Abstract. Neuroendocrine tumours (NETs) are rare pathologies which originate from neuroectodermic and endodermic cells and that can produce peptides and amino acids. About 70% of NETs derive from gastroenterohepatic (GEP) system and the other 30% from the different sites through the body. They are distinguished into single and multiple localizations and also into sporadic, familial multiple endocrine-related forms and recurrent forms. Moreover, when they produce hormones they usually are symptomatic; yet, they are characterized by the synthesis and secretion in the bloodstream of several tumor-specific markers or can express somatostatin receptors in their cellular surface. The diagnosis and follow-up of NETs rely on laboratory studies, histopathology and the combination of anatomical and functional imaging, with the latter being the main method for monitoring response to therapy. In recent years, nuclear medicine has contributed to the impressive development of the knowledge of NETs in terms of biology (receptor scintigraphy), pharmacology (development of new tracers) and therapy (radiometabolic therapy). Nuclear medicine procedures for diagnosis and treatment of NETs are based on the biological properties of these tumours: the expression of somatostatin receptors. Somatostatin receptor scintigraphy not only has a crucial role in diagnosis and staging of NETs, but also in assessing suitability for treatment with cold and radiolabelled somatostatin analogues, as well as in monitoring response to treatment and detecting recurrent disease. In conventional nuclear medicine, the two most important functional imaging modalities are $^{111}$In-octrescan and $^{123}$I-MIBG. Over the last 5 years, due to the development of new tracers, such as $^{68}$Ga labelled-DOTA-peptides PET and $^{18}$F-DOPA, PET has also been employed with significant benefits in the diagnosis and management of NETs.

Suspicion of the NETs or discovery of a known tumour is based on a patient’s family history, clinical manifestations, laboratory studies, histopathology and imaging studies.

Laboratory Studies and Histopathology

For each tumour type, characteristic clinical symptoms should lead to the measurement of specific markers such as serotonin, insulin, glucagon, gastrin, VIP, and somatostatin. An excessive amount of hormones leads to typical clinical signs. The type of symptoms will depend on where the tumour originated, where it may have spread to, and whether it produces a hormone such as serotonin. Carcinoid tumours produce excessive amounts of serotonin (1). When serotonin is broken down in the liver, it is excreted as 5-hydroxyindoleacetic acid (5HIAA) in the urine. A 24-hour urine collection is needed to check whether there are raised levels of 5HIAA. To establish the diagnosis of insulinoma a 12- to 72-hour fast is recommended, and a glucagon test may also be informative (2). For the diagnosis of gastrinoma, measurement of basal and maximal gastric acid output is recommended to exclude secondary hypergastrinaemia (3).

In histopathology, special stains are performed on the tumour for chromogranin A, neuron-specific enolase (NSE), protein gene product (PGP 9.5), Ki-67 protein, gastrin and other gut hormones. Chromogranin A serves as a sensitive but non-specific tumour marker in non-functioning and in functioning endocrine tumours. It is localized in secretory granules of neurons and endocrine cells and it is regarded as a powerful universal marker for NETs (4, 5). Excessively elevated levels (>1000 pg/ml) indicate unfavorable prognosis
defining the size and extent of soft tissue lesions and accurately relationship of masses and major vascular structures by development during the past years. It clearly shows the tumour size and extent (15). MRI has been an important reconstruction, when needed, and the accurate assessment of moreover, it improves the quality of three-dimensional image processing system allow qualitative and quantitative assessment of tumour vascularity. CT has better specificity than US and defines the anatomical borders of the lesion more clearly. It is essential in preoperative staging. Multi-slice CT allows multi-phase scanning (native, arterial, venous phase), which is mandatory for surgery and determining the extent of surgical resection (12). Nowadays, several anatomical imaging techniques are used: conventional X-ray, ultrasound (US) (endosonography, EUS; contrast-enhanced sonography; combined with colour Doppler sonography, intraoperative sonography), computed tomography (CT), magnetic resonance imaging (MRI), endoscopic retrograde cholangio-pancreatography (ERCP), magnetic resonance cholangio-pancreatography, CT-enteroclysis and arteriography (13). Conventional X-ray is still very useful to study particular tumour site (e.g. thorax). US has variable validity depending on the site of disease (excellent results have been obtained in detecting liver metastases). US generally shows more detail in children than in adults. Endosonography is useful in the identification of intraluminal tumours and has a very high accuracy for gastric and pancreatic tumours or when performed intraoperatively. Intraoperative palpation combined with intraoperative US has been shown to be more sensitive than other conventional techniques in the diagnosis of small pancreatic tumours, particularly insulinomas (14). Colour Doppler and power Doppler US combined with a computed image processing system allow qualitative and quantitative assessment of tumour vascularity. CT has better specificity than US and defines the anatomical borders of the lesion more clearly. It is essential in preoperative staging. Multi-slice CT allows multi-phase scanning (native, arterial, venous phase), which is mandatory to study liver lesions, for instance. Moreover, it improves the quality of three-dimensional reconstruction, when needed, and the accurate assessment of tumour size and extent (15). MRI has been an important development during the past years. It clearly shows the relationship of masses and major vascular structures by defining the size and extent of soft tissue lesions and accurately depicting displacement and invasion of adjacent organs. MRI and CT can identify larger primary tumours and organ metastases (16). Due to the small size of the tumours, morphological imaging techniques are sometimes unsuccessful at identifying sites of primary tumours or metastatic spread, which are both crucial for selection of the most appropriate therapy, such as surgery, radiofrequency ablation or other medical treatment. Therefore a combination of functional and morphological imaging is needed for initial diagnosis, localization of the tumour, staging, monitoring of the progress of the disease and assessment of the response to treatment.

