Histol Histopathol (2003) 18: 353-358

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

Proliferative activity of well differentiated neuroendocrine tumours of the gut

M.E. Zimmermann and F.T. Bosman

Institut Universitaire de Pathologie, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland

Summary. Neuroendocrine tumours of the gastrointestinal tract are relatively uncommon neoplasms with, in spite of their characteristic morphology, relatively unpredictable biological behaviour. In some sites, notably the appendix, these tumours are largely benign whereas at other localisations, such as the small bowel, metastases occur and the outcome is less favourable. Given the lack of discriminative power of histological parameters, immunohistochemical parameters have been proposed. Of these the Ki-67 index, as an indicator of proliferative activity, has shown some promise.

In order to assess their proliferative activity and the potential contribution of this parameter to defining biological behaviour, we performed Ki-67 immunostaining of a series of 64 well differentiated neuroendocrine tumours of the gut (stomach, small bowel, appendix, colon and rectum). Ki-67 labeling index, based upon counting of up to 5000 cells, ranged between 0 and 6.1‰. No difference was found according to age, gender, size, location or TNM classification. Ki-67 labeling index of midgut endocrine tumours of long term surviving patients did not differ from patients that died.

We conclude that Ki-67 labeling index as an indicator of proliferative activity of well differentiated neuroendocrine tumours of the digestive tract does not correlate with size nor site nor stage. Even though only small numbers of tumours could be analysed, which hampered appropriate statistical analysis, it seems unlikely that proliferative activity has potential as an independent prognostic parameter for this type of tumour.

Key words: Well differentiated neuroendocrine tumours, Digestive tract, Ki-67, Proliferation

Offprint requests to: Fred T. Bosman MD PhD, University Institute of Pathology, Bugnon 25, CH-1011 Lausanne, Switzerland. Fax: 0041 21 314 72 05. e-mail: Fred.Bosman@chuv.hospvd.ch.

Introduction

Carcinoid tumours of the bowel are uncommon neoplasms (Johnson et al., 1983) with a characteristic morphology. The term "karzinoid" (literally meaning carcinoma-like) was coined by Oberdorfer in 1907, to define tumours that, although resembling epithelial neoplasms, behaved in a more indolent fashion than usual carcinomas (Oberdorfer, 1907). They may secrete various peptides and biogenic amines including histamine, serotonin, gastrin, somatostatin, pancreatic peptide and glucagon. Neuroendocrine-specific proteins, such as chromogranin and synaptophysin, are useful targets for immunohisto-chemical verification of the diagnosis (Thomas et al., 1994; Burke et al., 1997; Portela-Gomes et al., 1999). It is now well acknowledged that, despite an apparent indolent behaviour, some of these tumours are quite capable of invading adjacent organs and metastasizing to distant sites. Recently, a new classification of these tumours has been proposed, which takes into consideration site, size, hormonal activity and histology (Capella et al., 1995). In this classification, the more general term neuroendocrine tumour is preferred over carcinoid and we have adopted this nomenclature for this paper. In this classification well differentiated tumours can be benign, or low grade malignant, size, functional activity and angio-invasion being important determinants of classification as either benign or low grade. Poorly differentiated tumours are invariably classified as high grade malignant. Angio-invasion is regarded as an important parameter for malignancy grading but this has not been substantiated extensively in clincopathological studies (Klöppel et al., 1999).

Size remains the best predictive factor for outcome, with significant differences according to location. For example, metastatic lymph nodes will be found in 44% of small bowel neuroendocrine tumours measuring less than 1 cm in size, while lymph node involvement is almost never found in appendiceal neuroendocrine tumours of the same size. Accordingly, overall 5-year

survival rates range from 50 to 60% for neuroendocrine tumours in the small bowel, compared to 90 to 100% for those in the appendix (Fenoglio-Preiser et al., 1989).

