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ORIGINAL ARTICLE – ENDOCRINE TUMORS

Pancreastatin Predicts Survival in Neuroendocrine Tumors

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ABSTRACT

Background. Serum neurokinin A, chromogranin A, serotonin, and pancreastatin reflect tumor burden in neuroendocrine tumors. We sought to determine whether their levels correlate with survival in surgically managed small bowel (SBNETs) and pancreatic neuroendocrine tumors (PNETs).

Methods. Clinical data were collected with Institutional Review Board approval for patients undergoing surgery at one center. Progression-free (PFS) and overall (OS) survival were from the time of surgery. Event times were estimated by the Kaplan–Meier method. Preoperative and postoperative laboratory values were tested for correlation with outcomes. A multivariate Cox model adjusted for confounders.

Results. Included were 98 SBNETs and 78 PNETs. Median follow-up was 3.8 years; 62 % had metastatic disease. SBNETs had lower median PFS than PNETs (2.0 vs. 5.6 years; $p < 0.01$). Median OS was 10.5 years for PNETs and was not reached for SBNETs. Preoperative neurokinin A did not correlate with PFS or OS. Preoperative serotonin correlated with PFS but not OS. Higher levels of preoperative chromogranin A and pancreastatin showed significant correlation with worse PFS and OS ($p < 0.05$). After multivariate adjustment for confounders, preoperative and postoperative pancreastatin remained independently predictive of worse PFS and OS ($p < 0.05$). Whether pancreastatin normalized postoperatively further discriminated outcomes. Median PFS was 1.7 years in patients with elevated preoperative pancreastatin versus 6.5 years in patients with normal levels ($p < 0.001$).

Conclusions. Higher pancreastatin levels are significantly associated with worse PFS and OS in SBNETs and PNETs. This effect is independent of age, primary tumor site, and presence of nodal or metastatic disease. Pancreastatin provides valuable prognostic information and identifies surgical patients at high risk of recurrence who could benefit most from novel therapies.

Small bowel (SBNETs) and pancreatic (PNETs) neuroendocrine tumors (NETs) have an annual incidence in the United States of 1–2 per 100,000.[1,2] Surgery is the primary treatment for SBNETs and PNETs and benefits even patients with advanced metastases.[2–13] Despite effective treatments and long overall survival (OS) times, tumor recurrence occurs frequently after resection.[7] Medical treatment with somatostatin analogs, such as octreotide, is indicated in patients with symptomatic or recurrent disease.[12] Octreotide promotes disease stabilization and prolongs survival in selected patients.[7,14] Additional treatments, such as peptide-receptor radionuclide therapy in SBNETs and PNETs and everolimus or sunitinib in PNETs, can help patients with recurrent, extensive, or refractory disease.[15–17]

The difficulty of distinguishing patients with indolent disease from those likely to experience early progression and death remains a major problem in neuroendocrine tumor management.[18] In addition to features visible on cross-sectional imaging, serum levels of tumor markers inform prognosis in SBNETs and PNETs. Neuroendocrine

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cells secrete proteins and amines such as chromogranin A, neurokinin A, pancreastatin, and serotonin, which reflect extent of disease and can herald progression.[19–23] Of these, chromogranin A is the most widely used and represents the only tumor marker recommended by current NET management guidelines.[2,3,12,24] Despite these endorsements, chromogranin A has important limitations for predicting NET prognosis, including false elevation due to comorbid conditions or medications and lack of assay standardization.[24–26] Pancreastatin has been proposed as an alternative biomarker, because its levels are less susceptible to nonspecific effects, the assay is more standardized, and early experience indicated a correlation with tumor burden and outcomes.[25–30]

Improving biomarker-based prognostication through long-term correlation with outcomes at specialized centers is identified as a priority in NET treatment.[18] In addition to improving the accuracy of discussions with patients, distinguishing high-risk patients before surgery allows inclusion in clinical trials of those most likely to benefit. To improve prognostication in neuroendocrine disease, we therefore sought to determine whether preoperative and postoperative serum levels of these four tumor markers correlate with outcomes in a large cohort of surgically managed SBNET and PNET patients with long-term follow-up.

