<div align="right">ORIGINAL ARTICLE</div>

Pancreatic polypeptide secreting tumors — an institutional experience and review of the literature

Angela Tatiana Alistar[1], Michelle Kang Kim[2], Richard Warner[2], Erin Moshier[3], Randall F Holcombe[1]

1. Tisch Cancer Institute, Mount Sinai School of Medicine, New York, USA. 2. Department of Gastroenterology, Mount Sinai School of Medicine, New York, USA. 3. Department of Preventive Medicine, Mount Sinai School of Medicine, New York, USA

Correspondence: Angela Tatiana Alistar. Address: Mount Sinai School of Medicine, Tisch Cancer Institute, One Gustave L. Levy Place, Box 1128, New York, USA. Telephone: 212-241-0354. Fax: 212-659-5599. E-mail: alistarangela@yahoo.com

Abstract

Objectives: We present a retrospective analysis of patients with pancreatic neuroendocrine tumors (PNETs) who have had Pancreatic Polypeptide testing in an attempt to better define Pancreatic Polypeptide producing tumors as an entity and the role of Pancreatic Polypeptide (PP) as a biomarker. To our knowledge, this is the first single center comprehensive review of Pancreatic Polypeptide producing tumors.

Methods: A retrospective study of patients with pancreatic neuroendocrine tumors seen at our institution from 1980 to 2011. All patients that have had PP concentrations measured at least once were evaluated. Data relating to diagnosis, pathology, surgery, liver directed therapies, chemotherapy and survival outcome were noted.

Results: 71 patients with PNETs fulfilled the inclusion criteria (8 PPomas, 22 PP producing tumors and 41 non -PP producing tumors). We identified a trend towards better survival for patients with PP producing tumors vs. non- PP producing tumors (p=0.19). There was no correlation between survival and a diagnosis of PPoma in relation to other PP producing tumors or non-PP producing tumors. There was a borderline significant positive correlation of PP in association with Chromogranin A in a postoperative setting (p=0.061).

Conclusions: Pancreatic Polypeptide is a biomarker that is worth prospective investigation and a standardized assay. Our analysis investigating Pancreatic Polypeptide as a prognostic and or predictive biomarker reveals a trend towards showing these characteristics. Using a standardized test and investigating this biomarker prospectively could lead to the validation of Pancreatic Polypeptide as a biomarker.

Key words

Pancreas, Neuroendocrine tumors, Pancreatic polypeptide, Biomarker

1 Background

The pancreatic neuroendocrine tumors (PNETs) are categorized on the basis of their clinical manifestation into functioning (F-PNET) and non-functioning tumors (NF-PNET). Functioning tumors (such as VIP-oma, insulinoma, gastrinoma, glucagonoma, somatostatinoma) are associated with clinical syndromes caused by inappropriate secretion of

hormones. Non-functioning tumors are not associated with a distinct hormonal syndrome, but may still have elevated hormone levels in the blood or immunoreactivity in tissue sections, that are clinically silent [1]. The biomarkers tested in PNETs can be divided into specific markers (such as: insulin for an insulin-producing tumor, gastrin for a gastrinoma, glucagon for glucagonoma) and general tumor markers, the most interesting being chromogranin A (CGA), pancreatic polypeptide (PP), and the α-subunits of hCG, VIP, glucagon, somatostatin, pancreastatin and substance P. However, at this time we do not have validation of the prognostic and predictive value of many of the biomarkers tested in PNETs. Measurement of detectable serum or plasma levels of various hormones can establish the diagnosis and assist in monitoring tumor response and disease progression of a PNET.

