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Accuracy of Pancreatic Neuroendocrine Tumour Grading by Endoscopic Ultrasound-Guided Fine Needle Aspiration: Analysis of a Large Cohort and Perspectives for Improvement

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Abstract
Introduction: Since the *WHO Classification of Tumours of the Digestive System* has been published in 2010, resected pancreatic neuroendocrine tumours (pNETs) are graded as grade 1 (G1), grade 2 (G2) or grade 3 (G3) using the Ki67 labelling index (Ki67-LI). Endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) is often used for diagnosis, but few studies have assessed its value for grading. ***Aims:*** The aims of this study were to compare the Ki67-LI obtained by cytological grading (cG) with that obtained by histological grading (hG) and to assess (1) the influence of tumour size and the number of counted cells on FNA grading as well as (2) the overall survival (OS) and progression-free survival based on cG. ***Materials and Methods:*** EUS-FNA was performed for 102 pNETs (57 resected). cG (200 cells counted) was done on all FNAs. For 29 FNAs, >2,000 cells were counted (14 resected). A comparison was made between hG and cG for the 57 resected patients. Patients were followed up until June 2016. ***Results:*** cG was consistent with hG in 39 of 57 patients with a concordance rate of 72% using a Ki67-LI cut-off of 5% for G1/G2. For Ki67-LI absolute values, the correlation was $r = 0.443$ and increased to $r = 0.824$ ($p < 0.001$) when only FNAs with >2,000 cells were counted. Twenty-one of 22 pNETs <2 cm had the same grading on cG and hG, whereas grading was discordant for 15 of 16 pNETs >2 cm. Thirty-eight patients died after 70.5 months of follow-up. OS for the whole cohort was 235 months and differed between cG1 (235 months), cG2 (36.3 months) and cG3 (10.9 months). ***Conclusion:*** cG of pNETs is more accurate when tumours measure <2 cm and more cells are counted on FNA. Discrepancies are seen between G2 tumours which are often considered G1 on FNA due to tumour heterogeneity. EUS-FNA is valuable to distinguish between patients with good (cG1) and poor (cG3) prognosis.

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Introduction

Pancreatic neuroendocrine tumours (pNETs) are nowadays easily recognized and diagnosed by endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) with an accuracy of >90% [1–3]. The grading of pNETs has been defined in the *WHO Classification of Tumours of the Digestive System* in 2010 [4] for resection specimens. It is based on counting the Ki67 labelling index (Ki67-LI) in 500–2,000 tumour cells in areas of highest labelling, so-called "hotspots." A grade 1 (G1) or well-differentiated low-grade endocrine tumour is therefore defined as a Ki67-LI of <3%, a grade 2 (G2) or well-differentiated intermediate-grade endocrine tumour as a Ki67-LI between 3 and 20% and, finally, a grade 3 (G3) or poorly differentiated high-grade endocrine tumour as a Ki67-LI of >20%. However, using this classification, several problems have been encountered. Firstly, the cut-off between G1 and G2 tumours has been a matter of debate in recent years, oscillating between 3% [5] and 5% [6, 7]. Secondly, different counting methods have been described, including eyeballing, cell count via the microscope or on digitalized and printed slides as well as automated methods. Two recent papers compared all or some of these methods on resection specimens [8] and EUS-FNA [9]. Both reached the same conclusion: manual count on a digitalized and printed document is the most accurate way to determine the Ki67-LI.

As EUS-FNA allows for cell block preparation from formalin-fixed, paraffin-embedded leftover cytological material, it is easy to perform immunocytochemistry to confirm the neuroendocrine nature of the tumour cells. It is therefore tempting to perform a Ki67 staining, in analogy with what is done for the resection specimen. To date, there are no well-defined guidelines for the evaluation of Ki67 on cytological material. Few studies have tried to address this question on small study populations, with not much detail on counting methods used and with contradictory results [1, 10–14]. Furthermore, in 2014, Hasegawa et al. [15] demonstrated the importance of counting >2,000 cells on FNA material to obtain a good correlation with the histological grade in a group of 20 pNETs. On the other hand, tumour size seems also an important factor for a good cytological-histological correlation [16].

