

Localization of hidden Insulinomas with ⁶⁸Ga-DOTA-exendin-4 PET/CT: A Pilot Study

Kwadwo Antwi¹, Melpomeni Fani¹, Guillaume Nicolas¹, Christof Rottenburger¹, Tobias Heye¹, Jean Claude Reubi², Beat Gloor³, Emanuel Christ^{*4}, Damian Wild^{*1}

* = shared last authors

¹Clinic of Radiology and Nuclear Medicine, University of Basel Hospital, Switzerland

²Division of Cell Biology and Experimental Cancer Research, Institute of Pathology, University of Bern, Switzerland

³Department of Visceral Surgery, University Hospital of Bern, Inselspital, Switzerland

⁴Division of Endocrinology, Diabetology and Clinical Nutrition, University Hospital of Bern, Inselspital, Switzerland

Address for correspondence: Damian Wild, Division of Nuclear Medicine, University of Basel Hospital, Petersgraben 4, CH-4031 Basel, Switzerland, Phone +4161 3286683, Fax +4161 2654925, damian.wild@usb.ch

First author: Kwadwo Antwi, Division of Nuclear Medicine, University of Basel Hospital, Petersgraben 4, CH-4031 Basel, Switzerland, Phone +4161 3286684, kwadwo.antwi@usb.ch

Word count: 2491

This work was supported in part by the Swiss National Science Foundation Grant No. 320030_152938/1 and the Desirée and Niels Yde's Foundation Grant No, 389-12.

Short running title: GLP-1 receptor PET/CT

ABSTRACT

^{111}In -DOTA-exendin-4 SPECT/CT has been shown to be highly efficient in the detection of insulinomas. We aimed at determining whether novel PET/CT imaging with $[\text{Nle}^{14}, \text{Lys}^{40}(\text{Ahx-DOTA-}^{68}\text{Ga})\text{NH}_2]\text{exendin-4}$ (^{68}Ga -DOTA-exendin-4) is feasible and sensitive in detecting benign insulinomas.

Methods: ^{68}Ga -DOTA-exendin-4 PET/CT and ^{111}In -DOTA-exendin-4 SPECT/CT were performed in a randomized cross-over order in 5 patients with endogenous hyperinsulinemic hypoglycemia. Gold standard for comparison was the histological diagnosis after surgery.

Results: In 4 patients histological diagnosis confirmed a benign insulinoma, whereas one patient refused surgery despite a positive ^{68}Ga -DOTA-exendin-4 PET/CT scan. In 4 out of 5 patients previously performed conventional imaging (CT/MRI) was not able to localize the insulinoma. ^{68}Ga -DOTA-exendin-4 PET/CT correctly identified the insulinoma in 4/4 patients whereas ^{111}In -DOTA-exendin-4 SPECT/CT correctly identified the insulinoma in 2/4 patients.

Conclusion: These preliminary data suggest that ^{68}Ga -DOTA-exendin-4 PET/CT is feasible in detecting hidden insulinomas.

Key Words: glucagon-like peptide-1 receptor targeting, exendin-4, insulinoma, gallium-68

INTRODUCTION

The most common cause of endogenous hyperinsulinemic hypoglycemia in adults is an insulinoma. Endogenous hyperinsulinemic hypoglycemia is biochemically diagnosed by a prolonged supervised fasting test in an inpatient setting (1). Approximately 5-10% of insulinomas are multiple, mainly in the context of multiple endocrine neoplasia type 1, less than 10% are malignant, and the majority are benign single insulinomas (1). The only curative treatment of an insulinoma is its surgical removal. Therefore the exact preoperative localization of the insulinoma is critical in order to plan the surgical intervention (1). Magnetic resonance imaging (MRI), computed tomography (CT) or endoscopic ultrasound are normally used to localize insulinomas (1). However the small size (often <1 cm), limits the sensitivity of these methods (1). The sensitivity can be increased by including methods such as angiography with selective arterial calcium stimulation and hepatic venous sampling. However, this procedure is invasive with concomitant risks for complications. The sensitivity of somatostatin receptor scintigraphy and SPECT/CT is usually low (33-50%) and inconsistent for ^{18}F -DOPA PET (90% in a prospective study and 20% in a retrospective study) (2).

