The Relationship Between MIB-1 Proliferation Index and Outcome in Pancreatic Neuroendocrine Tumors

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The expression of the cell-cycle–associated Ki-67 antigen by MIB-1 monoclonal antibody was retrospectively assessed in 35 surgically resected neuroendocrine tumor specimens of the pancreas embedded in paraffin. The MIB-1 proliferation index was correlated with the classification of the neuroendocrine tumors of the pancreas proposed by Klöppel et al. Four prognostic factors showed a significant correlation with MIB-1: local invasion, metastases, tumor differentiation, and production of insulin. However, analysis by the Cox Proportional Hazards Regression Model showed that only local invasion was an independent predictor of outcome. Finally, our study showed a statistically significant increase in the number of deaths and a statistically significant decrease in survival time when the MIB-1 proliferation index was higher than 4%. We conclude that MIB-1 proliferation index is a simple and reliable tool to predict the clinical outcome of the neuroendocrine tumors of the pancreas. The index might be useful for determining the prognosis for an individual because of the significant decrease in survival when the index is higher than 4%. (Key words: Pancreas; Neuroendocrine tumor; MIB-1; Proliferation index) Am J Clin Pathol 1998;109:286–293.

Neuroendocrine tumors of the pancreas are uncommon. They occur in less than 1 of 100,000 individuals.[1] The outcome of most neuroendocrine tumors of the pancreas is difficult to predict on histologic grounds. Nevertheless, some prognostic classifications have been developed to distinguish the benign neuroendocrine tumors of the pancreas from carcinomas. Klöppel and colleagues[2] introduced a new classification for an appropriate prognostic evaluation of the neuroendocrine tumors of the pancreas. This classification is based on the size of the tumor, the functional status, the differentiation grade, and the presence of local invasion or metastases, which are the classic features known to be of prognostic value in these tumors.[3] Hence, four groups of neuroendocrine tumors of the pancreas are distinguished: benign tumors, benign tumors with uncertain evolution, low-grade malignant tumors, and high-grade malignant tumors.

A variety of tests, such as nuclear morphometry,[4] immunohistochemical detection of the expression of human chorionic gonadotropin α,[5] and DNA ploidy[6] have been proposed as prognostic guides in neuroendocrine tumors of the pancreas, but the tests still await confirmation or better definition. Determining the proliferation index also provides adjunct objective information about the behavior of a neoplasm and, in some instances, a direct correlation has been shown between the proliferation index and the biologic aggressiveness.[7] Methods commonly performed to determine the kinetics of tumor growth include incorporation of DNA precursors, such as thymidine or bromodeoxyuridine,[8,9] flow cytometric DNA analysis,[10] and counts of silver-stained nucleolar organizer regions.[11] Immunohistochemical detection of cell-cycle–related antigens also deserves attention. A variety of monoclonal antibodies have been raised against cell-cycle–regulated antigens, including DNA polymerase α, proliferating cell nuclear antigen, and Ki-67. Antibody MIB-1 is known to react with the Ki-67 nuclear antigen, present throughout the cell cycle (G_1, S, G_2, M) but absent in resting G_0 cells.[12] Unlike the Ki-67 monoclonal antibody, which works only in frozen

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sections, the MIB-1 monoclonal antibody can be used in frozen and paraffin-embedded tissue specimens. Thus, MIB-1 can be used to determine the proliferation index on routinely processed surgical specimens.

In this study, we assessed the proliferation index of 35 neuroendocrine tumors of the pancreas by using an immunohistochemical detection of the cell-cycle–related antigen Ki-67 by the MIB-1 monoclonal antibody. The aim of this study was to compare the MIB-1 proliferation index of the neuroendocrine tumors of the pancreas with their staging evaluated according to the Klöppel classification and with their clinical outcome.

