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Endoscopic ultrasound-guided fine-needle aspiration for the diagnosis and grading of pancreatic neuroendocrine tumors: a retrospective analysis of 110 cases

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ABSTRACT

Background Data on the reliability of the Ki-67 index and grading calculations from endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) of pancreatic neuroendocrine tumors (PanNETs) are controversial. We aimed to assess the accuracy of these data compared with histology.

Methods Cytological analysis from EUS-FNA in patients with suspected PanNETs (n = 110) were compared with resection samples at a single institution. A minimum of 2000 cells were considered to be adequate for grading. Correlation and agreement between cytology and histology in grading and Ki-67 values, respectively, were investigated. Secondary outcomes included the diagnostic performance of EUS-FNA.

Results EUS-FNA samples were adequate for PanNET diagnosis and PanNET grading in 98/110 (89.1 %) and 77/110 (70.0 %) patients, respectively; thus, 77 samples were adequate for comparing cytology vs. histology. There were 67 (62.0 %), 40 (36.4 %), and 1 (0.9 %) patients with a final diagnosis of G1, G2, and G3 tumors, respectively. EUS-FNA grading was concordant with surgical pathology in 81.8 % of patients; under- and overgrading occurred in 15.6 % and 2.6 %, respectively. The overall level of agreement for grading was moderate (Cohen's κ = 0.59, 95 % confidence interval [CI] 0.34 – 0.78). Spearman's rho for Ki-67 in tumors ≤ 20 mm and > 20 mm was strong and moderate, respectively (rho = 0.68, 95 %CI 0.47 – 0.83; rho = 0.59, 95 % CI 0.35 – 0.75). The Bland – Altman plot showed that the Ki-67 values were comparable and reproducible between the two measurements.

Conclusions Although they were not available for a significant number of patients, grading and Ki-67 values from cytology correlated with histology moderately to strongly.

Introduction

Pancreatic neuroendocrine tumors (PanNETs) are rare malignancies with a good prognosis, especially when compared with their exocrine counterpart [1]. The biology of these tumors is complex [2] and there are currently few reliable markers of biological behavior. One of the most frequently used tools to assess tumor biology is the Ki-67 index. The World Health Organization (WHO) [3] and the European Neuroendocrine Tumor Society [4] guidelines rely on the Ki-67 index to distinguish between three tumor categories: G1 for of Ki-67 index < 2 %, G2 for values between 3 % and 20 %, and G3 for values > 20 %. Surgery is always recommended for functioning PanNETs. How-

ever, for resectable, nonmetastatic, nonfunctioning PanNETs, surgery is appropriate in the following situations: tumors larger than 20 mm in diameter; patients with symptoms; G2 or G3 tumors; or based on the patient's wishes. For tumors that are smaller than 20 mm and graded as G1, or those G2 tumors with a low Ki-67 value, surveillance seems to be safe [5], even if long-term results are lacking. The ability to obtain a cytological Ki-67 value at the time of diagnosis using fine-needle aspiration (FNA) is clinically invaluable and requires further investigation.

Considering that pancreatic surgery is still burdened by high rates of morbidity and mortality, tailored treatment based on a proper analysis of the tumor biology might improve patient outcomes. For example, small nonfunctioning PanNETs with high preoperative Ki-67 values may benefit from surgery regardless of the dimensions; high Ki-67 values might direct the surgeon to perform a standard pancreatic resection (over a parenchyma-sparing one) or an extended lymphadenectomy (over a regional one).

It remains unclear whether the preoperative Ki-67 index obtained by FNA accurately represents the postoperative Ki-67 index in the resected tumor, because some studies describe a good correlation while others show no correlation [6–9]. Weiss et al. reported the lack of correlation between preoperative FNA results and final histology for both Ki-67 and grading, with an average difference in the former of 5.9 % [10]. The authors state that preoperative FNA leads to undergrading of the tumor, which results in undertreatment [10]. Similar results have been recently reported by Leeds et al. [11]. Intratumor cellular heterogeneity is a confounding factor that may be responsible for this dissonance between the pre- and postoperative analyses [6, 12]. Furthermore, obtaining an adequate number of cells for reliable Ki-67 index determination is challenging using FNA. Sampling with a core biopsy needle (FNB) may overcome the limits of the FNA technique. A meta-analysis showed that FNB is superior to FNA for pancreatic masses, in terms of sampling adequacy and diagnostic accuracy, and it has the same risk of complications and rate of technical success [9]. Two multicenter randomized trials clearly demonstrated the benefits of FNB over FNA for the diagnosis of abdominal masses, including pancreatic masses [13, 14]. However, the studies included few cases of PanNETs, and thus, the results cannot be considered definitive. Leeds et al. reported that Ki-67 and grading information from FNB are more reliable than those obtained via FNA [11].