It has been shown that targeting the glucagon-like peptide-1 receptors (GLP-1R) using the specific ligand $[\text{Lys}^{40}(\text{Ahx-DOTA-}^{111}\text{In})\text{NH}_2]\text{exendin-4}$ or $[\text{Lys}^{40}(\text{Ahx-DTPA-}^{111}\text{In})\text{NH}_2]\text{exendin-4}$ or $[\text{Lys}^{40}(\text{Ahx-HYNIC-}^{99\text{m}}\text{Tc/EDDA})\text{NH}_2]\text{exendin-4}$ is a very sensitive ($\geq 95\%$ sensitivity), non-invasive, method to localize benign insulinomas with SPECT (2–4). In comparison to SPECT, PET possesses a higher spatial resolution and sensitivity and provides accurate quantification of tracer uptake (5). Recently, it has been shown that $^{68}\text{Ga-DO3A-VS-Cys}^{40}\text{-exendin-4}$ PET/CT can detect malignant insulinomas (6).

The aim of our study is to determine whether [Nle¹⁴,Lys⁴⁰(Ahx-DOTA-⁶⁸Ga)NH₂]exendin-4 (⁶⁸Ga-DOTA-exendin-4) PET/CT is feasible in the detection of benign insulinomas. Secondly to evaluate its detection rate compared to [Nle¹⁴,Lys⁴⁰(Ahx-DOTA-¹¹¹In)NH₂]exendin-4 (¹¹¹In-DOTA-exendin-4) SPECT/CT in the same patients.

MATERIALS AND METHODS

Patients

Five consecutive patients were screened and accepted for our prospective pilot study. Patients were referred from four tertiary centers in Switzerland. All patients fulfilled the following inclusion criteria: biochemically proven endogenous hyperinsulinemic hypoglycemia with neuroglycopenic symptoms, negative screening for sulfonylurea (exclusion of hypoglycemia factitia), contrast enhanced 3T MRI not older than 2 months and age above 18 years. Patients with evidence of malignant insulinoma in conventional imaging were excluded, as well as pregnant women, patients with allergies to exendin-4, and patients with renal insufficiency (blood creatinine concentrations >140 µmol/L).

The institutional review board approved this study and all subjects signed a written informed consent.

Procedures

¹¹¹In-DOTA-exendin-4 SPECT/CT and ⁶⁸Ga-DOTA-exendin-4 PET/CT were performed within 24-73 hours in randomized cross-over order. Synthesis and labelling of ⁶⁸Ga-DOTA-exendin-4 and ¹¹¹In-DOTA-exendin-4 were described elsewhere (7).

Total-body planar images and SPECT/CT of the abdomen were performed 4 h and 72 h after intravenous injection of 79.2 ± 9.3 MBq (range 66-90 MBq, 10.5-14.4 μ g) ^{111}In -DOTA-exendin-4. The SPECT/CT unit (Symbia Intevo, Siemens Healthcare) was equipped with a medium-energy, parallel-hole collimator (window setting 172 and 247 keV; width 15%; 2 x 180° rotation; 256 x 256 matrix; 60 projections with 24 sec acquisition time per projection). All patients received unenhanced low-dose CT (130 kVp, 40 mA) for attenuation correction and anatomical reference.

