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The value of the Ki-67 proliferation marker as a prognostic factor in gastroenteropancreatic neuroendocrine tumours

Znaczenie wskaźnika aktywności proliferacyjnej Ki-67 jako czynnika prognostycznego w nowotworach neuroendokrynnych układu pokarmowego

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Abstract

Introduction: Gastroenteropancreatic neuroendocrine tumours (GEP NETs) are a heterogenous group of tumours of various clinical presentations. Proliferative activity of tumour cells is an essential parameter determining the course of the disease and affecting the prognosis. The Ki-67 antigen is an important marker of cell proliferation, which shows activity in all the phases of the cell cycle, excluding the G0 phase.
Aim of the study: To assess the expression of Ki-67 in GEP NETs and to examine the association of Ki-67 with the stage of the tumour (tumour size, presence of metastases) and the hormonal function of the tumour.
Material and methods: We included 61 patients with GEP NETs (25 males and 36 females aged between 20 and 82 years [mean age: 56 years]). The proliferative activity was examined in paraffin blocks containing surgically removed tumour samples and in core-needle biopsies of primary and metastatic tumours. The presence of the Ki-67 antigen was assessed by immunohistochemistry using MIB-1 monoclonal antibodies. Based on the Ki-67 proliferative index we determined the tumour grade. In addition, we determined the tumour stage according to the TNM classification. In all the subjects we determined the levels of the non-specific NET marker (chromogranin A) and of specific NET markers (serotonin, insulin and gastrin in the blood and 5-hydroxyindoleacetic acid [5-HIAA] in 24-hour urine).
Results: The diagnoses of low-grade (Ki-67 ≤ 2%), intermediate-grade (Ki-67 3–20%) and high-grade (Ki-67 > 20%) NET were established in 38, 12 and 11 patients, respectively. Metastatic disease was diagnosed in 36/61 patients. A significantly higher expression of K-67 was observed in patients with metastatic disease ($p = 0.01$). A positive correlation was demonstrated between Ki-67 and the stage of the disease ($p = 0.01$) and between the histologic grade of the tumour and the stage of the disease ($p = 0.01$). No association between Ki-67 and the levels of chromogranin A, serotonin, insulin, gastrin and 5-HIAA was shown. There was also no difference in Ki-67 expression relative to the location of the primary tumour and the tumour size.
Conclusions: The Ki-67 proliferative index is an essential parameter predicting the course of GEP-NETs. **(Endokrynol Pol 2012; 63 (5): 362–366)**

Key words: *Ki-67 proliferative index, prognostic factor, gastroenteropancreatic neuroendocrine tumours*

