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^{18}F-Choline PET/CT as a New Tool for Functional Imaging of Non-Proliferating Secreting Neuroendocrine Tumors

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Abstract

Choline is an essential component for the formation of new cell membrane and the current understanding is that increased choline uptake in cancer lesions is explained by - and roughly correlates with - cellular proliferation.^{18}F-fluoromethylcholine, a radiolabeled PET-tracer, is increasingly used for the detection of proliferating cancers with PET/CT (choline PET), for example for restaging of recurrent prostate carcinoma. However, clinical findings have suggested that choline uptake may not always be related to proliferation. We present three cases with a carcinoid of the lung with high uptake of radiolabeled choline on PET/CT and a low Ki-67 proliferation index as demonstrated by immunostaining. Although the mechanism behind the enhanced uptake in well-differentiated neuroendocrine tumors is not yet fully understood, for clinical practice this finding means that not every highly choline-avid lesion represents an aggressive cancer; the diagnosis of a functional neuroendocrine tumor should be considered as well.

Keywords: Neuroendocrine tumor; Tumor proliferation; Cell secretion; ^{18}F-fluoromethylcholine; PET/CT

Introduction

Molecular imaging with ^{18}F-fluoromethylcholine PET/CT (choline PET) is increasingly used for the detection and (re-) staging of proliferating cancers, most commonly prostate cancer. Choline is an essential component for the formation of new cell membrane components and the current understanding is that malignancy-induced upregulation of choline kinase leads to the incorporation and trapping of choline in the tumor cell membrane. Therefore, increased choline uptake in cancer lesions is explained by - and roughly correlates with - cellular proliferation [1]. However, we present the histopathological analysis of a choline-positive secreting differentiated neuroendocrine tumor in three patients with very low proliferation rate, proving that high choline uptake in tumors can also be induced by other biological processes. This finding contributes to better understanding of the tumor biology behind the recently demonstrated applicability of choline PET for imaging of benign secreting tumors, such as parathyroid adenomas [2, 3].

Case Reports

Case 1

A 70-year-old man was referred to our hospital for a cT2b-NxM0 Gleason 4 + 4 = 8 adenocarcinoma of the prostate, with an initial PSA of 24.9 ng/mL. He underwent a laparoscopic radical prostatectomy combined with a lymph node dissection. Histopathological evaluation revealed a pT3bN1Mx Gleason 10 adenocarcinoma of the prostate, with metastases in one of seven resected lymph nodes. The patient received additional radiotherapy of the prostate bed. PSA responded with a decline to 0.9 ng/mL, but increased to 1.2 ng/mL 7 weeks later. Progressive disease was suspected and a choline PET was ordered to restage the patient. The PET images showed no signs of local recurrence but revealed three choline-avid parailiac lymph nodes suspicious for lymph node metastases. The largest node is indicated in Figure 1 with a green arrow.

In addition, we encountered an unexpected lesion in the upper lobe of the right lung, with a maximum diameter of 2 cm on low-dose CT and intense uptake of ^{18}F-choline (Fig. 1, red arrow). Based on the high choline metabolism a highly proliferative malignant process was suspected. However, fine needle aspiration of the pulmonary nodule revealed well-differentiated tumor cells of neuroendocrine origin. There were no signs of metastases in regional lymph nodes, and the patient underwent resection of the right upper lobe. Histopathological examination of the resected specimen confirmed the diagnosis of a typical carcinoid (Fig. 2A), with good vascularization, no