Determination of the proliferative fraction of a tumour has proven to be an additional prognostic factor for a variety of tumours. One of the most frequently used methods to determine the growth fraction of a tumour is the immunohistochemical detection of proliferation-associated antigens such as Ki-67 (Key et al., 1993). Ki-67 is a 350 kD non-histone protein, which is expressed at an increasing rate from late G1, through S and G2, till M phases of the cell cycle and with a half-life of about 20 minutes, which explains its high specificity for cycling cells (Duchrow et al., 1994). MIB-1 is a murine monoclonal antibody directed specifically against Ki-67, which has been used in a wide range of pathological settings, since its first description in 1983 by Gerdes et al. (1983). The Ki-67 labeling based proliferative activity has been analysed repeatedly in studies focusing on prognostic parameters of gastrointestinal neuroendocrine tumours. In general, poorly differentiated tumours show a fairly high Ki-67 labeling index. Well differentiated neuroendocrine tumours showed low Ki-67 labeling indices. Many studies reported lack of correlation between the Ki-67 labeling index and behaviour whereas others reported higher Ki-67 labeling in more aggressive tumours (Borstein-Quevedo and Gamboa-Dominguez, 2001; Canavese et al., 2001; Kawahara et al., 2002). In view of these discrepancies we decided to study Ki-67 labeling indices in well differentiated neuroendocrine tumours of the gastrointestinal tract.

Materials and methods

Case selection

The computerised files at the Institute of Pathology in Lausanne were surveyed for well differentiated neuroendocrine tumours arising from the appendix, jejunum and ileum (1987-1997), and from the stomach, duodenum, colon and rectum (1977-1997). From the indicated time periods, consecutive cases were included. We also included in the study all metastatic well differentiated neuroendocrine tumours diagnosed between 1977 and 1997.

Because of the number of cases available and the low proportion of cases with malignant behaviour, we attempted to evaluate outcome only for patients with jejuno-ileal (midgut) neuroendocrine tumours. Survival time was defined as the time elapsed from the date of the surgical intervention until death or until October 31, 1997 (the cut-off date for the study). Survival data were retrieved mainly from patient hospital records; when not available, last known family practitioners were contacted by mail; if they failed to answer or were unable to provide reliable information, local birth and death registries were contacted and copies of death certificates obtained for deceased patients.

Immunohistochemical staining

Four-μm-thick sections from paraffin-embedded tissues mounted on silane-coated slides were dried at 37 °C overnight, subsequently dewaxed in xylene and rehydrated in graded alcohol. Endogenous peroxidase was blocked with 0.3% hydrogen peroxide in methanol for 45 minutes. The sections were then heated for 20 minutes in 10 mM citrate buffer in a microwave oven (Moulinex, France) at 1300W. After microwave treatment, the sections were allowed to cool down at room temperature (RT) for 20 minutes were rinsed with Tris buffer for 2 minutes and successively incubated at RT for 30 minutes with normal goat serum (1:30), the MIB-1 antibody (Immunotech, Marseille, France;1:10) and biotinylated peroxidase complex (Sternberger, Maryland, USA;1:100). Peroxidase activity was then developed in the dark using diamino-benzidine as chromogen. Immunostained slides were counterstained with hematoxylin. As a negative control an unrelated mouse monoclonal antibody was used at a protein concentration equal to that of the MIB-1 antibody.

Counting procedure

Nuclei of tumour cells were considered to be immunostained if a dark diffuse or granular staining pattern was recognised throughout the nucleus. When staining was weak in comparison with crypt base cells or germinal centre cells, it was considered non-specific (which is a well known problem in microwave-treated paraffin sections) and was not taken into account for determination of the Ki-67 labelling index (LI). Immunostaining was validated using internal controls, i.e. nuclear staining in the crypt basis or germinal centres of lymphoid follicles, absence of nuclear staining in known non-cycling cell compartments.

We defined our Ki-67 LI as the percentage of labelled cells upon counting 1500 tumour cell nuclei in randomly selected high power fields (HPF, magnification x500). This number was chosen in order to obtain reproducible results, in view of the low numbers of labelled nuclei. On 1500 nuclei the data obtained for repetitive counts remained within ± 10% of the mean. When the number of labelled nuclei per 1500 was less than five, counting had to be continued up to 5000 nuclei in order to obtain the same reproducibility.