The primary aim of the current study was to establish the agreement and the correlation between cytological and histological grading and Ki-67 values. Secondary aims were to determine the diagnostic rate of EUS-FNA and concordance with histology.

Methods

Patient selection

The study followed the "STrengthening the Reporting of Observational Studies in Epidemiology" (STROBE) guidelines [15] (▶ **Fig. 1**). Patients who underwent pancreatic resection between January 2013 and July 2019 were selected from the prospectively maintained electronic database at the General and Pancreatic Surgery Unit, Pancreas Institute, University of Verona Hospital Trust, and the data were retrospectively analyzed. Only patients with preoperative FNAs for a suspected PanNET and postoperative histology that confirmed the PanNET nature were included. For this patient group, Ki-67 from cytology and histology, and grading from cytology and histology were compared. In addition, the diagnostic rate and the concordance rate for diagnosis were evaluated.

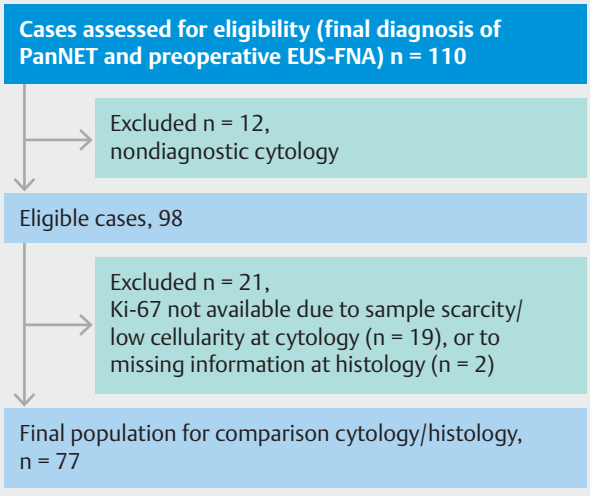
Ki-67 assessment

For the primary aim, the main inclusion criterion was the availability of Ki-67 at cytology and histology for the same patient. To ensure a homogeneous cohort, the following cases were excluded: metastatic neoplasms; multifocal neoplasms; PanNETs with preoperative FNA showing a mixed neoplastic component (mixed adeno-neuroendocrine carcinoma, neuroendocrine and acinar cell carcinoma); PanNETs treated preoperatively with neoadjuvant therapy.

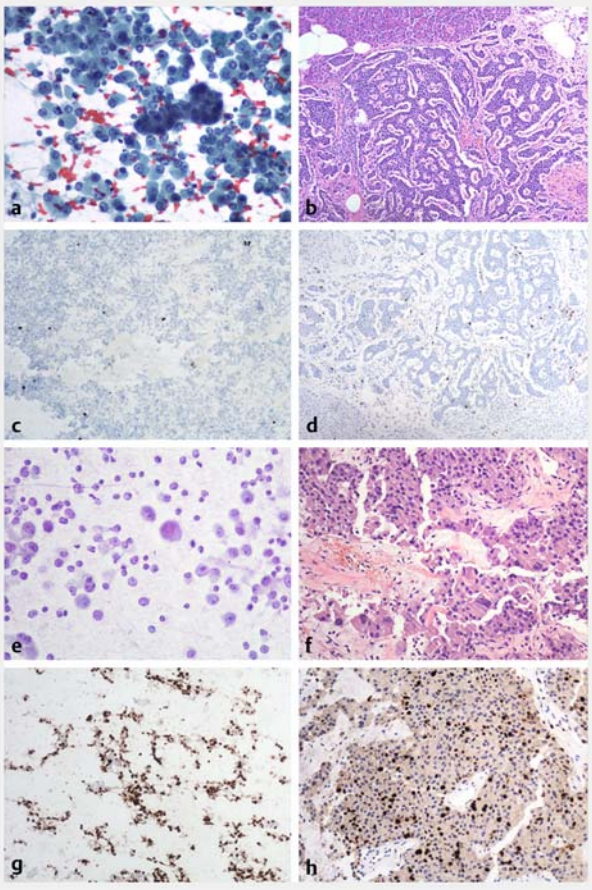
For histological slides, 500 cells in the region of the tumor with the highest labeling rate (hot spots) were counted as follows: Ki-67$^+$ cells/Ki-67$^+$ and Ki-67$^-$ [3]. The same procedure was used for cytological smears, and 2000 cells were counted (▶ **Fig. 2**). A cutoff of 2000 cells has been used by other groups [16], and it has been proposed as a reliable cutoff to provide a proper estimation of Ki-67 in cytological samples [6]. An immunocytochemical analysis with synaptophysin, chromogranin, and Ki-67 markers was always performed to determine the diagnostically relevant neuroendocrine nature of the samples.

