GUIDELINES FOR PET/CT IMAGING OF NEUROENDOCRINE NEOPLASMS WITH ^{68}Ga-DOTA-SOMATOSTATIN ANALOGUES AND ^{18}F-DOPA.

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Abstract

Neuroendocrine neoplasms are a heterogenous group of tumours, for which nuclear medicine plays an important role in the diagnostic work-up, follow-up and somatostatin receptor targeted therapies.These guidelines are aimed to assist nuclear medicine physicians in recommending, performing, interpreting and reporting the results of somatostatin receptor (SSTR) PET/CT imaging using ^{68}Ga-DOTA-somatostatin analogues (SSA), and ^{18}F-DOPA for various neuroendocrine neoplasms (NENs). The previous procedural guideline by EANM regarding the use of PET/CT tumour imaging with 68Ga-DOTA-SSAs has been revised and updated with the relevant and recent literature in the field with contribution of distinguished experts.

Keywords: Neuroendocrine tumour; neurendocrine neoplasm; carcinoid; PET/CT; ^{68}Ga-DOTATATE; ^{68}Ga-DOTATOC; ^{68}Ga-DOTANOC; ^{18}F-DOPA; ^{18}F-FDG; thyroid medullary cancer; pheochromocytoma; paraganglioma; foregut-NET; midgut-NET; hindgut-NET; hyperinsulism in infants

Background information and definitions

Neuroendocrine neoplasms (NENs) are a heterogenous group of neoplasms that originate from cells of neuroendocrine origin in many different organs but most frequently in the gastrointestinal tract and the lungs. Less common locations include thymus and other organs with endocrine function such as adrenal medulla, pituitary and thyroid.

NENs can be classified into three histopathological grades according to the World Health organisation (WHO) 2010 classification. This classification depends on the proliferation marker Ki-67 index and the mitotic index of the tumour **(Table 2).** According to a histopathological grading system, NENs can also be classified into four types. Type 1 refers to NENs with low Ki-67 and mitotic index, which are benign well-differentiated NENs. Type 2 refers to the well-differentiated NENs with an uncertain behaviour. Type 3 NENs are well-differentiated neuroendocrine carcinomas (NECs) with mostly low-grade malignant features. Type 4 refers to poorly-differentiated high-grade NECs with aggressive behaviour. Additionally, there is a tumour type consisting of adenocarcinoma with neurendocrine differentiation, which is called "MANEC" (mixed adenoneuroendocrine carcinomas).

The majority of NENs express somatostatin receptors (SSTR), which can be used as targets for radionuclide imaging and therapy. Somatostatin is a small cyclic neuropeptide that is found in neurons and endocrine cells and has a high density in the brain, peripheral neurons, endocrine pancreas and gastrointestinal tract **[1-10].** Since naturally occuring somatostatin is very unstable, synthetic more stable analogues have been developed [**1, 11**].

Scintigraphy with radiolabeled somatostatin analogues (SSA), first with ^{123}I and followed by ^{111}In and [99mTc], has been an important part of the imaging work up of patients with SSTR-positive NENs with a detection rate between 50 and 100% in different studies [**1-11**]. These varying detection rates between studies may at least partly be explained by the use of different SSA-preparations and varying acquisition techniques such as planar imaging only, combined planar imaging and SPECT or SPECT/CT. SSTR scintigraphy presents some limitations that may decrease the diagnostic efficacy. This is mostly due to high physiological uptake such as the liver as well as the lack of detection of smaller lesions because of the suboptimal physical characteristics of the radiopharmaceuticals and the relatively low spatial resolution of gamma cameras [**12-13**].

The transition to SSTR imaging by PET/CT with ^{68}Ga-DOTA-SSA, has brought a new vision with regard to spatial resolution, tumour-to-normal tissue contrast and patient comfort due to earlier and shorter acquisition times compared to SSTR planar and SPECT imaging . The most commonly used SSA-preparations as radiopeptides for PET/CT imaging are [^{68}Ga-DOTA^0 -Tyr^3]octreotide (^{68}Ga-DOTA-TOC, ^{68}Ga-edotreotide), [^{68}Ga-DOTA^0 -^{1}NaI^3]octreotide (^{68}Ga-DOTA-NOC) and [^{68}Ga-DOTA^0 -Tyr^3]octreotate (^{68}Ga-DOTA-TATE) [14-16]. All these radiopeptides bind to the SSTR subtype 2, which is predominantly expressed in NENs but each has different affinity profiles for other SSTR subtypes [17] (Table 1). ^{68}Ga-DOTA-NOC along with the highest affinity to SSTR2 shows a high affinity to SSTR3 and 5, ^{68}Ga-DOTA-TOC binds also to SSTR subtype 5 (with lower affinity compared to DOTA-NOC) while ^{68}Ga-DOTA-TATE predominantly binds to SSTR subtype 2. Finally, ^{68}Ga-DOTA-lanreotide binds to SSTR subtype 2 and 5 [18-20]. Recently, also ^{64}Cu-DOTA-TATE has been used for PET/CT imaging in NEN patients and was found superior to SPECT based somatostatin receptor imaging [19].

These above mentioned radiopeptides function as SSTR agonists. Recently some SSTR antagonists have, however, been introduced. Limited data on SSTR antagonist radiopeptides excist, especially for PET applications, which might more efficiently localise NENs due to binding to a higher number of receptor sites (practically all SSTR subtypes) and with more stable binding [21].

