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Review Article

# Ki-67 prognostic and therapeutic decision driven marker for pancreatic neuroendocrine neoplasms (PNENs): A systematic review

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ABSTRACT

*Background:* We systematically evaluate the current evidence regarding Ki-67 as a prognostic factor in pancreatic neuroendocrine neoplasms to evaluate the differences of this marker in primary tumors and in distant metastases as well as the values of Ki-67 obtained by fine needle aspiration and by histology.
*Methods:* The literature search was carried out using the MEDLINE/PubMed database, and only papers published in the last 10 years were selected.
*Results:* The pancreatic tissue suitable for Ki-67 evaluation was obtained from surgical specimens in the majority of the studies. There was a concordance of 83% between preoperative and postoperative Ki-67 evaluation. Pooling the data of the studies which compared the Ki-67 values obtained in both cytological and surgical specimens, we found that they were not related. The assessment of Ki-67 was manual in the majority of the papers considered for this review. In order to eliminate manual counting, several imaging methods have been developed but none of them are routinely used at present. Twenty-two studies also explored the role of Ki-67 utilized as a prognostic marker for pancreatic neuroendocrine neoplasms and the majority of them showed that Ki-67 is a good prognostic marker of disease progression. Three studies explored the Ki-67 value in metastatic sites and one study demonstrated that, in metachronous and synchronous liver metastases, there was no significant variation in the index of proliferation.
*Conclusions:* Ki-67 is a reliable prognostic marker for pancreatic neuroendocrine neoplasms.


**Contents**



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## 1. Introduction

The main adverse prognostic factors of neuroendocrine neoplasms (NENs) are the localization of the primary tumor (i.e. pancreatic NENs generally have a worse prognosis than intestinal NENs), the stage according to the tumor-node-metastasis (TNM) classification [1], and the histopathology according to the World Health Organization (WHO) classification, which expresses both the morphological appearance of the tumor and its proliferative activity (number of mitoses and proliferation index represented by Ki-67) [2].

In brief, The World Health Oraganization (WHO) 2010 classification distinguishes between well-differentiated neuroendocrine tumors (NETs) and poorly differentiated neuroendocrine carcinomas (NECs) of small or large cell type. NETs are then divided according to a grading scheme based on mitotic count or Ki 67 index in NETs-G1 (with a mitotic count $<2$ per 10 high-power fields (HPF) and/or $\leq 2\%$ Ki67 index), and NETs-G2 (with a mitotic count 2–20 per 10 HPF and/or 3–20% Ki67 index). All NECs are graded G3 (with a mitotic count $>20$ per 10 HPF and/or $>20\%$ Ki67 index) [2]. This classification has been also included in the guidelines of the European Neuroendocrine Tumor Society (ENETS) [3].

Ki-67 is an immunohistochemical stain and cells in G0 remain negative whereas cells in all other phases of the cell cycle stain positive since Ki-67 is a is a nucleolar antigen and diffuses all over the nuclear matrix only in G2 phase present only in the nuclei of cycling cells [4]. MIB-1 monoclonal antibody is the only antibody recommended by both ENETS and WHO and it is directed against different epitopes of the same proliferation-related antigen and may be used on fixed sections [5] to determine the Ki-67 labeling index which is a percentage of stained MIB-1 cells present in 500–2000 cells. This latter parameter distinguishes three different categories of grading patients, and it is now considered one of the strongest prognostic factors for NENs [6]. However, the best method to calculate the Ki67-labeling index remains controversial, as does the relative value of counting mitoses versus Ki67 labeling in determining the proliferative index [7,8]. Finally, comparison between the different studies should be carried out with caution because the immunohistochemistry method differs at different centers, and there is probably some inter-observer variability in determining the percentage of Ki-67-positive cells [4].

Our aims were to systematically review the current evidence regarding Ki-67 as a prognostic factor in pancreatic NENs (PNENs), to evaluate the differences of this marker in primary tumors and in distant metastases as well as the values of Ki-67 obtained by fine needle aspiration and by histology.

