Limited value for urinary 5-HIAA excretion as prognostic marker in gastrointestinal neuroendocrine tumors

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ABSTRACT

Introduction: To determine the prognostic value for overall survival (OS) of urinary 5-hydroxyindoleacetic acid (5-HIAA) excretion in patients with a gastrointestinal neuroendocrine tumor (NET) and to compare prognostic value with patient characteristics, ENETS/WHO grading, ENETS TNM staging and biomarkers.

Methods: Data collection from patients with a gastrointestinal NET or a NET with gastrointestinal metastases and available 5-HIAA excretion in 24 hour urine samples. Laboratory results were stratified for urinary 5-HIAA and chromogranin A (CgA): <2x upper limit of normal (ULN), 2-10x ULN, or >10x ULN. For neuron-specific enolase (NSE) this was reference range or >1x ULN. OS was compared using a Kaplan Meier with Log Rank test and hazard ratios were calculated using Cox-regression for univariate and multivariate analyses.

Results: 371 patients were included, 46.6% female, mean age 59.9 year. OS was shortest in patients with urinary 5-HIAA excretion >10x ULN vs reference range (median 83 months vs 141 months, p=0.002). In univariate analysis, urinary 5-HIAA excretion >10x ULN was a negative predictor (HR 1.62, 95% CI: 1.09-2.39). However in multivariate analysis only age (HR 1.04, 95% CI: 1.01-1.08) grade 3 disease (HR 5.09, 95% CI: 2.20-11.79), NSE >1xULN (HR 2.36, 95% CI: 1.34-4.14) and CgA >10xULN (HR 3.61, 95% CI: 1.56-8.34) remained predictors.

Conclusion: Urinary 5-HIAA excretion >10x ULN is a negative predictor for OS. However when added to other biomarkers and grading it is no longer a predictor for OS. It should therefore only be determined to assess carcinoid syndrome and not for prognostic value.

INTRODUCTION

While neuroendocrine tumors (NETs) are rare, they are well known for their production and secretion of polypeptide hormones which results in distinct clinical syndromes. NETs from midgut origin most often produce serotonin causing carcinoid syndrome with flushing and diarrhea, but also carcinoid heart disease which can result in right-sided heart failure.[1] Serotonin levels may vary over the day and therefore its metabolite 5-hydroxyindoleacetic acid (5-HIAA) is usually measured in 24-hour urine samples. The urinary 5-HIAA excretion has been shown to be a valuable tool for the diagnosis of small intestinal NETs (mainly in the presence of liver metastases) and it correlates with severity of heart disease in the carcinoid syndrome.[2,3] In the follow-up of patients with midgut NETs, urinary 5-HIAA excretion is usually determined in combination with chromogranin A (CgA) and neuron-specific enolase (NSE) to assess the status of the disease, especially in the so-called functioning (hypersecreting or syndromic) NETs.[3] Plasma 5-HIAA may become available in the near future, as it seems to be a good predictor of carcinoid heart disease, but it is at present hardly validated as prognostic marker in the follow-up of NETs.[4-6]

CgA is secreted by most NETs, including gastroenteropancreatic (GEP) NETs, pheochromocytomas and lung carcinoids and both functioning and non-functioning NETs.[7] It reflects tumor burden and it has been demonstrated to be an important marker for the diagnosis and prognosis in patients with GEP-NETs.[8] In several studies a high level of serum CgA has been shown to be an unfavorable factor for survival and, therefore, should be routinely measured during follow-up.[9-14] NSE is the third circulating tumor marker which can be used in the follow-up of NETs and is usually elevated in patients with poorly differentiated NETs and small-cell lung cancer. For the diagnosis of NETs, serum NSE is less specific, but in addition to serum CgA, sensitivity can increase and it as well can predict survival.[15,16]

