

Development of a Highly Sensitive and Specific Carboxy-Terminal Human Pancreastatin Assay to Monitor Neuroendocrine Tumor Behavior

Thomas M. O'Dorisio, MD,* Siegfried R. Krutzik, PhD,† Eugene A. Woltering, MD, FACS,‡ Erika Lindholm, BSE,§ Saju Joseph, MD,‡ Abby E. Gandolfi, BS, MNS,‡ Yi-Zarn Wang, DDS, MD, FACS,‡ J. Phillip Boudreaux, MD, FACS,‡ Aaron I. Vinik, MD, PhD,|| Vay Liang W. Go, MD,¶ James R. Howe, MD,# Thor Halfdanarson, MD,** M. Sue O'Dorisio, MD, PhD,†† and Gregg Mamikunian, MS†

Objective: Pancreastatin is a fragment of the chromogranin A (CgA) molecule. Existing pancreastatin assays, which depend on antibodies that cross-react in varying percents with the larger prohormone, may lack sensitivity and specificity to detect small changes in neuroendocrine tumor volume.

Methods: We developed a highly specific, sensitive pancreastatin assay. The antibody used recognizes the carboxyl terminal of the peptide hormone and was raised against a 17-amino acid porcine pancreastatin fragment with high homology with the carboxy-terminal amino acids 286-301 of the human CgA.

Results: Our assay measures more than 95% of circulating pancreastatin levels; has little or no cross-reactivity with CgA, even at plasma concentrations of 1000 ng/mL; and can detect pancreastatin levels of 17 pg/mL. Interassay reproducibility for the pancreastatin radioimmunoassay was determined from results of 3 quality control pools in 15 consecutive assays. Coefficients of variation for low, medium, and high pancreastatin levels were less than 20%. The sensitivity of serial pancreastatin assays to detect early liver tumor activity was demonstrated in 2 patients with slowly progressive neuroendocrine tumors and in patients undergoing surgical cytoreduction.

Conclusions: This highly specific, sensitive pancreastatin assay can detect small changes in liver tumor progression and is up to 100-fold more sensitive and specific than CgA assays in the United States.

Key Words: chromogranins, biomarkers, neuroendocrine tumors, carcinoid, islet cell tumors, cytoreduction, embolization, chemoembolization, pancreastatin assay

Abbreviations: NET - neuroendocrine tumor, CgA - chromogranin A (*Pancreas* 2010;39: 611-616)

Normal and malignant endocrine cells contain secretory granules that, in most cases, secrete chromogranin A (CgA).¹ Chromogranin A is an acidic secretory protein that is colocalized with both amines (eg, serotonin) and neuropeptides (eg, substance P) in the large, dense granules of neuroendocrine cells.² Serum or plasma levels of CgA have been demonstrated to be

an effective tumor volume marker for neuroendocrine tumors (NETs).¹ Chromogranin A is commonly used as a general marker of NETs³ and sequential CgA measurements are useful in determining changes in tumor volume. A fragment of CgA, pancreastatin, has been used as an indicator of the therapeutic success of tumor resection or embolic treatment of NET liver metastasis.⁴⁻⁶

Recent information suggests that CgA is a "prohormone" subject to intracellular and extracellular conversion to a smaller peptide, pancreastatin (49 amino acid residues).^{5,7} Prohormone convertase-1 (PC-1) seems to be an important endoprotease responsible for the processing of the precursor protein CgA to the smaller 49-amino acid residue, pancreastatin.^{5,8,9} In addition to multiple intermediary metabolic actions *in vivo*,⁷ recent articles suggest that, like CgA, pancreastatin may have value as both a NET marker and possibly as a prognostic indicator of malignant tumor behavior.^{6,10,11}

Although it has been suggested that pancreastatin may be an independent marker and may reflect the aggressiveness of NET metastasis to the liver, the pancreastatin assays published to date use antibodies that cross-react in varying percentages with the larger prohormone, CgA. This makes difficult the accurate interpretation of the impact that PC-1 may have in varying malignant stages of NETs. Furthermore, larger tumor burden may be necessary to detect changes in CgA that are measured in quantities of nanograms per milliliter (ng/mL). Thus, it is possible that the measurement of pancreastatin may better reflect both maturity of a NET and, perhaps, be a sensitive and specific marker of early increases in tumor activity/burden.²⁻⁴ Pancreastatin is measured in picograms per milliliter (pg/mL), rather than nanograms per milliliter and thus is 100-fold (mole-for-mole) more sensitive than CgA.

The purpose of the present study was to report the development of a pancreastatin radioimmunoassay (RIA) that is highly specific and sensitive (pg/mL) for the active carboxy-terminal region of human pancreastatin 1-52 with negligible CgA cross-reactivity. In this report, we will demonstrate the utility of pancreastatin as a marker of liver tumor progression and report parallelism of split patient samples of pancreastatin between our novel new assay and a previously published pancreastatin assay that measures both CgA and pancreastatin.^{6,11-13} Finally, we will demonstrate the utility of serial pancreastatin measurements as a sensitive marker of liver tumor progression in 2 patients with metastatic NETs and demonstrate its sensitivity in a series of patients undergoing extensive cytoreductive surgery.