PET/CT examination was performed on a GE Discovery ST PET/16-detector CT unit (GE Healthcare). One bed position of the upper abdomen was acquired during 8 min, 2.5 hours after intravenous injection of 79.8 ± 3.9 MBq (range 76-97 MBq, 12.0-15.3 μ g) ^{68}Ga -DOTA-exendin-4. All patients received unenhanced low-dose CT for attenuation correction and anatomical reference (120 kVp, 30-100 mAs). Blood samples were taken 2, 5, 15, 30, 60, 120 and 180 min after injection of ^{68}Ga -DOTA-exendin-4 and ^{111}In -DOTA-exendin-4 to measure blood glucose levels and blood clearance. An additional blood sample was taken 300 min after injection of ^{111}In -DOTA-exendin-4. All conventional scans were independently reported by experienced radiologists at the referral centers. GLP-1R SPECT/CT and PET/CT scans were independently assessed by 2 board-certified nuclear medicine physicians. Both readers were blinded to other imaging results and the patient's clinical history. In case of discordant finding, a consensus between the two nuclear medicine physicians was found. Tumor-to-background ratios were measured for ^{111}In -DOTA-exendin-4 (counts) and ^{68}Ga -DOTA-exendin-4 (maximal standardized uptake values).

Histological diagnosis was regarded as the standard for comparison. The pathologists were blinded to the results of other diagnostic tests but were aware of the patient's clinical history. Finally, GLP-1R expression was evaluated in vitro by GLP-1R autoradiography as previously described (8).

RESULTS

The clinical characteristics are summarized in Table 1. In all five patients a fasting test was performed. Symptoms of neuroglycopenia in association with low plasma glucose levels (mean 2.1, range 1.6-2.6 mmol/L) and inadequately increased insulin (mean 12.1, range 3.9-21.9 mU/L) and C-peptide (mean 0.714, range 0.5-0.98 nmol/L) levels was documented in all patients after 12–52 h of fasting.

The labeling yield of ^{111}In - and ^{68}Ga -DOTA-exendin-4 was >95% and the radiochemical purity was $\geq 95\%$ for ^{111}In -DOTA-exendin-4 and $\geq 93\%$ for ^{68}Ga -DOTA-exendin-4, at a specific activity of 30 MBq/nmol.

Blood sampling of ^{111}In -DOTA-exendin-4 revealed a biexponential blood clearance of $t_{1/2} = 16 \pm 2$ min and $t_{1/2} = 110 \pm 19$ min. ^{68}Ga -DOTA-exendin-4 revealed also a biexponential blood clearance of: $t_{1/2} = 14 \pm 3$ min and $t_{1/2} = 41 \pm 4$ min. Both compounds showed a plasma clearance of ~50% in the alpha phase (Supplemental Figure).

Imaging results are summarized in Table 2. In patient 4 focal uptake of ^{111}In -DOTA-exendin-4 and ^{68}Ga -DOTA-exendin-4 was highly suspicious for an insulinoma (Figure 1), however the patient has refused surgery so far.

All patients received exogenous glucose infusion (1000 mL, 10%) just before injection of the radiotracer for 5 hours. In doing so, no severe hypoglycemic episodes occurred. Two patients experienced nausea and two patients experienced nausea and vomiting after injection of ^{111}In -DOTA-exendin-4. One patient experienced nausea after the injection of ^{68}Ga -DOTA-exendin-4. No other adverse effects were observed.

In all four operated patients histology confirmed the diagnosis of a benign insulin producing tumor (Table 2) and symptoms of hypoglycemia resolved immediately after surgery.

Histopathological diagnosis was made at the local institution. In patient 5 imaging and additional *in vitro* GLP-1R autoradiography was performed (Figure 2 and 3).