SSTRs are not only targets for radionuclide imaging of NENs but also for peptide receptor radionuclide therapy (PRRT) for which the beta-emitters ^{177}Lu and ^{90}Y are currently used, generally conjugated to DOTA-TOC and DOTA-TATE. Confirmation of sufficient tumour receptor expression, traditionally with SSTR planar and SPECT imaging but increasingly with PET/CT, is needed to select patients eligible for PRRT. The treatment decision is also based on some factors such as the tumour load and location of the disease. For pre-therapy diagnostic ^{68}Ga-DOTA-SSA PET/CT imaging the appropriate radiopeptide should preferably be chosen i.e. ^{68}Ga-DOTA-TATE before PRRT with ^{177}Lu-/^{90}Y-DOTA-TATE and ^{68}Ga-DOTA-TOC when PRRT with ^{177}Lu-/^{90}Y- DOTA-TOC is considered [22]. Due to the short half-life of ^{68}Ga (68 minutes), ^{68}Ga-DOTA-SSA cannot be used for dosimetry, which is instead usually derived from scintigraphy during PPRT with ^{177}Lu-DOTA-labelled peptides.

Along with SSTR, NENs can be imaged by using other molecular and metabolic targets, based on their neuroendocrine functional features. Most widely studied for this application has been 3,4-dihydroxy-6-[18F]fluoro-l-phenylalanine (18F-DOPA). Higher accuracy for PET/CT with 18F-DOPA has been shown for imaging of well differentiated NENs as compared to conventional radiological and planar and/or SPECT imaging [23]. Currently, the main clinical indication for NEN imaging with 18F-DOPA is tumours with low/variable SSTR expression, such as neuroectodermal tumours [23]. Furthermore, since several types of malignant and non malignant lesions may show variable expression of SSTR, 18F-DOPA, as a tracer of catecholamine metabolic pathway, may be helpful in the characterisation of medullary thyroid cancer (MTC), jejuno-ileal (midgut) NEN, pheochromocytoma, neuroblastoma or paraganglioma in patients suspected of harbouring synchrone/metachrone metastatic malignancy (e.g. breast cancer). Although 18F-DOPA has no theranostic role in assessing the patient´s suitability for treatment with SSA, it may assist to identify lesions with low or absent SSTR expression, consequently predicting poor or no response to SSA therapy . Similarly, in therapy monitoring and surveillance, 18F-DOPA may be helpful in identifying new lesions to define disease progression. Major drawbacks with the use of 18F-DOPA are limited availability in several European countries and costly synthesis.

As a problem solving tool when 68Ga-DOTA-SSA- and 18F-DOPA-PET/CT does not suffice, PET/CT with the serotonine precursor 11C-5-hydroxy-tryptophan (5-HTP) has been shown suitable as a general tracer for NEN imaging and is currently available in two European centres [24,25].

Furthermore, radiolabelled peptide analogues targeting the cholecystokinin-2 receptor have been developed for NEN imaging. Initial clinical studies with 99mTc and 111In labelled gastrin analogues show very promising results in patients with MTC [26,27] as well as in patients with other NENs [28]. Recently, a new radiolabelled gastrin analogue with very promising characteristics for clinical translation, in terms of high metabolic stability, prolonged tumour uptake and low kidney retention, has been developed and will be studied in patients with metastatic MTC in the near future [29], however, evidence regarding possible PET-applications are still lacking.

PET/CT imaging with 2-[18F]fluoro-2-deoxy-D-glucose (18F-FDG) plays a role not only for lesion detection of G2 and G3 NENs, but additionally provides important prognostic information (28). While most of the low grade highly differentiated NENs show high uptake

of ^{68}Ga-DOTA-SSA and ^{18}F-DOPA, poorly differentiated NENs show preferential uptake of ^{18}F-FDG indicating a more aggressive behaviour and worse prognosis [**30**]**.**

Tumours that may be visualised with^{68}Ga-DOTA-SSA PET/CT include:

Tumours with high expression of somatostatin receptors [31-38)

- Gastro-entero-pancreatic tumours (GEP) functioning and non-functioning (e.g.: gastrinoma, insulinoma, glucagonoma, VIPoma, etc.)

- Broncho-pulmonary NENs

- Sympatho-adrenal system tumours (e.g. paraganglioma)

- Meningioma

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Tumours with low or varying expression of receptors [39-40]

- Breast carcinoma

- Melanoma

- Lymphoma

- Prostate carcinoma

- Non-small cell lung cancer

- Head and neck cancer

- Sarcoma

- Renal cell carcinoma

- Differentiated thyroid carcinoma

- Astrocytoma

Tumours with neuroendocrine/neuroectodermal features that may be visualised with ^{18}F-DOPA PET/CT include [33, 44-47]:

- Jejuno-ileal(midgut) NENs

- Pheochromocytoma

- Paraganglioma

- Neuroblastoma

- Medullary Thyroid Cancer

Other Tumours/tumour-like conditions with high ^{18}F-DOPA uptake include:

- Brain tumors

- Beta cell hyperplasia (especially for the indication of congenital hyperinsulinemic hypoglycemia).

Tumours with neuroendocrine features that show high[18]F-FDG uptake:

- Neuroendocrine carcinomas (NECs)

- Neuroendocrine neoplasms (NENs) with high histologic grade or G2 NENs with suspect aggressive behaviour.

