New imaging strategies in neuroendocrine tumors

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DOI:
10.1016/j.gie.2014.09.037

Chapter 4

Endoscopic ultrasound is superior for detection of pancreatic lesions compared to standard imaging in Multiple Endocrine Neoplasia type 1 patients

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Abstract

Objectives In Multiple Endocrine Neoplasia type 1 (MEN1), pancreatic neuroendocrine tumors (pNET) are the leading MEN1-related cause of death. Therefore, early detection is warranted. The best imaging technique for detection of pNETs in MEN1 is unknown. We performed a head-to-head evaluation of endoscopic ultrasound (EUS) and ^{11}C-5-hydroxytryptophan positron emission tomography (^{11}C-5-HTP PET), compared to the recommended screening techniques in MEN1 patients.

Design We conducted a cross sectional study in 41 patients at a tertiary care university medical center. Patients with proven MEN1 mutation or with one MEN1-manifestation and a mutation carrier as 1st grade family member, with recent screening by abdominal computed tomography (CT) or magnetic resonance imaging (MRI) and somatostatin receptor scintigraphy (SRS) were eligible. Patients underwent both linear EUS and ^{11}C-5-HTP PET. Patient- and lesion-based positivity was calculated for all imaging techniques.

Results In 35 out of the 41 patients, 107 pancreatic lesions were detected in total. EUS detected 101 pancreatic lesions in 34 patients, ^{11}C-5-HTP PET detected 35 lesions in 19 patients, and CT/MRI+ SRS 32 lesions in 18 patients (P <.001). ^{11}C-5-HTP PET performed similar to CT/MRI+ SRS, and better compared to SRS only (13 lesions in 12 patients), both at a patient- and lesion-based level (P < .05).

Conclusions EUS is superior to CT/MRI+ SRS for pancreatic lesion detection in MEN1 patients. In this setting ^{11}C-5-HTP PET is not useful. We recommend EUS as first choice pancreas imaging technique in MEN1 patients.

Introduction

Pancreatic neuroendocrine tumors (pNETs) are rare, with an incidence of 1-10 per 1 million in the general population.[1-2] pNETs can occur sporadically and as part of the hereditary multiple tumor syndromes Multiple Endocrine Neoplasia type 1 (MEN1) and von Hippel-Lindau disease. The prevalence of individuals with MEN1 is estimated at 1-10 per 100,000 in the general population.[3] MEN1 is characterized by a mutation in the *MEN1* gene located on chromosome 11q13. A mutation in this tumor suppressor gene can lead to endocrine tumors in various organs including the parathyroid gland, pituitary gland and pancreas. The prevalence of pNETs in MEN1 is 30-75%.[3-4] Non-functional pNETs are most common. pNETs can also produce hormones, and are called functional tumors if symptoms are present. Insulinomas and gastrinomas are the most frequent functional tumors in MEN1, of which gastrinomas are more often located in the duodenum than in the pancreas.[3] pNETs can have malignant potential and surgery is the only curative treatment.

Screening is performed for early detection of pNETs.[4-5] The 2001 expert-based MEN1 International Screening Guideline advised annual biochemical tests, including fasting glucose and insulin, starting at 5 years of age.[4] Additionally, at the age of 20 years, glucagon, pancreatic polypeptide, chromogranin A and pro-insulin are recommended annually together with somatostatin receptor scintigraphy (SRS) and abdominal computed tomography (CT) or magnetic resonance imaging (MRI) once every 3 years.[4] Despite screening, 28-46% of MEN1 patients die of a MEN1-associated manifestation, of which pNET is the leading cause of death.[6-8] Therefore, earlier detection of pNETs is warranted.

In a retrospective control study in patients without MEN1, EUS detected 82% of histological proven functional pNETs, which were not identified by trans-abdominal ultrasound and CT.[9] Several relatively small studies in MEN1 patients suggested that endoscopic ultrasound (EUS) is a sensitive method for early imaging of pNETs.[10-14] Given the lack of evidence, no screening modality for pNET is currently preferred in MEN1 patients; the recently revised MEN1 guideline recommends either CT, MRI or EUS annually.[5]

In addition to EUS, imaging with ^{11}C-5-hydroxytryptophan positron emission tomography (^{11}C-5-HTP PET) is potentially interesting for detection of pNETs in MEN1. ^{11}C-5-HTP is a precursor of serotonin, which can be decarboxylated by pNET cells to ^{11}C-serotonin.[15] ^{11}C-5-HTP PET in combination with CT is the most

sensitive method for imaging of advanced pNET, compared to CT combined with SRS or ^{18}F-6-fluoro-L-dihydroxyphenylalanin (^{18}F-DOPA) PET.[16]

To evaluate the imaging strategies EUS and ^{11}C-5-HTP PET for detecting pNETs, we performed a prospective head-to-head study of these strategies compared to the recommended conventional screening techniques CT/MRI and SRS in MEN1 patients. Since tumor markers are part of the current MEN1 guideline, we also evaluated serum chromogranin A and gastrin, and plasma glucagon and pancreatic polypeptide.

