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Habilitationsschrift

Radiolabeled Somatostatin Receptor Analogs in Neuroendocrine Tumor: Novel Applications and Clinical Indications For In-vivo Tumor Characterization

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Dr. med. Vikas Prasad
geboren in Patna Bihar Indien

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Dekan:	Professor Dr. med. A. Radlach-Pries
1. Gutachter/in:	Prof. Dr. Dirk Hellwig, Regensburg
2. Gutachter/in:	Prof. Dr. Andreas Buck, Würzburg

List of Abbreviations

Abb.	Meaning
5-HIAA	5-Hydroxyindoleacetic acid
APUD	Amine Precursos Uptake and Decarboxylation
BMI	Body Mass Index
CgA	Chromogranin A
CT	Computer Tomography
CUP	Carcinoma of Unknown Primary
DOTA	1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid
DOTANOC	DOTA-1-Nal3-octreotide
DOTATOC	DOTA-Phe(1)-Tyr(3)-octreotide
DOTATATE	DOTA-Tyr3-octreotate
DTPA	Diethelyne Triamin Pentate
CUP-NET	Carcinoma of Unknown Primary-Neuroendocrine Tumor
EANM	European Association of Nuclear Medicine
ENETS	European Neuroendocrine Tumor Society
EUS	Endosonography
F-18	Fluorine-18
FDG	Fluoro-deoxyglucose
Ga-68	Gallium-68
Ge-68	Germanium-68
GEP-NET	Gastoenteropancreatic Neuroendocrine Tumor
5-HIAA	5 Hydroxy Indole Acetic Acid
TACE	Trans Arterial Chemoembolisation
TAE	Trans Arterial Embolisation
HE	Hematoxylin Eosinophil
In-111	Indium-111
Ki67	Ki 67 Antigen
LAR Octreotide	Long Acting Release Octreotide
LSO	Lutetium Orthosilicate
Lu-177	Lutetium-177
MEN1	Multiple Endocrine Neoplasia
MRI	Multiple Resonance Imaging
NET	Neuroendocrine Tumor
NENs	Neuroendocrine Neoplasms
NSE	Neuron Specific Enolase

PET	Positron Emission Tomography
PET/CT	Positron EmissionTomography/Computed Tomography
pNET	Pancreatic NET
p.i.	Post injection
PRRT	Peptide Receptor Radionuclide Therapy
s.c.	Subcutaneous
SI-NET	Small Intestine-Neuroendocrine Tumor
SEER	Surveillance Epidemiology and End Results
SSR PET/CT	Somatostatin-Receptor PET/CT
SPECT	Single Photon Emission Computed Tomography
SRS	Somatostatin Receptor Scintigraphy
SUV	Standardised Uptake Value
SUVmax	Standardised Uptake Value (maximum)
Tc-99m	Technetium-99m
Thr	Threonine
TTF-1	Thyroid Transcription Factor 2
Tyr	Tyrosine
vHLD	von Hippel Lindau Disease
WHO	World Health Organisation
Y-90	Yttrium-90

1. Introduction

One of the hallmarks of neuroendocrine neoplasia (NEN) is their heterogeneity (1,2). This is one of the reasons that the available systemic treatment options in general fail to demonstrate persistent efficacy for more than 2-3 years (1,2). Apart from surgery, none of the systemic therapies including high-end targeted therapies have demonstrated curative efficacy of this relatively indolent tumor (1,2).

Stratification and sequencing of different treatment options, both systemic and locoregional, are based on the histopathology and tumor stage. Amongst several different proposed classifications for NEN, the European Neuroendocrine Tumor Society (ENETS) and World Health Organisation (WHO) classifications are widely accepted (3-5).

Table 1: Comparison of WHO 2010 and ENETS nomenclature and grading system for gastroenteropancreatic neuroendocrine tumor (GEP NET) and lung and thymus NEN (3-5).

Grade	Lung and Thymus (WHO 2010 / ENETS)		GEP NET (WHO 2010 / ENETS)	
	Nomenclature	Grading System	Nomenclature	Grading System
Low	Carcinoid tumor	<2% mitoses / 10 hpf and no necrosis	Neuroendocrine tumor grade 1	<2% mitoses / 10 hpf and Ki67 < 3%
Intermediate	Atypical carcinoid tumor	2-10 mitoses / 10 hpf or foci of necrosis	Neuroendocrine tumor grade 2	2-20 mitoses / 10 hpf or Ki67 3-20%
High	Small cell neuroendocrine carcinoma	> 10 mitoses / 10 hpf	Neuroendocrine carcinoma, grade 3 small cell carcinoma	> 20 mitoses / 10 hpf or Ki67 > 20%
	Large cell neuroendocrine carcinoma	> 10 mitoses / 10 hpf	Neuroendocrine carcinoma, grade 3 large cell	> 20 mitoses / 10 hpf or Ki67 > 20%

Pathologists play a central role in the management strategy; several immunohistochemical stainings are needed to characterize the neuroendocrine tumor (6). Immunohistochemistry for molecular profiling of tumour-tissues has taken centre stage in the management of NEN. Immunohistochemical staining for chromogranin A, synaptophysin and the related markers synaptic vesicle glycoprotein 2 and synaptobrevin 1, neural cell adhesion molecule 1, CDX2, transcription termination factor 1, somatostatin receptor subtype 2a, etc. allow molecular phenotyping and may

give an insight into the location of the primary tumor, an important aspect for choosing treatment strategies (6).