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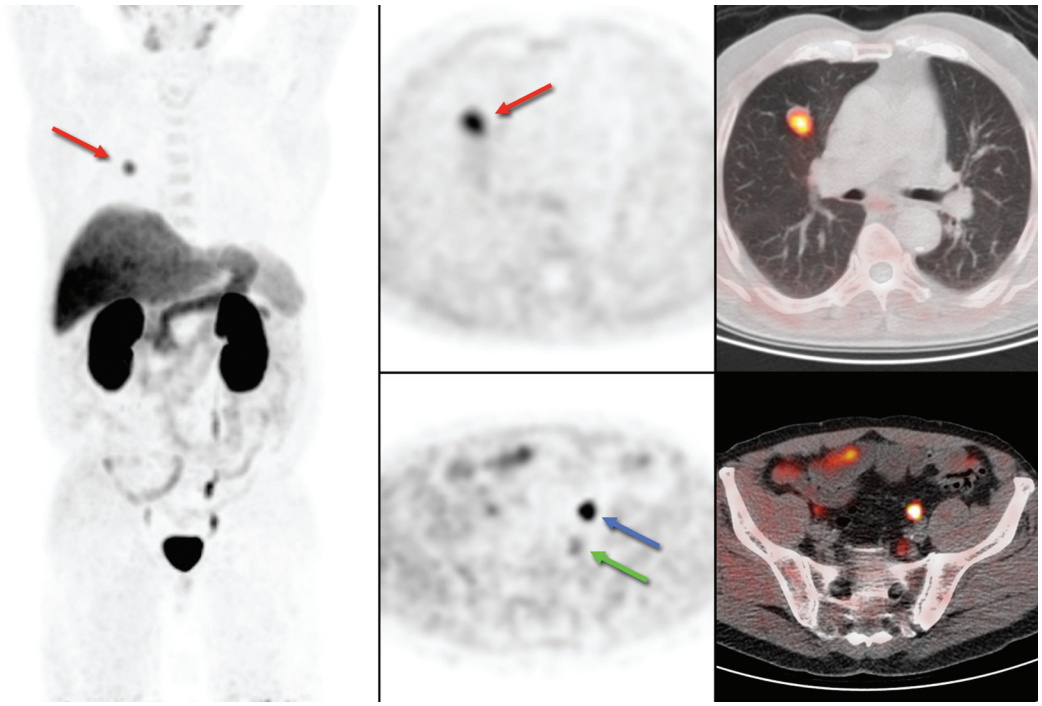

Figure 1. [18]F-choline PET/CT of case 1. Physiological uptake of [18]F-choline is seen in the salivary glands, liver, spleen and kidneys. The blue arrow shows excretion of radioactive urine in the left ureter and the green arrow indicates pathological uptake in a lymph node. In addition, we encountered an unexpected lung lesion in the right upper lobe with high uptake of [18]F-choline (red arrow).

necrosis, and hardly any mitotic activity (< 2 per 2 mm^2) or Ki-67 immunostaining (< 1%, Fig. 2B). Immunostaining for chromogranin, a general marker for neuroendocrine tumors, was 100%.

Case 2

A 73-year-old man presented with a 3 cm tumor in the right lung and biopsy revealed a typical carcinoid. Subsequently, the patient was referred for [68]Gallium-DOTATATE ([68]Ga-DOTATATE) PET revealing intense uptake in the tumor, but no metastasis (Fig. 3a). Two weeks after this scan, choline PET was performed to assess whether this tumor also demonstrated increased uptake as found in case 1. The scan revealed comparable intense uptake in the primary tumor and also no metastasis (Fig. 3b). Excision of the lesion revealed an atypical carcinoid, with good vascularization, no necrosis and mitotic activity of 6 per 2 mm^2. Ki-67 was 1-5%, and chromogranin staining was 100%.

Case 3

A 72-year-old man was referred for [68]Ga-DOTATATE PET because of a high suspicion on a carcinoid in the right lower lobe revealing intense uptake in the lesion (Fig. 4a). Based on this finding and the results of previous patients, a choline PET was performed demonstrating increased uptake in this lung lesion, but less intense as observed in the [68]Ga-DOTATATE PET (Fig. 4b). Histological examination after resection revealed an atypical carcinoid with a diameter of 2.5 cm, focal necrosis and mitotic activity of 2 per 2 mm^2. Ki-67 was 10%, and chromogranin staining was 100%.

In these three cases, there was a strong discrepancy between the imaging and pathology parameters of the lung carcinoids; the high uptake of radiolabeled choline on PET/CT could not be explained by proliferation as demonstrated by the mitotic activity and supported by Ki-67 immunostaining. This led to the conclusion that another biological process can also contribute to upregulation of the choline pathway.