EUS-FNA

EUS-FNA was carried out by two experienced endoscopists who each performed over 350 EUS-FNAs per year. In the absence of complications, three to four needle passes [17] were performed using a standard 25 gauge needle (EchoTip Ultra; Cook Medical,



▶ **Fig. 1** Study flow chart according to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement [15].



▶ **Fig. 2** Examples of grading and Ki-67 staining for pancreatic neuroendocrine tumors (PanNETs) in fine-needle aspiration (FNA) acquisition and specimen samples. **a – d** Pancreatic neuroendocrine tumor, G1. **a** Cytology shows highly cellular smear composed of loosely cohesive cells with granular chromatin and plasmacytoid morphology; in the center, an acinar cell aggregate can be seen. **b** On histology, solid (left side) and glandular type (right side) aggregates of well-differentiated endocrine cells. Immunohistochemical labeling for Ki-67 < 2 % on cytology (**c**) and histology (**d**). **e – h** Pancreatic neuroendocrine tumor, G3. **e** Cytology shows single dispersed atypical cells, that are more aggregated in sheets on histology (**f**). Immunohistochemical labeling for Ki-67 (dark nuclei) is consistent with G3 on cytology (**g**) and histology (**h**).

Limerick, Ireland). For each pass, 10 to 20 to-and-fro movements were performed while the stylet was slowly withdrawn (slow-pull technique) [18]. Whenever possible, different areas of the lesions were sampled in a fanning fashion (fanning technique) [19]. The whole collected material underwent cell-block handling [20], as rapid on-site evaluation after EUS-FNA was not available for logistical reasons.

Population characteristics

Demographic, clinical, radiological, cytological, and histological data were obtained. PanNETs were classified according to the WHO criteria, including data regarding differentiation status and Ki-67 index [3]. The institutional preoperative and surgical management of PanNETs has been described previously [21]. Patients underwent EUS-FNA for diagnosis, and grading according to the specialist's prescription, and the site, dimension, and shape of the tumor were considered.

Statistical analysis and study end points

Continuous variables were described as the mean and standard deviation for normally distributed variables, and as the median and interquartile range for variables with a skewed distribution. Pairs of categorical variables were compared using the chi-squared or Fisher's exact test. If Ki-67 was reported as < 1 %, then a value of zero was used. When Ki-67 was reported as a range, the greatest value was used.

For the primary aim, the agreement between preoperative (cytology from EUS-FNA) and postoperative (histology from resection specimen) grading was evaluated using Cohen's kappa, and the correlation between preoperative and postoperative Ki-67 values was assessed using Spearman's rho. A kappa value of 0.20 was considered to be poor, 0.21 – 0.40 was fair, 0.41 – 0.60 was moderate, 0.61 – 0.80 was good, and 0.81 – 1.00 was very good. A rho value of 0.00 – 0.19 was considered to be very weak, 0.20 – 0.39 was weak, 0.40 – 0.59 was moderate, 0.60 – 0.79 was strong, and 0.80 – 1.00 was very strong. Cases were defined as discordant when grading at cytology and histology differed, and differences in grading were further assessed using the McNemar – Bowker test.

For analysis of Ki-67 values, the Bland – Altman [22] plot was adopted to quantify the difference between measurements using a graphical method.

