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Tissue section thickness of Ki67 immunohistochemistry does not have a correlative effect on pancreatic neuroendocrine tumor grading

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BACKGROUND

Despite the widespread use of immunohistochemistry (IHC), there are debates on how pre-analytic variables influence diagnostic results, including the effect of varying tissue section thickness. Scant literature suggests that tissue thickness can affect stain interpretation, including nuclear stains that rely on assigning tumor cells to the positive category, such as Ki-67, and raise the question of whether tissue section thickness should be standardized for some diagnostic or prognostic tests. Grading of pancreatic neuroendocrine tumors (pNET) relies on Ki-67 score, with 3% being the threshold between grade 1 and 2. The goal of our study is to determine if variations in tissue section thickness affect calculation of Ki67 proliferation index that could affect tumor grade assignment in pNET.

METHODS

Five pNET resection specimens were selected. Sections for Ki67 were cut at 2um, 3um, 4um, and 5um, and stained with antibodies for Ki-67. Ki-67 score was determined by taking high-magnification photographs of a tumor and the corresponding area in slides cut at different microns. Using manual counting, the number of Ki-67 positive tumor nuclei was divided by the total number of tumor nuclei to calculate a Ki-67 score.

RESULTS

The total number of tumor nuclei present in a high-power photograph tended to decrease as the tissue thickness decreased; mean number of tumor cell nuclei counted across the 5 cases for 5um, 4um, 3um, and 2um sections was 1415, 1330, 1242, and 1319 nuclei, respectively. Ki67 score for each case by tissue section is provided in Table 1.

Table 1. Ki-67 score by slide section thickness for five cases of pNET.

	5 um	4 um	3 um	2 um
Case 1	5.59	5.21	4.38	4.12
Case 2	2.92	3.29	3.81	3.03
Case 3	0.90	1.04	1.25	1.00
Case 4	1.07	1.45	1.46	1.16
Case 5	5.29	6.74	7.80	6.30

CONCLUSIONS

Based on our study, thinner tissue sections did not correlate with lower Ki-67 score. Assignment of pNET grade does not require standardization of tissue thickness section for manual calculation of Ki-67 score.

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