METHODS

Clinical data for patients undergoing surgery for SBNETs and PNETs at a single center between 1999 and 2013 were retrospectively reviewed under an Institutional Review Board–approved protocol. The operative approach was as previously described.[31] Preoperative and postoperative laboratory values were recorded, and clinical notes and radiology reports were reviewed for dates of surgery, disease progression, last follow-up, and death. All event times were defined as from the date of surgery. Pancreastatin was measured with a C-terminal–specific radioimmunoassay as described.[25] Laboratory values were log-transformed because of skew and were tested both as continuous and categorical (normal range vs. elevated) variables for correlation with progression-free (PFS) and OS. Median event times were estimated by using the Kaplan–Meier method, and p-values were calculated by using the log-rank test.[32] Follow-up times were estimated by the reverse Kaplan–Meier method.[33] For laboratory values showing significant association with outcomes ($p < 0.05$) on univariate analysis, multivariate Cox models adjusted for effects of confounding factors.[34] Proportional hazards assumptions were verified. Patient characteristics were compared by using Fisher's exact or Wilcoxon rank-sum tests. Preoperative and postoperative laboratory values were compared by the Wilcoxon sign-rank test. All analyses used R v. 3.0.1 (Vienna, Austria).

RESULTS

Patient Characteristics

Included were 98 SBNET and 78 PNET patients ($n = 176$), 46 % of whom were female. The median age at surgery was 58 years, and 62 % had metastatic disease. The median time from diagnosis to surgery was 65 days. Significant differences existed between SBNET and PNET patients in median age at surgery, the proportion who were female, and the proportion with low-grade, node-positive, or metastatic disease (Table 1). Median follow-up was 3.8 years and was similar between SBNET and PNET patients. Median PFS was 3.3 years overall but was significantly shorter among patients with SBNETs compared with PNETs (2.0 vs. 5.6 years; $p < 0.01$). Despite high rates of tumor progression, estimated median OS was 10.5 years in patients with PNETs and was not reached in SBNET patients (Fig. 1). Five-year OS was 79 % in SBNETs and 80 % in PNETs. In 108 patients with metastatic disease at the time of surgery, 5-year OS was 76 % for SBNETs and 71 % for PNETs.

Laboratory Values and Outcomes

To understand their relation to outcomes, preoperative serum levels of tumor markers as well as clinical factors were tested for univariate association with PFS and OS. As expected, N and M stage, as well as tumor grade, showed significant correlations with PFS and OS (Table 2). Lymph node ratio, T-stage, and primary site showed significant associations with PFS, whereas age at surgery significantly correlated with OS. Preoperative labs were collected a median of 30 days before surgery (interquartile range 16–57 days). Of 176 patients, preoperative chromogranin A ($n = 121$), pancreastatin ($n = 130$), and serotonin ($n = 137$) were available for most patients, whereas neurokinin A ($n = 71$) was less commonly measured. Laboratory values were tested as continuous variables for association with outcomes. The risk of progression or death did not correlate with preoperative neurokinin A levels ($p > 0.4$; Table 2). Although neurokinin A was previously reported to correlate with OS in 35 midgut NET patients, even with analysis limited to SBNET patients with preoperative neurokinin A levels ($n = 52$), no association with PFS or OS existed ($p > 0.4$).[19] Preoperative serotonin levels were significantly associated with PFS, but not OS ($p = 0.02$ and 0.9, respectively). In contrast, preoperative chromogranin A (PreopCgA) and

TABLE 1 Patient characteristics and survival

Variable	Combined ($n = 176$)	SBNETs ($n = 98$)	PNETs ($n = 78$)	SBNETs versus PNETs (p value)
Age at surgery, years, median (range)	58.2 (22.2–85.3)	60.4 (27.6–85.3)	54.8 (22.2–81.5)	**<0.01**
Female (%)	46.0	38.8	55.1	**0.03**
Node-positive disease (%)	75.6	92.6	52.9	**<0.001**
Metastatic disease (%)	62.1	81.4	37.7	**<0.001**
Low-grade tumor (%)	76.4	85.2	65.2	**0.01**
Intermediate-grade tumor (%)	21.0	14.8	29.0	**0.047**
High-grade tumor (%)	2.5	0.0	5.8	**0.047**
Follow-up, years, median (95 % CI)	3.8 (3.0-4.5)	3.7 (2.7–4.4)	4.2 (3.2–5.6)	0.2
PFS, years, median (95 % CI)	3.3 (2.5–5.6)	2.0 (1.7–4.2)	5.6 (3.6–NA)	**<0.01**
OS, years, median (95 % CI)	10.5 (10.0–NA)	NA (9.1–NA)	10.5 (10.0–NA)	0.9

Bold values are statistically significant ($p < 0.05$)

SBNET small bowel neuroendocrine tumor, *PNET* pancreatic neuroendocrine tumor, *CI* confidence interval, *NA* cannot be estimated

