Endoscopic Ultrasound Features Associated with Malignancy and Aggressiveness of Nonhypovascular Solid Pancreatic Lesions: Results from a Prospective Observational Study

Endoskopische Ultraschallmerkmale assoziiert mit Malignität und Aggressivität bei nicht hypovaskulären soliden Pankreasläsionen: Ergebnisse einer prospektiven Beobachtungsstudie

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ABSTRACT

Background and Study Aims On contrast-enhanced imaging studies, nonhypovascular (i. e., isovascular and hypervascular) patterns can be observed in solid pancreatic lesions (SPLs) of different nature, prognosis, and management. We aimed to identify endoscopic ultrasound (EUS) features of nonhypovascular SPLs associated with malignancy/aggressiveness. The secondary aims were EUS tissue acquisition (EUS-TA) outcome and safety in this setting of patients.

Patients and Methods This prospective observational study included patients with nonhypovascular SPLs detected on cross-sectional imaging and referred for EUS-TA. Lesion features (size, site, margins, echotexture, vascular pattern, and upstream dilation of the main pancreatic duct) were recorded. Malignancy/aggressiveness was determined by evidence of carcinoma at biopsy/surgical pathology, signs of aggressiveness (perineural invasion, lymphovascular invasion, and/or microscopic tumor extension/infiltration or evidence of metastatic lymph nodes) in the surgical specimen, radiologic detection of lymph nodes or distant metastases, and/or tumor growth > 5 mm/6 months. Uni- and multivariate analyses were performed to assess the primary aim.

Results A total of 154 patients with 161 SPLs were enrolled. 40 (24.8 %) lesions were defined as malignant/aggressive. Irregular margins and size > 20 mm were independent factors associated with malignancy/aggressiveness ($p < 0.001$, OR = 5.2 and $p = 0.003$, OR = 2.1, respectively). However, size > 20 mm was not significant in the subgroup of other-than-neuroendocrine tumor (NET) lesions. The EUS-TA accuracy was 92 %, and the rate of adverse events was 4 %.

Conclusion Irregular margins on EUS are associated with malignancy/aggressiveness of nonhypovascular SPLs. Size > 20 mm should be considered a malignancy-related feature only in NET patients. EUS-TA is safe and highly accurate for differential diagnosis in this group of patients.

ZUSAMMENFASSUNG

Ziel In kontrastverstärkten Bildgebungsstudien werden in soliden Pankreasläsionen (SPLs) nicht hypovaskuläre (d. h. isovaskuläre und hypervaskuläre) Muster unterschiedlichsten Ursprungs, Prognose und Behandlung beobachtet. Unser Ziel war die Bestimmung der Merkmale des endoskopischen Ultraschalls (EUS) bei nicht hypovaskulären SPLs, die mit Malignität/Aggressivität assoziiert sind. Die sekundären Ziele waren das Outcome der EUS-Gewebeaufnahme (EUS-TA) und die Patientensicherheit.

Material und Methoden Diese prospektive Beobachtungsstudie umfasste Patienten mit nicht hypovaskulären SPLs, die in einem Schnittbildverfahren entdeckt wurden und zur EUS-TA einbestellt wurden. Läsionsmerkmale (Größe, Lage, Ränder, Echotextur, Gefäßmuster und stromaufwärts gerichtete Dilatation des Hauptpankreasgangs) wurden erfasst. Die Malignität/Aggressivität wurde durch Nachweis eines Karzinoms in der Biopsie/Pathologie, durch Zeichen von Aggressivität (perineurale Invasion, lymphovaskuläre Invasion und/oder mikroskopische Tumorausdehnung/-infiltration oder Nachweis metastasierter Lymphknoten) in der chirurgischen Probe, durch den radiologischer Nachweis von Lymphknoten oder entfernten Metastasen und/oder durch Tumorwachstum > 5 mm in 6 Monaten definiert. Zur Beurteilung des Primärziels wurden uni- und multivariate Analysen durchgeführt.

Ergebnisse Insgesamt wurden 154 Patienten mit 161 SPLs aufgenommen. 40 (24,8 %) Läsionen wurden als maligne/aggressiv definiert. Unabhängige, mit Malignität/Aggressivität assoziierte Faktoren waren unregelmäßige Ränder ($p < 0{,}001$; OR = 5,2) und eine Größe > 20 mm ($p = 0{,}003$; OR = 2,1). Allerdings war der Faktor Größe > 20 mm in der Untergruppe der nicht neuroendokrinen Tumor (NET) -Läsionen nicht signifikant. Die Genauigkeit der EUS-TA betrug 92 % und die Rate der unerwünschten Ereignisse 4 %.

Schlussfolgerung Unregelmäßige Ränder in EUS sind mit Malignität/Aggressivität von nicht hypovaskulären SPLs assoziiert. Eine Größe > 20 mm sollte nur bei NET-Patienten als Marker für Malignität angesehen werden. Die EUS-TA ist sicher und von hoher Genauigkeit für die Differenzialdiagnose in dieser Patientengruppe.

Introduction

Vascular patterns of solid pancreatic lesions (SPLs) have been investigated by different abdominal imaging modalities [1, 2] and by contrast-harmonic endoscopic ultrasound (CH-EUS) [3, 4]. By comparing the contrast enhancement of SPLs with that of the surrounding normal pancreatic parenchyma, three different main patterns can be identified: hypovascular, isovascular, and hypervascular. The hypovascular pattern is the most commonly observed [4, 5], and it predicts pancreatic ductal adenocarcinoma (PDAC) with a sensitivity of 92–96 % and an accuracy of 82–95 % [3, 4]. Non-hypovascular patterns (i. e., isovascular and hypervascular) are associated with diagnoses other than PDAC in approximately 95 % of cases [3], but they are both not disease-specific and can be observed in SPLs with different prognoses and levels of aggressiveness, thus requiring specific management [6–8]. The most common diagnoses among non-hypovascular SPLs are pancreatic neuroendocrine tumor (pNET) [4, 5], pancreatic metastasis [7, 8], acinar cell carcinoma [7, 8], pseudosolid serous cystadenoma (SCA) [9], intrapancreatic spleen [7, 8], solid pseudopapillary neoplasm [7, 8], mass-forming pancreatitis [5], and other rare tumors [7, 8, 10]. The positive predictive value of the hypervascular pattern in predicting a pNET is only approximately 56 % [4]. Moreover, neither iso- nor hypervascular behaviors have been associated with lesion aggressiveness. Therefore, the identification of concomitant features of the lesion associated with aggressiveness can be useful to guide clinical management. Finally, the diagnostic yield of EUS-tissue acquisition (EUS-TA) and the rate of EUS-TA-related adverse events (AEs) in this subset of patients is unknown.

