Eur J Nucl Med Mol Imaging (2009) 36:48–52
DOI 10.1007/s00259-008-0944-5

SHORT COMMUNICATION

Correlation of immunohistopathological expression of somatostatin receptor 2 with standardised uptake values in ^{68}Ga-DOTATOC PET/CT

Matthias Miederer · Stefan Seidl · Andreas Buck · Klemens Scheidhauer · Hans-Jürgen Wester · Markus Schwaiger · Aurel Perren

Received: 6 July 2008 / Accepted: 19 August 2008 / Published online: 20 September 2008

Abstract
Purpose In clinical routine somatostatin analogue positron emission tomography/computed tomography (PET/CT) such as ^{68}Ga-DOTA-Tyr-octreotide (DOTATOC)-PET/CT could substitute conventional ^{111}In-Octreotide scintigraphy. Immunohistochemistry (IHC) for somatostatin receptor 2 (SSTR2) might be a tool to predict positivity of ^{68}Ga-DOTATOC in patients where initial staging was not performed, e.g., in incidental findings. We therefore compared a score of SSTR2-IHC with the in vivo standard uptake value (SUV) of preoperative or prebiopsy ^{68}Ga-DOTATOC PET/CT.
Materials and methods In 18 patients, ^{68}Ga-DOTATOC PET/CT scans were quantified with SUV calculations and correlated to a cell membrane-based SSTR2-IHC score (ranging from 0 to 3).
Results Negative IHC scores were consistent with SUV values below 10. Furthermore, all score 2 and 3 specimens corresponded with high SUV values (above 15).
Conclusion SSTR2-IHC scores correlated well with SUV values and we propose to use SSTR2 immunohistochemistry in patients missing a preoperative PET scan to indicate ^{68}Ga-DOTATOC-PET/CT as method for restaging and follow-up in individual patients.

Keywords ^{68}Ga-DOTATOC · PET/CT · Somatostatin receptor · SSTR2 Immunohistochemistry · Endocrine oncology · Receptor imaging

M. Miederer (✉) · A. Buck · K. Scheidhauer · H.-J. Wester · M. Schwaiger
Department of Nuclear Medicine, Klinikum rechts der Isar, Technische Universität München,
Ismaningerstrasse 22,
81675 München, Germany
e-mail: mail@matthiasmiederer.de

S. Seidl · A. Perren
Department of Pathology, Klinikum rechts der Isar, Technische Universität München,
Ismaningerstrasse 22,
81675 München, Germany

Introduction

Although neuroendocrine tumours are generally slow progressing, in case of metastatic spread, therapeutic options are limited, and cure rates are low. For clinical management, somatostatin receptor (SSTR) scintigraphy such as ^{111}In-Octreotide scintigraphy has become obligatory in staging and restaging of most patients. It allows sensitive localisation of tumour manifestations, accurate follow-up and will select patients eligible for somatostatin receptor radiotherapy [1–3]. With availability of somatostatin analogues suitable as positron emission tomography (PET) tracers, improved sensitivity and the advantage of comparable quantification has been suggested. Many of the available PET tracers are utilizing the generator-derived positron emitter ^{68}Ga and display higher affinities to the somatostatin receptor 2 than conventionally used ^{111}In-DTPA-octreotide (Octreoscan). For example, ^{68}Ga-DOTA-Tyr-octreotide (DOTATOC) displays a very high SSTR2 receptor binding with an inhibitory concentration 50% (IC50, with ^{123}I-Tyr-octreotide as radioligand) of 2.5 nmol/L in comparison to 22 nmol/L of ^{111}In-DTPA-octreotide [4, 5]. Additionally, with significant shorter study times and increasing availability of hybrid technology including computed tomography (CT), PET has the potential of