Discussion

Bronchopulmonary carcinoids arise from bronchial mucosal cells known as enterochromaffin cells or Kulchitsky cells, which are part of the diffuse neuroendocrine system (DNES). These well-differentiated neuroendocrine tumors account for only 0.4-3% of all lung cancers, but for approximately 25% of all carcinoids [4, 5]. Carcinoid tumors are classified by pathological features as typical carcinoid or atypical carcinoid, depending on the amount of mitosis/2 mm^2 of viable tumor and the presence of necrosis or architectural disruption.

In clinical practice, computed tomography (CT) is used to identify and stage carcinoid tumors of the lung. Well-defined, centrally located tumors involving the airway with calcification, punctate or diffuse, are considered characteristic on CT

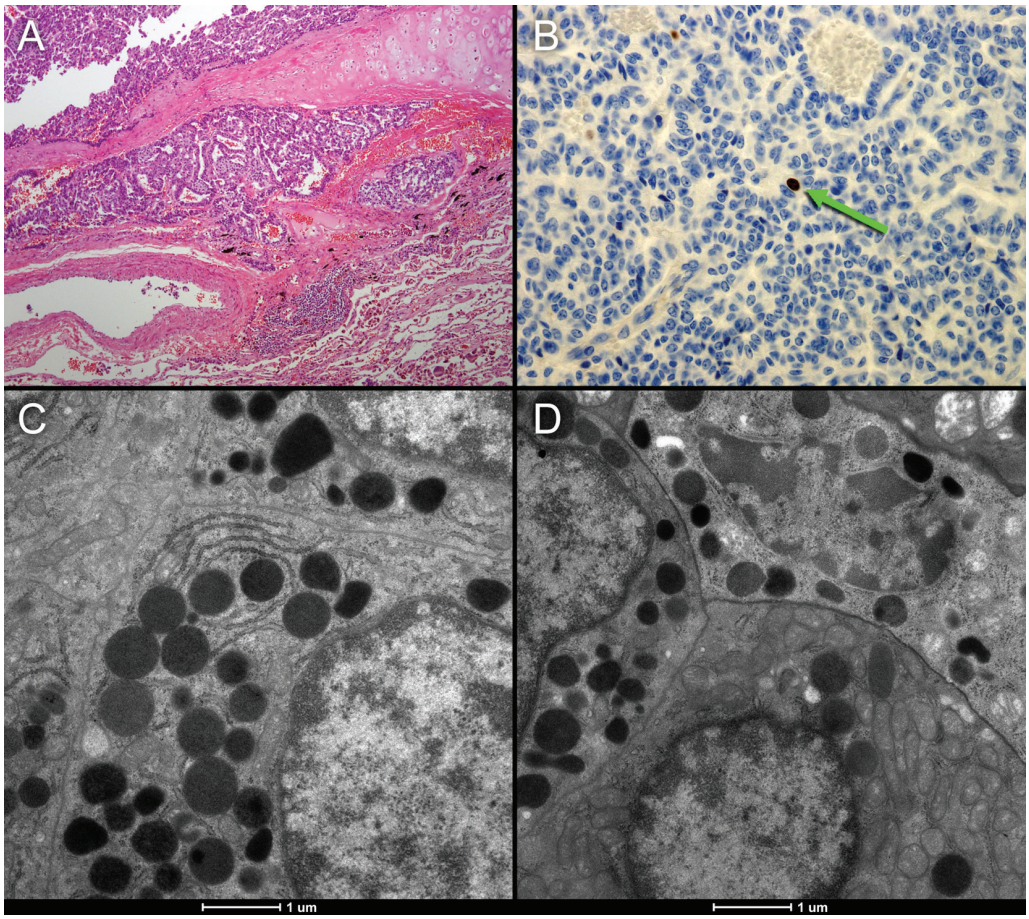

Figure 2. (A, B) Histopathological sample of the resected neuroendocrine tumor of case 1. The tumor shows good vascularization and hardly any necrosis (A). MIB1 immunostaining representing Ki-69 mitotic activity (B: brown colored cells) shows that there is hardly any mitotic activity (< 2 per 2 mm^2). The electron microscope images (C and D) of the neuroendocrine tumor show numerous voluminous electron dense vesicles in the cytoplasm (black circles).