PANCREASTATIN ASSAY

Materials and Methods

The pancreastatin antiserum (ISI-56) used in our assay recognizes the carboxyl terminal fragment 37-52 of the intact

From the *Department of Internal Medicine, University of Iowa Hospitals & Clinics, Iowa City, IA; †Inter Science Institute, Inglewood, CA; ‡Department of Surgery, §Louisiana State University Health Sciences Center, New Orleans, LA; ||Department of Internal Medicine, Eastern Virginia Medical School, Norfolk, VA; ¶Division of Digestive Diseases, Department of Medicine, University of California, Los Angeles, CA; and Departments of #Surgery, **Internal Medicine, and ††Pediatrics, University of Iowa, Iowa City, IA.

Received for publication June 5, 2009; accepted October 16, 2009.
Reprints: Thomas M. O'Dorisio, MD, Department of Internal Medicine, University of Iowa Hospitals & Clinics, 200 Hawkins Dr, Iowa City, IA 52242 (e-mail: Thomas-odorisio@uiowa.edu).
Copyright © 2010 by Lippincott Williams & Wilkins

human pancreastatin 1-52, corresponding to the amino acid sequence 286-301 and 250-301, respectively, of human CgA. This antiserum was raised to a 17-amino acid porcine pancreastatin fragment 33-49 (CgA 272-288) that possesses approximately 80% homology with amino acids 286-301 of the carboxyl terminal human CgA. Characterization studies of the antiserum have revealed equipotency between porcine and human carboxyl terminal fragments and also with an intact porcine 49-amino acid peptide (CgA 240-288) but found no immunoreactivity associated with the heterologous intact human 52-amino acid pancreastatin peptide. Similarly, ISI-56 antiserum does not react with recombinant human CgA 260-454. No inhibition of porcine pancreastatin tracer binding was detected with human CgA (260-454) at 1000 ng/mL and with intact human pancreastatin 1-52 present at 1280 pg/mL. Details of the antiporcine pancreastatin ISI-56 antiserum epitope studies, shown above, clearly point to differences in conformation on intact porcine and intact human pancreastatin as the source of observed discrepancies in the detection of the hormone in a subject's blood.

Our antiserum ISI-56-based RIA correlates well with the secretory and metabolic activities of neuroendocrine cells. This new pancreastatin assay closely parallels the pancreastatin "trends" generated with the previously published assay (developed by T.M.O. at the Ohio State University [OSU], see below) used to predict the tumor progression of NET.^{6,11,13}

The Pancreastatin RIA

The present pancreastatin RIA is based on a 1-step, extraction-free, 24-hour disequilibrium assay, followed by addition of a radiolabeled tracer and an additional 24-hour incubation at 4°C before the double antibody-based bound-free separation step. Antiporcine pancreastatin ISI-56 antiserum in a 1:100 stock solution was diluted in assay buffer to give a working dilution of 1:25,000. Porcine pancreastatin 1-49 peptide was used for preparation of standards covering the range of 10 to 1280 pg/mL of plasma-based matrix and also for the generation of a radioactive tracer. The in-house manufactured iodine 125 (¹²⁵I) pancreastatin tracer was purified by gel filtration on a Sephadex G-25 column. Radioiodine-labeled peptide fractions with the highest binding to the working dilution of the antibody were used as the tracer. When stored at 4°C, the tracer could be used for 8 to 10 weeks with retention of high immunoreactivity: 25% to 40% of the tracer was still bound to the working dilution of the antibody.

Quality control pools were prepared by supplementation of a pancreastatin-deficient biological matrix (hyposerum, hypoplasma, or RIA buffer) with known quantities of porcine pancreastatin 1-49 peptide. Three quality control pools were prepared which included the low, medium, and high ranges of the pancreastatin dose-response curves with target concentrations of 40, 200, and 600 pg/mL matrix, respectively.

Assay Procedure

All steps of the assay are performed at 4°C using sodium phosphate-buffered saline 0.2% bovine serum albumin as the assay buffer. Defibrinated, delipidized, and charcoal-treated human plasma was used as the matrix for standard and quality control pool preparation. Aprotinin was supplemented in each test tube to inactivate proteolytic enzymes potentially present in the analyzed patient samples. Two hundred microliters in duplicate of standard, patient sample, or quality control pool were preincubated with 100 μL of 1:25,000 diluted antipancrastatin antiserum (ISI-56) for 24 hours at 4°C followed by the addition

of 100 μL of porcine pancreastatin 1-49 radioiodine tracer, and a 24-hour 4°C incubation time. Finally, 200 μL of titrated normal rabbit serum and 200 μL of goat antirabbit immunoglobulin G were added to achieve separation of antibody-bound tracer from free tracer. Radioactivity in net counts per minute of tracer bound (*B*) to the antiserum in equilibrium with a specific dose of a standard (pg/mL) is expressed relative to the tracer bound (*B*₀) in the absence of any competing standard as percent (*B*/*B*₀).

