Research Article

Dual-color immunocytochemistry (Ki-67 with LCA) for precise grading of pancreatic neuroendocrine tumors with applicability to small biopsies and cell blocks

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ABSTRACT

Ki-67 (MIB-1) immunostaining to quantify the proliferative index of neuroendocrine tumors (NETs) has been recommended (especially for small biopsies). However, this has a number of challenges with nonrepresentative Ki-67 index due to interference by Ki-67 immunoreactive proliferating lymphocytes infiltrating the tumor and also some proliferating stromal cells including endothelial cells in the background. Our pilot project showed that dual-color immunostaining with inclusion of leukocyte common antigen (LCA) (Ki-67: nuclear brown; LCA: cytoplasmic red) can facilitate the weeding out of lymphocyte interference. We analyzed the results with 23 surgical cases of pancreatic NETs. This was followed by poststudy examination of 11 cases of endoscopic ultrasound-guided fine-needle aspiration of the pancreatic NETs (PanNETs) to evaluate the findings of the study.

Dual-color immunostaining for Ki-67 with LCA increased the precision of quantifying Ki-67 index, due to ability to exclude LCA immunoreactive lymphocytes. Other nontumor Ki-67 immunoreactive cells such as endothelial and stromal cells could be distinguished morphologically.

Digital methods were also attempted, but this approach could not distinguish infiltrating lymphocytes and other cells in sections resulting in erroneous results. This study demonstrated that grading of PanNET can be performed with increased precision with dual-color Ki-67 immunostaining protocol standardized in this study. As evaluated on a few cytopathology cases, this protocol is especially useful for the evaluation of small biopsies and cell block sections of fine-needle aspiration biopsy material where 50 high-power fields cannot be evaluated but have >500 tumor cell nuclei.

Keywords: Pancreas fine-needle aspiration, Endoscopic ultrasound-guided fine-needle aspiration, Pancreatic neuroendocrine tumors

INTRODUCTION

The pancreatic neuroendocrine tumors (PanNETs) are uncommon and represent 1%–2% of all pancreatic neoplasms (in adults between 40 and 60 years of age without sex predilection).¹ ⁵ The WHO grading system for PanNETs is essentially based on tumor mitotic activity with or without necrosis or the Ki-67 proliferative index (PI) expressed as the percentage of immunostained tumor cells with Ki-67 nuclear immunoreactivity.⁶ ⁷ Although PI correlates with clinical outcome⁸ ¹⁰ and is critical for its precise grading, it is relatively challenging to count them precisely with resultant nonreproducibility related to prognostic trends for a given case. Some approaches have recommended actual marking and counting of immunoreactive and nonimmunoreactive tumor cell nuclei on the printout of the image or on the screen with image.⁹
However, routine one-color Ki-67 immunostaining cannot achieve precise counting of actual tumor cell nuclei without correcting the interference due to infiltrating/contaminant reactive lymphoid population. This may grade any particular tumor into inappropriate grade, especially the tumors in gray zones.\[9-11\] There is a lack of consistent recommendations to overcome these issues.\[12,13\]

In this study, we evaluated methods to overcome counting interference due to reactive infiltrating lymphocytes in PanNETs using dual-color immunostaining protocol: Ki-67 – nuclear brown and LCA – cytoplasmic red reported recently by Shidham's group.\[14\] This protocol was reported to increase the accuracy of calculating Ki-67 index and prevent improper grading of neuroendocrine tumors (NETs). This study is for further evaluation of the protocol for routine application in surgical pathology, especially in smaller samples (biopsies and cytopathology cell blocks).

**MATERIAL AND METHODS**

The current study was performed after approval by the Institutional Review Board (IRB). Twenty-three surgical specimens (over a period of 6 years) interpreted as well-differentiated (Grade 1 and Grade 2) PanNETs were evaluated. This was followed by studying cell blocks of cytopathology specimens by evaluating 7 of 15 endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) of PanNETs as a final or differential diagnosis (over a period of 6 years). The cytology cases were correlated with surgical specimen grades and/or clinical outcome with follow-up periods ranging from 1 to 6 years. Because grading of low-grade tumors in gray zone with lower Ki-67 indices (<3% for Grade 1 and 3% or more for Grade 2) was more challenging, only these cases were studied.

**Immunostaining and evaluation**

Four-micrometer thick serial sections were cut from selected formalin-fixed, paraffin-embedded (FFPE) blocks from 23 cases of PanNETs. These FFPE blocks were selected based on the areas with worst grade for that lesion (based on number of mitotic figures and/or necrosis). Two adjacent sections were stained, respectively, by two methods [Table 1].