Almost all patients with PNETs will have elevation of CGA. In a series, 58% of the patients diagnosed with PNETs also had elevation of PP, 40% hGC-α and 20% hCG-β at diagnosis [2]. Increased levels of CGA have been reported in 50–80% of PNETs and sometimes correlate with the tumor burden. The highest CGA levels have been reported in NF-PNETs [3-5]. In a study by Panzuto et al, combination of CGA with measurement of PP increased the sensitivity from 84% to 96% in non-functioning tumors and from 74 to 94% in functioning tumors [6]. Serum PP alone shows a rather low sensitivity, somewhere between 40 and 55% [7].

Pancreatic polypeptide (PP), a 36-amino acid peptide, arises from both islet and acinar cells of the pancreas and may function as an important feedback inhibitor of pancreatic secretion after a meal (1). Release of PP by a meal, primarily protein, occurs in a biphasic manner. The first rapid release occurs as a result of vagal stimulation; the second, more prolonged rise (the so-called intestinal phase) occurs predominantly in response to hormonal stimulation [8]. Plasma PP levels increase with age; PP levels are elevated above those of age-controlled normal subjects in diabetic patients, after meal ingestion, cerebral stimulation, and hormone administration, after bowel resection, alcohol abuse, chronic noninfective inflammatory disorders ,chronic relapsing pancreatitis [9-12]. One study has shown an association between increased PP levels and increased intra-abdominal fat, but not subcutaneous fat, as measured by CT scan [13]. A clear cut biologic role for PP has not been established; however the only physiologic effects that are recognized in humans are the inhibition of gallbladder contraction and pancreatic enzyme secretion [14]. Thus, a tumor deriving from PP cells is predicted to be clinically silent, although this is not always the case.

The value of plasma PP as a possible biomarker for pancreatic neuroendocrine tumors is poorly defined. In a review of literature and statistical analysis of 58 cases of PP producing tumors, Soga et al (1994) proposed that PP may be involved in 3 different ways in pancreatic neuroendocrine tumors, such as : pure PPoma (tumors producing only PP ,without other known hormone), mixed PPoma (an endocrinoma that produces 2 gut pancreatic hormones ,PP and one other in significant competitive amounts) and multisecretory PPoma (that produces more than 2 types of gut pancreatic hormones in significant amounts) [15].

The role of PP as a screening biomarker was investigated in a prospective study of 26 patients including family members of patients with MEN I suggesting that elevated basal and responsive (postprandial) PP level is associated with asymptomatic pancreatic neuroendocrine tumors and normal basal plasma PP concentrations, but with exaggerated postprandial PP responses in 11 patients, were associated with various combinations of islet cell hyperplasia, antral G cell hyperplasia with moderate hypergastrinemia and parathyroid hyperplasia [16]. At this time the role of PP as a screening biomarker in asymptomatic patients remains investigational.

In our retrospective single institution study we aimed to describe the PP producing tumors and the PPomas as different entities in the heterogeneous group of PNETs and also to evaluate the predictive and prognostic value of PP as a biomarker.

2 Material and methods

2.1 Patient population

Institutional Review Board Approval was obtained to carry out this single center retrospective study. Patients were identified through query of existing Database at our institution. All patients with PNETs seen at our institution from 1980 to 2011 were identified. Patients had histopathologic confirmation of their diagnosis via primary surgical resection or biopsy. Presence of metastatic disease was assessed via cross sectional imaging, somatostatin receptor scintigraphy or tissue biopsy. We identified a total of 141 of such patients diagnosed with PNETs, of which patients 71 have had PP testing and 30 had abnormal values and met the inclusion criteria for this analysis. Medical record review of these 71 patients formed the basis for the study and was analyzed in 4 different subgroups: 41 non- PP producing PNETs, 30 PP producing PNETs, 8 PPomas and 22 PP producing tumors but non PPomas.