For the secondary aims, the diagnostic rate was assessed by dividing the number of diagnoses obtained by EUS-FNA over the total number of EUS-FNA procedures. The rate of concordance for diagnosis was considered as the proportion of agreement between diagnosis at cytology and the corresponding final histology.

All statistical tests were two-sided, and *P* values of < 0.05 were considered to be statistically significant. The statistical analyses were performed using SPSS v. 21 (IBM Corp., Armonk, New York, USA) and MedCalc (MedCalc Software, Ostend, Belgium).

Results

A total of 110 patients were included overall (▶ **Fig. 1**), including 97 with nonfunctioning PanNETs (88.2 %) and 13 with functioning tumors (11.8 %; all insulinomas). No periprocedural adverse events were reported. ▶ **Table 1** shows the demographic, clinical, pathological, surgical, and radiological features of the study population.

Pre- and postoperative grading

An EUS-FNA diagnosis was possible in 98 of the 110 patients (89.1 %); in 77 of these cases (78.6 %, 70.0 % of the whole cohort), a grade was obtained via cytology, and these 77 patients comprised the study population for the comparison of grading between cytology and histology. For two out of 110 cases (1.8 %), Ki-67/grading information at histology was not available.

▶ **Table 1** Demographic, clinical, and surgical features of the whole cohort (n = 110).

Age, median (IQR), years	**54 (18)**
Sex, male/female, n (%)	51/59 (46.4/53.6)
Symptoms, yes, n (%)	35 (31.8)
▪ Abdominal pain (any kind of)	17 (48.6)
▪ Symptoms of insulinoma	13 (37.1)
▪ Acute pancreatitis	2 (5.7)
▪ Jaundice	2 (5.7)
▪ Abdominal pain and weight loss	1 (2.9)
Site, n (%)	
▪ Head	48 (43.6)
▪ Body	33 (30.0)
▪ Tail	29 (26.4)
Syndromic cases, MEN1, n (%)	2 (1.8)
Tumor diameter, mean (SD), mm	24.5 (13.4)
▪ ≤/>20 mm, n (%)	51/59 (46.4/53.6)
Cytological grading, n (%)	77 (70)
▪ G1	58 (75.3)
▪ G2	18 (23.4)
▪ G3	1 (1.3)
Histological grading, n (%)	108 (98.2)
▪ G1	67 (62)
▪ G2	40 (37)
▪ G3	1 (1)
Surgery, n (%)	
▪ Pancreaticoduodenectomy	41 (37.3)
▪ Distal pancreatectomy with splenectomy	38 (34.5)
▪ Enucleation	12 (10.9)
▪ Spleen-preserving distal pancreatectomy	11 (10.0)
▪ Middle pancreatectomy	6 (5.5)
▪ Total pancreatectomy with splenectomy	2 (1.8)

IQR, interquartile range; MEN1, multiple endocrine neoplasia type 1.

The overall level of agreement between cytological and histological grading was moderate (Cohen's κ = 0.59, 95% confidence interval [CI] 0.34 – 0.78). When considering PanNET ≤ 20 mm (n = 33, 42.9%) and > 20 mm (n = 44, 57.1%), the agreement was good and moderate, respectively (κ = 0.64, 95%CI 0.34 – 0.93; κ = 0.56, 95%CI 0.31 – 0.82).

In the overall cohort, discordance was observed in 14 of 77 cases (18.2%), and thus, the overall correct grading rate at cytology was 58.3% (63/108). ▶ **Table 2** shows the distribution of concordant and discordant cases. The mean tumor diameter in the discordant cases was 30.5 mm (95%CI 22.40 – 37.7) and

▶ **Table 2** Concordance rate for grading between cytology from fine-needle aspiration and histology from specimen.

