A role for ^{11}C -methionine PET imaging in ACTH-dependent Cushing's syndrome

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Abstract

Objective: We report our experience of functional imaging with ^{11}C -methionine positron emission tomography–computed tomography (PET–CT) co-registered with 3D gradient echo (spoiled gradient recalled (SPGR)) magnetic resonance imaging (MRI) in the investigation of ACTH-dependent Cushing's syndrome.

Design: Twenty patients with i) *de novo* Cushing's disease (CD, $n=10$), ii) residual or recurrent hypercortisolism following first pituitary surgery ($\pm$ radiotherapy; $n=8$) or iii) ectopic Cushing's syndrome ($n=2$) were referred to our centre for functional imaging studies between 2010 and 2015. Six of the patients with *de novo* CD and five of those with persistent/relapsed disease had a suspected abnormality on conventional MRI.

Methods: All patients underwent ^{11}C -methionine PET–CT. For pituitary imaging, co-registration of PET–CT images with contemporaneous SPGR MRI (1 mm slice thickness) was performed, followed by detailed mapping of ^{11}C -methionine uptake across the sella in three planes (coronal, sagittal and axial). This allowed us to determine whether suspected adenomas seen on structural imaging exhibited focal tracer uptake on functional imaging.

Results: In seven of ten patients with *de novo* CD, asymmetric ^{11}C -methionine uptake was observed within the sella, which co-localized with the suspected site of a corticotroph microadenoma visualised on SPGR MRI (and which was subsequently confirmed histologically following successful transsphenoidal surgery (TSS)). Focal ^{11}C -methionine uptake that correlated with a suspected abnormality on pituitary MRI was seen in five of eight patients with residual or recurrent Cushing's syndrome following first TSS (and pituitary radiotherapy in two cases). Two patients elected to undergo repeat TSS with histology confirming a corticotroph tumour in each case. In two patients with the ectopic ACTH syndrome, ^{11}C -methionine was concentrated in sites of distant metastases, with minimal uptake in the sellar region.

Conclusions: ^{11}C -methionine PET–CT can aid the detection of ACTH-secreting tumours in Cushing's syndrome and facilitate targeted therapy.

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Invited Author's profile

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Introduction

Despite recent advances in imaging and surgical techniques, both immediate and long-term remission rates following transsphenoidal surgery (TSS) for Cushing's disease (CD) remain suboptimal for a condition that is associated with significant excess morbidity and mortality (1, 2, 3). This lack of success can be attributed in part to the challenge associated with reliably identifying the site of a corticotroph adenoma, which may be only a few millimetres in maximum diameter, coupled with the high rate of pituitary incidentalomas (10–15%) in the general population (4, 5). Accordingly, reliance on cross-sectional imaging alone (e.g., magnetic resonance imaging (MRI) or computed tomography (CT)) may fail to deliver a surgical target in up to 40% of CD patients (6), or erroneously implicate an incidentaloma as the source of adrenocorticotrophic hormone (ACTH) excess (7). Bilateral inferior petrosal sinus sampling (BIPSS) remains an important tool for discriminating central (pituitary) Cushing's from the ectopic ACTH syndrome (EAS), but its utility in lateralising a corticotroph adenoma is limited (findings at surgery correspond with BIPSS lateralisation in approximately two-thirds of cases, a rate only marginally better than chance alone) (8, 9, 10). Functional nuclear medicine imaging (e.g. octreotide scintigraphy, ¹⁸F-fluorodeoxyglucose positron emission tomography (¹⁸F-FDG-PET)) has been employed by some workers, but with variable success, which in part reflects the limited spatial resolution of traditional scintigraphy and pituitary CT and the relatively high background uptake of ¹⁸F-FDG by normal brain tissue.

¹¹C-methionine has been proposed as an alternative PET tracer for the localisation of pituitary adenomas (11, 12). In contrast to ¹⁸F-FDG, it is preferentially taken up by the normal pituitary gland, with relatively low uptake by the surrounding brain. Early studies confirmed that most types of pituitary adenoma exhibit enhanced ¹¹C-methionine uptake, thus offering potential advantages over both somatostatin receptor (SSTR) ligand and ¹⁸F-FDG tracers (11, 13). Although experience to date with ¹¹C-methionine in Cushing's syndrome is limited, preliminary findings appear promising. In a study of 12 patients with CD, Ikeda *et al.* (14) noted that ¹¹C-methionine was superior to ¹⁸F-FDG, detecting all microadenomas (100% sensitivity vs 67% for FDG). Moreover, co-registration of ¹¹C-methionine PET-CT with 3.0T MRI was reported to offer greater accuracy in localising an adenoma within the sella. Here, we expand these findings and present our experience in 20 patients