Subjects

Nineteen subjects with varying stages of NETs (mostly with liver metastasis) obtained from the Neuroendocrine Tumor Clinic at the University of Iowa were consecutively sampled over several weeks. All of the subjects gave written informed consent to have blood drawn for the purposes of detecting whether certain peptide values were useful markers of their disease state. The study was approved by the institutional review board of the University of Iowa, and the patients were treated in accordance with the Declaration of Helsinki. All specimens were collected with the specialized Z-tube prepared by Inter Science Institute (ISI, Englewood, Calif) (protecting the integrity of pancreastatin during collection), spun and aliquoted (within 30 minutes of collection), and split samples were sent frozen to either the OSU University Reference Laboratory (URL) or ISI. The OSU URL pancreastatin assay was established by author T.M.O. in 1989 and has been previously reported.¹¹ All samples were initially frozen at -70°C. Each sample was thawed 1 time and assayed within 1 week of receipt.

Figure 1 shows a composite pancreastatin dose-response curve consisting of 32 individual standard curve assays depicting mean values ± 2 SDs at each point of the standards that include the low, medium, and high ranges. Also shown in Figure 1 is the lack of cross-reactivity of recombinant human CgA 260-454 with competitive displacement detectable only at concentrations greater than 1000 ng/mL of incubate. Interassay reproducibility for the pancreastatin RIA was determined from the test results obtained from 3 quality control pools in 15 consecutive assays. The coefficient of variation for the low pool (mean value, 23 pg/mL) was 20%; the medium pool (mean value, 192 pg/mL), 14%; and the high pool (mean value, 526 pg/mL), 16%. The intra-assay coefficient of variation for the low pool (mean value, 21 pg/mL) was 12%; for the medium pool (mean value, 144 pg/mL), 6%; and for the high pool (mean value, 356 pg/mL), 8%.

Sensitivity of the Pancreastatin Assay

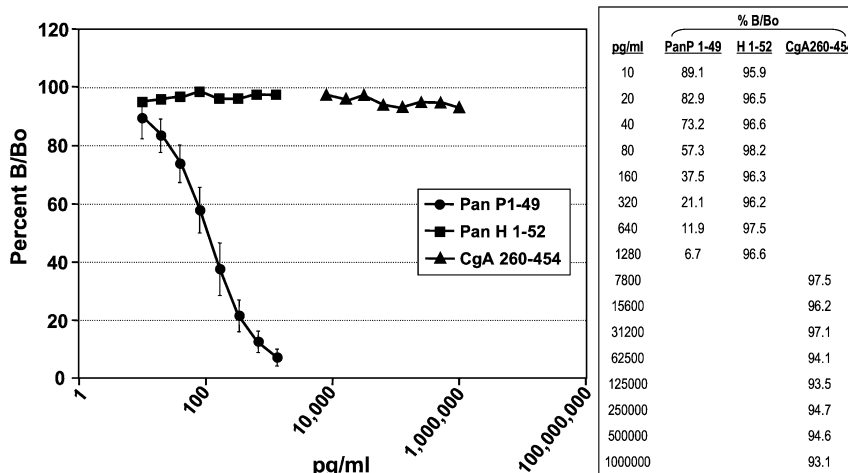
The sensitivity of our pancreastatin assay is defined by the concentration of porcine pancreastatin fragment 1-49 standards that yield a 10% displacement of tracer binding or greater relative to the binding given by an analyte-free sample as in *B*₀ tubes. The mean sensitivity of this assay was 17 pg/mL, with a range of 12 to 22 pg/mL of incubates.

Recovery and Stability of Pancreastatin

Experiments outlining the recovery and stability of the human pancreastatin fragment demonstrate that human pancreastatin incubated in various normal human sera and in pooled processed human sera and/or plasma very rapidly loses immunoreactivity at 4°C. Addition of aprotinin as an inhibitor of endogenous proteolytic enzymes to the assay tubes prevents loss of immunoreactive pancreastatin. With aprotinin added to the incubation tubes, recovery studies yielded an average recovery of 87% of the "spiked" immunoreactive pancreastatin.

Crossreaction of Anti P-Pan ISI-56 with Human Pan 1-52 and CgA 260-454

FIGURE 1. Composite dose-response curve generated from 32 assays run during the months March to December 2006, using the same antiserum, the same porcine pancreastatin 1-49 standard, and 5 lots of ¹²⁵I-labeled porcine pancreastatin 1-49 as tracer. Mean B/B₀ values obtained for each standard together with 2 SD (in B/B₀) are also given in the table. Also depicted is the lack of competitive inhibition by recombinant human chromogranin A 260-454, and human pancreastatin 1-52, corresponding to sequence 250-301 of human CgA. Refer to Sensitivity of the Pancreastatin Assay (Materials and Methods).