MATERIALS AND METHODS

Patients

Thirty-five patients with neuroendocrine tumors of the pancreas removed between 1985 and 1995 were identified. Their tumors were retrieved from the surgical pathology files of the hospitals of Saint-Etienne (Saint-Etienne, France) and Clermont-Ferrand, (Clermont-Ferrand, France). All data on age, sex, presenting symptoms, levels of serum gastrointestinal peptides, macroscopic features, and surgical procedure were available. Clinical charts or death registers were reviewed to determine patient outcome. Survival time was defined as the time elapsed from the date of the surgical operation until death or until August 30, 1996, which was the cutoff date for the study. If a patient's vital status as of the cutoff date was unknown, then the patient's condition was monitored as of the date of the last follow-up visit.

Methods

Histologic technique—Tumor tissues were fixed in Bouin's fixative or in 10% formalin. After dehydration, the specimens were routinely embedded in paraffin. Four-µm–thick sections were cut. The sections were stained with hematoxylin-eosin-safran and the Grimelius' argyrophil method.

Immunohistochemical staining—The immunohistochemical technique was performed in Bouin or formalin-fixed, paraffin-embedded tissue sections. The 4-µm–thick sections were mounted on silane-treated slides, dewaxed in xylene, and rehydrated in graded alcohols. Retrieval antigen procedures were performed before immunostaining for all the antibodies except chromogranin and CD57. Before adding the primary antibody, the sections were microwave irradiated for 5 minutes each in a citrate buffer (pH 6.0; 0.01 mol/L) and allowed to cool for 15 minutes in the same solution. After rinsing, sections were blocked for endogenous peroxidase with 3% hydrogen peroxide and water for 30 minutes at room temperature. Then, sections were washed in Tris buffer. Two different immunohistochemical techniques were used. The three-step immunoperoxidase technique described by Mason and Simmons[13] was performed for monoclonal antibodies. Tissue sections were incubated for 40 minutes each with the primary antibody in appropriate dilution, a rabbit peroxidase-labeled species-specific antimouse immunoglobulin (DAKO, Glostrup, Denmark) diluted 1:20 in Tris buffer, and a swine polyclonal peroxidase-labeled antirabbit immunoglobulin antibody (DAKO) diluted 1:20 in Tris buffer. The unconjugated peroxidase-antiperoxidase method described by Sternberger et al[14] was applied for polyclonal antibodies. Slides were incubated for 30 minutes with the normal goat serum (DAKO), followed by 40 minutes each with the primary polyclonal rabbit antibody, with goat antirabbit gammaglobulin (DAKO) and with peroxidase-antiperoxidase produced from rabbit (DAKO). The enzyme reaction was developed with diaminobenzidine and hydrogen peroxide (H_2O_2) for 10 minutes. Finally, the sections were counterstained with hematoxylin, dehydrated, cleared in xylene, and mounted. The antibodies used for this study were the following: antichromogranin A (Clone A11, Biosoft, Varilhes, France, monoclonal, 1:100), anti–neuron-specific enolase (DAKO, polyclonal, 1:100), CD57 (Clone NC1, Immunotech, Marseille, France, monoclonal, 1:25), anti-insulin (Clone E2E3, Immunotech, monoclonal, 1:50), antiglucagon (Clone K79bB10, Sigma, St Louis, Mo, monoclonal, 1:1,000), antigastrin (Clone 4C7A1, Immunotech, monoclonal, 1:50), antiserotonin (Clone 5HT-H2O9, DAKO, monoclonal, 1:10), anti–vasoactive intestinal polypeptide (Biogenex, San Ramon, Calif, polyclonal, 1:40), antisomatostatin (DAKO, polyclonal, 1:100), anti–pancreatic polypeptide (DAKO, polyclonal, 1:600), anticalcitonin (Biolyon, Lyon, France, polyclonal, 1:100), and MIB-1 antibody (Immunotech, monoclonal, 1:50).

