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Chapter XII

Diagnosis of Cushing's Disease

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Abstract

The major difficulty encountered when diagnosing Cushing's disease is the fact that the adenoma is too small to detect by conventional radiography. Moreover, meeting a relapsed patient during long term follow up cannot be ruled out and, because of fibrosis, the identification of a small recurrent adenoma within the remaining tumor nest is difficult.

Even if (with the aid of superconducting MRI) the morphological diagnosis of a minute disease nest can be made, the description rate is still low (25–57%). Even when dynamic MRI is used to detect such minute lesions, the diagnosis rate is practically the same, and super selective cavernous sinus sampling is only able to localize the lesion in 81% of cases, at most (although this method has a defect in diagnosing the exact localization and extent of the adenoma.).

L-methyl-^{11}C-methionine positron emission tomography (MET-PET) fusion 3T-MRI imaging improves diagnostic accuracy (up to 95%) by producing an image of the vigorous hormone synthesis occurring within the adenoma. In conclusion, MET-PET fusion 3T-MRI may herald a revolution in the diagnosis and treatment of Cushing's disease.

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Section 1. Useful Tests for Localizing Microadenomas in Cushing's Disease (MRI Fusion PET vs. Dynamic MRI vs. Cavernous Sinus Sampling)

[1] Clinical necessity for early diagnosis of microadenomas in Cushing's disease.

Cushing's disease has a poor prognosis, with a five year survival rate of less than 50% if left untreated [1]. The cause of death in Cushing's disease is determined by cardiovascular risk [2] and this risk, depending on disease duration, may continue even after the disease has been cured [3]. Therefore, there is an urgent need for new diagnostic methods that will enable the early diagnosis and treatment of Cushing's disease. Furthermore, as the number of cases of Cushing's disease presenting with atypical symptoms has recently increased, improvements in the accuracy of the methods currently used to obtain a localized diagnosis of the adenoma and establishment an early diagnosis are desirable.

[2] Difficulties in diagnosing microadenomas in Cushing's disease—an endocrinological perspective

The various endocrine test criteria and diagnostic standards for Cushing's disease can produce both false-negative and false-positive results. Reactivity typical of Cushing's disease is reported to be 60–80% [4–7]. The fact that there are more than a few exceptions, even in endocrinological terms, is a serious problem in the diagnosis of Cushing's disease. For example, 70% of cases test negative for Cushing's disease using the dexamethasone suppression test, but 76% test positive using the corticotrophin-releasing hormone (CRH) load test. That said, a clear differentiation between pituitary and ectopic lesions is difficult, even with tests that are important for determining Cushing's disease.

[3] Difficulties in diagnosing microadenomas in Cushing's disease—a diagnostic imaging perspective

The diagnostic rate of Cushing's disease is 42% when using diagnostic imaging techniques [8]. However, the diagnostic rate when using 1.5T-MRI is reported to be 52% [9]. When the author examined the diagnostic performance of both 1.5T-MRI and 3.0-MRI, it was found to be 57% and 25%, respectively (Table 1).

Although 3.0T-MRI has a superior signal-noise ratio (S/N), it is still difficult to argue that the diagnostic rate of 3.0T-MRI is better than that of 1.5T-MRI because MRI has limited diagnostic performance. As such, many cases of Cushing's disease are not detected by CT or MRI and, even when an abnormal signal is detected, it is not definite that the lesion identified is the cause of Cushing's disease because silent microadenomas are also present in 10–14% of normal persons [10, 11].

[4] "Blind spots" in MRI, dynamic MRI and cavernous sinus sampling tests

The method used to diagnose microadenomas in Cushing's disease include: (1) dynamic MRI, an improved imaging procedure that can identify tumors via the wash out of a radio-opaque contrast dye, (2) the cavernous sinus sampling test, which uses intravascular surgical techniques to measure the levels of pituitary derived hormones in the circulating blood, which are present at higher concentrations close to the pituitary gland [12, 13], and (3) methionine PET fusion 3T-MR imaging, which can provide accurate positional information. By

integrating the methionine PET with 3.0T-MRI imaging, functional information can be added to positional information, resulting in high sensitivity and accurate tumor localization [14].

Table 1. Diagnostic accuracy of microadenoma by 1.5T and 3.0T-MRI

Diagnostic accurcy of microadenoma on MRI		
Cushing's disease (N=30)	1.5T-MRI（N=14)	3.0T-MRI（N=16)
pre-clinical Cushing's disease (N＝12)	2/5 （40%）	1/7 （14%）
overt Cushing's disease (N=18)	6/9 （67%）	3/9 （33%）
Total accuracy	8/14 （57%）	4/16 （25%）

In this chapter, the characteristics, diagnostic performance, and false-positive findings of the following three methods are discussed:

a) MRI and dynamic MRI;
b) Super selective cavernous sinus sampling;
c) Combined 3.0T-MRI and ^{11}C-methionine positron emission tomography (MET-PET) imaging.

(a) Diagnostic performance of dynamic MRI vs. MRI

Although dynamic MRI is generally thought to yield improved diagnostic accuracy, a report examining the diagnostic rates of 1.0T-MRI and dynamic MRI found that the "true" positivity of MRI was 11/21 (52%), and that of dynamic MRI was 14/21 (67%). This difference was not statistically significant [15].

When analyzing the results from 14 Cushing's microadenomas that we had previously verified by surgical exploration, three cases diagnosed as positive by MRI were also found to be positive by dynamic MRI. However, one case that was negative by MRI was found to be positive by dynamic MRI. In other words, the positive diagnostic rate of dynamic MRI in the Cushing's adenoma group was 29%, compared with 21% for MRI. Thus, dynamic MRI has a slightly better diagnostic sensitivity.

Bias can have a significant influence on the results obtained from diagnostic imaging, and the presence of multiple pituitary adenomas has been reported [16–18]. This can result in false-positive results from diagnostic imaging and, indeed, multiple adenoma's were observed in 3/32 cases (9%) a study by the author [20]. Moreover, autopsy case reports documented pituitary adenomas in 316/3048 cases (10.4%) [11]. Also, other pituitary lesions, such as small pituitary hemorrhages, pituitary necrosis, and segmental atrophy of the pituitary due to age, may result in false-positive results. In the author's opinion, the accuracy of localized diagnosis of Cushing's adenoma using superconductive MRI is around 40%, and false-negative lesions, false-positive lesions, and double pituitary adenomas result in poor diagnostic accuracy.

Dynamic MRI proved to be better than MRI in both of the cases shown. However, although dynamic MRI suggested abnormal findings, it was still ineffective regarding the range of the abnormal findings. Because the technique involves imaging only a few ROI

(Regions of Interest), accurate mapping regarding the extent of an adenoma is virtually impossible.