2.2 Data collection

All data was recorded and stored in a Microsoft Access Database. Demographic data such as gender and age at diagnosis were noted .Pathologic data related to surgical resection including site and primary tumor's depth of invasion, Ki-67 index of primary tumor were recorded from operative and/or pathology reports when available. Biochemical data including serum Chromogranin A (CGA), serum serotonin, VIP, PP, gastrin and glucagon were recorded on a longitudinal basis. When available, biochemical data was classified as preoperative (0 to 12 months prior to resection of the primary tumor), immediate postoperative (0-3 months) and late postoperative (3-12 months). Clinical interventions including liver directed therapies, chemotherapies and somatostatin analogues were recorded as well. Survival information on our cohort was obtained from review of the clinical record and survival/follow-up interval was assessed form the date of diagnosis to date of death or last known follow-up. Follow-up was obtained in all the patients and ranged from 0.1 to 19 years (mean 4.5 years). Histological confirmation of tumor was obtained in all patients, either from biopsy or surgical specimen. All of the patients had biochemical confirmation. All patients had sporadic disease, none of them had multiple endocrine neoplasia type 1 (MEN 1).

2.3 Data analysis

All analyses were conducted using SAS Version 9.2 Copyright (c) 2002-2008 by SAS Institute Inc., Cary, NC, USA. Kaplan-Meier curves were constructed to demonstrate survival for our cohort [17]. Log rank tests were used to compare survival between groups. Throughout all analyses, statistical significance was determined by a criterion of $P<0.05$.

3 Results

3.1 Patients and primary tumor characteristics

The clinical characteristic of the 71 patients with PNETs who have had PP testing is described in Table1. The similarities among the 4 groups were: similar median age at diagnosis, mainly female patients and the functional status of the tumors. The differences among the 4 groups consisted of an earlier stage at diagnosis for PPomas (mainly stage III diseases) compared with the other groups that were mainly stage IV at diagnosis and consequently a lower rate of liver metastases for PPomas. The median survival for PP omas was slightly higher than the other subgroups.

Local therapeutic interventions were uniform among subgroups and ranged from curative resections for patients with locally advanced disease and palliative resection for symptomatic patients, refractory to medical management. Liver directed therapies such as hepatic artery chembolization, bland embolization and radiofrequency ablation were also commonly used for patients with liver metastases. When indicated, patients received systemic therapies as standard of care. The patients had various degrees of response rates with the different therapeutic interventions and are beyond the scope of the current analysis to evaluate the impact of the therapeutic intervention on survival of the cohort of patients analyzed.

Table1. Patient characteristics and treatment by sub-groups: PPomas, non PPomas PP producing tumors, non PP producing tumors, all PP tested PNETs

Characteristics	N=8 PP omas	N=22 Other PP producing	N=41 Non-PP producing	N=71 overall	P values *
Gender M	3 (37.5%)	12 (54%)	18 (42.8%)	33 (45%)	0.6938 (Fisher's exact test)
F	5 (62.5%)	10 (46%)	23 (57.2%)	39(55%)	
Mean age at diagnosis (yrs)	49	56	51	52	0.2357 (ANOVA)
Mean survival in yrs	5.7	3.8	4.9	4.6	0.6013 (ANOVA)
Median survival	18.01	**	12.03		0.3923 (log rank)
Mean survival	14.51	1.94	9.79		0.3923 log rank)
Functional status					
F	2 (25%)	11(50%)	14 (33.3%)	27 (37.5%)	0.4012 (Fisher's exact test)
NF	6 (75%)	11(50%)	28 (66.6%)	45 (62.5%)	
Stage /WHO					
I	1	1	2	5	
II	1	2	4	7	0.1476 (Fisher's exact test)
III	3	1	11	15	
IV	3(37.5%)	18 (81%)	25 (59.5%)	45 (62.5%)	
Hepatic metastases	3 (37.5%)	17 (77.2%)	24 (57.1%)	43 (59.7%)	0.1159 (Fisher's exact test)
Cytoreductive surgery	8 (100%)	15 (68.1%)	33 (78.5%)	66 (91.6%)	0.1850 (Fisher's exact test)
Palliative surgery	0	1	4	1	0.8109 (Fisher's exact test)
Liver directed therapies	2	9	14	26	0.7695 (Fisher's exact test)

 * *P* values for comparison of PPomas vs. other PP producing tumors vs. non PP producing tumors

 ** No median was calculated because 50% of patients did not die

The most common presenting findings of all the patients are outlined in Table 2. Unexpectedly, the PPomas were as symptomatic at presentation as the other subgroups; the most common symptoms were diarrhea, abdominal pain and flushing.