To address the above-mentioned issues, we decided to expand our study group, first published in 2014 [17], in order to increase the number of analysed tumours as well as their follow-up time.

Materials and Methods

We retrieved from our archives an additional group of 56 cases diagnosed between 2011 and 2013 which were added to the first series of 46 patients diagnosed between 1996 and 2010 and published in 2014 [17]. The study group (Fig. 1) includes, therefore, 102 pNETs from 101 patients diagnosed by EUS-FNA. Among them, 57 patients were operated, allowing for a comparison between cytology and histology. For all patients, we reviewed the medical records, EUS, and surgical and pathological reports to collect all data at diagnosis as well as follow-up data until June 2016.

For all newly included patients, FNAs were retrieved as well as a representative HE-stained slide from the resection specimen, and a Ki67 staining was systematically performed (clone MIB-1, dilution 1:150, Dako, Denmark).

Two-hundred tumour cells were manually counted by 2 independent operators on the cytological slides (B.W. and L.B. for the second series and B.W. and I.B. for the first), as well as at least 2,000 cells on the resection specimens (B.W. and A.J.-M.). In a subgroup of FNAs (n = 29), where sufficient material was available, 2,000 tumour cells were counted on digitalized and printed images by 1 operator (B.W.) (Philips Healthcare Ultra-Fast Scanner, at ×20).

Statistical Analysis

Statistical analyses were done with the SAS programme version 9.4 on Windows 7. The values of categorical variables are expressed in absolute numbers (n) and relative frequencies (% of corresponding total number). The continuous values are expressed as means ± standard deviations. Tumour grades were derived from the LI. Two different cut-offs were investigated for G1 and G2, namely >3 and >5%. As the Wilcoxon test showed that the counted values were homogeneous between operators, the mean value was used for further analyses.

For the inter-observer correlations, both the grade agreement and the absolute values of the counts were considered. The agreement between cytological grading (cG) and histological grading (hG) was evaluated with the Cohen κ test. For the absolute values, a non-parametrical Spearman test was applied. Tumour size of concordant (identical cG and hG) and discordant cases (different cG and hG) was compared using the Mann-Whitney test.

Overall survival (OS) and progression-free survival (PFS) were estimated using the Kaplan-Meier method and were compared following the cytological grading with a log-rank test. OS was defined as the time between the date of diagnosis and the date of death. In the event of the patient being alive at the data cut-off point, patients were censored on the date of last contact. PFS was defined as the time between the date of diagnosis and the date identifying first progression or death. If no such event occurred, the patient was censored on the date of last contact. All statistical tests are bilateral and a p value of <0.05 was considered statistically significant.

Results

Demographic and Tumour Characteristics

Demographic and tumour characteristics can be found in Tables 1 and 2. A total of 101 patients were included, presenting 102 tumours. The study population showed a