DISCUSSION

This report provides the proof of principle that ^{68}Ga -DOTA-exendin-4 PET/CT is feasible and sensitive in the preoperative detection of hidden insulinomas in patients. ^{68}Ga -DOTA-exendin-4 PET/CT detected the insulinoma in 4/4 operated patients already 2.5 hours after injection. GLP-1R imaging changed clinical management in 3/4 patients whereas in the first patient only ^{68}Ga -DOTA-exendin-4 PET/CT correctly localized the small insulinoma which was crucial for surgery planning. ^{68}Ga -DOTA-exendin-4 PET/CT revealed higher tumor-to-background ratios (2.5 hours p.i.) than ^{111}In -DOTA-exendin-4 SPECT/CT scans (4 and 72 hours p.i.) because of an advantageous partial volume effect in PET (9) and faster blood clearance of ^{68}Ga -DOTA-exendin-4 compared to ^{111}In -DOTA-exendin-4. Furthermore, PET has a higher spatial resolution than SPECT (5) which is highly relevant for the detection of insulinomas in close proximity to the highly active kidneys. The better spatial resolution of PET together with the higher tumor-to-background ratio of ^{68}Ga -DOTA-exendin-4 PET/CT revealed a higher insulinoma detection rate than with ^{111}In -DOTA-exendin-4 SPECT/CT.

Late ^{111}In -DOTA-exendin-4 SPECT scans (72 hours p.i.) showed a slightly higher tumor-to-background ratio than early SPECT scan. This is consistent with our previously published work (2) in which we suggested late scans 3-7 days after injection in patients with negative early scans. However, late scans may increase the risk for false positive results. This can be explained by the physiological expression of GLP-1R in pancreatic islets/acini and Brunner's gland of the duodenum (8).

The faster imaging procedure, higher tumor-to-background ratio, better spatial resolution (5) and lower radiation burden of ^{68}Ga -DOTA-exendin-4 PET/CT (7) favors this novel method over ^{111}In -DOTA-exendin-4 SPECT/CT. Furthermore, absolute quantification of tumor and background uptake, which might be useful to improve interobserver agreement and specificity, is better

evaluated with PET/CT than with SPECT/CT. GLP-1R PET/CT is expected to have a high clinical impact in the management of patients with endogenous hyperinsulinemic hypoglycemia. Previous experience (2) suggests that insulinoma in context with multiple endocrine neoplasia type 1 express GLP-1R. It is therefore likely that GLP-1R PET/CT will be a valuable tool to distinguish between insulin secreting lesions, gastrin or non-secreting pancreatic lesions in this genetic syndrome, thereby determining the surgical strategy. Whether GLP-1R PET/CT will be useful in diagnosing other conditions of endogenous hyperinsulinemic hypoglycemia, such as beta cell hyperplasia/hypertrophy (nesidioblastosis) in adults and children remains to be determined.

This study has limitations. First, conventional imaging could not be standardized because of differences in local availability. However, MRI was performed in all patients. Second, conventional imaging had the tendency to underperform compared with published literature (1,10). This might be explained by the fact that most patients were referred following negative conventional imaging and are therefore part of a negative selection. Finally only 5 patients have been investigated. However, this study was planned as a pilot study. Due to the promising results a larger study evaluating GLP-1R PET/CT is initiated.

CONCLUSION

This study proofs that ^{68}Ga -DOTA-exendin-4 PET/CT is feasible and a sensitive tool for the detection of insulinomas. The higher spatial resolution, the possibility of quantification and lower radiation burden favors ^{68}Ga -DOTA-exendin-4 PET/CT over ^{111}In -DOTA-exendin-4 SPECT/CT.

DISCLOSURE STATEMENT

We do not report a conflict of interest.

ACKNOWLEDGMENTS

We thank the respective doctors for referring the patients as well as University of Basel Hospital staff for excellent technical assistance.

