A Single Fasting Plasma 5-HIAA Value Correlates With 24-Hour Urinary 5-HIAA Values and Other Biomarkers in Midgut Neuroendocrine Tumors (NETs)

Maria R. Tellez, PhD, Gregg Mamikunian, MS,* Thomas M. O'Dorisio, MD,† Aaron I. Vinik, MD, PhD,‡ and Eugene A. Woltering, MD§*

Objectives: 5-Hydroxyindoleacetic acid (5-HIAA) is used for the evaluation of neuroendocrine tumors (NETs) but currently requires a 24-hour urine collection.

Methods: We developed a gas chromatography mass spectroscopy–based plasma 5-HIAA assay. We compared 24-hour urine 5-HIAA values against plasma 5-HIAA values in 115 mixed-variety patients with NETs and in a subset of 72 patients with only small bowel NETs. We also compared the information gained from urinary and plasma 5-HIAA values with other biomarkers of midgut NET activity to determine the plasma assay's clinical implications.

Results: In a group of 115 patients with all types of NETS, in a subset of patients with midgut NET and in a subgroup of midgut NETS with liver metastasis, the correlation between the urine and fasting plasma 5-HIAA values were statistically significant ($P \leq 0.0001$). Comparison of the proportion of normal or abnormal urinary and plasma 5-HIAA values to the proportion of chromogranin, serotonin, neurokinin, or pancreastatin values that were in the normal or abnormal range yielded essentially identical information.

Conclusions: Plasma fasting 5-HIAA values are proportional to urinary 5-HIAA values and yielded identical clinical correlation with other biomarkers.

Key Words: 5-hydroxyindoleacetic acid, 5-HIAA, neuroendocrine tumors, midgut, serotonin, carcinoid syndrome

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Neuroendocrine tumors (NETs) are derived from Kulchitsky cells and can originate from multiple sites. Small bowel NETs are rare and generally are not diagnosed until they are at an advanced stage.[1,2] Neuroendocrine tumors, especially of the midgut, have the ability to secrete serotonin and serotonin's degradation product, 5-hydroxyindoleacetic acid (5-HIAA). The latter is used as a biomarker for the diagnosis and follow-up of these tumors, especially when liver metastases are present.[3] The rate-limiting step for the synthesis of serotonin in NETs is the conversion of tryptophan into 5-hydroxytrytophan. In midgut (small intestinal) tumors, 5-hydroxytrytophan is rapidly converted to serotonin, which is then either stored in the neurosecretory granules or secreted directly into the vascular compartment. Most of the secreted serotonin is taken up and stored by platelets, whereas the rest of the circulating serotonin remains free in the plasma. This free serotonin is converted into the urinary metabolite 5-HIAA by the enzymes monoamine oxidase and aldehyde dehydrogenase[4] (Fig. 1). Most elevated 5-HIAA levels are seen in patients with midgut NETs that have metastasized to the liver.[5,6] Based on the potential influence of a multitude of confounding factors such as stress, exercise, and the dietary intake of tryptophan, many investigators recommend the measurement of 5-HIAA in 24-hour urine collections rather than spot urine collections of 5-HIAA, which might be influenced by a transient surge of serotonin.[3]

The reference range for a 24-hour urinary 5-HIAA varies between laboratories but is approximately 2 to 8 mg per day.[4] Measurement of plasma 5-HIAA is not a novel idea. Previous groups have attempted to measure 5-HIAA in plasma via high-performance liquid chromatography (HPLC) and liquid chromatography tandem mass spectrometry (LC-MS/MS). Both methods demonstrated that plasma 5-HIAA is a reliable alternative to urinary 5-HIAA and produces comparable, if not more sensitive, results. However, these studies were small, largely nonhomogeneous and did not produce a patient-accessible commercially viable testing method.[7–9] The half-life of free serotonin in plasma is 1 to 2 minutes, and the half-life of platelet serotonin mirrors the half-life of platelets themselves (5 days).[10,11] While we cannot be certain that a plasma 5-HIAA measurement is not affected by a transient surge in serotonin, we feel that this is highly unlikely. We hypothesized that urinary and plasma 5-HIAA values collected simultaneously in a NET patient would yield equivalent results. In this study, an overall group (n = 115) of patients with NET who might be screened with a urinary 5-HIAA test for tumor stage and functionality were evaluated. A subset of this group (n = 72) were patients with midgut primary NETs (all stages) who might either be initially screened with this test or followed with serial 5-HIAA values to determine tumor persistence or recurrence. A third group of patients (n = 47) included only individuals with midgut primaries metastatic to the liver. These patients would normally be followed with serial urinary 5-HIAA determinations to watch for the progression of their disease. This group of patients is expected to have the highest 5-HIAA values. The convenience of measuring 5-HIAA levels in plasma would decrease the use of the 24-hour urine assay. In turn, this should offer significant increases in patients' satisfaction and potentially an increase in patients' compliance in obtaining serial biomarkers.