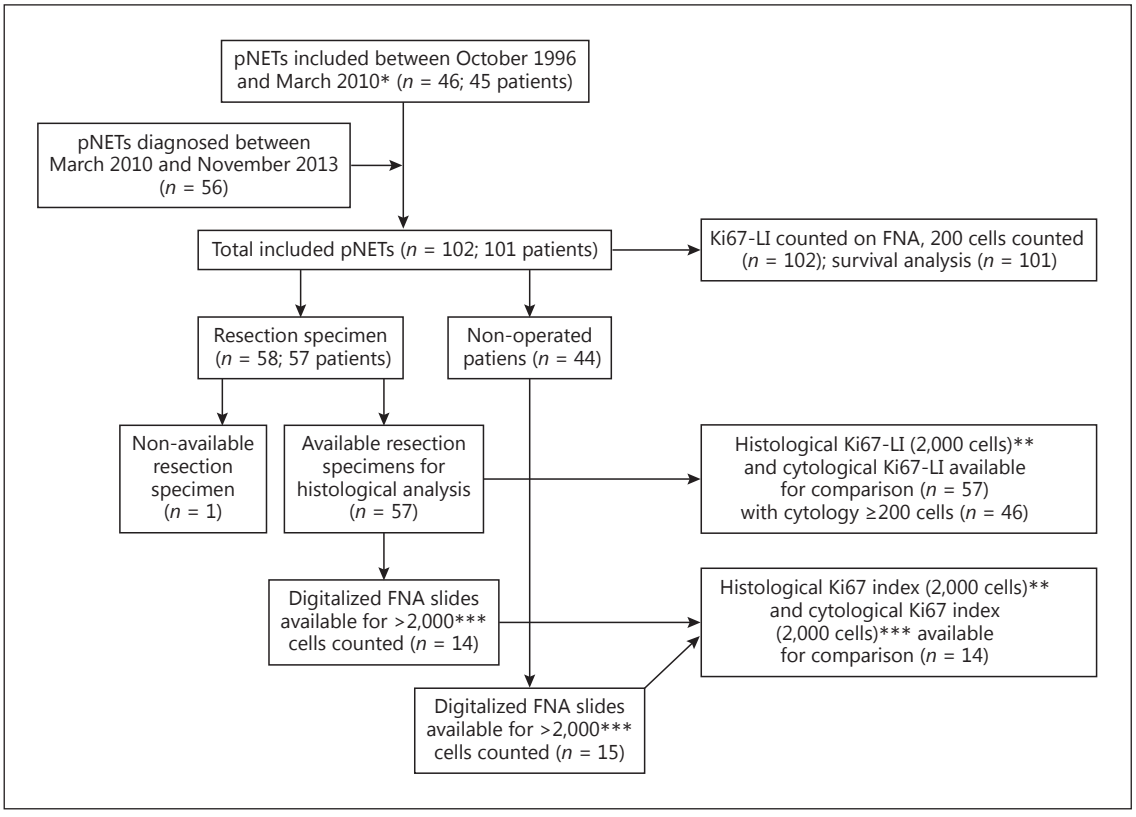

Fig. 1. Study group (*n* stands for the number of tumours). * Weynand et al. [17], 2014. ** Rindi et al. [4], 2010. *** Hasegawa et al. [15], 2014.

male preponderance (55.4%) and a mean age of 59.2 ± 12.8 years. Three tumours were diagnosed between 1996 and 2000, 53 tumours between 2001 and 2010 and 46 tumours between 2011 and 2013. Most cases were asymptomatic and sporadic. Most tumours were non-functioning, as expected. The main location was the head of the pancreas (48.0%), and the mean tumour size was 25.0 ± 23.9 mm.

After cytological diagnosis, 57 patients (56.4% of the study population) were resected after a mean period of 25 weeks. Their TNM staging (AJCC/UICC, 7th edition) can be found in Table 3. Most tumours, whether resected or not, were low stage (IA and IB). There were no stage III (or T4) tumours in our cohort. Resected stage IV patients had liver metastases, and more than half of them underwent a liver transplantation after the pancreatic resection.

Inter-Observer Correlation

The Wilcoxon test demonstrated homogeneous Ki67-LI values between the different operators ($p = 0.729$ for the Ki67-LI on FNA and $p = 0.898$ for the Ki67-LI on histological specimens). All values were compared between pairs of operators. Therefore, all subsequent analyses were done on the mean values of counts.

Ki67-LI on FNA

In all 102 FNAs, Ki67-LI could be quantified, with a mean of 301.5 ± 123.1 cells counted. Thirteen slides showed <200 tumour cells identified (most between 150 and 200 cells). Using the 3% cut-off, there were 65 G1, 28 G2 and 9 G3 tumours. Using the 5% cut-off, we observed 79 G1, 14 G2 and 9 G3 tumours. The 29 digitalized and printed cases where >2,000 cells were available were classified as 20 G1, 5 G2 and 4 G3 tumours (3% cut-off). Sixty-nine percent (20 of 29 cases) showed a similar grade when compared to the 200-cell count, 1 case was classified as G2 instead of G3, and 8 G2 tumours were reclassified as G1.