Split Sample Results

Figure 2 depicts the comparison of test results obtained by the 2 (ISI, URL) pancreastatin assays using split samples from 19 NET patients. There is a striking similarity between the 2 pancreastatin assays. The URL uses an antibody previously published^{6,11,13} that recognizes both the pancreastatin fragment and CgA, whereas the ISI antibody recognizes pancreastatin and has a very small amount of cross-reactivity with CgA at very high CgA levels (1000 ng/mL). There seems to be a 5-fold difference in the calculated/measured values between the 2 assays. However, the numerical trends are virtually identical. It is likely the URL values are higher owing to the high cross-reactivity with CgA.

The following 2 cases illustrate the potential for pancreastatin to be a better prognostic indicator of midgut NETs progression than CgA from which it is cleaved or 5-hydroxyindoleacetic acid (5-HIAA), a byproduct of serotonin metabolism.

SERIAL PANCREASTATIN MEASUREMENTS

Patient 1 is a 50-year-old white man who initially presented in late 2004 with progressive weight loss (30 lb for 6 months), flushing, diarrhea (5–8 watery stools per day), and intermittent crampy abdominal pain with occasional vomiting (Fig. 3). A computed tomographic (CT) scan of the abdomen with oral and intravenous contrast demonstrated a mass in the right lower abdomen that extended across the midline. The diagnosis of intermittent small bowel obstruction was made, and the area was explored through a midline incision. At the time of surgery, the patient was found to have a mass arising in the terminal ileum and extending into the sigmoid colon. Extensive tumor was found in the pelvis, along the diaphragm, and several liver metastases were observed. A frozen section of this tumor was consistent with well-differentiated neuroendocrine carcinoma. In October 2005, the patient underwent ileal and sigmoid resections with primary anastomoses. The gallbladder was removed in anticipation of subsequent octreotide therapy, and the patient recovered uneventfully.

Postoperatively, the patient was started on octreotide LAR therapy (120 mg/mo, 30 mg/wk) and followed up with serial (at 3-month intervals) 5-HIAA determinations, and CgA (reference, <36.5 ng/mL) and pancreastatin measurements (reference, <135 pg/mL) performed at the Inter Science Institute. Whereas the CgA and 5-HIAA values remained low,

the pancreastatin values progressively increased (Fig. 3). A OctreoScan and CT scan performed weeks after the increased pancreastatin (late 2005) demonstrated increasing tumor activity/burden in the liver and on diaphragm. Repeat exploration was undertaken 1 month later (November 2005); the diaphragm was debulked, several liver lesions were treated with radiofrequency ablation, and his pelvic floor tumors were completely resected.

Postoperatively, his pancreastatin values fell to a normal (273 to 113 pg/mL), whereas his CgA values remained low and unchanged (13.3 vs 11.0 ng/mL). The 5-HIAA likewise remained stable and unchanged. Abdominal CT scans obtained in February 2006 showed no tumor. Subsequently, his pancreastatin values

Comparison of ISI vs URL Pancreastatin Values

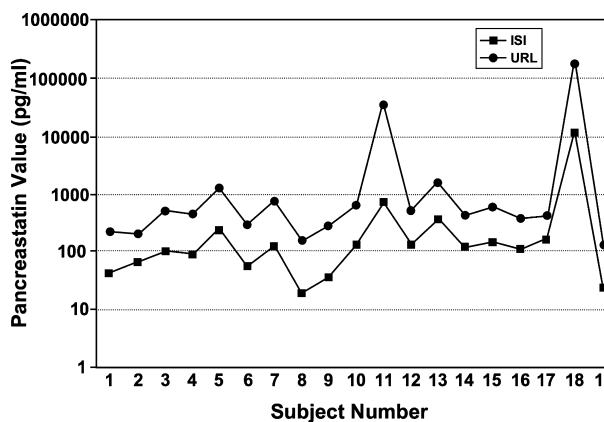


FIGURE 2. Comparison of pancreastatin levels obtained from 2 different pancreastatin assays. One pancreastatin assay has been previously published.^{11,14} This assay was performed at URL (the OSU, Columbus, Ohio) using a unique pancreastatin antibody. This antibody cross-reacts more than 95% with CgA. The second pancreastatin assay is from Inter Science Institute (ISI). The pancreastatin values were generated using ISI-56 porcine pancreastatin antibody that recognizes solely pancreastatin. The ISI-56 antibody has almost negligible cross-reactivity with chromogranin A as opposed to URL's pancreastatin antibody. As noted, the trends of the split samples are very similar, but the values are 5-fold greater with the OSU URL assay.