Case Study (Figures 1 and 2)

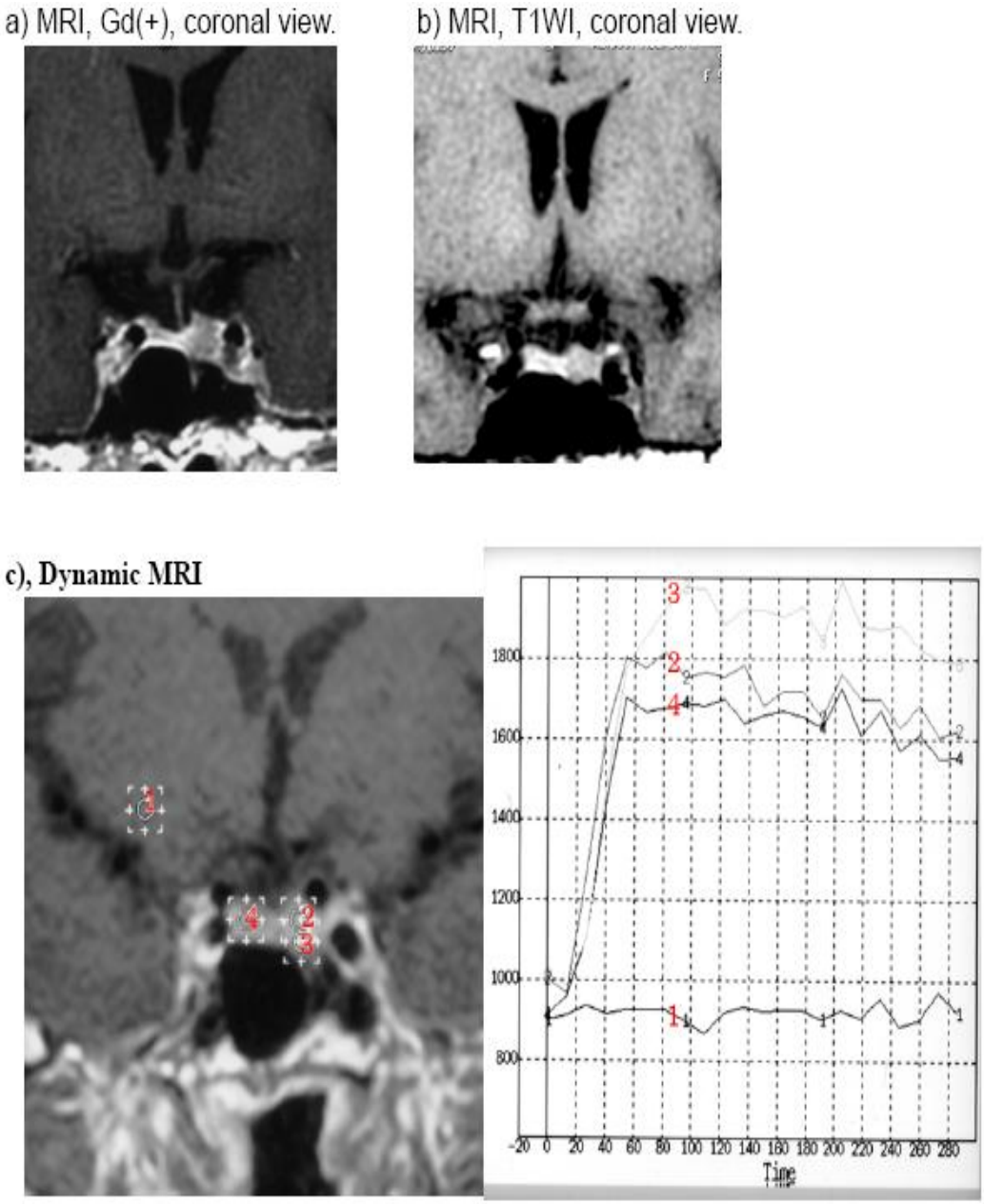


Figure 1. A 55 year-old female with pre-clinical Cushing's disease. This case shows the disagreement between MRI findings (a, b) and dynamic MRI findings (c). Surgical findings confirmed the accuracy of dynamic MRI.

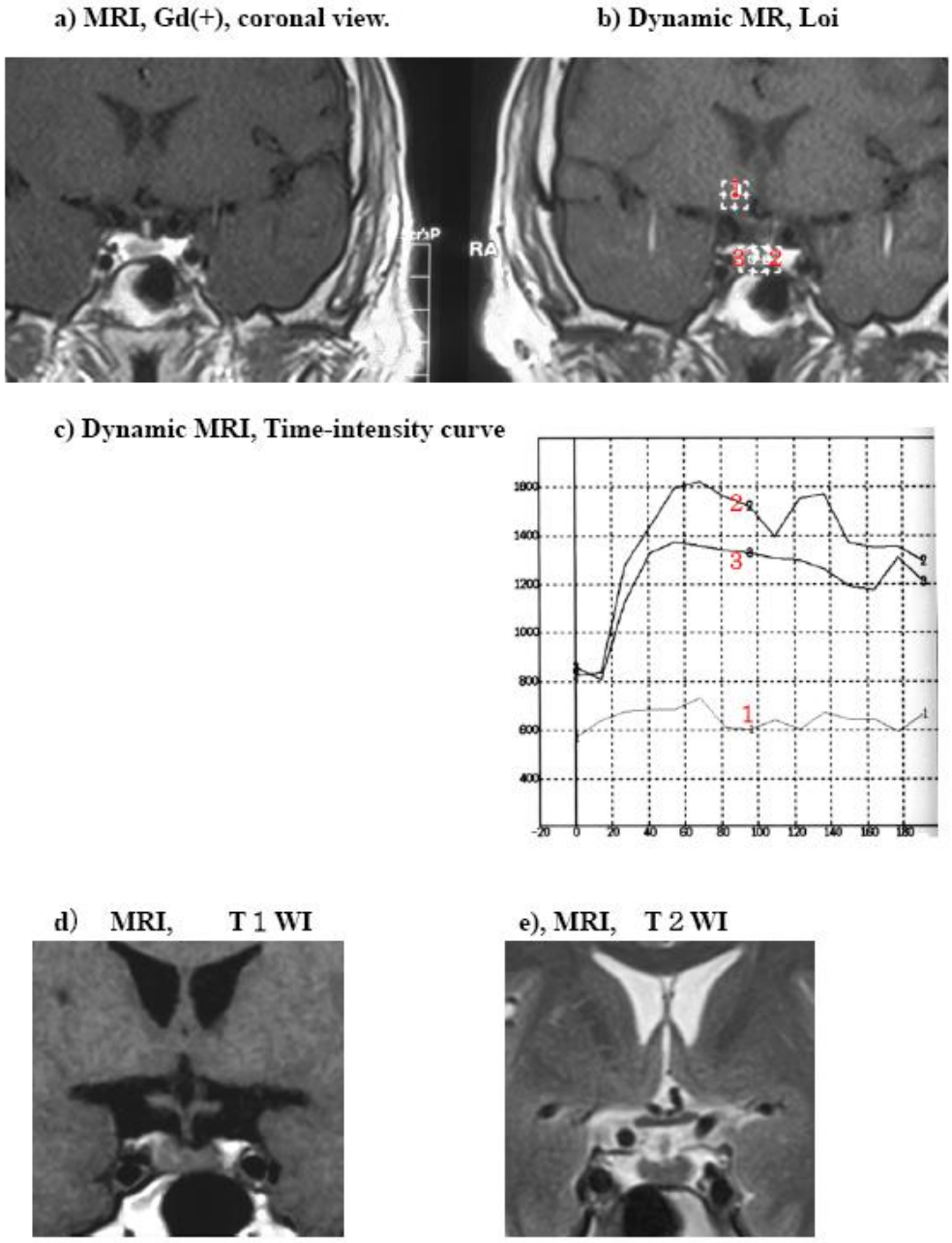


Figure 2. A 40 year-old male with Cushing's disease. A Cushing's adenoma was detected by MRI (a, d, e) and by dynamic MRI (b, c).