with ACTH-dependent Cushing's syndrome. In addition, we highlight a novel approach to image analysis that permits co-registration of ¹¹C-methionine PET-CT with spoiled gradient recalled (SPGR) acquisition MRI (Met-PET/MRI), and subsequent detailed profiling of tracer uptake across the sella. This technique has two important advantages: i) it allows the site of maximal ¹¹C-methionine uptake to be more accurately defined and ii) it addresses the important question of whether a lesion seen on structural imaging exhibits functional activity. Accordingly, we show how it can help confirm or reveal the site of autonomous ACTH secretion in patients with ACTH-dependent Cushing's syndrome.

Patients and methods

Patients

¹¹C-methionine PET-CT was performed in 20 patients (16 women, four men; mean age 43 years (range 17–77 years)) with ACTH-dependent Cushing's syndrome referred to our University Teaching Hospital between 2010 and 2015. Ten patients had newly diagnosed CD (confirmed biochemically by the demonstration of ≥ 2 of: raised 24-h urinary free cortisol excretion; failure of serum cortisol to suppress to < 50 nmol/l following dexamethasone 0.5 mg 6 h for eight doses; raised sleeping midnight serum (> 100 nmol/l) or late-night salivary (> 4.3 nmol/l) cortisol; with unsuppressed ACTH levels and with a clear central to peripheral gradient on BIPSS), with conventional MRI (see below) identifying a possible microadenoma in six cases. Seven patients had residual or recurrent disease following TSS, two of whom had also received adjunctive radiotherapy (fractionated in one case and stereotactic radiosurgery in the other). All were referred because of equivocal post-treatment MRI appearances. One patient had undergone TSS for presumed CD, but with resection of an incidental Rathke's cleft cyst; subsequent review of the case revealed inconclusive initial investigations with respect to the source of autonomous ACTH secretion. Two patients were referred for further investigation of possible EAS (one with a structural lesion on MRI, but no demonstrable gradient on BIPSS; the second with acute onset of florid Cushing's syndrome). All patients were managed in accordance with local clinical guidelines and all patients provided informed consent for ¹¹C-methionine PET-CT imaging. The decision to offer further treatment was undertaken on a case-by-case basis

following discussion by a specialist pituitary multidisciplinary team comprising pituitary neurosurgeons, endocrinologists, radiation oncologist, neuropathologist and neuroradiologist, who had full access to the Met-PET/MRI scans to aid decision-making.

Synthesis of ¹¹C-methionine

The PET tracer, L-[methyl-¹¹C]-methionine, was synthesised in compliance with good manufacturing practice using a captive solvent in loop methylation method without preparative HPLC, adapted from methods published previously (15, 16, 17). Briefly, [¹¹C]CO₂ was produced using a PETtrace cyclotron (GE Medical Systems, Milwaukee, WI, USA) via the ¹⁴N(p,α)¹¹C reaction before conversion to [¹¹C]MeI in the MeI MicroLab (GE Medical Systems). This was then transferred to the HPLC loop of a modified TracerLabFXC (GE Medical Systems) synthesiser containing an L-homocysteine precursor solution (0.5 M aqueous NaOH solution in ethanol). ¹¹C-methionine was produced in yields up to 15 GBq with a radiochemical purity of >96% and specific activity between 32.2 and 1564 GBq/μmol (average 205.5 GBq/μmol).

¹¹C-methionine PET-CT imaging

PET scans were acquired on a GE Discovery 690 PET-CT scanner (GE Medical Systems). Twenty minutes prior to each study, 300–400 MBq of L-[methyl-¹¹C]-methionine was administered intravenously. Attenuation correction was achieved using a low dose CT (140 kV, 220 mA, 0.5 s rotation, and 0.984 mm pitch). This was followed with a single bed position PET study of the head. Time-of-flight (ToF) PET data were acquired for a total acquisition time of 20 min. PET images were reconstructed with CT attenuation correction using fully 3D iterative reconstruction algorithms (three iterations, 24 subsets, 2 mm Gaussian post-filter) incorporating ToF and resolution recovery software (VUE Point FX and Sharp IR) to a 3.27 mm slice thickness. CT images were reconstructed at 1.25 mm slice thickness. All studies were independently reviewed by nuclear medicine physicians with expertise in PET-CT on the Xeleris workstation (GE Healthcare, Amersham, Buckinghamshire, UK).