MATERIALS AND METHODS

Development of Assay

Stock solutions of aqueous 5-HIAA were prepared in concentrations ranging from 1 mg/mL to 0.1 μg/mL. Calibrators were

From the *Inter Science Institute, Inglewood, CA; †Division of Endocrinology and Metabolism, University of Iowa, Iowa City, IA; ‡Strelitz Diabetes Institute, Eastern Virginia Medical School, Norfolk, VA; and §Section of Surgical Endocrinology, Department of Surgery, Louisiana State University Health Sciences Center, New Orleans, LA.

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Reprints: Eugene A. Woltering, MD, FACS, 200 W Esplanade Ave, Suite 200, Kenner, LA 70065 (e-mail: ewolte@lsuhsc.edu).

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Maria Tellez, PhD, is an employee of Inter Science Institute, Inglewood, CA. Gregg Mamikunian is the CEO of Inter Science Institute in Inglewood, CA. Drs. O'Dorisio, Vinik, and Woltering are members of the Inter Science Institute GI Council and act as consultants for Inter Science Institute.

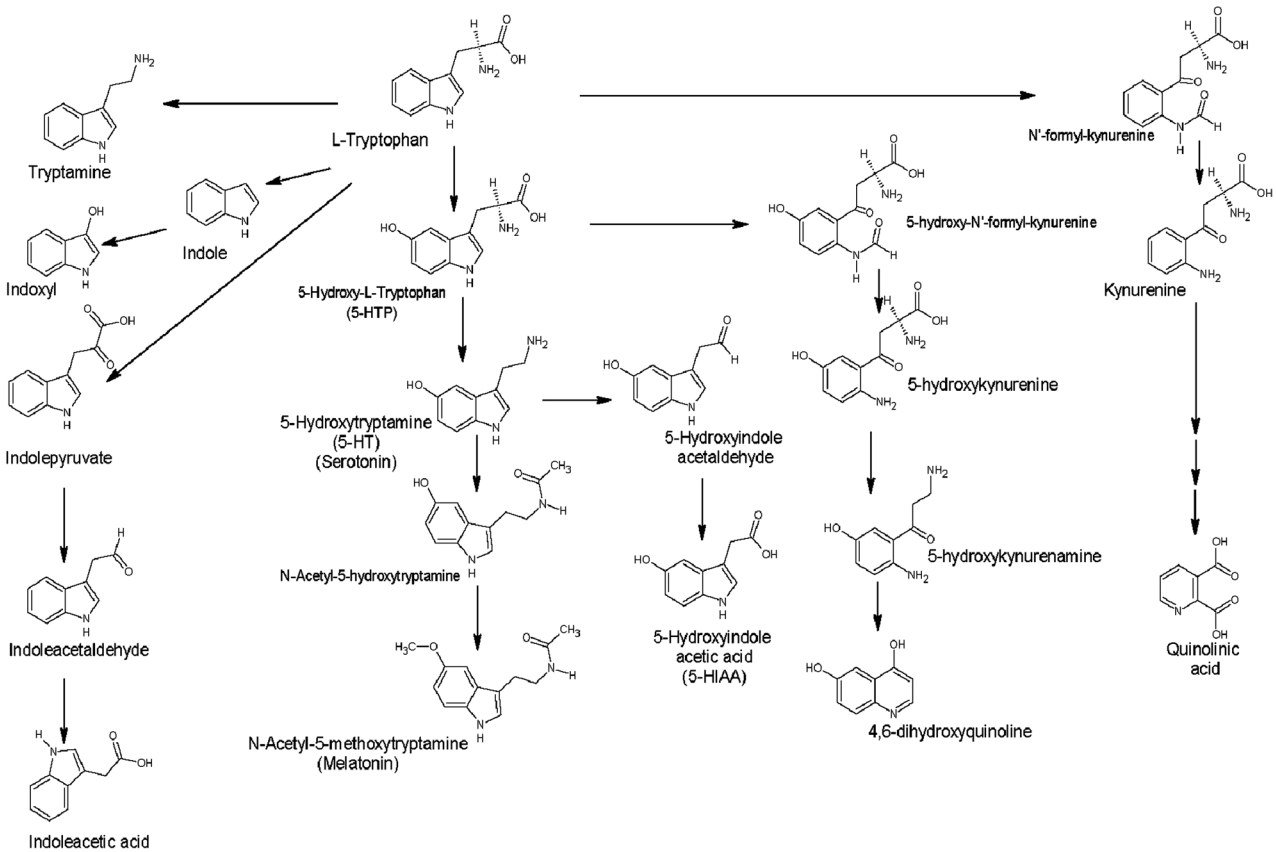

FIGURE 1. Biosynthesis of serotonin. Permission was received from Elsevier Limited (UK) and the figure author Gregg Mamikunian, MS. This figure was originally published in Endocrinology and Metabolism Clinics of North America. All permission requests for this image should be made to the copyright holder.

prepared by adding 5-HIAA from stock solutions to defibrillated, delipidized human plasma (DDHP) to give the following concentrations: 1, 5, 10, 25, 50, 100, 500, 1000, and 10,000 ng/mL. A 5-HIAA derivative was used as an internal standard. Each calibrator was added to acetonitrile and the resulting suspension was centrifuged. The acetonitrile/water supernatant was reduced in volume by evaporation. To the aqueous phase, sodium chloride was added and the suspension extracted with ether. The organic phase extract was derivatized using *N*-methyl-*N* (trimethylsilyl) trifluoroacetamide