Streszczenie

Wstęp: Nowotwory neuroendokrynne układu pokarmowego (GEP NETs, *gastroenteropancreatic neuroendocrine tumors*) stanowią heterogenną grupę nowotworów o różnym obrazie klinicznym. Istotnym parametrem decydującym o przebiegu choroby i wpływającym na jej rokowanie jest aktywność proliferacyjna komórek nowotworowych. Antygen Ki-67 jest ważnym markerem proliferacji komórkowej, wykazującym aktywność we wszystkich fazach cyklu komórkowego, z wyjątkiem fazy G0.
Cel pracy: Celem pracy była ocena ekspresji antygenu Ki-67 w nowotworach neuroendokrynnych układu pokarmowego oraz badanie związku pomiędzy Ki-67 i stopniem zaawansowania choroby (wielkość guza, obecność przerzutów) oraz czynnością hormonalną guza.
Materiał i metody: Badaniem objęto 61 chorych z GEP NET (36 kobiet i 25 mężczyzn w wieku 20–82 lat, śr. wieku 56 lat). Do badania aktywności proliferacyjnej wykorzystano bloczki parafinowe, zawierające zmiany nowotworowe usunięte operacyjnie oraz materiał pobrany z ognisk pierwotnych i przerzutowych za pomocą biopsji gruboigłowej. Obecność antygenu Ki-67 oceniono za pomocą badania immunohistochemicznego z użyciem przeciwciał monoklonalnych MIB-1. W oparciu o wartość indeksu proliferacyjnego Ki67 ustalono stopień dojrzałości histologicznej nowotworu. Dodatkowo określono stopień zaawansowania nowotworu w oparciu o cechy TNM. U wszystkich chorych oznaczono stężenie niespecyficznego markera nowotworów neuroendokrynnych (chromogranina A) oraz specyficznych markerów NETs (serotonina, insulina, gastryna we krwi oraz kwas 5-hydroksyindolooctowy w dobowej zbiórce moczu).
Wyniki. U 38 chorych rozpoznano nowotwór neuroendokrynny o niskim stopniu złośliwości Ki-67 ≤ 2%, u 12 — o pośrednim Ki-67 3–20%, u 11 — o wysokim stopniu złośliwości Ki-67 > 20%. Rozsianą chorobę nowotworową rozpoznano u 36/61 badanych. Stwierdzono znamiennie większą ekspresję antygenu Ki-67 w guzach neuroendokrynnych u chorych z przerzutami ($p = 0,01$). Wykazano dodatnią korelację pomiędzy Ki-67, a stopniem zawansowania choroby (*staging*) ($p = 0,01$) oraz dodatnią korelacją pomiędzy stopniem złośliwości histologicznej guza neuroendokrynnego a stopniem zaawansowania choroby ($p = 0,01$). Nie wykazano związku między Ki-67 a stężeniem chromograniany A, serotoniny, insuliny, gastryny i kwasu 5-hydroksyindolooctowego. Nie stwierdzono także różnicy w ekspresji antygenu Ki-67 w zależności od lokalizacji ogniska pierwotnego i wielkości guza neuroendokrynnego.
Wnioski: Wskaźnik aktywności proliferacyjnej Ki-67 jest istotnym parametrem pozwalającym przewidzieć przebieg nowotworów neuroendokrynnych układu pokarmowego. **(Endokrynol Pol 2012; 63 (5): 362–366)**

Słowa kluczowe: *indeks proliferacyjny Ki-67, czynnik prognostyczny, nowotwory neuroendokrynne układu pokarmowego*

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Introduction

Gastroenteropancreatic neuroendocrine tumours (GEP NETs) are a heterogenous group of tumours of diverse morphology, endocrine function, clinical course and response to treatment. The latest WHO histopathological classification published in 2010 divides neuroendocrine tumours (NETs) into well differentiated and poorly differentiated tumours [1]. The grade of the tumour is an essential factor determining the course of the disease and affecting the prognosis. It is an independent parameter whereby neuroendocrine tumours are divided into three prognostic groups: low grade (G1), intermediate grade (G2) and high grade (G3) tumours. Until recently the tumour grade was determined on the basis of mitotic index, which is the number of mitotic figures per 10 high-power fields at 400 × magnification. Mitotic index identifies proliferating cells only in the mitotic phase. For the past few years other methods have been investigated to assess the histological maturity of the tumours, including the determination of the Ki-67 proliferative index. This is an immunohistochemical method that assesses the expression of the nuclear antigen Ki-67 using MIB-1 monoclonal antibodies. Ki-67 is an important marker of cell proliferation which is active in cell cycle phases G1, S and G2 and during mitosis [2]. For this reason some authors consider this marker superior to mitotic index in the assessment of the proliferative activity of tumour cells [3]. In addition, the Ki-67 proliferative index is useful in the examination of tumour biopsies in which the amount of the tissue is too low to enable the grading of the tumour using mitotic index [1, 4]. Retrospective studies have demonstrated that Ki-67 shows a good correlation with tumour size, angioinvasion and behaviour of neuroendocrine tumours [5, 6].

Both the European Neuroendocrine Tumour Society (ENETS) and the World Health Organisation (WHO) have accepted mitotic index and Ki-67 as parameters to use in grading tumours [1]. Ki-67 has was also included in the Polish recommendations developed by the Polish Neuroendocrine Tumour Network in 2008 [7].

The aim of the study was to assess the expression of Ki-67 in GEP NETs and to examine the correlation of Ki-67 with the stage of the tumour (tumour size, presence of metastases) and with the hormonal function of the tumour.