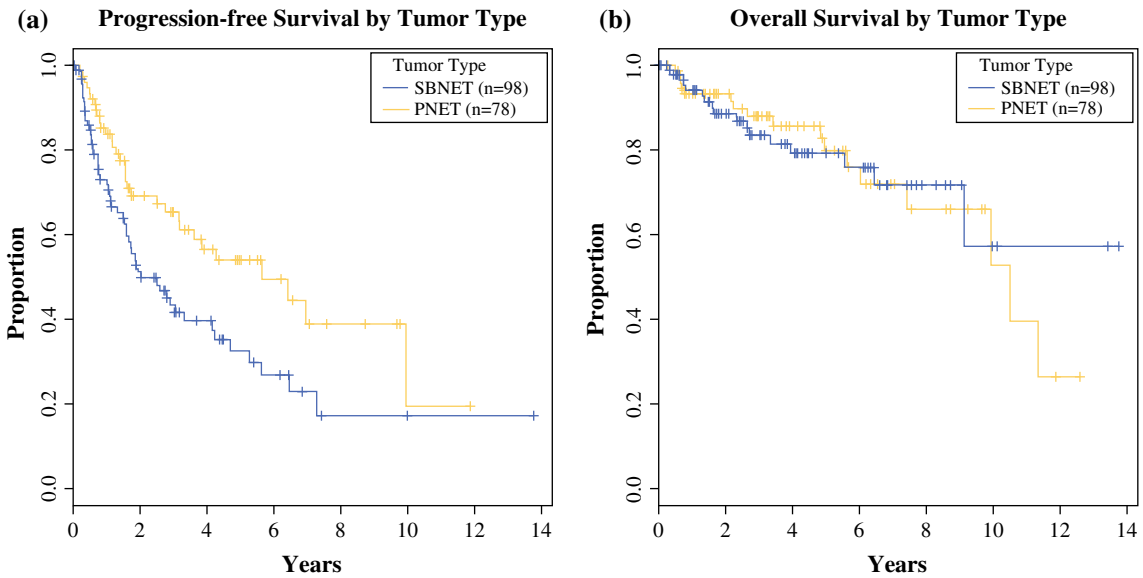

FIG. 1 Progression-free (**a**) and overall (**b**) survival by primary tumor type. SBNETs are shown with *darker lines* and PNETs with *lighter lines*. Progression-free survival was significantly lower among SBNET patients. *SBNET* small bowel neuroendocrine tumor, *PNET* pancreatic neuroendocrine tumor

pancreastatin (PreopPST) levels showed significant correlation with both PFS and OS ($p < 0.05$). These results suggested that PreopCgA and PreopPST offer prognostic insight in SBNETs and PNETs.

To investigate whether a simple distinction between elevated versus not-elevated tumor markers provided useful information, laboratory values were next considered as categorical variables. Preoperative levels of chromogranin A, pancreastatin, serotonin, and neurokinin A were elevated above their reference ranges in 66, 65, 69, and 39 % of patients tested, respectively. When considered as binary variables, high PreopPST, but no other marker, showed significant association with worse PFS and OS ($p < 0.05$; Table 2). When compared with those with normal levels, patients with PreopPST above the reference range of 135 pg/mL had significantly shorter median PFS (1.7 vs. 6.5 years) and OS (9.1 years vs. not reached; Table 3; Fig. 2a, b). Five-year PFS was 14.9 % among patients with elevated PreopPST, compared with 59.4 % among patients with normal levels, and 5-year OS fell to 72.6 % with elevated PreopPST from 88.3 % among patients with normal levels.

Multivariate Analysis

Univariate association of PreopCgA and PreopPST levels with outcomes suggested that these tests could be predictive

TABLE 2 Univariate analysis of association with progression-free (PFS) and overall survival (OS) by log-rank test

Clinical feature	Combined (n = 176)		SBNETs (n = 98)		PNETs (n = 78)	
	PFS (p value)	OS (p value)	PFS (p value)	OS (p value)	PFS (p value)	OS (p value)
Tumor site	**<0.01**	0.9	–	–	–	–
Sex	0.7	0.8	0.4	0.2	0.7	0.4
Age at surgery	0.11	**<0.01**	0.13	**<0.01**	0.8	0.3
T-stage	**<0.01**	0.08	0.7	0.6	**<0.01**	0.06
N-stage	**<0.001**	**0.023**	0.4	0.2	**<0.001**	**0.032**
M-stage	**<0.001**	**<0.01**	**<0.01**	0.08	**<0.01**	**0.038**
Low-grade	0.08	0.8	0.2	0.4	**0.02**	0.7
Lymph node ratio	**0.012**	0.5	0.4	0.3	**0.017**	0.7
Log preop neurokinin A	0.9	0.6	0.9	0.5	0.9	0.7
Log preop serotonin	**0.017**	0.9	**<0.01**	0.43	0.9	0.9
Log preop chromogranin A	**0.024**	**0.033**	0.08	**0.027**	0.2	0.4
Log preop pancreastatin	**<0.001**	**<0.001**	**<0.01**	**0.039**	**<0.001**	**<0.001**
Preop neurokinin A >40 ng/mL	0.9	0.6	0.9	0.3	0.8	0.2
Preop serotonin >200 ng/mL	0.3	0.3	0.10	0.9	0.5	0.8
Preop chromogranin A >95 ng/mL	0.4	0.3	0.8	0.6	0.5	0.3
Preop pancreastatin >135 pg/mL	**<0.001**	**0.048**	**<0.01**	0.15	0.054	0.066

