Histopathological Revision for Gastroenteropancreatic Neuroendocrine Tumors in Expert Centers: Does it Make the Difference?

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ABSTRACT

Introduction: The histopathological correct diagnosis of gastroenteropancreatic neuroendocrine tumors (GEP-NETs) is crucial for treatment selection and prognostication. It is also very challenging due to a limited experience in non-expert centers. Revision of pathology is standard of care for most patients who are referred to NET expert centers. Aim of this study was to describe the clinical impact of histopathological revision for GEP-NET patients referred to an expert center.

Methods: Retrospective multicenter analysis of all GEP-NETs receiving a histopathological revision in 6 European NEN expert centers (Jan 2016–Dec 2016) to evaluate the impact on patient management

Results: 175 patients were included of which 14.7% referred for a second opinion. The type of histological samples included biopsies, which made up 69.1%, followed by surgical specimens at 23.4% and endoscopic resections making up 7.5%. The revision had a clinical impact in 36.0% of patients, more frequently observed in patients with a higher Ki67 (Grade 3 vs. Grade 1: OR 3.52, $P < 0.01$) and with an unknown tumor primary site suspected to be GEP (OR 6.54, $P = 0.03$). The modification of the histopathology then gave rise to a new therapeutic indication which included resection or medical treatment in 26.3% of patients. Indication to then perform a new imaging test occurred in 21.1%, and a recommendation to follow-up with no further treatments in 6.3%.

Conclusions: Histopathological revision in expert centers for NETs can change the diagnosis, with a significant clinical impact in about one third of patients.

INTRODUCTION

Gastroenteropancreatic neuroendocrine neoplasms (GEP-NETs) are rare, although their incidence has significantly increased over the last 30 years.[1] The diagnosis of a NET can be suggested by a combination of imaging and biomarkers, but pathology is essential for the correct diagnosis and grading.

The NETs expert centers discuss clinical management decisions in a multidisciplinary setting (institutional tumor boards). The Guidelines of the European Neuroendocrine Tumor Society (ENETS) are followed to define diagnostic and therapeutic strategies for patients with NETs, including the histopathological diagnosis.[2-10] Pathology reports of NETs should include cell morphology (differentiation), proliferative index (Ki67 and mitotic count) and immunohistochemical expression of biomarkers such as chromogranin A (CgA) synaptophysin or CD56.[11-13] Although these diagnostic criteria have been validated in several populations,[14-16] a proportion of patients referred to expert centers still require histopathological revision. The indication to re-evaluate the tumoral specimen on site may be due to the standard operating procedure at the expert center with obligatory pathological review (as recommended in some countries such as France), a second opinion requested by the expert center, or due to discrepancies between pathologic report and clinical behavior. The impact of pathological reassessment on the management of NET patients has not been investigated to date.

The aim of this study was to investigate the clinical usefulness of histopathological revision, in a retrospective study of GEP-NETs collected in 6 European NET expert centers.

METHODS

A retrospective analysis was performed including GEP-NET patients receiving a revision of the pathology report in six European NET expert centers (Rotterdam, Paris, Amsterdam, Sheffield, Katowice, Ghent) between 1st January and 31th December 2016.

The exclusion criteria were: a primary tumor outside of the GEP system or unknown (unless suspected to be a GEP site), as well as the absence of the first histopathological report from the referring center.

The histopathological re-evaluation was performed on site by pathologists with a long-standing experience in NETs, and according to the diagnostic criteria reported by ENETS guidelines.[11-13]

Cases were considered “second opinions,” if histopathological revision (often associated to a therapeutic suggestion) was required by the referring center. “Fully referred,” cases were patients who continued to be managed in the NET expert center after the referral.

The following variables were collected in a common anonymized database: reason for referral, referring hospital (university/non-university), tumor primary site, and kind of specimen (surgical/endoscopic resection of biopsy; primary or metastasis). The histopathological parameters were recorded in detail including both the first histopathological report and in the revision (Ki67, grading, mitotic count, neuroendocrine immunostaining, WHO 2010 Classification,[17] tumor differentiation and morphology for G3, pTNM staging and R status in case of surgical specimen), as well as any change in patients' management due to the revision.