Patients and methods

Patients

In this prospective study, treating physicians in the MEN1 centers at the University Medical Centers of Rotterdam, Utrecht, Nijmegen and Groningen referred patients to the University Medical Center Groningen for study participation. Patients were included between February 2009 and August 2011. Eligible were those with genetically proven MEN1 or patients with clinically proven MEN1 with a 1st grade family member with genetically confirmed MEN1, with an age of ≥18 years. Standard MEN1 screening had to be performed at the patients' own MEN1 center. This screening included SRS within 6 months, abdominal CT or MRI and serum/plasma tumor marker assessment including serum chromogranin A and gastrin, and plasma glucagon and pancreatic polypeptide within 4 months before inclusion in the study.

Excluded were pregnant patients and patients known with alcohol abuse and/or chronic pancreatitis. This study was approved by the Medical Ethics Committee of Groningen, and all patients gave written informed consent. The study was registered in the Dutch trial register under http://www.trialregister.nl/trialreg/index.asp (NTR1668).

Conventional screening

CT/MRI

Depending on the preference of the treating physician, patients underwent abdominal CT or MRI. CT scans were performed with a multidetector CT scanner, before and after intravenous (IV) administration of iodine-containing contrast agent. MRI scans

were performed in T1 and T2-weighted sequences, with and without IV administration of gadolinium-containing contrast agent. The reconstruction interval varied between 0.75-5.0 mm. CT and MRI scans were reviewed by a radiologist (EJvdJ) blinded for the clinical information. In case of discrepancy between the radiologist of the referral and the research center, a second radiologist (AHHB) reviewed these scans, and consensus between both radiologists was reached after discussion.

SRS

According to guidelines of the Dutch Nuclear Medicine Association, 24 hours after administration of ~200 MBq ^{111}In-pentetreotide (Octreoscan; Mallinckrodt, Petten, the Netherlands) IV, planar total-body and 3D SPECT images were obtained using standard methods as described previously.[17] SRS scans were reviewed by a nuclear medicine physician (AHB) blinded for clinical information. In case of discrepancy between the nuclear medicine physician of the referral and the research center, a second nuclear medicine physician (KPK) reviewed the SRS, and after discussion between both nuclear medicine physicians, consensus was reached.

Tumor markers

Assessment methods of tumor markers differed between centers. Serum chromogranin A,[18] plasma pancreatic polypeptide,[19] serum gastrin [20] and plasma glucagon [21-22] were assessed with commercially available radioimmunoassays as described previously. Serum gastrin was also determined with an immunometric assay.[23] At one center, gastrin and pancreatic polypeptide were assessed with in-house radioimmunoassays. Since the use of proton pump inhibitors can lead to spurious elevation of chromogranin A and gastrin levels,[24-25] patients on proton pump inhibitors were excluded from analysis of these levels. To compare results obtained with various assays, ratios were calculated by dividing the value with the upper limit of normal. Ratio > 1 of one of the four tumor markers in one patient was defined as overproduction.

In patients with symptoms of an insulinoma (symptoms consistent with hypoglycemia, low plasma glucose concentration when symptoms are present, relief of symptoms when plasma glucose level is raised), a 72 hour fast test was performed at the referral center.[26]

EUS and ^{11}C-5-HTP PET

If possible, EUS and ^{11}C-5-HTP PET were performed on the same day. EUS was performed with a linear ultrasound endoscope (FG-34UX, Pentax GmbH, Hamburg, Germany) and a scanner system (EUB-525, Hitachi Ultrasound BV, Reeuwijk, the Netherlands). The endoscope had a 60º forward oblique viewing video camera, a 120º scanning ultrasound transducer with a 105º field of view, and a 2.0 mm working channel. The scanning frequency could be switched between 5 and 10 MHz. Presence of vascularity was assessed with power Doppler, and elasticity or rigidity of lesions were qualitatively assessed with elastography.[27] Patients underwent EUS with conscious sedation. EUS was performed by one endoscopist (HMvD) blinded for all other imaging. Number and location of pancreatic lesions were recorded on a standardized record form. Fine needle aspiration (FNA) was performed with a 22 or 25 G needle with a stylet (Sono Tip II 22 and 25 Gauge Medi-Globe GmbH, Germany) when cytological confirmation of a NET was desirable by the referred physician and/or the lesion was > 1 cm. A cytotechnologist was present during the FNA procedure for on-site assessment of the obtained material. All procedures were videotaped and photographs were obtained for all visualized lesions. For validation, videotapes of 22 non-selectively chosen lesions were reviewed by a second endoscopist (PF).