(b) Diagnostic performance of cavernous sinus sampling

Cavernous sinus sampling was developed to improve accuracy in the diagnosis of Cushing's disease [17]. This technique involves inserting a micro catheter into the cavernous sinus, where an adrenocorticotropic hormone(ACTH) blood sample is taken (Figure 3). Because this procedure is not covered by insurance in Japan, it is expensive, using on average

3–4 micro catheters (at 100,000 Yen each). Even so, the accuracy of diagnosis is much better than for conventional tests [12, 13].

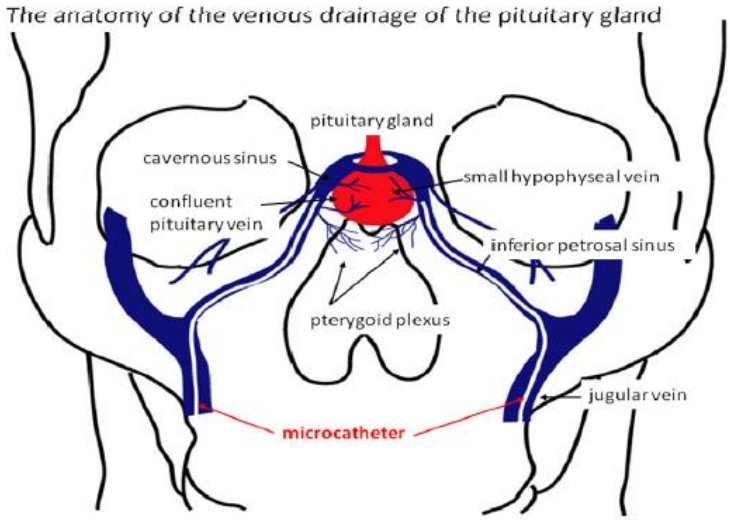


Figure 3. Development of a venous system at the base of the skull. The site of insertion of the micro catheter is shown.

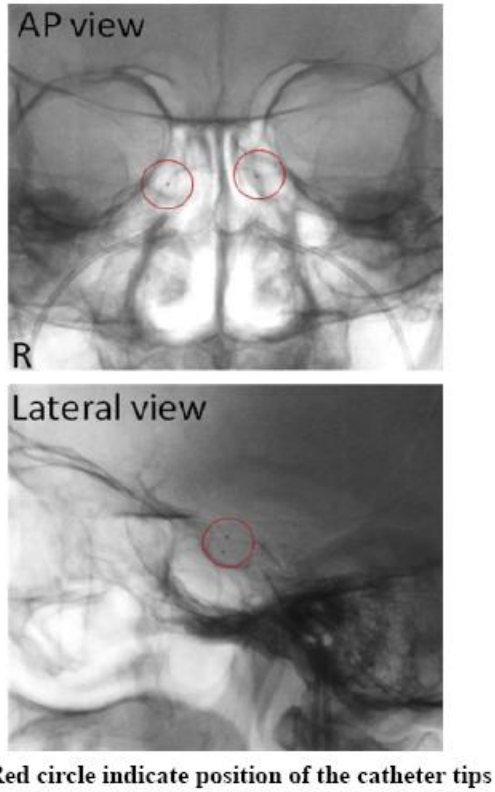


Figure 4. Plain film Xp confirming the location of the tip of the catheter. a) Anteroposterior view and b) lateral image.

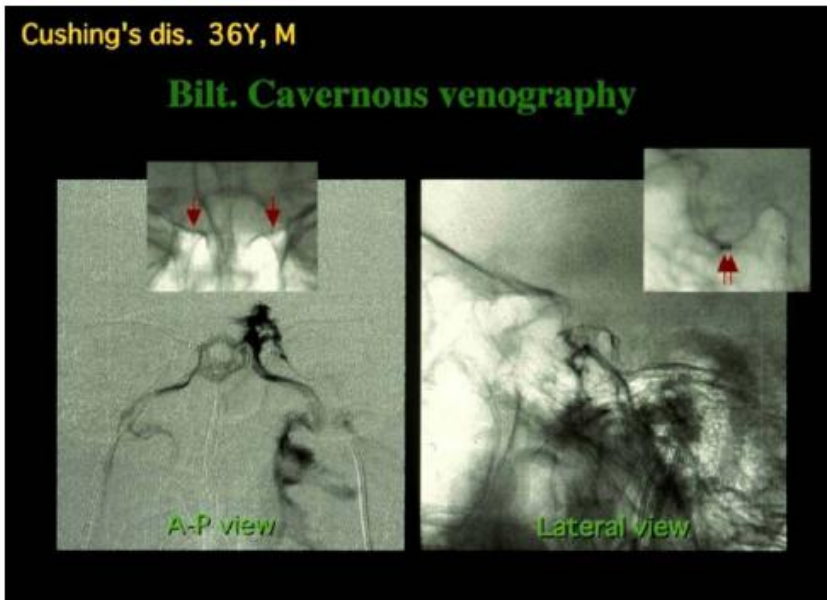


Figure 5. A 36 year-old male with Cushing's disease. Cavernous sinus venography confirming that the tip of the catheter is inside the targeted venous system. Anteroposterior and lateral XR images.

However, depending on how well the cavernous sinus sampling test is controlled and the evaluation methods used, the diagnosis rate for Cushing's disease and the sensitivity in localizing the adenoma may vary.

The different methods used to evaluate the blood samples obtained using this method and the diagnosis rates related to each method were examined by the author and the findings are outlined below:

The tip of the micro catheter is positioned so that it is almost at the center of the cavernous sinus (CS) (Figure 4a and b). In addition to confirming that the tip of the catheter is in position using fluoroscopic guidance, it is important to undertake CS venography to confirm that the catheter is in the CS (Figure 5).

The blood collection areas are as follows:

1) For sampling of ACTH and follicle-stimulating hormone (FSH), blood is collected close to the left and right CS. Peripheral ACTH blood is collected simultaneously;
2) ACTH and FSH blood is collected close to the left and right CS 15 minutes after CRH loading. Simultaneous collection of peripheral ACTH blood samples is essential. The CS/peripheral blood ACTH ratio before/after loading and the gradient of the ACTH/FSH ratio close to the left and right CS before/after loading are then calculated. Central Cushing's disease is diagnosed when the CS ACTH/peripheral ACTH ratio before or after CRH loading is ≥ 2 [19, 20]. To localize the adenoma to the left or right hand CS, a left and right CS ACTH/peripheral ACTH ratio ≥ 2 or a left and right CS ACTH ratio/CS FSH ratio of ≥ 2 is used. The results from 27 cases analyzed using these criteria are shown Table 2.