In addition to pathology, diagnosis of NEN is based on clinical features, tumor markers and imaging. NEN can be classified clinically into functional and non-functional tumors depending upon their ability to secrete active peptides and neuroamines which in turn leads to specific symptoms like flush, diarrhea, hypoglycemia, etc. (1,2). Tumor markers like chromogranin A, serum serotonin, urine 5-HIAA, neuron specific enolase, are most commonly used in the diagnosis as well as in follow-up. Other specific tumor markers like insulin and pro insulin for insulinoma, glucagon for glucagonoma, etc. are additionally measured depending upon the specific tumor entity (7).

Most of the GEP NETs are sporadic but some may be associated with some specific genetic mutations and might be hereditary like von Hippel Lindau syndrome, multiple endocrine neoplasia, etc. (7). That is why it is also essential to supplement pathology and serum tumor markers with genetic analysis in a specific subgroup of patients (7).

Aforementioned tools for diagnosis however are not enough for guiding treatment. Localizing the tumor (primary) as well as judging the extent of disease (staging and restaging) are prerequisites for treatment allocation. Morphological imaging (CT and MRI) with high degree of sensitivity plays a significant role in providing important information on the anatomy. For detection of liver metastasis, MRI with liver specific contrast is most sensitive (8). CT is excellent for detection of lung metastasis. Hypervascularisation, another hallmark of differentiated neuroendocrine tumor is often utilized for enhancing the detection rate of CT by acquiring images in the arterial as well as in the portovenous phase in addition to standard venous phase images (8). However, for the detection of primary tumor and staging and restaging of extrahepatic tumor as well as for verification of presence or absence of specific targets on NEN, imaging (scintigraphy, SPECT, PET), with functionalized radionuclides often are complementary and supplement CT and MRI (9).

Molecular imaging using radiolabeled probes utilizes tumor specific upregulated metabolic pathways or overexpression of specific receptors (9). The majority of neuroendocrine tumors express somatostatin receptor (SSR) subtype 2 (95%), SSR 1 (80%) and SSR 5 (75%) (10). Most of the somatostatin receptor targeting radiopharmaceuticals are receptor agonists and show high affinity to SSR 2 (11). Recent research has however shown that SSR antagonists have higher detection rate as compared to SSR agonists (12,13).

Amongst the tracers used in conventional nuclear medicine for imaging with gamma cameras, In-111 DTPA-D-Phe1-octreotide (In-111 pentetreotide; OctreoScan, Mallinckrodt, Inc, St. Louis, Missouri) as well as Tc-99m EDDA- HYNICTOC (Tektrotyd) are approved for imaging of NET based on several clinical studies (14-16). There are in addition several PET tracers with high affinity for SSR receptors, like DOTATOC [DOTA-D-Phe1-Tyr3-octreotide]), DOTATATE (DOTA-D-Phe1-Tyr3-Thr8-octreotide) and DOTANOC (DOTA-1-Nal3-octreotide) (9).

The superior diagnostic efficiency and resolution of PET favors the use of PET tracers over conventional SPECT tracers (9). In addition to tracers based on somatostatin receptor analogues, there are several other tracers for example F-18 DOPA and C-11 5HTP that have shown some benefit in selected types of neuroendocrine tumor (17,18). I-123 MIBG is another useful tracer for diagnosis of pheochromocytomas, paraganglioma and some sub-entities of NEN (19). However the aforementioned tracers are used only when radiolabeled somatostatin receptor analogues are either not available or did not allow establishing the diagnosis (9).

Apart from somatostatin receptor analogues, F-18 FDG also plays a significant role in the management of NET by allowing to differentiate and characterize between aggressive and non aggressive NETs. In addition, F-18 FDG allows determining the prognosis of a patient. In general however, F-18 FDG PET/CT is performed only in selected patients after imaging with radiolabeled somatostatin analogues and mostly in NEN with higher proliferation indices (9,20).

For labeling SSR analogues with Ga-68 for PET imaging, Ge-68/Ga-68 generator systems are used to provide Ga-68 on a daily basis. There are several commercially available Ge-68/Ga-68 generators on the market as for example from Eckert and Ziegler (Pharmgrade generator), ITG, Ithemba, etc. Since the development of DOTA (a strong chelator for Ga-68), somatostatin analogue ligands, mainly developed by Maecke et al. and in combination with industrially available Ge-68/Ga-68 generator system constructed by Rösch and Knapp et al. an increasing number of centers in Europe are using Ga-68- DOTA- somatostatin analogues for the diagnosis of NEN (with PET or PET- CT) (21).

2. Primary hypotheses relevant for the research work presented in this ‘Habilitationsschrift’

The major hurdles in diagnosis and management of neuroendocrine tumor arises from a) it's heterogeneity, b) lack of specificity of morphological imaging as well as available tumor markers and c) lack of validation and sensitivity of approved nuclear medicine imaging and therapy tools. Hybrid imaging such as Ga-68 DOTA-X PET/CT provides the combination of functional imaging with morphological imaging, inherently allowing maximization of information to overcome some of the aspects of heterogeneity of NEN. DOTA-X labeled with beta emitter Lu-177 enables perfect implementation of receptor based radionuclide therapy but the direct relationship to the biokinetics of Ga-68 DOTA-X still remains an open question.

