

On the value of Ki-67 in the prognostic grading of pancreatic neuroendocrine neoplasms: an update.

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Introduction

In 1983, Gerdes, Schwab, Lemke and Stein reported upon a new monoclonal antibody which was generated by immunizing mice with nuclei of the Hodgkin lymphoma cell line L428. This antibody was found to label nuclei of proliferating cells including tumor cells. In their report the authors therefore concluded that “Ki-67 (a name derived from the city of Kiel where the authors worked, and the number 67 of the original clone in the 96-well plate) may be a potent tool for easy and quick evaluation of the proportion of proliferating cells in a tumour”(1). As the exact function of the protein was not well characterized, the initial name Ki-67 was kept. The original antibody which worked only on frozen material, was subsequently replaced by antibodies such as the monoclonal MIB-1 which were found to react with Ki-67 in formalin-fixed and paraffin-embedded tissues. Using this antibody it was possible to perform large-scale studies to assess the proliferative activity in different neoplasms (2, 3).

The systematic assessment of the proliferative activity of pancreatic neuroendocrine neoplasms (PanNEN), started in the 1990s, when it was shown that the Ki-67 index predicted malignant behavior (4, 5). As it became clear that the Ki-67 index of NENs of the pancreas, and also the gut, has great prognostic value, along with mitotic count, it was included into the WHO's NEN classifications of the GI tract (in 2000) and pancreas (in 2004), which were both based on a classification put forward by Cappella, Heitz, Höfler, Solcia and Klöppel in 1995 (6, 7). The most recent WHO classification of gastroenteropancreatic (GEP-) NENs (8), published in 2010, includes a three tiered grading, mainly based on the Ki-67 index, that was proposed by ENETS in 2006 (9). In recent years the Ki-67 index has become one of the most reliable prognostic factors of grading PanNETs and thus it is now a requirement in the reporting protocols and guidelines used by CAP, ENETS, NANETS, and AJCC.

Nature of the Ki 67 antigen

The monoclonal antibody Ki-67 identifies a 359-kD non-histone nuclear protein that is involved in the control and timing of cell proliferation and expressed in G1, G2, S, and M-phase of the cell cycle, with a maximum in the G2 and M phases. During its expression it is redistributed from the interior of the nucleus/nucleolus to the perichromosomal layer and heterochromatin (10).

Methods to assess the Ki 67 index

The evaluation of the immunohistochemical staining of Ki-67 performed with the monoclonal antibody MIB1, is the basis for the calculation of the Ki-67 index. The number of stained nuclei is expressed as a percentage (index) of immunoreactive cells. It is recommended to count between 500 and 1,000 tumor cells in randomly selected fields. The highest score (“hot spot”) is chosen as corresponding index. All immunostained nuclei of tumor cells are counted as positive regardless of staining intensity to decrease the subjectivity of the scoring process.

Among the methods to determine the Ki-67 index, manual count of a camera-captured, printed image (CCPI) appears to be the most reliable procedure (11, 12) and shows good reproducibility, although the whole evaluation process takes on the average between 10 and 15 minutes. This recommendation is based on the results of a study comparing CCPI with three other counting methods. “Eye-ball” estimation was the fastest method (average time < 1 minute), but with the poorest reliability and reproducibility. Manual eye count proved to be a rather quick way to determine the Ki-67 index, averaging between 5 and 8 minutes, but had also a poor reproducibility. Automated count was the most expensive and least practical method with major impact on turnaround time (limited by the accessibility of machine and personnel), but more importantly, had significant inaccuracies in over-counting unwanted material. However, in a paper by Tang automated counting was shown to have comparable accuracy as counting on camera-captured printed images (12). However, this statement has to be viewed with some caution, since the machine might not only count labelled endocrine cells but also lymphocytes, endothelial cells and stromal cells and thus could lead to erroneously high Ki-67 indices. Also molding of tumor nuclei, overly thick sections and background pigment may contribute to miscalculations (11).

Problems of assessing Ki 67

The immunostaining procedures for Ki-67 may differ between laboratories, although the wide use of automated staining machines has reduced this bias in recent years and has led to a kind of standardization of staining intensity. However, in a recent paper it was shown that interlaboratory variability in tissue processing and fixation, as well as different reagents and pretreatment for immunohistochemical staining, may still be the reason for considerable interlaboratory differences (13). These differences were especially obvious in low-proliferating tumors. External standardization by quality measures may help to improve reliability and reproducibility

Other problems in assessing Ki-67 stainings and calculating a reproducible Ki-67 index are intratumoral heterogeneity and the interpretation of pale brown tumor nuclei as positive. Intratumoral heterogeneity may be found synchronously within different regions of an individual tumor or among different metastatic sites, or metachronously, as higher grade metastasis may develop in the course of disease progression. While the first problem is endogenous to tumors and can only be solved by the availability of sufficient tumor tissue for evaluation, the second problem may be solved, if the staining of the tumor nuclei is compared with the staining in background non-neoplastic tissue, since stromal cells should not stain positively.

Intratumoral heterogeneity is particularly an issue of the reliability of a Ki-67 index of a PanNET metastatic to the liver and diagnosed by a radiologically guided core needle biopsy from which the Ki-67 index is determined. However, it has been shown that Ki-67 staining of core biopsies usually provides an adequately reliable method of proliferation assessment for prognosis of metastatic NETs to the liver (14).

Ki 67 index and its prognostic significance in PanNENs

In recent years it has been shown that the Ki-67 index is the most significant factor in prognosis and survival of PanNENs. This has been revealed by a number of studies using the WHO 2010 classification (15-17) which grades the well-differentiated PanNENs, called PanNETs, by their proliferative activity into either G1 PanNETs (Ki-67 index \leq 2%) or G2 PanNETs (Ki-67 index

3%-20%), and the poorly differentiated PanNENs, called PanNECs, into G3 tumors (Ki-67 index >20%) (8). In one of these studies it was suggested to use a cut-off of 5% for the G1 PanNETs, since a higher risk of progression was observed when 5% was used instead of 2% (15). However, there is so far not sufficient evidence of differences in clinical management based on this higher cut-point to justify changing it.

Recently, the distinction of well from poorly differentiated PanNENs applying the World Health Organization 2010 classification has become difficult for those few well differentiated PanNENs whose Ki-67-index exceeds 20%. Although they have retained their well differentiated neuroendocrine growth pattern, their Ki-67 index greater than 20% gives them a grade G3 that shifts them into the poorly differentiated neuroendocrine neoplasm category (18) Although these tumors appear to have a somewhat worse prognosis than G2 NETs, their behavior is still better than that of the PanNECs. In addition, they show the features (i.e. hormone expression and hormonal syndromes) usually associated with PanNETs and they appear to lack the genetic abnormalities (i.e. changes in expression and mutation of TP53 and RB1) of PanNECs (19, 20). These PanNENs have been provisionally called PanNETs G3 and their clear distinction from PanNECs is currently the topic of a number of articles (18, 20) (Konukiewitz, *Modern Pathology* in press).

Summary

Ki-67 is a proliferation marker whose nuclear expression in neoplasms is closely related to prognosis and survival. Exact counting of Ki-67 is therefore of utmost importance. In PanNENs grading is mainly based on Ki-67 counts, and the grades of the individual tumors are the basis for their therapeutic management.

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