^{11}C-5-HTP PET scans were performed as described previously [15] either on an ECAT HR+ PET camera (n=12) or on a Siemens Biograph mCT camera (PET/CT 64 slices) (Siemens, Knoxville, TN) (n=29). PET scans were independently and randomly interpreted by two nuclear medicine physicians (AHB, KPK) blinded for all clinical information and other imaging. Number and location of positive lesions were listed on a standardized record form. In discrepant cases, consensus was reached between both physicians.

The results of the four imaging modalities were discussed in a multidisciplinary team, consisting of a radiologist (EJvdJ), nuclear medicine physician (AHB), gastroenterologist (HMvD), endocrinologist (TPL) and the clinical trial doctor (SJvA). The pancreatic lesions found on both EUS and ^{11}C-5-HTP PET were matched with CT/MRI+ SRS.

Statistical analysis

To demonstrate additional or new lesions in 12.5% of the patients with EUS and/or ^{11}C-5-HTP PET, 39 patients were needed for a statistically meaningful comparison. McNemar's test was used for comparison with 80% power and 5% two-sided

significance levels. Analysis was performed at the level of individual patients and lesions. Since EUS only images the pancreatic region, EUS and ^{11}C-5-HTP PET were compared with CT/MRI, SRS and CT/MRI+ SRS for the pancreatic region only. The percentage of detected pancreatic lesions was calculated for all four imaging techniques by using a composite reference standard. This standard included the four imaging outcomes. McNemar's test was used to compare the yield of the four imaging techniques. A *P* value <.05 was considered statistically significant. All authors had access to the study data and had reviewed and approved the final manuscript.

Results

Patient characteristics

In total 41 MEN1 patients were recruited for study participation. Characteristics of the included MEN1 patients are shown in table 1. The 41 patients carried 19 different MEN1 mutations (Supplementary Table 1).

Table 1. Patient characteristics (n=41)

Characteristics	Value
Sex: Female/Male (n of patients)	27/14
Median age in years (range)	44 (18-67)
Earlier pancreatic surgery: yes/no (n of patients)	7/34
Enucleation (n of patients)	2
Lymph node dissection (n of patients)	1
PPPD* (n of patients)	1
Pancreatic tail resection (n of patients)	3
Use of proton pump inhibitor: yes/no (n of patients)	11/30
Imaging: CT/MRI (n of patients)	23/18

Abbreviations: PPPD, pylorus preserving pancreaticoduodenectomy; CT, computed tomography; MRI, magnetic resonance imaging.

MEN1 conventional screening

With conventional screening, ≥ 1 pancreatic lesions were detected by CT/MRI in 14 patients (34%), by SRS in 12 patients (29%) and by CT/MRI+ SRS in 18 patients (44%). Of the 23 patients with CT and the 18 with MRI, 9 (39%) and 6 (33%) patients had positive imaging for pancreatic lesions, respectively. In total, 32 pancreatic lesions were detected with CT/MRI (24 lesions) and SRS (13 lesions).

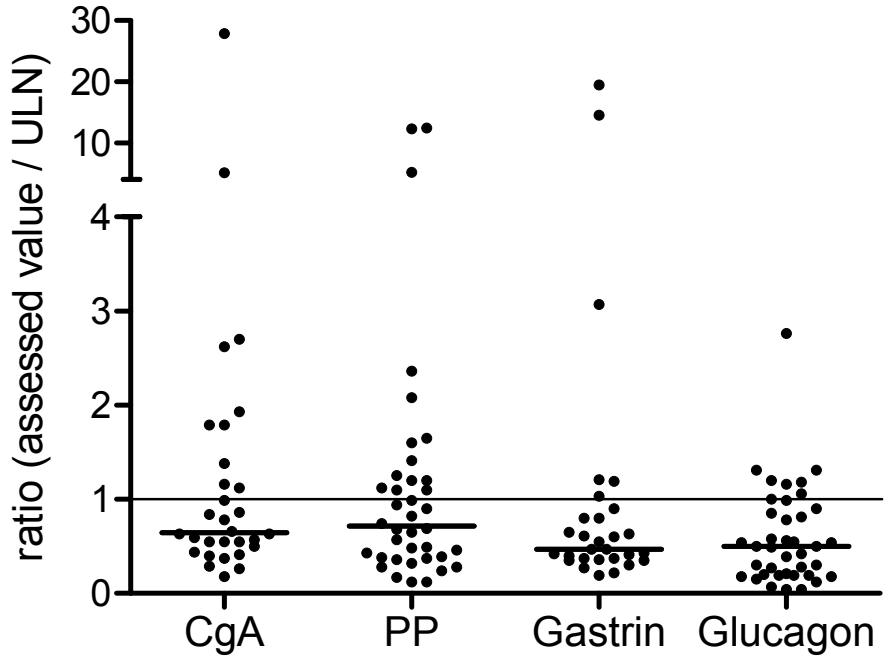

***Figure 1**. The y-axis shows the ratios of the tumor marker values for on the x-axis chromogranin A (n=30), pancreatic polypeptide (n=38), gastrin (n=29) and glucagon (n=39). Abbreviations: CgA, chromogranin A; PP, pancreatic polypeptide; ULN, upper limit of normal*

Of the 41 patients, 11 used proton pump inhibitors and were excluded from chromogranin A and gastrin analysis; 10 patients had elevated serum chromogranin A levels, 14 had elevated plasma pancreatic polypeptide levels, 6 had elevated serum gastrin levels, and 7 patients had elevated plasma glucagon levels (Figure 1). In 59% of the patients, levels were marginally elevated, being 1-2 times the upper limit of normal. Combining the 4 tumor markers resulted in 22 patients with elevated tumor markers, of whom 10 had a pancreatic lesion on CT/MRI and/or SRS.