Material and methods

The study population consisted of 61 patients with GEP NETs, including 25 males and 36 females aged between 20 and 82 years (mean age: 56 years). Fasting blood samples for determination of hormones were collected at 8.00am from an arm vein. We determined the levels of the non-specific NET marker (chromogranin A) and of specific NET markers (serotonin, insulin and gastrin in the blood and 5-hydroxyindoleacetic acid [5-HIAA] in 24-hour urine).

The proliferative activity of the NETs was assessed in paraffin blocks containing surgically removed tumour samples and in core-needle biopsies of primary and metastatic tumours. The proliferative activity involving the determination of the Ki-67 antigen was assessed by immunohistochemistry using MIB-1 monoclonal antibodies. The percentage of cells containing Ki-67 was calculated by examining 500–2000 tumour cells in a field demonstrating the most intensive nuclear staining (hot spot).

Grading and staging

In line with the most recent WHO classification published in 2010 [1] we determined the grade of the NET based on the Ki-67 proliferative index (Table I) and the stage of the disease based on the TNM classification (tumour size, nodal involvement and the presence or absence of distant metastases).

Inclusion criteria

We included adults over the age of 18 years with a diagnosis of a GEP NET after obtaining written informed consent from each of the patients.

Exclusion criteria

Patients with GEP NETs who refused consent, minors, pregnant women, breastfeeding women, patients with end-stage liver disease, patients with stage 4 or 5 chronic kidney disease and patients with advanced heart failure were excluded from the study.

Ethical approval

The study was approved by the Bioethics Committee at the Silesian Medical University in Katowice, Poland (Resolution No KNW/0022/KB1/63/10).

Statistical analysis

All the statistical calculations were done using MedCalc. Linear regression curves were constructed for the observed correlations. The differences between the variables in the specific groups were assessed using univariate analysis of variance. Results with a p value below 0.05 were considered statistically significant.

Results

The most common locations of the primary tumour in the group of 61 patients with NETs were the pancreas (16 patients) and the large intestine (15 patients) (Table II). In 11 patients, the location of the primary tumour

Table I. *Grading of GEP-NETs*

Tabela I. *Stopień histologicznej dojrzałości (grading) GEP -NET*

Grade (G)	Mitotic index	Ki-67 proliferative index (%)
G1	< 2	≤ 2
G2	2–20	3–20
G3	> 20	> 20

Table II. *Location of the primary tumour in the study population*

Tabela II. *Lokalizacja ogniska pierwotnego nowotworu neurendokrynnego w badanej grupie*

Location of the primary tumour	Number (%) of patients
Pancreas	16 (22.2%)
Stomach	7 (11.5%)
Small intestine	7 (11.5%)
Large intestine	15 (24.6%)
Appendix	7 (11.5%)
Unknown	9 (14.8%)
Total	**N = 61**

Table III. *Presence of metastases relative to the grade of the tumour*

Tabela III. *Obecność przerzutów w zależności od stopnia złośliwości GEP-NET*

Grade	Number (%) of patients N = 61	Number of patients without metastatic disease n = 25 (41%)	Number of patients with metastatic disease n = 36 (59%)
G1	38 (62.3%)	22	16
G2	12 (19.7%)	2	10
G3	11 (18.0%)	1	10

could not be established and the diagnosis was based on the results of a core biopsy of a metastatic tumour in the liver. A total of 38 patients (62.3%) were diagnosed with low-grade NET (Ki-67 ≤ 2%) and 12 patients (19.7%) with intermediate-grade NET (Ki-67 3–20%). The remaining 11 patients (18.0%) had high-grade tumours (Ki-67 > 20%). Metastatic disease was present in 36 patients (59%), while no metastases were identified in 25 patients (Table III).

