Pancreas: Original Paper

Digestive Diseases

Dig Dis 2019;37:325–333
DOI: 10.1159/000499172

Received: February 7, 2019
Accepted: February 11, 2019
Published online: March 21, 2019

Pancreatic Neuroendocrine Tumours: The Role of Endoscopic Ultrasound Biopsy in Diagnosis and Grading Based on the WHO 2017 Classification

Milena Di Leo[a, b] Laura Poliani[a] Daoud Rahal[c] Francesco Auriemma[a] Andrea Anderloni[a] Cristina Ridolfi[d] Paola Spaggiari[c] Giovanni Capretti[d] Luca Di Tommaso[b, c] Paoletta Preatoni[e] Alessandro Zerbi[b, d] Carlo Carnaghi[f] Andrea Lania[b, g] Alberto Malesci[b, e] Alessandro Repici[a, b] Silvia Carrara[a]

[a]Humanitas Clinical and Research Center, IRCCS, Digestive Endoscopy Unit, Division of Gastroenterology, Milan, Italy; [b]Humanitas University, Department of Biomedical Sciences, Milan, Italy; [c]Department of Pathology, Humanitas Clinical and Research Center, IRCCS, Milan, Italy; [d]Humanitas Clinical and Research Center, IRCCS, Pancreatic Surgery Unit, Milan, Italy; [e]Humanitas Clinical and Research Center, IRCCS, Division of Gastroenterology, Milan, Italy; [f]Humanitas Clinical and Research Center, IRCCS, Division of Oncology, Milan, Italy; [g]Humanitas Clinical and Research Center, IRCCS, Division of Endocrinology, Milan, Italy

Keywords
Pancreatic neuroendocrine tumor · Endoscopic ultrasound · Biopsy · Grading · Neuroendocrine tumour

Abstract
Background: One of the controversial issues in the diagnosis of pancreatic neuroendocrine tumours (pNETs) is the accurate prediction of their clinical behaviour. ***Objectives:*** The aim of the study was to evaluate the role of endoscopic ultrasound (EUS) biopsy in the diagnosis and grading of pNETs in a certified ENETS Center. ***Methods:*** A prospectively maintained database of EUS biopsy procedures was retrospectively reviewed to identify all consecutive patients referred to a certified ENETS Center with a suspicion of pNET between June 2014 and April 2017. The cytological and/or histological specimens were stained and the Ki-67 labeling index was evaluated. In patients undergoing surgery, the grade obtained with EUS-guided biopsy was compared with the final histological grade. The grade was evaluated according to the 2017 WHO classifications and grading. ***Results:*** The study population included 59 patients. EUS biopsy material reached an adequacy of 98.3% and was adequate for Ki-67 evaluation in 84.7% of cases. Twenty-nine patients (49.2%) underwent surgery. Of these, 25 patients had Ki-67 evaluated on EUS biopsy: the agreement between EUS biopsy grading and surgical specimen grading was 84%. ***Conclusion:*** EUS biopsy is an accurate method for the diagnosis and grading of pNETs based on the WHO 2017 Ki-67 labelling scheme.

KARGER

E-Mail karger@karger.com
www.karger.com/ddi

Milena Di Leo, MD
Digestive Endoscopy Unit, Division of Gastroenterology
Humanitas Research Hospital
Via Manzoni, 56, Rozzano, IT–20089 Milan (Italy)
E-Mail milena.di_leo@hunimed.eu

Introduction

Pancreatic neuroendocrine tumours (pNETs) are rare tumours that arise from neuroendocrine cells. The majority of pNETs are non-functioning and are found in asymptomatic patients, while most functioning pNETs are insulinomas and gastrinomas [1]. About 10–30% of patients with pNETs have multiple endocrine neoplasia type 1 syndrome [2].

One of the most controversial issues in the diagnosis of pNETs is the accurate prediction of their clinical behaviour, since they may present different morphologic features that do not necessarily reflect the degree of aggressive behaviour [3–6].

According to the ENETS and WHO 2017classifications, pNET grading should be expressed using the mitotic index and the Ki-67 proliferation index [7, 8]. The new WHO classification differs from the previous WHO 2010 classification in regard to the definition of the G1 cut off and includes a new tumour category of pNETG3 [7]. Although pNETs are often detected incidentally by cross-sectional imaging, endoscopic ultrasound (EUS) plays an important role in the detection and in confirmation of the diagnosis, and EUS biopsy is a valid and safe tool for reaching the final pathological diagnosis. In addition to the cytological or histological diagnosis of pNETs, the material obtained under EUS guidance can be a reliable source for providing grading based on the WHO Ki-67 labelling scheme [9], with high reproducibility and very good inter-observer agreement [10].

The aim of this study was to evaluate the role of EUS biopsy in the diagnosis and grading of pNETs in a certified ENETS Center.

Methods

Patients

A retrospective review of a prospectively maintained database of EUS procedures was carried out to identify all patients referred to EUS for suspected pNET. A computerized system was used to extrapolate the list of patients referred between June 2014 and April 2017.

The institutional review board of the hospital approved the observational study (NCT02855151) and the protocol was conducted according to the Declaration of Helsinki.