- Medullary thyroid cancer (MTC)

- Mixed adenoneuroendocrine cancers (MANEC)

- Synchrone/metachrone non-NEN malignancies

Clinical indications for PET/CT imaging of NENs with ^{68}Ga-DOTA-SSA

Clinical indications for ^{68}Ga-DOTA-peptide PET/CT imaging of NENs are primary tumour localisation and characterisation, tumour staging, detection of recurrent disease and restaging, as well as selecting patients eligible for PRRT. The results of these different imaging applications have been reported in the literature as follows:

Diagnosis and staging: Localise primary tumours and detect sites of metastasis (staging) [**34-41**, **48-49**]

- Re-staging: Follow-up of NEN patients to detect residual, recurrent or progressive disease (detection of recurrent disease and restaging) [**34-41**, **48-51**]

- Management decisions: Select patients with metastatic disease for PRRT [**34-41, 50**].

- Monitor the response to therapy (surgery, radiotherapy, chemotherapy or PRRT) [**52**].

- Detection of the primary occult NEN when there is biochemocal evidence and/or symptoms of NEN disease with no evidence of a primary tumour on conventional radiological imaging.

- Detection of the primary tumour in patients with metastasis from an unknown primary NEN on conventional radiological imaging **[48].**

- Characterisation of a broncho-pulmonary mass as a NEN when other diagnostic modalities were inconclusive.

- Detection, characterisation and restaging in case of biochemical NEN recurrence.

The sensitivity of ^{68}Ga-DOTA-SSA PET/CT is likely to vary among tumour types and patients, and even between lesions in the same patient, depending on their SSTR density. Due to the short half life of ^{68}Ga, conjugated peptides cannot be used for dosimetry, which is usually derived from ^{177}Lu-DOTA-labelled peptides. The sensitivity of ^{68}Ga-DOTA-SSA PET/CT may theoretically be reduced also in patients receiving therapeutic doses of somatostatin analogues such as octreotide, but this issue still needs to be clarified **[53].** On empirical grounds, prior to PET with ^{68}Ga-DOTA-SSA, it has been recommended to discontinue therapy with SSA (when possible and not contraindicated) to avoid possible

SSTR blockade **[54].** However, there are literature reports of improved tumour/non-tumour ratio, following pre-treatment with SSA as a consequence of non-saturability of SSTR expressed by malignant cells in contrast to SSTR expressed in normal tissues **[55,56].**

If discontinuation of SSA treatment is undertaken, it has been suggested that the time interval between interruption of therapy and ^{68}Ga-DOTA-SSA PET/CT depends on the type of SSA used: 1 day is suggested for short-lived SSA and 3–4 weeks for long-acting SSA preparations. However, this issue is still not definitely clarified and many centres do not require SSA withdrawal before PET/CT imaging. Some centers suggest that the best option is to perform the PET/CT study just prior to the scheduled monthly dose of long-acting octreotide **[54].** However, taken together there is no clear evidence that discontinuation of somatostatin analogues prior to PET imaging with ^{68}Ga-DOTA-SSA is necessary.

^{68}Ga-DOTA-SSA PET/CT imaging in non-NENs

Less frequently ^{68}Ga-DOTA-SSA PET/CT can be used in non-NEN imaging, particularly when PRRT is considered. Except for the determination of SSTR status ^{68}Ga-DOTA-SSA PET/CT cannot be considered as the first-choice functional modality in the management of patients with non-NENs.

Indications for ^{18}F-DOPA PET/CT for NENs:

PET/CT with ^{18}F-DOPA targets tumours with enhanced intracellular transport and decarboxylation of the amino acid DOPA and is for NEN imaging approved in several EU countries for the following indications:

Diagnosis

- Diagnosis and localisation of glomus tumours in patients with a gene mutation of the succinate dehydrogenase D variant

- Localisation of pheochromocytoma and paraganglioma

  Diagnosis and localisation of insulinomas in the case of hyperinsulinism in infants and children

Staging

- Phaeochromocytoma and paraganglioma

- Well differentiated NENs of the digestive tract

Detection in case of reasonable suspicion of recurrences or residual disease

- Phaeochromocytoma and paraganglioma

- Medullary thyroid cancer with elevated serum levels of calcitonin

- Well differentiated NENs of the digestive tract

- Other endocrine digestive tumours when SSTR imaging is negative

Indications for ^{18}F-FDG PET/CT imaging of NENs:

- Localisation of NECs and high-grade poorly-differentiated NETs with aggressive behaviour

- Prognosis

- Localisation of synchrone/metachrone non-NEN malignancy

The proposed PET/CT imaging strategies with the use of the abovementioned currently available radiopharmaceuticals and based on the different NEN types are tabulated in **Table 3**.

Precautions for PET/CT imaging

- Pregnancy (suspected or confirmed): In the case of a diagnostic procedure in a patient who is or may be pregnant, a clinical decision is necessary to consider the benefits against the possible harm of carrying out the procedure.

- Breastfeeding: If radiopharmaceutical administration is considered necessary, breastfeeding should be interrupted and can be restarted when 7 physical half-lives for the radionuclide in radiopharmaceutical has elapsed when the level of radiation in the milk will not result in a radiation dose to the child greater than 1 mSv.