However discussion remains on the usefulness of the urinary 5-HIAA excretion (and maybe plasma 5-HIAA levels) as a prognostic factor for survival in GEP-NET and lung NET patients, as it seems to be inferior to serum chromogranin A and only a few publications report on its prognostic value.[3] Elevated urinary 5-HIAA excretion was a negative predictor of survival in a study by Tiensuu-Janson and co-workers, but was no longer a predictor when compared with serum CgA.[17] Additional studies confirmed the predictive value of the urinary 5-HIAA excretion but it was not compared with serum CgA and serum NSE, as especially serum CgA is nowadays routinely used in the follow-up of NETs.[18-22]

In this retrospective study, our aim was to determine once more if the urinary 5-HIAA excretion is a prognostic marker for overall survival in patients with gastrointestinal NETs and to compare it with serum CgA, and serum NSE in combination with ENETS TNM staging, ENETS/WHO grading and other patient or tumor characteristics as nowadays routinely used in clinical practice.

METHODS

Patients

All records of patients who were treated for a NET between 1993 and 2012 in the ENETS Centre of Excellence for Neuroendocrine Tumors, Erasmus MC, Rotterdam, the Netherlands, were analyzed. Patients were included if they had a gastrointestinal NET with liver metastases and at least one 24 hour urine sample of 5-HIAA was available at the time of diagnosis or referral to our center. Patients with liver metastases from unknown primary origins were also included when they presented with a mesenterial lymph node deposit typical for midgut tumors or (functional) imaging showed no evidence of a lung, pancreas, kidney or ovarium NET. Patients diagnosed with the multiple endocrine neoplasia (MEN) type1, Von Hippel Lindau disease (VHL) were excluded.

Patient characteristics, pathology data, laboratory results and imaging findings were all recorded. Furthermore data on treatment modalities during follow-up were recorded, date of last visit or, if applicable, date of death. NETs were diagnosed on basis of a combination of markers, imaging and histology (including synaptophysin and chromogranin staining) according to current guidelines.[9,23]

Tumor markers

5-HIAA was determined in 24-hour urine samples and measured using the reversed-phase high-performance liquid chromatography (HPLC) with fluorimetric detection. The reference range is below 50µmol per 24 hours.[24] If available two 24 hour samples were used and the average was calculated, but if only one sample was available this was reported.

CgA in serum was measured using a solid-phase, two-site IRMA assay (Cisbio Bioassays) with an upper limit of normal (ULN) of 94µg/L. NSE in serum was measured using an electrochemoluminescence immunoassay on an immunoassay analyzer (Roche Diagnostics) and has an ULN of 16.2µg/L.

Results were stratified for levels of 24-hour urinary 5-HIAA excretion, serum CgA and serum NSE. Because slight elevation of 24-hour urinary 5-HIAA excretion and serum CgA is often due to interference or dietary incompliance, reference range was defined as values being below two times ULN. Furthermore urinary 5-HIAA excretion and serum CgA was stratified as, high (2-10x ULN) and very high (>10x ULN).

Patients with a 5-HIAA excretion below 10 µmol per 24 hours were excluded because of probable inappropriate 24-hour sampling.

Serum NSE was stratified for being either high (above ULN) or normal (within reference range).

Statistical analysis

All markers were recorded at diagnosis or referral to our center, to determine the prognostic value for survival. Primary outcome was overall survival and hazard-ratios of possible predictors of survival in univariate and multivariate analyses.

Differences between groups on baseline were tested with Chi-square for categorical data and with a one-way ANOVA for continuous data. Overall survival was analyzed with the Kaplan-Meier method, with Log Rank testing to determine significant differences between the mortality in the groups according to the range of 5-HIAA. Hazard ratios were calculated using a Cox-regression analysis. Both univariate and multivariate analyses were performed. A p-value of <0.05 was considered statistically significant.

Calculations were performed using SPSS for Windows software, (version 23.0, SPSS Inc.)

RESULTS

In total 374 patients were identified with a gastrointestinal NET or NET from unknown origin, with liver metastases and available 24 hours 5-HIAA urine samples. Three patients were excluded for having 5-HIAAs below 10µmol/L leaving 371 patients available for analyses. Of these patients, 84.6% had provided two urine samples at first visit. All patients were included in the baseline and univariate analyses, but 171 patients had one or more missing values and were excluded from the multivariate analysis.