Table 2. Primary clinical presentations of the 30 PP producing tumors, non PP producing tumors and PPoma

Primary presenting symptom	8 PP oma	22 PP producing tumor-non PPomas	30 PP producing tumors	41 non PP producing tumors	P value *
Diarrhea	2	8	10	11 (26.8%)	0.5531
Abdominal pain	2	6	8	10 (24.3%)	0.8276
Flushing	1	1	2	2 (4.8%)	1.00
Asymptomatic	3	7	10	14 (34.1%)	0.943

Note: chi square *p* value of PP producing vs. non PP producing comparison

3.2 Statistical analyses

4 subgroups were evaluated for survival: all 71 PP tested patients, 30 patients with PP producing tumors, 8 patients with PPomas, 22 PP with producing tumors that were non PPomas and 41 patients with PP non-producing tumors. PPomas were different from the other groups in respect to: stage (mostly loco-regional disease) and functional status (mostly NF). PPomas were comparable with the other groups regarding median age at diagnosis and gender. Survival comparison between the 4 groups did not reveal a statistically significant difference for PPomas vs. non-PP producing tumors and for PPomas vs. PP producing tumors (See Kaplan Meier curves, Figure.1A, and 1B). There was a trend towards better survival for patients with PP producing tumors vs. non PP producing (p=0.19) (see Kaplan Meier, Figure C).
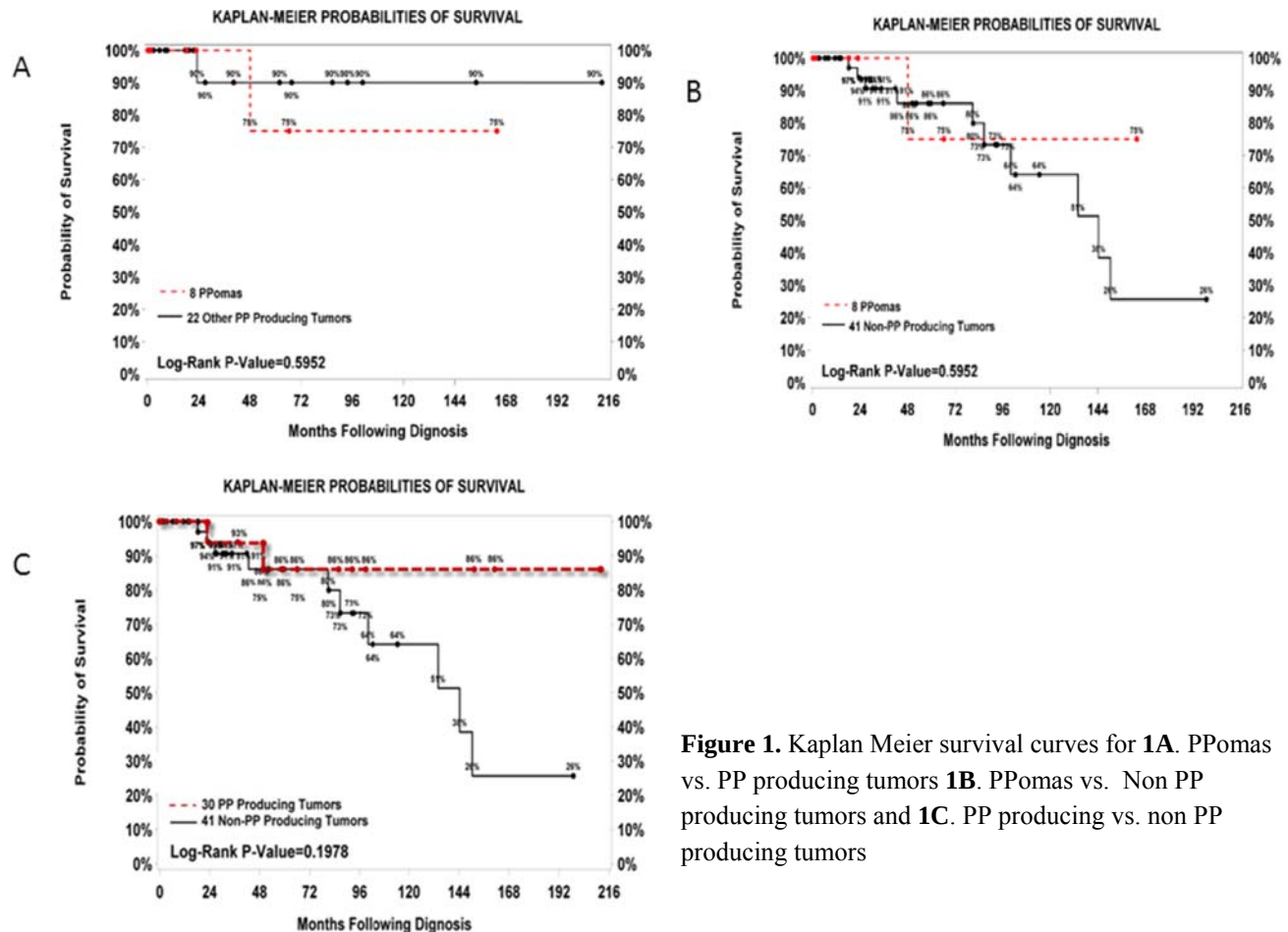
Figure 1. Kaplan Meier survival curves for **1A**. PPomas vs. PP producing tumors **1B**. PPomas vs. Non PP producing tumors and **1C**. PP producing vs. non PP producing tumors