EUS

With EUS, 101 pancreatic lesions were detected in 34 patients (83%) with a mean size of 9.1 ± 7.5 mm. Different lesion characteristics were identified (Table 2). Most lesions were homogeneous, hypoechoic and iso-elastic, and 42% of lesions were hypervascular. Of all lesions, 15 (15%) were cystic; 9 lesions had a thickened wall, which was hypervascular in 5 lesions (Figure 2). EUS was positive in the 18 patients with pancreatic lesions on CT/MRI+ SRS. In 11 of these patients (61%), EUS

detected 31 additional lesions. In the 23 patients without pancreatic lesions on CT/MRI+ SRS, EUS was positive in 16 patients (70%) and revealed 43 lesions. A second EUS expert (PF) reviewed 22 videotapes of lesions with sizes ranging from 2.5-25.8 mm. The presence of pancreatic lesions was confirmed in all cases.

In 10 patients, FNA was obtained for 12 lesions, with a median of 3 passes (range 1-4) per lesion. In 6 lesions the cytological diagnosis NET could be confirmed. The remaining samples did not yield enough cell material for a diagnosis. One patient was hospitalized because of abdominal pain after the FNA procedure due to acute pancreatitis. Plasma C-reactive protein was 114 mg/L (upper limit of normal 10 mmol/L) and plasma amylase was 82 U/L (upper limit of normal 220 U/L) and on the CT, infiltration of mesenterial fat close to the pancreatic head was seen. The patient recovered with conservative treatment within 6 days.

Table 2. Characteristics of pancreatic lesions detected with EUS

EUS characteristics	Total n (%) of lesions
Location	101 (100)
Pancreatic head	46 (46)
Pancreatic body-tail	55 (55)
Morphology	101 (100)
Solid	86 (85)
Cyst	6 (6)
Cyst with a thick wall	9 (9)
Margins	93 (100)
Sharp	68 (73)
Unsharp margins	25 (27)
Echogenic pattern	100 (100)
Hyperechoic	2 (2)
Hypoechoic	83 (83)
Anechoic	15 (15)
Ultrasonographic texture	94 (100)
Homogeneous	69 (73)
Heterogeneous	25 (27)
Power Doppler signal	88 (100)
Positive	37 (42)
Negative	51 (58)
Elastography	73 (100)
Rigid	19 (26)
Iso-elastic	54 (74)
Halophenomena	95 (100)
Yes	16 (17)
No	79 (83)

