Published in final edited form as:

Surgery. 2012 July ; 152(1): 107–113. doi:10.1016/j.surg.2012.02.011.

Ki-67 Predicts Disease Recurrence and Poor Prognosis in Pancreatic Neuroendocrine Tumors

Nicholas A. Hamilton, MD,

Department of Surgery, Washington University, Saint Louis, MO

Ta-Chiang Liu, MD, PhD,

Department of Pathology and Immunology, Washington University, Saint Louis, MO

Antonino Cavatiao, BS,

Department of Surgery, Washington University, Saint Louis, MO

Kareem Mawad, MD,

Department of Surgery, Washington University, Saint Louis, MO

Ling Chen, PhD,

Biostatistics Core, Siteman Cancer Center, Saint Louis, MO

Steven S. Strasberg, MD,

Department of Surgery, Washington University, Saint Louis, MO

David C. Linehan, MD, and

Department of Surgery, Washington University, Saint Louis, MO

Dengfeng Cao, MD, PhD

Assistant Professor, Department of Pathology and Immunology, Washington University, Saint Louis, MO

Abstract

Background—Pancreatic neuroendocrine tumors are rare malignancies for which the ideal staging method remains controversial. Ki-67 is a cell proliferation marker that has been shown to have some utility in predicting prognosis in neuroendocrine tumors. We sought to test the predictive ability of Ki-67 staining for disease recurrence and overall survival in pancreatic neuroendocrine tumors.

Methods—The medical records of patients who underwent pancreatic resection for pancreatic neuroendocrine tumors at a tertiary referral hospital from 1994–2009 were reviewed. The pathologic specimens of all were stained for Ki-67 and recorded as percentage of cells staining positive per high-powered field. The 10-year disease-free and overall survival was analyzed.

Results—One hundred forty patients were identified. Gender and age were not associated with increased risk of disease recurrence. Patients with tumors larger than 4cm or with Ki-67 staining >9% were more likely to have disease recurrence ($p=0.0454$, 0.047 , respectively) and have decreased overall survival ($p<0.0001$, 0.0007 , respectively).

Corresponding author: William G. Hawkins, MD, Associate Professor, Department of Surgery, Washington University, Saint Louis, MO, hawkinsw@wudosis.wustl.edu, 660 S. Euclid, Box 8109, Saint Louis, MO 63110, Office: (314) 362-7046, Fax: (314) 367-1943.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

This work was presented in part at the 6th Academic Surgical Congress February, 2011

Conclusions—Increasing tumor size and increasing Ki-67 staining both correlate with increased risk of disease recurrence and decreased overall survival. Designing a staging system that incorporates both of these clinical variables will enable better identification of patients at risk for recurrent pancreatic neuroendocrine neoplasm.

Introduction

Pancreatic neuroendocrine tumors (PNETs) are a rare group of neoplasms arising from the endocrine pancreas and incidence is on the rise [1]. Because of their relative rarity, classification of these tumors has been inconsistent. Some groups have attempted to apply the TNM (Tumor, Node, Metastasis) classification schema which is applied to the more virulent pancreatic adenocarcinoma [2], while others have attempted to classify them based on mitotic rate and necrosis, as is done in other endocrine tumors [3]. Still others have created a prognostic scale based on size, grade, node status and resection margins for prediction of prognosis [4].

Ki-67 is a cell-surface antigen with expression found in all proliferating cells. Grading neuroendocrine tumors by Ki-67 proliferation index has recently been added to the American Joint Committee on Cancer's (AJCC) carcinoid staging system [5]. Studies have shown percent Ki-67 expression correlates with malignant potential [6] and prognosis in PNETs [7]. Despite this, the widespread use of Ki-67 has not been implemented in the PNET staging guidelines by the AJCC and only considered in the earliest stage (stage I) by the World Health Organization's classification [8]. Recently, some groups have advocated for changing the staging from a tumor, node and metastasis (TNM) model to a tumor, grade and metastasis (TGM) model, with grades of well and poorly differentiated PNETs [9]. However, it is still unclear how Ki-67 proliferation index should fit into the staging of PNETs.

We sought to define the prognostic ability provided by Ki-67 staining in PNETs and compare that to other known prognostic factors.