The primary aim of this study was to evaluate the EUS features of non-hypovascular SPLs associated with malignancy/aggressiveness in a large cohort of patients. Secondary aims were to evaluate the performance of EUS-TA in this patient population.

Patients and Methods

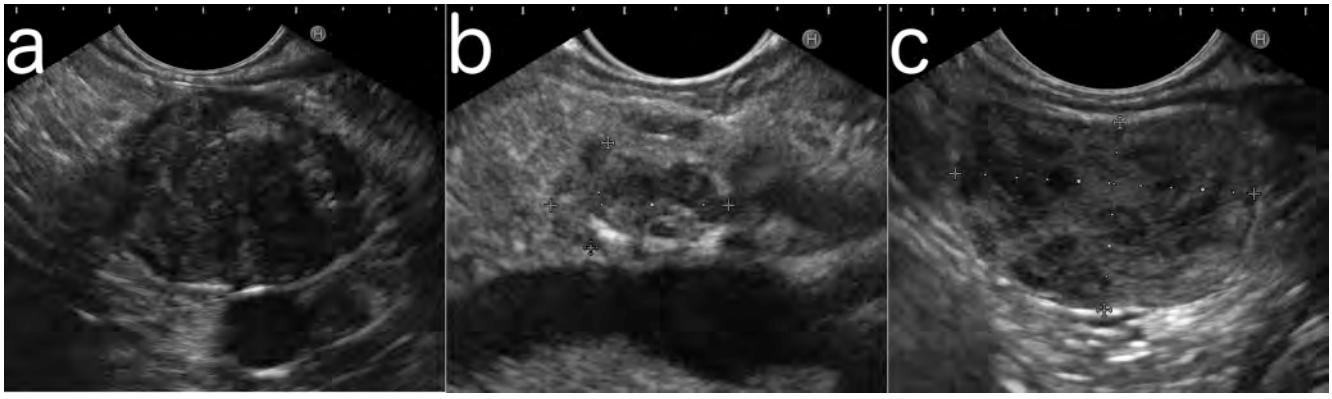
Study design, population and ethics

This is a prospective observational study that was approved by the local Institutional Ethics Committee (protocol n. 24360, 2016.05.17). The study was conducted in a tertiary pancreatic care center, including consecutive patients referred for EUS-TA between May 2016 and April 2018, fulfilling the following criteria:

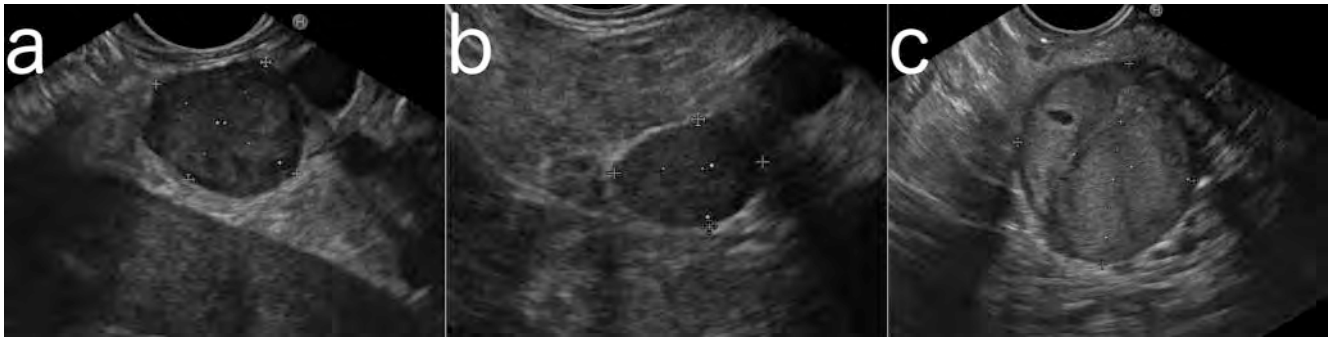
- Inclusion Criteria
  a) Patients with one or more SPLs showing a non-hypovascular contrast pattern (i. e., iso- or hypervascular in the arterial and venous phases) detected on contrast-enhanced computed tomography (CE-CT) and/or contrast-enhanced magnetic resonance imaging (CE-MRI).
- Exclusion Criteria
  a) Patients with features of diffuse chronic pancreatitis (e. g., pancreatic calcifications) [3].
  b) Hypovascular pattern observed during early and/or late phases on CH-EUS.
  c) Lesion not found or of nonpancreatic origin on EUS.
  d) Cystic lesion on EUS.
  e) Patients refusing to be included in the study.
- Drop-out criteria
  Patients lost to follow-up before 12 months.

EUS, CE-EUS and EUS-TA procedures

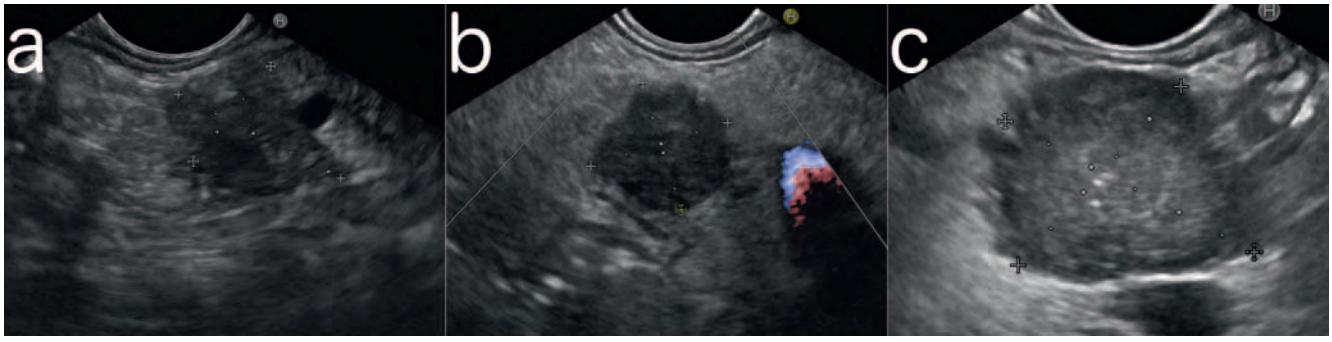
Endoscopic procedures were performed in deep sedation with the patient in the left lateral decubitus position by two expert endo-



▶ **Fig. 1** Examples of heterogeneous echotexture. **A** Heterogeneous mass in the tail of the pancreas turned out to be an inflammatory mass-forming lesion; **B** An 18-mm nodule in the pancreatic neck with heterogeneous texture and irregular margins diagnosed as an acinar carcinoma at surgical pathology; **C** Heterogeneous echotexture nodule measuring 24 mm in the pancreatic tail with smooth margins; surgical pathology confirmed a neuroendocrine tumor G1 without signs of aggressiveness or nodal involvement.