yielding fully silylated 5-HIAA. Derivatized extracts were analyzed by gas chromatography mass spectrometry on a Varian CP-3800 gas chromatograph coupled to a Varian Saturn 2000 MS/MS (Varian, Inc, Walnut Creek, Calif; now a part of Agilent Technologies, Palo Alto, Calif).

The derivatized extract yielded a single sharp peak. Quantification of this peak gave us a limit of detection that was defined as the amount that would be expected to afford a signal-to-noise (S/N) ratio of 5. The limit of detection was calculated from the S/N ratio generated by 10 replicate runs of the lowest calibrator (1 μg/L) to be 0.2 μg/L. The limit of quantification was set at 1 μg/L and gave an average S/N ratio of 26 (n = 10). The percent CV (coefficient of variation) of multiple runs, over separate days, of the 1-μg/L calibrator was 8.3%, which is well below the 15% maximum allowed to establish a valid limit of quantification.

Intra-assay variability was measured from 10 replicate spiked DDHP samples at 3 different concentrations (low, normal-high, and elevated). The percent CV calculated from these runs were 3.8% for low (3.7 μg/L) 5-HIAA concentrations, 2.3% for the normal-high (18.7 μg/L) sample, and 10% for the elevated (85.5 μg/L) sample. Interassay variability, calculated from assays performed on 3 separate days, led to percent CVs of 4.8%, 5.3%, and 4.3% for the low, normal-high, and elevated concentrations, respectively. Assay linearity was good. Means of the results of quadruplicate analysis of 10-, 100-, 1,000-, 5,000-, and 10,000-fold dilutions of a 10,000-μg/L spiked DDHP sample were never more than 20% different from the theoretically predicted values. The calibration curve had a relative SD of 16% over the whole calibration range (1–10,000 μg/L) and an R^2 of 0.9997. Spiked recoveries of a 2-ng/mL spiked DDHP sample ranged from 80% for a 4-ng/mL spike to 98% for a 100-ng/mL spike.