Functional Imaging Techniques

At present, it is impossible to plan the management of a patient affected by NETs without performing nuclear medicine examinations. Functional imaging techniques used in the evaluation of NETs is somatostatin receptor scintigraphy (SRS), whole body planar scintigraphy, WB and single photon emission computerized tomography (SPECT), and positron emission tomography (PET). SPECT imaging can visualize more lesions than planar imaging, so it is mandatory for an accurate evaluation (17). Nuclear medicine procedures for the diagnosis and treatment of NETs are based on the biological properties of these tumours, namely the expression of somatostatin receptors. Somatostatin, also known as growth hormone-inhibiting hormone (GHIH) or somatotropin release-inhibiting factor (SRIF), is a small peptide hormone of 14 amino acids that regulates the endocrine system and affects neurotransmission and cell proliferation via interaction with G-protein-coupled somatostatin receptors (18). Somatostatin receptors (SSTR) are integral membrane glycoproteins that are distributed in a variety of tissues throughout the body. Molecular studies have revealed the existence of five distinct somatostatin receptor types with different tissue distribution. These receptors have been cloned and chronologically termed SSTR1, SSTR2 (with 2 splice variants: SSTR2A and SSTR2B), SSTR3, SSTR4, and SSTR5. Many NETs are known to express SSTRs with varying intensity. The most common subtypes of SSTRs of NETs are SSTR2 and SSTR5, and their expression can be quite elevated (19, 20). Since NETs are well differentiated, they usually overexpress somatostatine receptors.

In patients with SSTRs-positive pathology, SSTR scintigraphy is a highly sensitive diagnostic method to localize NETs and can provide a functional assessment of the disease state. In addition, scintigraphy can add specificity and increased confidence in a diagnosis of a mass seen on anatomical imaging techniques. It is the most sensitive test for metastatic spread of NETs (21). Several somatostatin analogues have been developed both for imaging as well as therapy of NETs. Octreotide is a somatostatin analogue consisting of eight amino acids. Pentreotide (an octreotid analogue) has been shown to
bind to SSTRs on both tumour and non-tumour sites throughout the body. The agent is labelled with $^{111}$In-DTPA ($^{111}$In-diethylene triamine pentaacetic acid), commercially available as Octroscan® Mallinckrodt. Octreotide is excreted almost exclusively by the kidneys (via glomerular filtration) with 85-90% of the dose recovered in the urine in the first 24 hours. Uptake in the kidneys is for the most part from reabsorption of the radiolabeled peptide in the proximal renal tubular cells via megalin receptors after glomerular filtration. There is little hepatobiliary excretion (2-10%), but bowel activity may sometimes be visualized on delayed 24 hour images, and a bowel preparation should be given prior to imaging. The sensitivity of $^{111}$In pentetreotide may be reduced in patients concurrently receiving therapeutic doses of octreotide acetate (Sandostatin®) for control of symptoms from certain tumours, although this has not been identified in clinical trials. It is nonetheless recommended that octreotide therapy be withheld for at least 24 to 72 hours prior to the examination, if possible (23). Somatostatin analogues can be radiolabelled not only with $^{111}$In (e.g. Octroscan®) but also with $^{99m}$Tc e.g. $^{99m}$Tc-EDDA/HYNIC-TOC ($^{99m}$Tc-EDDA/HYNIC-D-Phe 1, Tyr 3] octreotide) (24). Diagnosis, localization and tumour spread can be determined using $^{99m}$Tc-EDDA/HYNIC-TOC at least as confidently as using $^{111}$In-octreotide. Its advantages are availability, low cost, decreased absorbed dose for the patients and high quality of scintigraphic images (24). Scintigraphy with $^{111}$In or $^{99m}$Tc-labelled somatostatin analogues has become the main imaging technique for NETs, particularly those expressing a high density of SSTRs. Combined with SPECT, it is currently the first choice imaging technique for these tumours.