[6]. Additional investigation and staging with ^{18}F-fluoro-deoxyglucose (FDG) PET scanning is described in literature, but remains controversial. Due to the generally low metabolic activity and limited proliferation rate of carcinoid tumors, FDG uptake in PET scanning often demonstrates low uptake (equivocal in comparison to mediastinal uptake) [7-9]. In general, well-differentiated neuroendocrine tumors demonstrate high expression of somatostatin receptors on their cell surface. In this respect, non-invasive tumor characterization and staging with radiolabeled somatostatin analogs, such as ^{111}Indium-octreotide or ^{68}Gallium-DOTATATE, have shown good results. Therefore, these tracers are regarded as first choice for imag-

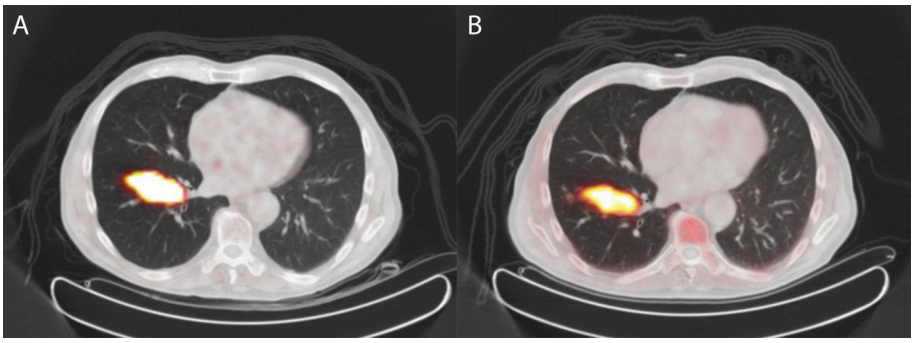

Figure 3. ^{68}Gallium-DOTATATE PET/CT (A) and ^{18}F-choline PET/CT (B) of a patient with an atypical carcinoid of the lung (case 2). The tumor shows increased somatostatin receptor expression on ^{68}Gallium-DOTATATE PET/CT but also intense uptake of ^{18}F-choline on ^{18}F-choline PET/CT.

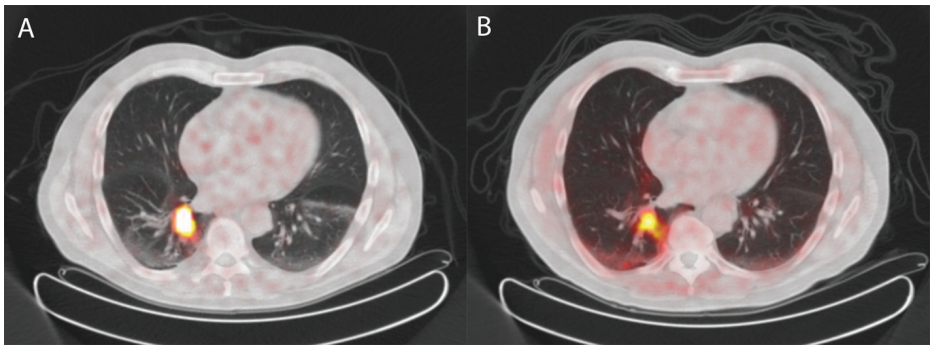

Figure 4. ^{68}Gallium-DOTATATE PET/CT (A) and ^{18}F-choline PET/CT (B) of a patient with an atypical carcinoid of the lung (case 3) revealing similar uptake of ^{68}Gallium-DOTATATE as well as ^{18}F-choline as compared to case 2.

ing in grade I and II tumors [10, 11].