Methods

The medical records of patients with PNETs treated surgically at Barnes-Jewish Hospital/ Washington University School of Medicine from 1994–2009 were retrospectively reviewed. Any patient with insufficient clinical follow up or whose pathologic specimen did not allow for Ki-67 staining (too little tissue remaining) was excluded. Demographic data was collected, including gender, ethnicity, age at operation, location of tumor (proximal vs distal), type of operation (enucleation, Whipple procedure, distal pancreatectomy, total pancreatectomy) and longitudinal outcome was assessed. Results are reported as means (standard error of the mean). We then examined the surgical specimen. Information about tumor size, grade, margin and the number of lymph nodes (collected and with metastatic disease) from each patient was collected. Standard hematoxylin and eosin staining was performed as well as immunohistochemical staining for Ki-67 on all patients. The Ki-67 proliferation index was graded by two pathologists (DC and TL) as the percentage of tumor cells per high powered field staining positive for Ki-67. Comparisons between groups were made using student t-tests on GraphPad Prism 5 (GraphPad Software, Inc., La Jolla, CA). We defined the date of disease recurrence by the first date of any radiographic evidence of local recurrence or metastatic disease. Disease-free survival (DFS) was defined as the time from operation to first recurrence. Those alive and free of disease were censored at last contact. Overall survival was determined primarily from the medical records and supplemented in a few cases by the Social Security Death Index. Overall survival (OS) was defined as the time from operation to time of death. Patients alive were censored at the date

of last clinical contact. Univariate Cox proportional hazard models were fitted to identify factors significantly related to OS or DFS. To assess whether any of these factors is an independent predictor of survival, multivariate Cox models were constructed via a backward selection procedure (using p-value of 0.25 for entering an explanatory variable and p-value of 0.05 for variable removal), while considering all factors showing $p < 0.2$ in the univariate analyses. All statistical tests were 2-sided and p-values < 0.05 were considered significant. All the analyses were performed with SAS version 9.2 (SAS Institute, Cary, NC).

Results

From 4/1/1994 to 4/1/2009, 156 patients were treated surgically for PNETs. Complete medical records and tissue samples were available in 140. Sixteen (11.4%) patients were found to have stage IV disease at the time of operation and were excluded from primary analysis. Patient demographics can be found in table 1. Twenty-nine (20.7%) patients experienced recurrence of disease. Nineteen (13.6%) patients had known Multiple Endocrine Neoplasia Type I Syndrome and 22 (15.7%) presented with symptoms consistent with functional endocrine tumor (flushing, hypoglycemia, dizziness). Mean time ($\pm$ standard error of mean) to recurrence was 3.7 $\pm$ 0.5 years. Overall 5-year and 10-year survival was 85.7% and 59.3% for patients without disease recurrence and 76% and 16.7% for patients in whom disease recurred, respectively. Average survival after disease recurrence was 2.34 $\pm$ 0.4 years.

The location of the tumor was not predictive of disease recurrence, as recurrence was seen in 25.7% of proximal lesions and 25.5% of distal lesions. ($p=0.80$). Age and gender were also not found to be predictive of disease recurrence ($p=0.0606$, 0.4776, respectively) or decreased overall survival ($p=0.0901$, 0.4580, respectively). The MEN status of patients was also not predictive of disease recurrence or overall survival ($p=0.483$, 0.393, respectively).

Factors associated with recurrence

Tumor Size—Tumor size was associated with disease recurrence, with mean sizes of 2.21 $\pm$ 1.7 cm and 3.73 $\pm$ 2.2 cm in patients without and with disease recurrence, respectively ($p=0.0002$). When stratified by the size of the tumor (grouped by tumors < 2 cm, 2–4 cm and > 4 cm), tumor size was associated with increased risk of recurrence and decreased overall survival ($p=0.0196$, <0.005 , respectively). Figure 1 demonstrates disease free and overall survival by tumor size.

Tumor Differentiation—The degree of tumor differentiation was not consistently reported on the pathology reports at our institution. This variable was present in 83 (59.3%) pathology reports (it was not a standard of reporting at our institution until recently). However, in those patients in whom it was reported, differentiation significantly predicted disease recurrence. Patients with moderately to poorly differentiated tumors were 6.23 times as likely to have disease recurrence as those with well-differentiated tumors (95% CI 1.88–20.63). Figure 2 demonstrates a Kaplan Meier survival curve stratified by tumor differentiation.