A significantly higher (p = 0.01) expression of Ki-67 was identified in the tumours in patients with metastatic disease (Fig. 1). The mean Ki-67 proliferative index was

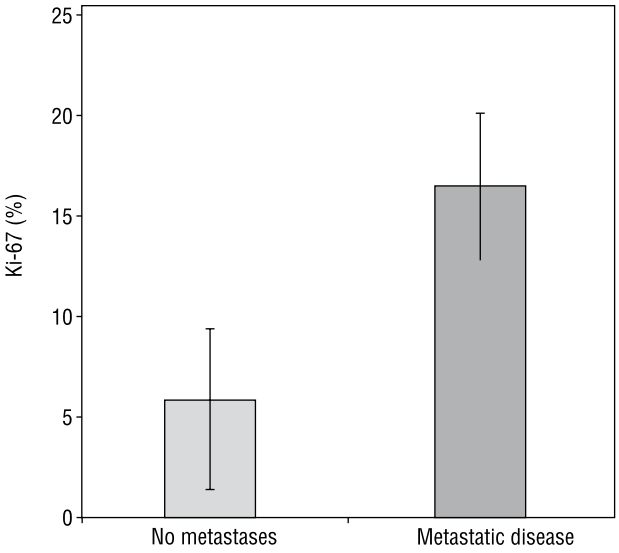

Figure 1. *Correlation between the Ki-67 proliferative index and the presence of metastases in patients with GEP-NETs*

Rycina 1. *Średnia wartość wskaźnika Ki-67 w guzach neuroendokrynnych u chorych bez przerzutów i z przerzutami*

5.4% in the group of patients without metastatic disease and 16.5% in the in the group of patients with metastatic disease. Using Spearman's rank correlation we demonstrated a positive correlation between the presence of metastases and the tumour grade (r = 0.44, p = 0.01). We also showed a significantly higher (p-0.024) rate of higher-grade NETs in the group of patients with metastatic disease (Fig. 2). We found a positive correlation between Ki-67 and the stage of the disease (r = 0.31, p = 0.01) and a positive correlation between the grade of the NET and the stage of the disease (r = 0.32, p = 0.01).

We found no correlations between Ki-67 and the levels of chromogranin A, insulin and gastrin in the blood and between Ki-67 and the level of 5-HIAA in 24-hour urine. We also found no difference in the expression of Ki-67 relative to the location of the primary tumour and the tumour size.

Discussion

For many years GEP NETs were treated as tumours of lower aggressiveness and a milder course (often of many years' duration) compared to other neoplasms [8]. Most of these tumours were highly differentiated on histopathologic examination. Further clinical observations revealed that some of the highly differentiated tumours were in fact characterised by a considerable aggressiveness: they invaded lymph nodes and internal organs and led to death in a relatively short time. For many years researchers looked for markers that would allow to predict the behaviour of NETs. Common acceptance

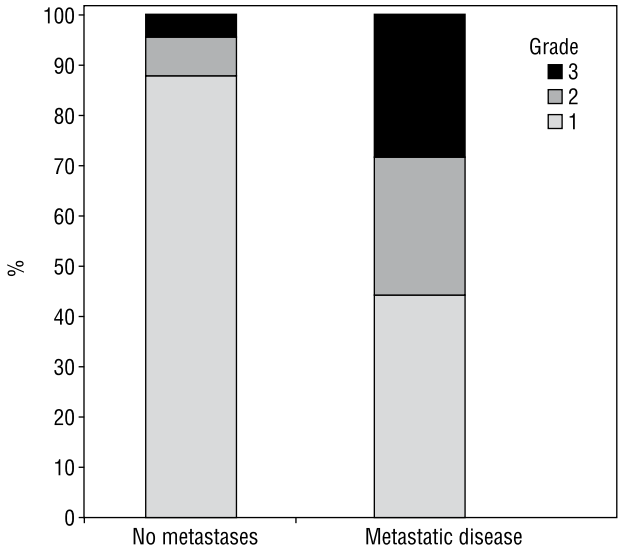

Figure 2. *A comparison of the grade of the NETs in the group of patients without metastatic disease and in the group of patients with metastatic disease*

Rycina 2. *Porównanie stopnia złośliwości histologicznej nowotworów neuroendokrynnych w grupie chorych z przerzutami i bez ognisk przerzutowych*

as such markers was gained by mitotic index and the presence of areas of necrosis in the tumour [9, 10] and, in the past few years, by the Ki-67 proliferative index [1, 5–7]. All these parameters define the histological maturity of the tumour (grade), which forms the basis for the division of NETs into three prognostic groups: low grade (G1), intermediate grade (G2) and high grade (G3) tumours [1, 11].