Reagents

Acetonitrile, diethyl ether, sodium chloride, glacial acetic acid, and 5-HIAA were obtained from Sigma-Aldrich. Ultra Resi-Analyzed water suitable for organic trace analysis was obtained from JT Baker (Avantor Performance Materials, Center Valley, Pa). *N*-methyl-*N* (trimethylsilyl) trifluoroacetamide activated with ethanethiol and ammonium iodide was obtained from Fluka (Sigma-Aldrich, St Louis, Mo). Defibrinated, delipidized human plasma was obtained from Golden West Biologicals (Temecula, Calif).

Analysis of Patients' Data

One hundred fifteen clinic patients with NETs were evaluated as part of a Louisiana State University Health Sciences

Center, New Orleans, Louisiana, and Ochsner Medical Center, New Orleans, Louisiana, Institutional Review Board–approved protocol. Seventy-two of the 115 patients had small bowel primaries of which 47 (65%) of the 72 patients had metastatic disease to their liver. The remaining 43 patients (of 115) had non–small bowel primaries and included primaries of the following: appendix, bile duct, cecum, colon, duodenum, lung, pancreas, rectum, and stomach. All specimens from the patients were collected as part of routine patient management, and the 5-HIAA plasma levels were measured on discarded clinical samples that were properly frozen and maintained. No additional blood or urine samples were collected as part of this study. For routine blood collections, these measurements followed an overnight fast and were taken just before the administration of long-acting somatostatin analogs such as octreotide or lanreotide in their depot formulations. No additional alterations in diet or drug administration were used during the traditional collection of these biomarkers. Routine clinical care was used in the collection of the urinary 5-HIAA values; instructions were given to the patients on the appropriate diet and drugs that could alter urinary 5-HIAA. Patients' urine and plasma 5-HIAA values were measured within ± 3 months of each other and were collected from January 2011 to September 2011.

Patients' charts were used to collect their neurokinin A (NKA), pancreastatin, chromogranin A (CGA), and serotonin values from within the study period. Only pancreastatin values and NKA values from Inter Science Institute (Inglewood, Calif) were allowed in this study. In contrast, the urinary 5-HIAA, CGA, and serotonin values were performed by multiple commercial laboratories.[12–15]

Patients' biomarker levels were entered into a Microsoft Excel spreadsheet (version 97-2003), and scatter plots were created using the log of the biomarker value. Data were transferred from Excel into MedCalc (version 11.2.1, Mariakerke, Belgium) to obtain coefficients of determination (r^2) and P values.

The following cutoffs were used to delineate "normal" from "abnormal" marker values: urinary 5-HIAA, less than 6 mg/24 hours; plasma 5-HIAA, less than 22 ng/mL (as previously described in the "Materials and Methods" section); pancreastatin, less than 135 pg/mL; NKA, less than 40 pg/mL. 5-Hydroxyindoleacetic acid, NKA, and pancreastatin values were used "as is". Six milligrams per 24 hours is the most common upper normal limit for urinary 5-HIAA. However, some laboratories use 10 mg/24 hours or 15 mg/24 hours. Owing to this variability in upper normal 5-HIAA limits, we expressed our results using 6 mg/24 hours, 10 mg/24 hours, and 15 mg/24 hours as cutoffs. In this study, CGA and serotonin had variable "high normal" cutoffs; therefore, if a patient's value fell within the assay's normal limits, it was given a score of "1". If the biomarker's value was greater than the test's upper normal limit, the clinical value was normalized against the individual laboratory's high normal value (clinical result/high normal laboratory value).

RESULTS

Demographics

For the 72 patients with small bowel primaries, the mean ± SD age was 60 ± 10 years, ranging from 41 to 89 years. Thirty-three patients were men, and 39 patients were women. Sixty-eight patients were white, and 4 patients were African American. Two patients were classified as having localized disease, 14 patients had regional disease, and 56 patients had distant disease. Of the 56 patients with distant disease, 47 patients had metastatic disease to the liver.