▶ **Fig. 2** Representative endoscopic ultrasound images of lesions with regular margins. **A** A 24-mm nodule in the tail of the pancreas showing heterogeneous texture and smooth borders; at surgical pathology, a neuroendocrine tumor (NET) G1 without signs of aggressiveness or lymph node involvement was diagnosed; **B** A 16-mm homogeneous nodule in the tail of the pancreas with regular margins diagnosed as an intrapancreatic spleen at endoscopic ultrasound biopsy; **C** A large (38-mm) heterogeneous nodule in the uncinate process with hypoechoic rim and lateral shadowing suggesting the presence of a capsule; a NET G2 with nodal involvement was confirmed at surgical pathology.



▶ **Fig. 3** Examples of lesions with irregular margins. **A** An 18 mm heterogeneous nodule in the neck of the pancreas showing poorly defined margins diagnosed as ductal adenocarcinoma; **B** A lesion with irregular borders in the pancreatic head measuring 19 mm. Endoscopic ultrasound sampling demonstrated a NET G2 with cytologically confirmed hepatic metastasis. **C** A large (28 mm) tumor in the pancreatic tail with poorly defined margins diagnosed as a solid pseudopapillary neoplasm.

sonographers with a volume of more than 500 EUS/yr. A Pentax linear echoendoscope (EG-3870 UTK) with a Hitachi ultrasound processor was used for all examinations.

The EUS features recorded for each lesion were a) lesion location (head/uncinate process, neck/body, or tail); b) size (mm); c) ultrasonographic texture (homogeneous or heterogeneous [▶ **Fig. 1**]); d) margins (smooth [▶ **Fig. 2**] or irregular/poorly

defined [▶ **Fig. 3**]) [11]; and e) evidence of main pancreatic duct (MPD) upstream dilation (compared with downstream duct size).

CH-EUS was performed using the standard contrast harmonic procedure [12]. Sonovue™ 4.8 mL (Bracco Imaging, Milan, Italy) was the contrast agent used in all patients. The vascular pattern of the lesion was established in comparison with the surrounding normal pancreatic parenchyma. Echotexture was defined after CH-EUS to accurately detect small cystic changes or inhomogeneous enhancement [11]. In the case of multiple SPLs, CH-EUS was performed targeting each single lesion by awaiting a washout period of 10 minutes between Sonovue™ administration.

The needle choice for EUS-TA was based on the endosonographer's preference. Histologically designed needles, fork-tip (SharkCore, Medtronic Inc., Sunnyvale, CA [13]) or side fenestrated (ProCore, Cook Medical, Limerick, Ireland [14]), were mainly employed. The slow pull technique [13] in all cases and the fanning technique [15], whenever possible, were used. Rapid-on-site evaluation (ROSE) was not available in any case. Three needle passes, if possible, were performed. The acquired sample was macroscopically evaluated by endosonographer [16] and, if judged insufficient, additional passes up to a maximum of seven were done. Samples were processed as histological specimens or using the cell-block processing method [17]. E&E staining was routinely adopted for histology. The immunohistochemical panel to be used was chosen according to histology and clinical suspicion.

Malignancy/aggressiveness definition and follow-up

For the purpose of this study, lesions were defined as malignant/aggressive in the presence of one or more of the following criteria:

a) Evidence of carcinoma at biopsy or surgical histology;
b) Signs of aggressiveness (perineural invasion, lymphovascular invasion, and/or microscopic tumor extension/infiltration or evidence of metastatic lymph nodes) at surgical pathology [18, 19];
c) Radiologically detected (on CE-CT or CE-MRI or ^{68}Ga-somatostatin receptor positron emission tomography [^{68}Ga-PET] or ^{18}fluoro-deoxyglucose-PET [FDG-PET]) lymph nodes or distant metastases at baseline evaluation or during a follow-up of at least 12 months;
d) tumor growth > 5 mm/6 months [20].

Imaging follow-up was carried out according to current guidelines for pNETs [20] every 6 months by CE-MRI (or CE-CT). A last telephone contact with all patients was made at the end of the study. Lesions without evidence of the abovementioned features were classified as benign/non-aggressive.

EUS-TA outcome measurements and final diagnoses

EUS-TA adequacy was defined as the presence of tissue sufficient to define a specific diagnosis at cyto-histological evaluation. Cyto-histological samples were classified using the Bethesda classification [21]. For the classification of malignancy, cytological and histological interpretations "suspicious for malignancy" were deemed malignant. The presence of atypia was regarded as benign. The final diagnosis was established on surgical pathology whenever available, while in nonresected patients it was assessed on EUS-TA cytology/histology combined with a compatible clinical course during a follow-up of at least 12 months.

EUS-related AEs were assessed 3 days and 15 days after biopsy by outpatient visits or telephone contact and were classified according to Cotton et al. [22].

Statistical analysis

Continuous variables were assessed for normality and are expressed as means with standard deviations (SD). Categorical variables are expressed as frequencies with percentages. The chi-square test with Yates's correction in 2 × 2 contingency tables and Fisher's exact test for cases with small expected frequencies (< 5) were used for univariate analysis of categorical data. Statistical significance was determined by a p-value < 0.05. All tests were 2-tailed. Multivariate analyses to investigate the association between EUS features and malignancy/aggressiveness and factors impacting EUS-TA accuracy was carried out employing logistic regression. Factors with a p-value < 0.05 in the univariate analysis were included in the multivariate analysis. Data are expressed with odds ratios (ORs) and their relative 95 % confidence intervals (CIs).