In 71 patients with PNETs, 30 have had PP producing cells (42.6%) and of the 30 patients PP secreting tumors, 23 have had surgery. We performed one analysis evaluating the correlation between PP and CGA changes in relation to surgery. Among the patients who have had abnormal PP levels the following analysis was performed: linear regression analyzing the relationship between CGA and PP pre-surgery and post-surgery. Due to different assays used at different times during their clinical care we used normalized values of each biomarker and we natural log transformed both variables. The PP levels were assayed with different assays throughout the course of the disease. The normal range varied from 70-430 pg/ml, 0-418 pg/ml, 100-780 pg/ml, 0-150 pg/ml, 51-326 pg/ml, and 64-234 pg/ml. The CGA levels were assayed with different assays throughout and the normal range varied such as: 0-100pg/ml, 0-36.4pg/ml, 0-76 pg/ml,1.9-15pg/ml, 6-39pg/ml, 2.3-14.3 pg/ml. The sensitivity, inter and intra-assay variation of a normal, intermediate elevated and markedly

elevated value were not performed as this information was not made available by the tests providing companies .The analysis for PP was not adjusted for patients with renal failure as this is not standardly performed in practice .It appears that the relationship between natural log transformed PP and natural log transformed CGA is a positive linear association. For post-op analysis, the slope is borderline significant with a *p* value of 0.061 while for pre-op the correlation was not statistically significant (Figure 2). Possible explanations of these findings could be the relative small sample size and a non-standardized assay for PP.
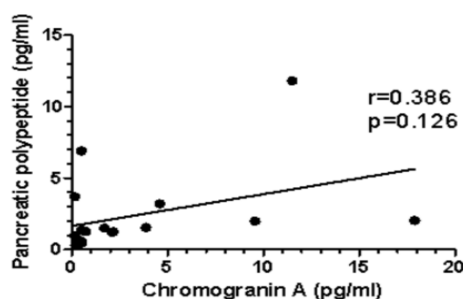
Figure 2. Linear regressions of PP and CGA pre and post-surgery for the PP producing tumors