All changes between the original and the reviewed histopathological report were recorded in a secure databases, in accordance with the local centers confidentiality guidance. The significance of the histopathological change was correlated to the consequent clinical impact. Clinical usefulness was defined as the occurrence of clinical changes due to the histopathological revision, such as recommending the performance of new diagnostic imaging tests, new therapy (surgical or endoscopic resection, medical treatment) or to active surveillance via follow-up only.

Statistical analyses were performed using a dedicated software program (Medcalc 15.6.1, http://www.medcalc.be). Continuous variables were reported as medians and ranges, and qualitative variables as frequencies and percentages. A comparison between the subgroups was carried out using Fisher's exact test or the Chi-squared test for non-continuous variables, while the Mann-Whitney U test was adopted for continuous variables. A *P* value was considered statistically significant when < 0.05.

Logistic regression was adopted to investigate a possible correlation of clinical usefulness of the histopathological revision with tumor features (Table 3). Results were expressed as odds ratios (OR) and 95% confidence intervals (CI). A multivariate model was constructed by the "enter method," including all variables that had shown significance at the univariate analysis.

RESULTS

Overall, 175 patients were included in this study. Tumor features at referral i.e. prior to histopathological revision at the expert center are presented in Table 1. The expression of chromogranin A and synaptophysin had been immunohistochemically assessed in 134/175 (76.6%) of cases, and were positive in 121/134 (90.3%) and 127/134 (94.7%), respectively. Transcription factors CDX2, Islet1 or TTF1 had been stained in of 33/175 patients (18.8%). Other evaluated tumor markers included neuron-specific enolase (NSE) (described in 2.3% of samples, and positive in 50.0%) and CD56 (assessed in 41.7%, positive in 95.9%). The Ki67 was available in 145/175 cases (82.8%). The mitotic count was reported in 68/175 cases (38.8%), including 9 cases for whom the Ki67 value had not been assessed. The tumor differentiation of G3 tumors was described in only 17/26 (65.4%) of them.

Table 1: Patients' features before the tumor board discussion: stratification according to clinical usefulness of the revision.

Features	All patients (*n* = 175)	Clinical usefulness (*n* = 63)	No clinical usefulness (*n* = 112)	*P*-value
Referred from University Hospitals, *n* (%)	39 (22.3)	13 (20.6)	26 (23.2)	0.85
Referred from non-University Hospitals, *n* (%)	136 (77.7)	50 (79.4)	86 (76.8)	
Reason for referral				1.00
Full referral, *n* (%)	151 (86.3)	54 (85.7)	97 (86.6)	
Second opinion, *n* (%)	24 (14.7)	9 (14.3)	15 (13.4)	
Pathological specimen				0.36
Biopsy, *n* (%)	101 (69.1)	34 (54.0)	67 (59.8)	
Endoscopic resection, *n* (%)	13 (7.5)	7 (11.1)	6 (5.4)	
Surgical specimen, *n* (%)	61 (23.4)	22 (34.9)	39 (34.8)	
Sample site				0.06
Primary tumor, *n* (%)	118 (67.4)	48 (76.2)	70 (62.5)	
Metastasis, *n* (%)	57 (32.6)	15 (23.8)	42 (37.5)	
Tumor primary site				< 0.01
Pancreas, *n* (%)	54 (30.8)	21 (33.3)	33 (29.4)	
Small bowel, *n* (%)	50 (28.6)	7 (11.1)	43 (38.4)	
Others, *n* (%)	63 (36.0)	29 (46.1)	34 (30.4)	
Unknown, *n* (%) *	8 (4.6)	6 (9.5)	2 (1.8)	
pTNM Staging †				0.25
Stage I, *n* (%)	4 (5.6)	1 (10.0)	3 (4.9)	
Stage II, *n* (%)	5 (7.0)	2 (20.0)	3 (4.9)	
Stage III, *n* (%)	27 (38.0)	4 (40.0)	23 (37.8)	
Stage IV, *n* (%)	35 (49.4)	3 (30.0)	32 (52.4)	
Ki67 [%; median (range)] ‡	4 (1-100)	6 (1-100)	3 (1-90)	0.01
G Grading ‡				< 0.01
G1, *n* (%)	67 (46.2)	17 (37.8)	50 (50.0)	
G2, *n* (%)	51 (35.2)	13 (28.9)	38 (38.0)	
G3, *n* (%)	27 (18.6)	15 (33.3)	12 (12.0)	