Statistical evaluation

The Kruskall-Wallis or the Mann-Whitney tests were used to analyse statistical significance of the differences in proliferation index between different clinico-pathological categories. Overall survival, defined as the time from diagnosis to death was used as a measure of prognosis. Given the small numbers and/or the high percentage of survivors only the midgut tumours were analysed with regard to differences in LI relative to survival. Due to the limited number of cases,

multivariate analysis could not be performed.

Results

After elimination of cases with qualitatively or quantitatively insufficient material, 64 well differentiated neuroendocrine tumours of the digestive tract, originating from the stomach (6), duodenum (3), ileum (27), appendix (20), colon (1) and rectum (7), were available. Low numbers of Ki-67 immunolabelled cells were found in most cases. Labelling cells were randomly distributed. Examples of the obtained Ki-67 immunolabelling are given in Fig. 1. The main results are summarized in table 1.

Of 9 foregut (stomach, duodenum) neuroendocrine tumours (6 males, 3 females; mean age 57, range: 40-81) size was unknown in one case, ≤ 1 cm in two cases, > 1 cm in 6 cases. Four cases were classified pT2, three pT3 and two pT4. One gastric and one duodenal neuroendocrine tumour had developed lymph node metastases. The highest Ki-67 LI observed in a primary tumour was 3.4‰. This tumour showed striking nuclear pleomorphism, as may be encountered in well differentiated neuroendocrine carcinomas. All other primaries had a very low Ki-67 LI (median 0.45‰, range: 0-3.4). Ki-67 LI was also low in both lymph node metastases (0.2 and 1.0 respectively).

Of 27 small bowel well differentiated neuroendocrine tumours studied (14 males, 13 females; mean age 65, range: 39-90), 24 were >1 cm in size, 1 ≤1 cm and size was undetermined in another two cases. One case was classified pTx, 2 pT2, 1 pT3 and 23 pT4. Eighteen cases were pN1, and seven pM1. Median Ki-67 LI was 0.20‰ (range 0-3.8).

Of 20 well differentiated neuroendocrine tumours of the appendix (6 males, 14 females; mean age 33; range: 10-82), 18 measured ≤1 cm, 2 ≥1 cm. Two cases were classified pT2, another two pT3 and sixteen pT4. Lymph nodes were available in only 2 cases, and both were without tumour.

The highest Ki-67 LI observed was 6.1‰; in the other tumours, the LI was much lower (median 0.85‰, range: 0-6.1).

Of the 8 cases of well differentiated neuroendocrine tumour of the colon and rectum (hindgut) mean Ki-67 labeling index was 0.80‰ (range 0-3.8). There were 6 pT2, 1 pT4 and 1 pTx cases. One case had lymphnode and liver metastases. Of this case Ki-67 LI was 0.2‰.

The differences in Ki-67 LI between different groups of cases is presented in Table 1. There were no age-related differences in Ki-67 LI. Labeling indexes were

Table 1. Ki-67 labeling index and clinicopathological characteristics of gastrointestinal neuroendocrine tumours.

CLINICO-PATHOLOGICAL CHARACTERISTICS	n*	Ki-67 LABELING INDEX median**	range**	p
Age				
≤ 55	29	0.94	(0-6.1)	n.s.
>55	28	1.51	(0-1.7)	
Location				
foregut	9	0.45	0-3.4	n.s.
midgut	27	0.20	0-3.8	
appendix	20	0.85	0-6.1	
hindgut	8	0.80	0-3.8	
Size				
≤ 1 cm	23	1.25	0-6.1	
>1 cm	30	0.76	0-3.8	p=0.06
T stage				
T2/3	21	1.18	0-6.1	n.s.
T4	41	0.80	0-3.8	
Tumour type				
primary	56	0.6	0-6.1	n.s.
lymph node metastasis	14	0.7	0-9.2	
liver metastasis	6	0.9	0.2-3.3	
Follow-up				
died	7	0.92	0-3.8	
alive	10	0.34	0-0.6	n.s.