Ki67-LI on Resection Specimen

A mean of 2,158.9 ± 429.4 cells could be counted on the 57 resected tumours. With a 3% cut-off, 30 tumours

Table 1. Demographics of the study group

	Whole population (n = 101)	Surgical population (n = 57)
Total number of patients (tumours)	101 (102)	57 (58)
Sex, *n* (%)		
Women	45 (44.6)	26 (45.6)
Men	56 (55.4)	31 (54.4)
Age at diagnosis, years		
Mean ± SD	59.4±12.84	56.3±12.99
Min.	25	25
Max.	85	82
Age at diagnosis, *n* (%)		
<70 years	80 (79.2)	48 (84.2)
≥70 years	21 (20.8)	9 (15.8)
Symptoms at diagnosis, *n* (%)		
Fortuitous finding	52 (51.2)	26 (45.6)
Hypoglycaemia	6 (5.9)	6 (10.5)
Abdominal pain	6 (5.9)	4 (7.0)
Familial screening	4 (4.0)	3 (5.3)
Diarrhoea	3 (3.0)	3 (5.3)
Weight loss	3 (3.0)	1 (1.7)
Liver metastases	3 (3.0)	0 (0.0)
Jaundice	4 (4.0)	0 (0.0)
De novo diabetes	1 (1.0)	1 (1.7)
Unknown	19 (18.9)	13 (22.8)
Hereditary syndrome, *n* (%)		
Sporadic	95 (94.1)	54 (94.7)
MEN1	4 (3.9)	2 (3.5)
VHL	2 (1.9)	1 (1.7)

MEN1, multiple endocrine neoplasia type 1; VHL, von Hippel-Lindau syndrome.

Table 2. Tumour characteristics

	Whole population (n = 102)	Surgical population (n = 58)
Total number of tumours	102	58
Functional status, *n* (%)		
Non-functioning	90 (88.2)	47 (81.1)
Insulinomas	6 (5.9)	6 (10.3)
Gastrinomas	5 (4.9)	4 (6.9)
Glucagonoma	1 (1.0)	1 (1.7)
Location in the pancreas, *n* (%)		
Corpus	12 (11.8)	4 (6.9)
Head	49 (48.0)	28 (48.3)
Overlapping[a]	9 (8.9)	4 (6.9)
Tail	31 (30.4)	22 (37.9)
Unknown	1 (1.0)	0 (0.0)
Tumour size, mm		
Mean ± SD	25.0±23.9	28.5±21.6
Median	18.0	20.0
Max.	160	110
Min.	2	2
Unknown	6	0

[a] Overlapping either corpus and head or corpus and tail.

Table 3. TNM staging (AJCC/UICC, 7th edition)

				Non-resected tumours (cTNM)	Resected tumours (pTNM)
Stage IA	T1	N0	M0	24 (54.6%)	22 (37.9%)
Stage IB	T2	N0	M0	1 (2.3%)	13 (22.4%)
Stage IIA	T3	N0	M0	2 (4.5%)	3 (5.2%)
Stage IIB	T1–T3	N1	M0	3 (6.8%)	12 (20.7%)
Stage III	T4	N0 or N1	M0	0	0
Stage IV	T1–T4	N0 or N1	M1	14 (31.8%)	8 (13.8%)
Total				44	58

were G1, 23 G2 and 4 G3, whereas with a 5% cut-off, there were 39 G1, 14 G2 and 4 G3 tumours.

Comparison of Cytology and Histology Grading

When comparing grades (Table 4), there was a moderate agreement between Ki67-LI on FNA and resection specimen regardless of whether the 3% (κ coefficient 0.434) or the 5% cut-off (κ coefficient 0.354) was used. For G1 tumours, the Ki67-LI was correctly evaluated on FNA in 90% of cases (27 of 30 cases) with the 3% cut-off and in 94.9% of cases (37 of 39 cases) with the 5% cut-off. For G2 tumours, we found only 43.5% (10 of 23) and 14% (2 of 14) correctly graded tumours with the 3 and 5% cut-offs, respectively. For G3 tumours, only 2 of 4 were correctly graded, whereas the 2 others were graded G2 and G1, respectively. Figure 2 shows the distribution of Ki67-LI counts, using the histological grade as gold standard and showing the cytological grade of the corresponding FNAs, demonstrating clearly that cG3 tumours were underestimated on the FNA counts. The median (min.–max.) values were 1.4% (0.0–4.0%) for cG1 versus 1.9% (0.2–2.8%) for hG1, 2.5% (0.0–7.9%) for cG2 versus 5.5% (3.0–9.2%) for hG2 and 14.4% (1.1–23.0%) for cG3 versus 24% (21.1–27.6%) for hG3.