REFERENCES

1. De Herder WW, Niederle B, Scoazec J-Y, et al. Well-differentiated pancreatic tumor/carcinoma: insulinoma. *Neuroendocrinology*. 2006;84:183-188.
2. Christ E, Wild D, Ederer S, et al. Glucagon-like peptide-1 receptor imaging for the localisation of insulinomas: a prospective multicentre imaging study. *Lancet Diabetes Endocrinol*. 2013;1:115-122.
3. Christ E, Wild D, Forrer F, et al. Glucagon-like peptide-1 receptor imaging for localization of insulinomas. *J Clin Endocrinol Metab*. 2009;94:4398-4405.
4. Sowa-Staszczak A, Pach D, Mikołajczak R, et al. Glucagon-like peptide-1 receptor imaging with [Lys40(Ahx-HYNIC- 99mTc/EDDA)NH2]-exendin-4 for the detection of insulinoma. *Eur J Nucl Med Mol Imaging*. 2013;40:524-531.
5. Martin WH, Delbeke D, Patton JA, Sandler MP. Detection of malignancies with SPECT versus PET, with 2-[fluorine-18]fluoro-2-deoxy-D-glucose. *Radiology*. 1996;198:225-231.
6. Eriksson O, Velikyan I, Selvaraju RK, et al. Detection of metastatic insulinoma by positron emission tomography with [(68)ga]exendin-4-a case report. *J Clin Endocrinol Metab*. 2014;99:1519-1524.
7. Wild D, Wicki A, Mansi R, et al. Exendin-4-based radiopharmaceuticals for glucagonlike peptide-1 receptor PET/CT and SPECT/CT. *J Nucl Med*. 2010;51:1059-1067.
8. Körner M, Stöckli M, Waser B, Reubi JC. GLP-1 receptor expression in human tumors and human normal tissues: potential for in vivo targeting. *J Nucl Med*. 2007;48:736-743.

9. Soret M, Bacharach SL, Buvat I. Partial-volume effect in PET tumor imaging. *J Nucl Med.* 2007;48:932-945.
10. Druce MR, Muthuppalaniappan VM, O'Leary B, et al. Diagnosis and localisation of insulinoma: the value of modern magnetic resonance imaging in conjunction with calcium stimulation catheterisation. *Eur J Endocrinol.* 2010;162:971-978.

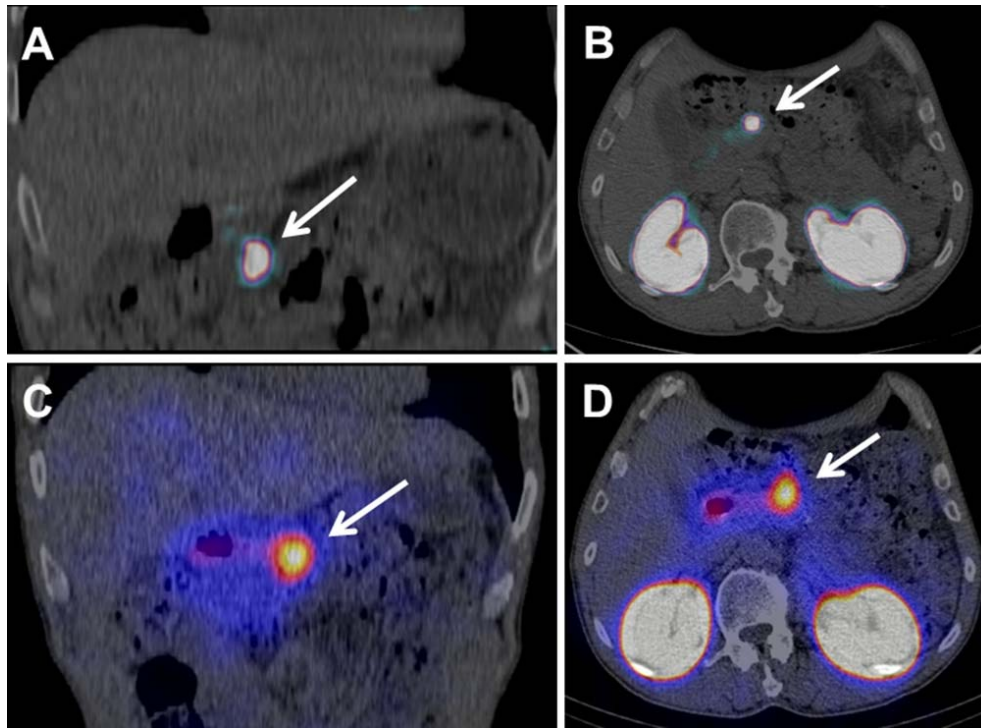


FIGURE 1. Coronal (A) and transaxial (B) PET/CT images from patient 4, 2.5 hours after injection of 80 MBq ^{68}Ga -DOTA-exendin-4. Coronal (C) and transaxial (D) SPECT/CT images of the same patient 72 hours after injection of 90 MBq ^{111}In -DOTA-exendin-4. There is focal uptake of ^{111}In -DOTA-exendin-4 and ^{68}Ga -DOTA-exendin-4 in the pancreatic body (arrow); the patient refuses surgery so far.