**Method I [Table 1]**

Step-by-step protocol for dual-color immunostaining with Ki-67 and LCA: Shidham's protocol on one of the sections:

1. Deparaffinization on line at 72°C
2. Heat-induced epitope retrieval (HIER): Roche/Ventana Cell Conditioner #1 (pH 8.0) ready to use for 36 min at 95°C
3. Ki-67 (clone 30-9) ready to use Roche/Ventana antibody incubated at 37°C for 32 min
4. Roche/Ventana ultraView Universal DAB Detection Kit, as per encrypted Roche/Ventana protocol
5. LCA (clone 2B11 and PD7/26) ready to use Roche/Ventana antibody incubated at 37°C for 32 min

**Table 1: The details of the immunostaining protocols.**

| Method | Antibody details | Chromogen | Staining pattern |
|--------|------------------|-----------|------------------|
| Method I: Dual color immunostaining with Ki-67 and LCA | First antibody Brown nuclear - Ki-67 Clone: 30-9 Source: V Ventana Corporation Tucson, AZ, USA Dilution: Ready to use Duration: 32 min Second antibody Red cytoplasmic LCA Clone: 2B11 and PD7/26 Source: Ventana Dilution: Ready to use Duration: 32 min | First DAB chromogen staining ultraView Universal DAB Detection Kit (Ventana™) | Ki-67: Nuclear brown LCA: Cytoplasmic red |
| Method II: Single-color immunostaining with Ki-67 | Conventional single-color immunostaining for Ki-67 (without LCA) Clone: 30-9 Source: Ventana Dilution: Ready to use Duration: 32 min | DAB chromogen staining ultraView Universal DAB Detection Kit (Ventana™) | Ki-67: Nuclear brown |

DAB: Diaminobenzidine tetrahydrochloride, LCA: Leukocyte common antigen
6. Roche/Ventana ultraView Universal Alkaline Phosphatase Red Detection Kit, as per encrypted Roche/Ventana protocol
7. Counterstain on line with Roche/Ventana Hematoxylin II for 12 min
8. Postcounterstain with Roche/Ventana Bluing Reagent for 4 min

All the steps 1–8 were performed on line, on the instrument, Ventana Ultra BenchMark.

Immunostaining pattern: Ki-67 – nuclear brown and LCA – red cytoplasmic.

This protocol was finalized after evaluating a few other combinations as discussed later under discussion.

Method II [Table 1]
Single-color immunostaining for Ki-67 (without LCA) on adjacent section:
1. Deparaffinization on line at 72°C
2. HIER: Roche/Ventana Cell Conditioner #1 (pH 8.0) ready to use for 36 min at 95°C
3. Ki-67 (clone 30-9) ready to use Roche/Ventana antibody incubated at 37°C for 32 min
4. Roche/Ventana ultraView Universal DAB Detection Kit, as per encrypted Roche/Ventana protocol
5. Counterstain on line with Roche/Ventana Hematoxylin II for 12 min
6. Postcounterstain with Roche/Ventana Bluing Reagent for 4 min

All the steps 1–6 were performed on line, on the instrument, Ventana Ultra BenchMark.

Immunostaining pattern: Ki-67 – nuclear brown.

Tonsillar tissue was used as a positive control. The negative controls were run simultaneously with the primary antibody replaced by a buffer.

All of the surgical cases were initially graded by counting mitotic figures in 50 high-power fields (HPF) with or without necrosis [Table 2].[13] Preliminarily histomorphological grading was based on the WHO 2010 classification system: grade 1 (well differentiated) – mitotic figures 0–2/10 HPF without necrosis or Ki-67 proliferation index <3%; Grade 2 (intermediate grade) – mitotic figures 2–20/10 HPF with or without necrosis or Ki-67 proliferation index 3%–20%; and Grade 3 (high-grade neuroendocrine carcinomas) = mitotic figures >20/10 HPF with or without necrosis or Ki-67 proliferation index >20% [Figures 1-3 and Table 2].

Seven of the 23 cases were classified as Grade 1 and 16 of the 23 cases were classified as Grade 2. Of these, 15 cases of EUS-FNA with PanNET in differential diagnosis from January 1, 2011, to January 9, 2017, were also evaluated. Of these, 11 were PanNETs, and dual-color immunostaining could be evaluated in 7 of these 11 cases with appropriately cellular cell blocks [Table 3].