Standard and 3D gradient echo MRI

MR imaging was performed on a 1.5T superconducting unit (GE Signa, Milwaukee, WI, USA) using a circularly-polarised head coil. Coronal T1-weighted spin echo (SE) images were obtained before and after intravenous

injection of 0.1 mmol/kg gadopentetate dimeglumine. A SPGR acquisition sequence was also performed to optimise co-registration with the PET/CT dataset. In brief, sagittal T1-weighted fast spoiled gradient echo images (TR (repetition time) 11.5 ms, TE (echo time) 4.2 ms, slice thickness 1 mm, 0 mm gap, 256×256 matrix) of the whole head were obtained after i.v. injection of 0.1 mmol/kg gadopentetate dimeglumine.

Image processing and analysis

Image processing was performed in the commercial software package MATLAB (version R2013a, the MathWorks, Inc., Natick, MA, USA, 2013). PET data sets (ToF or SharpIR reconstruction) were co-registered with the SPGR MR images using cubic interpolation. The PET uptake of one image set was normalised to the corresponding (background) cerebellar uptake. The registered MR and PET images were read into the profiling software, which overlays the PET onto the MRI, and produces a two-dimensional profile of the pituitary uptake on a selected orientation (axial, coronal or sagittal) and slice. The reference position was established as follows: using the coronal slices, the midpoint of the profile was centred on the hypothalamic origin of the pituitary stalk with left and right limits set as equidistant (10 mm) from the centre of the profile. The software outputs three-dimensional surface plots of the pituitary uptake and PET/MRI overlays to allow correlation of site(s) of tracer uptake with site(s) of possible structural abnormalities.

Results

Patients with *de novo* CD

Ten patients with newly diagnosed CD underwent ¹¹C-methionine PET-CT, and in seven of these profiling revealed an asymmetric peak in tracer uptake within the sella, which co-localized with a possible adenoma identified on SPGR MRI (illustrative cases 1–3; Table 1; Figs 1, 2 and 3). In four patients initial conventional SE MRI failed to identify a structural lesion, which was subsequently visualised on SPGR sequences. No abnormal pattern or focus of tracer uptake was seen in three patients, all of whom subsequently underwent transsphenoidal exploration, but only one attained post-operative remission.

Case 1 ► A 47-year-old woman with typical symptoms and signs of hypercortisolism was found to have ACTH-dependent Cushing's syndrome (Table 1). Conventional T1W SE coronal MRI pre- (Fig. 1a) and post-gadolinium

Table 1 Radiological, intraoperative and histological findings in illustrative cases 1–6.

Case	Gender/age (years)	UFC (nmol/24 h)	0900 h ACTH (ng/l)	Pituitary deficits ^a	BIPSS max C:P ratio	BIPSS max IS ratio	BIPSS laterality	SE MRI	SPGR MRI	PET	TSS findings/histology
1	F/47	529	39	LH/FSH	78	2.4	Right	Right	Right	Right	Right CA
2	F/48	370 (active phase)	58	Nil	45	40	Right	? Right	Right	Right	Right CA
3	F/31	1542	34	LH/FSH	245	146	Right	No lesion	Left	Left	Left CA
4	F/43	202	55	Nil	NA	NA	NA	Left	Left	Left	Left CA
5	F/42	620	15	Nil	27	7.4	Right	? Left	Left	Left	Left CA
6	F/42	1628	113	Nil	1.7	–	–	? Right (dynamic sequences)	? Right	No focal tracer uptake	NA (NET)

ACTH, adrenocorticotrophic hormone (reference range <50 ng/l); BIPSS, bilateral inferior petrosal sinus sampling; BIPSS max C:P ratio, maximum ratio between central (right or left sinus) and peripheral ACTH concentrations at any time point; BIPSS max IS ratio, maximum inter-sinus ACTH ratio at any time point; CA, corticotroph adenoma; FSH, follicle-stimulating hormone; LH, luteinizing hormone; PET, ¹¹C-methionine PET/MRI; NA, not available; NET, neuroendocrine tumour; SE, spin echo MRI; SPGR, spoiled gradient recalled MRI; TSS, transsphenoidal surgery, UFC, urinary free cortisol (reference range 0–146 nmol/24 h).

^aFormal testing for growth hormone deficiency was not performed, but all patients had insulin-like growth factor 1 levels within the respective age- and gender-matched reference ranges.