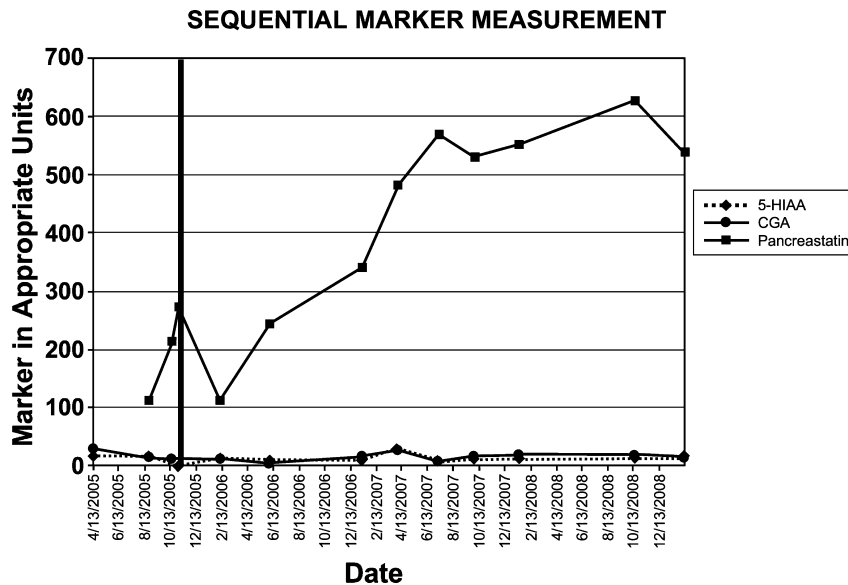


FIGURE 3. Sequential (April 2005 to December 2008) pancreastatin, 5-HIAA, and chromogranin A measurements in a 50-year-old man who initially presented with progressive weight loss, flushing, and diarrhea. After surgical debulking in October 2005 and again in November of 2005 (single bold vertical line), the pancreastatin value fell dramatically to reference levels, although the 5-HIAA and CgA remained essentially unchanged. Restaging in February 2006 showed no evidence of tumor on CT or OctreoScan. By May 2006, his pancreastatin values rose into the abnormal range and his scans demonstrated low-volume tumor recurrence. In the ensuing follow-up period, his 5-HIAA and CgA values did not reflect tumor progression, although his pancreastatin levels predicted his tumor's growth was confirmed by repeated CT's.

have progressively increased to more than 600 pg/mL, whereas his CgA and 5-HIAA values have remained relatively stable. Since February 2006, he has undergone serial CT scans; these have shown slowly progressive liver metastasis.

Patient 2 is a 48-year-old white woman who presented in 2004 having undergone a right hemicolectomy and right hepatic lobectomy. Her symptoms were well controlled by continuous subcutaneous infusion of octreotide at a dosage of 2 mg/d. On serial CT scans and bone scans, she demonstrated slow progression of her hepatic and bony disease. She underwent the placement of a hepatic artery port for intra-arterial infusion with 5-fluorouracil and was being treated with intravenous clodronate. Subsequently, the patient underwent a series of 2 intra-arterial infusions with 5-fluorouracil followed by chemoembolization with doxorubicin, cisplatin, and mitomycin C. The hepatic arterial port clotted and was removed. The patient underwent her third chemoembolization after 2 weeks of oral capecitabine. Tumor growth stabilized, and she remained essentially asymptomatic. Serial marker determinations show an essentially flat curve for serotonin, 5-HIAA, CgA, and progressively increasing pancreastatin values (Fig. 4). Her symptoms remained well controlled on her continuous octreotide subcutaneous infusion at a dosage of 2 mg/d. A careful review of her serial hepatic CT scans demonstrated slow growth of several of her hepatic lesions between February 2006 and July 2007 (Fig. 4). By April 2008, her tumor had progressed to the point that additional therapy was warranted. Her pancreastatin value had risen to over 4000 pg/mL. Oral capecitabine therapy was administered for 1 week before repeat chemoembolization. After her fourth chemoembolization, her pancreastatin value dropped dramatically to 587 pg/mL by February 2009 and then slowly began to increase again.

These cases support the sensitivity and predictive value of serial pancreastatin determinations in detecting small changes in tumor volume and tumor progression.

PANCREASTATIN AND CYTOREDUCTIVE SURGERY

Further support of the sensitivity of pancreastatin change in demonstrating effectiveness of therapeutic intervention can be made from an ongoing study of hepatic cytorreduction procedures in patients with metastatic NET from Louisiana State University (authors E.L., S.J., Y.Z.W., J.P.B., and E.A.W.). Thirty-nine patients with metastatic carcinoid tumor of the small intestine and elevated preoperative pancreastatin and CgA levels underwent greater than 90% hepatic cytorreduction. Determination of the percent of tumor cytorreduced was made independently by 2 surgeons who estimated tumor burden after reduction. Of the 39 patients with elevated preoperative pancreastatin levels, 38 (97%) exhibited greater than 25% decrease in pancreastatin after 90% hepatic cytorreduction. The overall mean \pm SD pancreastatin decrease in the debulked population was 53% \pm 44%.