## 2. Material and methods

### 2.1. Literature search and data extraction

A search was carried out on September 11, 2014 using the MEDLINE/PubMed database (United States National Library of Medicine National Institutes of Health) with the following search strategy in order to select the data existing in the literature: (((((“Gastro-enteropancreatic neuroendocrine tumor”[Supplementary Concept] OR “Pancreatic Neoplasms”[Mesh] OR ((GEP[tiab] OR gastroenteropancreatic[tiab] OR gastro-enteropancreatic[tiab] OR gastro-entero-pancreatic[tiab]) AND neoplasms[mesh]) OR (GEP-NEN*[tiab] OR GEP OR (pancrea*[tiab] AND gastro*[tiab])) OR (pancrea*[tiab] OR NET[tiab])) AND (“Ki-67 Antigen”[Mesh] OR ki67[tiab] OR ki-67[tiab] OR MIB-1[tiab] OR “MIB 1” OR MIB1[tiab]) AND pancrea*) AND human[mesh] NOT (review[pt] OR case reports[pt]) AND English[la]) AND “last 10 years”[PDat])) OR gep-nens[tw]) Filters: published in the last 10 years.

The search was limited to human studies written in English excluding review articles and case reports. We also evaluated the possible presence of additional studies by means of a hand search of the bibliographies from the primary studies, review articles and key journals. A total of 348 citations were found in MEDLINE/PubMed. The investigators independently screened all articles for those meeting the broad inclusion criteria. As shown in Fig. 1, of the 348 papers, five were excluded because duplicate papers; thus, 343 records were screened and 231 were excluded because they contained data regarding diseases other than those pertinent to the purpose of this review, because they were review articles not containing data useful for the analyses or because they were comments on articles without new data/cases. Therefore, 112 papers with available data remained. Of these 112 papers, 61 were also excluded because they did not contain original data or are duplicate publication, and, finally, 51 papers were considered for the present study [7–58]. Of these 51 studies, 36 were used to evaluate the data on Ki-67 in patients with PNENs [10–14,16,18,19,22,23,27–36,38–46,48,50,51,54,56]; 12 were utilized to evaluate the method of obtaining tissue for analysis from the primary pancreatic neoplasm in order to evaluate the Ki-67 [8,9,15,17,21,25,26,37,47,49,55,57] and three were utilized to evaluate the data on Ki-67 in distant metastases of PNEN patients [19,24,58].

### 2.2. Data analysis

Data are reported as absolute numbers or percentages; data regarding the comparative analysis between the cytological and the histological assessment of Ki-67 was analyzed using the Wilcoxon Signed Rank Test. Statistical analysis was carried out using SPSS for Windows rev. 13.0. $P$ values $<0.05$ were considered statistically significant.

## 3. Results

The characteristic of the studies utilized for clinical assessment of Ki-67 is summarized in Table 1. It should be noted that the quality of statistic applied in all studies is good but the sample size has been not evaluated in all the studies.

### 3.1. Obtaining the tissue for Ki-67 evaluation

Pancreatic tissue suitable for Ki-67 evaluation was obtained from surgical specimens in 20 studies [10,13,18,20,23,27–29,32,33,35,36,38,39,41,50,52–54,56], from surgical specimens or biopsies in 12 studies [8,9,11,19,27,28,31,37,40,43,45,48], and from surgical specimens or biopsies or at autopsy in the remaining two studies [16,42]. In two studies the method used for obtaining the pancreatic tissue was not reported [44,45]. Preoperative and postoperative Ki-67 evaluation concordance was 83%. These data were also confirmed by another study [15]. Pooling the data of the three studies which compared the Ki-67 value obtained both in cytological and surgical specimens [33,47,53], it was found that the values were not related (Fig. 2). The mean $\pm$ SD of Ki-67 was $6.1 \pm 10.8$ on cytological specimens and $5.6 \pm 8.2$ on histological specimens. Of the 53 cases evaluated, Ki-67 determined histologically was lower than that cytologically assessed in 16 cases (30.2%), higher in 14 cases (26.4%) and it was equal in the remaining 23 cases (43.4%). There was a clear non concordance between the cytological and the histological assessment of Ki-67 in only three cases of which all had a value of Ki-67 higher than 30% (Fig. 2).

### 3.2. Ki-67 evaluation on pancreatic tissue

The assessment of Ki-67 was manual in the majority of the papers considered for this review. However, the major question