Incidence and prognostic value of serotonin secretion in pancreatic neuroendocrine tumors

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ABSTRACT

Introduction: Serotonin secretion occurs in approximately 1-4% of patients with a pancreatic neuroendocrine tumor (pNET), but the incidence is not well defined. Aim of this study is to determine incidence of serotonin secretion with and without carcinoid syndrome, and the prognostic value for overall survival (OS).

Methods: Data were collected from 255 patients with a pNET if 24-hour urinary 5-hydroxy-indoleacitic acid excretion (5-HIAA) was assessed. Patients were diagnosed with serotonin secretion if 24-hour urinary 5-HIAA excretion was more than 3x upper limit of normal (ULN) of 50 µmol/24 hours during follow-up. Effect of serotonin secretion on OS was estimated with uni- and multivariate analyses using a Cox-regression.

Results: 2 (0.8%) patients were diagnosed with carcinoid syndrome and another 20 (7.8%) had a serotonin-secreting pNET without symptoms. These patients mostly had ENETS stage IV disease with high chromogranin A (CgA). Serotonin secretion was a negative prognostic factor in univariate analysis (HR 2.2, 95% CI: 1.27-3.81), but in multi-variate analysis only CgA >10x ULN (HR: 1.81, 95% CI 1.10-2.98) and neuron-specific enolase (NSE) >ULN (HR: 3.51, 95% CI 2.26-5.46) were predictors for OS. Immuohistochemical staining for serotonin was positive in 28.6% of serotonin-secreting pNETs (1 with carcinoid syndrome) and negative in all controls.

Conclusion: carcinoid syndrome is rare in patients with a pNET, but serotonin secretion occurs often. This is a negative prognostic factor for OS, but after correction for CgA and NSE it is no longer a predictor and probably only a “not-so innocent bystander” in patients with high tumor burden.

INTRODUCTION

The carcinoid syndrome is most often observed in patients with liver metastases from low-grade small intestinal neuroendocrine tumors (NETs). This can result in significant morbidity like heart failure and extensive diarrhea. Small intestinal NETs present with flushing and diarrhea in 60-80% of patients but this has only been sporadically described for pancreatic neuroendocrine tumors (pNETs).[1]

Occurrence of the carcinoid syndrome in a patient with a pNET was first described by Peart in 1963 with more case reports appearing shortly thereafter.[2-4] In the Netherlands Cancer Registry, 68 patients with a serotonin-secreting pNET were reported, comprising 2.8% of all carcinoid tumors reported from 1989-1996.[5] Other epidemiological studies report that serotonin-secreting pNETs represent approximately 1-4% of all pNETs.[6-10] A reliable estimation, however, is difficult to make as nomenclature has changed over the past decades. Until recently, these pNETs were named carcinoids but currently it is recommended to describe the tumors as serotonin-producing or serotonin-secreting pNETs.[11] Still, different standards for diagnosis are being used. Sometimes serotonin-secreting pNETs are classified with use of tissue samples (serotonin staining), however this does not always reflect clinical functionality of the tumor as assessed by determining the 24-hour urinary 5-hydroxyindole acetic acid (5-HIAA) excretion.[10,11] In gastrointestinal NETs 24-hour urinary 5-HIAA excretion has a high sensitivity and specificity for detecting metastatic midgut NETs, but it is less useful for predicting survival in follow-up.[12-14] In pNETs, the incidence and the effect of elevated 24-hour urinary 5-HIAA excretion on survival is unknown.

Three categories of patients can be identified which can potentially demonstrate serotonin secretion by a pNET. First of all, of course, the patient with symptoms of the carcinoid syndrome, but as described they are relatively rare. Secondly, the largest group of patients presents with a non-functioning pNET, meaning that no specific hormonal syndrome is present. These patients most often present with a pancreatic incidentaloma, or with abdominal pain and weight loss instead of hormonal syndromes.[15,16] Currently, it is not recommended to screen for hormonal secretion when no specific symptoms are present.[17] Thereby little is known on (sub-clinical) secretion of serotonin in non-syndromic pNETs and its prognostic relevance. The third category of patients have a pNET causing a hormonal syndrome, like an insulinoma, VIPoma, gastrinoma, or glucagonoma. Recently *Crona, et al.* reported co-secretion of multiple hormones in patients with pNETs, but although screening with urinary 5-HIAA excretion was performed, co-secretion of serotonin was not found in this series of 323 pNET patients.[18]

The aim of this study was to determine the incidence of the carcinoid syndrome in pNET patients in our tertiary referral center, but also to assess serotonin secretion in patients without the carcinoid syndrome. This included non-syndromic as well as syndromic pNETs. Furthermore, we aimed to assess the risk-factors for serotonin secretion, including the serotonin-staining in pathology and the effects of serotonin secretion on overall survival.