When Cushing's disease was diagnosed as central, the positive rate both before and after CRH loading was 23/27 (85%). Table 2 shows that an increase (up to 67%) in the sensitivity

for localized diagnosis of microadenomas is possible by comparing the amount of ACTH in blood collected from the CS with that in the peripheral blood collected at the same time, comparing this with the FSH value obtained for the CS blood, and then correcting the ratio. Furthermore, the sensitivity of localized diagnosis could be increased to 81% by collecting blood in the same manner after CRH loading.

Table 2. Diagnostic reliability of data obtained by cavernous sinus sampling evaluated by various indicater. (N=27)

	cavernou sinus－ACTH/ peripheral ACTH ratio	cavernou sinus－ACTH ratio／ cavernous FSH value.
Pre CRH load	63%	67%
Post CRH load	78%	81%

Selective cavernous sinus sampling is a highly invasive test and is very painful for the patient. However, it is still considered to be inadequate for the accurate localization of a pituitary lesion (Figures 6 and 7). Moreover, some patients have a poorly developed internal petrous sinus and, in such cases, the test cannot be completed. Thus, this diagnostic method has anatomical limitations.

Case study

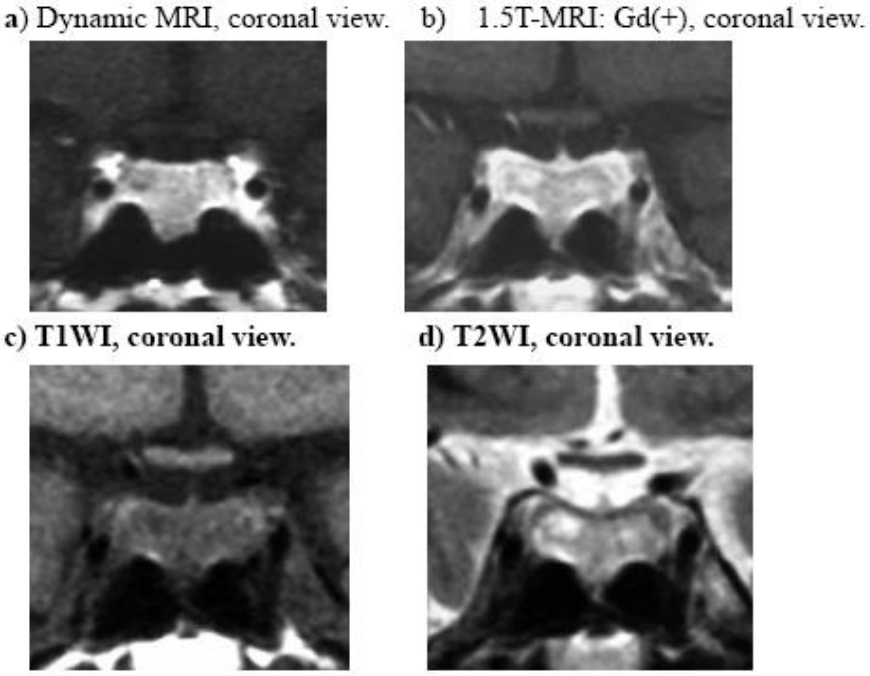


Figure 6. (Continued)

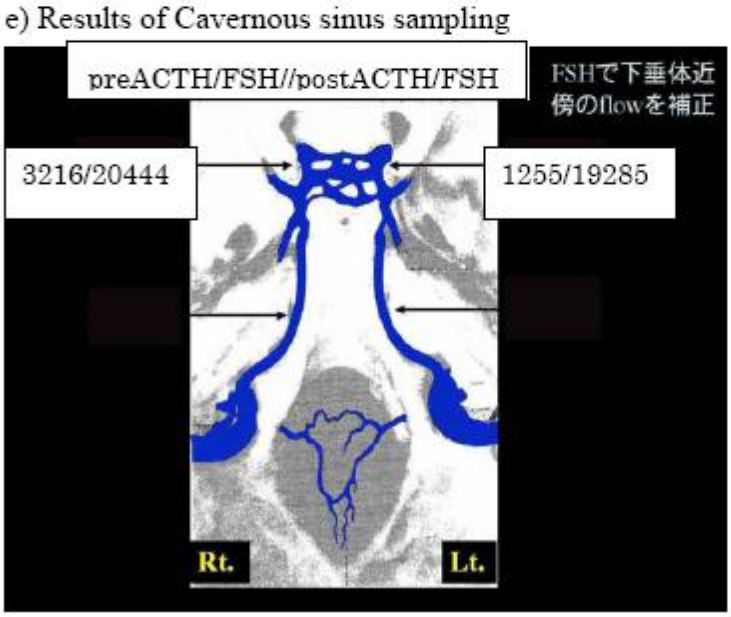


Figure 6. A 49 year-old female with Cushing's Disease. The localization of the tumor is not clear in the MRI scans (a–d). Data from the sampling test before CRH loading (e) shows that the tumor was localized on the right side. Sampling data obtained after CRH loading show that the tumor was in the center. The surgical findings confirmed a tumor occupying the sella turcica. This case illustrates that CRH load sampling diagnosis is better than non-load sampling diagnosis.

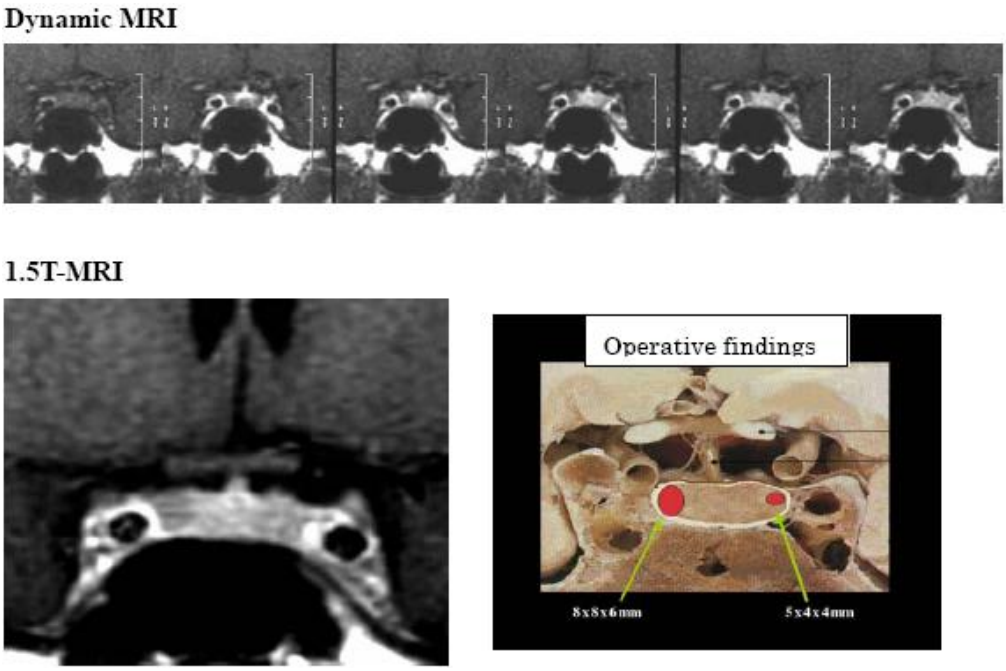


Figure 7. (Continued).