When comparing absolute numbers of the whole cohort, the Spearman r coefficient was 0.443 (p = 0.001), showing a moderate correlation with a positive curve. When excluding the cases with <200 cells on FNA, the

Table 4. Comparison of cytological and histological grading

	Cytological grades			
	G1	G2	G3	Total
Grades, cut-off 3%				
Histological grades				
G1	**27**	3	0	30
G2	13	**10**	0	23
G3	1	1	**2**	4
Total	41	14	2	**57**
Statistics				
κ coefficient	0.434			
PABAK-OS	0.368			
p value	<0.001			
Grades, cut-off 5%				
Histological grades				
G1	**37**	2	0	39
G2	12	**2**	0	14
G3	1	1	**2**	4
Total	50	5	2	**57**
Statistics				
κ coefficient	0.354			
PABAK-OS	0.439			
p value	<0.001			

G1, grade 1; G2, grade 2; G3, grade 3; PABAK-OS, Prevalence- and bias-adjusted Kappa-ordinal scale.

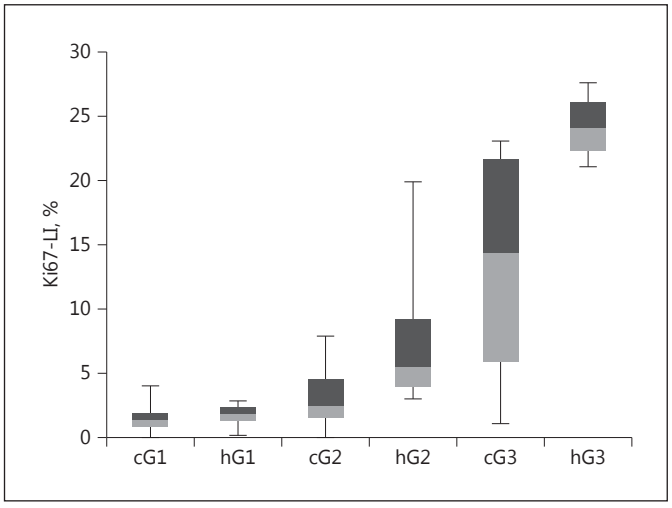

Fig. 2. Distribution of the Ki67 labelling index (Ki67-LI). Dark grey box, from median to 75th percentile; light grey box, from median to 25th percentile; plain lines, values from minimum to maximum. cG, cytological grade; hG, histological grade.

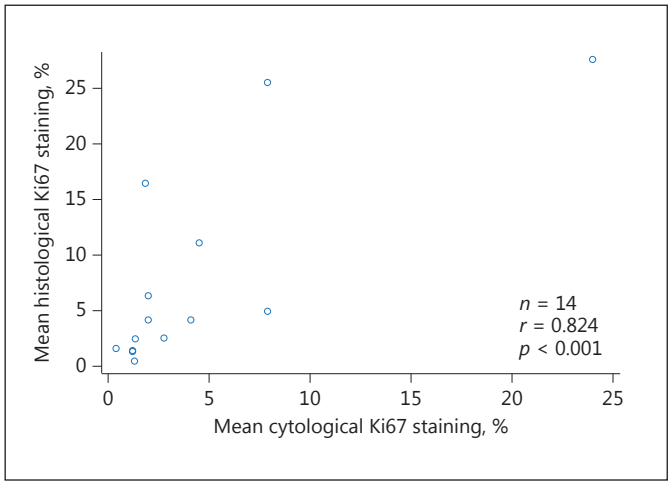

Fig. 3. Correlation between absolute numbers of the Ki67 labelling index on cytology and histology (group of 2,000 counted cells).

correlation was still moderate but increased to an *r* coefficient of 0.574 ($p < 0.001$). When counting 2,000 cells on FNA, the correlation became very high with an *r* coefficient of 0.824 ($p < 0.001$) (Fig. 3).