Ki-67 Proliferation Index—The degree of Ki-67 proliferation in specimens was strongly correlated with both disease free and overall survival. Patients with a proliferation index $> 9\%$ were 6.9 times as likely to have recurrent disease as those with a Ki-67 index $< 9\%$ (95% CI 3.0–15.7) and 8.5 times as likely to have disease recurrence when compared to patients with a Ki-67 index $< 5\%$ (95% CI 3.4–21.4). Representative pathologic staining of each group can be seen in Figure 3. Figures 4a and 4b show disease free survival by two different stratification groupings, one suggested by the AJCC grading guidelines ($<2\%$, 2–

20% and > 20%, $p < 0.01$) for non-pancreatic neuroendocrine tumors and one optimized to separate out low, intermediate and high risk patients (<5%, 5–9% and >9%, $p < 0.0001$), respectively. Five-year disease-free survival for low, moderate and high risk Ki-67 staining is 92%, 75% and 52% by AJCC stratification and 95%, 80% and 42%, respectively, by our optimized stratification. Ten-year disease-free survival is 80%, 70% and 52% by AJCC stratification and 92%, 75% and 52% by our stratification for low, moderate and high risk levels of Ki-67 present.

The amount of Ki-67 present in the resected tumor also predicts overall survival. Both the AJCC and our proposed stratification systems identified the three groups with statistical significance (See figures 5A, 5B). Five- and 10-year overall survival by AJCC stratification was 90% and 85% for low risk, 88% and 70% for moderate risk and 57% and 0% for high risk Ki-67 proliferation index. Using our stratification system, we found similar results, with overall survival rates of 90% and 88% for low risk, 90% and 80% for moderate risk and 61 and 20% for high risk 5- and 10- year survival, respectively. Figure 5 demonstrates overall survival by Ki-67 staining stratification.

Lymph node status—The presence of lymph node disease was predictive of disease recurrence. Only 27.6% of patients without disease recurrence had nodal disease, compared to 66.7% of patients with recurrent disease ($p < 0.005$), and the odds ratio of having disease recurrence if nodal disease was present was 5.25 (95% CI 1.89–14.59). Table 2 shows the relationship between the lymph node status and the incidence of disease recurrence when compared to both tumor size and Ki-67 staining.

WHO Classification—Since the WHO classification of PNETs is the only classification system currently including Ki-67 in the staging criteria, we staged our patients by the WHO classification and plotted the disease free and overall survival. This is illustrated in Figure 6. As with tumor differentiation, the lymphovascular and perineural invasion data was limited. Table 3 illustrates the different clinical data associated with disease recurrence and poor outcomes.

Multivariate analysis—Multivariate analysis showed tumor size is independently associated with overall survival (p -value= 0.0085). No other single clinical variables were statistically significant for overall survival and no clinical variables were statistically significant for disease free survival. There was no correlation between tumor size, tumor differentiation or Ki-67 proliferation index.

Discussion

The incidence of neuroendocrine tumors of the pancreas is on the rise. This may be due in large part to increased recognition of the disease. Many small tumors are identified incidentally because of increased utilization of cross sectional imaging. The likelihood of finding small non-functional PNETs is likely rising at a faster rate than that of functional PNETs. It may be that many of these small tumors in elderly individuals might never become symptomatic. Identifying risk factors and creating a prognostic staging system that accurately predicts those patients at elevated risk for disease progression, recurrence and death would allow for improved long-term monitoring and selective, surgical and adjuvant therapies. Evidence is mounting that biochemical markers like Ki-67, MIB-1 [10], and others add in identifying patients at risk for disease progression recurrence. We are hopeful that molecular characterization of tumors will help direct therapy to a greater extent. Adding molecular characterization to the staging schema will likely prove an objective, reproducible event that could potentially be used as a surrogate for lymph node status when few nodes are obtained at the time of a resection. Further study would be required to determine if Ki-67

staining in combination with other molecular markers on preoperative biopsy could eventually guide treatment planning by helping to define the extent of resection needed (enucleation as opposed to a formal resection), particularly in patients with small, minimally-Ki-67 positive tumors. Finally, Ki67 staining might prove useful in determining which patients should undergo adjuvant investigational therapies [11].