Each case was reviewed with an Olympus BX40 microscope by three reviewers (LMB, NS, and VS). Two methodologies were used: (a) manual counting of camera-captured printed image[9,10] and (b) automated counting with digital method (Ventana Image Analysis System [VIAS], Ventana Medical Systems, Inc; Oro Valley, AZ, USA).

For the automated counting, the Ki-67 labeling index was calculated with digital method (VIAS). The entire slide was scanned at ×4 objective. The area with the highest nuclear labeling for the section with only Ki-67 was selected, and for the dual-stained sections (LCA – red cytoplasmic/Ki-67 – nuclear brown), the area with less red cytoplasmic staining and higher number of Ki-67 brown immunostaining was selected. Then, a quantitative analysis was performed for each.

For the manual count method, each slide was manually scanned at ×10 objective, and the area with the greatest Ki-67 nuclear labeling was selected for photographing and printing. Similarly, for the sections that were dual-color immunostained (LCA with Ki-67), areas with the least red cytoplasmic labeling and higher number of Ki-67 brown immunostaining were selected for photographing and printing properly labeled printout of the images.

A count of 500 tumor nuclei was performed for both conventional Ki-67 single-color method with nuclear brown immunostaining and for the dual-color immunostained slides. In the single-color stained slides, the cells showing nuclear immunostaining were crossed off (nontumor cells were excluded). Light brown or pale staining nuclei as well as cells that morphologically did not resemble a tumor cell were not counted. For the dual-stained slides, only the cells showing nuclear positivity with Ki-67 without cytoplasmic red LCA immunostaining were counted. All the cells (leukocytes) showing red cytoplasmic staining were not counted. Any morphologically identifiable nontumor/stromal cells (proliferating endothelial cells/myofibroblasts) were also excluded.

Ki-67 index was calculated as a percentage (number of cells with nuclear immunoreactivity for Ki-67 per 100 tumor

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Table 2: Grading system for pancreatic neuroendocrine tumors.[14,15]

| Grade                  | Morphologic criteria                      | Ki-67 index (%) |
|------------------------|------------------------------------------|-----------------|
| Low grade well differentiated | <2 mitoses/50 hpf without necrosis                  | <3              |
| Intermediate grade well differentiated | 2–50 mitoses/50 hpf OR foci of necrosis | 3–2             |
| High grade poorly differentiated | >50 mitoses/50 hpf                        | >20             |
cell nuclei) with both the single- and dual-color protocols. The results were compared using the Wilcoxon test for two staining methods and correlated with final grading.

RESULTS

Since the software of the program used in the study could not distinguish the nuclear staining of tumor cells from the nuclear staining of leukocytes, the digital automated method could not distinguish tumor cells infiltrating lymphocytes/leukocytes and other cells. With the dual staining, the available software could not correct for the Ki-67 immunoreactive nontumor cell nuclei with red cytoplasmic immunostaining for LCA (leukocytes). The digital automated method overestimated the Ki-67 counts in the sections stained by both the methods and so digital approach was abandoned and was not evaluated further because of the limitation of the currently available software.

Dual-color immunostaining with Ki-67 and LCA (Method I) estimated Ki-67 index with higher precision (average of 1%, range: 0%–3%) for Grade 1 cases. The important observation was that the range with routine single immunostaining protocol (Method II) was 0%–4% with potential miscategorization of some of these Grade 1 cases into Grade 2. This difference was statistically significant ($P = 0.038$). Higher precision with dual-color immunostaining protocol was due to the ability to exclude lymphocytes/leukocytes (with cytoplasmic red LCA immunoreactivity) infiltrating the tumor with dual-color protocol [Table 4].

Similarly, for Grade 2 PanNET cases, dual-color immunostaining with Ki-67 and LCA (Method I) also estimated Ki-67 index with higher precision with an average of 6% (range: 0%–31%). Ki-67 index with conventional single staining protocol (Method II) was slightly higher (average: 7%, range: 4–40) due to the ability to exclude lymphocytes/leukocytes with cytoplasmic red LCA immunoreactivity. This difference was statistically significant ($P = 0.004$) [Table 4]. The most important observation was that the range for Method II (conventional single staining) was