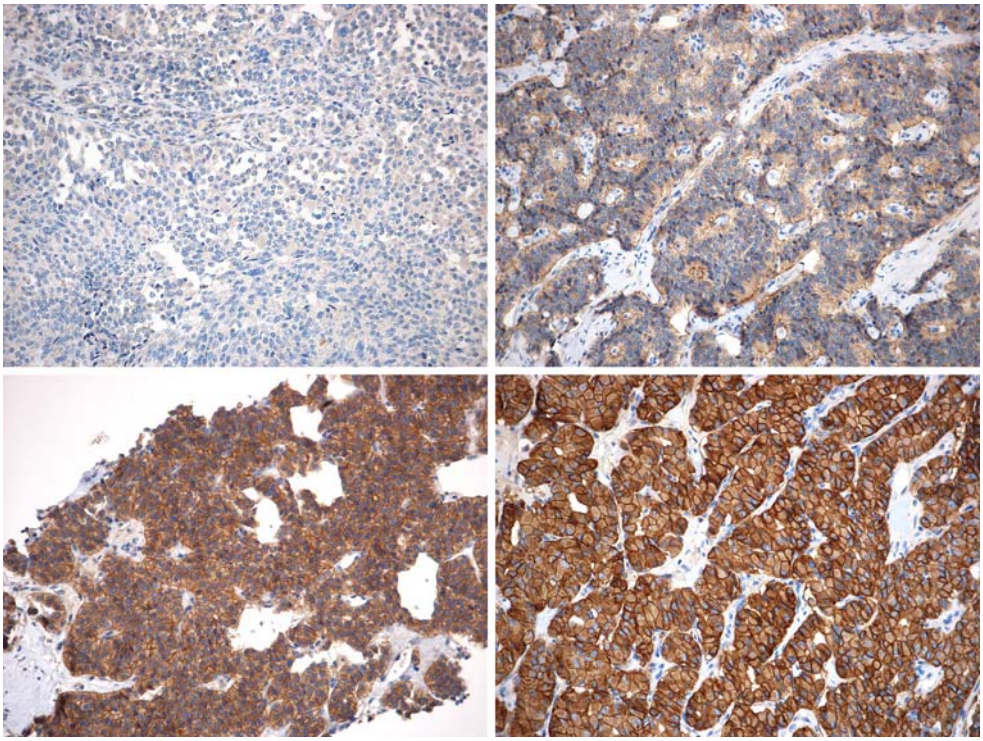

Fig. 1 Immunohistochemistry using an anti-SSTR2 antibody: *Upper row left*: score 0, *upper row right*: score 1, *lower row left*: score 2; *lower row right*: score 3

substituting conventional ^{111}In-DTPA-octreotide scintigraphy and single photon emission tomography (SPECT) when available [6]. Direct comparison already showed an increased diagnostic effectiveness of ^{68}Ga-DOTATOC PET over ^{111}In-DTPA-octreotide SPECT [7].

The indication of SSTR imaging for restaging and follow-up is based on a preoperative scan proving the expression of the SSTR2. However, the neuroendocrine phenotype of tumours is frequently detected only after surgery by histopathological analysis of the resection specimen. In this clinical setting, the use of SSTR imaging for restaging is uncertain, as a baseline scan is not available, and the tumour might not sufficiently express somatostatin receptors. Immunohistochemistry is able to detect SSTR2 on tumour specimens. In one retrospective study, immunohistochemical analysis of SSTR2 showed a 77% concordance when compared to ^{111}In-DTPA-octreotide scintigraphy [8]. Translation of these findings to PET using a

Table 1 Patient characteristics including results of immunohistochemistry and respective standardised uptake values of ^{68}Ga-DOTATOC

Diagnosis	Score	Mean SUV	Max SUV	Ki67 (%)
Thymoma	0	1.8	2.5	N/A
Pulmonary metastasis from medullary thyroid carcinoma	0	1.8	4.4	5
Large cell NEC of the right thoracic wall	0	4.0	6.6	60
Lymph node metastasis from medullary thyroid carcinoma	0	4.8	6.8	<1
Liver metastasis form a NEC of the coecum	0	6.2	9.5	30
Pulmonary carcinoid	1	8.6	11.6	<1
NEC of the ileum	2	18.4	19.5	<1
Lymph node metastasis from a gastrinoma	3	15.8	22.9	<1
NEC of the ileum	1	18.3	25.3	<1%
NEC of the bladder	1	5.9	27.8	30%
NEC of the ileum	2	19.4	27.8	1%
NEC of the pancreas	3	21.4	28.5	7%
Liver metastasis from NEC of pancreas or colon	3	21.0	31.8	30%
Liver metastasis from a rectum NEC	1	25.0	37.0	10%
Liver metastasis from a pancreatic glucagonoma	3	29.8	43.9	2%
Primary presacral NEC (probably arising in a tailgut cyst)	3	31.0	49.0	3–4%
NEC of the pancreas	3	19.3	57.0	4%
NEC of the pancreas	3	48.0	67.5	1%