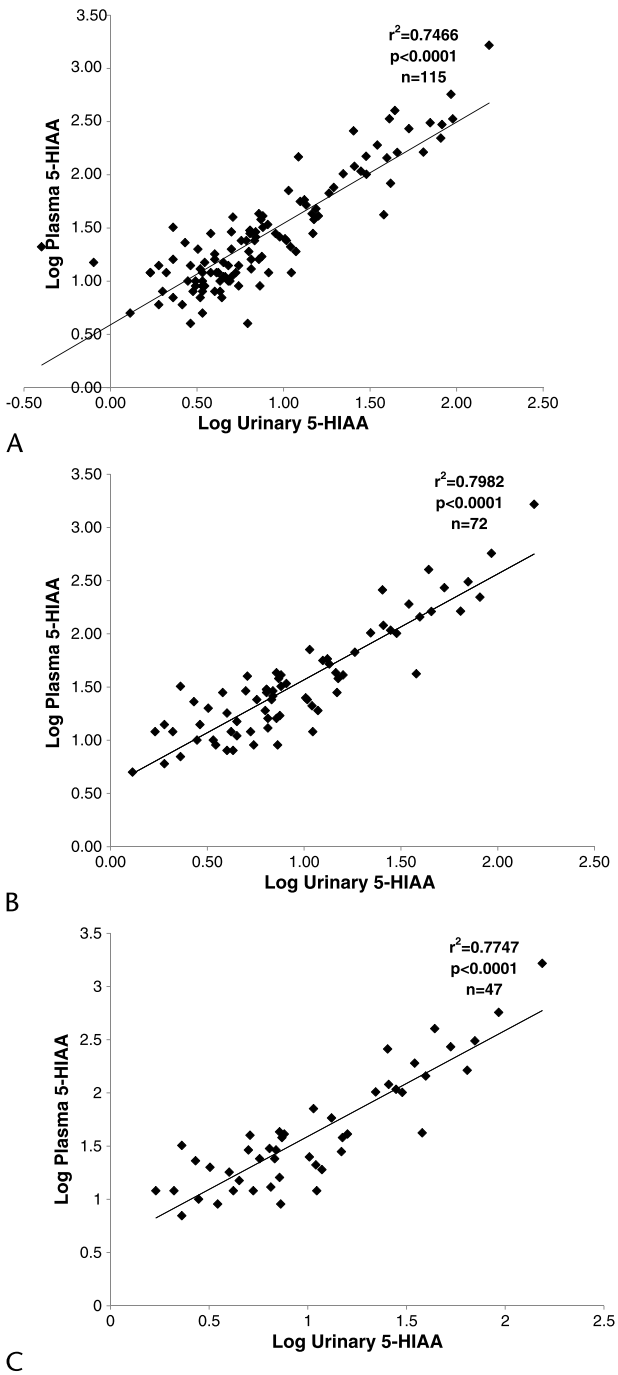

FIGURE 2. A, Urinary 5-HIAA values versus plasma 5-HIAA values. Log-scale scatterplot of urinary 5-HIAA values versus plasma 5-HIAA values for any primary NET (n = 115). B, Urinary 5-HIAA values versus plasma 5-HIAA values for small bowel primaries. Log-scale scatterplot of urinary 5-HIAA values versus plasma 5-HIAA values for small bowel NET primaries only (n = 72). C, Urinary 5-HIAA values versus plasma 5-HIAA values for small bowel primaries with liver metastasis. Log-scale scatterplot of urinary 5-HIAA values versus plasma 5-HIAA values for patients with small bowel primary NETs with liver metastasis (n = 47).

TABLE 1. Comparison of r and r^2 Values

Patient Group	No. Patients	Coefficient of Correlation (r)	Coefficient of Determination (r^2)*	Slope*
All primaries	115	0.8638	0.7466	0.9527
Small bowel primaries	72	0.8862	0.7982	0.9947
Small bowel primaries with liver metastasis	47	0.8802	0.7747	0.9953

*Log-scale graph values used for these calculations.

