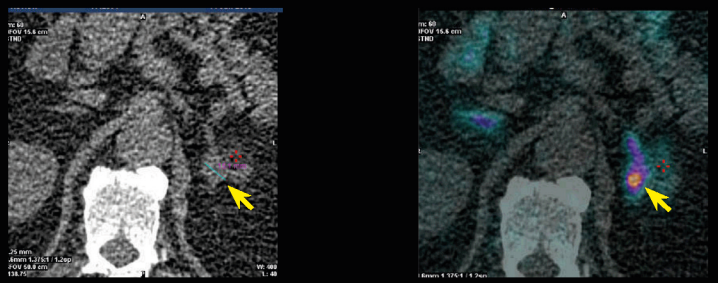
Case 2

D



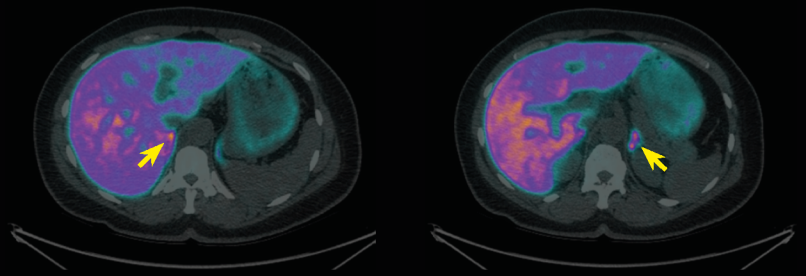
Case 15

E



Case 13

F



Case 4