somatostatin analogue as PET tracer is likely to be feasible; however, ^{68}Ga-DOTATOC binds more specifically to SSTR2, and therefore, the correlation might even be better. We therefore correlated a semiquantitative SSTR2 scoring of immunohistochemistry with the standardised uptake value calculated from ^{68}Ga-DOTATOC PET/CT scans performed in a retrospective series of 17 patients with neuroendocrine tumours and one thymoma patient.

Materials and methods

In 18 patients (mean age 53 years, eight female, ten male) with available tumour specimens and ^{68}Ga-DOTATOC PET/CT scans, specific ^{68}Ga-DOTATOC uptake in tumour lesions was quantified by calculation of standardized uptake values (SUV). PET/CT scans were acquired on a Siemens Biograph 16 at 20 min after application of approximately 120 MBq of ^{68}Ga-DOTATOC (mean 112 MBq, SD 15 MBq). Seven or eight bed positions with 4 min emission time were acquired. PET images were reconstructed using CT attenuation correction (OSEM). SUV calculations were performed on a Siemens Syngo workstation, and mean and maximum SUV (activity concentration corrected for patient weight and total injected dose) were determined using individual 50% of maximum isocontours in tumour lesions. Histopathologic specimens from either operation (14 patients) or biopsy (four patients) were formalin fixed and embedded in paraffin according to standard procedures. Thin paraffin sections, 4 µm, were immunostained after deparaffinisation and protease-based antigen retrieval (6 min, Benchmark, Ventana) with antibodies against SSTR2 (1:600; Gramsch laboratories, Schwabhausen, Germany) on an automated immunostainer (Benchmark, Ventana) using the ABC method. For semiquantitative analysis of the SSTR2 staining, a four-tiered grading system (Fig. 1) was used in analogy to the DAKO-score for her2-neu: 0 (negative), 1+ (weak partial membrane positivity), 2+ (intermediate complete membrane positivity) and 3+ (strong complete membrane positivity). According to this system, only membranous (not cytoplasmic) staining was scored. Two investigators (A.P., S.S.) performed the analysis independently, blinded to the SUV values. Statistical analysis [one-way analysis of variance (ANOVA)] was done with GraphPad Prism 3.0.

Results

The patients included had the following tumours: 11 neuroendocrine carcinomas (NEC) of the digestive tract, two metastasised medullary thyroid carcinomas, one pulmonary carcinoid, one NEC each of the thoracic wall and the bladder and a primary presacral neuroendocrine carcinoma (probably arising in a tailgut cyst), as well as one thymoma (Table 1). Semiquantitative scoring of the SSTR2 immunohistochemistry revealed five tumours with score 0, four tumours with score 1, two tumours with score 2 and seven tumours with score 3. Of the five histopathologically SSTR2-negative tumours, all had low mean standard uptake values on ^{68}Ga-DOTATOC PET ranging from 1.8 in the thymoma to 6.2 in liver metastases of a grade 3 coecal NEC (Fig. 2). However, all these tumours were still visible on the PET images. For all SSTR2-positive tumours (score 1–3), SUV values were significant-