Normal pancreas (for chromogranin, neuron-specific enolase, CD57, insulin, glucagon, somatostatin, and pancreatic polypeptide), gastric antrum (for gastrin), and colon (for vasoactive intestinal polypeptide) were used as positive controls. Negative control tissue sections were included for each tumor with the same steps except for deletion of the primary antibody.

Counting procedure—The MIB-1 proliferation indexes were calculated based on a count of 1,000 tumor nuclei at a magnification of 400. Nuclei of tumor cells were considered to be immunostained for MIB-1 if a pale to dark diffuse or granular staining pattern was recognized throughout the nuclear area. If MIB-1 reactivity was limited to a few minute particles of nuclear staining, it was considered a trace reaction and not computed in the MIB-1 index. Nuclei immunolabeled for MIB-1 were counted in each tumor by two independent observers using a standard light microscope equipped with an ocular reticle. The means of the two percentages of MIB-1-labeled nuclei were used in the statistical analysis described in the "Statistical analysis" section. After visual inspection, areas within the section presenting the highest number of labeled cells were counted, regardless of the staining intensity.

Classification of the tumors—Tumors were divided into two groups according to their clinical and laboratory findings, ie, functioning and nonfunctioning tumors. Functioning tumors were defined by the occurrence of a compatible clinical syndrome associated with the serum elevation and the immunohistochemical detection of the corresponding hormones. Nonfunctioning tumors were identified by the absence of clinical symptoms and the lack of serum elevation of the gastrointestinal peptides, regardless of the presence of immunostaining for any hormone. Tumors were also classified according to the Klöppel

CLINICOPATHOLOGIC DATA FOR THE 35 CASES OF NEUROENDOCRINE TUMORS OF THE PANCREAS*

Case No.	*Tumor Type*	*Tumor Size (cm)*	*Metastases*	*Local Invasion*	*Klöppel Stage*	*MIB-1 Proliferation Index (%)*	*Follow-Up (mo)*	*Outcome*
1	Nonfunctioning	4	Yes	Yes	III	7.80	0	PO
2	Gastrinoma	8	Yes	Yes	III	5.65	17	AW
3	Nonfunctioning	5	Yes	Yes	III	5.25	7	AW
4	Calcitoninoma	13	No	Yes	III	8.40	0	PO
5	Nonfunctioning	8	Yes	Yes	IV	7.80	78	M
6	Nonfunctioning	5	No	Yes	III	6.10	48	AW
7	Insulinoma	7.5	Yes	Yes	III	3.50	132	AW
8	Insulinoma	1.5	No	No	I	0.50	30	AW
9	Nonfunctioning	1	No	No	I	1.80	30	AW
10	Nonfunctioning	3.5	No	Yes	III	9.00	1	PO
11	Nonfunctioning	5	Yes	Yes	III	5.70	30	AW
12	Glucagonoma	2.5	Yes	Yes	III	8.40	0	PO
13	Vipoma	4.5	Yes	Yes	III	5.15	43	AW
14	Nonfunctioning	4	Yes	Yes	III	5.20	0	M
15	Nonfunctioning	4	Yes	Yes	III	4.95	18	AW
16	Nonfunctioning	6	Yes	Yes	III	12.5	12	AW
17	Gastrinoma	1	No	No	I	0.90	56	AW
18	Carcinoid	2	No	No	II	2.10	13	AW
19	Nonfunctioning	6	Yes	Yes	III	3.80	90	AW
20	Gastrinoma	1.5	No	No	II	3.50	77	AW
21	Insulinoma	1.5	No	No	I	1.15	97	AW
22	Nonfunctioning	6	No	No	III	0.45	12	AW
23	Nonfunctioning	2	Yes	Yes	II	8.40	13	AW
24	Nonfunctioning	4	Yes	Yes	III	4.35	5	M
25	Nonfunctioning	2	No	No	II	2.30	3	AW
26	Nonfunctioning	4	No	No	III	4.70	12	AW
27	Insulinoma	6	Yes	Yes	III	5.50	3	M
28	Nonfunctioning	9	Yes	Yes	III	10.00	10	AW
29	Nonfunctioning	7	Yes	No	III	4.30	7	AW
30	Insulinoma	1	No	No	I	0.66	18	AW
31	Calcitoninoma	10	Yes	No	III	7.35	36	AW
32	Nonfunctioning	4.5	No	No	III	4.50	36	AW
33	Nonfunctioning	5	Yes	Yes	IV	16.10	4	M
34	Nonfunctioning	4	No	Yes	III	8.70	10	AW
35	Glucagonoma	5	Yes	Yes	III	10.00	3	M