Tumor size, positive lymph nodes and Ki-67 staining were found to be predictive of disease recurrence and poor overall survival. We found that defining low, intermediate and high risk groups by Ki-67 proliferation indices of < 5%, 5–9% and > 9% optimizes the ability to distinguish between patients who are at risk of having recurrent disease and those who are not. In patients with disease recurrence, Ki-67 expression was elevated in patients with large tumors or positive lymph nodes, suggesting elevated expression can be found in both locally advancing malignancy as well as metastatic tumors.

When ANOVA was performed to check for the relationship between these three factors, it was found that patients with nodal metastases were more likely to have larger tumors ($p=0.04$), but we did not identify a strong correlation between Ki-67 proliferation index and nodal metastases, suggesting each serves as an independent prognostic factor for potential disease recurrence.

Multivariate analysis identified tumor size as the only independent predictor of overall survival and no variable was individually predictive of disease recurrence. This illustrates the importance of including each factor in the staging of PNETs, rather than relying on any one predictor of disease recurrence.

There were several limitations to our study. First and foremost was the retrospective nature of the data collection. The medical records were not complete in all categories, making it impossible to analyze all the known predictors of disease recurrence and survival. For example perineural and lymphovascular invasion were not included in the analysis. The disease is relatively rare, and the patients have slow disease progression making it likely that we are underpowered to observe all the important correlations between the analyzed factors. Our patient population might not be representative of all centers caring for patients with PNETs, as we see a disproportionally high number of patients with MEN. While there did not seem to be a difference in disease recurrence in patients with and without MEN, the biology and multifocality of their tumors is likely different from that of spontaneous PNETs.

In summary, we found that the percentage of Ki-67 positive cells in PNETs predicts disease recurrence and overall survival. We are strongly in favor of further investigations into this and other molecular markers in guiding the management of this disease. Inclusion of Ki67 in future grading schemes will allow for a more objective and reproducible staging schema and might be an important element in deciding the future management of patients with PNETs.

Acknowledgments

This work was funded by the Washington University Surgical Oncology Training Grant (NIH-5T32CA00962122), the Doug Phillips Fund for Pancreatic Cancer Research and the Siteman Cancer Center Support Grant (NIH-2P30CA09184209).

References

1. Franko J, Feng W, Yip L, Genovese E, Moser AJ. Non-functional neuroendocrine carcinoma of the pancreas: incidence, tumor biology and outcomes in 2,158 patients. *J Gastrointest Surg*. 2010; 14:541–548. [PubMed: 19997980]

2. Bilimoria KY, Bentrem DJ, Merkow RP, et al. Application of the pancreatic adenocarcinoma staging system to pancreatic neuroendocrine tumors. *J Am Coll Surg*. 2007; 205:558–563. [PubMed: 17903729]
3. Hochwald SN, Zee S, Conlon KC, et al. Prognostic factors in pancreatic endocrine neoplasms: an analysis of 136 cases with a proposal for low-grade and intermediate-grade groups. *J Clin Oncol*. 2002; 11:2633–2642. [PubMed: 12039924]
4. Ballian N, Loeffler AG, Rajamanickam V, Norstedt PA, Weber SM, Cho CS. A simplified prognostic system for resected pancreatic neuroendocrine neoplasms. *HPB*. 2009; 11:422–428. [PubMed: 19768147]
5. Edge, SB.; Fritz, AG.; Byrd, DR., et al., editors. *AJCC Cancer Staging Manual*. 7th ed.. 2010. Ch 24: Exocrine and Endocrine Pancreas; p. 241-249.
6. Pelosi G, Bresaola E, Bogina G, Pasini F, Rodella S, Castelli P, et al. Endocrine tumors of the pancreas: Ki-67 immunoreactivity 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*. 1996; 27:1124–1134. [PubMed: 8912819]
7. Jamali M, Chetty R. Predicting prognosis in gastroentero-pancreatic neuroendocrine tumors: an overview and the value of Ki-67 immunostaining. *Endocr Pathol*. 2008; 19:282–288. [PubMed: 18931958]
8. Eneha F, Saeger HD, Schmidt CM, Grutzmann R. Neuroendocrine tumors of the pancreas. *The Oncologist*. 2009; 14:456–467. [PubMed: 19411317]
9. Martin RCG, Kooby DA, Weber SM, Merchant NB, Parikh AA, Cho CS, et al. Analysis of 6,747 pancreatic neuroendocrine tumors for a proposed staging system. *J Gastrointest Surg*. 2011; 15:175–183. [PubMed: 21103949]
10. Gentil-Perret A, Mosnier JF, Buono JO, Berthelot P, Chipponi J, Balique JG, et al. The relationship between MIB-1 proliferation index and outcome in pancreatic neuroendocrine tumors. *Am J Clin Pathol*. 1998; 109:286–293. [PubMed: 9495200]
11. Vilar E, Salazar R, Perez-Garcia J, Cortes J, Oberg K, Tabernero J. Chemotherapy and role of the proliferation marker Ki-67 in digestive neuroendocrine tumors. *Endocrine-Related Cancer*. 2007; 14:221–232. [PubMed: 17639039]