Urinary 5-HIAA Versus Plasma 5-HIAA

Correlation between urinary and plasma 5-HIAA values was assessed for all patients (n = 115), regardless of their primary tumor site. Urinary 5-HIAA values were obtained from a variety of commercial laboratories, and we chose 6 mg/24 hours as the cutoff for the normal value. However, we recognize that some laboratories' normal values go up to as high as 15 mg/d. Therefore, an additional subanalysis was performed using 10 mg/24 hours and 15 mg/24 hours as the normal cutoffs. For the plasma 5-HIAA results, we used an upper limit of 22 ng/mL as the normal limit. With 6 mg/24 hours as the upper normal limit for urinary 5-HIAA, in 98 (85%) of the 115 patients, the urinary and plasma 5-HIAA levels were in agreement (ie, if patients had a normal urinary 5-HIAA, then they also had a normal plasma 5-HIAA; conversely, if patients had an elevated urinary 5-HIAA, then they also had elevated plasma 5-HIAA). In 92 (80%) of the 115 patients, urinary and plasma 5-HIAA were in agreement when 10 mg/24 hours was used as the upper normal limit for urinary 5-HIAA. In 86 (75%) of the 115 patients, urinary and plasma 5-HIAA were in agreement when 15 mg/24 hours was used as the upper normal limit for urinary 5-HIAA. The correlation between the urinary and plasma 5-HIAA values for the entire group of 115 patients (regardless of urinary 5-HIAA cutoff value) was statistically significant ($P < 0.0001$), with an r^2 value of 0.7466 (Fig. 2A).

The correlation of the 2 assay results for the 72 patients with small bowel primaries was also assessed. Again, the correlation between urinary 5-HIAA and plasma 5-HIAA was significant ($P < 0.0001$), with an r^2 value of 0.7982 (Fig. 2B).

Lastly, the correlation between urinary and plasma 5-HIAA for the 47 patients with small bowel primaries and liver metastasis was assessed. The P value for the correlation between urinary 5-HIAA and plasma 5-HIAA values was significant ($P < 0.0001$), with an r^2 value of 0.7747 (Fig. 2C; Table 1).

Urinary 5-HIAA and Plasma 5-HIAA Versus Other Biomarkers

For patients with small bowel primaries, their urinary and plasma 5-HIAA values were compared to their plasma NKA value within the same time period (n = 71). A patient with an elevated urinary 5-HIAA (>6 mg/24 hours) had an elevated NKA 14% of the time, whereas a patient with an elevated plasma 5-HIAA had an elevated NKA 13% of the time (Table 2).

For patients with small bowel primaries, their urinary and plasma 5-HIAA values were compared to their plasma pancreastatin values from within the same time period (n = 71). A patient with an elevated urinary 5-HIAA (>6 mg/24 hours) had an elevated pancreastatin 47% of the time, whereas a patient with an elevated plasma 5-HIAA had an elevated pancreastatin 53% of the time (Table 3).

In patients with small bowel primaries, their urinary and plasma 5-HIAA values were compared to their plasma CGA values from within the same time period. Chromogranin A values were not available for one patient (n = 70). A patient with an elevated urinary 5-HIAA (>6 mg/24 hours) had an elevated CGA 42% of the time, and a patient with an elevated plasma 5-HIAA had an elevated CGA 41% of the time (Table 4).

For patients with small bowel primaries, their urinary and plasma 5-HIAA values were compared to their plasma serotonin values from within the same time period. Serotonin values were not available for 11 patients (n = 61). A patient with an elevated urinary 5-HIAA (>6 mg/24 hours) had an elevated serotonin 44% of the time, whereas a patient with an elevated plasma 5-HIAA had an elevated serotonin 48% of the time (Table 5).

DISCUSSION

Neuroendocrine tumors are generally not diagnosed until they are at an advanced stage.[2] Neuroendocrine tumors, especially metastatic midgut NETs, have the ability to secrete serotonin. The

TABLE 2. Comparison of the Percent of Patients With Normal and Elevated NKA Values, Compared to Urinary (<6 mg/24 Hours, Normal) or Plasma 5-HIAA Values

		Neurokinin A		
		Normal	Elevated	
Urinary 5-HIAA	Normal	25 (34%)	0 (0%)	34.7%
	Elevated	37 (51%)	10 (14%)	65.3%
		86.1%	13.9%	
Plasma 5-HIAA	Normal	27 (38%)	1 (1%)	38.9%
	Elevated	35 (49%)	9 (13%)	61.1%
		86.1%	13.9%	

Small bowel primaries only (n = 72).