PO = died during the early postoperative period; AW = alive and well; M = died of metastatic disease.
*All tumors were well differentiated, except in cases 5 and 33.

classification as follows: stage I, insulinomas or nonfunctioning tumors less than 2 cm in size or functioning tumors less than 1 cm; stage II, insulinomas or nonfunctioning tumors ranging from 2 to 3 cm or functioning tumors ranging from 1 to 2 cm; stage III, all other histologically well-differentiated tumors; and stage IV, all histologically poorly differentiated carcinomas. All the neuroendocrine tumors of the pancreas associated with local invasion or distant metastases were classified as stage III or stage IV, regardless of their histologic differentiation.

Statistical analysis—The Spearman correlation rank test was used to correlate the Klöppel tumor stage with the clinical outcome. The outcome was defined as dead or alive at the cutoff date of the study. Correlations between clinical outcome and the different prognostic factors included in the Klöppel classification (ie, diameter, metastases, local invasion, functional status, and production of insulin) were examined by dividing the population into two groups corresponding to the character of the prognostic factor. The groups then were tested for the common mean value by using the Student's test. Interobserver reproducibility for the MIB-1 count was determined by the Spearman test. Correlations between the MIB-1 index and the tumor stage, the grade of histologic differentiation, metastases, hormonal synthesis (insulin *vs* others or no hormone) were evaluated by analysis of variance (ANOVA). Correlation of tumor size with MIB-1 was performed by multiple regression. A receiver operator characteristic (ROC) curve[15] was established to specify the MIB-1 index associated with the best sensitivity and specificity in prediction of death. The probability of survival for the different proliferation indexes was calculated by using the Kaplan-Meier method.[16] To establish the Kaplan-Meier curve, the patients were divided into two groups: the first included patients with an MIB-1 proliferation index lower than or equal to 4%; the second included patients with an MIB-1 proliferation index higher than 4%. The Cox Proportional Hazards Regression Model was used to determine independent predictors of survival.[17]

RESULTS

The clinicopathologic data for the patients included in the study groups are summarized in the Table. Ages ranged from 27 to 77 years (mean, 55 3/4 years), and the male/female ratio was 4:5. According to the Klöppel classification, 5 tumors were stage I or benign tumors, 4 were stage II or tumors of uncertain prognosis, 24 were stage III or low-grade malignant tumors, and 2 were stage IV or high-grade malignant tumors. The size of the tumors ranged from 1 to 16 cm (mean, 4.7 cm). Microscopic examination showed that 33 tumors were well-differentiated neuroendocrine tumors of the pancreas and 2 were poorly differentiated neuroendocrine tumors of the pancreas. Twenty tumors were associated with distant metastases at the time of operation. Fourteen tumors were considered functioning. These 14 tumors were classified as follows: insulinoma, 5; gastrinoma, 3; glucagonoma, 2 (Fig 1); calcitoninoma, 2; vipoma, 1; and carcinoid, 1. Twenty-one tumors were considered nonfunctioning. Immunohistochemical study showed that insulin was produced by 8 tumors, glucagon by 14, somatostatin by 6, pancreatic polypeptide by 10, serotonin by 4, gastrin by 7, vasoactive intestinal peptide by 1, and calcitonin by 3. Twenty-one tumors exhibited production of more than 1 hormone.