Baseline clinical characteristics are shown in Table 1. Patients had an average age of 59.9 ± 10.5 years and 46.6% were female. Patients mostly presented with a small intestinal NET or only with a mesenterial node metastasis and with grade 1 or 2 tumors. 47 patients (12.7%) presented with liver metastases with unknown primary tumor. At referral 136 (36.7%) patients were already using a somatostatin analogue. Unfortunately data on tumor grade were only available in 59.5% of patients.

Table 1: Overall baseline characteristics (n=371)

Female, *n* (%)	173 (46.6)
Age (years ± SD)	59.9 ± 10.5
Primary Tumor, *n* (%)	
Small Intestine	195 (52.5)
Ileocecal	34 (9.2)
Colon	36 (9.7)
Appendix	6 (1.6)
Mesenterial Node	53 (14.3)
Unknown	47 (12.7)
Grade, *n* (%)	
Grade 1 (Ki67 ≤ 2%)	108 (29.1)
Grade 2 (Ki67 3-20%)	98 (26.4)
Grade 3 (Ki67 >20%)	11 (3.0)
Missing	154 (41.5)
Use of Somatostatin-analogue, *n* (%)	136 (36.6%)

Patients with very high 24-hour urinary 5-HIAA excretion had the highest incidence of somatostatin analogue use (p=0.002) and the highest serum NSE and CgA levels, however this was not significant. (Table 2) During follow-up 27 patients (23.9%) who had a normal 24-h urinary 5-HIAA excretion at baseline developed elevated 5-HIAA excretion during follow-up, mainly between 2-10x ULN.

Table 2: Baseline characteristics of groups based on 5-HIAA values

	Ref Range (n=165)	2-10x ULN (n=159)	>10xULN (n=119)	P-value
Age	58.3 ± 11.7	60.9 ± 10.6	60.4 ± 8.9	0.11
CgA	5210.8 ± 34109	2321.7 ± 13784	7778.4 ± 18096	0.17
NSE	28.0 ± 87.8	19.5 ± 37.6	40.3 ± 117.0	0.19
Use of SSA	28 (24.8%)	54 (37.2%)	54 (47.8%)	0.002

Numerical data are mean ± SD.
Differences were tested with X^2 for categorical data and with ANOVA for numerical data.
ULN: Upper Limit of Normal, CgA: Chromogranin A,
NSE: Neuron-specific enolase
SSA: somatostatin-analogue

Overall survival

Overall 159 patients died during a median follow-up of 115 months. When stratified for the 24-hour urinary 5-HIAA excretion, overall survival was shortest in patients with very high 24-hour urinary 5-HIAA excretion and longest in patients with normal 5-HIAA excretion (Figure 1, p=0.03). Median survival varied from 141 months in the group with normal 24-hour urinary 5-HIAA excretion and 83 months in the group with very high 24-hour urinary 5-HIAA excretion, corresponding with five year survivals of respectively 74.0% and 63.0%.

Prognostic factors

In univariate analysis very high 24-hour urinary 5-HIAA excretion was associated with a shorter survival when compared to the reference range with hazard ratios of 1.09 (95% CI: 0.73-1.63) for high 5-HIAA and 1.62 (95% CI: 1.09-2.39) for very high 5-HIAA (Table 3). However in a multivariate analysis 24-hour urinary 5-HIAA excretion is no longer a prognostic marker in this population, with hazard ratios of 0.76 (95% CI: 0.45-1.88) and 0.92 (95% CI: 056-1.61) for high and very high 5-HIAA (Table 4). Sex was also not associated with survival, but age at diagnosis, serum CgA and NSE were negative predictors of survival. Furthermore, grade 3 tumors were associated with the highest hazard ratio of 5.09 (95% CI: 2.20-11.79). Adding baseline use of a somatostatin analogue to the multivariate analysis did not significantly change the model.