(Fig. 1b) identified an 8 mm right-sided lesion, which was confirmed on SPGR sequences (Fig. 1c). Profiling of ¹¹C-methionine uptake across the sella revealed a clear right-sided peak (Fig. 1d), which co-localised with the suspected adenoma (Fig. 1e; PET overlaid onto coronal SPGR MRI sequence shown in Fig. 1c). Surgical and histological findings confirmed a corticotroph adenoma, and 0900 h serum cortisol on day 1 postoperatively was undetectable (<25 nmol/l).

Case 2 ► A 48-year-old woman with intermittent symptoms of hypercortisolism was diagnosed with periodic/cyclical Cushing's syndrome. BIPSS performed during an active phase confirmed a pituitary origin with a right-sided gradient (Table 1). Conventional T1W SE coronal MRI pre- (Fig. 2a) and post-gadolinium (Fig. 2b) raised the possibility of an inferior right-sided microadenoma, which was visualised more easily on coronal (Fig. 2c) and axial (Fig. 2d) SPGR sequences. ¹¹C-methionine profiling revealed an asymmetric right-sided peak of tracer uptake within the sella (Fig. 2e) corresponding to the abnormality identified on MRI (Fig. 2f; PET overlaid onto axial SPGR MRI sequence shown in Fig. 2d). At surgery a right-sided corticotroph adenoma was resected from the site of the suspected lesion. The patient remains in remission 4 years later.

Case 3 ► A 31-year-old woman was diagnosed with ACTH-dependent Cushing's syndrome. Conventional T1W SE coronal MRI pre- (Fig. 3a) and post-gadolinium (Fig. 3b) was unremarkable, but axial SPGR sequences identified a possible left-sided microadenoma (Fig. 3c). BIPSS confirmed a pituitary origin, but with a strongly positive right-sided gradient (Table 1). Profiling of ¹¹C-methionine uptake across the sella revealed asymmetric tracer uptake skewed to the left (Fig. 3d) and concordant with the site of the suspected lesion on SPGR MRI (Fig. 3e; PET overlaid onto axial SPGR MRI sequence shown in panel Fig. 3c). Surgery confirmed a corticotroph adenoma adjacent to the left cavernous sinus. The patient remains in remission 1 year later.

Patients with recurrent/persistent ACTH-dependent Cushing's syndrome following previous pituitary surgery (± radiotherapy)

Eight patients with recurrent or persistent ACTH-dependent Cushing's syndrome underwent ¹¹C-methionine PET-CT. In five cases ¹¹C-methionine profiling demonstrated tracer uptake which co-localized with an

Figure 1

Case 1: ^{11}C -methionine PET-CT co-registered with SPGR MRI confirms functionality within an easily visualised right-sided corticotroph microadenoma. (a and b) T1-weighted coronal SE MRI pre- and post-gadolinium. (c) Coronal SPGR MRI following gadolinium. (d) Sella profiling of ^{11}C -methionine uptake in the coronal plane. (e) PET profile overlaid onto coronal SPGR MRI

area identified on SE and/or SPGR MRI as the most likely site of residual or recurrent adenoma (illustrative cases 4 and 5; Table 1; Figs 4 and 5). In three patients no clear abnormal focus of ^{11}C -methionine uptake was seen, and none of these had an abnormality on SE or SPGR MRI.

Case 4 ► A 43-year-old woman who had previously undergone successful TSS for CD caused by a pituitary

shown in c. L, left; M, midline (based on the origin of the pituitary stalk at the level of the hypothalamus); R, right. Arrows denote the site of the adenoma. The ratio of ^{11}C -methionine pituitary uptake relative to background cerebellar uptake is shown using a scale of 0 (blue) to maximum 5 (red).

macroadenoma presented with features suggestive of recurrence 2 years after her initial operation. Conventional T1W SE coronal MRI pre- (Fig. 4a) and post-gadolinium (Fig. 4b) revealed no change compared to earlier post-operative scans, showing an area of low signal intensity on the left side of the fossa, extending in to the cavernous sinus, which was confirmed on SPGR sequences (Fig. 4c). This had previously been interpreted as consistent with postoperative change/scar tissue as the patient

Figure 2

Case 2: ^{11}C -methionine PET-CT co-registered with SPGR MRI aids identification of a small microadenoma that is suspected on initial MRI. (a and b) T1-weighted coronal SE MRI pre- and post-gadolinium. (c and d) Coronal and axial SPGR MRI following gadolinium. (e) Sella profiling of ^{11}C -methionine uptake in the

was in full clinical and biochemical remission and glucocorticoid-dependent. Profiling of ^{11}C -methionine uptake across the sella revealed the expected concentration of tracer within the normal gland (Fig. 4d and e – right sella), but a second discrete focus of tracer uptake on the left (Fig. 4d and e – left sella/parasellar region). At surgery, ACTH-staining tumour was resected from the site of the suspected recurrence on the left, and the patient went back in to remission.