| Patient | Sex | Final grading (C/S) | Grade (PI) | Clinical course |
|---------|-----|---------------------|------------|----------------|
|         |     | Grading on surgical specimen with routine one-color Ki-67 (%) | Grading on cell block (cytopathology) dual color Ki-67/LCA (%) | |
| 1       | Male| Grade 1 (C)         | NA         | 1 (1)          | Developed colon cancer (adenocarcinoma) |
| 2       | Female | Grade 1 (C/S) | 1 (1)     | 1 (2)          | Surgical with PNI, LVI, Ki-67 PI (1%) flushing/diarrhea |
| 3       | Male | Grade 1 (C)         | NA         | 1 (2)          | No progression |
| 4       | Male | Grade 1 (C)         | NA         | 1 (1)          | No progression |
| 5       | Female | Grade 2 (C) | 1 (1) | 2 (18.50)     | Liver metastasis, LVI, peripancreatic fat invasion, Ki-67 PI: 1% (Note: Ampulla of Vater biopsy - Grade 2 (Ki-67 PI: 5%–10%) |
| 6       | Male | Grade 2 (S)         | 2 (5–10)  | No block      | Surgical biopsy Ki-67 PI: 5%–10% |
| 7       | Female | Grade 2 (C) | NA       | 2 (6)          | Succumb to disease (retropertioneal invasion), treated with Xeloda and Afinitor |
| 8       | Female | Grade 2 (C) | NA       | 2 (5)          | Liver metastasis with radioembolization |
| 9       | Male | Grade 3 (C)         | NA       | NA            | Died from MVA |
| 10      | Female | NET (Grade NA) | NA       | NA            | No follow up |
| 11      | Female | NET (Grade NA) | NA       | Too scant diagnostic material | Liver metastasis on capcitabine |

MVA: Motor vehicle accident, NET: Neuroendocrine tumor, NA: Not available, PI: Proliferation index, C: Cytopathology, S: Surgical pathology, PNI: Perineural invasion, LVI: Lymphovascular invasion, LCA: Leukocyte common antigen

Table 3: Cases with endoscopic ultrasound-guided fine-needle aspiration of pancreatic neuroendocrine tumors (in which cell blocks with adequate diagnostic material were available).

Table 4: Comparison of Ki-67 proliferative index with two methods on surgical specimens.

| PanNET grade* | Average Ki-67 labeling | $P$ |
|---------------|------------------------|-----|
|               | Method I (%) | Method II (%) |   |
| 1 (7 cases)   | 1 (0–3) | 3 (0–4) | 0.038 |
| 2 (16 cases)  | 6 (0–31) | 7 (2–40) | 0.004 |

*Based on histomorphological evaluation. Method I: Dual-color immunostaining with Ki-67 (brown nuclear) and LCA (red cytoplasmic), Method II: Single-color immunostaining for Ki-67 (without LCA) on adjacent section. PanNET: Pancreatic neuroendocrine tumor, LCA: Leukocyte common antigen
quite broad with the highest count up to 40% with potential miscategorization of some these Grade 2 cases into Grade 3. Dual-color Ki-67 immunostaining simplified the interpretation and improved the precision of manual counting. Figures 1 and 2 show PanNETs immunostained with dual- and single-color immunostaining protocols for Ki-67 PI. Routine single-color protocol with brown nuclear staining for Ki-67 (MIB-1) could not facilitate the identification of leukocytes from tumor cells. Dual-color immunostaining, Ki-67 (nuclear brown) and LCA (cytoplasmic red), however, could allow easy weeding out of leukocytes. Other nontumor Ki-67 immunoreactive cells such as endothelial cells could be distinguished morphologically by both manual methods.

In general, Method II (conventional single-color Ki-67 immunostaining) had a tendency for falsely higher values with potential for miscategorization with a tendency to upgrade the tumor.

Of 15 EUS-FNA with differential diagnosis of PanNETs, 11 retained final diagnosis of PanNETs after clinical correlation. Eight of these cases had cell blocks with high enough cellularity to perform Shidham's dual-color immunostaining protocol for Ki-67 PI. Grade 1 PanNETs accounted for 4 cases, of which 3 were treated nonsurgically and 1 case was treated with surgical resection. The surgical resection in this case [patient #2, Table 3] of Grade 1 PanNET retained its initial low-grade status (graded based on single-color immunostaining for Ki-67 without LCA). Grade 2 PanNETs accounted for 4 cases, of which 2 were treated surgically and 2 cases were treated nonsurgically. Correlation with surgical resection in one of the cases (patient #5) showed Grade 1 on surgical resection. The ampulla of Vater biopsy, however, showed Grade 2 NET similar to grading on EUS-FNA cell block with dual-color protocol. This highlights the issue related to sampling artifact because only one randomly selected block from surgical resection was immunostained. In contrast, FNA procedure with multiple passes sampled different areas of lesion with better sampling of representative areas with correct grading which matched with ampulla of Vater biopsy and the clinical progression in this case #5. Grade 3 PanNETs accounted for 1 case which did not have a surgical resection due to unrelated death. Two cases could not be graded due to low cellularity of cell block, and surgical resection was not performed.