With this background, the present research work tried to answer the following questions:

- **Characterization and validation of Ga-68 labeled somatostatin receptor agonists for detection of somatostatin receptor expression on neuroendocrine tumor metastasis**
    - Does SSR PET/CT actually measure the somatostatin receptor expression?
    - Do normal tissue / organs and tumor tissue have different uptake of Ga-68 DOTA-X?
    - Is there a difference in the sensitivity and detection rate of Ga-68 DOTA-X PET/CT and Lu-177 DOTA-X scintigraphy post therapy on dosimetry images?
- **Detection of unknown primaries in neuroendocrine tumors using molecular imaging**
    - Does SSR PET/CT has a role in the detection of primary neuroendocrine tumors in patients with NET metastasis and unknown primary tumor?
    - Can genetic fingerprints of metastasis be used for predicting the primary tumor entity and localization in addition to SSR PET/CT?
- **Validation of higher sensitivity of Ga-68 labeled somatostatin receptor agonists in comparison to historical data on In-111 Octreoscan**
    - Does Ga-68 SSR PET/CT have any role in historically of the somatostatin receptor negative benign insulinomas and nesiodioblastosis?
    - Can the high sensitivity of Ga-68 DOTA-TOC be used for screening of pancreatic neuroendocrine tumor in vHLD patients?

- **Can Ga-68 DOTA-X be useful in radioguided surgery of gastroenteropancreatic (GEP) neuroendocrine tumor**?

3. Authors own work included in this 'Habilitationsschrift'

3.1. Characterization and validation of Ga-68 labeled somatostatin receptor agonists for detection of somatostatin receptor expression on neuroendocrine tumor metastasis

3.1.1 Immunohistochemical correlation with SUVmax (co-author)

Maxmimum standardized uptake value (SUVmax) is one of the most studied, often clinically relevant semiquantitative parameter in the analysis of tracer uptake on PET/CT. It was hypothesized that SUVmax is a potential marker for the quantification of somatostatin receptor expression on neuroendocrine tumor (22). To validate this, 44 surgically resected paraffin embedded metastasis were generated from 34 histologically documented GEP-NET patients. Somatostatin receptor density on tumor specimens was measured immunohistochemically using the immunoreactive score (IRS) of Remmele and Stegner (23,24). For SSR2a staining, monoclonal antibody (clone UMB-1) was used whereas for SSR1 and 3-5, polycloncal antibodies were used (25). For in-vivo SSR density quantification by Ga-68 DOTANOC PET, maximum standardized uptake values (SUVmax) were used. Significant correlations were observed between IRS for SSR2A and SSR5 and the SUVmax of PET/CT ($p< 0.05$, each). Based upon these findings, it could be concluded that tumor SUVmax on Ga-68 DOTANOC PET can be used as a non-invasive surrogate marker for prediction of SSR2a density, the most important receptor subtype for diagnosis and treatment of NEN with somatostatin analogs.

3.1.2 Tissue uptake of Ga-68 DOTANOC

The radiopharmaceuticals used in PET/CT imaging are injected intravenously and physiologically they are also taken up by non-NET normal cells, e.g. the pituitary glands, the adrenal glands etc. For correct interpretation of PET/CT it is important to understand and characterize the distribution of the somatostatin receptor targeting radiopharmaceuticals in the body. For this purpose, the biodistribution of Ga-68 DOTANOC in patients with neuroendocrine tumors was studied (26). The primary aims of this study were to a) characterize the normal biodistribution of Ga-68 DOTANOC, b) to ascertain the normal range of Ga-68 DOTANOC uptake in metastasis to liver, bone and lymph nodes and c) to generate a cut-off value using ROC for differentiating tumor related SSR expression from the normal physiological uptake in the processus uncinatus of pancreas, which is one of the major pitfalls for false positive NEN findings in the pancreas. The SUVmax in normal organs, primary pancreatic neuroendocrine tumors (pNET) as well as in metastatic lesions to the liver

liver, bone and lymph nodes of 89 NET patients undergoing Ga-68 DOTANOC PET/CT were analysed. The results showed that there is a broad range of SSR expressions in metastastic lesions and in pNET (see table 2). The uptake of Ga-68 DOTANOC in

spleen was found to be highly variable. Because of excellent target non-target ratio of 3.4±2.3, 14.5 ± 19.1, and 11.3±8.9 for liver, lymph node and bone metastases respectively, Ga-68 DOTANOC was found to be an excellent tracer for imaging somatostatin receptor positive tumors.

Table-2 SUVmax Ga-68 DOTANOC in normal organs and tumor lesions

Organ	SUVmax ± SD
Pituitary gland	2.6±1.3
Thyroid gland	3.4±1.4
Lung parenchyma	0.9±0.8
Normal liver	6.9±2.0
Spleen	22.0±10.0
Adrenal	6.0±2.5
Kidney	12.9±3.8
Intestine	2.3±1.0
Gluteal	1.0±0.3
Femur	0.8±0.3
Blood pool	2.6±1.2
Uncinatus	5.8±2.0
Pancreas NET	20.8±10.8
Liver Metastasis	19.6±13.4
Lymph node	12.5±10
Bone metastases	9.5±6

3.1.3 Comparison of Ga-68 DOTATATE and Lu-177 DOTATATE (shared first authorship)

The other important issue in the theranostic application of radiolabeled somatostatin receptor analogs is the influence of the peptide concentration and the kind of radiometal on tracer biodistribution and diagnostic performance. In addition, it was important to know if the images acquired on Lu-177 DOTATATE post therapy scans are comparable to PET images despite lower spatial resolution of gamma camera based scintigraphy in comparison to PET techniques. To answer this question, we compared post therapy Lu-177 DOTATATE scintigraphy containing approximately 150 µg peptide with the pre-therapeutic maximum intensity projection (MIP) images of Ga-68 DOTATATE PET/CT containing approximately 10 µg in 44 patients with histologically confirmed NET (27). Ga-68 DOTATATE and Lu-177 DOTATATE imaging was performed within 7.9 ± 7.5 days of each other. Post therapy Lu-177 DOTATATE planar scintigraphy was acquired at 0.5, 2, 24, 48 and 72 h. Tumor uptake on scintigraphy were given a score from 0 to 4; 0 being lowest and 4 highest. The number of lesions identified on Lu-177 DOTATATE scans was compared to those detected on Ga-68 DOTATATE studies obtained before