METHODS

Patients

In the Erasmus MC Cancer Institute, ENETS Centre of Excellence, Rotterdam, neuroendocrine tumors have been registered in a database since January 1st, 1993. For this study, all patients diagnosed with a neuroendocrine tumor of the pancreas were included up to December 31st 2015, if at least one sample of 24-hour urinary 5-HIAA excretion was available. Patients diagnosed with multiple endocrine neoplasia (MEN) type1, or von Hippel Lindau disease (VHL) were excluded. pNETs were diagnosed on basis of biomarkers and imaging, after which diagnosis was confirmed with pathology, according to ENETS guidelines. When only a tissue biopsy was available of a metastasis, the diagnosis of the primary pancreatic tumor was confirmed by (functional) imaging. Patients were diagnosed with a pNET when there was biopsy of the pancreas with a neuroendocrine tumor or of a (liver) metastasis showing a NET and patients had a pancreatic tumor on additional imaging consistent with a NET (reported by radiologist). To rule out NETs from other origin, all radiological imaging of patients with elevated 24-hour urinary 5-HIAA excretion was once more reviewed by 2 of the authors (WTZ and WWdH). Patients were excluded if they had other intestinal localizations inconsistent with a pNET (e.g. mesenterial nodes, peritoneal metastases or other intestinal tumors). Concurrently, other patient characteristics as age and gender were recorded, as well as ENETS grading (Mitotic count and Ki67-index), staging (ENETS/WHO 2010) and tumor markers (serum chromogranin A (CgA: upper limit of normal 94 µg/L) and neuron-specific enolase (NSE: upper limit of normal 16.2 µg/L)).

Diagnosis of functional tumors

Serotonin secretion was analyzed by determining the 24-hour urinary 5-HIAA excretion. Patients were diagnosed with a serotonin-secreting tumor if 24-hour urinary 5-HIAA excretion was above three times the upper limit of normal of 50 µmol per 24 hours at any time during follow-up. This limit was chosen so that small elevations due to dietary incompliance would not directly be interpreted as serotonin secretion by the pNET. Urinary 5-HIAA excretion is determined routinely when patients are referred for Peptide Radionuclide Receptor Therapy (PRRT) with radiolabeled somatostatin analogues, or at discretion of the treating endocrinologist. 5-HIAA was determined in 24-hour urine samples and measured using the reversed-phase HPLC with fluorimetric detection.[19] All samples were analyzed in the same laboratory. If available two 24 hour samples were used and the average was calculated, but if only one sample was available this was reported, as this seems also to be reliable in small intestinal NET.[20] For this study the first measurement of 24-hour urinary 5-HIAA excretion (usually at referral) and highest urinary 5-HIAA during follow-up were registered. Other functional/syndromic tumors were diagnosed according to ENETS guidelines.[17] Diagnosis of the ectopic ACTH syndrome and PTHrP-secretion in our population has been described

earlier.[21,22] When none of the above syndromes was diagnosed the pNET was classified as non-functional/syndromic.

Serotonin-staining

Immunohistochemical staining on hormones is not routinely performed during the work-up of NETs in our centre. To assess the value of the serotonin stain we selected all patients with elevated 24-hour urinary 5-HIAA excretion and matched the same number of controls on the basis of ENETS Stage and serum CgA. Tissue samples from time of diagnosis were acquired with use of PALGA, the nationwide network and registry of histo- and cytopathology in the Netherlands.[23] Immunohistochemical staining was performed after antigen retrieval with CC1 for 92 minutes and pronase P1 for 4 minutes, using anti-serotonin clone 5Ht-H209 from DAKO in a Ventana Benchmark Ultra stainer. Staining was visualized with Ultra View DAB and reviewed by an expert pathologist.

Statistics

Baseline characteristics were compared with an independent sample t-test for continuous data and with a Chi-square for categorical data. A Kaplan Meier was used to estimate overall survival and a log-rank test was used to test for significant differences between groups. Furthermore the effect of serotonin secretion on survival was compared with other predictors (age, sex, stage IV disease, serum chromogranin A and neuron-specific enolase) with a Cox-proportional hazard model. A P-value smaller than 0.05 was considered statistically significant. Calculations were performed with SPSS for Windows (version 23.0, SPPS Inc.)