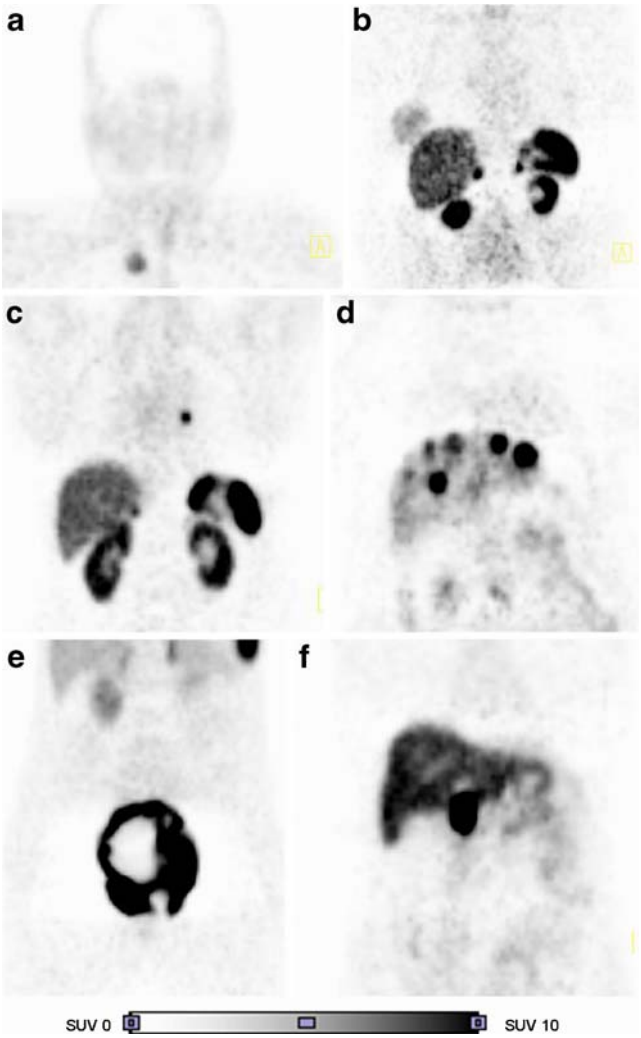

Fig. 2 Coronal ^{68}Ga-DOTATOC PET images of tumour lesions (*upper row*: low uptake, *middle and lower row*: high uptake). **a** Lymph node metastasis of a medullary thyroid carcinoma (SUV mean=5, IHC score=0); **b** NEC of the thoracic wall (SUV mean=4, IHC score=0); **c** pulmonary carcinoid (SUV mean=9, IHC score=1); **d** liver metastasis of a NEC of the ileum (SUV mean=19, IHC score=2); **e** primary presacral NEC (SUV mean=31, IHC score=3); **f** NEC of the pancreas (SUV mean=48, IHC score=3)

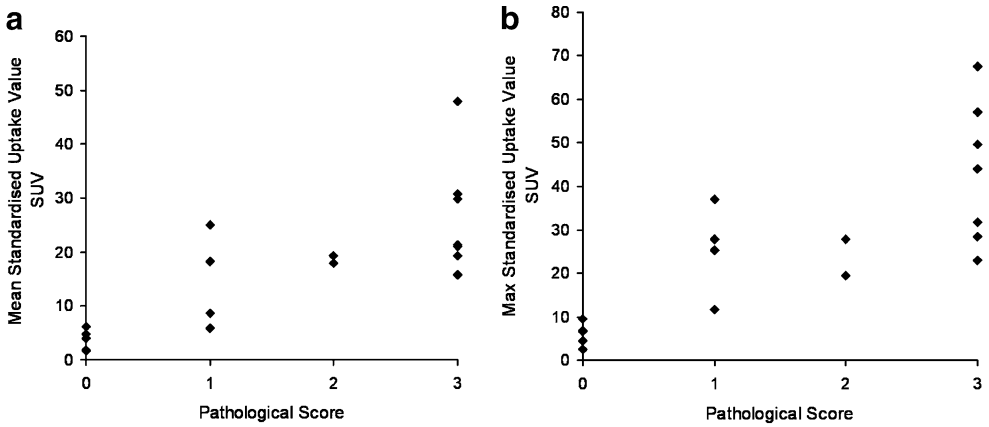

Fig. 3 Correlation between **a** SUV mean and **b** SUV max with a immunohistochemical score ranging from 0 to 3

ly higher, except for a NEC of the bladder with score 1 at immunohistochemistry and a measured mean SUV of 5.9. A correlation between pathological score and SUV was evident (Fig. 3) and one-way ANOVA showed significant differences between the SUV (mean and max) for the different pathological scores ($P<0.05$) and a linear trend ($P<0.05$) in posttest analysis. Two tumours with strong cytoplasmic and weak membranous immunopositivity both showed a weak SUV confirming the approach to only score membranous positivity. A correlation of SUV with the proliferation index (Ki67) showed that the four grade 3 NECs were only partly associated with low SUV values. However, no clear correlation between SUV and proliferation index was evident (Fig. 4).