Figure 1.
(A) Disease free and (B) Overall survival by tumor size

Figure 2.
10-Year Disease Free Survival by Tumor Differentiation

Figure 3.

Figure 4.
Disease Free Survival by Ki-67 Staining (A)-AJCC neuroendocrine tumor stratification (B)
our proposed stratification

Figure 6.
(A) Disease free and (B) Overall survival by WHO Classification

Table 1

Patient, Tumor and Surgical Characteristics

		No. (%)
Sex	Male	74 (52.9)
	Female	66 (47.1)
Patient age		54.2 +/- 1.3
Tumor type	Nonfunctioning	104 (74.3)
	Insulinoma	20 (14.3)
	Gastrinoma	9 (6.4)
	Glucagonoma	1 (0.7)
	VIPoma	6 (4.3)
Tumor Site	Head	64 (45.7)
	Body-Tail	76 (54.3)
Hereditary Status	Sporadic	120 (85.7)
	MEN 1	20 (14.3)
WHO grade	I	92 (65.7)
	II	42 (30)
	III	6 (4.3)
	Not recorded	57 (40.7)
Ki-67	<2	43 (30.7)
	2–20	78 (55.7)
	>20	19 (13.6)
Tumor Size mean (median)		2.97 (2.0)
Operation	Enucleation	14 (10)
	Anatomic resection (whipple or DP)	110 (78.6)
	Anatomic Resection and Metastasectomy	16 (11.4)
Surgery technique	Open Surgery	126 (90)
	Laparoscopic	14 (10)

Table 2

Exploring the relationship between increasing tumor size, increasing percent of Ki67 positivity, lymph node positivity and incidence of recurrence

Tumor Size (primary)	Lymph Node Positive (percent)	Recurrence (percent)
<1cm	1	0 (0)
1–2cm	9	3 (33.3)
2–3cm	8	1 (12.5)
3–4cm	7	3 (42.9)
>4cm	16	7 (43.8)
Ki67 positive (primary)	Lymph Node Positive (percent)	Recurrence (percent)
<2	12	1 (8.3)
2–9	14	4 (28.6)
10–20	5	3 (60)
>20	12	5 (41.7)

Table 3

Prognostic factors following resection of locoregional disease

		Number with recurrent disease (%)	Univariate significance	
			OS	DFS
Sex	Male	17 (23)	0.1066	0.1009
	Female	11 (17)		
Tumor type	Functioning	4 (11)	0.22	
	Nonfunctioning	22 (21)		
Ki-67 (AJCC)	<2	5 (10)	<0.0002	0.0454
	2–20	16 (21)		
	>20	5 (26)		
Ki-67 (Proposed)	<5		< 0.0001	<0.0001
	5–9			
	>9			
Tumor Size	<2cm	4 (8.5)	0.0007	0.0196
	2–4cm	5 (15)		
	>4cm	9 (24)		
Patient age	<40	4 (18)	0.6124	0.6471
	40–60	14 (16)		
	>60	5 (17)		
Ki-67 (continuous)			< 0.0001	0.0362
Tumor Size (continuous)			< 0.0001	0.0337
Patient age (continuous)			0.0880	0.6829