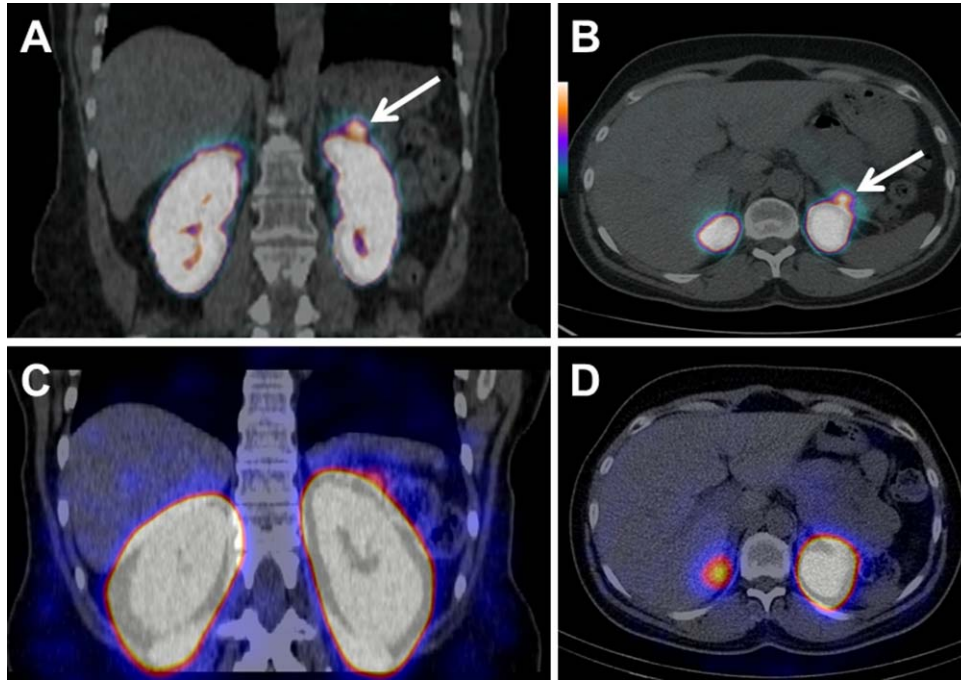


FIGURE 2. Coronal (A) and trans-axial (B) PET/CT images from patient 5, 2.5 hours after injection of 76 MBq ^{68}Ga -DOTA-exendin-4. Coronal (C) and trans-axial (D) SPECT/CT images of the same patient, 72 hours after injection of 66 MBq ^{111}In -DOTA-exendin-4. The arrow (A, B) shows focal ^{68}Ga -DOTA-exendin-4 uptake in the distal portion of the pancreatic tail consistent with the surgically removed insulinoma. SPECT/CT did not visualize the insulinoma.