PRRT. Out of a total of 318 detected lesions; 280 (88%) lesions were concordant on both imaging modalities. Among the discordant lesions (n=38), 29 (76.3%) were detected only on Ga-68 DOTATATE PET, whereas 9 (23.7%) were only visible on Lu-177 DOTATATE. The positive predictive value, accuracy and sensitivity of post therapy Lu-177 DOTATATE scintigraphy were 97%, 88% and 91% respectively. On 72 h Lu-177 DOTATATE images (delayed images) significantly more lesions (91%) were detected as compared to the 30 min (immediate) images (68%; $p<0.05$). Scintigraphy and PET showed highest concordance for bone metastasis (97%) whereas lowest concordance was observed for head and neck lesions (75%). The sizes of concordant lesions were significantly larger than discordant lesions (mean size 3.8 vs 1.6 cm; $p < 0.05$). The concordant liver lesions having a visual score of 1 to 3 in the 72-h Lu-177 DOTATATE post therapy scintigraphy had a lower SUVmax than those metastasis with a score of 4 (mean SUVmax 10.9 vs 18; $p < 0.05$).

Relevant Original Works

1. Kaemmerer D, Peter L, Lupp A, Schulz S, Sänger J, **Prasad V**, Kulkarni H, Haugvik SP, Hommann M, Baum RP. Molecular imaging with $^{Ga\text{-}68}$-SSR PET/CT and correlation to immunohistochemistry of somatostatin receptors in neuroendocrine tumours. Eur J Nucl Med Mol Imaging. 2011 Sep; 38(9): 1659-68 **(co-author)**.
2. **Prasad V**, Baum RP. Biodistribution of the Ga-68 labeled somatostatin analogue DOTA-NOC in patients with neuroendocrine tumors: characterization of uptake in normal organs and tumor lesions. Q J Nucl Med Mol Imaging. **2010** Feb; 54(1):61-7.
3. Sainz-Esteban A, **Prasad V**, Schuchardt C, Zachert C, Carril JM, Baum RP. Comparison of sequential planar Lu-177-DOTATATE dosimetry scans with Ga-68 DOTATATE PET/CT images in patients with metastasized neuroendocrine tumours undergoing peptide receptor radionuclide therapy. Eur J Nucl Med Mol Imaging. 2012 Mar;39 (3):501-11 **(shared first authorship).**

Molecular imaging with $^{Ga\text{-}68}$-SSR PET/CT and correlation to immunohistochemistry of somatostatin receptors in neuroendocrine tumours. Eur J Nucl Med Mol Imaging. 2011 Sep; 38(9): 1659-68. doi: 10.1007/s00259-011-1846-5.

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Comparison of sequential planar Lu-177-DOTATATE dosimetry scans with Ga-68 DOTATATE PET/CT images in patients with metastasized neuroendocrine tumours undergoing peptide receptor radionuclide therapy. Eur J Nucl Med Mol Imaging. 2012 Mar;39 (3):501-11. https://doi.org/10.1007/s00259-011-2003-x.

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3.2. Detection of unknown primaries in neuroendocrine tumors using molecular imaging

3.2.1 Ga-68 DOTANOC PET/CT and CUP NET

Management of NEN is largely dependent on the localization of the primary tumor and its grade. Most of the gastroenteropancreatic neuroendocrine tumors show increased somatostatin receptor expression. That is why it was hypothesized that Ga-68 DOTA-X PET/CT should be able to pick up the unknown primary in patients with histologically confirmed metastasis of NEN. In a bi-centric study consisting of 59 patients (33 M: 26 F, age: 65 ± 9 years) with unknown primary neuroendocrine tumor, the sensitivity of Ga-68 DOTANOC PET/CT was retrospectively analysed (28). The SUVmax of primary tumors detected on PET/CT were compared with SUVmax of known pancreatic NET (pNET) and small intestinal NET (SI-NET). The results of PET/CT were also correlated with CT alone. Ga-68 DOTANOC PET/CT localised the site of the primary in 59% (35/59) of patients: ileum/jejunum-14, pancreas-16, rectum/colon-2, lungs-2 and paraganglioma-1. On retrospective analysis, in conjunction with PET findings, CT confirmed the findings in 20% of patients (n=12). The SUVmax of unknown pNET (18.6± 9.8) and SI-NET (9.1± 6.0) were significantly lower ($p < 0.05$) as compared to the ones with known primary tumour sites (pNET 26.1± 14.5; SI NET 11.3± 3.7); 81% of the patients had low-grade and 19% high-grade NET. Ga-68 DOTANOC PET/CT findings lead to surgical resection of 6 patients (10.1%; 4 pancreatic, 1 ileal and 1 rectal tumour). In one patient a primary tumor of the lungs was confirmed by bronchoscopy and histopathology. In comparison to the historical data on In-111 Octreoscan (39% detection rate for CUP), Ga-68 DOTANOC was found to be superior in detecting primary tumors in CUP-NET.