* Suspected to be gastroenteropancreatic
† Available in 71 cases
‡ Available in 145 cases

Table 2 describes the significant histopathological changes determined by the local revision, observed in 63/175 (36.0%) of included patients. In 21/175 (12.0%) cases more than one histopathological change was determined by the revision. In 5/175 (2.8%) cases a NET was excluded. More specifically, the new diagnoses included a colon adenocarcinoma, and 4 cases of pancreatic malignancies (poorly differentiated pancreatic squamous cell carcinoma,

Table 2: Significant changes in the histopathological diagnosis after the revision at the expert center.

Features	All patients (*n* = 175)
Exclusion of a NET, *n* (%)	5 (2.8)
First diagnosis of a NET, *n* (%)	4 (2.3)
Proliferative index (ki67)	41 (23.4)
Change in the value, *n* (%)	26 (14.8)
First assessment, *n* (%)	15 (8.6)
Tumor invasion, *n* (%) *	19 (10.8)
Tumor differentiation, *n* (%) †	3 (1.7)
Cell type, *n* (%) ‡	1 (0.05)
MiNEN, *n* (%)	6 (3.4)
Additional neurohormone staining, *n* (%)	4 (2.3)
Concomitant atrophic gastritis, *n* (%) §	1 (0.05)

* invasion parameters: TNM, R, or lymphovascular (L, V)
† For the 27 G3 NEN
‡ For the 11 G3 NEC
§ For the 16 gastric primary sites
NEN: neuroendocrine neoplasm; NEC: neuroendocrine carcinoma; MiNEN: Mixed neuroendocrine/non-neuroendocrine.

two adenocarcinomas and one solid pseudopapillary neoplasm). In 4/175 (2.3%) patients, the diagnosis of exocrine carcinoma (with primary of esophagus, pancreas, intestinal in 2 cases) was changed by the revision, into a neuroendocrine histopathological type. The Ki67 reassessment changed the value in 41/175 (23.4%) cases, with a median change of 4%. In detail, Ki67 was evaluated for the first time in 15/175 (8.6%) of cases. Ki67 reassessment changed the value in 26/175 (14.8%). This modification determined also a change in the grading classification in 20/175 (11.4%): in 7/20 (35.0%) a higher grading was calculated, in 13/45 (65.0%) a lower grading. In two G2 cases immunohistochemistry was positive only at NSE, and negative for both CgA and synaptophysin. G3 histology was confirmed in 17/27 (62.9%), with changes in differentiation in 3/17 (17.6%).

Clinical usefulness was characterized by the consequent change in indications in 63/175 (36.0%) patients. Specifically, a change in therapeutic management occurred due to the revision in 46/175 (26.3%) patients: in 8 patients leading to a resection (1 endoscopic) and in 38 to a change in medical treatment. The change in pathological diagnosis led to an indication for an additional imaging test in 37/175 (21.1%) patients, and of active surveillance or follow-up without any further treatment in 11/175 (6.3%). This last group included 4 gastric NETs, 2 appendiceal NET, 1 pancreatic NET smaller than 2 cm in size, 1 case of small bowel and 3 rectal NET that had been curatively resected.

Clinically significant changes were more frequently observed in patients with higher Ki67 (NEC G3 vs. NET G1: OR 3.52, $P < 0.01$), and with an unknown tumor primary site suspected

to be GEP (OR 6.54, *P* = 0.03) (Model 2, Table 3). For cases with a small-bowel primary tumor the local revision had significantly less impact on patient management (OR 0.25, *P* < 0.01) (Model 1, Table 3).