RESULTS

In the period from 1993 through 2015, 403 patients with a pNET were treated in our centre and in 255 patients one or more available samples of 24-hour urinary 5-HIAA excretion were available. At diagnosis patients were on average 56.3 ± 12.5 years old and 48.6% was female. They presented mainly with stage IV disease and were treated with PRRT in 78.4% of cases. Average urinary 24-hour 5-HIAA excretion at referral was 71.0 ± 134µmol per 24-hours (median: 35.2µmol per 24 hours). 44 patients had a functional tumor (other than serotonin-secreting pNET), mainly insulinomas. (Table 1)

Of all patients, 22 (8.6%) had 24-hour urinary 5-HIAA excretion above 3xULN and thus were diagnosed with a serotonin-secreting pNET. However, only two patients (0.8%) had symptoms compatible with the carcinoid syndrome. 18 patients (81.8%) had elevated 24-hour urinary 5-HIAA excretion at referral and 4 patients developed serotonin secretion later at follow-up.

Table 1: Baseline characteristics

	All patients (n=255)	5-HIAA normal (n=233)	5-HIAA elevated (n=22)	
Age (years ± SD)	56.3 ± 12.5	56.3 ± 12.1	56.4 ± 16.7	p=0.99 (NS)
Female, *n* (%)	124 (48.6)	115 (49.4)	9 (40.9)	p=0.45 (NS)
Stage 4, *n* (%)	218 (85.5)	197 (84.5)	21 (95.5)	p=0.16 (NS)
Grade, *n* (%)				p=0.89 (NS)
Grade 1	46 (18.0)	43 (18.4)	3 (13.6)	
Grade 2	90 (35.3)	82 (35.2)	8 (36.4)	
Grade 3	13 (5.1)	12 (5.2)	1 (4.5)	
Unknown	106 (41.6)	96 (41.2)	10 (45.5)	
Co-secretion, *n* (%)				p=0.69 (NS)
ACTH	4 (1.6)	4 (1.7)	0	
Insulin	14 (5.5)	10 (4.3)	2 (9.1)	
Gastrin	5 (2.4)	4 (1.7)	1 (4.5)	
PTHrP	7 (2.7)	7 (3.0)	0	
Glucagon	6 (2.4)	6 (2.6)	0	
VIP	6 (2.4)	6 (2.6)	0	
NSE (µg/L ± SD)	39.3 ± 93 (n=220)	35.7 ± 86 (n=201)	77.3 ± 105 (n=19)	p=0.25 (NS)
CgA (µg/L ± SD)	2497.5 ± 8419	1406.5 ± 3255	14002.7 ± 24200	p=0.02
PRRT, *n* (%)	200 (78.4)	180 (77.3)	21 (95.5)	p=0.13 (NS)

Numerical data are mean ± SD.
Differences were tested with X^2 for categorical data and with an independent t-test for numerical data.
Neuron-specific Enolase (NSE): not assed in all patients

Serotonin-secreting pNETs presented more often in males with distant metastases (ENETS Stage IV), but this was not significant. Serum chromogranin A (CgA) was significantly higher in patients with serotonin-secreting pNETs (Table 1). 78% of patients with serotonin producing pNETs had a serum CgA of more than 20 times ULN, corresponding with an incidence of serotonin secretion of 23.8% in this group (Figure 1). 95% of patients with serotonin-secreting pNETs were also diagnosed with liver metastases. One patient only had retroperitoneal lymph node metastases. The primary tumor in the pancreas had a mean size of 4.7cm (range: 2.5-8.0cm) at diagnosis or referral to our centre. There were no differences in tumor grade between serotonin-secreting pNET and non-serotonin-secreting pNET. An echocardiography was not routinely performed, but no patients had clinical significant cardiac valve disease.

Secondary hormone secretion

Three patients were diagnosed with co-secretion of serotonin while they were already diagnosed with secretion of another pancreatic hormone. Two patients had ENETS stage IV insulinomas and one patient an ENETS stage IV gastrinoma. All patients were referred for

PRRT with radiolabeled somatostatin analogues in our centre and only had symptomology fitting with an insulinoma or gastrinoma and not fitting with the carcinoid syndrome (Table 2). These patients all had liver metastases and 2 had bone metastases. Serum CgA ranged from 2189 to 22600 µg/L and serum neuron-specific enolase (NSE) was elevated in two patients.