A positive statistical correlation was found between the Klöppel stage and the clinical outcome (ie, death or no death; $P = .005$). There was also a significant statistical correlation between the clinical outcome and three of the individual parameters of the Klöppel classification: (1) the production of insulin ($P = .002$), (2) the local invasion ($P = .03$), and (3) the occurrence of metastases ($P = .02$). These significant correlations were obtained only if early postoperative deaths were excluded. Conversely, no correlation was found between the functional status or the tumor size and the clinical outcome.

The immunohistochemical staining patterns of the neuroendocrine tumors of the pancreas and the MIB-1 proliferation indexes are summarized in the Table. MIB-1 immunostaining was confined to the nuclei of tumor cells exhibiting a diffuse or a granular pattern (Fig 1, C). The nucleoli were sometimes immunolabeled. All the mitotic figures were immunolabeled. The immunolabeled tumoral cells were usually irregularly distributed in the tumors. There was no preferential distribution of immunoreactive cells in perivascular, central, or peripheral areas of the tumors.

The mean of the MIB-1 proliferation indexes of the 35 neuroendocrine tumors of the pancreas was 5.6% (index range, 0.45%–16.1%). The evaluation of interobserver reproducibility showed a high correlation between the two observers ($P < .0001$, confidence interval, 98%–100%). The means of MIB-1 proliferation indexes for each tumoral stage were as follows: I, 1%; II, 4.1%; III, 6.3%; and IV, 11.9%. The MIB-1 proliferation index was significantly correlated with the tumor stage ($P = .001$, ANOVA). There was a statistically significant correlation