Bold values are statistically significant ($p < 0.05$)

Tumor markers were analyzed both as continuous log-transformed variables and as categorical elevated versus normal variables. Preoperative pancreastatin shows significant correlation with PFS and OS

SBNET small bowel neuroendocrine tumor, *PNET* pancreatic neuroendocrine tumor

of earlier progression and death. However, clinical characteristics predictive of PFS and OS were not equally represented in SBNET and PNET patients. To investigate whether differences in preoperative laboratory values provided independent prognostic information, multivariate Cox models adjusted for confounding factors. After accounting for the primary tumor site, age at surgery, and presence of nodal or metastatic disease, higher PreopPST, but not PreopCgA, remained independently predictive of worse PFS and OS ($p < 0.001$ and $p = 0.01$ for PreopPST; $p = 0.27$ and $p = 0.29$ for PreopCgA; Table 4). These results did not change when tumor grade was added to the model (Table S1). Because of changes in grading criteria over time and low numbers of non-low-grade tumors, grade was omitted from the final model. Estimated median Cox-adjusted PFS was 2.0 years among patients with elevated PreopPST versus 5.6 years among patients with normal PreopPST (Fig. 2c). Thus, independent of known prognostic factors, a 3.6-year difference in median PFS is attributable to whether PreopPST is elevated.

The location of the primary tumor also remained independently predictive of PFS and OS after multivariate adjustment. To confirm that differences in survival by PreopPST levels were not due to differences inherent in SBNET versus PNET tumors, PFS and OS were compared by primary tumor site in patients with elevated versus normal PreopPST. No significant differences existed. In patients with normal PreopPST, PFS and OS were similar in SBNET and PNET patients (median PFS, 6.5 and 5.6 years, $p = 0.4$; OS not reached for both, $p = 0.4$; Table 3). In patients with elevated PreopPST, PFS and OS were lower than in patients with normal PreopPST but again were similar between SBNET and PNET patients (median PFS, 1.7 and 1.6 years, $p = 0.6$; OS, 9.1 and 5.6 years, $p = 0.3$). From these analyses we conclude that elevated PreopPST is associated with a sharp decrease in predicted PFS and OS regardless of SBNET or PNET origin.

Postoperative Tumor Marker Levels

Postoperative laboratory values were next tested for association with PFS and OS. Postoperative levels were drawn at a median of 124 days after surgery and were recorded for most patients. In these patients, surgery reduced serum tumor markers, and postoperative chromogranin A (PostopCgA; $n = 117$), pancreastatin (PostopPST; $n = 124$), and serotonin ($n = 129$) were significantly lower than preoperative levels (median changes: −30.5 ng/mL, −55.0 pg/mL, −88.0 ng/mL, respectively; $p < 0.01$ for all). Postoperative neurokinin A ($n = 54$) did not differ from PreopNKA ($p = 0.09$). Correlations of these values with PFS and OS mirrored those of preoperative values, with postoperative serotonin showing significant association with PFS but not OS ($p = 0.01$ and 0.9) and with PostopCgA and PostopPST showing significant correlation with PFS and OS ($p < 0.05$). Both PostopCgA and PostopPST remained independently correlated with PFS and OS after multivariate adjustment for patient age, tumor site, and presence of nodal and distant metastases ($p < 0.01$). Increased levels of either

TABLE 3 Survival differences based on tumor marker elevation, shown in all patients and in small bowel (SBNET) and pancreatic neuroendocrine tumor (PNET) subgroups