Patients with suspected pNET were enrolled based on clinical history (symptoms such as hypoglycaemia, diarrhoea), genetic syndrome (multiple endocrine neoplasia) and/or typical imaging findings (hypervascularized lesions on CT and/or MRI with contrast medium and/or lesions with hypercaptationat 68Ga-PET scan and/or somatostatin receptor scintigraphy). Patients undergoing EUS follow-up of pNETs without biopsy performed (histological diagnosis already obtained in the past), as well as patients with final pathological diagnosis different from pNET were excluded.

EUS-Guided Biopsy

The Olympus GF-UCT180 series linear array echoendoscope (Olympus Europa SE & CO. KG, Hamburg, Germany) in combination with the new EU-ME2 echoprocessor (Olympus SE & CO. KG, Hamburg, Germany) were used to stage the lesions and to guide the pancreatic biopsies.

All the EUS were performed by 2 experienced endosonographers (S.C. and A.A.) in a third level Endoscopic Center (>1,000 EUS/year).

The EUS-guided biopsy was performed with 22-gauge or 25-gauge needles (respectively, Expect™ Slimline Needle, Boston Scientific, MA, United States; SharkCore™ and Beacon BNX™ Needle, Medtronic, Newton, MA, USA), chosen at the discretion of the endosonographer. The SharkCore™ needle was available after June 2015 in our hospital.

The biopsies were performed combining the fanning technique [11] and the slow pull technique [12]. Since pNETs are usually hypervascularized, in order to reduce the contamination of the specimen with blood, no suction was applied to the needle.

At each pass, the material was expressed onto a smear slide for macroscopic on-site quality evaluation. If drop-like material was obtained, it was smeared between 2 glass slides, fixed with ethanol, and stained with a Hematoxylin and Eosin (H&E) for cytological analysis, and a rapid on-site evaluation (ROSE) was performed to determine adequacy. If a micro-fragment or "worm-like" material, tan-pink or red, thick and granular, was observed on the slide at gross visual assessment, all the material was placed in a container of 10% neutral buffered formalin fixative to create a tissue block for the final histological examination. When bloody material and clots were obtained, they were also collected in formalin.

When both a liquid part and a solid micro-fragment were obtained, both cytological and histological evaluations were performed (Fig. 1).

Pathological Evaluation and Analysis of the Ki-67 Index

After adequacy was obtained, the specimens were stained with an H&E for cytological analysis the others were stained with H&E. Immunohistochemical studies were performed in all cases that had adequate histological specimens for staining. The cases were graded according to the WHO 2017 classification [7].

Two experienced pathologists performed cytological and histological evaluations and grading.

Manual counting of camera-captured images was used to determine the Ki-67 index.

When both cytology and histology samples were obtained from the EUS biopsy, the Ki-67 index was evaluated only on the histological specimen.

If the patient underwent surgery, Ki-67 and mitotic count were evaluated in the histological specimen as dictated by the WHO 2017 classification. In this study population, the EUS biopsy results were compared with the final histological diagnosis, and agreement between the EUS biopsy grade and the surgical specimen grade was evaluated using per cent agreement and k-statistics.

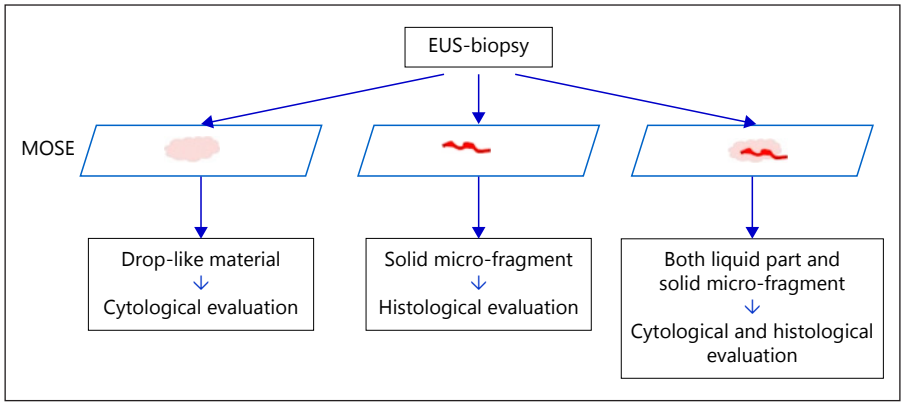


Fig. 1. How the specimens from EUS biopsies were processed. EUS, endoscopic ultrasound; MOSE, macroscopic on-site quality evaluation.

Table 1. Features of the study population (n = 59)

Features	
Gender, male, *n* (%)	34 (57.6)
Age, years, mean (SD)	57.6 (15.6)
Presence of symptoms, *n* (%)	
No	42 (71.2)
Yes, not specific	9 (15.2)
Yes, genetic syndrome	5 (8.5)
Yes, endocrinological symptoms	3 (5.1)
Diameter, mm, mean (SD)	21 (14.9)
Lesion location, *n* (%)	
Head	9 (15.3)
Uncinate process	8 (13.6)
Neck	5 (8.5)
Body	22 (37.3)
Tail	14 (23.7)
Multiple locations	1 (1.6)

Statistical Analysis

Data was entered into an Excel spreadsheet (Microsoft Excel 2010; Microsoft Corporation, Redmond, Washington, USA). Results for continuous variables were summarized using mean ± SD, and categorical variables using proportions. Chi-square test was used to compare categorical outcomes. Statistical analyses were performed using SPSS version 17 (SPSS Inc., Chicago, IL, USA). Statistical significance was determined at two-sided p values <0.05.