Finally, when analysing the concordant ($n = 41$) and discordant ($n = 16$) cases with a 5% cut-off, taking the size of the tumour into account (22 tumours measuring <2 cm and 35 tumours measuring >2 cm), we could show that in the discordant group all but 1 tumour (15 of 16, 93.8%) measured >2 cm, whereas 21 of the 22 (95.5%) tumours measuring <2 cm were concordantly graded tumours.

Survival Analysis

Of the 101 patients, 38 died. Of these, 26 deaths were attributable to the progression of pNETs; 8 patients died of other causes (2 concomitant pancreatic adenocarcinomas, 1 metastatic lung adenocarcinoma, 2 post-operative deaths, 1 septic shock, 1 multiple organ failure and 1 sudden death). Four patients died of unknown causes. Of the 68 survivors, 7 have progressive disease.

The median follow-up time of the whole group was 70.5 months, with a minimum of 1 day and a maximum of 238 months. For the surviving patients, the median follow-up period was 74.3 ± 41.5 months. No patient has been lost to follow-up.

The median OS of the whole group was 235.3 months (95% confidence interval 68.7–235.30). When considering the 3% cut-off, the OS was significantly ($p < 0.001$) different between cG1 (median 235.3 months) and cG3 (median 10.95 months) tumour patients but not between cG1 and cG2 tumour patients. With the 5% cut-off

Table 5. Progression-free survival

	Grade 1	Grade 2	Grade 3
Whole population	NE	39.80 ($p = 0.017$)	10.07 ($p < 0.001$)
HR		2.61 (95% CI 1.174–5.806)	14.70 (95% CI 5.787–37.35)
Non-resected patients	NE	39.80 ($p = 0.004$)	10.07 ($p < 0.001$)
HR		11.21 (95% CI 2.149–58.45)	37.01 (95% CI 6.606–207.4)
Resected patients	NE	51.18 ($p = 0.007$)	14.90 ($p < 0.001$)
HR		8.45 (95% CI 1.845–38.73)	40.47 (95% CI 6.687–244.9)

Values are median progression-free survival, expressed in months, with p values unless otherwise indicated. HR, hazard ratio; NE, non-evaluable; CI, confidence interval.

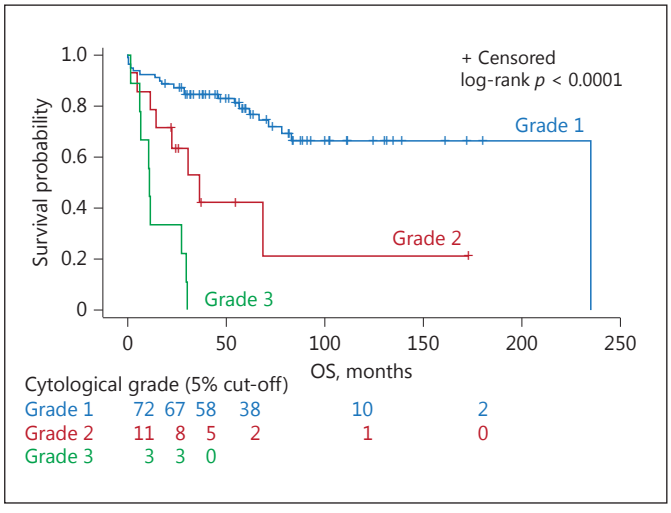

Fig. 4. Kaplan-Meier curve of overall survival of the whole population according to cytological grade (5% cut-off).

(Fig. 4), the median survival of cG1 tumour patients (235.30 months) was significantly ($p < 0.001$) different from cG2 (36.35 months) and from cG3 (10.95 months) tumour patients. No significant difference was observed between cG2 and cG3 tumour patients. The hazard ratio for OS was 3.78 for cG2 and 12.55 for cG3 tumour patients compared to cG1 tumour patients.