Discussion

We show that positive somatostatin receptor-2 immunostaining correlates with ^{68}Ga-DOTATOC PET positivity. While SSTR2 immunonegative tumours could not be visualized in ^{111}In-DTPA-octreotide scans [9], we demonstrate evidence that they can still be visualized when somatostatin analogue PET is performed. However, negative membranous SSTR2 immunhistochemistry is associated with a low mean standardised uptake value (approximately 5) and a low maximum SUV (below 10) in these lesions (this might represent weak binding to somatostatin receptor 5). Therefore, we propose to include SUV measurements into the interpretation of ^{68}Ga-DOTA TOC PET studies. In an earlier immunohistochemical study, grouping of scores 0 and 1 as negative lead to best concordance with ^{111}In-DTPA-octreotide scintigraphy [9]. In the present comparison with ^{68}Ga-DOTATOC PET also, two of the four specimens displaying a immunohistochemical score of 1 showed very intense uptake. This result might be explained by the higher affinity of ^{68}Ga-DOTATOC to SSTR2. As the number of score 1 tumours is small, further data are needed to clarify this issue. Of note, all tumours exhibiting an immunohistochemical SSTR2 score 2 and 3 showed high SUV (above 15). This correlation of immunohistochemical SSTR2 expression with ^{68}Ga-DOTATOC PET has implications: In patients where the compulsory initial (preoperative) somatostatin receptor imaging was not performed because of the unknown neuroendocrine tumour phenotype, a strong SSTR2 immunohistochemistry (score 2/3) makes ^{68}Ga-DOTATOC PET the imaging method of choice for restaging and follow-up. In this setting, SSTR2 immunohistochemistry might therefore be the best substitute for a positive preoperative somatostatin receptor imaging. Additionally, the correlation of the pathological score with ^{68}Ga-DOTATOC accumulation provides evidence that SSTR2 immunohistochemistry could assist in identifying patients appropriate for further diagnostic workup potentially leading to somatostatin receptor radiotherapy. Alternatively, somatostatin receptor autoradiography would allow a receptor subtype quantification and is therefore superior to immunohistochemistry. However, this method is not widely available and needs fresh frozen tissue. Immunohistochemistry has the advantage to be feasible on formalin-fixed paraffin-embedded tissue, which is always available. One 1.4 cm lymph node metastasis of a gastrinoma with a tracer accumulation in the medium range showed an immunohistochemical score 3. In such small lesions (below 2.5 cm), a

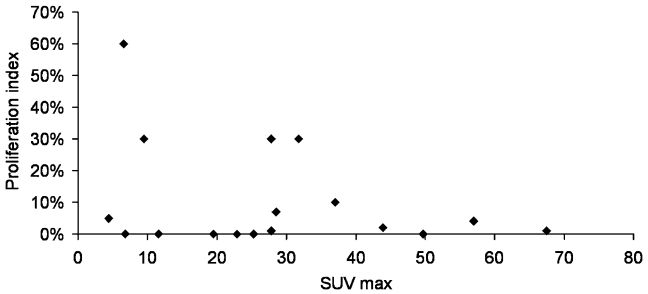

Fig. 4 Correlation between proliferation index (Ki67) and SUV max

partial volume effect of the PET leading to low SUVs should be considered [10]. On the other hand, one large liver metastasis from a rectal NEC with intermediate tracer uptake displayed a pathological score of 1. This might be explained by heterogeneity of tumour tissue with regard to SSTR2 expression.

Conclusion

In neuroendocrine tumours, we detected a significant correlation of membranous SSTR2 expression as determined by immunohistochemistry and tumour uptake of the SSTR2 analogue ^{68}Ga-DOTATOC as determined by PET and calculation of standardised uptake values. Therefore, we propose to use SSTR2 immunohistochemistry in patients missing a preoperative PET scan to indicate ^{68}Ga-DOTATOC PET/CT as method of choice for restaging and follow-up in individual patients.

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