The EUS-TA yield was calculated per lesion in an intention-to-treat (ITT) analysis (inadequate/nondiagnostic samples were counted as false-negative). The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of EUS-TA were calculated in comparison with the final diagnosis and in relation to the specific tumor diagnosis.

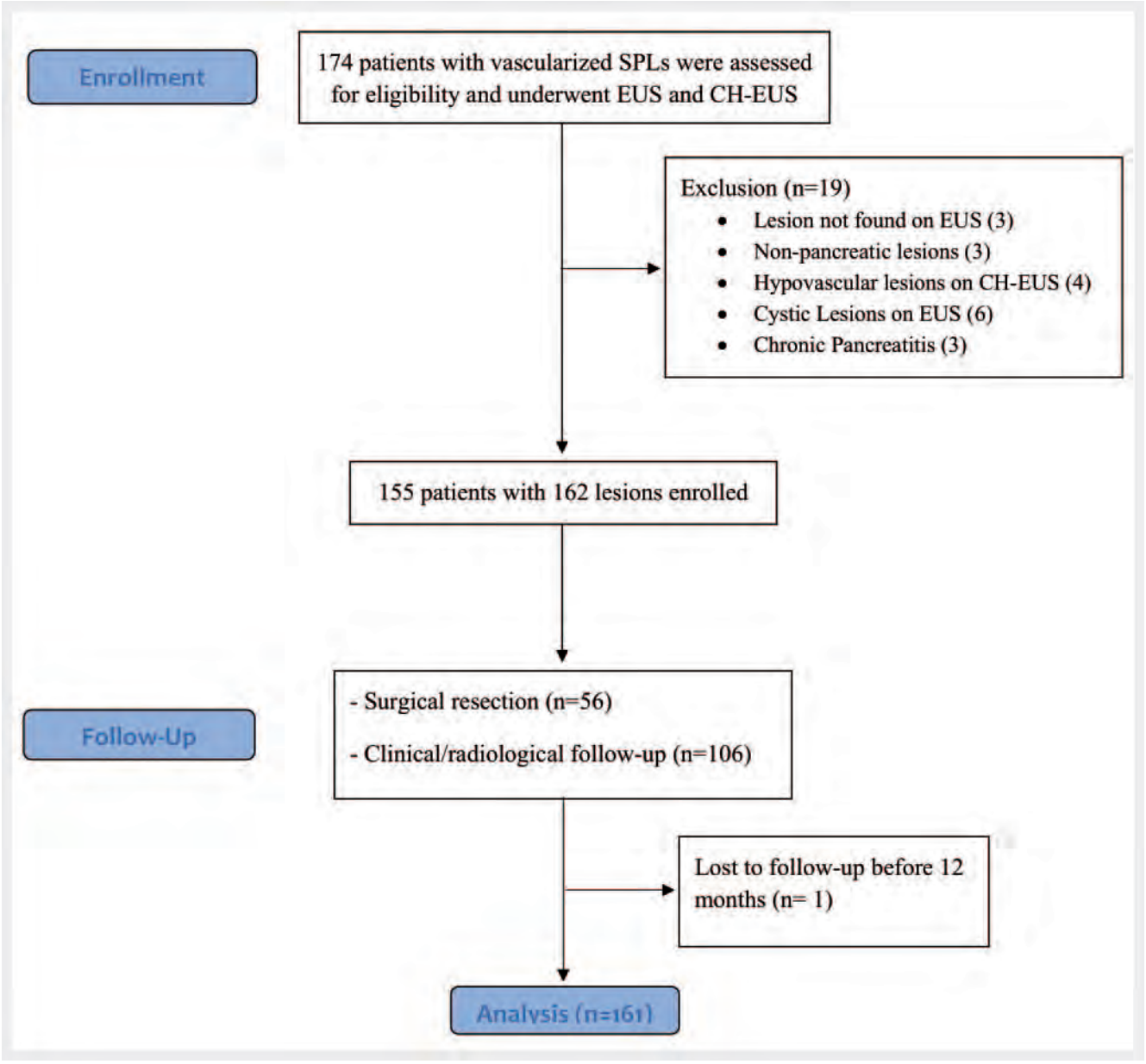
Data were analyzed using SPSS 22 software (SPSS, an IBM company, Chicago, USA).

Results

Overall, 174 consecutive patients with vascularized SPLs on cross-sectional imaging studies were assessed for eligibility. 20 patients (11 %) were excluded (four cases because of hypovascular pattern on CH-EUS), while the remaining 154 (M/F 70/84, mean age 58.7 years ± 13.1) with 161 SPLs were included (▶ **Fig. 4**). Demographics, EUS features, final diagnoses and patient outcomes are summarized in ▶ **Table 1, 2**.

50 lesions were located in the pancreatic head/uncinate (31.1 %), 55 in the neck/body (34.1 %), and 56 in the tail (34.8 %). The mean lesion size was 18.8 mm ± 10.6 (range 4–60 mm). The vascular pattern was hypervascular in 110 (68.3 %) cases and isovascular in 51 (31.7 %). The ultrasound echotexture was homogeneous in 89 (55.3 %) cases and heterogeneous in 72 (44.7 %). The margins were classified as regular in 117 (72.2 %) cases and irregular in 45 (27.8 %). Upstream dilation of the MPD was observed in 20 (12.4 %) cases. Overall, ^{68}Ga-PET, FDG-PET, and double-tracer PET (^{68}Ga and FDG) were performed in 129 (83.8 %), 122 (79.2 %), and 118 (76.6 %) patients, respectively.

56 lesions (34.8 %) were resected, and 105 (65.2 %) underwent follow-up for a median period of 20 months (range 12–32 months). Among the resected lesions, 28 (50 %) were malignant/aggressive (16 carcinomas, sign of aggressiveness in 11 cases, distant metastasis in one case). Nonresected lesions



▶ **Fig. 4** STROBE study flowchart.

were defined as malignant/aggressive in 12 (11.4 %) cases (evidence of carcinoma on EUS-TA in 8 cases, distant metastasis in 3 cases, and tumor growth > 5 mm/6 months in one pNET). 9 lesions (5.6 %) (mean size 10.2 mm, range 7–17) in which EUS-TA resulted in inadequate samples (N = 8) or was not feasible (N = 1) were finally classified as benign/non-aggressive, despite an undefined diagnosis, because they remained stable in size without the appearance of suspected metastasis on imaging studies during a median follow-up of 20 months (range 14–27 months). Overall, 40 (24.8 %) lesions were defined as malignant/aggressive, and 121 (75.2 %) were defined as benign/non-aggressive.