3.2.2 Gene Expression and CUP NET (co-author)

Because there are still up to 40% of the patients where the primary can not be picked up on Ga-68 DOTA-X scans a further study was undertaken to explore the genetic fingerprints of the metastasis to predict the location of the primary tumor. In this follow-up study, the cryopreserved tissues from metastases of NET were collected in three different institutions (A, n=29; B, n=50, C, n=132) (29). The specimens from A and B were examined with comparative genomic hybridization (Agilent 105 K) and gene expression analysis (Agilent 44 K) whereas immunohistochemistry was performed in the specimens from C. The laboratory analysis was performed by personal without any knowledge of the site of the primary. Ileum primary could be

detected with the help of gene expression analysis correctly in 94 % of the cases of A and in 58 % of B; pancreatic primary was predicted in 83 % A and 20 % of B patients, respectively. The pooled sensitivity of gene analysis in patient samples from A and B for prediction of ileal NETs was 75% and for pancreatic NETs 38%. Immunohistochemical profiling of specimens from C showed an overall sensitivity of 80 % for prediction of the primary tumor; CD302 was found to be the best marker for ileal NET whereas PPWD1 was found to be the best marker for pancreatic NET detection.

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3.3 Validation of higher sensitivity of Ga-68 labeled somatostatin receptor agonists in comparison to historical data on In-111 Octreoscan

3.3.1 Ga-68 DOTATOC and Ga-68 DOTATATE for detection of insulinoma

In almost all the guidelines, the role of somatostatin receptor scintigraphy with In-111 Octreoscan in insulinoma is critically discussed because of its poor sensitivity. With this background a pilot study was undertaken with the hypothesis that Ga-68 DOTATOC / DOTATATE (SSR) PET/CT should be more sensitive for the detection of insulinoma or nesidioblastosis in patients with a high degree of suspicion of pancreatogenic hypoglycemia (30). We included 13 patients with histopathologically proven or a high clinical suspicion of pancreatogenic hyperinsulinaemia. The results of SSR PET/CT were then correlated with the histopathological findings. Following criteria were considered as gold standard for the final diagnosis of insulinoma: a) normalization of blood glucose levels after surgical resection of the suspected lesion b) histopathological confirmation. For the diagnosis of nesidioblastosis, it was found to be sufficient to exclude insulinoma in addition to conclusive histopathological examination of a segment of the pancreas. Malignant insulinoma were defined by the presence of locoregional or distant metastasis.

Out of 13 patients, 2 patients had malignant insulinoma, 8 had nonmetastasized insulinoma, and 3 had nesidioblastosis. SSR PET/CT detected the focus of endogenous pancreatic hypoglycaemia in 11/13 patients (84.6%). Histopathological staining confirmed 16 insulin positive foci. On SSR PET 14/16 lesions were detected. On SSR PET, one intrapancreatic spleen was misdiagnosed as insulinoma focus. The resulting sensitivity and positive predictive value of SSR PET was found to be 87% and 93.3 %, respectively. SSR2a immunohistochemistry was performed in 10 specimens (2 nesidioblastosis, 7 benign and 1 malignant insulinoma): 8/10 (80 %) of specimens stained positive for SSR2a; out of them 7 lesions were detected on SSR PET/CT. SSR PET/CT directed therapy resulted in complete remission of the hypoglycaemic events. This explorative study suggests that SSR PET/CT can play a significant role in the management of patients with endogeneous pancreatogenic hyperinsulinism.

3.3.2 Ga-68 SSR PET for the screening of vHLD

We hypothesized that the high sensitivity of Ga-68 DOTA-X PET/CT is also of importance for screening of patients with genetic predisposition for pancreatic NET (pNET) as in von Hippel Lindau (vHLD) disease.

The National Cancer Comprehensive Network suggests use of high resolution MRI, CT as well as In-111 Octreoscan for the detection of pNET and characterization of pancreatic lesions in patients with von Hippel-Lindau disease (vHLD) (31). However, the diagnostic performance of CT and MRI is often diminished because of the presence of cystic lesions in the pancreas of vHL patients (32). Octreoscan has limited spatial resolution and causes higher radiation burden in comparison to Ga-68 SSR PET (33). The performance of Ga-68 DOTATOC PET/CT in contrast depends on the presence of somatostation receptor expression, and demonstrates a high sensitivity for the diagnosis and staging of neuroendocrine tumors (9). To ascertain the hypothesis that Ga-68 DOTATOC PET/CT will also be very sensitive for screening pNET in vHLD, we analysed Ga-68 DOTATOC PET / 3-phase contrast-enhanced CT performed in 20 consecutive vHLD patients (M:F:8:12; 44.7±11.1 years) between 01/2013-11/2015 (34). In addition, patients were examined with MRI of the abdomen, spine, and head. A focal circumscribed uptake of Ga-68 DOTATOC PET in the pancreas more than the immediate background was defined as positive. Genetically, 12 patients had type 1 and 8 had type 2 vHLD. Ga-68 DOTATOC PET/CT detected pNET in 11 (55%; 8 type 1, 3 type 2) and morphological imaging (CT or MRI) detected pNET in 9 patients (45%; 6 type 1, 3 type 2). Serum CgA was mildly elevated in 2/11 patients with pNET. In 4/11 patients (36.4%) imaging detected multiple pNETs; size of the lesions was 10.4±8.3 mm with 41.1% having pNET size > 10 mm. One of the patients presented with lymph node metastases. In addition, PET detected SSR positive (SUVmax 2.1-10.1) cerebellar and spinal hemangioblastomas in 3 patients. The frequency of pNET in vHLD-T1 was more than in vHLD-T2 (66.7% vs. 37.5%, p=0.089). None of the patients showed progression of pNET during the duration of follow-up. Based upon these results, we could support the hypothesis that even for screening purpose, in vHLD patients, Ga-68 DOTATOC PET/CT was more sensitive than historical data of 13.6% and 41% for 111In Octreoscan and CT+MRI, respectively. However, as none of the patients showed disease progression during the duration of follow-up (12-37 months), further imaging with SSR PET should only be performed based on the clinical need.