Abbreviation: EUS, endoscopic ultrasound

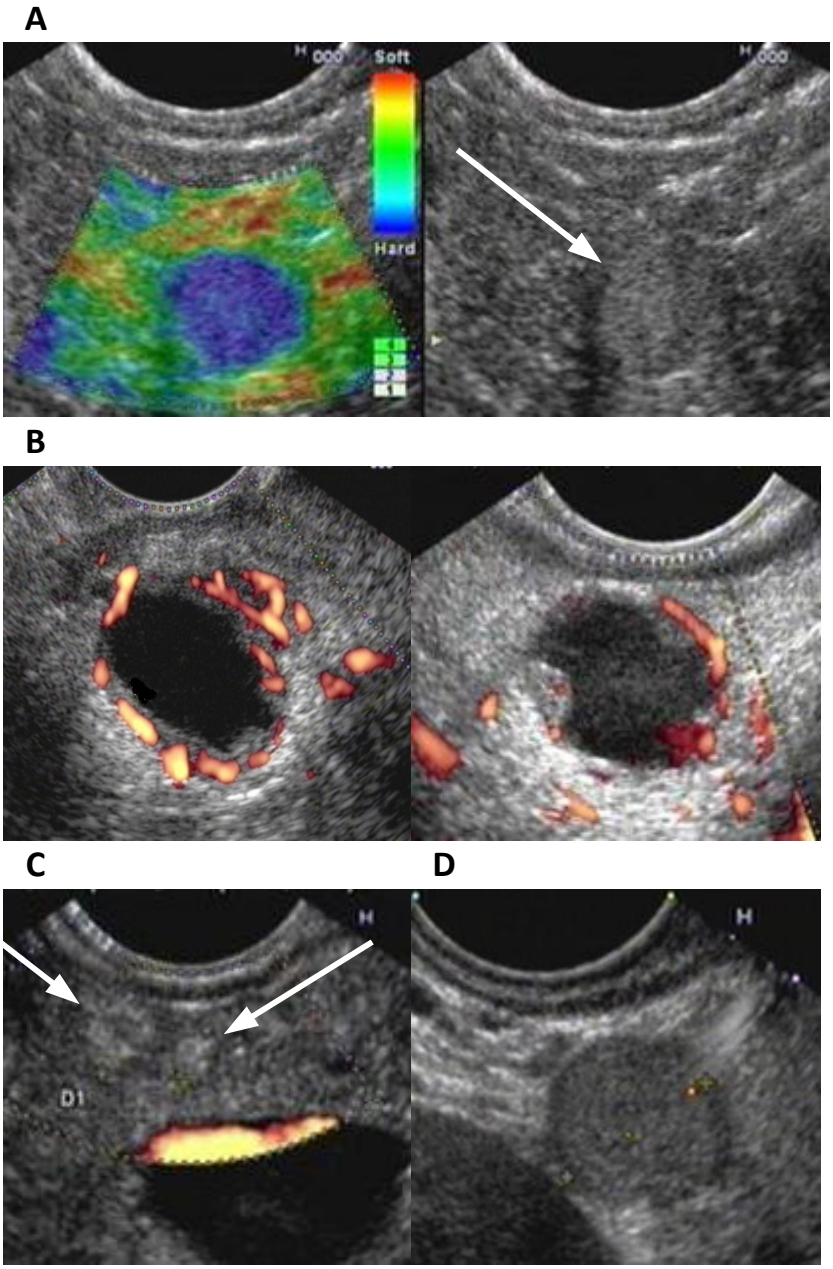

Figure 2. *EUS images of pancreatic lesions.* ***A*** *Elastography image of a pancreatic solid lesion (arrow). Blue color indicates rigidity.* ***B*** *Pancreatic cystic lesions with a thick hypervascular wall. Flow is present based on power Doppler, which indicates hypervascularity.* ***C*** *Two solid hyperechoic pancreatic lesions (arrows).* ***D*** *Pancreatic solid lesion with halo phenomena (black surrounding).*

^{11}C-5-HTP PET

^{11}C-5-HTP PET showed uptake in the pancreas in 19 patients (46%) and detected 35 pancreatic lesions with focal increased uptake. In the 18 patients with pancreatic lesions on CT/MRI+ SRS, ^{11}C-5-HTP PET detected 28 pancreatic lesions in 15 patients. In the 23 patients without pancreatic lesions on CT/MRI+ SRS, ^{11}C-5-HTP PET was positive in 4 patients, and detected 7 pancreatic lesions.

Surgery

Two patients underwent surgery. One patient with a pancreatic lesion on CT, SRS, ^{11}C-5-HTP PET and EUS underwent a pancreatic tail resection of a 7.5 cm NET, confirmed with histology. The other patient with 6 lesions of 0.3-2.6 cm underwent subtotal pancreatectomy. All 6 were initially visualized with EUS, 4 with ^{11}C-5-HTP PET, 2 with SRS and 1 with CT. The 2.6 cm lesion in the pancreatic head and multiple lesions in pancreatic body and tail showed NET at histology.

Table 3. Positive imaging for pancreatic lesions per pancreatic region

Pancreas	**Total number (%) of lesions detected:**					
Location	CT/MRI	SRS	CT/MRI+SRS	^{11}C-5-HTP PET	EUS	Total
Head	8 (17%)	7 (15%)	14 (29%)	12 (25%)	46 (96%)	48 (100%)
Body-tail	16 (27%)	6 (10%)	18 (31%)	23 (39%)	55 (93%)	59 (100%)

Abbreviations: CT, computed tomography; MRI, magnetic resonance imaging; SRS, somatostatin receptor scintigraphy; ^{11}C-5-HTP, ^{11}C-5-hydroxytryptophan positron emission tomography; EUS, endoscopic ultrasound

EUS and ^{11}C-5-HTP PET compared to MEN1 conventional screening

In 35 of 41 patients (85%), at least one of the imaging techniques was positive for a pancreatic lesion. At a patient-based level, EUS showed pancreatic lesions in more patients compared to CT, SRS and CT/MRI+ SRS (all P <.001) (Table 3). ^{11}C-5-HTP PET performed similar to CT/MRI and CT/MRI+ SRS, but was superior compared to SRS only (P <.05). Regarding pancreatic lesions, 8 patients had 1 lesion, 9 patients had 2 lesions, 6 patients had 3 lesions, 4 patients had 4 lesions and 8 patients had 5 or more lesions. In total, 107 pancreatic lesions were detected: 48 were located in the pancreatic head and 59 in the pancreatic body-tail region (Table 3). Compared to CT/MRI, SRS and CT/MRI+ SRS, EUS found the most lesions (all P <.001). This

was also the case for lesions > 1 cm, (all P <.01) (Table 4). In contrast, ^{11}C-5-HTP PET performed similar to CT and CT/MRI+ SRS, but found more lesions compared to SRS only (P <.05). At a patient- and lesion-based level (also lesions > 1 cm), EUS performed better compared to ^{11}C-5-HTP PET (P <.01). Figure 3 shows an example of a MEN1 patient with results of the four imaging techniques.