EUS features associated with malignancy/aggressiveness

Univariate analysis showed that lesion diameter > 20 mm, irregular margins, heterogeneous echotexture, and upstream dilation of the MPD were significantly associated with malignancy/aggressiveness ($p < 0.001$ for all features). In the multivariate analysis, however, only size > 20 mm ($p = 0.03$, OR = 2.1) and irregular margins ($p < 0.001$, OR = 5.2) were found to be independent factors. The sensitivity, specificity, PPV, NPV, and accuracy in predicting malignancy/aggressiveness were 61.5 % (95 % CI, 44.6–76.6), 77.9 % (95 % CI, 69–84.8), 47 % (95 % CI, 37–57–4), 86.4 % (95 % CI, 80.8–90.5) and 73.9 % (95 % CI, 66.4–80.5) for size > 20 mm, respectively, and 65 % (95 % CI, 48.3–79.4), 85.9 % (95 % CI, 78.5–91.6), 60.5 % (95 % CI, 48.2–71.5), 88.1 % (95 % CI, 82.9–

▶ **Table 1** Demographic and endoscopic ultrasound features of study cohort (154 patients with 161 lesions).

features	values
sex, N (%)	
▪ male	70 (45.2)
▪ female	84 (54.8)
age, yr ± SD	58.7 ± 13.1
lesion size, mm ± SD	18.8 ± 10.6
lesion Site, N (%)	
▪ head/Uncinate	50 (31.1)
▪ neck/Body	55 (34.1)
▪ tail	56 (34.8)
ultrasound texture, n (%)	
▪ homogeneous	89 (55.3)
▪ heterogeneous	72 (44.7)
margins, n (%)	
▪ regular	117 (72.2)
▪ irregular	45 (27.8)
upstream MPD dilation, N (%)	20 (12.4)
vascular Patter, n (%)	
▪ isovascular	51 (31.7)
▪ hypervascular	110 (68.3)
outcome, N (%)	
▪ surgery	56 (34.8)
▪ follow-up	105 (65.2)
final diagnoses, N (%)	
▪ pNET	92 (57.1)
▪ MTS-RCC	13 (8)
▪ intrapancreatic spleen	8 (5)
▪ PDAC	7 (4.3)
▪ inflammatory	7 (4.3)
▪ SCA	6 (3.7)
▪ lymphoid tissue NOS	6 (3.7)
▪ SPN	4 (2.5)
▪ others	9 (5.6)
▪ undefined	9 (5.6)

MPD, main pancreatic duct; NET, neuroendocrine tumor; MTS-RCC, metastasis from renal cell carcinoma; PDAC, pancreatic ductal adenocarcinoma; SCA, serous cystic adenoma; SPN, solid pseudopapillary neoplasm; NOS, not otherwise specified. Others include: 2 PEComas, 2 autoimmune pancreatitis, 2 lymphomas, 2 acinar carcinoma, and 1 schwannoma.

91.9) and 80.8 % (95 % CI, 73.8–86.5), respectively, for irregular margins.

After excluding the 9 patients with undefined diagnoses, a subgroup analysis separating pNETs (N = 92, 60.5 %) from other-than-pNET diagnoses was performed (N = 60, 39.5 %). In the pNET group, the results were similar to those found in the overall population, with size > 20 mm and irregular margins as independent factors in the multivariate analysis (p = 0.013, OR = 2.5 and p < 0.001, OR = 3.9, respectively). In contrast, in the other-than-pNET group, only irregular margins were independently associated with malignancy/aggressiveness (p < 0.001, OR = 3.9) (▶ **Table 3**). These results were independent of the endosonographer who performed the procedure.

EUS-TA outcomes and adverse events

EUS-TA was feasible in all but one lesion (99.4 %) due to interposed vessels. An EUS-FNB needle was used in 145/160 (90.6 %) cases, whereas in 15/160 (9.4 %) cases, a standard FNA needle was employed. The mean number of passes per lesion was 3.5 ± 0.96 (range 1–7 passes). Overall, EUS-TA resulted in adequate specimens in 149/161 (92 %) cases. The sensitivity, specificity, PPV, NPV, and accuracy were 91.6 % (95 % CI, 86–95.4), 100 % (95 % CI, 59–100), 100 % (95 % CI, 97.3–100), 35 % (95 % CI, 24.2–47.5), and 91.9 % (95 % CI, 86.6–95.6), respectively. When only resected patients were evaluated, the sensitivity, specificity, PPV, NPV, and accuracy were 96.3 % (95 % CI, 87.5–99), 100 % (95 % CI, 34.2–100), 100 % (95 % CI, 93.1–100), 50 % (95 % CI, 15–85), and 96.4 % (95 % CI, 87.7–99.6), respectively. The needle type employed (fork-tip versus side-fenestrated reverse-bevel versus side-fenestrated anterograde-bevel versus standard) was significantly associated with diagnostic accuracy (p = 0.021) (▶ **Table 4**).

Among 92 pNET lesions, the Ki-67 index value on EUS-TA specimens could be assessed in 86 cases (93.5 %). According to the 2017 WHO classification [23], pNETs were classified as G1, G2 and G3 in 76 (88.4 %), 9 (10.5 %), and 1 (1.2 %) cases, respectively. The grade G2 / G3 on EUS-TA specimens was associated with both irregular margins (p = 0.04) and size > 20 mm (p = 0.0008). Considering pNETs that underwent resection, the Ki-67 index value on EUS-TA specimens was available in 30 cases. The concordance rate for grading between EUS-TA and surgical pathology was 76.7 % (▶ **Table 5**).

Six (3.9 %) AEs were observed: four (2.6 %) cases of acute pancreatitis, all managed conservatively (in one case complicated by fluid collection); one (0.65 %) case of post-EUS atrial fibrillation, medically treated without sequela; and one (0.65 %) case of fever without an obvious cause, self-resolving in a few days without any treatment.