Choline is a precursor for the biosynthesis of phospholipids, amongst others phosphatidylcholine (PtdCho), which are major components of the cell membrane [12]. After intracellular uptake, phosphorylation by choline-kinase is the first step in the biosynthesis of choline phospholipids such as PtdCho. Especially PtdCho is required for the build-up and maintenance of cell membranes. As a result, increased cell proliferation causes an increase of choline uptake, and this is the corner stone for PET scanning using radiolabeled choline in prostate cancer [13, 14]. So far, data in literature have shown promising results in restaging prostate cancer patients, and it was shown that ^{18}F-choline PET yielded better results than ^{18}F-FDG PET [15, 16].

Because of the very low proliferation rate of neuroendocrine tumors, the uptake of choline in present cases is probably not explained by an increased production of PtdCho. Other mechanisms may therefore be the explanation for the finding in these patients. It is known that choline is also involved in other metabolic pathways. For example in lung tissue, choline is an important precursor in the synthesis of surfactant, which is produced in alveolar cells, and for the production of acetylcholine (ACh) [17].

In a recent review, choline metabolite concentrations and enzyme expression in different human cancers were presented, demonstrating an increased choline-transport and choline-kinase (CK) activity in many of them [1]. In lung cancer, not only an increased CK activity was observed, but also an increased expression of choline transporters. The high-affinity choline transporter (CHT)1 which is expressed in neurons and is required for the synthesis of ACh, is not expressed by some lung tumors, amongst others small cell lung carcinoma (SCLC). Instead, these tumors express choline-transporter-like protein 1 (CTL1), by which the uptake and metabolism of choline is increased [18]. Whether this is also applicable to all lung neuroendocrine tumors is not clear and has to be elucidated.

Lung cancer cells create a cholinergic autocrine loop by syntheses and secretion of ACh and it responds to endogenous ACh. This loop has been best characterized in SCLC, a type of neuroendocrine lung tumor [19]. In cholinergic neurons, the neurotransmitter ACh is synthesized from choline and acetyl-CoA by choline acetyltransferase (ChAT) and is then translocated into synaptic vesicles by vesicular ACh transporter (VAChT). In contrast, the role of VAChT in lung cancer cells is unclear and the secretion of ACh is less tightly regulated and not necessarily vesicular [20]. In our first case, the electron microscope images of the neuroendocrine tumor in Figure 2 show numerous voluminous electron dense vesicles in the cytoplasm (C and D: black circles). Furthermore, active reticulo endoplasmic system (RES) organelles are present. These findings of neuroendocrine activity may indicate an overexpression of the cholinergic autocrine loop with a high turnover of intracellular vesicles probably requiring significant quantities of cell membrane components including choline. This may be a possible alternative mechanism of high choline uptake, not related to proliferation. These findings may lead to further investigation of new molecular imaging purposes for choline-PET.

ACh interacts with muscarinic and nicotinic ACh receptors. Data in literature have shown that choline may also interact directly as an agonist with the nicotinic acetylcholine receptors, and, consequently, ^{18}F-choline PET scanning may be feasible to visualize increased expression of these receptors in non-proliferating tissues [21, 22].

These considerations also apply to functional imaging of benign secreting tissues that do not proliferate. After several incidental discoveries of functioning parathyroid gland adenomas [23-25], choline PET has been advocated as a new tool for the detection and localization of these hyperfunctioning lesions [2, 3, 26]. However, the biological processes behind the uptake of choline in parathyroid adenomas have not been evaluated or described. Based on the described theoretical considerations and the histopathological findings in our cases with NET, we assume that choline uptake in benign secreting tumors is also not related to proliferation but might be based on upregulation of the cholinergic autocrine loop, increased turnover of intracellular vesicles, and a corresponding increased expression of choline transporters. This hypothesis needs to be evaluated in further research.

Conclusion

Although the mechanism behind the enhanced uptake in well-differentiated, non-proliferating and secreting tumors is not yet fully understood, we propose that choline uptake might be related to an upregulation of the cholinergic autocrine loop and

a corresponding increased expression of choline transporters. For clinical practice, this means that not every highly choline-avid lesion represents an aggressive cancer; the diagnosis of a functional neuroendocrine tumor however should be considered as well. These findings support attempts to apply ^{18}F-choline PET for functional imaging of parathyroid adenomas and other benign non-proliferating secreting tissues.

Conflict of Interest

The authors declare that they have no conflict of interest.

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