In total, 6 lesions were missed with EUS: 3 were detected with CT/MRI (1 lesion > 1 cm), 2 with ^{11}C-5-HTP PET and 1 both with CT and ^{11}C-5-HTP PET; 4 of the 6 lesions were located in the pancreatic tail.

Of the 35 patients with pancreatic lesions, 18 (51%) did have elevated tumor markers, of which 8 had less than 2-fold elevation of tumor markers, 4 between 2-fold and 3-fold, and 6 had > 3-fold elevation. Of the 6 patients without visualized pancreatic lesions, 4 (67%) also had elevated tumor markers, all with less than 2-fold elevation. Of these 4 patients, 2 had another MEN1-related manifestation: 1 patient had a pituitary macro-adenoma and 1 patient had a hyperparathyroidism, which can also be responsible for the elevated levels. Next to the pancreatic lesions, 15 extra-pancreatic lesions were found. (Supplementary Table 2).

Table 4. Positive imaging for pancreatic lesions in 35 patients

Imaging Modality	Patients n (%)	*P* value	Lesions n (%)	*P* value	Lesions >1 cm n (%)	*P* value
CT/MRI	14 (40)	-	24 (22)	-	17 (46)	-
SRS	12 (34)	-	13 (12)	-	11 (30)	-
CT/MRI+SRS	18 (51)	-	32 (30)	-	23 (62)	-
^{11}C-5-HTP PET	19 (54)	1*	35 (32)	0.74*	19 (51)	0.45*
EUS	34 (97)	<0.001*	101 (94)	<0.001*	36 (97)	<0.01*

* For ^{11}C-5-HTP and EUS, discordance was calculated for the number of visualized lesions, compared to CT/MRI plus SRS.

Abbreviations: CT, computed tomography; MRI, magnetic resonance imaging; SRS, somatostatin receptor scintigraphy; ^{11}C-5-HTP PET, ^{11}C-5-hydroxytryptophan positron emission tomography; EUS, endoscopic ultrasound

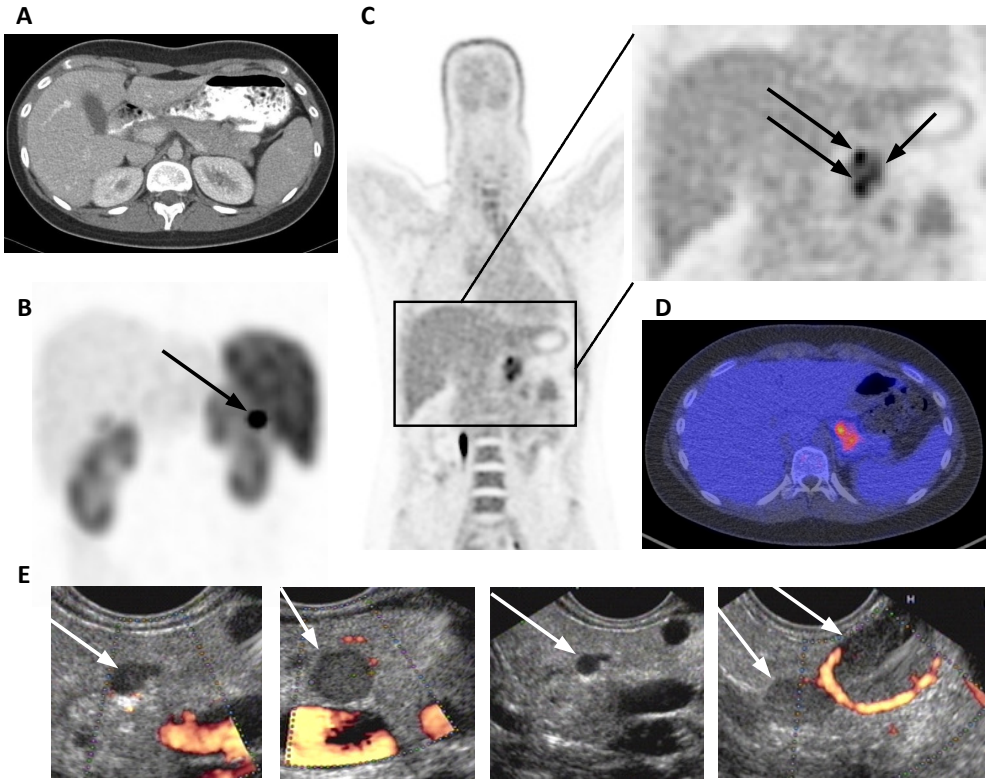

Figure 3. ***A*** *Axial image of CT scan,* ***B*** *maximum intensity projection image of SRS and* ***C*** *coronal image of* ^{11}*C-5-HTP PET and* ***D*** *an axial fusion image of* ^{11}*C-5-HTP PET with low-dose CT and* ***E*** *images of pancreatic lesions visualized with EUS. With CT no lesion was found and SRS showed 1 lesion located in the pancreatic tail. With* ^{11}*C-5-HTP PET 3 lesions were visualized in the pancreatic body/tail region. However, EUS visualized 5 hypoechoic lesions: 4 in pancreatic body/tail region and 1 in the pancreatic head, with size ranging from 3.5-14 mm. The lesion of 14 mm showed a clear hypervascular wall with power Doppler.*