- The ionising radiation from ^{68}Ga-DOTA-SSA must be carefully evaluated in subjects under 18 years of age. However, the dosimetry of ^{68}Ga- SSA is more favorable than that of ^{111}ln-pentetreotide.

It has been recommended to temporarily withdraw SSA therapy (when possible) to avoid possible SSTR blockade (see patient preparation). In some patients the withdrawal of therapy might not be tolerated **[54-56]** .

Pre-examination procedures for ^{68}Ga-SSA PET/CT Imaging

1) Patient preparation

• The physician or the technologist should give the patient a detailed information about the procedure.

• It has been advocated by some authors to withdraw "cold" octreotide therapy (when possible and not contraindicated) to avoid possible SSTR blockade. The time interval between interruption of therapy and ^{68}Ga-DOTA-SSA PET/CT depends on the type of SSA used: one day is suggested for short-lived SSAs and at least 4-7 weeks for long-acting SSA preparations **[54-56].**

• There is no need for fasting before the procedure.

2) Pre-injection

All information useful for optimal interpretation of the study should be considered by the nuclear medicine physician:

- relevant history of suspected or known primary tumour

- absence or presence of functional symptoms

- laboratory test results (hormones and tumour markers)

- results of other imaging modalities (CT, MRI, US, X-ray)

- results of recent biopsy, (including tumour grading and ki-67), surgery, chemotherapy, radiotherapy or radionuclide therapy

- history of recent SSA therapy and of PRRT

3) Administration of ^{68}Ga-DOTA-SSA (DOTA-TOC, DOTA-NOC, DOTA-TATE)

- The radiopharmaceutical should be administered as a short bolus injection using an indwelling catheter to avoid extravasation and immediately followed by injection of about 50 mL of physiological saline.

- The administered amount of activity should be determined by taking into account the Directive 97/43/EURATOM. It is expected that Diagnostic Reference Levels (DRL) for the radiopharmaceuticals will not to be exceeded for standard procedures when good and normal practice regarding diagnostic and technical performance is applied. It should be noted that in each country Nuclear Medicine physicians should respect the DRLs and the rules stated by the local regulations. Activities higher than the DRLs must be justified. For the aforementioned reasons, the following activity for ^{68}Ga-DOTA-TOC, ^{68}Ga-DOTA-NOC, ^{68}Ga-DOTA-TATE should be considered only as a general indication, based on literature data and current experience.

- The activity administered ranges from 100 to 200 MBq, depending on the PET scanner technical characteristics and the patient´s body weight. As with other PET-tracers the injected activity should be calculated according to the patients body-weight. The recommended activity is 2 MBq/kg body-weight and can be reduced in fat patients because fat accumulate very little ^{68}Ga-DOTA-SSA. In adult patients, as a rule at least 100 MBq of ^{68}Ga-DOTA-SSA needs to be administered to obtain good image quality. The experience in paediatric patients is very limited. When the use of ^{68}Ga-DOTA-SSA is considered necessary in a child 2 MBq/kg

is similarly recommended in order to reduce the administered activity according to the recommendations of the **EANM Paediatric Task Group [57].**

Definitive dosimetric data for ^{68}Ga-DOTA-TOC, DOTA-NOC and DOTA-TATE are available in the literature and the mean effective dose is 0.023, 0.025, and 0.026 mSv/MBq, respectively, in several dosimetric studies **[58-60].** The organ receiving the largest radiation dose is the spleen, followed by kidneys and bladder.

The amount of peptide in the injected ^{68}Ga-DOTA-SSA preparation (DOTA-TOC, DOTA-NOC, DOTA-TATE) should be below 50 µg (in discussion in PharmEur); this amount is not expected to have any clinically significant pharmacological effect. The radiopharmaceutical should not be injected into intravenous lines together with solutions for parenteral nutrition.

4) Post-injection

Patients should void before scanning. Elimination of the extra fluid intake will help to flush out any radioactivity (either ^{68}Ga labelled DOTA-SSA or ^{68}Ga transferrin) from the circulation and by glomerular filtration. This will reduce the background activity as well as the radiation dose to kidneys and bladder.

Pre-examination procedures for ^{18}F-DOPA PET Imaging

1) Patient preparation

•   The physician or the technologist should give the patient detailed information about the procedure

•   Oral premedication with carbidopa (L-alpha-hydrazino-alpha-methyl-b-3,4-dihydroxyphenyl propionic acid), an inhibitor of the aromatic aminoacid decarboxylase enzyme, is controversial. The posology of carbidopa usually ranges between 100 and 200 mg (or 2 mg/kg of body weight) **[61,62].** Timmers et al. **[63]** reported that, compared with baseline ^{18}F-DOPA PET, carbidopa pre-treatment resulted in the detection of 3 additional lesions in 3 of 11 patients with pheochromocytoma or extra-adrenal paraganglioma. In contrast, in one infant of the Ribeiro's series the diffuse uptake of ^{18}F-DOPA in the pancreas completely disappeared under carbidopa treatment while the kidney activity was still present:

the patient had histologically proven diffuse abnormal pancreatic cells scattered in the whole pancreas [64]. Similar findings have been reported by Kauhanen et al. in 2 of 3 adults with insulinoma [65]. These findings do not favour the use of carbidopa in patients with pancreatic tumours since pancreatic physiological uptake disappears, and tumour uptake could also disappear along with this. Carbidopa effect on ^{18}F-DOPA uptake in insulinomas is not fully elucidated. No final consensus has been reached about the usefulness of carbidopa in patients with insulinoma-related hyperinsulinemic hypoglycaemia [66,67]. Currently, no preparation of carbidopa (without levodopa) is commercially available.