In contrast, of the 39 patients with elevated preoperative CgA levels, only 17 (44%) exhibited greater than 25% CgA decreases after 90% hepatic cytorreductive surgery.

Clearly, pancreastatin was a much more sensitive correlative to the percent tumor removed during cytorreductive surgery than was CgA. Chromogranin A values in this study were measured at Inter Science Institute or at Quest Laboratories. A previously published study has shown essentially identical values between these 2 CgA assays.¹⁴ Whether greater than 25% decrease in pancreastatin levels after major liver tumor cytorreduction predicts stabilization of tumor burden as was previously reported^{11,13} remains to be determined.

DISCUSSION

Classically, serial measurement of 5-HIAA and CgA values has been considered the criterion standard for the detection of progressive functional midgut NET. We have demonstrated that

Markers Trends in Patient 2 Following Surgery and Octreotide Therapy

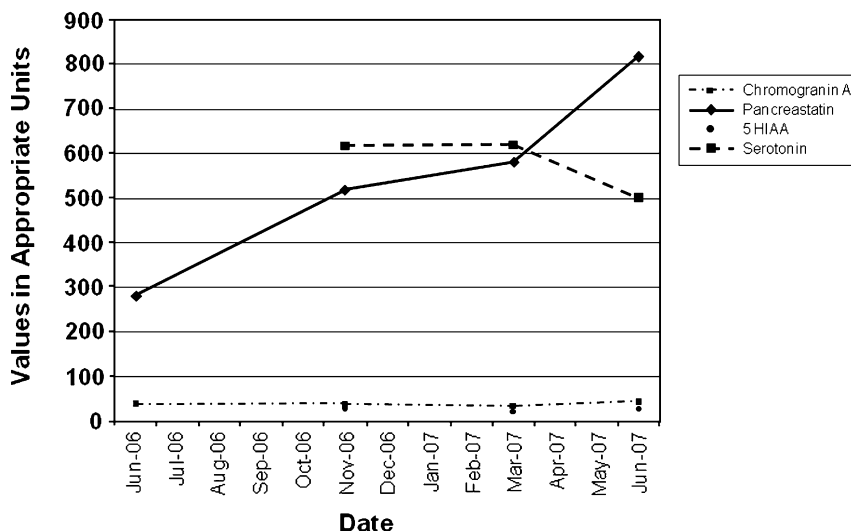


FIGURE 4. Changes in sequential measurements of multiple NET markers in a 48-year-old woman with a slowly progressing ileal carcinoid metastatic to the bone and liver. Pancreastatin values rise progressively, whereas serotonin, 5-HIAA, and CgA values are not nearly as sensitive to changes in tumor volume. As in patient 1, pancreastatin heralded CT progression of her tumor. The bold vertical line indicates the timing of a 1-week course of capecitabine followed by a chemoembolization using mitomycin C, doxorubicin, and cisplatin. Pancreastatin accurately reflects the efficacy of the chemoembolization, whereas serotonin rose transiently reflecting lability and unreliability as a stable tumor marker.

our novel pancreastatin assay may be a more sensitive predictor of early changes in tumor volume. All pancreastatin samples in these studies were assayed by ISI and all CgA assays were performed by either ISI or Quest. The 5-HIAA assays were performed by a number of local laboratories across the nation. To the best of our knowledge, only 2 CgA assays available in the United States have undergone split sample testing and are both sensitive and parallel each other's results.¹⁴ Other commercial laboratories in the United States report large reference ranges, which make serial measurements of early tumor burden inaccurate or meaningless. These huge differences in the reference range of these assays also make comparison of individual patient biomarkers across laboratories impossible. These include ARUP (reference range, 0–50 ng/mL; reported as <200 ng/mL), Laboratory Corp (reference range, 0–5 nmol/L; reported as <5 nmol/L or 225 ng/mL), Mayo (reference range, ≤225 ng/mL), Quest (reference range, ≤36.4 ng/mL), and ISI (reference range, 6–40 ng/mL). With CgA, patients' results are reported and measured above the lower limit of sensitivity rather than the normal reference range in order for them to be more useful tumor burden markers. A recent article by Arnold et al¹⁵ supports the value of CgA determinations reported in several European studies and alludes to the problems found during CgA determinations being performed in the United States. The article of Arnold et al¹⁵ used a CgA assay that was in place at Phillips University (Marburg, Germany) since 1993 and had been standardized by Stridsberg et al in 2003.¹⁶

As noted previously, with exception of the comparisons between CgA from ISI assay and Quest's CgA assay,¹⁴ standardization of the many other commercially available CgA assays in the United States is not feasible because different NET centers and their respective hospitals use different commercially available laboratories. The laboratory chosen to provide CgA determination for a NET Center is often mandated by contractual agreements and is not driven by assay quality.