Case 5 ► A 42-year-old woman had persistent CD after a ‘failed’ TSS, during which a central (4 mm) microadenoma was identified and resected, but proved to be an incidental (non-secretory) prolactinoma. She was treated with

axial plane. (f) PET profile overlaid onto axial SPGR MRI shown in d. L, left; M, midline; R, right. Arrows denote the site of the adenoma. The ratio of ^{11}C -methionine pituitary uptake relative to background cerebellar uptake is shown using a scale of 0 (blue) to maximum 3 (red).

metirapone and hydrocortisone (‘block and replace regimen’) and followed up with serial imaging. One year later, T1W SE MRI (Fig. 5a and b) identified a suspicious area on the post-gadolinium images adjacent to the left cavernous sinus (Fig. 5b), which was confirmed on SPGR MRI (Fig. 5c). This lesion had not been noted previously. Profiling of ^{11}C -methionine uptake across the sella revealed the expected concentration of tracer within the remaining normal gland (Fig. 5d and e – right sella), but a second, subtle, focus of low level tracer uptake on the left corresponding to the hypointense lesion seen on MRI (Fig. 5d and e – left sella). The patient underwent a second TSS targeting the left-sided lesion, which was subsequently confirmed to be a corticotroph microadenoma.

Figure 3

Case 3: ^{11}C -methionine PET-CT co-registered with SPGR MRI identifies a left-sided microadenoma that is not readily visualised on initial MRI, in a patient with clear BIPPS lateralisation to the right. (a and b) T1-weighted coronal SE MRI pre- and post-gadolinium. (c) Axial SPGR MRI following gadolinium. (d) Sella profiling of ^{11}C -methionine uptake in the

axial plane. (e) PET profile overlaid onto axial SPGR MRI shown in c. L, left; M, midline; R, right. Arrows denote the site of the adenoma. The ratio of ^{11}C -methionine pituitary uptake relative to background cerebellar uptake is shown using a scale of 0 (blue) to maximum 3 (red).

Postoperatively, the patient is in full remission and remains glucocorticoid-dependent.

Patients with EAS

^{11}C -methionine PET-CT was performed in two patients with presumed EAS. In both cases very little tracer uptake was seen within the pituitary fossa, but distant sites of metastasis were detected, with subsequent histology

confirming ACTH-staining neuroendocrine tumours (NETs): small bowel primary tumour in one patient (illustrative case 6; Table 1; Fig. 6) and primary breast tumour in the other.

Case 6 ► A 42-year-old woman was diagnosed with ACTH-dependent Cushing's syndrome. Although conventional T1W SE MRI was considered unremarkable (Fig. 6a and b), dynamic sequences raised the possibility of a right-sided

Figure 4

Case 4: ^{11}C -methionine PET-CT co-registered with SPGR MRI confirms the site of recurrence in a patient with relapsed Cushing's disease following first TSS. (a and b) T1-weighted coronal SE MRI pre- and post-gadolinium. (c) Coronal SPGR MRI following gadolinium. (d) Sella profiling of ^{11}C -methionine uptake in the coronal plane. (e) PET profile overlaid onto coronal SPGR MRI shown in c. L, left; M, midline; R, right. Yellow

arrows show residual normal gland, which exhibits physiological tracer uptake (the patient was otherwise eupituitary). White arrows show the site of the recurrent adenoma, which corresponds with a second discrete focus of tracer uptake. The ratio of ^{11}C -methionine pituitary uptake relative to background cerebellar uptake is shown using a scale of 0 (blue) to maximum 3 (red).

microadenoma (Fig. 6c). However, BIPSS failed to show a central:peripheral ACTH gradient, suggesting EAS (Table 1). In light of these discordant findings, the patient underwent whole body ^{11}C -methionine PET-CT. ^{11}C -methionine pituitary profiling revealed minimal tracer uptake throughout the sella, with no focus at the site of the suspected right-sided microadenoma (Fig. 6d and e). However, a left supraclavicular lymph node exhibited

methionine uptake (Fig. 6f), and biopsy demonstrated features in keeping with a NET. Further investigation revealed a small bowel primary NET with liver metastases.