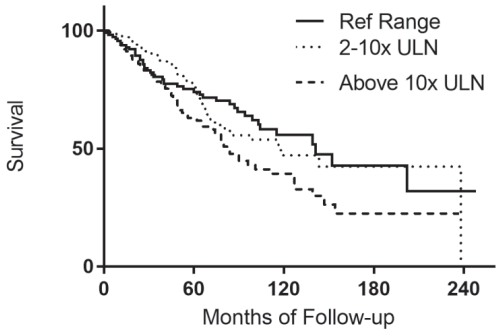

Figure 1: Overall survival curves stratified for urinary 5-HIAA excretion
Kaplan Meier estimate of overall survival for patients with a gastrointestinal NET, stratified for urinary 5-HIAA excretion. Curves were compared with a log-rank test (p=0.002)

Table 3: Univariate analysis of 5-HIAA

	Hazard ratio (95% CI)
Reference range (<100µmol/24h)	1.0 (reference)
2-10x ULN (100-500µmol/24h)	1.09 (0.73-1.63)
>10x ULN (>500µmol/24h)	1.62 (1.09-2.39)

ULN: Upper Limit of Normal, 5-HIAA: 5-hydroxyindoleacetic acid

Table 4: Multivariate analysis of prognostic factors (n=200)

	Hazard ratio (95% CI)
Female	0.95 (0.56-1.61)
Age	1.04 (1.01-1.08)
Grade	
Grade 1	*Reference*
Grade 2	1.40 (0.79-3.34)
Grade 3	5.09 (2.20-11.79)
5-HIAA	
Reference Range (100µmol/24h)	*Reference*
2-10x ULN (100-500µmol/24h)	0.76 (0.45-1.88)
>10x ULN (>500µmol/24h)	0.92 (0.56-1.61)
Chromogranin A	
Reference Range (<188µg/L)	*Reference*
2-10x ULN (188-940µg/L)	1.59 (0.75-3.34)
>10x ULN (>940µg/L)	3.61 (1.56-8.34)
NSE above ULN (>16.2µg/L)	2.36 (1.34-4.14)

ULN: Upper Limit of Normal, 5-HIAA: 5-hydroxyindoleacetic acid, NSE: Neuron-specific enolase

DISCUSSION

Current ENETS guidelines are inconclusive on the value of the 24-hour urinary 5-HIAA excretion to determine prognosis in patients with GEP-NETs.[3] While serum CgA and NSE are widely reported as predictors for survival, few studies show any additional value for urinary 5-HIAA excretion.[17-22] 24-hour urinary 5-HIAA excretion was studied as a predictor for survival in our study. We were able to predict survival stratified for urinary 5-HIAA excretion with the Kaplan-Meier method and very high 24-hour urinary 5-HIAA excretion was identified as a negative predictor for survival in patients with a midgut NET. However in multivariate analysis other tumor markers (serum CgA and serum NSE) and tumor grade were far more powerful predictors and the 24-hour urinary 5-HIAA excretion was no longer a significant predictor in this multivariate analysis. Therefore, 24-hour urinary 5-HIAA excretion seems to have no additional value for predicting prognosis in follow-up when serum CgA and serum NSE are already used.

A limited number of other studies in GEP-NET patients have shown that elevated 24-hour urinary 5-HIAA excretion is associated with a shorter survival, but these studies did not compare the urinary 5-HIAA excretion with serum CgA or NSE.[18-22] Only Janson and colleagues have compared urinary 5-HIAA excretion with serum CgA in GEP-NET patients using a multivariate analysis and demonstrated, as in our study, that urinary 5-HIAA excretion had no additional value for the prognosis of patients with GEP-NETs when other contemporary biomarkers are used.[17] We confirmed these results, but additionally using serum NSE, in combination with staging and grading.