•   Fasting: On an empirical basis, to avoid interaction with amino acids from food, ^{18}F-DOPA should be administered to patients fasting for a minimum of 4 hours without limiting water intake.

.   Medication Withdrawal: No special interactions have been reported and no therapeutic discontinuation of is needed.

.   Posology and time of acquisition: According to extensive literature data, the recommended activity of ^{18}F-DOPA for adults is 2 to 4 MBq/kg body-weight administered by slow intravenous injection over approximately one minute [68, 69].

The use of ^{18}F-DOPA in children and adolescents has to be considered carefully, based upon clinical needs and assessing the risk/benefit ratio in this patient group. The activities to be administered to children and adolescents may be calculated according to the recommendations of the European Association of Nuclear Medicine (EANM) paediatric dosage card (http://www.eanm.org/publications/dosage_calculator.php?navId=285).

To detect foci in the liver, intestine or pancreas area, early "static" images can be acquired starting 5 minutes after injection, or a "dynamic" acquisition starting right after the injection during 10 minutes. Whole-body images are usually acquired 60 minutes after injection.

Physiological biodistribution of ^{68}Ga-DOTA-SSAs

^{68}Ga-DOTA-SSAs are rapidly cleared from the blood. Arterial activity elimination is bi-exponential and no radioactive metabolites are detected within 4 h in serum and urine. Maximal tumour activity accumulation is reached 70±20 min post injection. Kidney uptake averages <50% of that of the spleen. Excretion is almost entirely through the kidneys [13].

SSTRs are expressed by many neuroendocrine and non-neuroendocrine cells of the body, and many normal organs show ^{68}Ga-DOTA-SSA accumulation including the liver, spleen, pituitary, thyroid, kidneys, adrenal glands, salivary glands, stomach wall and bowel. The pancreas shows variable uptake of ^{68}Ga-DOTA-SSAs and although all five SSTR subtypes are present, the SSTR receptor subtype 2 is preferably found and is located in the islets of Langerhans. A well known potential pitfall is a variable but sometimes very high focal ^{68}Ga-DOTA-SSA accumulation in the uncinate process of the pancreas that may mimic a pancreatic NEN [70]. Also, the prostate gland and the glandular tissue in the breast may show diffuse low-grade physiological ^{68}Ga-DOTA- SSA uptake.

The biodistribution of the various ^{68}Ga-DOTA-SSA preparations may vary depending on the receptor coverage of the peptide. According to literature data, ^{68}Ga-DOTA-TATE, which has mainly affinity to SSTR subtype 2, shows more intense physiological uptake in the pituitary and salivary glands [60], than ^{68}Ga-DOTANOC, binding to the SSTR subtypes 2, 3 and 5. Although the SSTR subtype binding profile of ^{68}Ga-DOTANOC is wider than that of ^{68}Ga-DOTATATE, the NEN uptake of ^{68}Ga-DOTA-TATE was in a PET/CT imaging study shown more intense and with higher lesion-to-background ratio compared with ^{68}Ga-DOTA-NOC [71]. By contrast, PET/CT imaging with ^{68}Ga-DOTA-NOC in another study detected significantly more lesions than using ^{68}Ga-DOTA-TATE in patients with gastroenteropancreatic-NENs [72]. However, there is still a debate in the literature whether the uptake pattern differences due to the dissimilar SSTR subtype profiles of the various ^{68}Ga-DOTA-SSA significantly affects the tumour imaging yield. In direct comparisons of ^{68}Ga-DOTA-SSA preparations in the same patients, small imaging differenses have been found, usully for merely singular or a small number of lesions. Thus, to date there are no studies in larger patient groups that convincingly shows that the diagnostic performance of ^{68}Ga-DOTA-TOC, ^{68}Ga-DOTA-TATE and ^{68}Ga-DOTA-NOC are dissimilar.

Physiological Biodistribution of ^{18}F-DOPA

The biodistribution of ^{18}F-DOPA was investigated in a cohort of 107 patients and showed physiological uptake in the basal ganglia, liver, adrenal glands which was very variable [68, 73]. In the pancreas the accumulation was most prominent in the uncinate process and less intense in the body and tail. Further, very intense and variable ^{18}F-DOPA uptake was seen in the excretory organs such as the gallbladder and biliary tract. The tracer was excreted through

the kidneys, in which the highest activity was found together that in the urine. Bowel uptake was an unusual finding and when present, was low and diffuse. Low uptakes were also found in the myocardium, muscles and in some cases a very faint uptake in the mammary glands, the oral cavity, the esophagus. In the literature it is reported that children can present ^{18}F-DOPA uptake in the growth plates [74].

Effect of carbidopa premedication on the biodistribution of ^{18}F-DOPA

 Oral premedication with carbidopa, to block the aromatic amino acid decarboxylase enzyme, is controversial and is for NEN visualisation of less common use than for neurologic imaging purposes. The pre-administration of carbidopa increases the ^{18}F-DOPA uptake in the basal ganglia, lungs, myocardium and liver and decreases the pancreatic uptake **[63].** Physiological excretion into the biliary tract and the urinary system is unaffected by carbidopa pre-medication. Similar effects of carbidopa premedication are reported in children [74].