Discussion

We have shown that ^{11}C -methionine PET-CT co-registered with SPGR MRI is a potentially important addition to

Figure 5

Case 5: ^{11}C -methionine PET-CT co-registered with SPGR MRI identifies the site of a previously unsuspected corticotroph microadenoma in a patient with persistent Cushing's disease following transsphenoidal resection of a pituitary incidentaloma. (a and b) T1-weighted coronal SE MRI pre- and post-gadolinium. (c) Coronal SPGR MRI following gadolinium. (d) Sella profiling of ^{11}C -methionine uptake in the coronal plane. (e) PET profile overlaid onto coronal SPGR MRI shown in c. L, left; M, midline; R,

right. Yellow arrows show residual normal gland, which exhibits physiological tracer uptake (the patient was otherwise eutipuitary). White arrows show the site of the adenoma (Note the dumb-bell appearance of ^{11}C -methionine uptake across the sella, with a left-sided tail of low grade tracer uptake corresponding to the site of the suspected lesion). The ratio of ^{11}C -methionine pituitary uptake relative to background cerebellar uptake is shown using a scale of 0 (blue) to maximum 3 (red).

the clinician's armamentarium for the investigation of ACTH-dependent Cushing's syndrome. Using a novel approach to image analysis, which allows more accurate mapping of tracer uptake within the sella, it appears to have particular utility when a structural lesion is identified/queried on MRI, either in a patient with *de novo* Cushing's syndrome or when previous pituitary surgery has failed to achieve sustained remission from

hypercortisolism (Figs 1, 2, 3, 4 and 5). It may also identify sites of ectopic ACTH secretion (Fig. 6).

In many centres, pituitary MRI is performed early in the diagnostic algorithm for ACTH-dependent Cushing's syndrome, because demonstration of an obvious macroadenoma simplifies management; however, macroadenomas account for <10% of all corticotroph adenomas (18, 19). In contrast, many microadenomas are not reliably visualised using

Figure 6

Case 6: ^{11}C -methionine PET-CT co-registered with SPGR MRI shows minimal tracer uptake in the sella in a patient with a possible lesion on dynamic MRI, but BIPSS suggestive of ectopic Cushing's syndrome. (a and b) T1-weighted coronal SE MRI pre- and post-gadolinium. (c) Dynamic MRI. (d) Sella profiling of ^{11}C -methionine uptake in the coronal plane. (e) PET profile overlaid onto coronal SPGR MRI (Note the absence of tracer

uptake at the site of the suspected focal lesion seen on dynamic and SPGR MRI). (f) Coronal and axial PET-CT of the upper thorax. L, left; M, midline; R, right. Dashed arrow denotes the site of a pituitary incidentaloma. Solid arrows show ^{11}C -methionine uptake within a supraclavicular lymph node. The ratio of ^{11}C -methionine pituitary uptake relative to background cerebellar uptake is shown using a scale of 0 (blue) to maximum 3 (red).

conventional (SE) MRI sequences, with several case series suggesting that 40% or more of patients with CD have 'negative scans' (6, 10). Accordingly, more detailed sequences (e.g., dynamic and/or SPGR MRI) are often employed, leading to a reported improvement in sensitivity from 50–55% (SE) to 70–75% (dynamic) and 70–90% (SPGR), but with the important caveat of a high detection rate of incidental pituitary lesions (6, 20, 21, 22). This presents a significant challenge, with the risk that a coincidental pituitary lesion will be erroneously identified as the source of excess ACTH, either in a patient with genuine CD due to a non-visualised microadenoma (illustrative case 5, Fig. 5c) or in a patient with EAS (illustrative case 6, Fig. 6c). In one series of 65

patients with proven ectopic disease, 17 (26%) were shown to have subtle abnormalities on pituitary MRI (23).