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3. 4 High sensitivity of Ga-68 DOTATOC is useful for radioguided surgery (shared first authorship)

Use of handheld gamma probes intraoperatively, termed as radioguided surgery (RGS) for the localization of a suspect focus has been reported previously (35). However their utilization in the field of neuroendocrine tumor using positron emitters like Ga-68 has not been tested. It was hypothesized that the high sensitivity and specificity of Ga-68 DOTANOC will be of help to the surgeons in localising a metastasis or primary tumor through hand held gamma probes (36).

In an explorative pilot study performed in 9 NEN patients scheduled for surgery due to various indications, the technique of RGS was used. All patients received preoperatively Ga-68 somatostatin receptor PET/CT to confirm the presence of NEN metastasis or primary tumors. On the day of the operation, 1-3 hours before the start of the operative procedure, another injection of Ga-68 DOTANOC was given. Thereafter, the impact of RGS on the planned operation procedure and also on the tumor detection were analysed. Overall, 72 locations were scanned with a hand held gamma probe intraoperatively. The gamma probe could detect 94% of the histologically confirmed lesions, SSR PET/CT could detect 69% and surgical palpation could localize only 50% of the lesions. RGS had a major impact on surgical management, leading to a change in the operative procedure in 56% of the patients. This supported the hypothesis that the intraoperative use of hand-held gamma probes is feasible and could also be useful in surgeries of NEN, specially in detecting small metastasis in the abdomen.

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4. Discussion

Discovery of somatostatin receptors on neuroendocrine neoplasia has revolutionized the management of these tumors (37). This important target has been the mainstay for diagnosis and treatment of NET for the last 2 decades (38). Radiolabeled peptides targeted towards somatostatin receptors have also played an equally important role in the management of NET as is also accepted by the ENETS guidelines (6-8). In-111 Octreoscan was one of the first tracers to be approved for confirmation of somatostatin receptors. Indeed, very first autoradiography studies performed confirmed that In-111 Octreoscan binds directly to the somatostatin receptors (39). The similar kind of confirmatory study was expected for Ga-68 labeleld somatostatin analogs used for PET imaging. The advantages of PET over gamma camera imaging are its higher sensitivity, its higher spatial resolution and the possibility to quantify tracer uptake more reliably. It was for the first time that we showed that the SSR expression on tumors as measured by semiquantitative PET parameter SUVmax can be reliably used for estimation of immunohistochemically measured SSR density (22). Having said that, one obvious limitation of the study was its retrsospective nature and dependency of IHC scoring on observer's experience. In addition, differential SSR subtypes can only be assessed through IHC because SUVmax cannot differentiate tracer binding to different subtypes. SUVmax of Ga-68 DOTANOC however clearly gives an idea of the SSR2a receptor density, the most predominant receptor subtype on well differentiated neuroendocrine tumors. This is a very important information for treatment with peptide receptor radionuclide therapy or with cold somatostatin receptor analogs, because the higher the SSR expression the greater is the probability of tumor response (40-42). In addition, there are 5-10% of well differentiated gastroenteropancreatic neuroendocrine tumors which do not show any expression of SSR. In these patients, treatment with somatostatin analogs like Sandostatin® or Lanreotide® should be critically discussed. Previous studies have shown that the intensity of the uptake on Octreoscan is predictive of response to Sandostatin® (40).

Another important aspect of the somatostatin receptor guided imaging is to characterize the biodistribution and uptake in normal organs as well as in tumor lesions as SSRs are physiologically expressed in several other organs / tissues like pituitary gland, adrenal glands, spleen, kidneys, etc. (9,26). Even active inflammation shows increased somatostatin receptor expression, specially on activated

macrophages (9,26). That is why we initiated a study to characterize the normal biodistribution of Ga-68 DOTANOC (26). It was indeed found that the uptake in tumors as well as in normal organs like spleen, adrenal and pituitary glands was highly variable. Infact, spleen showed in general a very high tracer uptake. Subsequent studies have shown that this uptake is mostly localized in the activated macrophages, lymphocytes of the spleen and can be suppressed with cold somatostatin analogs like Sandostatin® (43). This allows spleen as a good reference for visual interpretation of images as well as for internal quality control of radiolabeleld peptides. In contrast to spleen, the uptake of Ga-68 DOTANOC in liver is most likely due to the metabolism of radiolabeled peptides in hepatocytes. High lipophilicty of Ga-68 DOTANOC in comparison to other tracers like Ga-68 DOTATOC or Ga-68 DOTATATE results in lower target/ non-target ratios (26). This is a disadvantage for detecting small lesions in the liver because the PET scanner underestimates tracer uptake in lesions smaller than two times of the spatial resolution due to partial volume effects.

Apart from spleen, the other organ which shows a very high uptake of Ga-68 DOTANOC, is the kidney. This uptake in kidney is predominantly due to the excretion of tracer; a small percentage of tracer however binds to the peritubular vessels, too. Indeed studies have shown that previous treatment with cold somatostatin analogs like Sandostatin® results in reduction of tracer uptake in the kidneys up to 15% (44). High uptake in the kidney is a major limitation for treatment with PRRT because of the fear of radiation induced nephrotoxicity and renal failure.