TABLE 3. Comparison of the Percent of Patients With Normal and Elevated Pancreastatin Values, Compared to Urinary (<6 mg/24 Hours, Normal) or Plasma 5-HIAA Values

		Pancreastatin		
		Normal	Elevated	
Urinary 5-HIAA	Normal	17 (24%)	8 (11%)	34.7%
	Elevated	13 (18%)	34 (47%)	65.3%
		41.7%	58.3%	
Plasma 5-HIAA	Normal	24 (33%)	4 (6%)	38.9%
	Elevated	6 (8%)	38 (53%)	61.1%
		41.7%	58.3%	

Small bowel primaries only (n = 72).

24-hour urinary concentration of the degradation product of serotonin, 5-HIAA, is widely used as a critical biomarker for the diagnosis and follow-up of these tumors.[3] The collection of a 24-hour urine specimen is inconvenient and, for most individuals, embarrassing, especially if they have to collect and store the urine container at work. Therefore, a different way to measure 5-HIAA is desirable and would be beneficial to both patients and clinicians.

Previously, other groups have successfully measured 5-HIAA in plasma and found it to be a reliable method that gives results proportional to urinary 5-HIAA values. However, these studies were small and performed in groups of patients with different tumor types, and ultimately, they did not produce a feasible or a commercially viable plasma 5-HIAA test.[7–9] To test the equivalence of the our 5 plasma 5-HIAA assay to the widely used urinary 5-HIAA assay, we performed regression analysis of the two assays in an overall group of 115 individuals, in a subset of 72 individuals with all stages of midgut carcinoids and finally, in a group of 47 patients with midgut NETs and distant metastatic disease to the liver. All of these yielded statistically significant correlations ($P < 0.001$, $P < 0.001$, and $P < 0.001$, respectively). We feel this is compelling evidence that these assays are equivalent.

This study demonstrates that the results of simultaneous urinary and plasma 5-HIAA assays from the same individual are proportional. However, the clinical implications of a normal or abnormal marker value also has to be viewed in context of the other biomarkers used to diagnose the disease in new patients or to follow existing patients who have the potential for tumor persistence or recurrence after therapy. The choice of markers useful for diagnosis and follow-up of NETs are often based on recommendations from multiple national and international groups such as the North American Neuroendocrine Tumor Society and the European Neuroendocrine Tumor Society.[12–17] To determine the clinical relevance of the plasma and urinary 5-HIAA values, we compared the proportion of individuals with normal or abnormal urinary or plasma 5-HIAA values to the proportion of patients with normal or abnormal levels of NKA, pancreastatin, CGA, and serotonin. The recent guidelines published by the European Neuroendocrine Tumor Society states that CGA and 5-HIAA are the minimally necessary biochemical tests useful for diagnosis and long-term follow-up of midgut NETs.[17] Based on the information gained from our comparison of traditional markers to urinary and plasma 5-HIAA levels, we believe that the two have equivalent clinical relevance. A prospective study of serially measured biomarker levels in patients with midgut NET is needed to determine the relative sensitivity and specificity of each marker: to detect primary tumors, to detect early-stage persistence or recurrence, or to monitor the progression of metastatic disease over time.

TABLE 4. Comparison of the Percent of Patients With Normal and Elevated CGA Values, Compared to Urinary (<6 mg/24 Hours, Normal) or Plasma 5-HIAA Values

		Chromogranin A		
		Normal	**Elevated**	
Urinary 5-HIAA	Normal	23 (32%)	2 (3%)	35.2%
	Elevated	16 (23%)	30 (42%)	64.8%
		54.9%	45.1%	
Plasma 5-HIAA	Normal	25 (35%)	3 (4%)	39.4%
	Elevated	14 (20%)	29 (41%)	60.6%
		54.9%	45.1%	

Small bowel primaries only. Note that CGA assayed by different methods and the values were normalized. A CGA value was not available on one patient (n = 71).