Preparation of ^{68}Ga-DOTA-SSAs:

Currently different types of [68]Ge/^{68}Ga-generators are being used, all of them providing ^{68}Ga in strongly acidic hydrochloric acid solutions (0.05-0.6 M HCl) **[65]**. For radiolabelling of DOTA-SSAs, different techniques are being employed, usually using semi- or fully automated systems. These are either based on prepurification and concentration of the generator eluate using an anion-exchange or cation-exchange technique. Alternatively, a fraction of the generator eluate may be used directly for radiolabelling. The procedure is performed using a suitable buffer at elevated temperature followed by purification of the preparation solution on a C-18 cartridge and appropriate aseptic formulation. Either method employed must ensure that the level of germanium-68 ([68]Ge) in the final preparation is lower than 0.001% of the total ^{68}Ga radioactivity. Quality parameters to be tested may vary dependent on the technique applied and are currently defined within a monograph of the European Pharmacopeia for [68]GaDOTA-TOC (*Gallium- (^{68}Ga) Edotreotide Injection, No. 2482*). Quality control protocols may include tests for radionuclidic purity, radiochemical purity (HPLC, TLC), chemical purity (buffer, solvents) as well as sterility and endotoxin testing using validated methods. Generally, quality control should be performed according to the governing monograph or national regulations; whichever is applicable. A review on production technologies and quality aspects can be found in previous publications **[75,76].** Recently, generators with a marketing authorisation have become available and radiolabelling

kits are in the pipeline. This will simplify kit-based preparation of ^{68}Ga-DOTA-SSAs, potentially reducing the requirements for purification, GMP compliance and quality control.

PET/CT scanner quality control

A strict quality control programme should be routinely performed according to the rules of each country, as stated in the Council Directives 97/43/ EURATOM.

Image acquisition of ^{68}Ga-DOTA conjugate peptides

Data acquisition is performed by means of a dedicated PET/CT scanner in 3D mode. The timing for image acquisition ranges between 45 and 90 minutes after injection and varies on the basis of the ^{68}Ga-DOTA-SSA used. There are no unequivocal data in the literature in this respect, but according to the experience of various centres the best results are achieved with image acquisition at 45 to 60 minutes after injection for ^{68}Ga-DOTA-TATE and 60 to 90 minutes for ^{68}Ga-DOTA-TOC and ^{68}Ga-DOTA-NOC. The acquisition is performed as a whole body scan from head to mid thighs. Image reconstruction should be performed by an iterative reconstruction algorithm using the system's implementation and settings. Reconstructions may be performed with or without time of flight information, depending on the systems capabilities. When possible it is, however, recommended to acquire and reconstruct data with time of flight information. Reconstructions should be performed including all regular corrections, such as normalisation, (CT based) attenuation correction, dead time, decay correction and, preferably, model based scatter correction.

Image interpretation for PET/CT imaging with ^{68}Ga-DOTA-SSAs

Normal biodistribution and abnormal accumulations should be evaluated by a nuclear medicine physician. Tracer accumulations other that physiological or areas of accumulations higher than that of the background activity can be considered as pathological. Clearly demarkated findings with higher tracer uptake as compared to that of the liver are classified as definitely positive for enhanced SSTR expression and thus indicative for a SSTR expressing neoplasm. Linear, non-focal intestinal uptake with moderate intensity is considered physiological. Pancreas may show variable physiological tracer uptake and a well known pitfall is physiological uptake in the uncinate process [70].

Interpretation criteria

To evaluate ^{68}Ga-DOTA-SSA PET/CT studies, the following issues should be taken into consideration:

• clinical question raised in the request for ^{68}Ga-DOTA-SSA PET/CT imaging

• clinical patient history, recent biochemical test results

• comprehension of the physiological tracer distribution

• anatomical localisation of the ^{68}Ga-DOTA-SSA uptake with corresponding separate and fused CT images; correlation with other imaging modalities (CT, MRI) is strongly recommended

• intensity grading of the ^{68}Ga-DOTA-SSA uptake that can also be expressed semi-quantatively (SUVmax)

• ^{68}Ga-DOTA-SSAs may show variable uptake in different tumour types, with respect to tumour histology, SSTR expression and density, anatomical site and size of the lesion(s)

• causes of false negative results

• causes of false positive results

Image interpretation for ^{18}F-DOPA PET/CT

^{18}F-DOPA visualises a very specific metabolic process and presents non-specific accumulation only corresponding to its excretory pathways. In other normal tissues, ^{18}F-DOPA has minimal uptake and therefore provides high lesion-to-background contrast. Patients who are referred for ^{18}F-DOPA PET/CT generally have a clinical suspicion of disease, based on their clinical records and/or biochemical results and/or previous imaging findings. Thus, it is helpful to be a priori aware of this information, to be aware of the physiological tracer uptake patterns, and to have knowledge of the ^{18}F-DOPA PET/CT apparence of the different tumour types, their patterns of spread and of the anatomical sites for recurrent disese.