Apart from the aforementioned organs / tissues with highly variable tracer uptake, primary tumor and metastatic lesions also showed very high tracer uptake. This high tracer uptake allows reliable detection of lesions as small as 5-8 mm; of course the sensitivity of PET is highest for detection of lymph nodes and bone metastasis as compared to liver metastasis (45). As already mentioned above, this lower sensitivity of PET for liver metastasis in comparison to MRI for example is due to higher background activity in normal liver as compared to the background around an involved lymph node or bone (46). The high target to non-target ratio for the detection of tumor lesions in general is one of the main advantages of Ga-68 SSR PET.

Encouraged by these promising results, it was also hypothesized that Ga-68 DOTANOC PET should be able to detect primary tumors in patients with CUP. Treatment of neuroendocrine tumor is primarily guided through the site of the primary

tumor (6-8). In general, patients with CUP NET have poorer prognosis (6-8). The detection of the primary tumor allows better treatment stratification. Surgery is the only curative treatment option for NET. In the absence of visible primary tumors on imaging, surgeons often have to undertake explorative laparotomy (6-8). Some primary NET can be multifocal in origin, which also has an influence on the surgical management. In our bicentric study, we could show for the first time that Ga-68 DOTANOC PET/CT allows detection of primary NET in 59% of the patients. One of the limitations of the study was that the PET was not correlated with three phase CT, which is one of the standard imaging tool in NET (6-8). However, subsequent studies have further supported the data that SSR PET detects primaries in 50-59% of patients with CUP NET (47-49). ENETS guidelines and other upcoming guidelines suggest performing specific immunohistochemistry like TTF-1, CD-X2, Islet-1 staining to further help predict the primary site of NET. This approach is equally important and attractive because tissue specimens are almost always available for confirmation of NET. Besides immunohistochemistry, genetic fingerprints of tumors might also help in detecting the primary as already proven in lung cancer, breast cancer and colon cancer (50,51). In contrast, primary NET of gastropancreatic origin are a challenge as the data is not well established. In our work, we looked at genetic fingerprints in surgical specimens collected from three centres. Interestingly, the pilot study, despite its limitations, showed three gene expression patterns, which could localize primaries in ileum, pancreas and stomach. Moreover, the results also showed that there is still some work to be done before the method can be used routinely. The primary concern in genetic analysis on tissue specimens is the quality of specimen; if not properly stored, the information gleamed out of genetic mining may be misleading or incorrect as shown in our study. One centre could detect primaries in the ileum or pancreas with a sensitivity of 94% and 83%, respectively whereas in specimens from other centre B the sensitivity dropped down to 48% and 64% for ileum and pancreas primaries respectively. As a next step in further validation of genetic profling for management of NET patients, we looked into 51 marker genes of NET and analysed the different ‘omes’ SSRome, proliferome, metabolome, secretome, epigenome and pluromes (52). The results were analysed with an artificial intelligence programme and named as NETest. We could demonstrate both very high sensitivity and specificity of NETest as biomarker in comparison to chromogranin A; and we showed a high correlation between NETest and SRS PET for detection of tumor in NET

patients. Indeed, NETest was found to detect disease occurrence long before the lesions were detected on CT or MRI. This again underscores the need to have a proper place and sequence of using molecular imaging with highly sensitive PET tracers, which can detect changes at picomolar levels in the management of NET (9).

The higher sensitivity of SSR PET, compared to the historical data on In-111 Octreoscan is further supported by two of our studies on insulinoma and vHLD. The role of In-111 Octreoscan in the detection of endogeneous hyperinsulinimic focus has been extensively studied. A poor sensitivity of Octreoscan ranging between 24-60% and an excellent spatial resolution of 3-Phase CT or MRI, with sensitivities ranging between 80-95% has been the primary reason for not recommending SRS for diagnosis of insulinoma or endogeneous hyperinsulinemic foci (53,54). We could demonstrate, in an explorative study, that SSR PET/CT could be very useful and sensitive for localizing the focus of insulin overproduction in pancreas. Its sensitivity of 87% coupled with the high resolution of 3-phase CT, performed as state of the art PET/CT procedures, opens the possibility to use this technique upfront (30). In contrast to neuroendocrine tumor of the pancreas, insulin producing foci in the pancreas are often very small and show mild to moderate SSR expression which necessitates more precise and detailed evaluation of PET images. One of the differential diagnoses, which should be kept in mind, is an intrapancreatic spleen. In case of suspicion, the pattern of uptake and correlation with spleen uptake and CT information might help to achieve a proper diagnosis. Furthermore, the presence of small pNET in the processus uncinatus can sometimes be masked because of the high physiological uptake in that region of the pancreas. In such cases, the results should be correlated with endosonography. The development of new tracers like Ga-68 Exendin 4 might ease up the localization of benign insulinomas even further (55).