	Histology			
	G1	**G2**	**G3**	**Total**
Cytology overall, n = 77				
▪ G1	46	12	0	58
▪ G2	2	16	0	18
▪ G3	0	0	1	1
▪ Total	48	28	1	77
Cytology PanNETs ≤ 20 mm, n = 33				
▪ G1	21	4	0	25
▪ G2	1	7	0	8
▪ G3	0	0	0	0
▪ Total	22	11	0	33

PanNET, pancreatic neuroendocrine tumor.

that of the concordant cases was 24.9 mm (95%CI 21.6 – 28.2). The overall sensitivity for grading was 81.8% (95%CI 71.4 – 89.7), whereas for G1, G2, and G3, it was 79.3% (95%CI 66.6 – 88.2), 88.9% (95%CI 65.3 – 98.6), and 100% (95%CI 2.5 – 100), respectively. When considering tumors ≤ 20 mm, the sensitivity of the overall cohort, and of G1 and G2 tumors was 84.8% (95% CI 68.1 – 94.9), 84.0% (95%CI 63.9 – 95.5), and 87.5% (95%CI 47.3 – 99.7), respectively. A McNemar – Bowker test revealed that the changes in grading at cytology and histology were not symmetrical ($P < 0.001$).

A univariate analysis was performed to analyze crude associations for cases that were discordant for grading. None of the associations considered (sex, age [≤ or > 50 years], symptoms [yes/no], tumor dimensions [≤ or > 20 mm], tumor site [head, neck, tail]) showed a statistical significance.

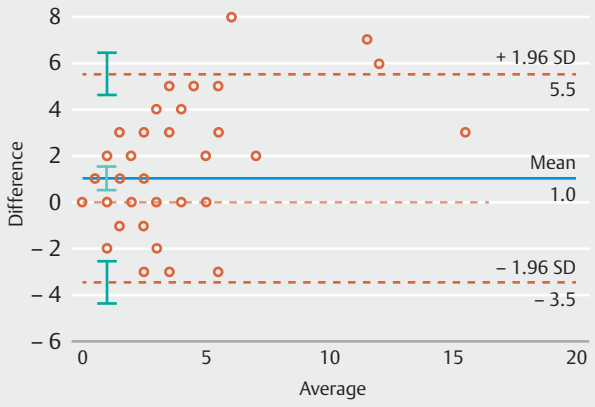
Pre- and postoperative Ki-67 values

Spearman's rho was strong for the overall population (rho = 0.62, 95%CI 0.46 – 0.74). When analyzing tumors ≤ 20 mm and > 20 mm, it was strong and moderate, respectively (rho = 0.68, 95%CI 0.47 – 0.83; rho = 0.59, 95%CI 0.35 – 0.75).

The Bland – Altman plot showed the Ki-67 values were comparable and reproducible between cytology and histology (▶ **Fig. 3**).

Secondary aims

The diagnostic rate of EUS-FNA was 89.1% (98/110; 95%CI 83.2% – 95%), and the rate of concordance for diagnosis was 100%. ▶ **Table 3** presents the pathological data and concordance rates for diagnosis considering the final histology.



▶ **Fig. 3** Bland–Altman plot. One outlier was removed to correct the skewness of the data. The plot demonstrates an excellent agreement between cytology and histology Ki-67 values. SD, standard deviation.

▶ **Table 3** Pathological data.

Diagnosis from FNA	n (%)	Final histology, n (%)
Nondiagnostic	12 (10.9)	Nonfunctioning PanNET, 10 (83.3) Insulinoma, 1 (8.3) NEC and acinar cell carcinoma, 1 (8.3)
Diagnostic	98 (89.1)	All confirmed
▪ Nonfunctioning PanNET	86 (87.7)	
▪ Insulinoma	12 (12.3)	
Nodal status (N+)*		
▪ Overall	20 (20.4)	
▪ G1	9 (45, 15.5 % of G1)	
▪ G2	10 (50, 26.3 % of G2)	
▪ G3	1 (5, 100 % of G3)	

FNA, fine-needle aspiration; PanNET: pancreatic neuroendocrine tumor; NEC: neuroendocrine carcinoma.

* n = 96 (in 12 cases (10.9 %) the nodal status was not assessable due to parenchyma-sparing surgeries; in 2 cases grading was not available at histology).

Discussion

In this study, we investigated whether Ki-67 values and grading from cytology accurately represents histology. We also assessed the diagnostic rate of EUS-FNA and its concordance with histology.

Generally, we found a very good EUS-FNA diagnostic rate of 89.1 %, with all diagnoses confirmed by final histology. These numbers are consistent with previous findings [23 – 25] and they highlight the reliability of the FNA-based diagnosis of PanNETs.