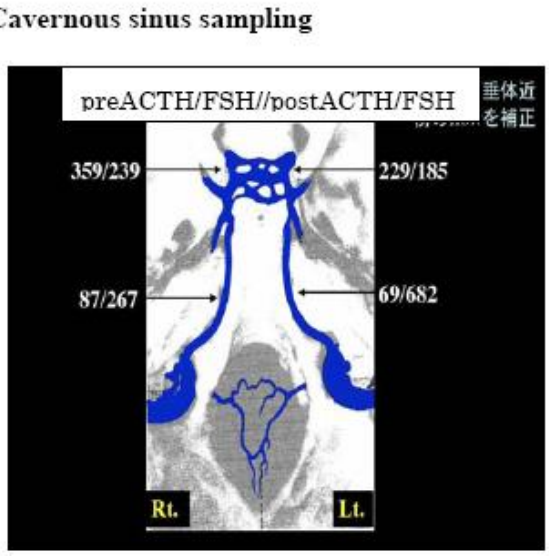


Figure 7. A 30 year-old female with Cushing's disease. According to MRI and dynamic MRI, an adenoma was suspected on the right side of the pituitary. Data from the sampling test before CRH loading showed the presence of a tumor on the right side. Data from the sampling test after CRH loading showed that the tumor was in the center. During surgery, two ACTH-producing tumors were confirmed on the right and left. Sampling under these conditions will result in the conclusion that there is no difference between the right and left (i.e. center). Hence, sampling with CRH loading is considered to be better than sampling without CRH loading, but it is important to consider the possibility of a sampling result that does not necessarily show the localized presence of an adenoma.

(c) Diagnostic performance of methionine PET Fusion 3.0T-MRI imaging.

PET has been used clinically since the 1990s, but image resolution is poor and insufficient for the localized diagnosis of pituitary lesions [21, 22]. Therefore, highly accurate and functional diagnostic imaging of localized adenomas in Cushing's disease is needed. If an imaging method could confirm the presence of a viable adenomatous cell group within the pituitary, it may be possible to ascertain the conditions required to induce pathological changes in a neoplastic lesion. If the lesions could be detected, their removal may alleviate Cushing's disease. PET fusion 3T-MRI is the only method capable of such imaging.

When images obtained using fluoro-2-deoxy-D-glucose/positron-emission tomography (FDG-PET) 3.0T-MRI or methionine PET imaging were compared with surgical findings, the results obtained with methionine PET imaging regarding adenoma localization were consistent with those from surgery. This technique is very useful for the localized diagnosis of Cushing's disease, and excellent progress in surgical treatment is expected.

Although there are reports that FDG-PET is effective for the diagnosis of microadenomas in Cushing's disease [22], methionine PET provides superior diagnostic information with respect to tumor localization and has high diagnostic accuracy (95%). The diagnostic accuracy of FDG-PET is 52% (Table 3).

The advantage of MET-PET fusion 3T-MRI is that the image provides clear and accurate spatial information(Figures 8, 9 and 10). Furthermore, when comparing the standardized uptake value (SUVmax) for preclinical Cushing's disease and overt Cushing's disease using methionine PET, no significant difference was observed (Table 3).

Table 3. Summary of clinical data and MET and FDG SUVmax in patients with Cushing disea and pre-clinical Cushing'S disease

	patient	sex	age	MET-SUVmax	FDG-SUVmax	Pathology	adenoma size
1	Y.I.	F	73	1.8	Negativ	Cushing disease	microadenoma
2	K.S.	M	32	2.5	5.9	pre-clinical Cushing disease	microadenoma
3	J.N.	F	51	-	3.7	Cushing disease	microadenoma
4	A.O.	F	11	3.3	5.7	Cushing disease	microadenoma
5	S,E.	F	39	3.5	5.4	Cushing disease	microadenoma
6	T.I.	F	60	2.5	4.4	Cushing disease	microadenoma
7	N.A.	F	38	3.4	3	Cushing disease	microadenoma
8	T.M.	M	26	1.9	Negativ	pre-clinical Cushing disease	microadenoma
9	R.S.	F	55	1.9	Negativ	Cushing disease	macroadenoma
10	M.K.	F	21	4.1	2.6	pre-clinical Cushing disease	microadenoma
11	Y.T.	F	26	2.7	3.9	pre-clinical Cushing disease	microadenoma
12	N.W.	F	62	1.6	Negativ	pre-clinical Cushing disease	microadenoma
13	K.S.	F	49	3.5	8.2	Cushing disease	microadenoma
14	H.K.	M	39	2.5	Negativ	pre-clinical Cushing disease	microadenoma
15	T.H.	M	67	1.1	Negativ	pre-clinical Cushing disease	microadenoma
16	M.Y.	F	35	3.9	5	Cushing disease	microadenoma
17	Y.M.	F	35	2.5	Negativ	Cushing disease	microadenoma
18	K.H.	F	40	2.1	Negativ	Cushing disease	microadenoma
19	F.A.	F	51	1.9	Negativ	Cushing disease	microadenoma
20	Y.I.	F	74	4.3	3.1	Cushing disease	microadenoma
21	I.N.	F	63	3.1	Negativ	Cushing disease	microadenoma