Results

Clinical Characteristics

Sixty-four patients underwent EUS for suspected pNET. In 4 patients, the suspicion of pNET was not confirmed after EUS biopsy: 1 patient had a solid pseudopapillary tumour, 1 had a solid variant of serous cystadenoma and 2 had metastasis from renal cancer. These 4 patients were excluded from the study. In 1 patient, the histological diagnosis after EUS biopsy (pNET with a low proliferative index) was not confirmed after surgery (serous cystadenoma).

The study population included 59 patients (Table 1): 25 females and 34 males, mean age 57.6 years (SD 15.6 years).

Forty-two patients (71.2%) had an incidental finding of pancreatic mass, 9 (15.2%) had symptoms such as pain, diarrhoea, weight loss, pancreatitis or jaundice, and 5 (8.5%) had genetic syndrome. Three patients (5.1%) had endocrinological symptoms such as hypoglycaemia or Cushing syndrome (CT scan and PET scan negative before EUS).

The mean size of the lesions was 21 mm (SD 14.9) and the location was the body and tail in 36 patients (61%), head in 9 (15.3%), uncinate process in 8 (13.6%) and neck in 5 (8.5%). One patient had multiple pancreatic tumours.

On EUS imaging, the solid pNETs were mostly identified as homogeneously hypoechoic, hypervascularized lesions, with well-defined margins. Less frequently, they showed hyperechoic features and/or irregular margins, and inhomogeneous echostructure. In 15 cases (25.4%), the pNETs presented cystic spaces or had the appearance of a unilocular cystic lesion with thickened hypervascularized wall.

EUS Biopsy Results

A 25G needle was used in the majority of patients (47 cases, 79.7%). A22G needle was used in the remaining 12 patients (20.3%). The mean number of needle passes was 1.9 (SD 0.8; range 1–4; Table 2).

In 17 out of 59 cases (28.8%), the tumour size was ≤10 mm. In 16 of these small pNETs, the EUS biopsy material was adequate for final diagnosis (94.1%) and Ki-67 was evaluable on the cytological or histological specimen in 14 cases (82.4%).

