Editorial

Grading of EUS-FNA cytologic specimens from patients with pancreatic neuroendocrine neoplasms: it is time move to tissue core biopsy?

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Abstract: Pancreatic neuroendocrine neoplasms (p-NENs) are rare and characterized by an indolent course, with a much better prognosis than non-neuroendocrine tumors of the pancreas. In the non-functional class of p-NENS, surgery remains the only curative treatment for early localized disease, but there are few therapeutic options for advanced disease. The prognosis of non-functional p-NENs is determined by many clinical criteria. In 2010, however, the World Health Organization (WHO) introduced a grading system in which determination of the Ki-67 proliferative index has become essential with key role in determining therapeutic decision in both advanced and early diseases. Conventionally, Ki-67 has been assessed on surgical specimens. In last decade, however, the availability of EUS-guided fine needle aspiration (EUS-FNA) has provided the opportunity to sample pancreatic lesions and to assess the value of this parameter pre-operatively. The few studies reporting the use of EUS-FNA cytological specimens for Ki-67 measurement showed promising results. As shown by Weynand and colleagues FNA-cytology may underestimate the staging and caution in using this method to classify tumors as low-grade (G1) should be adopted. Thus, Ki-67 expression on cytological specimens remains unsatisfactory and the need for tissue biopsy specimens has been strongly advocated. Based on a recent study that has reported a high concordance of EUS-guided core biopsy for histologic examination and surgical specimens, especially when a cut-off of 5% is used to differentiate G1 and G2 tumors, EUS tissue acquisition by core biopsy is ready for prime time and should be adopted as a standard of practice.

Keywords: Pancreatic neuroendocrine tumors; grading; proliferation index; EUS-guided fine needle aspiration (EUS-FNA); EUS-guide biopsy

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Pancreatic neuroendocrine neoplasms (p-NENs) are rare, but their incidence has significantly increased in the last decades (1). Although they represent ~1% of all pancreatic neoplasms, their prevalence is about 10%, mostly accounting for low to intermediate grade p-NENs with a relatively "indolent" clinical course (1,2). Only a minor fraction of p-NENs are high-grade neuroendocrine carcinomas (p-NEC). p-NENs are classified as functional or non-functional depending on the presence or absence of a clinical hormonal hypersecretion syndrome, and clinical management of these lesions can be very challenging.

In non-functional p-NENs, surgery remains the only curative treatment for localized disease, while there are few therapeutic options for advanced disease (3). The prognosis of patients with non-functional p-NENs can be predicted by the World Health Organization (WHO) 2000 classification, and by a specific TNM staging system adopted by the European Neuroendocrine Tumors Society (ENETS) and

more recently by the American Joint Cancer Committee (AJCC) and the new WHO 2010 classification (4,5). The clinical value and applicability of this ENETS-TNM system has been extensively validated (5,6), and the central importance of the grade of the tumor as determined by the Ki-67 proliferative index for prognosis within each TNM stage has been confirmed (6).

Because of its prognostic significance, Ki-67 proliferative index has a key role in determining therapeutic decision in both advanced and early diseases. In patients with unresectable tumors, the most appropriate first-line therapeutic regimen is chosen based on the degree of cell proliferation index and may include different somatostatin analogs, targeted therapies (i.e., everolimus and sunitinib), peptide receptors targeted therapy, and different chemotherapeutic schedules (5,7). Similarly, in patients with non-functional p-NENs less than 2 cm, where no study has demonstrated a survival benefit of surgery and the risk of malignancy is low, the choice between surgery with the associated morbidity and mortality and clinical follow up strongly depends on the tumor site and the value of Ki-67 proliferation index (5). In this scenario, knowledge of Ki-67 determination may prove fundamental for the discussion with a patient regarding pros and cons of available therapeutic options.