Tumor Marker	Level	Median years PFS (95 % CI)	PFS *p* value	5-year PFS (%)	Median years OS (95 % CI)	OS *p* value	5-year OS (%)
All patients							
Preoperative pancreastatin	High (>135 pg/mL; $n = 84$)	1.7 (1.3–2.6)	**<0.001**	14.9	9.1 (5.6–NA)	**0.048**	72.6
	Normal ($n = 46$)	6.5 (4.7–NA)		59.4	NA (7.5–NA)		88.3
Preoperative chromogranin A	High (>95 ng/mL; $n = 80$)	2.0 (1.7–5.3)	0.4	33.8	NA (6.5–NA)	0.3	77.0
	Not elevated ($n = 41$)	3.2 (2.5–NA)		28.1	NA (5.6–NA)		88.5
Postoperative pancreastatin	High (>135 pg/mL; $n = 57$)	1.6 (1.1–1.9)	**<0.001**	16.4	7.5 (5.6–NA)	**<0.01**	67.2
	Normal ($n = 67$)	7.3 (6.5–NA)		64.3	NA (9.1–NA)		90.3
Postoperative chromogranin A	High (>95 ng/mL; $n = 61$)	1.9 (1.6–3.3)	**<0.01**	27.8	NA (NA–NA)	**0.03**	72.8
	Normal ($n = 56$)	5.6 (3.2–NA)		57.3	NA (6.5–NA)		87.1
Preoperative, postoperative pancreastatin	High, high (>135 pg/mL; $n = 44$)	1.6 (0.8–1.9)	**<0.01**	8.6	6.5 (4.0–NA)	0.15	63.8
	High, normal ($n = 17$)	3.9 (2.6–NA)		32.1	9.1 (5.6–NA)		100
Preoperative, postoperative chromogranin A	High, high (>95 ng/mL; $n = 49$)	1.6 (1.3–3.3)	**0.02**	24.5	6.5 (6.1–NA)	**0.03**	71.0
	High, normal ($n = 16$)	6.5 (2.5–NA)		57.1	NA (NA–NA)		100
SBNETs only							
Preoperative pancreastatin	High (>135 pg/mL; $n = 64$)	1.7 (1.3–2.6)	**<0.01**	15.0	9.1 (6.5–NA)	0.15	74.7
	Normal ($n = 15$)	6.5 (4.7–NA)		70.9	NA (NA–NA)		92.3
Postoperative pancreastatin	High (>135 pg/mL; $n = 42$)	1.5 (0.8–1.9)	**<0.001**	8.8	NA (6.5–NA)	**0.03**	68.9
	Normal ($n = 32$)	7.3 (6.5–NA)		74.5	NA (9.1–NA)		100
Preoperative, postoperative pancreastatin	High, high (>135 pg/mL; $n = 37$)	1.6 (0.8–2.0)	**0.02**	11.0	NA (6.5–NA)	0.6	69.4
	High, normal ($n = 11$)	4.2 (2.6–NA)		32.5	9.1 (5.6–NA)		100
PNETs only							
Preoperative pancreastatin	High (>135 pg/mL; $n = 20$)	1.6 (1.2–NA)	0.054	16.4	5.6 (2.7–NA)	0.07	68.6
	Normal ($n = 31$)	5.6 (2.5–NA)		56.1	NA (7.5–NA)		86.6
Postoperative pancreastatin	High (>135 pg/mL; $n = 15$)	1.6 (1.3–NA)	**0.02**	32.6	6.1 (5.0–NA)	0.053	66.5
	Normal ($n = 35$)	NA (3.2–NA)		56.5	NA (NA–NA)		82.7
Preoperative, postoperative pancreastatin	High, high (>135 pg/mL; $n = 7$)	1.3 (0.5–NA)	0.14	0.0	2.7 (2.2–NA)	**0.04**	41.7
	High, normal ($n = 6$)	3.9 (3.2–NA)		27.8	NA (NA–NA)		100

Bold values are statistically significant ($p < 0.05$)

Both preoperative and postoperative pancreastatin levels provide strong discrimination of patient outcomes. Among those with elevated preoperative tumor markers, postoperative pancreastatin levels provide additional prognostic information

PFS progression-free survival, *OS* overall survival, *CI* confidence interval, *NA* cannot be estimated

permitted strong discrimination between patients more and less likely to have early progression or death (Table 3).

The impact of postsurgical normalization of tumor markers was assessed by considering outcomes in patients with elevated PreopPST by whether PostopPST remained elevated. Among 84 patients with elevated PreopPST, PostopPST levels were available for 61. In these patients, PostopPST levels remained significantly predictive of both PFS and OS ($p < 0.01$). Whereas elevated PreopPST by itself indicates predicted median PFS and 5-year OS of 1.7 years and 73 %, among those whose PreopPST levels normalized after surgery, median PFS and 5-year OS improved to 3.9 years and 100 % (Table 3; Fig. 2d). In those whose PostopPST remained elevated, 5-year PFS was