Table 2. Results of EUS biopsy

N	Needle passes	G	ROSE adequacy	Cytology	Histology	Cytologic Ki-67, %	Histologic Ki-67, %	Cytologic Grade	Histologic Grade	Final adequacy	Final Ki-67	Final grade
1	1	22	NP	Not adequate	NET	NA	<1	NA	1	Y	Y	1
2	1	25	Y	NET	NP	<5	NA	2	NA	Y	Y	2
3	2	25	Y	NET	NP	NA	NA	NA	NA	Y	NA	NA
4	2	25	NP	NP	NET	NA	<1	NA	1	Y	Y	1
5	4	25	Y	NET	NET	NA	<1	NA	1	Y	Y	1
6	2	25	Y	NET	NP	10	NA	2	NA	Y	Y	1
7	1	22	Y	NET	NET	NA	<1	NA	1	Y	Y	1
8	2	25	Y	NET	NET	NA	<1	NA	1	Y	Y	1
9	2	25	Y	NET	NET	NA	2	NA	1	Y	Y	1
10	2	22	Y	NET	NET	NA	30	NA	3	Y	Y	3
11	2	25	Y	NET	NET	NA	1	NA	1	Y	Y	1
12	2	25	Y	NET	NET	NA	1	NA	1	Y	Y	1
13	3	25	Y	NET	NP	NA	NA	NA	NA	Y	NA	NA
14	1	25	Y	NET	NET	NA	2	NA	1	Y	Y	1
15	1	25	Y	NET	NP	NA	NA	NA	NA	Y	NA	NA
16	1	25	Y	NET	NP	<1	NA	1	NA	Y	Y	1
17	2	22	Y	NET	NET	NA	4	NA	2	Y	Y	2
18	2	25	Y	NET	NET	NA	<1	NA	1	Y	Y	1
19	1	25	Y	NET	NP	<1	NA	1	NA	Y	Y	1
20	2	25	Y	NET	NET	NA	8	NA	2	Y	Y	2
21	2	25S	Y	NET	NET	<1	NA	1	NA	Y	Y	1
22	1	25	Y	NET	NET	NA	<1	NA	1	Y	Y	1
23	2	25	Y	NET	NET	<1	NA	1	NA	Y	Y	1
24	1	25	Y	NET	NP	5	NA	2	NA	Y	Y	2
25	1	22	Y	NET	NET	NA	<1	NA	1	Y	Y	1
26	2	22	Y	NET	NET	NA	2	NA	1	Y	Y	1
27	1	25	Y	NET	NET	NA	2	NA	1	Y	Y	1
28	3	25S	NP	NP	NET	NA	2	NA	1	Y	Y	1
29	1	22	Y	NET	NP	NA	NA	NA	NA	Y	NA	NA
30	1	25	Y	NET	NET	NA	2	NA	1	Y	Y	1
31	2	25	Y	NET	NET	NA	5	NA	2	Y	Y	2
32	1	25S	Y	NET	NET	NA	1	NA	1	Y	Y	1
33	1	25S	NP	Not adequate	NET	NA	2	NA	1	Y	Y	1
34	1	25S	Y	NET	NET	NA	<1	NA	1	Y	Y	1
35	2	22	Y	NET	NET	NA	<1	NA	1	Y	Y	1
36	3	25S	Y	NET	NET	2	NA	1	NA	Y	Y	1
37	2	25	NP	NET	NP	NA	NA	NA	NA	Y	NA	NA
38	2	25S	Y	NET	NP	NA	NP	NA	NP	Y	NA	NA
39	1	25S	Y	NET	NP	5	NA	2	NP	Y	Y	2
40	2	25	Y	NET	NP	NA	NA	NA	NA	Y	NA	NA
41	2	25S	Y	NET	NET	NA	<1	NA	1	Y	Y	1
42	3	25	NP	NET	NET	NA	<1	NA	1	Y	Y	1
43	3	25S	NP	NP	NET	NA	2	NA	1	Y	Y	1
44	3	25S	NP	NP	NET	NA	4	NA	2	Y	Y	2
45	4	25S	Y	NET	NET	NA	2	NA	1	Y	Y	1
46	1	22	Y	NET	NET	NA	2	NA	1	Y	Y	1
47	3	25S	NP	NP	NET	NA	2	NA	1	Y	Y	1
48	1	25S	NP	NET	NP	<1	NA	1	NA	Y	Y	1
49	3	25S	Y	NET	NET	NA	2	NA	2	Y	Y	2
50	3	22	NP	NET	NET	NA	NA	NA	NA	Y	NA	NA
51	3	25S	Y	NET	NET	NA	2	NA	2	Y	Y	2
52	3	25S	Y	NET	NET	NA	2	NA	1	Y	Y	1
53	1	22	NP	Not adequate	NP	NA	NA	NA	NA	N	NA	NA
54	3	25S	Y	NET	NET	<1	NA	1	NA	N	Y	1
55	2	25S	Y	NET	NET	NA	2	NA	1	Y	Y	1
56	2	25S	Y	NET	NP	<1	NA	1	NA	Y	Y	1
57	1	25S	Y	NET	NP	1	NA	1	NA	Y	Y	1
58	2	25S	Y	NET	NP	2	NA	1	NA	Y	Y	1
59	1	22	Y	NET	NET	NA	80	NA	3	Y	Y	3

NET, neuroendocrine tumor; NA, not applicable; S, SharkCore needle; NP, not performed; Y, YES; EUS, endoscopic ultrasound.

Table 3. Adequacy of pathological specimens according to needle type

Type of needle	*n* (%)	Final adequacy, *n* (%)	*p* value	Final Ki-67, *n* (%)	*p* value
25G, Beacon BNX™ Needle, Medtronic, *n* (%)	25 (40.7)	25 (100)	0.394	20 (80)	0.195
25G, SharkCore, SharkCore, Medtronics, *n* (%)	22 (39.0)	21 (95.5)		21 (100)	
22G, Expect™ Slimline, Boston Scientific, *n* (%)	12 (20.3)	11 (91.7)		9 (75)	

Prior to performing ROSE, after each needle passage, a macroscopic on-site quality evaluation was made as the material was released onto the slide.

In 54 cases (91.5%), drop-like material was stained for cytological evaluation. Fifty-one cases were adequate after staining (92.6%), and final diagnosis of pNET was made.

In 41 patients (69.5%), the specimens were judged by the endosonographer to be representative of micro-fragments and they were collected in formalin. All of these histological specimens were diagnostic (100%). In 30 of 41 histological specimens (73.2%), the biopsy was performed with a 25G needle.

Combining the results of cytology, histology and cytology plus histology, the overall EUS biopsy adequacy was 98.3%. No difference in terms of outcomes was found according to the different types of needles (Table 3).

Mild self-limiting bleeding, defined as hyperechoic leakage of at least 3 mm and less than 10 mm in thickness, was observed in 4 cases of puncture of a solid lesion. Self-limiting bleeding was also observed in a cystic lesion, as well as in the pancreatic duct after EUS biopsy of a solid lesion infiltrating the pancreatic duct wall. None of the patients with mild bleeding had symptoms or required any therapy. No major complications such as acute pancreatitis or bleeding that required blood transfusion or hospitalization occurred.

Ki-67 Labelling Index

Overall, the EUS biopsy material (from both cytology and histology) was adequate for Ki-67 evaluation in 50 of 59 patients (84.7%). When a histological specimen was obtained from EUS biopsy, the Ki-67evaluation was feasible in 36 out of 41 histological samples (87.8%). In 14 (28%) patients, the Ki-67 index was calculated only on the cytological sample (Fig. 2, 3).