Of special notice is the large variability in ^{18}F-DOPA uptake in the pancreas, especially in the uncinate process that, in some cases, can minic a pancreatic NEN. Similarly, highly variable accumulation in the adrenal glands must be taken into consideration to avoid misinterpretation

of a normal adrenal as a pheochromocytoma. A high adrenal ^{18}F-DOPA uptake, when homogeneous and symmetrical, and not associated with a morphological finding of a tumour on CT/MRI, should be considered as physiological. Because the liver accumulation of ^{18}F-DOPA is relatively constant this can serve as a normal tissue reference and be helpful for semiquantitative measurements of the lesion-to-liver ratio.

In the ^{18}F-DOPA PET/CT image reading, any focal uptake besides areas of physiologic tracer distribution can be considered as pathological. When paragangliomas/pheochromocytomas are suspected, either a non-physiological extraadrenal focal uptake, or asymmetrical adrenal uptake together with a concordant enlarged gland, or an adrenal uptake higher than that of the liver together with a concordant enlarged gland on CT/MRI, can be considered as pathological.

Reporting of the Scans

For both ^{68}Ga-DOTA-SSA and ^{18}F-DOPA PET/CT imaging, the nuclear medicine physician should record: the clinical question, a concise patient's clinical history, type and date of examination, administered activity and route of administration, CT parameters and dosimetry, relevant medications (patient preparation, previous therapy with cold somatostatin analogues, Carbidopa premedication, withdrawal period, chemotherapy, etc.), laboratory and other imaging studies results.

The report should describe:

 1. the procedure (the type of ^{68}Ga-DOTA-SSA and its administered activity, the administered activity of ^{18}F-DOPA, acquisition time, duration of imaging, the area imaged)

2. the findings (site and size of the lesion(s), uptake intensity, SUVmax etc.)

3. comparative data - the findings should be related to previous PET/CT scans performed with the same tracer or to ^{18}FDG PET/CT, if performed, or to results of other imaging modalities such as CT/MRI when appropriate

4. interpretation: a clear diagnosis should be made if possible, accompanied - when appropriate - by a description of the study limitations (potential causes of false negative or false positive results). Additional diagnostic examinations or an adequate follow-up should be suggested, when required.

Potential Pitfalls in ^{68}Ga-DOTA-SSA PET/CT Imaging

• A usually intense but sometimes variable tracer accumulation is seen in the spleen and accessory spleen(s) if present, in kidneys, adrenals and pituitary. After spleenectomy, accumulation in accessory spleen(s) may be misinterpreted. Benign adrenocortical adenomas may also show ^{68}Ga-DOTA-SSA uptake. The liver uptake is generally lower than that of the spleen. The thyroid and salivary glands are (mostly) faintly visible. A variable and sometimes very high tracer uptake in the uncinate process of the pancreas should not be misinterpreted. Particularly in patients referred for ^{68}Ga-DOTA-SSA PET/CT because of a supsected pancreatic NEN, the finding of a high tracer uptake in the uncinate process should be thouroughly correlated to radiological imaging (CT/MRI).

• Contamination with urine of clothes and/or skin may cause false positive images.

• SSA therapy or endogenous production of somatostatin by a tumour may interfere with tumour detection by reducing or enhancing tumour detectability.

• Variable tumour differentiation and heterogeneous SSTR expression may influence the affinity for ^{68}Ga-DOTA-SSAs and thereby the diagnostic accuracy

• False negative findings may be due to tumour dedifferentiation or small lesion size

• False positive findings can be encountered in the presence of activated lymphocytes that can express SSTR at sites of inflammation/infection.

• Syncronus tumours other than NENs may express SSTR and can be detected

Potential Pitfalls in ^{18}F-DOPA PET/CT Imaging

An intense focal accumulation in the gallbladder and, in some patients, also in the common bile duct may mimic an intestinal tumour or a hepatic metastasis [77]. Knowledge of the normal tracer biodistribution and its physiological excretion together with the use of correlative radiological imaging (CT/MRI) should help the reader to avoid misinterpretation in this respect.

The urinary excretion of the tracer can also be the cause of several pitfalls. The intense uptake of the tracer in the kidneys can mask a pathologic uptake in the tail of the pancreas. Moreover, the kidney activity can hide a pathologic uptake in the adrenals, especially in patients with dilatation of the urinary collective system (superior caliceal groups and kidney pelvis). Activity in the ureters, especially if focal as opposed to the usually segmental pattern, can mimic retroperitoneal uptake and should be correlated to radiological imaging (CT/MRI). In order to minimise possible image interpretation problems from urinary bladder activity, the patients are always ask to void immediately before start of the PET/CT examination. If needed, the 110 minutes half life of ^{18}F offers the possibility to acquire late ^{18}F-DOPA images after diuretic administration or after ambulation and hydration to help discriminating between pathological and physiological image findings.

The physiologically intense and very variable ^{18}F-DOPA uptake in the pancreas can lead to pitfalls. A physiological uptake in the uncinate process can be interpreted as a pancreatic NEN or confused with a retroperitoneal lymph node metastasis (false positive). Alternatively, a pancreatic NEN with the same uptake intensity as that of the pancreas may be overlooked (false negative). Moreover, physiological pancreatic uptake may potentially interfere with visualisation of the left adrenal. When ^{18}F-DOPA is performed because of suspected adrenal pathology, premedication with carbidopa can in these cases prevents masking of an adrenal lesion by blocking the pancreatic uptake and also increases the uptake in the lesions.