4 Discussion

Measurement of circulating biomarkers is noninvasive and relatively inexpensive but there are significant problems with false-positive results across many tumor types. PNETs are a fascinating group of diseases that secrete many different proteins, whose role as screening, predictive or prognostic biomarkers is yet to be elucidated. In the current paper we discuss the role of PP as a biomarker and we describe our institutional experience with PP producing tumors and PPomas.

Our database analyses of the pancreatic polypeptide's role as a biomarker yielded interesting results, despite the limitations of a nonstandardized assay and the limitations of a retrospective analysis.

According to the literature, patients with PP-omas are asymptomatic and only secrete Pancreatic Polypeptide. Patients with PPomas were diagnosed mainly with loco regional disease, in contrast with the other subgroups of patients that presented mainly with stage IV at diagnosis. This finding is somewhat counterintuitive being that PP omas are considered to be in general nonfunctioning tumors and one would expected that their diagnosis would be prompted by advanced disease. However, in our cohort of patients only 37% of the PPomas were asymptomatic. As far as presenting symptoms, PPomas have had very similar clinical presentation with the other subgroups. Most common presenting symptoms such as diarrhea, flushing and abdominal pain were comparable among the 4 subgroups suggesting that clinicians cannot rely on symptomatology to distinguish among these tumor types. Our 8 patients with PPoma met the criteria for a PP only secreting tumor but exhibited clinical symptoms that were not explained by their normal biormarkers (CGA, Serotonin, 5HIAA, glucagon, VIP, pancreastatin, somatostatin and gastrin). We are not describing a new clinical syndrome for PP-omas.

The survival analysis suggests that patients with PP producing tumors may have a better survival and prognosis compared with patients with non-PP producing tumors. Patients diagnosed with PPomas seem to behave as a homogeneous entity in the heterogeneous group of PNETs. Although the median overall survival for PPomas (5.7 yrs) was higher than the other subgroups, the statistical analysis did not reveal a better overall survival for PP omas vs. the other subgroups. It is possible that the small size is responsible for this result.

In our review of literature and institution database we identified a number of issues regarding PP testing. One of the unresolved problems is the diagnostic accuracy of elevated basal PP concentrations as a marker for endocrine-secreting tumors and the lack of a standardized validated assay. There is evidence in the literature that healthy subjects can have an elevated PP level; currently there is no clinically approved validation assay of a high PP level.

A second problem is the lack of a clear definition of what a PPoma as an entity may be. Our recommended definition of a PPoma at this time is a pancreatic neuroendocrine tumor that only secretes abnormal levels of PP. The confirmatory role of the IHC for PP is questionable since it would be positive for islet cell hyperplasia also. This definition holds valid in the context of a standardized test for pancreatic polypeptide and eliminating confounders such as impaired renal function, diabetes, postprandial status. Differentiation of a high basal concentration in a healthy subject from that appearing in patients with tumor has been difficult. Schwartz suggested that administration of atropine would suppress concentrations in healthy subjects and would fail to do so in patients with tumors, but this has not been subjected to extensive examination [18]. This assay is not currently commonly used in practice and has not been validated, but is worth prospective investigation.

Regarding the role of PP as a biomarker, while our results are not conclusive they are hypothesis generating. For further investigation we recommend prospective testing of PP to be done on a fasting state and controlling for impaired renal function, age and diabetes. PP should be included in therapeutic clinical trials to better understand the interaction between therapeutic agents and PP levels. Ideally, a standardized assay should be implemented and used uniformly. At this time, based on our experience, we do not recommend PP as a prognostic or predictive biomarker in clinical practice but we recommend that PP should be further investigated.

Acknowledgements

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