Discussion

The vascular pattern is a key feature of the diagnostic workup of SPLs. In the case of hypovascular behavior, the diagnosis of PDAC is highly probable. In contrast, the isovascular and hypervascular patterns are not disease-specific, and they are not significantly associated with lesion malignancy or aggressiveness. In the present prospective study, we aimed to identify EUS features associated with malignancy or with signs of aggressiveness in a large cohort of patients with non-hypovascular SPLs. The availability of such information can be of paramount importance in helping physicians to proceed in the diagnostic/therapeutic

▶ **Table 2** Endoscopic ultrasound features and outcomes stratified for diagnosis.

diagnosis (n)	mean size (mm)	site, n (%)			vascular pattern, n (%)		borders, n(%)		echotexture, n (%)		MPD dilation, n (%)	outcome, n (%)	
		head	body	tail	iso	hyper	smooth	irregular	homogeneous	heterogeneous		surgery	FU
NET (92)	18.7	32 (34.4)	35 (37.6)	26 (28)	20 (21.5)	73 (78.5)	79 (84.9)	14 (15.1)	20 (21.7)	72 (78.3)	8 (8.6)	32 (34.8)	60 (64.5)
MTS-RCC (13)	18.7	4 (30.8)	5 (38.5)	4 (30.8)	2 (15.4)	11 (84.6)	5 (38.5)	8 (61.5)	8 (61.5)	5 (38.5)	1 (7.7)	6 (46.2)	7[1] (53.8)
intrapancreatic Spleen (8)	14.6	0	0	8 (100)	2 (25)	6 (75)	8 (100)	0	8 (100)	0	0	0	8 (100)
PDAC (7)	26.9	4 (57.1)	2 (28.6)	1 (14.3)	5 (71.4)	2 (28.6)	0	7 (100)	1 (14.3)	6 (85.7)	6 (85.7)	7 (100)	0
inflammatory (7)	20.3	3 (42.8)	2 (28.6)	2 (28.6)	6 (85.7)	1 (14.3)	2 (28.6)	5 (71.4)	3 (42.9)	4 (57.1)	2 (28.6)	0	7 (100)
SCA (6)	20.2	1 (16.6)	4 (66.6)	1 (16.6)	0	6 (100)	4 (66.6)	2 (33.3)	1 (16.7)	5 (83.3)	0	0	6 (100)
lymphoid tissue NOS (6)	10	1 (16.7)	1 (16.7)	4 (66.6)	3 (50)	3 (50)	6 (100)	0	6 (100)	0	0	0	6 (100)
SPN (4)	24.5	0	2 (50)	2 (50)	4 (100)	0	2 (50)	2 (50)	0	4 (100)	1 (25)	3 (75)	1 (25)
others (9)	27.8	4 (44.4)	4 (44.4)	1 (11.1)	6 (66.7)	3 (33.3)	4 (44.4)	5 (55.6)	2 (22.2)	7 (77.8)	2 (22.2)	6 (66.7)	3 (33.3)
undefined (9)	10.2	2 (22.2)	0	7 (77.8)	3 (33.3)	6 (66.7)	8 (88.9)	1 (11.1)	8 (88.9)	1 (11.1)	0	0	9 (100)

MPD, main pancreatic duct; NET, neuroendocrine tumor; MTS-RCC, metastasis from renal cell carcinoma; PDAC, pancreatic ductal adenocarcinoma; SCA, serous cystic adenoma; SPN, solid pseudopapillary neoplasm; NOS, not otherwise specified; Others include: 2 PEComas, 2 autoimmune pancreatitis, 2 lymphomas, 2 acinar carcinoma, and 1 schwannoma.

[1] Chemotherapy.

▶ **Table 3** Factors associated with malignancy/aggressiveness of non-hypovascular solid pancreatic lesions.

population / feature	overall population		NET		other lesions	
	univariate, p value	multivariate, p value, OR (95 % CI)	univariate, p value	multivariate, p value, OR (95 % CI)	Univariate p value	Multivariate, p value, OR (95 % CI)
sex	0.850	–	1	–	0.583	–
age ▪ < 60 ▪ > 60	0.568	–	0.764	–	0.283	–
lesion size ▪ ≤ 20 mm ▪ > 20 mm	**< 0.001**	**0.03, 2.1** (0.15;3.01)	**< 0.001**	**0.013, 2.5** (0.68;5.49)	0.787	–
lesion site ▪ head/uncinate ▪ neck/body ▪ tail	0.151	–	0.212	–	0.498	–
margins ▪ smooth ▪ irregular	**< 0.001**	**< 0.001, 5.2** (2.42;5.33)	**< 0.001**	**< 0.001, 3.9** (1.67;5.30)	**0.001**	**< 0.001, 3.7** (2.01;6.66)
echotexture ▪ homogeneous ▪ heterogeneous	**< 0.001**	0.454, 0.75 (0.86;1.91)	**< 0.001**	0.1, 1.6 (–0.22;2.47)	0.124	–
MPD dilation	**< 0.001**	0.082, 1.7 (–0.22;3.59)	**0.003**	0.150, 1.4 (–0.63;4.01)	0.099	–
vascular pattern ▪ isovascular ▪ hypervascular	0.433	–	1	–	1	–

NET, neuroendocrine tumor; MPD, main pancreatic duct; OR, odds ratio.

workup. Moreover, when multiple lesions are present, awareness of suspicious features may help the endosonographer to choose the lesion to be punctured. In univariate analysis, four features, such as lesion size > 20 mm, irregular margins, heterogeneous echotexture, and upstream dilation of the MPD, were found to be associated with malignancy/aggressiveness. However, only size > 20 mm and irregular margins were independent factors in the multivariate analysis.

Size is a well-known risk factor for tumor aggressiveness. Current guidelines for pancreatic NET management suggest resection when a tumor is larger than 2 cm [20], and the results of our study are in agreement with this suggestion. Indeed, a size > 2 cm was an independent factor associated with malignancy/aggressiveness in the pNET group. However, when a diagnosis other than pNET was confirmed, the size was not significant. The reason may be found in the heterogeneity of diagnoses included in this subgroup of patients, including small malignant lesions (e. g., metastases) and large benign tumors (e. g., SCA or rare tumors). This finding could suggest the need for preoperative sampling prior to resection, even for a vascularized nodule larger than 2 cm suspicious for pNET. Indeed, inappropriate pancreatic surgery for benign lesions mimicking pNET was reported by several recent studies [9, 23].