Discussion

This is the first imaging study in MEN1 patients in which a prospective head-to-head comparison of EUS and ^{11}C-5-HTP PET was performed relative to the standard screening for pNET detection. Compared to CT/MRI and SRS – separately or combined – EUS is superior for early detection of pancreatic lesions at both a patient and lesion-based level. In this screening setting, ^{11}C-5-HTP PET is not useful.

Prominent in this study is the excellent performance of EUS. In our detailed analysis, most of the pancreatic lesions (83%) had a hypoechoic appearance on EUS. But not only solid lesions were detected; 15 lesions (15%) were cystic, with 9 having a thick, solid wall. Previously, cystic lesions were detected in 8% of MEN1 cases with EUS, but cysts with a thick wall were not described in that series.[13] In contrast to other studies which only looked at the echogenic pattern, we also checked for the presence of hypervascularity with power Doppler, which showed that 42% of pancreatic lesions were hypervascular. Moreover, differences were seen with elastography: most lesions had an iso-elastic consistency. Differences in EUS characteristics might reflect differences in growth behavior or malignant potential. In a retrospective EUS study with pNETs,[28] a univariate analysis of EUS findings showed that – in addition to larger size – heterogeneity and cystic changes are predictors of malignancy in pNET, which also might apply in MEN1 patients.

Until now MEN1 pancreatic imaging studies did not collect EUS and conventional imaging data prospectively. Moreover, not all standard screening imaging were available for a head-to-head comparison. However, the findings of the multicenter study in 90 MEN1 patients in which EUS was compared with MRI for pancreatic lesions is of interest.[29] In that study, 268 pancreatic lesions were detected with EUS and 158 with MRI. Of the 106 lesions ≥ 10 mm, 20 lesions (19%) were not detected by EUS, 19 of which were located in the pancreatic body-tail region. The authors therefore concluded that EUS and MRI are complementary and that both should be performed in the pancreas work-up for MEN1 patients.[29] In our study, EUS performed equally well in both the pancreatic head and body-tail region. This might be explained by the fact that EUS was performed in one center by an endoscopist who structurally mapped the pancreas. Only a linear scope was used with high quality devices. Since in the majority of cases EUS findings could not be confirmed by other imaging or pathologically, 22 EUS movies were reviewed by a second endoscopist at another academic center (PF). All lesions were considered as valid, which further supports EUS being superior compared to standard imaging.

In our study, EUS identified 101 lesions in 35 patients. Multiplicity of pancreatic lesions in MEN1 has also been confirmed by others in histological reports.[30-31] In pancreatic specimens of 28 MEN1 patients, the number of NETs (size > 5 mm) varied from 1–8 per patient.[31] Although cytological assessment was not performed in most pancreatic lesions in our study, the a priori chance of a present pNET is high in this patient group.

In one MEN1 imaging study for pNET screening, ^{11}C-5-HTP PET was retrospectively evaluated in 16 patients and detected lesions in 6 (38%) patients, of which 2 lesions corresponded with CT.[12] In our study, ^{11}C-5-HTP PET was of no value compared to standard screening. ^{11}C-5-HTP PET detected only 51% of lesions > 1 cm, indicating that lack of detection is not only a result of the resolution of the PET camera. However, compared to SRS alone, ^{11}C-5-HTP PET performed better, which indicates that SRS is not useful in the surveillance of MEN1. This outcome is in line with an earlier head-to-head comparison study in patients with advanced pNET, which showed ^{11}C-5-HTP PET being superior to SRS.[16]

After the initial imaging, 2 patients had surgery, and NETs were histologically confirmed. Moreover, in 50% of the FNA procedures, the diagnosis NET could be confirmed. In previous retrospective study using EUS FNA for the diagnosis pNET, the yield was 53%.[32] Similar to Voss and colleagues,[32] our samples were often hemorrhagic, with only few cells available for cytology. In our study, one patient required hospitalization after the FNA procedure because of pancreatitis. In patients with pNETs, complications due to EUS guided FNA occurred in 1%.[33] Due to the risk of complications, it is questionable whether FNA in MEN1 patients is indicated, considering the high a priori chance of pNET. In the MEN1 setting, FNA could be reserved for a firm indication or whether diagnostic uncertainty exists.