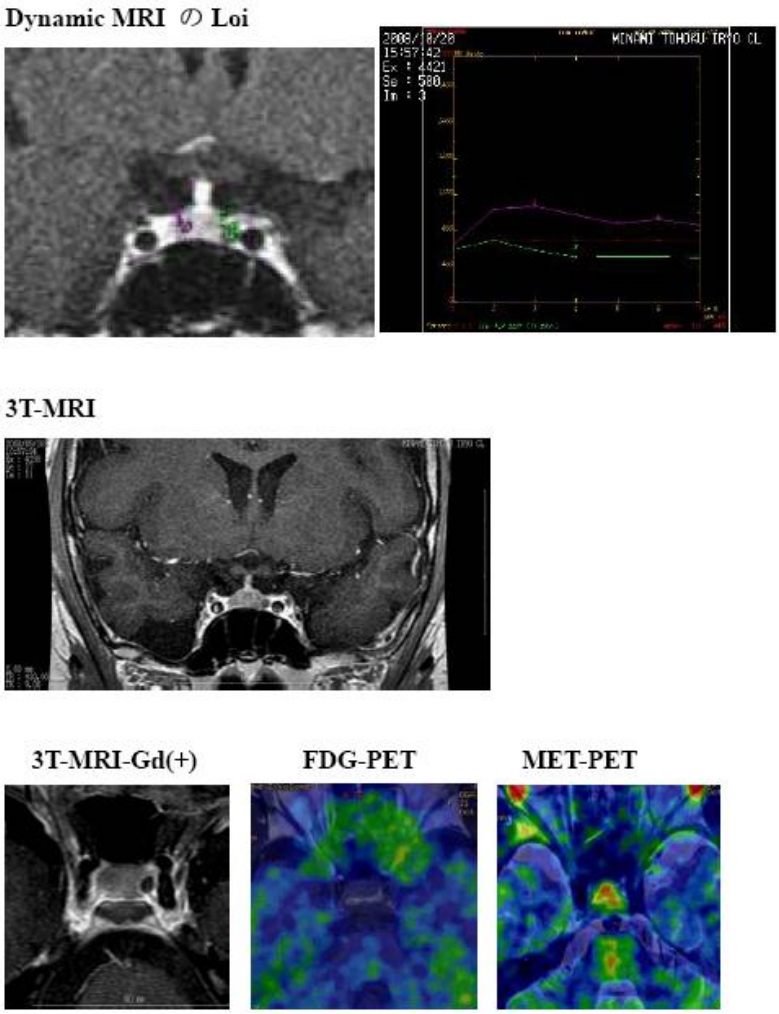


Figure 8. An 11 year-old female with Cushing's disease. A false-positive, with the lesion on the left, was assumed after MRI, but neoplastic findings were detected by dynamic MRI on the right. MET-PET identified the tumor as a localized adenoma on the right side of center.

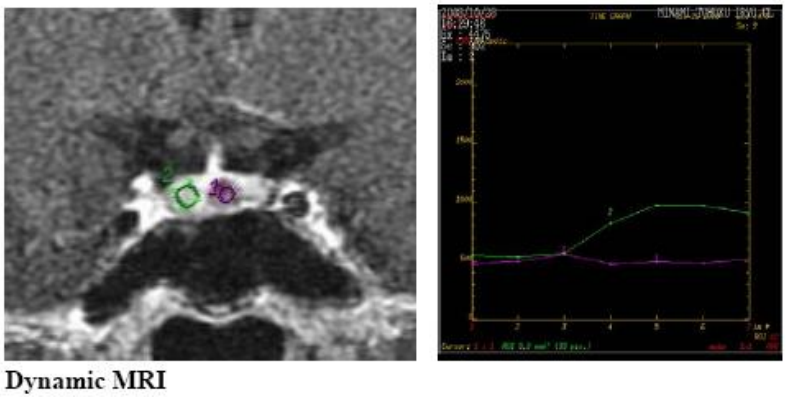


Figure 9. (Continued)

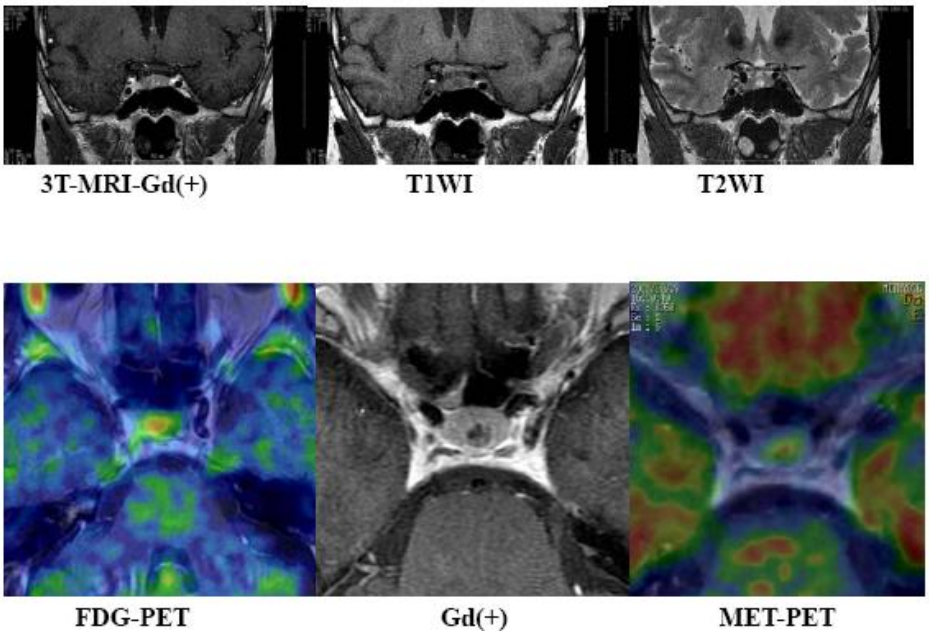
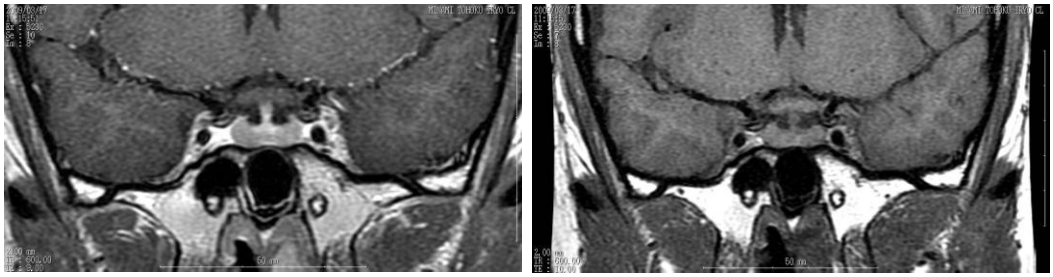
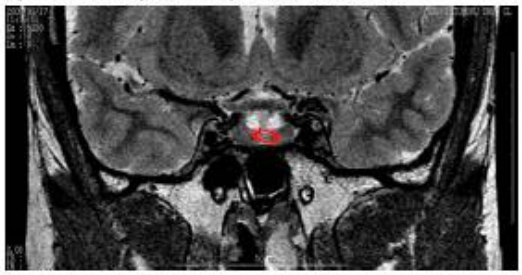


Figure 9. A 39 year-old female with Cushing's disease. Although MRI findings showed a false-positive finding of an adenoma to the left of center, dynamic MRI indicated a localized adenoma on the right. MET-PET confirmed a right-side adenoma. However, according to FDG-PET, FDG uptake was focused in the center. This proved to be hemorrhage and necrosis and the findings were, therefore, a false-positive.

a) 3T-MRI, Gd(+), coronal view. **b)3T- MRI, T1WI, coronal view.**



c) 3T-MRI, T2WI, coronal view.



Red circle indicate actual localization of adenoma.