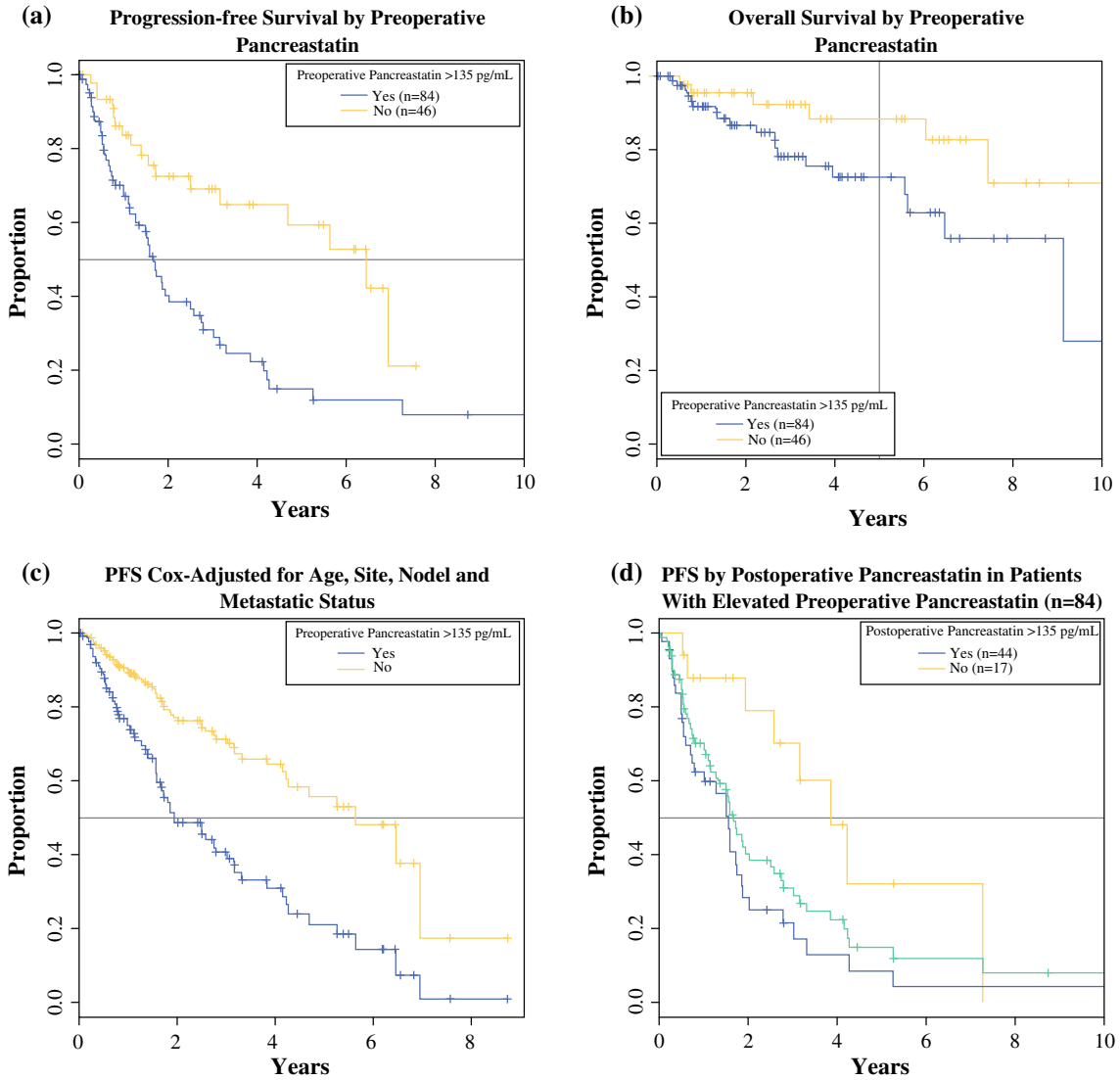

FIG. 2 Median progression-free (PFS) (**a**) and 5-year overall survival (OS) (**b**) were higher in patients with normal (*upper lighter line*, $n = 46$) versus elevated preoperative pancreastatin (*lower darker line*, $n = 84$; median PFS, 6.5 vs. 1.7 years; 5-year OS, 88 vs. 73 %). **c** Multivariate Cox model-adjusted PFS. Estimated median PFS was significantly longer in patients with normal preoperative pancreastatin (*upper lighter line*) compared with elevated (*lower darker line*) even after adjustment for confounding factors. **d** PFS by postoperative pancreastatin in patients with elevated preoperative pancreastatin levels. Patients with elevated preoperative pancreastatin (*middle line*; same as the darker line in **a**; $n = 84$) can be further stratified by elevated (*lower darker line*, $n = 44$) versus normalized (*upper lighter line*, $n = 17$) postoperative pancreastatin levels

only 8.6 % (vs. 14.9 % as predicted by elevated PreopPST alone), and median OS dropped to 6.5 years. Deceptively, combining PreopCgA with PostopCgA information resulted in statistically significant differences in OS (Table 3), but this was because elevated PreopCgA failed to select a high-risk subset of patients. Because of the nonsignificant influence of elevated PreopCgA, little additional information was gained by combining preoperative and postoperative measurements, and differences in outcome were similar to those predicted by PostopCgA alone. Survival by normalization of PostopPST in SBNET and PNET subgroups was similar to the combined results, although larger sample sizes made results in the combined group more robust.