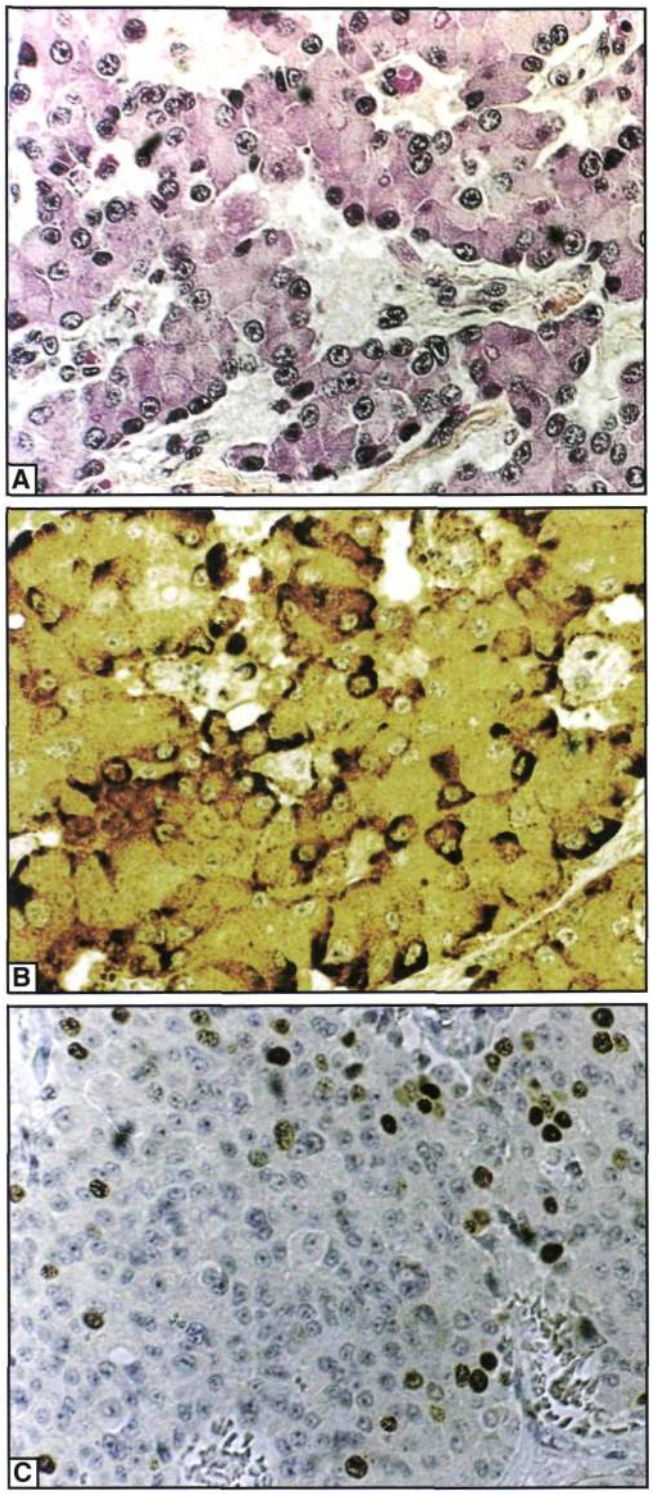

FIG 1. Malignant glucagonoma. A, Predominantly trabecular growth pattern (hematoxylin-eosin-safran, original magnification ×400). B, Numerous tumoral cells were immunolabeled (immunohistochemical stain with antiglucagon monoclonal antibody, original magnification ×400). C, Of the tumoral cells, 10% were immunolabeled (immunohistochemical stain with monoclonal antibody MIB-1, original magnification ×400).

between the MIB-1 proliferation index and the tumor size ($P < .01$). The means of the MIB-1 proliferation indexes were 5.2% for well-differentiated neuroendocrine tumors of the pancreas and 11.9% for poorly differentiated neuroendocrine tumors of the pancreas. A statistically significant correlation was found between the MIB-1 proliferation index and the histologic differentiation of the neuroendocrine tumors of the pancreas ($P = .007$, ANOVA).

The occurrence of local invasion was strongly correlated with the MIB-1 proliferation index ($P < .001$). Similarly, the mean of the MIB-1 proliferation index of the tumors associated with metastases (7%) was higher than that of the tumors without metastases (3.7%), and a statistically significant correlation was found between the occurrence of metastases and the MIB-1 proliferation index ($P = .002$, ANOVA). The mean of the MIB-1 proliferation index was 4.5% in functioning tumors and 6.4% in nonfunctioning tumors. No statistical difference of the MIB-1 proliferation index was found between the functioning tumors and the nonfunctioning tumors or between the neuroendocrine tumors of the pancreas without production of insulin and the neuroendocrine tumors of the pancreas with production of insulin.

Only 31 cases were included for the Cox Proportional Hazards Regression Model because 4 cases associated with early postoperative deaths were censored. The classic prognostic factors and the statistically significant variables in univariate analysis, ie, diameter, Klöppel stage, occurrence of metastases or local invasion, differentiation grade, and MIB-1 proliferation index, were considered in the multivariate analysis. Local invasion was the strongest predictor ($P = .002$; confidence interval, 95%) over other variables.

The construction of an ROC curve showed that an MIB-1 proliferation index higher than 4% predicted a significant increase in the risk of death due to the tumor.

Overall survival estimates were obtained according to the actuarial techniques of Kaplan and Meier. This study showed that the patients with an MIB-1 proliferation index higher than 4% had a statistically significant poorer outcome ($P = .03$; Fig 2).