To date, the value of the tumor markers chromogranin A, pancreatic polypeptide, gastrin and/or glucagon for screening of pNET is unknown. They are part of the MEN1 guidelines,[4-5] but without a known level of evidence. In our study, tumor markers were elevated in 51% of patients with pancreatic lesions on imaging, of which 44% had only marginally elevated levels (< 2 times the upper limit of normal). Moreover, 4 of the 6 patients without pancreatic lesions had marginally elevated levels; 2 of them had another MEN1-related manifestation. A large number of clinical conditions can cause false-positive levels, making interpretation difficult.[25-26, 34] Moreover, elevated chromogranin A levels were detected in 27% of patients with sporadic primary hyperparathyroidism or sporadic pituitary adenomas,[35] illustrating that chromogranin A can be elevated in the presence of other MEN1-related manifestations. In that same study, chromogranin A was elevated in 44% of MEN1

patients without NETs.[35] Overall, no clear data support that these markers are useful for pNET screening in MEN1.

pNET is the leading cause of MEN1-related death. To date, no markers are available that can predict malignant potential of pNET in MEN1. Size positively correlates with metastatic potential and is used as one of the most important arguments for pancreatic surgery. The MEN1 guideline recommends considering surgery for a lesion size ≥ 1 cm. However, no evidence exist concerning the best timing for surgery. Since EUS identified most lesions, follow-up with EUS will reveal insight into their growth velocity.[13] For smaller lesions are visualized, significant growth of pNETs can be detected. This may support surgery and may decline morbidity and mortality rates due to pNETs in MEN1.

Our study strongly supports EUS being superior for detection of pancreatic lesions in MEN1 patients compared to CT/MRI+ SRS and ^{11}C-5-HTP PET. Based on our results, we suggest EUS as first choice imaging technique for pNET detection in MEN1.

Acknowledgements We would like to thank Drs D.J. Gouma and D. O'Toole for their contributions as members of the external monitoring committee.

Funding Supported by a Grant of the Dutch Cancer Society (RUG 2008-4188).

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Supplementary data

Supplementary Table 1. Overview of mutations in the MEN1 patients

Patients (n)	Mutation	Location	Type mutation
2	c.112delT (p.Ser38fs)	exon 2	Frameshift
1	c.249_252del (p.Ile85fs)	exon 2	Frameshift
1	c.322C>T (p.Arg108X)	exon 2	Nonsense
5	c.358_360del (p.Lys120del)	exon 2	In-frame deletion
1	c.506C>A (p.Ala169Asp)	exon 3	Missense
3	c.517del (p.Leu173fs)	exon 3	Frameshift
2	c.545T>C9 (p.Leu182Pro)	exon 3	Missense
3	c.631del (p.Arg211fs)	exon 3	Frameshift
1	c.810G>A (p.Trp270X)	exon 5	Nonsense
1	c.965A>G (p.His322Arg)	exon 7	Missense
1	c.1024G>C (p.Ala342Pro)	exon 7	Missense
2	c.1074C>G (p.Tyr358X)	exon 8	Nonsense
3	c.1099A>T (p.Lys367X)	exon 8	Nonsense
1	c.1192C>T (p.Gln398X)	exon 8	Nonsense
3	c.1430dupG (p.Glu478fs)	exon 10	Frameshift
4	c.1561dup (p.Arg521fs)	exon 10	Frameshift
1	c.1594C>T (p.Arg532X)	exon 10	Nonsense
1	c.1677_16845dup8 (p.Lys562fs)	exon 10	Frameshift
5	c.-110-?1848+?del (p.?)	whole gene	Deletion

Supplementary Table 2. Overview of extra-pancreatic (suspicious) NET lesions

Location	Lesion visualized on imaging			
	CT/MRI	SRS	^{11}C-5-HTP PET	EUS
Duodenum	Yes	No	No	No
Duodenum[#]	No	No	No	Yes
Duodenum[#]	No	No	No	Yes
Adjacent to pancreas LN	No	No	Yes	Yes
Adjacent to pancreas LN	No	No	No	Yes
Adjacent to pancreas LN	No	Yes	No	Yes
Adjacent to pancreas LN	Yes	Yes	No	No
Liver[^]	Yes	No	No	No
Liver	No	Yes	No	No
Liver	No	Yes	Yes	No
Omentum	Retrosp	No	Yes	No
Left adrenal gland	Yes	Yes	No	Yes
Pulmonary LN	Retrosp	No	Yes	No
Pulmonary LN	Retrosp	No	Yes	No
Pulmonary LN	Retrosp	No	Yes	No

[#] Histological confirmed NET

[^] Classified as benign lesion based on imaging

Abbreviations: LN, lymph node; retrosp, retrospectively seen; CT, computed tomography; MRI, magnetic resonance imaging; SRS, somatostatin receptor scintigraphy; ^{11}C-5-HTP, ^{11}C-5-hydroxytryptophan PET; EUS, endoscopic ultrasound.