DISCUSSION

In this study we demonstrate that PreopPST provides significant prognostic information in SBNET and PNET patients, with higher levels independently predicting worse PFS and OS. Although considering the degree of tumor marker elevation allowed the strongest correlations with outcomes, a binary distinction between pancreastatin elevated above the reference range versus normal allowed

TABLE 4 Multivariate Cox model results

Factor	Progression-free survival			Overall survival		
	HR	95 % CI	*p* value	HR	95 % CI	*p* value
Results in all patients						
Log preoperative pancreastatin (per doubling)	1.40	1.18–1.67	**<0.001**	1.54	1.11–2.14	**0.01**
Node-positive disease	2.40	1.03–5.58	**0.043**	12.7	1.59–109	**0.02**
Metastatic disease	2.32	1.01–5.32	**0.047**	3.07	0.60–15.7	0.18
Age at surgery (per year)	1.02	1.00–1.04	0.066	1.07	1.03–1.12	**<0.01**
Primary site (if SBNET)	0.48	0.26–0.89	**0.02**	0.17	0.06–0.47	**<0.001**
Results in PNETs only						
Log preoperative pancreastatin (per doubling)	1.57	1.08–2.27	**0.02**	1.85	1.05–3.28	**0.03**
Node-positive disease	2.66	0.88–8.00	0.08	5.27	0.53–52.4	0.16
Metastatic disease	2.27	0.79–6.52	0.13	1.55	0.23–10.4	0.7
Age at surgery (per year)	1.01	0.98–1.04	0.6	1.02	0.96–1.08	0.6
Results in SBNETs only						
Log preoperative pancreastatin (per doubling)	1.36	1.11–1.67	**<0.01**	Insufficient events for multivariate analysis in this subgroup		
Node-positive disease	1.52	0.43–5.35	0.5			
Metastatic disease	1.77	0.41–7.57	0.4			
Age at surgery (per year)	1.03	0.99–1.06	0.10			

Bold values are statistically significant ($p < 0.05$)

Preoperative pancreastatin levels remained independently predictive of progression-free and overall survival after adjustment for confounding factors

HR hazard ratio, *CI* confidence interval

separation of patients into groups at high and low risk for progression and death. Incorporating PostopPST measurements further refines prognostic predictions.

Pancreastatin is a fragment of the 439-amino acid chromogranin A peptide produced by the peptidase prohormone convertase-2.[25,35,36] Its predominant human form contains 52 amino acids, although tumors may secrete additional shorter N-terminal-truncated fragments.[37] Stored in secretory granules, pancreastatin inhibits glucose-stimulated insulin release and pancreatic and gastric secretion while it promotes glycogenolysis and impairs glucose uptake in muscle, fat, and liver.[37,38] Although high pancreastatin has been recognized as a feature of neuroendocrine tumors for some time, pancreastatin's role in normal physiology remains poorly understood.[36,39] Pancreastatin seems to exert its effects through activity at membrane-associated G-proteins and phospholipase C, but a specific membrane-bound pancreastatin receptor (PSTR) has not been identified.[37,39] Attempts to identify the PSTR have focused on affinity purification from rodent liver.[37,39] NETs overexpress many hormone receptors, such as those for somatostatin and gastric-inhibitory polypeptide, making it tempting to speculate that the putative PSTR might be more abundant in, and more readily isolated from, NET tissue specimens.[40–42] Pancreastatin causes Ras-independent activation of the mitogen-activated protein kinase pathway and also activates the phosphatidyl-inositol-3-kinase/Akt pathway.[37] Whether pancreastatin represents a potential pharmacologic target in addition to reflecting NET disease burden is unknown.