The utility of ^{18}F-DOPA PET/CT in adult patients with hyperinsulinemic hypoglycemia can be cumbersome since there is few difference between pathologic or non pathologic areas of the pancreas (which show a very variable physiologic uptake of the tracer. Moreover premedication with carbidopa could lead to another possible methodological pitfall when considering patients with hyperinsulinemic hypoglycemia since carbidopa (a peripheral AADC inhibitor) decreases the whole pancreatic uptake decreasing also the lesion to background ratio [**78**]. Disappearance of ^{18}F-DOPA focal pancreatic hot spots has been reported after premedication with carbidopa in patients with hyperinsulinemic hypoglycemia [**64,74,78**].

Pitfalls related to pathology

Possible sources of false-negative ^{18}F-DOPA PET/CT results can be related to small lesion size and tumor de-differentiation. Genetic factors may also affect the ^{18}F-DOPA uptake in paraganglioma. On one hand, succinate dehydrogenase B-subunit (SDHB) gene mutations may result in extra-adrenal paragangliomas for which ^{18}F-DOPA PET shows a lower sensitivity than for non-SDHB-related lesions [63]. On the other hand, the fact that only neuroendocrine cells are able to take up and decarboxylate ^{18}F-DOPA and store the resulting amine, leads to few false-positive ^{18}F-DOPA PET findings.

Koopmans et al. studied prospectively 53 patients with GEP-NENs and recorded 100% patient based sensitivity, 95% region based sensitivity and 96% lesion-based sensitivity, which were better than CT, SRS and CT & SRS combined and without false positive results [79].

In a meta-analysis on PET/CT imaging in recurrent MTC, ^{18}F-DOPA have shown better results in terms of sensitivity and specificity but with a complementary role for ^{18}F-FDG in more agressive tumours, thus reflecting the two different metabolic pathways of these tracers. It was reported that false positive ^{18}F-DOPA findings were uncommon, and that false negative results could be mainly related to small lesions or to de-differentiation. The diagnostic performance of ^{18}F-DOPA in recurrent MTC improved in patients with higher serum calcitonin levels [80, 81].

Technical pitfalls

PET/CT represents a major technologic advance, consisting of two complementary modalities which provide both functional and anatomic information and whose combined strength tends to overcome their respective weaknesses. With combined PET/CT, the superimposition of the precise structural detail provided by CT allows an accurate anatomical localisation of the PET findings and a correlation of the PET findings with the morphological findings.

With CT based attenuation correction there is a potential risk of overestimating the true activity concentration in very high attenuating materials such as metallic implants [82]. In these instances, comparison with the non-attenuated PET images is usually helpful to differ attenuation correction induced from true tracer accumulations.

Another possible pitfall can be caused by misregistration between the PET and CT image volumes. Superimposition of a focal radiotracer uptake on the wrong anatomic position in the

CT can be caused by breathing, patient motion, bowel motility, etc and can lead to misinterpretation.

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Table 1: Somatostatin receptor (SSTR) subtype affinity profiles of different ^{68}Ga-DOTA-somatostatin analouges

Peptide /receptor subtype	SSTR1	SSTR2	SSTR3	SSTR4	SSTR5
68Ga-DOTATOC	>10.000	2.5±0.5	613±140	>1000	73±21
68Ga-DOTATATE	>10.000	0.2±0.004	>1000	300±140	377±18
68Ga-DOTANOC	>10.000	1.9±0.4	40.0±5.8	260±74	7.2±1.6

The table lists the inhibitory constant (nmol/L) for ^{68}Ga-DOTA-somatostatin analouges. The IC50 value indicates the concentration when 50% of binding is inhibited.

Table 2: Grading for NETs according to the WHO 2010 Classification.

Grade	Mitotic count (/2 mm2)*	Ki-67 index (%)**
G1	<2	≤2
G2	2-20	3-20
G3	>20	>20

*10 high power field (HPF) 40x magnification=2 mm2. It is recommended to count mitoses in at least 50 fields at x40 magnification in areas of highest mitotic density and to divide the total number of mitoses by 5.

**MIB 1 Antibody, % of 500-2000 tumour cells in areas of highest labelling.

Table 3: Proposed diagnostic strategy based on the NEN type for PET/CT imaging with ^{68}Ga-DOTA-SSA, ^{18}F-DOPA and ^{18}F-FDG as the first choice (I), second choice (II) and third choice (III).

Type of NEN	Place in diagnostic strategy (I-II-III)*		
	^{68}Ga-DOTA-SSA	^{18}F-DOPA	^{18}F-FDG
Medullary thyroid cancer	III mainly when treatment with SSAs is an option	In patients with high serum calcitonin levels : I In patients with high serum CEA levels II	In patients with high serum calcitonin levels II In patients with high serum CEA levels I
Foregut NEN	I	Not indicated	I
Midgut NEN	I	I	II
Hindgut-NEN	II	II	I
Pheochromocytoma	II/III	With SDHD mutation I With SDHB mutation II-	With SDHD mutation II With SDHB mutation I

Paraganglioma	Head and neck I Abdomen/pelvis II	Head and neck II Abdomen/pelvis I	Head and neck III Abdomen/pelvis III
NEN CUP*	If suspected primary foregut I	İf suspected primary midgut I	To localise the primary tumour and suspected non-NEN malignancy I
Neuroblastoma		I	Older age, advanced stages or MYCN amplification I
Hyperinsulinism in infants and in children		I	

*CUP: carcinoma of unknown primary