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Fine-needle aspiration–based grading of pancreatic neuroendocrine neoplasms using Ki-67: is accurate WHO grading possible on cytologic material?

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Introduction The World Health Organization (WHO) has provided grading guidelines for pancreatic neuroendocrine neoplasms (PanNENs) based on mitotic count and Ki-67 proliferation index. Driven by the desire to provide earlier tumor grading for clinical management decisions, some groups have proposed grading PanNENs at the time of fine-needle aspiration (FNA) using Ki-67 proliferation rates. Although a Ki-67 rate can be performed on FNA cell blocks, there are potential sampling limitations with this technique that may affect the reliability of the Ki-67 result.

Materials and Methods Forty-nine PanNENs with FNA cell blocks and corresponding resection material were evaluated by immunohistochemistry for expression of Ki-67. Ki-67 proliferation rate was calculated based on cell counts >500 cells in the highest-staining areas. Ki-67 scores from FNA cell blocks were correlated with Ki-67 scores from resection specimens.

Results The FNA Ki-67 proliferation rates overall did not correlate well with the resection specimen. A linear regression analysis of the correlation between FNA %Ki-67 and resection %Ki-67 showed a slope of 3.2 and an $R^2 = 0.58$. The average difference in Ki-67 proliferation rate between FNA and resection was 5.9%. Thirty-nine percent (19 of 49 cases) of PanNENs showed discordant grading between the FNA cell

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block and resection specimen. Almost all (18 of 19) discordant cases demonstrated a lower FNA-based grade than the resection grade.

Conclusions FNA cell block grading using Ki-67 rates frequently led to under-grading of the tumor. This finding is consistent with concerns that FNA may not provide accurate grading because of the limited sampling of the tumor.

Introduction

Pancreatic neuroendocrine neoplasms (PanNENs) are rare tumors that represent approximately 2% of pancreatic primary tumors.[1] For many years it has been felt that both the stage and grade of these tumors are important for predicting recurrence and metastasis. The mitotic count and Ki-67 proliferative index have been consistently found to be markers of patient prognosis.[2] As a result, the European Neuroendocrine Tumour Society (ENETS) developed a grading system of PanNENs based on the mitotic count and Ki-67 proliferative index.[3] The World Health Organization (WHO) adapted these recommendations for the 2010 classification.[4] Under this current classification system, grade 1 neuroendocrine tumor is defined by <2 mitoses per 2 mm^2 in 10 high-power fields and/or Ki-67 labeling index $<3\%$; grade 2 is defined as 2 to 20 mitoses per 2 mm^2 and/or 3-20% Ki-67 labeling index; and neuroendocrine carcinoma is defined by >20 mitoses per 2 mm^2 and a Ki-67 proliferative index $>20\%$. The Ki-67 proliferative index should be defined by the area with the highest percent nuclear staining and recommends at least 500 to 2000 nuclei to be counted.

Standard of care for pancreatic lesions now involves the use of endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) to sample the lesion and provide an accurate diagnosis. There has been much debate, however, as to whether these small FNA biopsies are sufficient for the accurate grading of PanNENs. Given the small amount of material aspirated in these biopsies, obtaining an accurate representation of heterogeneous and large neoplasms can be challenging. On the other hand, surgical resection and resection-based grading of the primary tumor may not always be feasible. A handful of studies have attempted to address the concordance of FNA-based grading and surgical resection-based grading.

To date, the majority of studies of PanNEN EUS-FNA have demonstrated concordance between the FNA grading using Ki-67% and the resection grading. One study of 22 PanNENs demonstrated 86% concordance between FNA grading and resection grading and suggested that EUS-FNA samples could provide accurate grading of the tumor.[5] Of the 22 PanNENs evaluated, 3 showed discordance of the FNA grading with 2 of 3 being underestimated of the grade. Unno et al evaluated 19 PanNENs and showed that 13 patients were concordant and only 6 patients had discrepant FNA grading. They argue that the discrepant cases had significantly larger tumor size.[6]

We propose that a larger study is needed to evaluate an increased number of cases using a more systematic and reproducible Ki-67% evaluation method. We present 49 patients with both PanNEN FNA cell blocks and resection specimens to evaluate the concordance between FNA-based PanNEN grading and surgical resection-based grading. We propose that using larger case numbers, as well as systematic and manageable counting regimens, will help to more accurately predict the concordance of PanNEN FNA-based grading in clinical practice.

Materials and methods

Patient selection

In this institutional review board–approved study, paired FNA cell blocks and resection material collected between 1998 and 2015 were retrieved from Vanderbilt University Medical Center pathology archives. Forty-nine patients were identified in the archives with both adequate FNA cell block and resection material. FNA cell blocks were considered adequate if they contained at least 500 tumor cells. Clinical information including the patient age, sex, whether syndromes were diagnosed, and outcome were recorded. Tumor characteristics including WHO grade, size, functionality, presence of lymphovascular invasion, presence of perineural invasion, necrosis, degree of infiltration, and metastases were recorded.

Hematoxylin and eosin–stained 4 μm sections from corresponding cell block and surgical resection specimens were reviewed by two pathologists (VW, CS) to confirm the cytopathologic and histopathologic diagnoses. Tumor differentiation was assessed using the 2010 WHO classification. On cytologic specimens, the diagnosis was confirmed using neuroendocrine markers including synaptophysin and chromogranin.

Immunohistochemistry

Four-micron sections from both the FNA and corresponding resection material were evaluated by immunohistochemistry for expression of Ki-67. Ki-67 staining was performed according to manufacturer's protocols. FNA cell blocks were also stained using CD45 to confirm that the Ki-67 labeled cellularity represented tumor cellularity rather than tumor-infiltrating immune cells.