Recent research highlights pancreastatin's advantages in assessing neuroendocrine disease. Pancreastatin assays are more standardized than those for chromogranin A, and pancreastatin levels do not vary with proton pump inhibitor exposure.[25,30] Pancreastatin has greater sensitivity and specificity for diagnosing NETs than chromogranin A and might better reflect neuroendocrine disease burden.[25,28,29] Pancreastatin also correlates with outcomes. In 122 NET patients undergoing hepatic artery chemoembolization, pancreastatin predicted response to therapy, and elevated levels independently correlated with lower survival (1.9 vs. 3.4 years).[29] Pretreatment pancreastatin independently predicted worse survival in 59 NET patients beginning somatostatin analog therapy and also closely paralleled tumor burden.[27]

Our results showing dramatic differences in outcomes based on pancreastatin levels in 176 surgically managed patients extend these findings and support pancreastatin's utility for predicting NET behavior. Elevated PreopPST predicted a median PFS of 4.8 years less than with normal PreopPST and a 5-year OS more than 15 % lower (Fig. 2a, b). Adding PostopPST measurements allowed further separation of these estimates (Fig. 2d). Patients whose

elevated PreopPST remained high after surgery had a greater than 90 % chance of progression and nearly 40 % chance of death within 5 years, whereas none of the patients whose pancreastatin normalized after surgery died during the same period, and median PFS more than doubled (Table 3). It is unknown whether serial pancreastatin measurements during follow-up add additional information; however, Pre- and PostopPST offer significant prognostic power. Multivariate analysis and investigation of results stratified by tumor type confirm that these effects do not reflect the status of other prognostic markers but constitute independent information. Furthermore, if PreopPST levels are not available, this study demonstrates that isolated PostopPST or PostopCgA serve as strong indicators of probable outcomes.

The divergent prognoses of patients with elevated and normal PreopPST recommend updates in NET management and in future research. First, its strong prognostic implications support using pancreastatin as part of SBNET and PNET initial work-up and subsequent monitoring. Next, although somatostatin analogs are effective in progressive disease and are well tolerated, they are expensive, and many patients enjoy long periods of PFS after surgery without additional treatment.[12,14] Current guidelines do not recommend adjuvant octreotide in asymptomatic patients. Instead, patients begin additional therapeutics upon evidence of progression.[2,3] Whether early initiation of octreotide or other treatments before tumor progression would impact survival is unknown. The currently enrolling Eastern Cooperative Oncology Group E2212 phase II trial (NCT02031536) will determine whether adjuvant everolimus prolongs PFS in metastatic PNETs. As identification of novel NET therapeutic targets and development of new agents proceeds, pancreastatin's ability to discriminate outcomes even in patients with metastases suggests that future trials for advanced disease should consider monitoring pancreastatin.[40,43] Our results support that elevated PreopPST selects patients with median PFS nearly 5 years lower than patients with normal levels, who could benefit most from more aggressive therapy. Incorporation of pancreastatin in new clinical trial inclusion criteria could help identify patients most likely to benefit and reduce required sample sizes by selecting patients at the highest risk, in whom researchers could best discern treatment effects.

The independent association of PostopCgA levels with survival agrees with earlier data. Extensive evidence supports chromogranin A for NET evaluation, but few studies specifically address PreopCgA.[20,22,23,44–47] In the present study, PreopCgA was not significantly associated with outcomes after adjusting for prognostic covariates. The reason for this result is unclear but could relate to the influence of factors beyond tumor burden, such as proton pump inhibitor use, kidney disease, inflammation, and hypertension, on PreopCgA levels.[24,26,30,44] Thus, although PostopCgA is helpful, our results suggest lower utility of PreopCgA in prognostication. In terms of price, tests of CgA and PST are comparable, with the Interscience Institute quoting a list price of $125 for clinical CgA testing compared with $225 for PST, although actual prices vary depending on testing volume (ISI, Inglewood, CA, USA; unpublished communication, Interscience Institute Pricing, 2014).

Limitations of this analysis include its retrospective nature and that correlations were stronger in terms of PFS than OS. This may be due to a low number of deaths, which limit our study's power to detect differences in OS. The 5-year survival rates for metastatic SBNETs and PNETs of 76 and 71 % in this cohort compare favorably with results from Surveillance, Epidemiology, and End Results (5-year survival of approximately 45 % in SBNETs and 25 % in PNETs with metastases).[1] It is likely that longer follow-up and accumulation of more OS events will augment the significant correlation between pancreastatin and survival. A strength of this study is that because pancreastatin predicted similar differences in outcome in SBNETs and PNETs (Table 3), these groups could reasonably be analyzed together, increasing sample sizes and statistical power to detect pancreastatin's effects on survival.

In summary, preoperative and postoperative pancreastatin levels constitute strong independent predictors of PFS and OS in SBNET and PNET patients. PreopPST identifies high-risk patients before surgery independent of patient age, tumor site, and presence of nodal or metastatic disease. Combining PreopPST with PostopPST stratifies patients into low and extremely high-risk groups for progression and death. Pancreastatin levels should be included in initial work-up and subsequent follow-up of SBNETs and PNETs and can select high-risk patients for inclusion in prospective trials of novel therapeutic approaches.

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