*: numbers do not always add up to 64 because in some cases some data were missing; **: numbers are given in o/oo of cells labelled

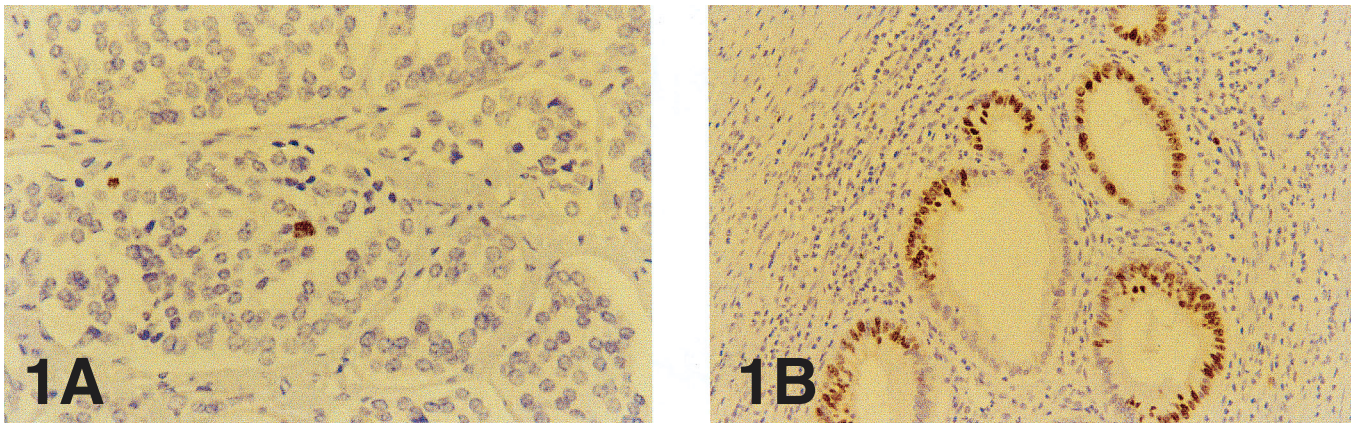

Fig. 1. Ki-67 immunolabelling on an appendiceal carcinoid. **A.** Labelling of scattered isolated nucle. **B.** Internal control, showing intense labelling of mucosal crypts in the same section. x 200

somewhat lower in midgut and foregut tumours but this was not significant. There was no significant difference in LI according to size (cut-off 1 cm), pT4 tumours did not show a higher LI than pT2/3 tumours. Lymph node metastases and liver metastases had a higher median LI than primary tumours, but this difference was not significant.

The number of foregut and hindgut tumours was too low for adequate follow-up analysis and all patients with an appendiceal tumour survived. We therefore limited the study of the outcome to the 21 small bowel well differentiated neuroendocrine tumours. All but two were pT4. Four patients were lost for follow-up. Nine patients had deceased, one during the early postoperative period, the eight others 56±37 months after diagnosis (range: 5-105). All had lymph node metastases and all but one also liver metastases. Fourteen patients were still alive, after a mean follow-up of 89±54 months (range: 21-245). We compared Ki-67 LI of the cases with at least 60 months follow-up. Seven patients had died (5 tumour-related; in 2 cases cause of death unknown), 10 patients were still alive. In the former group, mean Ki-67 LI was 0.92‰ and in the latter 0.34‰; this difference was not statistically significant.

Discussion

We determined the proliferation rate of 64 well differentiated neuroendocrine tumours of the gut by immunohistochemistry, using the monoclonal antibody MIB-1 which recognizes the Ki-67 antigen, and compared Ki-67 labeling index with size, site, stage and age. The overall proliferative activity, as reflected in the Ki-67 LI, in our study was particularly low, mostly not ranging higher than 3 to 4‰. We regard the results as reliable because staining of the sections was done batchwise and positive as well as negative controls were adequate. There was no indication of poorer immunostaining in older cases.