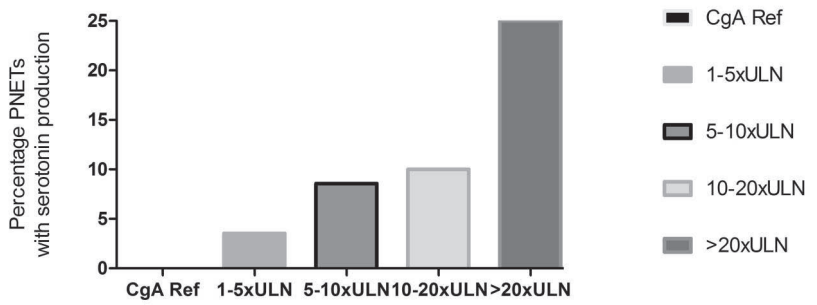

Figure 1: Incidence of serotonin secretion per range chromogranin A (CgA)
ULN: upper limit of normal (94.0 µg/L)

Table 2. Multivariate analysis for survival in patients with pNET

	Hazard ratio (95% CI)
Age	1.00 (0.98-1.02)
Male	1.02 (0.69-1.52)
Serotonin Secretion	1.35 (0.72-2.53)
Chromogranin A	
Reference Range (<188 µg/L)	*Reference*
2-10x ULN (188-940 µg/L)	1.63 (0.97-2.74)
>10x ULN (>940 µg/L)	1.81 (1.10-2.98)
NSE above ULN (>16.2 µg/L)	3.51 (2.26-5.46)
ENETS Stage IV	2.00 (0.92-2.740

Chromogranin A: upper limit of normal (ULN) 94.0 µg/L
Neuron-specific enolase (NSE): ULN 16. 2 µg/L

Overall Survival

In the entire cohort, 118 patients died after a median follow-up of 114 months. Survival was significantly shorter for patients with serotonin-secreting pNETs. Median survival was 116 months in patients with normal, non-elevated 24-hour 5-HIAA excretion, but decreased to 42 months when patients had serotonin-secreting pNETs (Figure 2: HR 2.2, 95% CI: 1.27-3.81) Five year survival was respectively 71.5% vs 46.0%.

In multivariate analysis, serotonin secretion was no longer a significant predictor for overall survival (HR: 1.35, 95% CI: 0.72-2.53), but serum CgA elevated above 10 times ULN

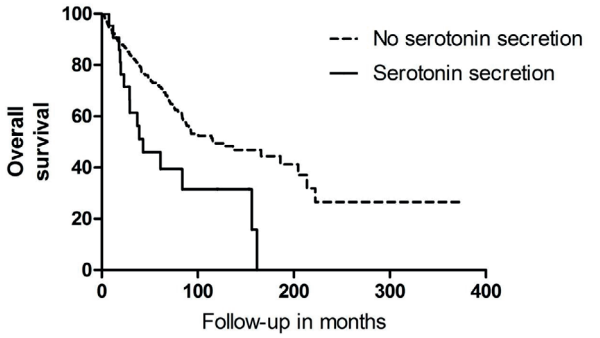

Figure 2: Kaplan-Meier estimate for survival stratified for serotonin secretion (log-rank: p=0.003)

(HR: 1.81 95% CI: 1.10-2.98) and serum NSE (HR: 3.51, 95% CI: 2.26-5.46) were significant predictors for survival. (Table 2)

Serotonin Stain

It was possible to perform serotonin-staining on 14 serotonin-secreting pNETs and 12 of the 22 matched controls. For 8 cases and 10 controls no remaining tissue was available. No controls demonstrated a positive serotonin stain, but 4 serotonin-secreting pNETs stained positive for serotonin resulting in a specificity of 100% and a sensitivity of 28.6%. One of the