Surgical Findings

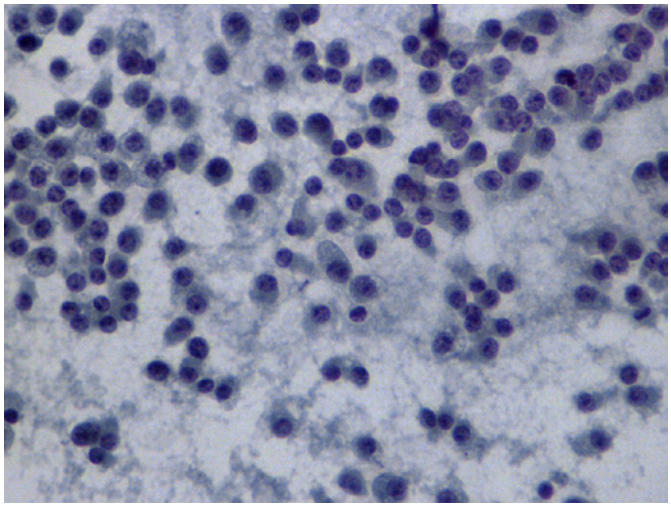
Twenty-nine of 59 patients with pNETs (49.2%) underwent surgery, with a mean time of 3.8 months after EUS (SD 3.9; range 0.2–15 months). Five had chemotherapy; 26 are in follow-up with stable disease. Twenty-five of the operated patients also had Ki-67 calculated on EUS biopsy: in 21 of these patients (84%), there was concordance between EUS biopsy grading and surgical specimen grading.

In 2 cases, an upgrading was observed in the surgical specimen one from G1 to G2 because of a final Ki-67 of 5% compared to 2% in the biopsy, and the other one from G1 with a Ki-67 of 2% to G3 with a Ki-67 of 30%. In the latter case, the surgery was performed 15 months after the biopsy due to evidence of dimensional growth of the lesion during the follow-up. Although the mean time between EUS and surgery was higher in these 2 patients comparing the remaining patients (8.5 months, SD 9.2 and 3.4 months, SD 3.2 respectively), this difference was not considered statistically significant (p = 0.07).

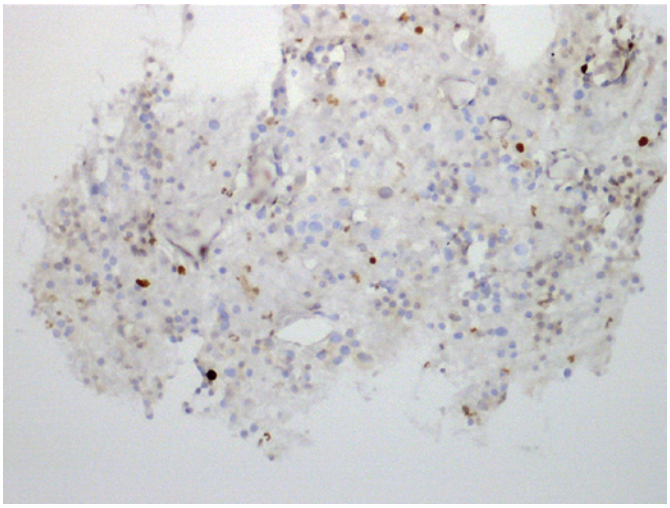
Two patients had a downstaging at the surgical specimens. In one patient with a 10 mm pNET of the tail, the Ki-67 index was 10% on the cytological specimen compared to 2% on the surgical specimen. On review after surgery, this case showed a Ki-67 between 2 and 10%, and the 2 pathologists agreed that the distinction between G1 and G2 was very difficult to express on the basis of the cytological specimen. In another patient, the surgical specimen showed a Ki-67 of 2% (G1) compared to 5% (G2) in the biopsy (Table 4).

Discussion

In this study, we aimed to evaluate the role of EUS biopsy in the diagnosis and grading of pancreatic NETs in a certified ENETS Center.



Color version available online



Color version available online

Fig. 2. Cytological specimen from EUS biopsy of a pNET. Papanicolau 40×.

Fig. 3. Micro-core from EUS biopsy (same case as Fig. 2) showing small neuroendocrine cells, 20×. Ki-67 5% (G2).

Table 4. Concordance between EUS biopsy grading and surgical specimen grading

n	EUS biopsy Ki-67, %	Surgery Ki-67, %	EUS biopsy Grade	Surgery grade	Grading concordance
1	<1	1	1	1	Y
2	<5	7	2	2	Y
6	10	2	2	1	N
8	<1	1	1	1	Y
12	1	1	1	1	Y
16	<1	<1	1	1	Y
17	4	7	2	2	Y
18	<1	1	1	1	Y
19	<1	1	1	1	Y
20	8	14	2	2	Y
23	<1	<2	1	1	Y
25	<1	1	1	1	Y
26	2	3	1	1	Y
33	2	3	1	1	Y
35	<1	1	1	1	Y
36	2	2	1	1	Y
39	5	3	2	1	N
41	<1	1	1	1	Y
42	<1	3	1	1	Y
43	2	1	1	1	Y
47	2	30	1	3	N
49	2	3	1	1	Y
51	2	5	1	2	N
52	2	1	1	1	Y
54	<1	2	1	1	Y

Y, yes; N, no; EUS, endoscopic ultrasound.