The mean KI-67 LI in our cases was about ten times lower than that published by Al Khafaji et al. (1998). They found in gastrointestinal neuroendocrine tumours, derived from the foregut, midgut and hindgut, a Ki-67 LI <1% in 50% of the cases, 1-10% in 43% of the cases and 10-50% in 'atypical' neuroendocrine tumours. The paper unfortunately provides only semiquantitative scores and criteria for positivity are not detailed. Differences in methodology as well as a different case selection might explain the discrepancy with our own results. We took only into account strongly stained nuclei and did random tumour cell counts. In our series, well differentiated neuroendocrine tumours with nuclear pleomorphism (atypical carcinoids), which reportedly tend to have higher mitotic indices than monomorphic well differentiated neuroendocrine tumours, accounted for only 2 out of 64 cases. In the series of Al-Khafaji et al. (1998) 19% of the cases showed nuclear atypia. The reliability of our counting approach is indicated by the relatively high Ki-67 LI we found for neuroendocrine tumours with nuclear pleomorphism (3.4‰ and 1.71‰) respectively, which is in the order of magnitude of the data of Costes et al. (1995) for bronchopulmonary neuroendocrine tumours. These authors expressed KI-67 LI as the percentage of stained nuclear surface relative to the total nuclear surface. This is, although significantly different from ours, an objective quantitative approach. Gentil Perret et al. (1998) reported on KI-67 LI in 35 pancreatic neuroendocrine tumours. Areas were not selected randomly; those presenting the highest number of labelled cells were counted. The overall mean KI-67 LI was 5.6% (range 0.5-16.1%), 5.2 for well-differentiated and 11.9% for poorly differentiated tumours. In yet another study, Rindi et al. (1999) evaluated Ki-67 staining in 102 neuroendocrine tumours originating from the stomach. They counted the number of labelled nuclei per 10 HPF (x400), which renders any comparison with our results impossible. One of the most recent reports on proliferative activity in gastrointestinal neuroendocrine tumours (Li et al., 2002) shows, in spite of some differences in methodology, labeling indices which are partly in the same range as ours: from 0 to 2.5%. Another recent paper (Kawahara et al., 2002) even reports Ki-67 as mostly negative in well differentiated gastrointestinal neuroendocrine tumours. We conclude that KI-67 LI as an indicator of proliferative activity in neuroendocrine tumours varies widely between different reports, in relation to important methodological differences. Based on rigorous methodology and similar data from quantitative studies on bronchopulmonary neuroendocrine tumours (Costes et al., 1995) we consider our data reliable. It is important to stress that only random counting of sufficient numbers of tumour cells will yield the reproducibility necessary for a robust classification parameter.

In order to analyse potential correlation of proliferative activity with age, size and stage, we felt justified to group well differentiated gut neuroendocrine tumours together, reasoning that if for individual sites these parameters correlate (as some authors propose), they should also correlate when taken together. Overall, there were no significant differences in LI according to age and gender. Likewise, we did not find significant differences in LI according to size or the pT stage. Lymph node and liver metastases showed higher LI than primary tumours, but this did not reach statistical significance. Compared to other sites, appendiceal and hindgut tumours showed a higher median LI but this was not statistically significant. Nonetheless, the higher LI of appendiceal cases is striking, as all patients with an appendiceal well differentiated neuroendocrine tumour survived, whereas of patients with a midgut well differentiated neuroendocrine tumour a significant number died. Of the patients with a midgut tumour that died the LI was not significantly higher than of patients that survived. Non-survivors all had lymph node and/or liver metastases, of which the LI was not higher than that of primary tumours.

Al-Khafaji et al. (1998) did not find differences in

KI-67 LI between tumours that had metastasised and those that had not. Shimizu et al. (2000) recently reported similar findings for rectal neuroendocrine tumours. In their series small (<5mm) tumours had a lower Ki-67 LI than large (>5mm) tumours. Rindi et al. (1999) investigated 102 neuroendocrine tumours originating from the stomach and found mitotic index, Ki-67 LI, histological grade, size and stage to be strongly interrelated. Ki-67 index did show correlation with prognosis, although vascular invasion was the only factor with independent value as a predictor of malignancy. Moyana et al. (2000) reported a positive correlation between Ki-67 positivity and metastatic behaviour but used only semiquantitative estimates. In a study of pancreatic neuroendocrine tumours Pelosi et al. (1996) and Gentil Perret et al. (1998) reported that tumours that had metastasized showed higher KI-67 LI than tumours without metastases. A KI-67 LI ≤ 4% was associated with a 100% survival rate at 60 months, while an LI >4% predicted significantly poorer outcome with only a 70% 5-year survival rate. These results confirm the findings of earlier studies of pancreatic endocrine tumours, using a KI-67 LI of 5% or of 2% (La Rosa et al., 1996; Losi et al., 1996) as a cut-off value in predicting outcome.