The second feature we found to be an independent factor associated with malignancy/aggressiveness was the irregularity of lesion margins. The aspect of tumor margins may reflect the growth pattern of the tumor. Smooth borders are generally observed in well-encapsulated, not-aggressive nodules. Differently irregular/poorly defined margins can be an expression of tumor pseudopodia or infiltrative growth. In the 2017, Zhu et al. reported a significant association between the aspect of lesion borders (well-defined or ill-defined) on EUS and the grading of pNET [25]. Moreover, the association between irregular shape on CT scan and aggressiveness has been previously reported also in two large retrospective surgical series of pNET [26, 27]. In our study, this feature was significant in the multivariate analysis in both pNETs and the other-than-pNET group, which indicates that this finding could reflect the infiltrative behavior of the nodule independently of its nature.

Another ultrasound feature previously identified to be associated with NET aggressiveness is the heterogeneous texture, which can be related to the presence of small calcifications, heterogeneous structure, or hemorrhagic/necrotic phenomenon. The latter may determine small cystic changes into the tumor that may appear inhomogeneous at basic EUS examination and/or after contrast injection [11, 28]. In 2009, a retrospective EUS

▶ **Table 4** Factors associated with endoscopic ultrasound tissue acquisition accuracy.

variable (n)	n (%)	p value
size		0.111
▪ ≤ 20 mm (112)	100 (89.3 %)	
▪ > 20 mm (49)	48 (97.9 %)	
site		0.055
▪ head/uncinate (50)	46 (92 %)	
▪ neck/body (55)	54 (98.2 %)	
▪ tail (56)	48 (85.7 %)	
needle type		**0.021**
▪ fork-tip (63)	61 (96.8 %)	
▪ side-fenestrated reverse bevel (58)	54 (93.1 %)	
▪ side-fenestrated anterograde bevel (24)	22 (91.7 %)	
▪ standard (15)	11 (73.3 %)	
number of passes		0.378
▪ ≤ 3 (72)	65 (90.3 %)	
▪ > 3 (88)	83 (94.3 %)	
vascular pattern		1
▪ isovascular (51)	47 (92.2 %)	
▪ hypervascular (110)	101 (91.8 %)	

study [11] found heterogeneous texture to be an independent factor associated with the aggressiveness of pNET. More recently, Palazzo et al. [28] confirmed this finding in another retrospective study including all histologically proven pNETs with available CH-EUS videos at the beginning of the diagnostic workup. The results of the present study seem to be in contrast with previous reports because we found heterogeneous texture not to be an independent factor for disease malignancy/aggressiveness in multivariate analysis. Previous studies, however, were retrospective and included only pancreatic NETs, whereas our study was prospective and evaluated all types of vascularized lesions. Moreover, our definition of aggressiveness based on histological findings and tumor behavior during follow-up was different from the study by Ishigawa [11], where the 2004 WHO classification was used, or from the one by Palazzo [28], in which the tumor was defined as aggressive if there was evidence of metastatic disease or when Ki67 > 20 %. Finally, no follow-up for "non-aggressive" tumors was available in any of these studies, thus making their conclusions based on baseline determination only.

Another warning sign we evaluated was the upstream dilation of the MPD. A recent study by Nanno et al. found MPD dilation to be an independent factor associated with pNET aggressiveness (nodal involvement and recurrence after surgery) [29]. In contrast, despite being significant in the univariate analysis, in our study, the MPD dilation did not remain an independent factor in the multivariate analysis. However, the study by Nanno et al. was a retrospective surgical series of pancreatic NETs, which was most likely burdened by population selection biases. Moreover, as previously demonstrated [30], MPD dilation strictly depends on lesion size (larger lesions are associated with MPD dilation) and location (lesion in the tail may not determine MPD dilation). In the study by Nanno et al. [29], the cut-off utilized for lesion size was 15 mm and not 20 mm as in our study, and the lesions located in the body and tail were evaluated in the same group. Finally, we evaluated only vascularized lesions, excluding those with hypovascular patterns. These differences could explain the contrasting findings with the previous study in multivariate analysis.

The present study confirmed the heterogeneity of diagnoses among non-hypovascular SPLs. Our findings are in agreement with the previous literature. In a recent study Dietrich et al. included 219 small (< 15 mm) SPLs evaluated by CH-EUS or CH ultrasound. Among them, 137 were iso- or hypervascular and were benign in 77 % of cases and malignant in 23 % with great etiological variability (5 PDACs, 91 pNETs, 13 metastases, etc.) [31]. The assumption that a vascularized SPL can be presumptively considered to be a pNET should be abandoned. Indeed, the rate of benign lesions resected for suspicion of pNET remains high [23]. ^{68}Ga- PET, commonly used during the diagnostic workup of vascularized SPLs, also cannot accurately guide the decision-making process: lesions with varying levels of aggressiveness requiring different management can show either positive (e. g., metastasis from clear cell renal cancer [32] or intrapancreatic spleen [33]) or negative (e. g., schwannomas [10] or SCA [9]) uptake. Moreover, in our study, the commonly used indirect signs of pNET aggressiveness (i. e., size > 20 mm and upstream dilation of MDP) were not associated with lesion aggressiveness in the subgroup of other-than-pNET patients. Therefore, an accurate preoperative diagnosis of its nature should be attempted in any case by EUS-TA, especially given the potential morbidity of pancreatic surgery. Of note, the vascular behavior of the intrapancreatic spleen on CH-EUS is often different from pNETs, with typical progressive and intense enhancement even during the late phase that reflects that of the major spleen (▶ **Fig. 5**). Overall, in this setting of patients, we found EUS-TA reached a definitive diagnosis with an overall accuracy of approximately 92 % with an acceptable rate of AEs (approximately 4 %). Regarding EUS-TA, despite the observational nature of this study, our findings suggest that the use of a core biopsy needle is associated with a better outcome than EUS-FNA. In particular, fork-tip needles had the highest accuracy (approximately 97 %), followed by side-fenestrated needles (accuracy approximately 93 %). However, randomized studies are needed to confirm this finding.