Table 3: Factors associated to clinical usefulness of the histopathological revision: univariate and multivariate analysis

Univariate analysis			
Variables	**OR**	**95% CI**	***P***
Referred from University Hospitals	0.88	0.41 – 1.86	0.73
Full referral vs. second opinion	0.93	0.38 – 2.26	0.87
Surgical specimen vs. others	1.00	0.52 - 1.92	0.99
Sample site: primary vs. metastasis	1.92	0.96 – 3.84	0.06
Tumor primary site			
Pancreas vs. others	1.19	0.62 – 2.32	0.59
Small bowel vs. others	0.20	0.08 – 0.48	< 0.01
Unknown vs. others (suspected GEP)	5,79	1.13 – 29.61	0.02
pTNM Staging *			
Stage II	2.00	0.11 – 35.80	0.64
Stage III	0.52	0.04 – 6.35	0.61
Stage IV	0.28	0.02 – 3.61	0.33
Ki67 †	1.01	1.00 – 1.03	0.03
G Grading *			
G2	1.03	0.44 – 2.39	0.94
G3	3.16	1.24 – 8.06	0.01
Multivariate analysis			
Model 1			
Variables	**HR**	**95% CI**	***P***
Small bowel tumor primary site vs. others	0.25	0.08 – 0.71	< 0.01
G Grading *			
G2	0.87	0.36 – 2.08	0.76
G3	2.08	0.78 – 5.55	0.14
Model 2			
Variables	**HR**	**95% CI**	***P***
Unknown tumor primary site vs. others	6.54	1.13 – 37.80	0.03
G Grading *			
G2	0.99	0.42 – 2.36	0.99
G3	3.52	1.36 – 9.06	< 0.01

† Continuous variable

* Categorical variable (respectively, Stage I and G1 as referral category)

GEP: gastroenteropancreatic; OR: Odds ratio; CI: confidence interval.

DISCUSSION

The importance of histological revision in expert centers has been seen in many neoplasms, with an impact on clinical decisions ranging between 7.5%-23.3%.[18-27] This is the first study investigating the clinical usefulness of histopathological re-evaluation after tumor board discussion in NET expert centers. Our results show that in 36.0% of histologically revised NETs the additional information offered by the "on site" re-assessment significantly affected patient management. In details, a surgical/endoscopic resection was proposed in 4.6% of the revised cases and a change in medical treatment in 21.7%. In an additional 21.1% of patients the histopathological re-assessment led to the need for further diagnostic tests, such as an additional ^{18}F-fluorodeoxyglucose positron emission tomography/ computerized tomography (^{18}FDG-PET/CT) for complete staging of patients with G3 NET.

According to the literature for other neoplasms, the original histological report is changed by a second evaluation in expert centers in 18.0%-33.3% of cases, and in our study a similar rate of significant discordance was observed (36.0%). This observation supports the importance of referring NET patients to expert centers in order to benefit from the long-standing experience of pathologists and the interdisciplinary team working in these sites.

In our series the proliferative index (Ki67) is the element that most often differed between original and revised reports (23.4%, Table 2). The Ki67 value represents a main prognostic factor for these neoplasms [28,29] and significant changes in its value, leading to a change in WHO 2010 classification,[12,13] may translate to a different prognosis with a differing therapeutic approach.[9] In our series an additional neuroendocrine immunostaining was performed in 2.3% of cases. These analyses have a clinical impact on patients' management, in particular to confirm the suspicion of the tumor primary location, such as CDX2 positivity for small bowel or Islet-1 for pancreas (Table 3).[30,31]

The description of cell morphology in our series determined the exclusion of a NET in 3.4% of cases and led to the diagnosis of a mixed neuroendocrine/non-neuroendocrine neoplasm in 2.9%. Cell morphology changed the histopathology report in 17.6% of NEC G3 cases confirmed by the expert revision, improving the diagnostic information available for these patients. In fact the heterogeneity of G3 NETs has been recently proven. The new WHO 2017 classification proposes two subgroups: well-differentiated NET G3; poorly differentiated NEC G3, characterized by a different prognosis and benefiting from different therapeutic approaches.[32] In expert centers, this distinction has been adopted prior to the introduction of this new classification. The addition to the report, along with the more precise Ki67 definition, was the main factor which determined a clinical impact in these patients. It is this feature, allied with the fact that only 62.9% of G3 cases were confirmed, that explains why clinically significant changes were more frequently observed in G3 than in G1 cases (Table 3).

In conclusion, histopathological revision in expert centers for NETs can change the pathological characterization and have an impact on patients' management in about one third

of cases. A multidisciplinary approach for these patients, involving the support of expert centers, should be considered for this rare entity.

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