The use of 24-hour urinary 5-HIAA excretion in the follow-up of GEP-NET patients can, on the other hand, not completely be abolished. The determination of plasma or 24-hour urinary 5-HIAA excretion has been shown to be positively correlated with the severity of the carcinoid syndrome.[6,25] However, simple history taking from patients should suffice during follow-up and, moreover, current clinical trials prefer questionnaires to determine flushing episodes and diarrhea frequency and volumes over this biochemical marker. Its value, therefore, lies mainly in its predictive value for carcinoid heart disease. In this context it should still be measured at diagnosis and at follow-up.[2,6,25] Current ENETS guidelines recommend annual cardiac screening in patients with the carcinoid syndrome, but in clinical practice there is diversity in local screening protocols for carcinoid heart disease.[9,26] It is currently unclear if one urinary sample with non-elevated urinary 5-HIAA excretion values at baseline is sufficient to rule out the development of carcinoid heart disease. Also it is not determined how often during follow-up re-evaluation of the urinary 5-HIAA excretion should be undertaken for a timely decision for further cardiac testing. In this respect the measurement of plasma NT-proBNP levels (maybe including serum CgA and plasma 5-HIAA) might be more relevant also.[6,27] In our study cohort, 23.9% of patients with normal 5-HIAA at baseline had elevated 5-HIAA at follow-up, showing that one negative screening at baseline isn't sufficient

to rule out serotonin production at follow-up and therefore minimizing the risk of carcinoid heart disease on the basis of 24-hour urinary 5-HIAA excretion.

Major limitation of our study is the non-protocolled, retrospective design of this study. Not all patients included had supplied two urine samples, but in the light of recent studies and our own unpublished observations, this is probably not a limitation as even shorter collection periods also seem to be reliable.[28,29] For the multivariate analysis a large number of patients had to be excluded, mainly due to missing data on Ki67 staining of tumor samples. However since this showed to be one of the most powerful predictors of survival we decided to accept this, but this results in a selection of more recently diagnosed patients, because the MIB-1 staining of tumor samples was introduced around 2010.

In conclusion, the determination of 24-hour urinary 5-HIAA excretion is inferior to other available serum biomarkers for predicting survival in patients with gastrointestinal NETs. Serum CgA and NSE have a higher predictive value and there is no need for dietary restriction or 24-hour urine collections. Urinary 5-HIAA might still be important for determining the potential risk for the development of carcinoid heart disease. However their might be an important role for NT-proBNP, possibly in combination with plasma 5-HIAA and serum CgA, in predicting carcinoid heart disease. For this indication, it is still unclear whether a negative screening for urinary 5-HIAA excretion is sufficient, or that repeated 5-HIAA is required during follow-up for the early determination of the carcinoid heart disease risk.