DISCUSSION

This retrospective study assessed the cell proliferation in 35 neuroendocrine tumors of the pancreas using an immunohistochemical technique on dewaxed slides with MIB-1 antibody. The monoclonal antibody MIB-1 has been proved to be a Ki-67 equivalent, with the advantage over the classic Ki-67 of efficiently

working in archival formalin-fixed tissue.[18–23] The Ki-67 monoclonal antibody reacts with a proliferation-associated antigen expressed in all active parts of the cell cycle; the expression of Ki-67 antigen begins during the S phase, progressively increases through the S and G_2 phases, and reaches a maximum with mitosis. After the division, the cells return to G_1 with a stock of Ki-67 antigen. The level of Ki-67 decreases progressively during the G_1 phase and finally disappears in cells with a long G_1 phase.[24,25] However, although labeling of proliferating cells with Ki-67 and MIB-1 antibodies is essentially equivalent, there are limited differences depending on the binding capacity of nuclear proteins to DNA.[26]

In our study, the method of counting was strictly defined as follows to avoid subjectivity in the scoring process: (1) All the immunostained nuclei were counted regardless of staining intensity. (2) The nuclei immunolabeled for MIB-1 among 1,000 tumoral cells were counted in the areas with the highest detectable staining. By using this method, we obtained good interobserver reproducibility. This method was described in other reports.[27] In these studies, the interobserver reproducibility was similar to that in our study. It has been demonstrated that in neuroendocrine tumors of the digestive tract, subjective scoring of MIB-1 immunochemistry achieved results comparable with those obtained with DNA flow cytometry.[28] However, the flow cytometry technique does not allow the distinction of the MIB-1 positive tumoral cells from other MIB-1 positive cells present in the stroma, such as lymphoid cells, vascular cells, or adjacent reactive cells.[29] Conversely, the immunohistochemical method of MIB-1 labeling likely is more time-consuming. Finally, different studies also showed that the MIB-1 proliferation index is an alternative tool to bromodeoxyuridine for the determination of the proliferation index in different tumors,[30] thus avoiding the administration of a potentially mutagenic drug.

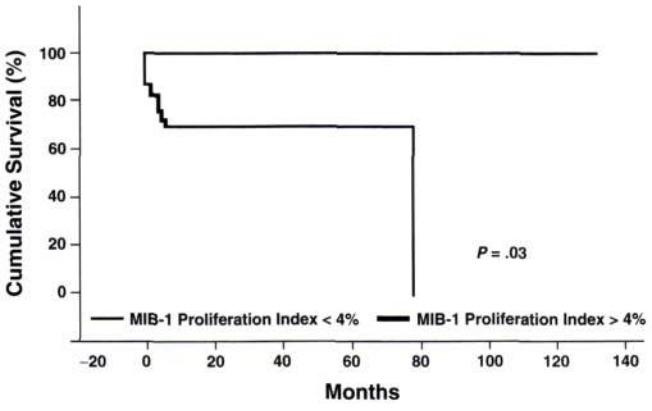

FIG 2. Actuarial survival of 35 patients with neuroendocrine tumors of the pancreas according to the MIB-1 index. Tumors with an MIB-1 index greater than 4% showed a statistically significant decreased percentage of cumulative survival.

A revised classification of neuroendocrine tumors of the pancreas was proposed by Klöppel et al in 1995.[2] It was based on several studies dealing with the prognostic evaluation of the neuroendocrine tumors of the pancreas. To our knowledge, this classification has not yet been evaluated to confirm its prognostic value. So, our first objective was to compare the Klöppel stages with the clinical outcome. We observed a significant correlation. Furthermore, the clinical outcome was correlated with most of the parameters constituting the Klöppel classification. Therefore, this classification seems to be of interest for the prognostic evaluation of the neuroendocrine tumors of the pancreas (and may be used as a reference in the present study).

Our study showed a statistical correlation between the MIB-1 proliferation index and the stages of the prognostic classification proposed by Klöppel et al.[2] Indeed, each stage of the classification is associated with a statistically different MIB-1 proliferation index. The prognostic classification established by Klöppel and colleagues is based on five parameters of neuroendocrine tumors of the pancreas: size, functional status (particularly based on their production of insulin), grade of histologic differentiation, the occurrence of local invasion, and the existence of distant metastases.