Since its initial report in 1992 (8), EUS-guided fine needle aspiration (EUS-FNA) has progressively become the procedure of choice to obtain definitive diagnosis of pancreatic lesions, including p-NENs (9). Several studies have shown that EUS-FNA has sensitivity and accuracy ranging from 80-90% for the diagnosis of p-NENs (9). Importantly, not only can EUS-FNA confirm the neuroendocrine nature of the pancreatic lesion, but can also give prognostic information by predicting the 5-year survival of these patients (10,11) and by assessing the grading of the neoplasia by determining the Ki-67 proliferation index (11-15). However, it remains unclear whether the Ki-67 index obtained by EUS-FNA specimens is truly representative of the Ki-67 index in the resected tumor (16). Other areas of uncertainty are whether tumor cells in the highest Ki-67 index cluster on the slide or all tumor cells on the slide should be measured, and what factors influence the concordance rates between the Ki-67 index determinations in EUS-FNA specimens and those from resected specimens.

In a recently published study by Weynand and colleagues (17), the authors tried to answer some of the above mentioned questions. They retrospectively evaluated the accuracy of Ki-67 on EUS-FNA specimens to predict the definitive grade of p-NENs, and determined the inter-observer agreement and the relationship between cytologic tumor grading and progression-free survival (PFS). Forty-six p-NENs (57% located in the pancreatic head) from 45 patients that were diagnosed by EUS-FNA over a 14-year period (mostly over an 8-year period) were analyzed. Thirty-three (50%) of the punctured lesions (mean diameter 33±25 mm) were resected. A mean of 330±180 cells were counted on cytologic specimens (min 100, max 950), with at least 200 cells counted in 37/46 (80%) of the samples. On surgical specimens, a mean number of 2,001±49 cells were counted (min 1,480, max 2,130). A very good inter-observer agreement in the grading evaluation of both EUS-FNA (kappa index =0.93) and surgical (kappa index =0.94) specimens between two investigators with an expertise and experience in p-NENs pathology was found. On the other hand, a poor correlation between cytologic and surgical histologic samples was found (kappa index =0.21). Discrepancies were observed mainly for histologic G2 p-NENs, where cytology underestimated grading in 10 out of the 14 cases (71%). Moreover, two of seven tumors classified as G2 on EUS-FNA were G1 on surgical specimens, while of the three patients with G3 on the surgical specimen two were classified as G1 and G2 on EUS-FNA, respectively. This poor correlation remained unchanged even in the subgroup of lesions where more than 200 cells could be counted and when a cut-off of 5% was used to distinguish G1 from G2 tumors.

The discrepancy between cytology and histology was attributed to the fact that cellularity of EUS-FNA is variable and that EUS-FNA may not always sample the most mitotically active tumor areas, whereas on a histologic specimen one can easily determine the area of strongest nuclear labelling and count the minimum number of neoplastic cells as defined by ENETS and WHO. The heterogeneity of mitotic activity in p-NENs may clearly explain this sampling error (16). The ENETS and WHO grading system recommend that at least 40-50 high power fields should be counted for mitoses and the area with highest labeling should be used to determine the Ki-67 index, with at least 2,000 tumor cells counted (18).

The authors discussed that as the FNA-cytology may tender to underestimate the staging obtained on surgical specimen, one should be very cautious in using this method to classify a tumor as low-grade (G1) (17). On the other hand, a G3 on cytology is very meaningful and indicates a poor prognosis. Overall, the study is interesting and well

conducted and it is currently the largest study where roust analysis of Ki-67 between FNA-cytology and histology of surgical specimen was performed, with a good inter-observer correlation.

The study pointed out the inadequacy of FNA cytology especially in G2 tumors. The recent availability of better needles for tissue acquisition may circumvent this problem. We have recently evaluated the feasibility, yield, and clinical impact of EUS-guided fine-needle tissue acquisition (EUS-FNTA) with a 19-gauge needle to obtain tissue core biopsy samples for histologic diagnosis and Ki-67 analysis in a prospective series of patients with suspected non-functional p-NENs (19). The sample was adequate in 93% of patients, and, notably, the concordance rate for the grading of the tumor based on Ki-67 index between the FNTA histology and histology of the surgical specimen was 83%, and only 2/12 patients were upstaged from G1 to G2 or downstaged from G2 to G1 after surgery, respectively. Moreover, when a cut-off of 5% was used to distinguish G1 and G2 tumors a 100% concordance was found. As these results were obtained in a relatively small cohort of patients, further larger multicentre studies should be performed to evaluate the reproducibility of Ki-67 proliferation index in p-NENs patients and to analyze the relationship between preoperative grading and PFS, and stratify patients for appropriate treatment modalities based upon their preoperative grading.