The OS for the resected group was not analysable, as these low-grade tumours have, by definition, a long survival, especially after resection, and the number of deaths was insufficient to perform the analysis. The survival was significantly better for cG1 tumour patients (the median could not be determined because of the low death rate in the group; 6 out of 23 patients) than for cG2 (28.82 months, $p = 0.003$) and cG3 tumour patients (10.46 months, $p < 0.001$). This means that non-resected patients with cG2 and cG3 tumours have a 8.41 and 27.69 times higher risk to die than cG1 tumour patients, respectively.

The 5-year and 10-year survival rates of the whole group with a 3% cut-off were 80.3 and 65.7% for G1 tumours and 56.1 and 46.8% for G2 tumours, respectively. All G3 tumour patients were already dead at 5 years.

Progression-Free Survival

In all 3 cohorts (whole group, resected and non-resected patients), PFS was significantly better for G1 than for G2 and G3 tumours (Table 5). When looking at the whole cohort, cytological G1 tumour patients (median not evaluable because only 14 of the 51 cG1 patients showed a progression) did much better than G2 (39.80 months) and G3 (10.07 months) tumour patients with a progression risk of 2.61 and 14.70 times, respectively, using the 3% cut-off.

Discussion

pNETs are rare tumours whose incidence has been rising in the last years because of better diagnostic methods including EUS. Their prognosis is much better than that for conventional adenocarcinoma, but their clinical evolution is variable, difficult to predict and sometimes pejorative [18, 19]. One of the best-established prognostic criteria of pNETs is the degree of cellular proliferation measured by the evaluation of the Ki67 index, as implemented in the 2010 WHO classification [4]. The proposed grading system has been established on resection specimens, and although EUS-FNA is the most frequently used diagnostic method for pNETs, there are no guide-

lines on how to evaluate this tumour grade on cytological material. Since 2014 and the publication of our first paper [17], a few groups have done comparative studies between the Ki67-LI obtained on FNA and the corresponding resection specimens [2, 9, 15, 16, 20–24]. Our study represents the largest to date, not only in terms of comparison of grading, but also in terms of Ki67-LI absolute values, between cytology and corresponding resection specimens, studying the influence of tumour size, the number of counted cells on FNA and overall and progression-free survival.

The concordance rate between Ki67-LI on FNA and the corresponding resection specimens of 68.4% is moderate ($\kappa = 0.434$) when using the 3% cut-off between G1 and G2 tumours and does not significantly differ when using the 5% cut-off. These numbers are in line with our previous paper [17]. Nevertheless, increasing the number of counted cells on FNA increases the correlation significantly. When excluding the FNAs where <200 cells were available for counting, the correlation coefficient was $r = 0.574$, and when 2,000 cells were counted, the correlation became strong with $r = 0.824$. These values correspond to those found in the literature where concordance varies between 44 and 89% and the κ coefficient between 0.29 (feeble) and 0.82 (strong) [16, 24]. This implies that although we could not define cut-offs between grades, absolute values nicely correlate between Ki67-LI on FNA and resection specimens when counting a maximum of cells on our routine clinical material, confirming the study by Hasegawa et al. [15].

Further analysing our results, we observed that G2 tumours are the most discordant in our series, with FNA under-staging the tumours. This is also largely described in the recent literature [2, 15, 16, 21–24] and seems to be due to tumour heterogeneity. Yang et al. [25], studying tumour heterogeneity on 45 liver metastases from pNETs, showed that G1 tumours are relatively homogeneous, whereas 91% of their G2 cases harboured areas with <3% Ki67-LI. This has also been demonstrated by Hasegawa et al. [15] who analysed whole slides of 40 resected pNETs. The tumour areas with a Ki67-LI >3% represented only 18.9% of the total tumour area. It is therefore easy to calculate that an FNA has 4 out of 5 times the chance to miss G2 areas. This is probably also an explanation for under-grading of G3 tumours. In our study, although we had only 4 resected G3 tumours, 2 were under-staged even after counting 2,000 cells for one of both. In both resection specimens, we found low-proliferating areas. The grading of resection specimens is based on the count of hotspot areas, implying that in higher-grade tumours there might be areas with a lower Ki67-LI. G3 tumours tend also to be larger at diagnosis, which influences the overall area punctured by FNA, and furthermore, they dilute the hotspot areas. Over-grading has also been described in the literature [9, 20] and can be explained by the fact that some pNETs are contaminated by proliferating inflammatory cells, mostly lymphocytes or endothelial cells, which can sometimes be confounding on FNA material.