Figure 10. (Continued)

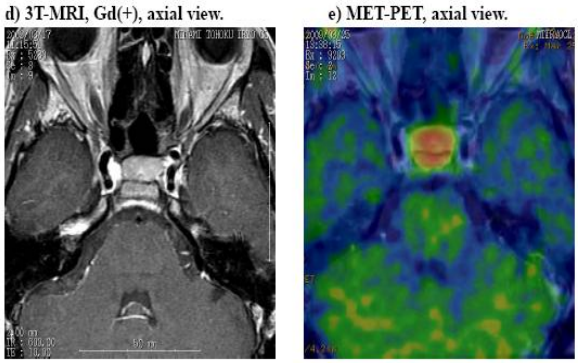


Figure 10. A 21 year-old female with pre-clinical Cushing's disease. A lesion that could not be clearly identified by MRI (a, b) was identified, along with its localization and extent by MET-PET (c). The red circle in (c) shows the localized range of the adenoma determined by surgical findings.

Case Study

A single exception from 21 cases is presented below. In this case, an adenoma causing Cushing's disease could not be identified by MET-PET, but was diagnosed from the surgical sample.

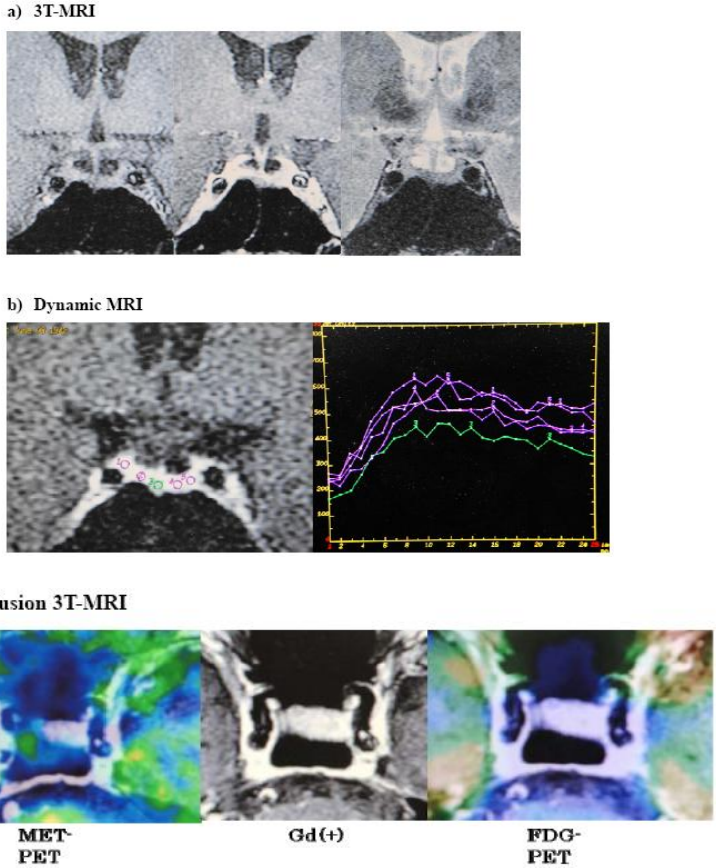


Figure 11. A 66 year-old male with pre-clinical Cushing's disease. A localized adenoma is shown in the lower center of the 3T-MRI (a) and dynamic MRI scans (b). No uptake was observed in MET-PET or FDG-PET (c). This is the only case in which uptake could not be confirmed by MET-PET.

The case is that of a 67 year-old male. Endocrinological findings were consistent with pre-clinical Cushing's disease, although there were no clinical symptoms. No significant uptake was observed by either MET-PET or FDG-PET. However, an area of reduced contrast (2–3mm diameter) in the lower central pituitary was detected by MRI, and a delay in the wash-out of barium contrast media was observed by dynamic MRI. Thus, an adenoma was suspected (Figure 11). Histological examination of a surgically excised sample from this region confirmed an ACTH-producing adenoma.

Why is MET-PET effective?

Methionine passes through endothelial cells via an amino acid transporter and is taken up by tumor cells through protein synthesis. Since the half-life of MET is 20 minutes, the MET detected by MET-PET can be regarded as the level of MET uptake by the tissue. In other words, the MET uptake in tissues actively undergoing protein synthesis is presumed to be high. Various factors influence the uptake of MET, but the level of protein synthesis within a single cell and the cell distribution density, are considered to be particularly important. Corticotroph cell adenomas are known to contain very few interstitial cells and to have a high cell density. Also, an outstanding characteristic of Cushing's adenoma is that almost 100% of adenoma cells are ACTH immunopositive. Our *in vitro* ultrastructural experimental data show that pituitary adenoma cells are undergoing active protein synthesis, regardless of whether the adenoma is functional [23], and the amount of protein synthesis within corticotroph cell adenomas greatly exceeds that in normal pituitary tissue. For this reason, MET uptake by the adenoma provides a good contrast to normal pituitary tissues (Figure 12).

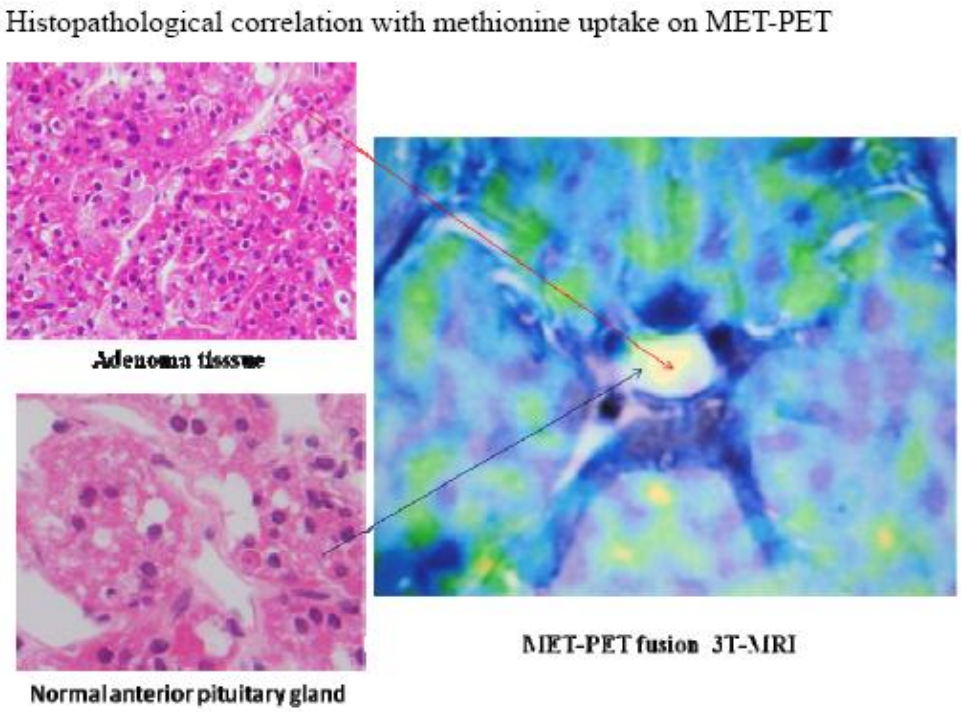


Figure 12. The cell density in normal pituitary tissue is lower than that in adenomas. The degree of MET uptake correlates with the cell density within the adenoma.