Pancreastatin is a 49-amino acid residue that is formed by the action of PC-1 on the prohormone polypeptide CgA.⁹ Chromogranin A is a large 439-amino acid acidic secretory protein present in normal neuroendocrine cells and their tumor cells.⁵ The pancreastatin values reported^{6,11,13} and as measured in our present pancreastatin assay are in picograms per milliliter

(pg/mL) of the subject's plasma. The prohormone CgA circulates at levels that are 100-fold higher (taking into account molar differences; ng/mL levels) than pancreastatin (pg/mL).^{2,4} Thus, with antibodies that recognize both the larger-molecular weight hormone and the pancreastatin split fragment, it is not certain whether the pancreastatin levels actually reflect the true predictive phenomenon of tumor progression behavior/volume versus the known higher levels of CgA, which may raise the pancreastatin level if the antibody recognizes both the pancreastatin fragment and the CgA. Such seems to be the case for the higher pancreastatin levels measured at the OSU URL where the pancreastatin antibody is reported to have high (>95%) cross-reactivity with CgA. Most importantly, the pancreastatin assay (from ISI) reported here demonstrates excellent parallelism with the previously reported URL pancreastatin assay (Fig. 3). Previously, all pancreastatin assay values reported have used the OSU URL assay (developed by T.M.O. in 1989 at OSU).

The parallelism between the rising pancreastatin levels and clinical status of our 2 patients is in agreement with the 3 articles previously published in the United States, which used the pancreastatin assay from the OSU URL.^{6,11,13} We believe the pancreastatin blood levels reflect liver tumor status and progression in patients with both midgut carcinoid tumors and NETs of the pancreas. Indeed, early pancreatic NET metastasis to the liver is accurately reflected as well. In a nonprospective review of 38 pancreatic NET patients, 25 had liver metastasis. Pancreastatin was observed to be as sensitive as CgA in reflecting liver metastasis and was not necessarily dependent on an elevated CgA level. Both CgA and pancreastatin may have been attenuated by concurrent use of octreotide acetate.

We believe the assay heralds tumor activity/progression before changes are seen by CT or magnetic resonance imaging. A recent article by Bloomston et al¹³ from the OSU using the previously published pancreastatin assay (Fig. 2) supports the value of pancreastatin levels before and after hepatic artery chemoembolization intervention using pancreastatin as a predictor of liver tumor progression.¹³ In this retrospective analysis of 122 patients with metastatic carcinoid tumor undergoing hepatic artery chemoembolization, 14% and greater than 20% reduction of pancreastatin (after vs before embolization) were

associated with an additional radiographic regression or stabilization of disease in 80% of the patients. These authors also noted that absolute pancreastatin levels of 5000 pg/mL or greater (≥ 1000 pg/mL using the ISI pancreastatin value) were associated with decreased survival ($P < 0.07$, not significant).¹⁷ Our present pancreastatin assay clearly parallels the pancreastatin assay used at the OSU¹³; therefore, our ISI pancreastatin values would be expected to have similar predictive results, albeit, at a different magnitude (Figs. 3 and 4) than those reported by OSU URL.¹³

Our new pancreastatin assay is unique because it recognizes mostly pancreastatin (vs both CgA and pancreastatin as seen in the OSU assay). The sensitivity of our pancreastatin assay alludes to the utility and importance that PC-1 may have in defining the potentially malignant behavior of NET that metastasizes to the liver. Although it is not entirely clear where the PC-1 exerts its action on CgA to form pancreastatin, it may resemble the action of monoamine oxidase that produces the metabolite of serotonin, 5-HIAA, in the urine in higher amounts when there is large liver tumor burden from midgut carcinoid. Thus, the source of PC-1 may well be the liver because the most sensitive changes seem to be associated with liver tumor progression and/or burden.

In light of the work of Desai et al,¹¹ Calhoun et al,⁶ the recent work of Bloomston et al,¹³ and our present work, we conclude that C-terminally directed pancreastatin assays may provide us with an important early marker of changes in NET burden, especially those in the liver. This concept was recently alluded to by Stronge et al from Belfast, UK.¹⁸ Using antibodies raised in rabbits to synthetic human pancreastatin and specific to the C-terminal region of pancreastatin (as in the present assay in the United States), these authors demonstrated that an immediate increase in pancreastatin after institution of somatostatin analog therapy was associated with poor patient survival.

Most recently, we observed a rise of pancreastatin in a patient with no evidence of active NET, but with severe renal failure. In that pancreastatin is considered a hormone, it is not surprising that its renal clearance, like other peptide hormones (eg, gastrin, insulin), is dependent in part on renal function.