The analysis of our results shows that the MIB-1 proliferation index correlated with all the parameters of the Klöppel classification except the functional status. In our study, only the tumors associated with a clinical hormonal syndrome were considered as functioning. This definition is in accordance with most clinical reports, but different from that used by Klöppel et al, who considered as functioning all tumors associated with hormonal production that were detectable by immunochemistry.[2] In earlier reports, functioning neuroendocrine tumors of the pancreas are often associated with a better prognosis than nonfunctioning tumors. This was likely because of their earlier diagnosis. In our study, we did not observe a statistical difference between the prognosis for functioning and nonfunctioning neuroendocrine tumors of the pancreas. This might explain the

absence of statistical differences between the means of MIB-1 proliferation indexes in functioning and nonfunctioning neuroendocrine tumors of the pancreas.

Many studies demonstrated that true insulinomas, particularly those associated with a clinical syndrome, have a better prognosis than other neuroendocrine tumors of the pancreas.[31] In our study, we observed no significant difference between the MIB-1 proliferation index in insulinomas and the MIB-1 proliferation index in other neuroendocrine tumors of the pancreas, whereas the prognosis of insulinomas was better. However, among patients with insulinoma, only one died of the tumor. Interestingly, this patient had the highest MIB-1 proliferation index (5.5%).

Conversely, the size of the tumors correlated with the MIB-1 proliferation index and the prognosis. This correlation is the strongest for the small neuroendocrine tumors of the pancreas (data not shown). In one report, the correlation between the size of the tumors and the proliferation indexes was demonstrated only in functioning tumors, without evident explanation.[27]

In our study, we noted that MIB-1 proliferation index was significantly higher in poorly differentiated neuroendocrine tumors of the pancreas than in well-differentiated neuroendocrine tumors of the pancreas. This difference is associated with a different outcome. The correlation between the MIB-1 proliferation index, the clinical outcome and the histologic differentiation has been reported in other neuroendocrine tumors, particularly those of pulmonary derivation. In studies of typical carcinoids, atypical carcinoids, large cell neuroendocrine carcinomas, and small cell neuroendocrine carcinomas, a significant difference in MIB-1 proliferation indexes has been demonstrated between carcinoids and carcinomas. Likewise, the survival rate was significantly different between pulmonary neuroendocrine tumors with a low and those with a high proliferation index.[32]

Finally, there is a high correlation between local invasion, occurrence of metastases, clinical outcome, and MIB-1 proliferation indexes. According to the Klöppel classification, stage III and stage IV neuroendocrine tumors of the pancreas are associated with a high proliferative activity and a poor outcome. It is interesting that the Cox multivariate model shows that the only variable with an independent effect on survival is local invasion.

Our study showed a statistically significant increase in the number of deaths and a statistically significant decrease in survival time with an MIB-1 proliferation index higher than 4%. This result is similar to that determined by another study using proliferating cell nuclear antigen for measuring the proliferation index in neuroendocrine tumors of the pancreas.[27] Pelosi et al[33] recently published a report of the study of the expression of MIB-1 antigen in neuroendocrine tumors of the pancreas. They considered that tumors with an MIB-1 proliferation index greater than 5% were likely to have an unfavorable prognosis. It is also interesting that an MIB-1 proliferation index of 4% is claimed to have prognostic value in neuroendocrine tumors of the bronchopulmonary tract.[32]

Our data indicate that the count of nuclei immunolabeled for MIB-1 performed by using a rigorous method is a simple and reproducible method to determine the growth fraction of neuroendocrine tumors of the pancreas. This method, available in any laboratory, can be applied to routinely processed samples. The MIB-1 proliferation index seems to be a reliable tool to assess the prognosis of neuroendocrine tumors of the pancreas. It might provide information about the clinical outcome for individual patients. Because large series of neuroendocrine tumors of the pancreas are difficult to obtain, other studies throughout the world would be mandatory to perform a reliable meta-analysis of the validity of MIB-1 index.

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