The combination of cytology and histology obtained from EUS biopsy reached an adequacy of 98.3% in the diagnosis of pNET. We confirmed EUS-guided biopsy as a reliable, safe and effective technique for obtaining samples from pancreatic masses [13].

Once a pNET is diagnosed, the role of EUS biopsy in grading is very important in order to plan the best therapeutic approach [14, 15]. According to the ENETS and WHO 2017 criteria, the grading for pancreatic NETs should be expressed using the Ki-67 proliferation index [7, 8]. In our study, the overall adequacy of EUS biopsy for Ki-67 evaluation was 84.7%. When both cytology and histology samples were obtained from EUS biopsy, the Ki-67 index was evaluated only on the histological specimen as a choice of the pathologists. An interesting feature of pNETs is the intratumoral heterogeneity of the Ki-67 index, and the sampling variability of Ki-67 could be a potential limitation to the real prediction of the grade of the whole tumour. Moreover, the question has been raised as to whether the Ki-67 index obtained from needle biopsy represents the whole tumour [16–18]. Comparing the Ki-67 index and grading, we obtained a concordance of 84% between surgery and EUS biopsy. Our results are in line with a recent systematic review [19] that showed a concordance of 83% between preoperative and postoperative Ki-67 evaluation.

In our series, we observed one case with an overgrading based on cytology (10% compared to 2% in the resected specimen) and 1 patient with underestimation of G2 pNET, where EUS biopsy showed a Ki-67 of 2% compared to 5% in the resected specimen. In cases of very small nonfunctioning pNETs, in which the pretest probability of a G1 tumour is high, the bioptic finding of higher grade should be weighted together with other clinical and radiological information (size, location, distance from Wirsung, acute pancreatitis), and it should be discussed with the patient in a multidisciplinary team whether to perform surgical resection or not. Although the fanning technique is routinely used to obtain material, the EUS biopsy may not always be representative of the most mitotically active tumour areas, as compared to a histological specimen that represents the whole tumour, on which the pathologist can determine the area of higher nuclear labelling and count the minimum number of neoplastic cells. Since G2 pNETs are a heterogeneous group of tumours, in which Ki-67 ranges between 3 and 20%, some authors have suggested using a 5% cut off for distinguishing between G1 and G2 [20]. Applying the suggested cut off of 5%, our preoperative grading of pNETs was equal to the grading evaluated in the resected material in 95.8% of cases.

The same results were obtained by Hasegawa et al. [21]. The authors demonstrated a concordance of 74% between EUS-FNA and resected specimens when they used the mean Ki-67 index in EUS-FNA, while concordance rose to 77.8% when they used the higher Ki-67 index. Moreover, when they excluded EUS-FNA samples with less than 2,000 tumour cells (26% of EUS-FNA), concordance rose to 90%. On the contrary, Laskiewicz et al. [22] obtained 84.6% concordance in the evaluation of grading between FNA and tumour resection specimens, including 26.9% of FNA samples with less than 1,000 cells.

In 1 patient, the histological diagnosis after EUS biopsy (pNET with a low proliferative index) was not confirmed after surgery and the final diagnosis was a solid variant of serous cystadenoma. The possibility of false positive in EUS biopsy is well known, and a previous retrospective study [23] described a false positive rate of 1.1% in a cohort of patients who underwent EUS-guided biopsy for pancreatic masses.

The role of EUS stands out in the evaluation of small tumours (<2 cm). Thanks to the improvement of high-resolution imaging diagnostic capabilities, pancreatic masses smaller than 2 cm are often detected during routine cross-imaging studies. Several studies have shown that parenchymal-sparing surgery is effective and safe for patients with G1 and G2 pNETs, and the key to the therapeutic decision is an accurate pre-operative evaluation (location, relationships with nearby structures, diagnosis and grading) [16, 17]. This therapeutic approach is in accordance with ENET guidelines that encourage parenchymal-sparing surgery, specifying how EUS is fundamental to therapeutic planning [18]. In this setting, EUS can provide detailed information on lesion relationships with surrounding structures, such as the main pancreatic duct and vessels, becoming an instrument of surgical enucleation [15]. EUS biopsy is a powerful tool for obtaining samples from small lesions, as reported in the results of a study carried out by Jhala et al. [24], where a mean of 2.5 passes was needed to obtain adequate samples from lesions ≤25 mm, whereas a mean of 4.5 passes was needed to obtain adequate samples from lesions >25 mm. The rationale is that small masses have fewer necrotic areas, and fewer passes are necessary to obtain adequate material for final diagnosis [25]. In our study, if we consider the patients with very small lesions (≤10 mm), the adequacy of the EUS biopsy was 94.1%, and no more than 2 passes were needed to reach adequacy.