Finally, in bronchopulmonary carcinoid tumours several indications have been published as to the prognostic significance of the Ki-67 LI. Costes et al. (1995) found 7% of typical neuroendocrine bronchopulmonary tumours with a Ki-67 LI between 0 and 2‰, range similar to our cases. In their material a KI-67 LI of >4% predicted poor outcome. Similar findings were reported for lung neuroendocrine tumours by Böhm et al. (1996) and Laitinen et al. (2000).

In summary, proliferative activity of well differentiated gut neuroendocrine tumours, as determined by the KI-67 LI, is low and can only be reproducibly assessed by random counting of a sufficient number of cells. Ki-67 LI does not correlate with size, site or pT-stage and is not higher in metastatic than in primary tumours. Ki-67 LI of midgut well differentiated neuroendocrine tumours of surviving patients did not differ from those of patients that died. In spite of the limited number of cases available for analysis we feel justified to conclude that proliferative activity does not appear to be related to behaviour in well differentiated gut neuroendocrine tumours.

References

Al-Khafaji B., Noffsinger A.E., Miller M.A., DeVoe G., Stemmermann G.N. and Fenoglio-Preiser C. (1998). Immunohistologic analysis of gastrointestinal and pulmonary carcinoid tumours. Hum. Pathol. 29, 992-999.

Böhm J., Koch S., Gais P., Jutting U., Prauer H.W. and Hofler H. (1996). Prognostic value of MIB-1 in neuroendocrine tumours of the lung. J. Pathol. 178, 402-409.

Bornstein-Quevedo L. and Gamboa-Dominguez A. (2001). Carcinoid tumors of the duodenum and ampulla of vater: a clinicomorphologic, immunohistochemical, and cell kinetic comparison. Hum. Pathol. 32, 1252-1256.

Burke A.P., Thomas R.M., Elsayed A.M. and Sobin L.H. (1997). Carcinoids of the jejunum and ileum: an immunohistochemical and clinicopathologic study of 167 cases. Cancer 79, 1086-1093.

Canavese G., Azzoni C., Pizzi S., Corleto V.D., Pasquali C., Davoli C., Crafa P., Delle Fave G. and Bordi C. (2001). p17: a potential main inhibitor of cell proliferation in digestive endocrine tumors but not a marker of benign behavior. Hum. Pathol. 32, 1094-1101.

Capella C., Heitz P.U., Hofler H., Solcia E and Kloppel G. (1995). Revised classification of neuroendocrine tumours of the lung, pancreas and gut. Virchows Archiv. 425, 547-560.

Costes V., Marty-Ane C., Picot M.C., Serre I., Pujol J.L., Maury H. and Baldet P. (1995). Typical and atypical bronchopulmonary carcinoid tumours: a clinicopathologic and KI-67-labeling study. Hum. Pathol. 26, 740-745.

Duchrow M., Gerdes J. and Schluter C. (1994). The proliferation-associated Ki-67 protein: definition in molecular terms. Cell Proliferation 27, 235-242.

Fenoglio-Preiser C.M., Pascal R.R. and Perzin K.H. (1989). Carcinoid tumours. In: Tumours of the intestines. Armed Forces Institutes of Pathology. pp 265-335.

Gentil Perret A., Mosnier J.F., Buono J.P., Berthelot P., Cipponi J., Ballique J.G., Cuillerck J. Dechelotte P. and Boucheron S. (1998). The relationship between MIB-1 proliferation index and outcome in pancreatic neuroendocrine tumours. Am. J. Clin. Pathol. 109, 286-293.