TABLE 5. Comparison of the Percent of Patients With Normal and Elevated Serotonin Values, Compared to Urinary (<6 mg/24 Hours, Normal) or Plasma 5-HIAA Values

		Serotonin		
		Normal	**Elevated**	
Urinary 5-HIAA	Normal	15 (25%)	8 (13%)	37.7%
	Elevated	11 (18%)	27 (44%)	62.3%
		42.6%	57.4%	
Plasma 5-HIAA	Normal	17 (28%)	6 (10%)	37.7%
	Elevated	9 (15%)	29 (48%)	62.3%
		42.6%	57.4%	

Small bowel primaries only. Serotonin assayed by different methods and the values were normalized. Serotonin values were not available in 11 patients (n = 61).

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REFERENCES

1. Öberg KE. Management of neuroendocrine tumors: current and future therapies. *Expert Rev Endocrinol Metab.* 2011;6:49–62.
2. Ahmed A, Turner G, King B, et al. Midgut neuroendocrine tumours with liver metastases: results of the UKINETS study. *Endocr Relat Cancer.* 2009;16:885–894.
3. Zuetenhorst JM, Korse CM, Bonfrer JMG, et al. Daily cyclic changes in urinary excretion of 5-hydroxyindoleacetic acid in patients with carcinoid tumors. *Clin Chem*. 2004;50:1634–1639.
4. Mamikunian G, Vinik AI, O'Dorisio TM, et al. *Neuroendocrine Tumors: A Comprehensive Guide to Diagnosis and Management*. Inglewood, CA: Inter Science Institute; 2009.
5. Feldman JM, Jones RS. Carcinoid syndrome from gastrointestinal carcinoids without liver metastasis. *Ann Surg*. 1982;196:33–37.
6. Norheim I, Oberg K, Theodorsson-Norheim E, et al. Malignant carcinoid tumors: an analysis of 103 patients with regard to tumor localization, hormone production, and survival. *Ann Surg*. 1987;206:115–125.
7. Cai HL, Zhu RH, Li HD. Determination of dansylated monoamine and amino acid neurotransmitters and their metabolites in human plasma by liquid chromatography-electrospray ionization tandem mass spectrometry. *Anal Biochem*. 2010;396:103–111.
8. Degg TJ, Allen KR, Barth JH. Measurement of plasma 5-hydroxyindoleacetic acid in carcinoid disease: an alternative to 24-h urine collections? *Ann Clin Biochem*. 2000;37:724–726.
9. Miller AG, Brown H, Degg T, et al. Measurement of plasma 5-hydroxyindoleacetic acid by liquid chromatography tandem mass spectrometry—comparison with HPLC methodology. *J Chromatogr B*. 2010;878:695–699.
10. Thomas DP, Vane IR. 5-Hydroxytryptamine in the circulation of the dog. *Nature*. 1967;216:335–338.

11. Anderson GM, Stevenson JM, Cohen DJ. Steady-state model for plasma free and platelet serotonin in man. *Life Sci*. 1987;41:1777–1785.
12. Vinik AI, Silva MP, Woltering G, et al. Biochemical testing for neuroendocrine tumors. *Pancreas*. 2009;38:876–889.
13. O'Dorisio T, Krutzik SR, Woltering EA, et al. Development of a highly sensitive and specific carboxy-terminal human pancreastatin assay to monitor neuroendocrine tumor behavior. *Pancreas*. 2010;39:611–616.
14. Woltering EA, Hilton RS, Zolfoghary CM, et al. Validation of serum versus plasma measurements of chromogranin a levels in patients with carcinoid tumors. *Pancreas*. 2006;33:250–254.
15. Mamikunian P, Ardill JE, O'Dorisio TM, et al. Validation of neurokinin A assays in the United States and Europe. *Pancreas*. 2011;40:1000–1005.
16. Boudreaux JP, Klimstra DS, Hassan MM, et al. The NANETS consensus guidelines for the diagnosis and management of Neuroendocrine Tumors. *Pancreas*. 2010;39:753–766.
17. Pape UF, Perren A, Niderle B, et al. ENETS consensus guidelines for the management of patients with neuroendocrine neoplasms from the jejuno-ileum and he appendix including goble cell carcinomas. *Neuroendocrinology.* 2012;95:135–156.