**PET and PET Radiotracers in Diagnosis of NETs**

PET studies reflect the different metabolic pathways of NETs, such as glucose metabolism ($^{18}$F-Fluorodeoxyglucose), the uptake of hormone precursors ($^{11}$C-5-hydroxytryptophan ($^{11}$C-5HTP); $^{11}$C- or $^{18}$F-dihydroxyphenylalanine; $^{18}$F-fluorodopamine), the expression of receptors ($^{68}$Ga-labelled somatostatin analogues), as well as the synthesis, storage, and release of hormones ($^{11}$C-hydroxyephedrine and others) (25, 26, 27).

$^{18}$F-Fluorodeoxyglucose ($^{18}$F-FDG) PET with localizing or diagnostic CT, is the most common technique combining functional and morphological information in oncology today. Uptake of the FDG depends on intensity of glycolysis in the malignant cell; this is rather low in highly differentiated and slowly growing tumours, such as NETs. Therefore, FDG PET is not the ideal diagnostic method for evaluation of NETs. It can be useful, however, in demonstrating that dedifferentiation occurred; this has an important prognostic implication and can lead to change in therapy (chemotherapy). Recently, $^{18}$F-deoxyphenylalanine ($^{18}$F-DOPA) PET has emerged as a new diagnostic tool for the imaging of NETs. $^{18}$F-DOPA is a radiolabelled amino acid precursor of dopamine that was originally synthesized for the *in vivo* evaluation of receptor uptake in the caudate and putamen in Parkinson’s disease. In oncology it has been employed for the detection of carcinoid, pheochromocytoma, neuroblastoma, medullary thyroid carcinoma, microcytoma, carotid glomus tumours and melanoma. Hoegerle et al. (26, 28) compared whole-body $^{18}$F-DOPA PET with $^{18}$F-FDG PET, $^{111}$In-octreotide and morphologic imaging (CT and/or MRI) in patients with gastrointestinal carcinoid tumours. $^{18}$F-DOPA PET had the highest sensitivity for detecting the primary tumour and lymph node metastases. The study also examined the role of $^{18}$F-DOPA PET/CT in comparison to $^{111}$In-octreotide scintigraphy in patients with gastroenteropancreatic NETs. $^{18}$F-DOPA PET/CT identified the primary tumour in all patients with negative or inconclusive conventional imaging and $^{111}$In-octreotide scintigraphy and was able to detect additional unsuspected lesions, the majority of which were confirmed as metastatic deposits. Management was changed in 84% of patients who underwent $^{18}$F-DOPA PET/CT (29).

Many NETs, including some non-functional tumours, take up L-dihydroxyphenylalanine (L-DOPA) and 5-hydroxytryptophan (5-HTP) which then undergo decarboxylation by the enzyme amino acid decarboxylase to produce dopamine and serotonin (5-hydroxytryptamine) respectively. This amine uptake and decarboxylation provides an important target for functional imaging with PET. In comparative studies of patients with a variety of NETs, $^{11}$C-5HTP PET proved better than CT and SRS by visualizing additional small lesions. $^{11}$C-5HTP is selectively taken up by neuroendocrine tumour cells, decarboxylated and irreversibly trapped as $^{11}$C serotonin. With carbidopa premedication orally before $^{11}$C-5HTP PET examination, the tumour uptake can be increased and the urinary radioactivity concentration considerably reduced (30). Whole-body $^{11}$C-5HTP PET may therefore be useful as a universal imaging technique for gastroenteropancreatic NETs, imaging more tumour lesions than $^{111}$In-octreotide and CT in the majority of patients (31).

Gallium-68 ($^{68}$Ga) is a positron emitter with a suitable half life of 68 minutes and has been utilized for the labelling of somatostatin analogues for PET imaging of NETs with promising results in a small number of patients. The synthetic somatostatin analogues such as tetraazacyclododecane-tetraacetic acid-d-Phe(1)-Tyr(3)-octreotide (DOTA-TOC) can be readily labelled with $^{68}$Ga. $^{68}$Ga-labelled peptides are rapidly accumulated in tumours but not within tissues that do not express SSTRs, providing high contrast imaging. They have high affinity for SSTR2, -3, -5 and intermediate affinity for SSTR4. Kidney uptake for both radiopharmaceuticals is also much lower than $^{111}$In octreotide. The pre-clinical and clinical applications of this technique have been successful in a variety of tumours, particularly NETs and its labelling with
other ligands and molecules will improve the management of other tumours and the assessment of infection (32). Gabriel et al. (33) compared $^{68}$Ga-DOTA-TOC PET using conventional scintigraphy with $^{99m}$Tc-HYNIC-TOC and $^{111}$In-DOTA-TOC. $^{68}$Ga-DOTA-TOC PET showed a significantly higher detection rate compared with conventional SSTR scintigraphy and diagnostic CT with clinical impact in a considerable number of patients. The introduction of radiopharmaceuticals for PET imaging has therefore improved the quality of imaging of NETs.

References


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