We also acknowledge some limitations. First, this is a retrospective case series and we recognize that prospective trials are the methodological reference standard for investigating the diagnostic yield of EUS-guided biopsy in the management of pNETs. Second, different needle types and sizes were used. As pointed out by Larghi et al. [26], the use of a 19G needle in patients with nonfunctioning pNETs is safe, feasible and accurate. With this large-gauge needle, the adequacy of the specimens for histological examination was obtained in 93.3% of cases, and Ki-67 determination was made in 92.9% of cases with histological specimen (86.6% overall). Our results did not show statistical differences in terms of final adequacy, Ki-67 evaluation and correlation with surgery according to type of needle. This is in contrast to a recent pilot study [27] comparing SharkCore needle with standard Echotip needle in patients with known or suspected PNETs: the study concluded that the SharkCore® needle shows better results in obtaining tissue suitable for ancillary tests with fewer passes. Probably, the small sample size and the non-prospective randomized design hampered the value of our data, so further studies are needed to clarify the role of new EUS-guided biopsy needle in the evaluation of NET. Literature data show that most pNETs are found in young and asymptomatic subjects, and the variability of the biological behaviour of these cancers suggests the need for accurate diagnosis and grading even in the case of small lesion size. The main point of discussion regarding pNET management is small tumours, especially if they are asymptomatic and if found in young people. Current guidelines [8] stated that appropriate management of pancreatic NETs <2 cm is still debated and ENETS proposed a "wait and see" approach for small non-functioning pNETs. Results from large studies such as Asymptomatic Small Pancreatic Endocrine Neoplasms will help to choose the best strategy (NCT03084770). The Ki-67 index evaluation could lead to personalized management of these patients in a multidisciplinary approach. As our results showed, the EUS biopsy gave a very high concordance with the final histological grade, but undergrading and overgrading were both described, and this should be taken into consideration in a multidisciplinary team when surgery is proposed for a patient. In cases of EUS undergrading, the timing intercurred between EUS and surgery could play an important role, although other data are needed to clarify this point.

In conclusion, according to the results of the present study, EUS biopsy may be considered an accurate tool for the diagnosis and grading of pNETs based on the WHO Ki-67 labelling scheme. It should be proposed as a complementary test to aid in stratifying patients according to tumour grade and determining the best therapeutic approach or follow-up.

Disclosure Statement

The authors declare that they have no conflicts of interest to disclose.

Authors Contribution

M.D.L. and S.C.: designed the research. S.C. and A.A.: performed the procedures. M.D.L., L.P., D.R., A.A., P.S., L.D.T., F.A., and S.C.: performed the research and analyzed the data. L.P., C.R., G.L.C., and P.P.: followed up the patients. M.D.L., L.P., and S.C.: wrote the paper. D.R., P.S., A.L., C.C., A.Z., A.M., and A.R.: critically revised the manuscript for important intellectual content. All authors approved of the paper.

Funding Source

No funding was received for this study.

References

1 Vagefi PA, Razo O, Deshpande V, McGrath DJ, Lauwers GY, Thayer SP, et al. Evolving patterns in the detection and outcomes of pancreatic neuroendocrine neoplasms: the Massachusetts General Hospital experience from 1977 to 2005. Arch Surg. 2007 Apr;142(4):347–54.

2 Alexakis N, Neoptolemos JP. Pancreatic neuroendocrine tumours. Best Pract Res Clin Gastroenterol. 2008;22(1):183–205.

3 Adsay V. Ki67 labeling index in neuroendocrine tumors of the gastrointestinal and pancreatobiliary tract: to count or not to count is not the question, but rather how to count. Am J Surg Pathol. 2012 Dec;36(12):1743–6.

4 Ekeblad S, Skogseid B, Dunder K, Oberg K, Eriksson B. Prognostic factors and survival in 324 patients with pancreatic endocrine tumor treated at a single institution. Clin Cancer Res. 2008 Dec;14(23):7798–803.

5 La Rosa S, Klersy C, Uccella S, Dainese L, Albarello L, Sonzogni A, et al. Improved histologic and clinicopathologic criteria for prognostic evaluation of pancreatic endocrine tumors. Hum Pathol. 2009 Jan;40(1):30–40.

6 Pape UF, Berndt U, Müller-Nordhorn J, Böhmig M, Roll S, Koch M, et al. Prognostic factors of long-term outcome in gastroenteropancreatic neuroendocrine tumours. Endocr Relat Cancer. 2008 Dec;15(4):1083–97.

7 Bosman FT, Hruban RH, Theise ND. WHO classification of tumours of the digestive system. 4th ed. Lyon: International Agency for Research on Cancer; 2010.