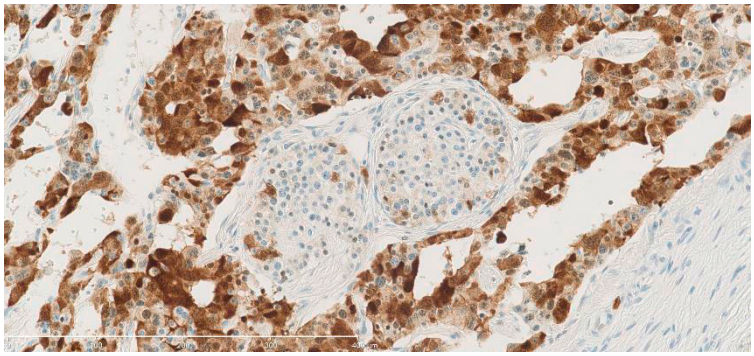

Figure 3: Serotonin staining of pancreatic neuroendocrine tumor

two cases with carcinoid syndrome demonstrated a positive serotonin stain. Mean 24-hour urinary 5-HIAA excretion was 601.9 ± 268 for positive serotonin stains and 230.0 ± 295 for negative stains (p=0.03).

DISCUSSION

Neuroendocrine tumors of the pancreas can secrete a large amount of hormones, sometimes even more than one concurrently.[18] Many of the syndromes associated with these hormones have been described widely in the literature and clear diagnostic standards have been set in international guidelines.[17] With regard to serotonin-secreting pNETs no clear diagnostic standards have been included in guidelines. Epidemiological series report a prevalence of 1-4% but different definitions for diagnosis are used.[5-8] In this study we defined serotonin secretion as having 24-hour urinary 5-HIAA excretion above three times the upper limit of normal of 50µmol per 24 hour and in this way 8.6% of patients were found to have serotonin secretion. Therefore, in this cohort, serotonin-secreting pNETs occur more frequently than previously reported. However we found only 2 patients in a total of 255 patients (0.8%) with a pNET who presented with the carcinoid syndrome, so this remains extremely rare. These patients had 24-hour urinary 5-HIAA excretion between 3-5 times upper limit of normal and presented with diarrhea. We did not see patients with carcinoid heart disease in our series. This is of importance because it is unknown if patients with a pNET need screening for serotonin secretion to minimize the risk of carcinoid heart disease. Case series analyzing the incidence of carcinoid heart disease rarely include pNETs and also carcinoid heart disease associated with pNETs has not been described up to present.[24-27] Screening of pNET patients for serotonin secretion is not currently incorporated in the guidelines.[17] With the incidence of serotonin secretion reported here one could advocate this should be done, but with the lack of clinical consequences (detecting carcinoid heart disease) this seems unnecessary. A positive immunohistochemical stain for serotonin predicts secretion well, but it is a poor screening tool due to its low sensitivity.

The population screened in this study is a population with mainly stage IV pNETs with highly elevated other biomarkers like serum CgA. A large subgroup was referred for PRRT. We did not asses the tumor burden of these patients with radiological studies, but in previous studies, serum CgA correlated to tumor burden.[28] At referral to our centre, patients with serotonin secretion had a significantly higher serum CgA reflecting high tumor burden. So while serotonin secretion was prevalent in this population this might be only one of many peptides these large tumors produce. This is also reflected when analyzing overall survival: patients with serotonin secretion have a shorter survival. But when correcting for other known predictors only serum NSE and serum CgA remain significant predictors. Serotonin secretion in large pNETs, which already have a poor prognosis, can therefore be considered an epiphenomenon with no additional value.

A review of literature reveals a very limited number of pNET cases with serotonin secretion in combination with other hormones.[29,30] Here we report 2 patients with cosecretion or consecutive secretion of insulin and serotonin and 1 patient with cosecretion of gastrin and serotonin. These patients did not have symptoms of the carcinoid syndrome and again these

patients had stage 4 disease with a very high serum CgA levels of more than 20 times ULN. Once more, elevated urinary 5-HIAA excretion seems to be a not so “innocent bystander” in patients with high tumor burden.

While the incidence of serotonin secretion was higher than previously reported, some caution is warranted because of the selected population in this study. Most patients received PRRT and had stage IV disease causing a selection bias. This makes it difficult extrapolate these results to the entire population pNETs, especially in stage I-III disease. In addition, the results might be flawed by midgut NETs that were incorrectly classified as pNETs. We addressed this issue by carefully reassessing all patients with elevated urinary 5-HIAA excretion and, thereby excluding all non-pNET patients.

However, the incidence of serotonin secretion remains higher than expected based on reported numbers and can also occur in patients with a pNET already secreting other hormones. It does seem to be an epiphenomenon rather than a solitary prognostic factor in pNET patients with high tumor burden. The carcinoid syndrome still remains very rare in pNETs.

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