For the comparison of cytology/histology, only 77 of 110 cases (70 %) were adequate for a Ki-67 evaluation, with a correct grading rate of 58.3 % (63/108). These proportions are similar to previously reported results [26 – 28], but lower than those shown by many other authors [9]. A mixture of three hypotheses may explain our results. First, a small needle was used for FNA and no histology was prepared. Second, more than 30 % of the tumors were larger than 30 mm, and they may have had cellular heterogeneity. Third, if there was scarce cellularity, as per internal policy, a value of Ki-67 was not attributed.

For grading concordance, we found an acceptable overall rate of 81.8 %, which is similar to the rate reported by other groups [9, 29 – 31]. This rate was higher for G2 tumors (about 89 %), and it increased to 84.8 % when tumors ≤ 20 mm were considered. The overall agreement was moderate and became good when considering PanNETs ≤ 20 mm; undergrading (n = 12) was more frequent than overgrading (n = 2). Additionally, 12 of 58 samples (20.7 %) graded as G1 at cytology were actually found to be G2 at final histology, and two of 18 (11.1 %) G2 tumors at cytology were actually G1 at histology. Among these, four PanNETs that were ≤ 20 mm were misgraded as G1 by cytology, whereas only one had G1 at histology after having been identified as G2 by FNA (▶ **Table 2**). The overall agreement between cytology and histology for grading was moderate. We have therefore confirmed what has previously been reported about the non-negligible possibility to undergrade a G2 tumor using FNA [6, 8, 11, 16]. Leeds et al. also reported this phenomenon with FNB [11], and it is likely that this is an intrinsic feature of this subtype of PanNET, which is probably related to the tumor tissue architecture. This should be considered when making therapeutic decisions to select the optimal treatment course, especially for PanNETs ≤ 20 mm, where the therapeutic choice may be controversial.

In terms of Ki-67 evaluation, Spearman's rho was strong for the overall population (rho = 0.62, 95 %CI 0.46 – 0.74). When analyzing tumors ≤ 20 mm and > 20 mm, correlation was strong and moderate, respectively (rho = 0.68, 95 %CI 0.47 – 0.83; rho = 0.59, 95 %CI 0.35 – 0.75). Cytology and histology tended to reach the same estimation. These findings have already been described by other authors who found a negative correlation between the concordance rate for Ki-67 and the tumor dimensions, where the bigger the tumor, the higher the cellular heterogeneity and the higher the risk of misestimation [6, 32, 33].

Multiple lessons can be learned from the results of the present investigation, which represents the largest single-center study reported to date that compared cytology- and histology-based determination of PanNETs. First, FNA is an effective diagnostic tool when PanNET is suspected. It enables a high rate of diagnosis, excellent diagnostic concordance, and negligible to no side-effects. Second, the grading assessment was satisfactory because it was accurate in about 80 % of cases; however, undergrading is possible and must be taken into account. Third, we found good agreement for Ki-67 values when considering tumors ≤ 20 mm. This finding may help with therapeutic management and future research of this subcohort of PanNET patients where the therapy might be controversial (surgery vs. fol-

low-up). Fourth, larger PanNETs were more prone to misdiagnosis and had the highest ΔKi-67 levels.

Considering the direct relationship between the diagnostic performance of sampling and the amount of tissue collected, it is likely that EUS-FNA will be substituted with EUS-FNB in the future. FNB allows more cellularity in the cell-block preparations, with a higher diagnostic performance, and, presumably, better reliability in terms of Ki-67 and grading data [9, 13, 14]. However, published data are currently inconclusive. Leeds et al. recently reported that FNB correlated with histology more strongly than FNA. However, the correlation reported for Ki-67 and grading ranged from moderate to strong based on the needle used to perform FNB sampling ($rho_{max} = 0.788$) [11]. Thus, other important factors, such as tumor heterogeneity and operator factors, may always prevent FNB (and FNA) from reaching correlation values that are close to perfection (e. g. rho = 1). The FNB technique will soon completely replace FNA at our institution.

This study has some limitations. The sample size was not adequately powered because 105 cases for comparison were required for adequate statistical power. This may reduce the strength of our conclusions. We did not perform a survival analysis comparing the data from FNA and histology because we considered that it was beyond the scope of this study. The pathology review was not blinded, and the same pathologists may have performed both the cytology and histology review of individual cases. Not all resected PanNETs underwent FNA, which was the surgeon's choice, and this may have also generated a selection bias. We did not consider patients with PanNETs ≤ 20 mm who underwent FNA and were then enrolled in a surveillance program as a control group. Finally, a review of the discordant and nondiagnostic cases, which might have identified possible influencing factors, was not performed.