We could confirm the study by Unno et al. [16] which has already elaborated on the importance of tumour size. When using the 5% cut-off, we showed that among the 22 tumours measuring <20 mm, 21 (95.5%) had a concordant grade between cytology and histology. Twenty concordant tumours were G1 and 1 was a G3. The discordant tumour was considered G2 on cytology and G1 on histology. Only 57% of tumours measuring >20 mm were concordant. From a clinical point of view, a size of >20 mm is considered the cut-off towards a more aggressive lesion. On the other hand, in the study by Fujimori et al. [2], EUS G2 and G3 tumours were larger and more heterogeneous than G1 tumours.

Our study reveals that the grading done on EUS-FNA can predict OS for patients with pNETs. The survival analysis of the whole study population, including 101 patients with a mean follow-up of 70.5 months (the longest follow-up among published results), could show an OS that was significantly different between cG1 and cG3 tumour patients, regardless of which cut-off was used. PFS showed the same results.

These results are in concordance with the literature on resection specimens. Scarpa et al. [26] assessed the OS of 237 pNET patients with the Ki67-LI, applying a 5% cut-off; they showed that G1 tumour patients had a 5-year OS of 91%, G2 tumour patients had a 5-year OS of 52% and G3 tumour patients had a 5-year OS of 12%. They could demonstrate that G2 and G3 tumour patients had a 2 and 11 times higher risk to die from their tumour, respectively, which is concordant with our results.

With these interesting findings in mind, it appears that the evaluation of the tumour grade on EUS-FNA material with the Ki67-LI should be included in the workup of each patient with a pNET. Nevertheless, the proposed grade should not be interpreted without integrating the clinical situation of each individual patient and should be discussed in a multidisciplinary meeting. Clinicians should be aware of the risk of cytological under-grading due to the heterogeneity of pNETs, especially in cG2 tumours and when tumours measure >2 cm. The best way to assess the Ki67-LI on cytological material is the manu-

al count, directly on the microscope or on prints from digitalized slides, of as many cells as possible, with a goal to reach 2,000 cells. In this case, cG1 tumours measuring <2 cm might be simply followed up, as advocated in the recent literature [27]. In our study, 73.9% of non-resected G1 tumour patients are alive with a lower limit of the 95% confidence interval of survival of 56.61 months. Those who died had either a metastatic tumour at diagnosis (8 patients), developed another malignancy (3 patients), died immediately after surgery (3 patients) or died of unknown reasons (4 patients).

In conclusion, our study includes the largest patient cohort comparing cytological and histological Ki67-LI, including a survival analysis based on cytological results with the longest median follow-up of 70.5 months. Grading errors persist due to tumour heterogeneity, especially in G2 tumours. Nevertheless, it appears that a group of patients with a good prognosis (G1) can be distinguished from a group of patients with a bad prognosis (G3) in terms of OS and PFS. Cytological grading of pNETs is especially accurate when the tumour size is small (<2 cm) and at least 2,000 tumour cells are counted. In this specific situation, the algorithm proposed for the treatment of resectable non-functional pNETs by the recently published ENETS consensus guidelines update can easily be applied, allowing for the distinction between G1, low G2 and G2 tumours during workup. For tumours measuring >2 cm, the general recommendation should be used [27]. Although we were not able to determine universal cut-offs for grading on EUS-FNA, we showed the importance of determining the Ki67-LI and, therefore, advise clinicians to base their treatment decisions on absolute numbers of cytological Ki67-LI.

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