**DISCUSSION**

It has been acknowledged that Ki-67 labeling index correlates with prognosis and predicts disease recurrence/overall survival in PanNETs. It is a requirement in the College of American Pathologists, European Neuroendocrine Tumor Society, North American Neuroendocrine Tumor Society, and American Joint Commission on Cancer protocols to use Ki-67 labeling index for the grading of PanNETs. In addition, for small biopsies and cell blocks of cytopathology specimens, calculation of number of mitotic figures in 50 hpf is frequently not possible and focal necrosis may not be sampled due to sampling artifact related to the chance factor. Thus, PI with Ki-67 is a better approach for
grading PanNETs than parameters based only on number of mitotic figures with/without necrosis. However, precise calculation of Ki-67 PI by routine one-color immunostaining is also plagued with challenges. Reid et al. and Adsay have already described that counting by eyeballing is no longer an option and that the best method of counting is the manual count by marking on the computer screen or paper print. The main problem is that routine one-color Ki-67 immunostaining cannot distinguish the PanNET cells from nonneoplastic leukocytes. This is true not only for small specimens such as biopsies or cell blocks of FNA but also for resection specimens.

Care should be taken to distinguish Ki-67 labeling in tumor cells from Ki-67 labeling in leukocytes and also, in stromal cells, histiocytes, and epithelial cells, which may also display proliferative activity. A principle challenge in calculating Ki-67 index precisely and reproducibly with conventional one-color immunostaining is an inability to weed out Ki-67 immunoreactive leukocytes from PanNET cells. Due to lack of morphological limitations, this may lead to nonrepresentative PI count (usually falsely higher count) with conventional single-color Ki-67 immunostaining.

Prior to finalization of the current Shidham’s dual-color Ki-67 protocol, we also tested various combinations of

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**Figure 3:** (Case #5, Table 3). (a) Low magnification (×10) of Pap-stained cytology preparation of fine-needle aspiration of neuroendocrine tumor. (b) Cell-block section immunostained with dual color Ki-67 (Brown, nuclear) with LCA (Red, cytoplasmic). (c) Low magnification (×10) shows a cell block section immunostained with, dual-color immunohistochemistry with inclusion of LCA (Ki-67 nuclear brown and LCA cytoplasmic red) which allows ID of leukocytes in small cell block sections (higher magnification – e). (d) High magnification (×40) of dual-color immunohistochemistry showing LAC immunoreactive leukocytes with red cytoplasmic immunohistochemistry ((for zoomed higher magnification – f) and Ki-67 immunoreactive tumor nuclei of neuroendocrine tumor without red cytoplasmic LCA immunoreactivity (higher magnification - g).
dual-color immunostains for precise counting of tumor cell nuclei. Dual-color Ki-67 (nuclear brown) with synaptophysin (cytoplasmic red) allowed confirmation of tumor cells, but the red background of tumor was too busy and distracting for precise counting of Ki-67 immunoreactive brown tumor cell nuclei, thus missing the lymphocytes if any in the busy background. Similarly, other combinations with other neuroendocrine immunomarkers such as (a) dual-color Ki-67 (nuclear brown) with chromogranin (cytoplasmic red) or (b) dual-color Ki-67 (nuclear brown) with CD56 (cytoplasmic red) were also not suitable. The most suitable combination was Ki-67 (brown nuclear) and LCA (red cytoplasmic) as it allowed identification and weeding out of Ki-67 immunoreactive lymphocytes with dual nuclear brown and red cytoplasmic immunostaining. We are recommending the application of Shidham's dual-color Ki-67 protocol (dual-color immunostaining – Ki-67: brown nuclear with LCA: red cytoplasmic) to distinguish Ki-67 immunoreactive tumor cells (without red cytoplasmic immunostaining) from leukocytes (cells with both nuclear brown and red cytoplasmic immunostaining). The protocol details are summarized in Table 1.