Gerdes J., Schwab U., Lemke H. and Stein H. (1983). Production of a mouse monoclonal antibody reactive with a human nuclear antigen associated with cell proliferation. Int. J. Cancer 31, 13-20.

Johnson L.A., Lavin P., Moertel C.G., Weiland L., Dayal Y., Doos W.G., Geller S.A., Cooper H.S., Nime F., Masse S., Simson I.W., Summer H., Folsch E. and Engstrom P. (1983). Carcinoids: the association of histologic growth pattern and survival. Cancer 51, 882-889.

Kawahara M., Kammori M., Kanauchi H., Noguchi C., Kuramoto S., Kaminishi M, Endo H. and Takubo K. (2002). Immunohistochemical prognostic indicators of gastrointestinal carcinoid tumours. Eur. J. Surg. Oncol. 28, 140-146.

Key G., Becker M.H., Baron B., Duchrow M., Schluter C., Askaa J. and Gerdes J. (1993). New Ki-67-equivalent murine monoclonal antibodies (MIB 1-3) generated against bacterially expressed parts of the Ki-67 cDNA containing three 62 base pair repetitive elements encoding for the Ki-67 epitope. Lab. Invest. 68, 629-636.

Klöppel G., Solcia E., Capella C. and Heitc Ph. (1999). Classification of neuroendocrine tumours. Ital. J. Gastroenterol. Hepatol. Suppl. 2, 111-116.

La Rosa S., Sessa F., Cva C., Leone B.E., Klersy C., Rindi G. and Solcia E. (1996). Prognostic criteria in non-functioning pancreatic endocrine tumours. Virchows Arch. 429, 323-333.

Laitinen K.L., Soini Y., Mattila J. and Paakko P. (2000). Atypical bronchopulmonary carcinoids show a tendency toward increased apoptotitc and proliferative activity. Cancer 88, 1590-1598.

Li C.C., Xu B., Hirokawa M., Qian Z., Yoshimoto K., Horiguchi H., Tashiro T. and Sano T. (2002). Alterations of E-cadherin, α-catenin and ß-catenin expression in neuroendocrine tumors of the gastrointestinal tract. Virchows Arch. 440, 145-154.

Moyana T.N., Xiang J., Senthilselvan A. and Kulaga A. (2000). The spectrum of neuroendocrine differentiation among gastrointestinal carcinoids: importance of histologic grading, MIB-1, p53, and bcl-2

immunoreactivity. Arch. Pathol. Lab. Med. 124, 570-576.

Oberdorfer S. (1907). Karzinoide Tumouren des Dünndarms. Frankfurt Zeitschr. Pathol. 1, 426-432.

Pelosi G., Bresaola E., Bogina G., Pasini F., Rodella S., Castelli P., Iacono C., Serio G. and Zamboni G. (1996). Endocrine tumours of the pancreas: Ki-67 immuno-reactivity on paraffin sections is an independent predictor for malignancy: a comparative study with proliferating-cell nuclear antigen and progesterone receptor protein immunostaining, mitotic index, and other clinicopathologic variables. Hum. Pathol. 27, 1124-1134.

Portela-Gomes G.M., Stridsberg M., Johansson H. and Grimelius L. (1999). Co-localization of synaptophysin with different neuroendocrine hormones in the human gastrointestinal tract. Histochem. Cell Biol. 111, 49-54.

Rindi G., Azzoni C., La Rosa S., Klresy C., Paolotti D., Rappel S., Stolte M., Capella C., Bordi C. and Solcia E. (1999). ECL cell tumour and poorly differentiated endocrine carcinoma of the stomach: prognostic evaluation by pathological analysis. Gastroenterology 116, 532-542.

Shimizu T., Tanaka S., Haruma K., Kitadai Y., Yoshihara M., Sumii K., Kajiyama G. and Shimamoto F. (2000). Growth characteristics of rectal carcinoid tumors. Oncol. 5, 229-237

Thomas R.M., Baybick J.H., Elsayed A.M. and Sobin L.H. (1994). Gastric carcinoids. An immunohistochemical and clinicopathologic study of 104 patients. Cancer 73, 2053-2058.

Accepted November 4, 2002