BIPSS remains the gold standard for distinguishing pituitary and ectopic sources of ACTH secretion (8, 24). However, it does not reliably lateralize the side of an adenoma in up to a third of patients with CD. Indeed, over-reliance on BIPSS to define lateralization is fraught with challenges that have been described in several reports including a large series of patients ($n=501$) from the National Institute of Health (10). The strict dichotomy of right vs left does not hold true in all cases, as up to 20% of adenomas are central or bilateral. In addition, interpretation can be difficult or impossible when lateralization varies at

different time points of the procedure. Wind *et al.* reported that a majority (58%) of cases lateralized to the right by BIPSS criteria, but this was not reflected in the findings at subsequent surgery (just 38% had a right-sided adenoma) (10). Intercavernous sinus mixing or right-sided dominance in venous drainage may confound BIPSS interpretation, and unfortunately venography during catheterization does not reliably predict these anatomical variations. Simultaneous measurement of prolactin to confirm catheter position has been proposed as a means for aiding interpretation, but is not universally applied (25, 26). In addition, care must be exercised when performing BIPSS in patients with suspected cyclical/periodic Cushing's, to ensure sampling is carried out during an active phase (24).

Nuclear imaging modalities have assumed increasing prominence in ACTH-dependent Cushing's syndrome in recent years, especially in EAS where tumour localization with conventional cross-sectional imaging can prove particularly challenging. The two modalities most commonly used in clinical practice are ¹¹¹In-pentetreotide scintigraphy ('octreoscan') and ⁶⁸Ga-DOTATATE or ⁶⁸Ga-DOTATOC PET/CT. Although both can theoretically be employed to detect ACTH-secreting tumours at any site, it is predominantly in EAS that their role has been established (27). While conventional SSTR scintigraphy lacks spatial resolution (especially for lesions <2 cm), this can be partly overcome through the use of SPECT (single-photon emission computerised tomography) for image acquisition. However, PET/CT is generally considered to offer superior anatomical definition of sites of tracer uptake. Crucially, both techniques depend on tumoral expression of SSTRs (especially SSTR subtypes 2 and 5) and this, coupled with the difficulties inherent in visualising very small lesions, explains why they have found only limited use in CD. Even in EAS there is considerable heterogeneity of SSTR2 and SSTR5 expression with scan sensitivity reported to be of the order of 40–70% (27).

¹⁸F-FDG uptake is linked to tumour glucose metabolism, as opposed to receptor binding, and hence lesions that do not express SSTRs, and are not visualized with octreotide scans, may theoretically be detected with ¹⁸F-FDG-PET (PET/CT) (28). Indeed, ¹⁸F-FDG-PET/CT has been shown to detect thymomas, thymic and bronchial carcinoids and pancreatic NETs causing EAS, although some clinicians argue that its use is limited when cross-sectional imaging is truly negative (29). However, as with SSTR imaging, reports exist of the detection of lesions not previously visualised on CT (30, 31, 32, 33).

In pituitary Cushing's ¹⁸F-FDG-PET/CT has found limited use to date, potentially reflecting the relatively low

metabolic rate of most corticotroph adenomas. In a recent prospective study of ten consecutive patients with subsequently confirmed CD, high resolution ¹⁸F-FDG-PET (¹⁸F-FDG-hrPET) was performed together with SE and SPGR MRI (34). ¹⁸F-FDG-hrPET revealed increased tracer uptake in four patients, two of whom had no demonstrable lesion on SE MRI. However, SPGR proved more sensitive than ¹⁸F-FDG-hrPET, identifying a pituitary adenoma in seven cases and, importantly, no adenomas were detected by ¹⁸F-FDG-hrPET that were not visualised on SPGR MRI. Indices of adenoma metabolic activity (ACTH concentration and ACTH response to CRH stimulation), rather than adenoma size, were proposed as potential predictors of ¹⁸F-FDG-hrPET positivity (34). Adenoma location on ¹⁸F-FDG-hrPET was reported to correspond with surgical findings in the four patients with positive PET scans, although the lack of co-registration of PET images with CT or MRI makes it difficult to be certain that the foci of increased ¹⁸F-FDG uptake corresponded with the lesions seen on MRI. A comparable detection rate (58%) was reported by Alzahrani *et al.* (35) in their retrospective study of 12 patients who underwent conventional ¹⁸F-FDG-PET/CT.

¹¹C-methionine has been employed as an alternative PET tracer for the localisation of pituitary adenomas by several groups (11, 12). Methionine, as the first amino acid incorporated into all peptides, is an ideal substrate for ¹¹C-labelling to allow identification of sites of increased peptide/protein synthesis. In contrast to ¹⁸F-FDG, it is preferentially taken up by the normal pituitary gland compared to surrounding brain [the ratio of standardized uptake values (SUVmax) in normal pituitary compared with background brain has been reported to be as high 2.0 in some studies (11)]. Physiological uptake is also seen in the nasopharynx, salivary and lacrimal glands, bone marrow, liver and pancreas.