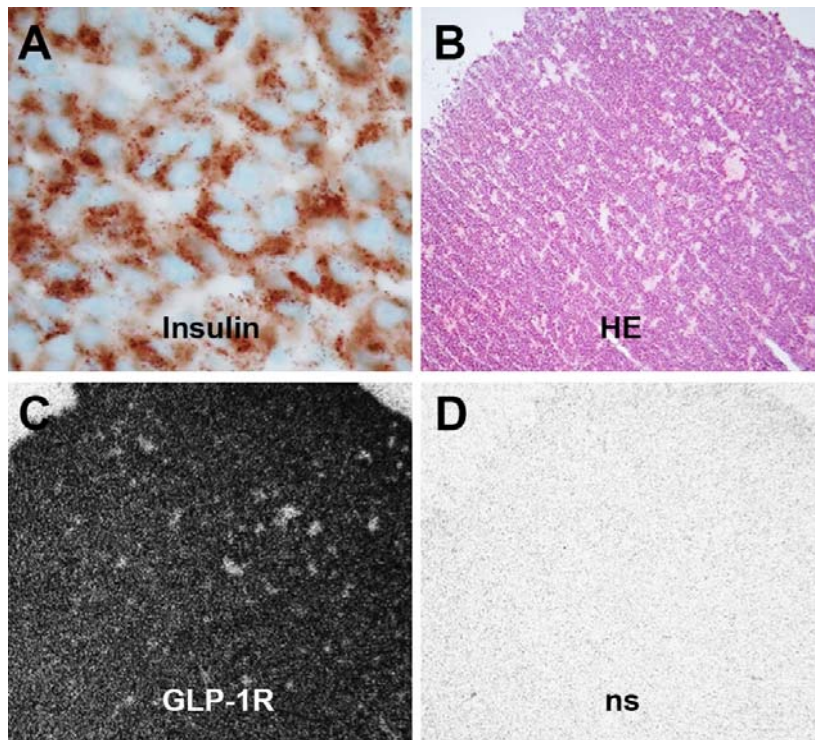


FIGURE 3. Hormone and receptor evaluation of resected insulinoma in patient 5. (A), Immunohistochemistry for insulin showing strongly labeled tumor cells. (B), Hematoxylin-eosin-stained tumor tissue. (C), In vitro autoradiography revealed a high GLP-1R density (mean 5766 dpm/mg tissue). (D), Autoradiogram showing nonspecific binding of ^{125}I -GLP-1 (7–36) amide in the presence of 100 nM GLP-1 (7–36) amide.

TABLE 1. Clinical characteristics

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Age (years)	39	48	64	63	56
Gender	F	F	M	M	F
Presenting symptoms	Hypoglycemia, palpitation, hyperhidrosis	Hypoglycemia, dizziness, hyperhidrosis	Hypoglycemia, palpitation, hyperhidrosis	Hypoglycemia, palpitation, hyperhidrosis	Hypoglycemia, tachypnea, hyperhidrosis
Duration of symptoms (months)	20	39	48	24	24

F= female; M=male

TABLE 2. Preoperative imaging and operative procedures

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Conventional imaging					
CT	FN	N/A	N/A	N/A	N/A
ASVS	FN	FN	N/A	N/A	N/A
EUS	FN	FN	FN	N/A	N/C
MRI	FN	FN	FN	-	TP
¹¹¹In-DOTA-exendin-4 SPECT/CT					
Detection of Tumor 4h p.i.	FN	TP	TP	+	FN
Tumor-to- background ratio	N/A	1.04	3.87	1.96	N/A
Detection of Tumor 72h p.i.	FP	TP	TP	+	FP
Tumor-to- background ratio	NA	1.12	3.93	2.04	NA
⁶⁸Ga-DOTA-exendin-4 PET/CT					
Detection of Tumor	TP	TP	TP	+	TP
Tumor-to- background ratio	2.00	3.79	6.87	5.95	32.42
SUVmax of tumor	5.5	10.8	49.1	25	30.8
Surgery and histology					
Surgical procedure	Enucleation	Pancreas tail resection	Pancreas tail resection	Not operated	Enucleation
Localization	Body/tail transition	Tail	Tail	N/A	Tail
Dimension (mm)	8	10	10	N/A	18
Histopathology	Insulinoma	Insulinoma	Insulinoma	N/A	Insulinoma

ASVS = selective arterial calcium stimulation and hepatic venous sampling; EUS = endoscopic ultrasound;

N/A = not applicable; N/C = not conclusive; FN = false negative; FP = false positive; TP = true positive; - = negative result; + = positive result