The digital automated method overestimated Ki-67 counts in the sections stained by both the methods. Hence, the digital approach could not be applied at this stage. In the future, imaging software for calculating Ki-67 proliferation index on dual-color immunostained sections is recommended to be standardized and evaluated if digital Ki-67 proliferation index can be reliably precise for dual-color protocol.

Further scrutiny with 11 EUS-FNA of PanNETs showed that our dual-color immunostaining approach for determining Ki-67 index can be applied to cytology cell blocks. The results correlated with finally resected specimens and/or clinical outcomes. In one case (patient #5), the cytology specimen correlated with the clinical outcome better than the grading on surgical resection (based on randomly selected single block). Patient #5 showed a Grade 2 NET (dual staining Ki-67 PI: 18.5%) on the EUS-FNA specimen. However, the resection specimen in this case showed Grade 1 (single staining Ki-67 PI: 1%–2%). An ampulla of Vater biopsy showed Grade 2 PanNET with a Ki-67 index of 5%–10%. Patient #5 developed a metastatic disease to the liver, two regional lymph nodes, and invasion into the peripancreatic adipose tissue with clinical progression pattern more in line with Grade 2 tumors than with Grade 1. The discrepancy between grading on surgical pathology and the cytology specimen may be due to technical aspects of how cytology specimens are obtained. A better sampling of larger areas of the tumor with cytology specimen concentrating all areas in one cell block may explain why higher grade areas were picked up representatively than by a single block of surgical resection. On all remaining cytology cases evaluated with dual-color Ki-67 immunostaining, the surgical and clinical outcomes correlated with expected with grade.

Dual-color immunostaining facilitated the distinction of Ki-67 immunoreactive tumor cells from Ki-67 immunoreactive nontumor cells with red cytoplasmic LCA immunoreactivity. Other nontumor Ki-67 immunoreactive cells such as endothelial cells could be distinguished morphologically by both manual methods. Dual-color immunostaining approach showed a higher precision due to the ability to exclude lymphocytes/leukocytes (with LCA immunoreactivity) infiltrating the tumor [Table 4]. We thus recommend Ki-67 index to be estimated based on Ki-67/LCA dual-color immunostaining.

There are a few reports highlighting the role of tumor-infiltrating lymphocytes in NETs.[20] Better prognosis associated with many lymphocytes infiltrating NET may be due to false upgrading of the tumors. Some of the tumor-infiltrating lymphocytes in a truly lower grade NET may be Ki-67 immunoreactive and could be counted in with falsely higher Ki-67 index (especially in tumors with borderline Ki-67 counts in gray zones). The true role tumor-infiltrating lymphocytes in NETs should be evaluated further by applying Shidham's dual-color Ki-67 protocol to study the potential false upgrading due to infiltrating lymphocytes. This technique may also be applicable to other similar lesions including other NETs, sarcomas, melanocytic lesions, and gastrointestinal stromal tumor where Ki-67 index is required to be estimated.

In summary, dual-color immunostaining with Ki-67 and LCA for grading NETs is an easy-to-perform precise method. Routine application of dual-color immunostaining is highly recommended, especially when the amount of tumor tissue is limited for evaluation. Based on confirmation with some cell blocks of EUS-FNA of PanNETs, small biopsies and cytopathology cell blocks of FNA material (where 50 hpf cannot be evaluated but have >500 tumor cell nuclei) should be routinely immunostained by dual-color protocol.

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COMPETING INTERESTS STATEMENT BY ALL AUTHORS

The authors declare that they have no competing interests.
AUTHORSHIP STATEMENT BY ALL AUTHORS

All authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and meet the conditions listed by the International Committee of Medical Journal Editors (ICMJE http://www.icmje.org).

Each author acknowledges that this final version was read and approved.

ETHICS STATEMENT BY ALL AUTHORS

This study was conducted with approval from the IRB (or its equivalent) of all the institutions associated with this study as applicable.

The authors take responsibility to maintain relevant documentation in this respect.

LIST OF ABBREVIATIONS (IN ALPHABETIC ORDER)

- EUS-FNA - Endoscopic ultrasound-guided fine-needle aspiration
- FFPE - Formalin-fixed, paraffin-embedded
- FNA - Fine-needle aspiration
- HPF - High-power field
- ID - Identification
- NETs - Neuroendocrine tumors
- PanNET - Pancreatic neuroendocrine tumors
- PD - Poorly differentiated
- PI - Proliferation index
- WD - Well differentiated.

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