Pancreas in itself poses a challenge for the confirmation of NET, specially if the tumors are very small. This problem of detecting small NET is also a challenge for CT or MRI, specially if pancreas shows cystic changes as is often the case in vHLD. Diagnosing a small pNET between multiple cysts in vHLD is a real challenge by means of CT or MRI. Detection of pNET in vHLD does have relevance as patients can develop metastasis (56). The recommendation of Octreoscan for characterization of lesions in the pancreas by the NCCN guideline has two major limitations and that are higher radiation burden and lower spatial with In-111 Octreoscan in comparison to SRS PET. The high sensitivity of Ga-68 DOTATOC

PET/CT for pNET detection in our study population argues favourably for performing at least one SSR PET/CT during the course of restaging of vHLD patients. Frequent intervals of SSR PET/CT in patients with genetic predisposition however should be avoided because there is no consensus as to how often imaging with SRS should be performed in vHLD patients. We showed in our study population that only 1 patient out of 20 presented with lymph node metastasis on SSR PET/CT. Importantly, in none of the patients tumor was found to be progressive.

Surgery of NET is the only curative option. Although SSR PET/CT has been shown to improve surgical management of NET patients, intraoperatively small lesions can be overlooked by naked eye. A high specificity and excellent resolution of hand held gamma probes have been shown to be useful in RGS. We could demonstrate that RGS detected a significantly higher percentage of lesions as compared to PET or the surgeons' hand (36).

More often than not, NET patients present themselves in stage IV. Although surgery can still be performed with palliative intent, systemic therapies are often called upon to manage the inoperable metastatic disease. Beta emitter labeled somatostatin receptor analogs like Lu-177 DOTATOC and Lu-177 DOTATATE have shown to be very effective in the treatment of progressive metastasized gastroenteropancreatic neuroendocrine tumors. The prerequisite for PRRT is the presence of sufficient tracer binding on tumor lesions as shown by SRS or SSR PET/CT. Although Ga-68 and Lu-177 used for diagnosis and therapy respectively are bound to the same peptide, the radiometal effect can have an influence on the tracer distribution and receptor binding. In addition, 10-15 times higher peptide concentrations are used in PRRT, which can itself lead to differential distribution. We could demonstrate that Lu-177 DOTATATE post therapy scans showed a high concordance of approximately 90% of the lesions seen on Ga-68 DOTATATE PET (27). Interestingly, Lu-177 DOTATATE did detect some lesions, which were missed by the PET images. This could be due to the peptide-induced internalization, which is much higher in the therapeutic setting using higher peptide amounts.

5. Conclusions

Ga-68 labeled somatostatin receptor analogs have an important role in the management of patients with NET. A close correlation between receptor expression as measured by immunohistochemistry and in-vivo molecular imaging (SRS PET)

allows reliable prediction of the presence of somatostatin receptors on NET. The higher sensitivity of SRS PET in comparison to historical data on In-111 Octreoscan enables a) screening for small pNETs in vHLD b) detection of unknown primary NET in 59% of patients and c) localization of pathologic foci of insulin production in the pancreas. Furthermore, SRS PET correlates well with tumor specific genetic fingerprints on metastases as well as in serum. Interestingly, the radiometal used for radiolabeling, Lu-177 or Ga-68, as well as the peptide concentration can also have an influence on the distribution of DOTA chelated synthetic somatostatin agonists. By handheld gamma probes, a procedure called as radioguided surgery, Ga-68 labeled tracers can also be used efficiently for intraoperative detection of small lesions.

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7. List of the publications included in this ‚Habilitationsschrift'

1. Kaemmerer D, Peter L, Lupp A, Schulz S, Sänger J, **Prasad V**, Kulkarni H, Haugvik SP, Hommann M, Baum RP. Molecular imaging with $^{Ga-68}$-SSTR PET/CT and correlation to immunohistochemistry of somatostatin receptors in neuroendocrine tumours. Eur J Nucl Med Mol Imaging. 2011 Sep; 38(9): 1659-68 (co-author).
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3. Sainz-Esteban A, **Prasad V**, Schuchardt C, Zachert C, Carril JM, Baum RP. Comparison of sequential planar Lu-177-DOTATATE dosimetry scans with68 Ga-DOTATATE PET/CT images in patients with metastasized neuroendocrine tumours undergoing peptide receptor radionuclide therapy. Eur J Nucl Med Mol Imaging. 2012 Mar;39 (3):501-11 **(shared first authorship).**
4. **Prasad V**, Ambrosini V, Hommann M, Hoersch D, Fanti S, Baum RP. Detection of unknown primary neuroendocrine tumours (CUP-NET) using (68)Ga-DOTA-NOC receptor PET/CT. Eur J Nucl Med Mol Imaging. **2010** Jan; 37(1):67-77
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8. Kaemmerer D, **Prasad V**, Daffner W, Haugvik SP, Senftleben S, Baum RP, Hommann M. Radioguided surgery in neuroendocrine tumors using Ga-68-labeled somatostatin analogs: a pilot study. Clin Nucl Med. **2012** Feb;37(2):142-7 **(shared first authorship)**

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9. Statutory Declaration

§ 4 Abs. 3 (k) der HabOMed der Charité

Hiermit erkläre ich, dass

- weder früher noch gleichzeitig ein Habilitationsverfahren durchgeführt oder angemeldet wurde
- die vorgelegte Habilitationsschrift ohne fremde Hilfe verfasst, die beschriebenen Ergebnisse selbst gewonnen sowie die verwendeten Hilfsmittel, die Zusammenarbeit mit anderen Wissenschaftlern/Wissenschaftlerinnen und mit technischen Hilfskräften sowie die verwendete Literatur vollständig in der Habilitationsschrift angegeben wurden.
- mir die geltende Habilitationsordnung bekannt ist.

Ich erkläre ferner, dass mir die Satzung der Charité-Universitätsmedizin Berlin zur sicherung Guter Wissenschaftler Praxis bekannt ist und ich mich zur Einhaltung dieser satzung verpflichte.

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