Prospects for future diagnosis and treatment of Cushing's disease

The above cases illustrate the advantages and disadvantages of each test method and their limits can be clearly seen. If positive MET-PET or FDG-PET findings are not observed in areas other than the pituitary, positive MET-PET findings are observed within the pituitary, and Cushing's disease is suspected from endocrinological tests, then surgery is justified. This means that selective cavernous sinus sampling is not necessary, except in special cases.

Since no significant difference was observed in MET uptake between preclinical Cushing's disease and overt Cushing's disease, or in the SUVmax from FDG-PET, methionine-PET may be a powerful tool for the early detection of Cushing's disease [14].

Thus, methionine PET fusion MRI can determine whether surgery is required to treat Cushing's disease, and can clarify the presence, location and size of abnormal lesions

Section 2. A Useful Test for the Localized Diagnosis of Adenoma in Recurrent Cushing's Disease

Difficulty in diagnosing recurrent Cushing's disease can be assumed in cases in which: (1) an abnormal MRI signal is obtained due to granulation tissue arising from previous surgery and is not directly due to a pathological lesion; and (2) a gamma knife or radiation was used and distinguishing degenerated fibrous tissue from a residual tumor is, therefore, difficult. In other words, even if a residual adenomatous lesion is seen on MRI, the tissue may not be the cause of active Cushing's disease. High MET uptake measured by PET is more reliable because of the minimal background signal generated by brain or pituitary tissue and (unlike FDG) it is not usually affected by inflammation caused by radiation treatment.

A case in which MET-PET was extremely useful for localized diagnosis of adenoma in recurrent Cushing's is presented below:

Case Study

The patient was a 45 year-old female with a prior history of hypertension.

Present illness: 2007: the cause of her high blood pressure was examined closely in a local hospital, although Cushing disease was not diagnosed (ACTH: 243 pg/ml, cortisol: 33 μg/ml). June 20th, 2007: A pituitary adenoma was identified on MRI. July 11th, 2007: A transsphenoidal operation was carried out at a local Hospital, but only partial resection of the tumor was achieved.

Pathological findings at first operation: Only ACTH was densely immuno-reactive within the tumor cells. Other pituitary hormones were negative. Ring-like cytokeratin immunoreactivity in the tumor cytoplasm indicated Crook's cell adenoma. The Ki-67 labeling index (L.I.) was as high as 29%. September 26th, 2007: The patient was treated with a gamma knife at the Gamma House Center to remove the residual tumor. The tumor marginal dose was 18Gy.

The symptoms of Cushing's disease progressed rapidly and she had difficulty in walking and seeing. December 24th, 2008: The patient was admitted to our clinic for surgical treatment (Figure 13a).

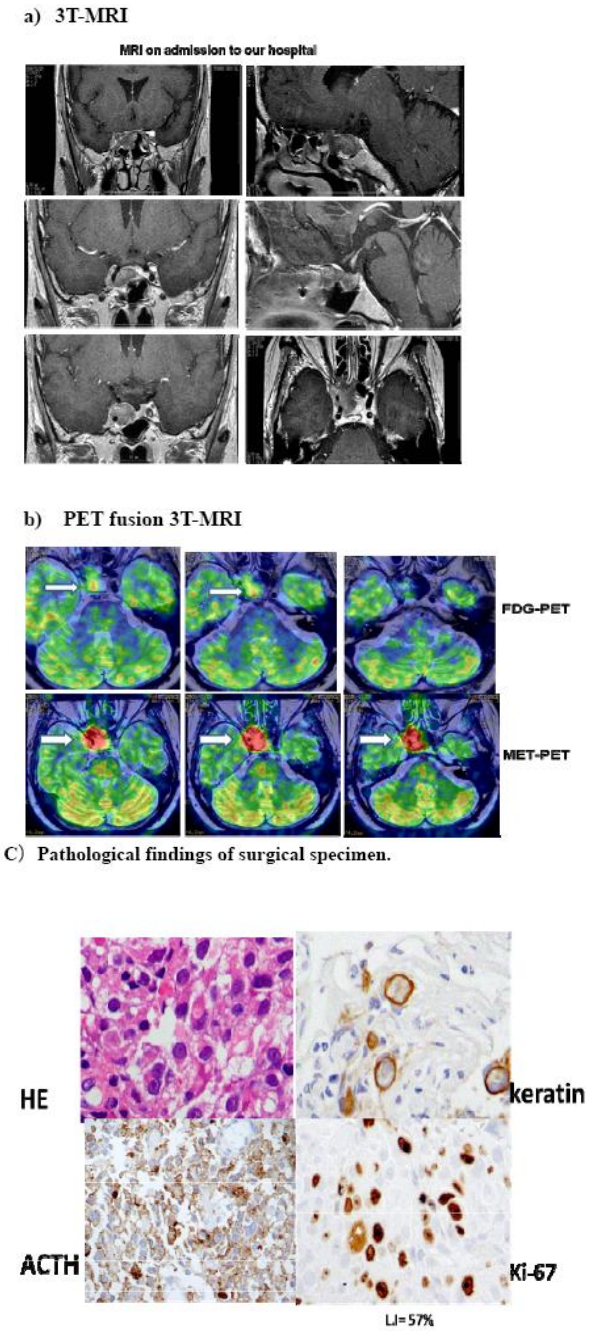


Figure 13. A 54 year-old female with recurrent Cushing's disease. (a) MRI image after surgery and radiation therapy. (b) FDG-PET, MET-PET fusion 3T-MRI. The uptake area of methionine is determined to be the active part of the tumor, regardless of previous surgery and irradiation. (c) The surgically-removed sample containing the area of methionine uptake had high tumor growth potential (Ki-67 L.I = 57%).

28th January, 2009: MET-PET and FDG-PET revealed high uptake of methionine and glucose by the residual adenoma (Figure 13b), suggesting that the adenoma was actively proliferating (ACTH: 196 pg/ml, cortisol 22.3 μg/ml).

January 18th, 2009: A second transsphenoidal operation was performed and 79–80% of the tumor was removed. Pathological examination of the surgical specimen revealed that the Ki-67 L.I. was extremely high (57%) (Figure 13c).

After surgery, the presence and extent of the active lesions in recurrent cases of Cushing's disease can be identified using MET-PET fusion 3T-MRI. Thus, this method is also useful for planing further surgery and for planning the dose of radiation required for gamma knife treatment of the active lesion.

Conclusion

Of the various diagnostic methods used for Cushing's adenoma, MET-PET fusion 3.0T-MRI is best able to detect and visualize the location and extent of adenomas. Using this method, the early diagnosis of Cushing's adenoma and early detection of active lesions in recurrent Cushing's adenoma are both possible. This method is, therefore, expected to make a significant contribution to improving the success of surgical treatments for Cushing's disease.

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