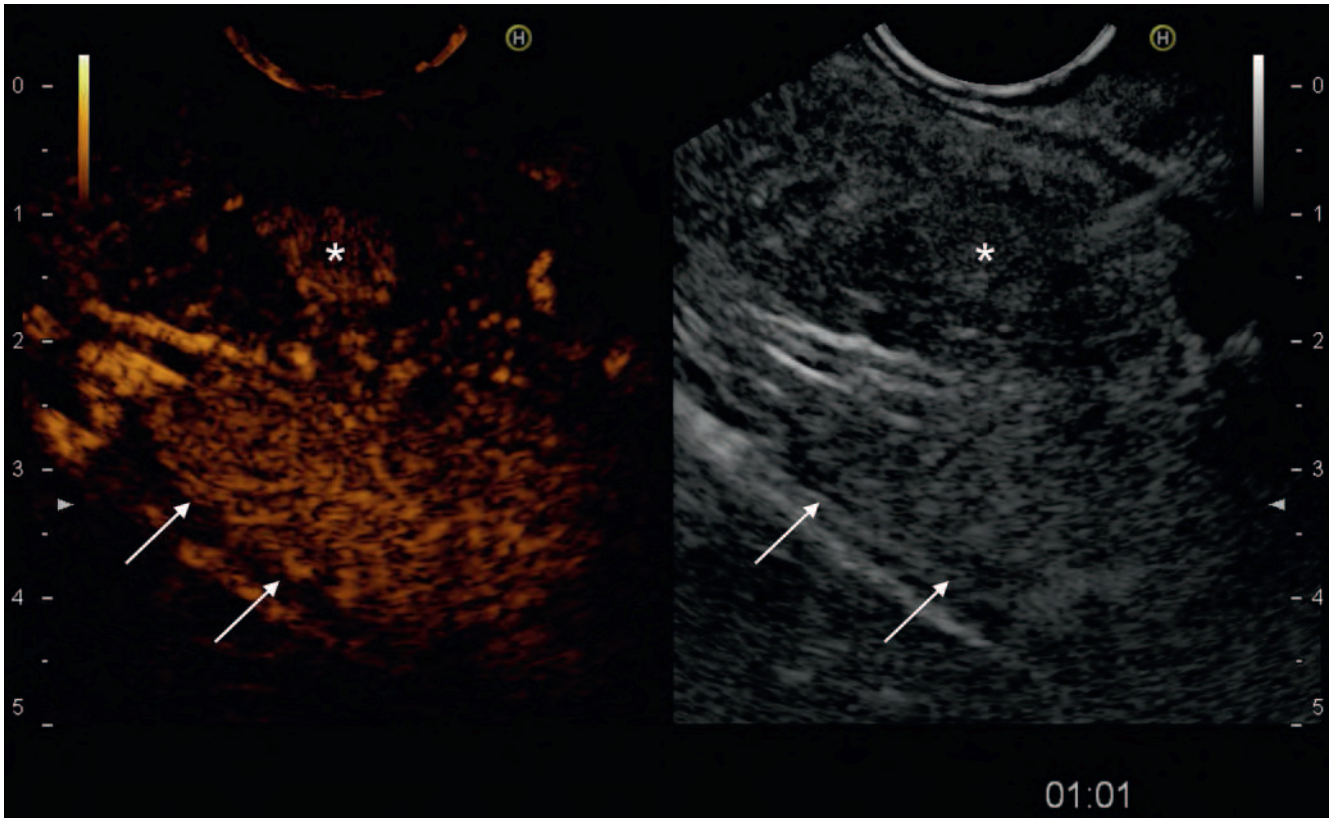
Despite the high accuracy of EUS-TA, some lesions (approximately 5 % in the present study) could have remained undefined because of inadequate samples. This finding highlights the importance of identifying the "warning" features on EUS (e. g., irregular margins) to guide the decision-making process in cases of inconclusive EUS-TA. For example, in our study, undefined lesions were small (mean size 10 mm), with regular borders, without upstream dilation of the MPD, and with homogeneous texture in all cases. A follow-up strategy was decided, and no changes were observed during a median follow-up of approximately 2 years.

We are aware that this study has some limitations. First, the interpretation of lesion features (e. g., lesion margin and echotexture) could be different among endoscopists in non-univocal

▶ **Table 5** Concordance rate of grading (Ki-67 index values) between EUS-TA specimens and surgical pathology in 30 resected pNETs.

		surgical pathology specimens			
		G1	**G2**	**G3**	**total**
EUS-TA specimens	**G1**	**17**	5	0	22
	G2	1	**6**	0	7
	G3	0	1	**0**	1
	total	18	12	0	**30**

pNETs, pancreatic neuroendocrine tumors; EUS-TA, endoscopic ultrasound tissue acquisition; G, grade (according to the 2017 WHO classification).



▶ **Fig. 5** Typical appearance of intrapancreatic spleen on contrast-enhanced endoscopic ultrasound (CH-EUS). A round, well-defined nodule is observed within the pancreatic tail (asterisk). On CH-EUS, one minute after the administration of Sonovue™, an intense hypervascular pattern similar to that of the spleen (arrows) is still visible.

cases. In our study, EUS procedures were performed independently by two endosonographers. Although we did not perform an interobserver agreement evaluation, we investigated the association between EUS features and lesion malignancy/aggressiveness by stratifying our population based on the performing endoscopist (LB or SFC), without any significant difference between them. Moreover, a very good interobserver agreement for lesion borders (Kappa = 0.84) and echotexture (Kappa = 0.82) on EUS was reported in previous studies [25, 28]. Second, the assessment of tumor aggressiveness could be weak, especially considering that only 35 % of our patients underwent surgery. However, unresected patients were followed-up by imaging for a minimum of 1 year (median follow-up of 20 months) to detect any changes or disease progression (lymph node appearance or tumor growth). Moreover, almost all patients (90/92, 97.8 %) with a diagnosis of NET underwent ^{68}Ga-PET at the beginning of diagnostic workup to exclude lymph nodes or distant metastases. Third, endosonographers were not blinded to the findings on imaging studies performed before EUS. Fourth, this was a single-center study performed in a tertiary referral center, and our results could not be confirmed in a community hospital setting.

In conclusion, our study demonstrated that irregular lesion margins are an independent factor useful in predicting malignancy/aggressiveness of iso- and hypervascular SPLs. Size > 20 mm should be considered a warning feature only when a diagnosis of NET is confirmed. EUS-TA is safe and highly accurate in this setting and should be largely adopted for the differential diagnosis of these lesions.

Conflict of Interest

The authors declare that they have no conflict of interest.

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