In conclusion, with the major limitation that cytological information may not be available at the time of FNA (in our experience this happened in almost one-third of the cases), we believe that an attempt to obtain this information should be made. We herein confirm the tendency of cytology toward undergrading compared with histology. Future adequately powered prospective multicenter studies with FNB are needed to obtain more definitive results.

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Competing interests

Dr. Scarpa is a speaker and consultant for Ypsen and Astra Zeneca. The remaining authors declare that they have no conflicts of interest.

References

[1] Halfdanarson TR, Rabe KG, Rubin J et al. Pancreatic neuroendocrine tumors (PNETs): incidence, prognosis and recent trend toward improved survival. Ann Oncol 2008; 19: 1727–1733

[2] Scarpa A, Chang DK, Nones K et al. Whole-genome landscape of pancreatic neuroendocrine tumours. Nature 2017; 543: 65–71

[3] Klöppel G, Adsay NV, Couvelard A et al. Tumours of the pancreas. Neuroendocrine neoplasms. In: WHO classification of tumours. Digestive system tumours.5th edn International Agency for Research on Cancer; 2019: 343–372

[4] Falconi M, Eriksson B, Kaltsas G et al. ENETS consensus guidelines update for the management of patients with functional pancreatic neuroendocrine tumors and nonfunctional pancreatic neuroendocrine tumors. Neuroendocrinology 2016; 103: 153–171

[5] Partelli S, Cirocchi R, Crippa S et al. Systematic review of active surveillance versus surgical management of asymptomatic small nonfunctioning pancreatic neuroendocrine neoplasms. Br J Surg 2017; 104: 34–41

[6] Hasegawa T, Yamao K, Hijioka S et al. Evaluation of Ki-67 index in EUS-FNA specimens for the assessment of malignancy risk in pancreatic neuroendocrine tumors. Endoscopy 2014; 46: 32–38

[7] Li J, Lin JP, Shi LH et al. How reliable is the Ki-67 cytological index in grading pancreatic neuroendocrine tumors? A meta-analysis J Dig Dis 2016; 17: 95–103

[8] Weynand B, Borbath I, Bernard V 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; 25: 389–395

[9] Li H, Li W, Zhou QY et al. Fine needle biopsy is superior to fine needle aspiration in endoscopic ultrasound guided sampling of pancreatic masses: a meta-analysis of randomized controlled trials. Medicine (Baltimore) 2018; 97: e0207

[10] Weiss VL, Kiernan C, Wright J et al. Fine-needle aspiration-based grading of pancreatic neuroendocrine neoplasms using Ki-67: is accurate WHO grading possible on cytologic material? J Am Soc Cytopathol 2018; 7: 154–159

[11] Leeds JS, Nayar MK, Bekkali NLH et al. Endoscopic ultrasound-guided fine-needle biopsy is superior to fine-needle aspiration in assessing pancreatic neuroendocrine tumors. Endosc Int Open 2019; 7: E1281–E1287

[12] Liszka Ł. Tissue heterogeneity contributes to suboptimal precision of WHO 2010 scoring criteria for Ki67 labeling index in a subset of neuroendocrine neoplasms of the pancreas. Pol J Pathol 2017; 67: 318–331

[13] Cheng B, Zhang Y, Chen Q et al. Analysis of fine-needle biopsy vs fine-needle aspiration in diagnosis of pancreatic and abdominal masses: a prospective, multicenter, randomized controlled trial. Clin Gastroenterol Hepatol 2018; 16: 1314–1321

[14] van Riet PA, Larghi A, Attili F et al. A multicenter randomized trial comparing a 25-gauge EUS fine-needle aspiration device with a 20-gauge EUS fine-needle biopsy device. Gastrointest Endosc 2019; 89: 329–339

[15] von Elm E, Altman DG, Egger M et al. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. Lancet 2007; 370: 1453–1457