8 Falconi M, Eriksson B, Kaltsas G, Bartsch DK, Capdevila J, Caplin M, et al.; Vienna Consensus Conference participants. ENETS Consensus Guidelines Update for the Management of Patients with Functional Pancreatic Neuroendocrine Tumors and Non-Functional Pancreatic Neuroendocrine Tumors. Neuroendocrinology. 2016;103(2):153–71.
9 Farrell JM, Pang JC, Kim GE, Tabatabai ZL. Pancreatic neuroendocrine tumors: accurate grading with Ki-67 index on fine-needle aspiration specimens using the WHO 2010/ENETS criteria. Cancer Cytopathol. 2014 Oct;122(10):770–8.
10 Weynand B, Borbath I, Bernard V, Sempoux C, Gigot JF, Hubert C, et al. Pancreatic neuroendocrine tumour grading on endoscopic ultrasound-guided fine needle aspiration: high reproducibility and inter-observer agreement of the Ki-67 labelling index. Cytopathology. 2014 Dec;25(6):389–95.
11 Bang JY, Magee SH, Ramesh J, Trevino JM, Varadarajulu S. Randomized trial comparing fanning with standard technique for endoscopic ultrasound-guided fine-needle aspiration of solid pancreatic mass lesions. Endoscopy. 2013 Jun;45(6):445–50.
12 Nakai Y, Isayama H, Chang KJ, Yamamoto N, Hamada T, Uchino R, et al. Slow pull versus suction in endoscopic ultrasound-guided fine-needle aspiration of pancreatic solid masses. Dig Dis Sci. 2014 Jul;59(7):1578–85.
13 Hewitt MJ, McPhail MJ, Possamai L, Dhar A, Vlavianos P, Monahan KJ. EUS-guided FNA for diagnosis of solid pancreatic neoplasms: a meta-analysis. Gastrointest Endosc. 2012 Feb;75(2):319–31.
14 Hosoda W, Takagi T, Mizuno N, Shimizu Y, Sano T, Yamao K, et al. Diagnostic approach to pancreatic tumors with the specimens of endoscopic ultrasound-guided fine needle aspiration. Pathol Int. 2010 May;60(5):358–64.
15 Artale S, Giannetta L, Cerea G, Maggioni D, Pedrazzoli P, Schiavetto I, et al. Treatment of metastatic neuroendocrine carcinomas based on WHO classification. Anticancer Res. 2005 Nov-Dec;25 6C:4463–9.
16 Chaudhry A, Oberg K, Wilander E. A study of biological behavior based on the expression of a proliferating antigen in neuroendocrine tumors of the digestive system. Tumour Biol. 1992;13(1-2):27–35.
17 Couvelard A, Deschamps L, Ravaud P, Baron G, Sauvanet A, Hentic O, et al. Heterogeneity of tumor prognostic markers: a reproducibility study applied to liver metastases of pancreatic endocrine tumors. Mod Pathol. 2009 Feb;22(2):273–81.
18 Yang Z, Tang LH, Klimstra DS. Effect of tumor heterogeneity on the assessment of Ki67 labeling index in well-differentiated neuroendocrine tumors metastatic to the liver: implications for prognostic stratification. Am J Surg Pathol. 2011 Jun;35(6):853–60.
19 Pezzilli R, Partelli S, Cannizzaro R, Pagano N, Crippa S, Pagnanelli M, et al. Ki-67 prognostic and therapeutic decision driven marker for pancreatic neuroendocrine neoplasms (PNENs): A systematic review. Adv Med Sci. 2016 Mar;61(1):147–53.
20 Scarpa A, Mantovani W, Capelli P, Beghelli S, Boninsegna L, Bettini R, et al. Pancreatic endocrine tumors: improved TNM staging and histopathological grading permit a clinically efficient prognostic stratification of patients. Mod Pathol. 2010 Jun;23(6):824–33.
21 Hasegawa T, Yamao K, Hijioka S, Bhatia V, Mizuno N, Hara K, et al. Evaluation of Ki-67 index in EUS-FNA specimens for the assessment of malignancy risk in pancreatic neuroendocrine tumors. Endoscopy. 2014 Jan;46(1):32–8.
22 Laskiewicz L, Jamshed S, Gong Y, Ainechi S, LaFemina J, Wang X. The diagnostic value of FNA biopsy in grading pancreatic neuroendocrine tumors. Cancer Cytopathol. 2018 Mar;126(3):170–8.
23 Siddiqui AA, Kowalski TE, Shahid H, O'Donnell S, Tolin J, Loren DE, et al. False-positive EUS-guided FNA cytology for solid pancreatic lesions. Gastrointest Endosc. 2011 Sep;74(3):535–40.
24 Jhala NC, Jhala D, Eltoum I, Vickers SM, Wilcox CM, Chhieng DC, et al. Endoscopic ultrasound-guided fine-needle aspiration biopsy: a powerful tool to obtain samples from small lesions. Cancer. 2004 Aug;102(4):239–46.
25 Jhala NC, Jhala D, Eloubeidi MA, Chhieng DC, Crowe DR, Roberson J, et al. Endoscopic ultrasound-guided fine-needle aspiration biopsy of the adrenal glands: analysis of 24 patients. Cancer. 2004 Oct;102(5):308–14.
26 Larghi A, Capurso G, Carnuccio A, Ricci R, Alfieri S, Galasso D, et al. Ki-67 grading of nonfunctioning pancreatic neuroendocrine tumors on histologic samples obtained by EUS-guided fine-needle tissue acquisition: a prospective study. Gastrointest Endosc. 2012 Sep;76(3):570–7.
27 Witt BL, Factor RE, Chadwick BE, Caron J, Siddiqui AA, Adler DG. Evaluation of the SharkCore® needle for EUS-guided core biopsy of pancreatic neuroendocrine tumors. Endosc Ultrasound. 2018 Sep-Oct;7(5):323–8.