Early studies have confirmed that most pituitary adenomas exhibit enhanced ¹¹C-methionine uptake, thus offering potential advantages over both SSTR ligand and ¹⁸F-FDG tracers (11, 13). It is perhaps somewhat surprising then that experience with ¹¹C-methionine imaging in CD is limited to a small number of cases. Ikeda *et al.*, describing their findings in 12 patients with CD who underwent 1.5T and 3.0T MRI, ¹¹C-methionine PET/CT (in 11 cases) and ¹⁸F-FDG-PET/CT, concluded that methionine was superior to FDG as a tracer, detecting all eleven microadenomas (100% sensitivity vs 67% (eight of 12) for FDG) (14). Moreover, co-registration of ¹¹C-methionine PET/CT with 3.0T MRI SE sequences was reported to offer greater accuracy in localising adenoma

within the sella. However, findings from this study must be interpreted with caution as the authors provided only limited information on how the localization of adenomas was defined (particularly with reference to background tracer uptake by remaining normal pituitary tissue), and little data was provided to allow correlation of imaging appearances with intraoperative findings and postoperative endocrine/clinical outcomes (14).

We have performed ¹¹C-methionine PET/CT in 20 patients with ACTH-dependent Cushing's syndrome who fall in to one of three subgroups: i) *de novo* CD (*n*=10), (ii) residual or recurrent hypercortisolism following first pituitary surgery ($\pm$ radiotherapy; *n*=8) or iii) ectopic Cushing's syndrome (*n*=2). In all but one case, images obtained from PET/CT were co-registered to the contemporaneous SPGR MRI (1 mm slices) using mutual information between the high resolution CT and MRI scans. In this way, CT acts as a bridge to allow registration of the PET and MR images (Met-PET/MRI). In addition, we have developed image analysis software that allows the distribution of ¹¹C-methionine uptake across the sella and adjacent structures to be profiled in three planes (axial, coronal and sagittal), thereby helping to confirm whether tracer uptake and, where evident, a structural abnormality are colocalized.

Our preliminary findings suggest that Met-PET/MRI may help inform decision-making in the following situations:

- i) in patients with *de novo* CD and:
 - a) a suspected lesion on SE MRI (Figs 1 and 2).
 - b) Normal SE MRI, but a suspected lesion on dynamic or SPGR MRI (Fig. 3).

In both of these situations PET confirms functionality within the visualised lesion;

- ii) following non-curative TSS, or in patients with recurrent disease (including Nelson's syndrome), when further surgery or radiotherapy is being considered – here, it can help distinguish residual/recurrent disease from post-treatment change/scar tissue (Figs 4 and 5) and
- iii) in EAS with an incidental non-functioning pituitary lesion (Fig. 6).

In addition, the source of ectopic ACTH secretion (including metastases) may be identified (Fig. 6f).

In contrast to the findings of Ikeda *et al.*, Met-PET/MRI did not identify a focus of abnormal tracer uptake in all cases: six (30%) had non-diagnostic scans. However, it is important to note that the majority of the 20 patients studied were selected for Met-PET/MRI on the basis of

indeterminate MRI appearances. It is likely therefore that the true sensitivity for detecting functioning corticotroph adenoma in an unselected cohort lies somewhere in between, but further studies are required to confirm these findings.

Several factors may contribute to the failure to localise a microadenoma or to identify the site(s) of residual/recurrent disease in a previously treated patient. These include relatively low levels of tracer uptake by corticotroph tumours – in our experience they accumulate less ¹¹C-methionine than their somatotroph, lactotroph or thyrotroph counterparts – which, particularly when combined with a central location within the sella, may result in tumour uptake merging with physiological uptake into the remaining normal gland. Consistent with this, lesions that are more laterally situated are relatively easily visualised by virtue of producing a skewed pattern of tracer uptake across the sella (Figs 1, 2 and 3). Failure to recognise or acknowledge cyclical/periodic CD may also theoretically yield a false negative result if the scan is performed during an inactive phase.

The application of image analysis techniques with detailed profiling of tracer uptake across the sella allows subtle patterns of abnormal tracer uptake to be identified, and this may have particular application in CD where low-level ¹¹C-methionine uptake by a small microadenoma may otherwise not be easily distinguished from background uptake by remaining normal pituitary tissue. We speculate therefore that a multi-modal pituitary imaging approach using SE and/or SPGR pituitary MRI and ¹¹C-methionine PET/CT could be adopted for 'difficult' pituitary Cushing's cases, in order to maximize the chance of adenoma detection and localization.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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