As many small p-NENs are diagnosed incidentally, surgery might not be indicated and observation is generally safe (5). In this scenario, the initial correct classification in terms of proliferative activity might be crucial information to discuss with the patient further management. Furthermore, as immunohistochemical analyses to evaluate the role of activity of relevant pathways might predict the response to targeted agents, EUS-FNTA in p-NENs might also prove relevant to plan appropriate treatments in more advanced patients (20).

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References

1. Yao JC, Hassan M, Phan A, et al. One hundred years after "carcinoid": epidemiology of and prognostic factors for neuroendocrine tumors in 35,825 cases in the United States. J Clin Oncol 2008;26:3063-72.
2. Fitzgerald TL, Hickner ZJ, Schmitz M, et al. Changing incidence of pancreatic neoplasms: a 16-year review of statewide tumor registry. Pancreas 2008;37:134-8.
3. Panzuto F, Nasoni S, Falconi M, et al. Prognostic factors and survival in endocrine tumor patients: comparison between gastrointestinal and pancreatic localization. Endocr Relat Cancer 2005;12:1083-92.
4. Rindi G, Petrone G, Inzani F. The 2010 WHO classification of digestive neuroendocrine neoplasms: a critical appraisal four years after its introduction. Endocr Pathol 2014;25:186-92.
5. Falconi M, Bartsch DK, Eriksson B, et al. ENETS Consensus Guidelines for the management of patients with digestive neuroendocrine neoplasms of the digestive system: well-differentiated pancreatic non-functioning tumors. Neuroendocrinology 2012;95:120-34.
6. Rindi G, Falconi M, Klersy C, et al. TNM staging of neoplasms of the endocrine pancreas: results from a large international cohort study. J Natl Cancer Inst 2012;104:764-77.
7. Ganetsky A, Bhatt V. Gastroenteropancreatic neuroendocrine tumors: update on therapeutics. Ann Pharmacother 2012;46:851-62.
8. Vilmann P, Jacobsen GK, Henriksen FW, et al. Endoscopic ultrasonography with guided fine needle aspiration biopsy in pancreatic disease. Gastrointest Endosc 1992;38:172-3.
9. Attili F, Capurso G, Vanella G, et al. Diagnostic and therapeutic role of endoscopy in gastroenteropancreatic neuroendocrine neoplasms. Dig Liver Dis 2014;46:9-17.
10. Figueiredo FA, Giovannini M, Monges G, et al. EUS-FNA predicts 5-year survival in pancreatic endocrine tumors. Gastrointest Endosc 2009;70:907-14.
11. 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;46:32-8.
12. Figueiredo FA, Giovannini M, Monges G, et al. Pancreatic endocrine tumors: a large single-center experience. Pancreas 2009;38:936-40.
13. Piani C, Franchi GM, Cappelletti C, et al. Cytological Ki-67 in pancreatic endocrine tumours: an opportunity for pre-operative grading. Endocr Relat Cancer 2008;15:175-81.
14. Chatzipantelis P, Konstantinou P, Kaklamanos M, et al. The role of cytomorphology and proliferative activity in predicting biologic behavior of pancreatic neuroendocrine tumors: a study by endoscopic ultrasound-guided fine-needle aspiration cytology. Cancer 2009;117:211-6.

15. Alexiev BA, Darwin PE, Goloubeva O, et al. Proliferative rate in endoscopic ultrasound fine-needle aspiration of pancreatic endocrine tumors: correlation with clinical behavior. Cancer 2009;117:40-5.
16. 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;35:853-60.
17. 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 2013. [Epub ahead of print].
18. Klöppel G, Couvelard A, Perren A, et al. ENETS Consensus Guidelines for the Standards of Care in Neuroendocrine Tumors: towards a standardized approach to the diagnosis of gastroenteropancreatic neuroendocrine tumors and their prognostic stratification. Neuroendocrinology 2009;90:162-6.
19. 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;76:570-7.
20. Capurso G, Fendrich V, Rinzivillo M, et al. Novel molecular targets for the treatment of gastroenteropancreatic endocrine tumors: answers and unsolved problems. Int J Mol Sci 2012;14:30-45.

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