In our study we investigated the usefulness of the Ki-67 proliferative index for predicting the course of GEP-NETs. We mainly searched for an association between the tumour grade and the stage of the disease (in terms of tumour size and the presence of nodal involvement and distant metastases). We also investigated the relationship between the proliferative activity of the tumour cells and the location of the tumour, tumour size and endocrine function. As expected, we observed a significantly higher expression of Ki-67 in the tumours of patients with metastatic disease (mean Ki-67: 5.5%). These findings are consisted with those of other authors [12, 13]. Among the 25 patients without metastatic disease 22 (88%) had a low grade (G1) tumour, 2 had an intermediate grade (G2) tumour and 1 had a high grade (G3) tumour. In the literature, there is still controversy as to the threshold values for Ki-67 that would separate tumours of low proliferative activity from those of high proliferative activity. In the ENETS and WHO classifications Ki-67 ranges of $\leq 2\%$, 3–20% and $> 20\%$ were adopted to form the basis for the division of NETs into three degrees of histological maturity: G1 (highly differentiated tumours of low proliferative activity and low grade), G2 (highly differentiated tumours of intermediate grade) and G3 (poorly differentiated tumours of high proliferative activity and aggressive clinical course) [1].

According to other authors, a Ki-67 value of 5% should be adopted as the threshold value that separates tumours of low proliferative activity from those of high proliferative activity and that determines a different response to chemotherapy. In a study published in 2008 in which 180 non-functioning pancreatic NETs were investigated, Betting et al. showed prognostic value of Ki-67 proliferative index values exceeding 5% [14]. Better responses to systemic treatment in patients with NETs characterised by Ki-67 proliferative index values exceeding 5% have also been reported [15], which is why in this group of patients chemotherapy is recommended as the treatment of choice. On the other hand, in GEP NETs with Ki-67 proliferative index values below 5%, the response to systemic treatment is poor and in these patients other treatment modalities are preferred (somatostatin analogues, interferon alfa, angiogenesis inhibitors and mTOR inhibitors).

In our study, the mean Ki-67 in the group of patients with GEP NETs without metastases was 5.4%, which is consistent with the above studies. A notable finding of our study is the presence of multiple nodal and distant metastases in as many as 16 out of 38 (42%) patients with highly differentiated GEP NETs and a low Ki-67 proliferative index of $\leq 2\%$ (low grade [G1] tumours). When analysing our results one should take into account the heterogenous architecture of the tumours and the differences in the proliferative activity of the tumour cells between the primary tumour and the metastatic tumours. In some of the patients, the diagnosis was established on the basis of a core biopsy of a metastatic tumour, which may have limited the prognostic value of the Ki-67 proliferative index. There may be other factors that affect the course of the tumour independently of the proliferative activity of the tumour cells. Our findings are consistent with other reports [16–21]. Of note is the fact that all the patients with metastatic disease who participated in our study were in a good clinical condition and showed no signs of progression.

Other findings in our study included a positive correlation between the grade of the tumour and the presence of metastases, a positive correlation between Ki-67 and the stage of the disease and a positive correlation between the grade of the tumour and the stage of the disease, which supports the usefulness of the Ki-67 proliferative index for predicting the behaviour of the tumour. These findings are consistent with those by other authors [22–24].

We showed no relationship between Ki-67 and the levels of chromogranin A, serotonin, insulin, gastrin

and 5-HIAA. We also found no relationship between the hormonal function of the tumour and the stage of the disease. According to some authors, the absence of hormonal function of GEP NETs is a poor prognostic factor, especially in pancreatic NETs, while other researchers, us included, have found no differences in the course of GEP NETs between functioning and non-functioning tumours [17]. We also found no difference in the expression of Ki-67 relative to the location of the primary tumour and the tumour size.

Conclusions

The Ki-67 proliferative index may be a helpful parameter in the prediction of the course of the disease in patients with GEP NETs. This parameter shows a correlation with the stage of the disease but no correlation with the levels of chromogranin A and other selected biochemical markers of NETs.

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