[16] Boutsen L, Jouret-Mourin A, Borbath I et al. Accuracy of pancreatic neuroendocrine tumour grading by endoscopic ultrasound-guided fine needle aspiration: analysis of a large cohort and perspectives for improvement. Neuroendocrinology 2018; 106: 158–166

[17] Polkowski M, Jenssen C, Kaye P et al. Technical aspects of endoscopic ultrasound (EUS)-guided sampling in gastroenterology: European Society of Gastrointestinal Endoscopy (ESGE) Technical Guideline – March 2017. Endoscopy 2017; 49: 989–1006

[18] Nakai Y, Isayama H, Chang KJ et al. Slow pull versus suction in endoscopic ultrasound-guided fine-needle aspiration of pancreatic solid masses. Dig Dis Sci 2014; 59: 1578–1585

[19] Bang JY, Magee SH, Ramesh J et al. Randomized trial comparing fanning with standard technique for endoscopic ultrasound-guided fine-needle aspiration of solid pancreatic mass lesions. Endoscopy 2013; 45: 445–450

[20] Ieni A, Todaro P, Crino SF et al. Endoscopic ultrasound-guided fine-needle aspiration cytology in pancreaticobiliary carcinomas: diagnostic efficacy of cell-block immunocytochemistry. Hepatobiliary Pancreat Dis Int 2015; 14: 305–312

[21] Landoni L, Marchegiani G, Pollini T et al. The evolution of surgical strategies for pancreatic neuroendocrine tumors (Pan-NENs): time-trend and outcome analysis from 587 consecutive resections at a high-volume institution. Ann Surg 2019; 269: 725–732

[22] Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1986; 1: 307–310

[23] Hooper K, Mukhtar F, Li S et al. Diagnostic error assessment and associated harm of endoscopic ultrasound-guided fine-needle aspiration of neuroendocrine neoplasms of the pancreas. Cancer Cytopathol 2013; 121: 653–660

[24] Larghi A, Capurso G, Carnuccio A 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; 76: 570–577

[25] Manta R, Nardi E, Pagano N et al. Pre-operative diagnosis of pancreatic neuroendocrine tumors with endoscopic ultrasonography and computed tomography in a large series. J Gastrointestin Liver Dis 2016; 25: 317–321

[26] Diaz Del Arco C, Esteban Lopez-Jamar JM, Ortega Medina L et al. Fine-needle aspiration biopsy of pancreatic neuroendocrine tumors: correlation between Ki-67 index in cytological samples and clinical behavior. Diagn Cytopathol 2017; 45: 29–35

[27] Cui Y, Khanna LG, Saqi A et al. The role of endoscopic ultrasound-guided Ki67 in the management of non-functioning pancreatic neuroendocrine tumors. Clin Endosc 2020; 53: 213–220

[28] Baruah A, Adsay V, Pant M et al. Mo1329 diagnostic yield and predictors of adequate cytology sampling for ki67 on endoscopic ultrasound guided fine needle aspiration for grading of pancreatic neuroendocrine tumors. Gastrointest Endosc 2018; 87: AB448

[29] Farrell JM, Pang JC, Kim GE et al. Pancreatic neuroendocrine tumors: accurate grading with Ki-67 index on fine-needle aspiration specimens using the WHO 2010/ENETS criteria. Cancer Cytopathol 2014; 122: 770–778

[30] Di Leo M, Poliani L, Rahal D et al. Pancreatic neuroendocrine tumours: the role of endoscopic ultrasound biopsy in diagnosis and grading based on the WHO 2017 Classification. Dig Dis 2019; 37: 325–333

[31] Hijioka S, Hara K, Mizuno N et al. Diagnostic performance and factors influencing the accuracy of EUS-FNA of pancreatic neuroendocrine neoplasms. J Gastroenterol 2016; 51: 923–930

[32] Unno J, Kanno A, Masamune A et al. The usefulness of endoscopic ultrasound-guided fine-needle aspiration for the diagnosis of pancreatic neuroendocrine tumors based on the World Health Organization classification. Scand J Gastroenterol 2014; 49: 1367–1374

[33] Fujimori N, Osoegawa T, Lee L et al. Efficacy of endoscopic ultrasonography and endoscopic ultrasonography-guided fine-needle aspiration for the diagnosis and grading of pancreatic neuroendocrine tumors. Scand J Gastroenterol 2016; 51: 245–252