In summary, we have established a highly sensitive and specific pancreastatin assay that measures greater than 95% of the circulating pancreastatin level. Furthermore, we have characterized this assay and compared it to the only other commercially available pancreastatin assay in the United States (the OSU URL). Results from these 2 assays were parallel, but the OSU assay had a great deal of cross-reactivity with CgA as reflected in 5-fold higher serum values. Our antiserum recognizes the biologically active sequence of pancreastatin and therefore is more likely to reflect the pathology and physiologic changes that occur with this molecule.¹⁹ We believe that because the sensitivity of pancreastatin, versus CgA (>100 -fold (on a per-mole basis)), pancreastatin may well reflect smaller, earlier changes of NET liver progression/burden.

ACKNOWLEDGMENTS

The authors thank the work of Mr Brent A. Howe and Mr Joseph R. Stewart at The Ohio State University Core Peptide Laboratory. Their assistance in helping us compare assay sensitivity was invaluable. The authors also thank the technical assistance of A. Okonko who performed the pancreastatin assay at Inter Science Institute, Mackenzie L. Pedersen for manuscript preparation and formatting, and Trisha Chiasson for manuscript editing.

REFERENCES

- O'Connor DT, Deftos LJ. Secretion of chromogranin A by peptide-producing endocrine neoplasms. *N Engl J Med.* 1986;314:1145–1151.
- Kolby L, Bernhardt P, Swärd C, et al. Chromogranin A as a determinant of midgut carcinoid tumour volume. *Regul Pept.* 2004;120:269–273.
- Nobels FRE, Kwekkeboom DV, Bouillon R, et al. Chromogranin A: its clinical value as marker of neuroendocrine tumors. *Eur J Clin Invest.* 1998;8:431–440.
- Sondenaa K, Sen J, Heinle F, et al. Chromogranin A, a marker of the therapeutic success of resection of neuroendocrine liver metastases: preliminary report. *World J Surg.* 2004;28:890–895.
- Udupi V, Lee HM, Kurosky A, et al. Prohormone convertase-1 is essential for conversion of chromogranin A to pancreastatin. *Regul Pept.* 1999;83:123–127.
- Calhoun K, Toth-Fejel S, Cheek J, et al. Serum peptide profiles in patients with carcinoid tumors. *Am J Surg.* 2003;186:28–31.
- Iacangelo AL, Fischer-Colbrie R, Koller KJ, et al. The sequence of porcine chromogranin A messenger RNA demonstrates chromogranin A can serve as the precursor for the biologically active hormone, pancreastatin. *Endocrinology.* 1988;122:2339–2341.
- O'Connor DT, Cadman PE, Smiley C, et al. Pancreastatin: multiple actions on human intermediary metabolism in vivo, variation in disease, and naturally occurring functional genetic polymorphism. *J Clin Endocrinol Metabol.* 2005;90:5414–5425.
- Tatemoto K, Efendic S, Mutt V, et al. Pancreastatin, a novel pancreatic peptide that inhibits insulin secretion. *Nature.* 1986;324:476–478.
- Kogner P, Bjellerup P, Svensson T, et al. Pancreastatin immunoreactivity in favourable childhood neuroblastoma and ganglioneuroma. *Eur J Cancer.* 1995;31A:557–560.
- Desai DC, O'Dorisio TM, Schirmer WJ, et al. Serum pancreastatin levels predict response to hepatic artery chemoembolization and somatostatin analogue therapy in metastatic neuroendocrine tumors. *Regul Pept.* 2001;96:113–117.
- O'Dorisio MS, Hauger M, O'Dorisio TM. Age-dependent levels of neuropeptides in normal children. *Regul Pept.* 2002;109:189–192.
- Bloomston M, Al-Saif O, Klemanski D, et al. Hepatic artery chemoembolization in 122 patients with metastatic carcinoid tumor: lessons learned. *J Gastrointest Surg.* 2007;11:264–271.
- Woltering EA, Hilton RS, Zolfoghary CM, et al. Validation of serum versus plasma measurements of chromogranin A levels in patients with carcinoid tumors: lack of correlation between absolute chromogranin A levels and symptom frequency. *Pancreas.* 2006;33:250–254.
- Arnold R, Wilke A, Rinke A, et al. Plasma chromogranin A as marker for survival in patients with metastatic endocrine gastroenteropancreatic tumor. *Clin Gastroenterol Hepatol.* 2008;6:820–827.
- Stridsberg M, Eriksson B, Oberg K, et al. A comparison between three commercial kits for chromogranin A measurements. *J Endocrinol.* 2003;177:337–341.
- Sanchez-Margalet V, Lucas M, Goberna R. Pancreastatin: further evidence for its consideration as a regulatory peptide. *J Mol Endocrinol.* 1996;16:1–8.
- Stronge RL, Turner GB, Johnston BT, et al. A rapid rise in circulating pancreastatin in response to somatostatin analogue therapy is associated with poor survival in patients with neuroendocrine tumours. *Ann Clin Biochem.* 2008;45:560–566.
- Zhang K, Rao F, Wen G, et al. Catecholamine storage vesicles and the metabolic syndrome: the role of the chromogranin A fragment pancreastatin. *Diab Obes Metab.* 2006;8:621–623.