REFERENCES

1. de Herder WW. Biochemistry of neuroendocrine tumours. *Best Pract Res Clin Endocrinol Metab* 2007; **21**(1): 33-41.
2. Zuetenhorst JM, Bonfrer JM, Korse CM, Bakker R, van Tinteren H, Taal BG. Carcinoid heart disease: the role of urinary 5-hydroxyindoleacetic acid excretion and plasma levels of atrial natriuretic peptide, transforming growth factor-beta and fibroblast growth factor. *Cancer* 2003; **97**(7): 1609-15.
3. O'Toole D, Grossman A, Gross D, et al. ENETS Consensus Guidelines for the Standards of Care in Neuroendocrine Tumors: biochemical markers. *Neuroendocrinology* 2009; **90**(2): 194-202.
4. Tellez MR, Mamikunian G, O'Dorisio TM, Vinik AI, Woltering EA. A single fasting plasma 5-HIAA value correlates with 24-hour urinary 5-HIAA values and other biomarkers in midgut neuroendocrine tumors (NETs). *Pancreas* 2013; **42**(3): 405-10.
5. Adaway JE, Dobson R, Walsh J, et al. Serum and plasma 5-hydroxyindoleacetic acid as an alternative to 24-h urine 5-hydroxyindoleacetic acid measurement. *Ann Clin Biochem* 2015.
6. Dobson R, Burgess MI, Banks M, et al. The association of a panel of biomarkers with the presence and severity of carcinoid heart disease: a cross-sectional study. *PLoS One* 2013; **8**(9): e73679.
7. Taupenot L, Harper KL, O'Connor DT. The chromogranin-secretogranin family. *N Engl J Med* 2003; **348**(12): 1134-49.
8. Modlin IM, Gustafsson BI, Moss SF, Pavel M, Tsolakis AV, Kidd M. Chromogranin A--biological function and clinical utility in neuro endocrine tumor disease. *Ann Surg Oncol* 2010; **17**(9): 2427-43.
9. Pape UF, Perren A, Niederle B, et al. ENETS Consensus Guidelines for the management of patients with neuroendocrine neoplasms from the jejuno-ileum and the appendix including goblet cell carcinomas. *Neuroendocrinology* 2012; **95**(2): 135-56.
10. Rossi RE, Garcia-Hernandez J, Meyer T, et al. Chromogranin A as a predictor of radiological disease progression in neuroendocrine tumours. *Ann Transl Med* 2015; **3**(9): 118.
11. Han X, Zhang C, Tang M, et al. The value of serum chromogranin A as a predictor of tumor burden, therapeutic response, and nomogram-based survival in well-moderate nonfunctional pancreatic neuroendocrine tumors with liver metastases. *Eur J Gastroenterol Hepatol* 2015; **27**(5): 527-35.
12. Yao JC, Hassan M, Phan A, et al. One hundred years after "carcinoid": epidemiology of and prognostic factors for neuroendocrine tumors in 35,825 cases in the United States. *J Clin Oncol* 2008; **26**(18): 3063-72.
13. Seregni E, Ferrari L, Bajetta E, Martinetti A, Bombardieri E. Clinical significance of blood chromogranin A measurement in neuroendocrine tumours. *Ann Oncol* 2001; **12 Suppl 2**: S69-72.
14. Arnold R, Wilke A, Rinke A, et al. Plasma chromogranin A as marker for survival in patients with metastatic endocrine gastroenteropancreatic tumors. *Clin Gastroenterol Hepatol* 2008; **6**(7): 820-7.
15. Oberg K. Circulating biomarkers in gastroenteropancreatic neuroendocrine tumours. *Endocr Relat Cancer* 2011; **18 Suppl 1**: S17-25.
16. van Adrichem RC, Kamp K, Vandamme T, Peeters M, Feelders RA, de Herder WW. Serum neuron-specific enolase level is an independent predictor of overall survival in patients with gastroenteropancreatic neuroendocrine tumors. *Ann Oncol* 2015.
17. Janson ET, Holmberg L, Stridsberg M, et al. Carcinoid tumors: analysis of prognostic factors and survival in 301 patients from a referral center. *Ann Oncol* 1997; **8**(7): 685-90.
18. Turner GB, Johnston BT, McCance DR, et al. Circulating markers of prognosis and response to treatment in patients with midgut carcinoid tumours. *Gut* 2006; **55**(11): 1586-91.

19. van der Horst-Schrivers AN, Post WJ, Kema IP, et al. Persistent low urinary excretion of 5-HIAA is a marker for favourable survival during follow-up in patients with disseminated midgut carcinoid tumours. *Eur J Cancer* 2007; **43**(18): 2651-7.
20. Formica V, Wotherspoon A, Cunningham D, et al. The prognostic role of WHO classification, urinary 5-hydroxyindoleacetic acid and liver function tests in metastatic neuroendocrine carcinomas of the gastroenteropancreatic tract. *Br J Cancer* 2007; **96**(8): 1178-82.
21. Agranovich AL, Anderson GH, Manji M, Acker BD, Macdonald WC, Threlfall WJ. Carcinoid tumour of the gastrointestinal tract: prognostic factors and disease outcome. *J Surg Oncol* 1991; **47**(1): 45-52.
22. Hellman P, Lundstrom T, Ohrvall U, et al. Effect of surgery on the outcome of midgut carcinoid disease with lymph node and liver metastases. *World J Surg* 2002; **26**(8): 991-7.
23. Falconi M, Bartsch DK, Eriksson B, et al. ENETS Consensus Guidelines for the management of patients with digestive neuroendocrine neoplasms of the digestive system: well-differentiated pancreatic non-functioning tumors. *Neuroendocrinology* 2012; **95**(2): 120-34.
24. van Haard PM. Chromatography of urinary indole derivatives. *J Chromatogr* 1988; **429**: 59-94.
25. Bhattacharyya S, Toumpanakis C, Chilkunda D, Caplin ME, Davar J. Risk factors for the development and progression of carcinoid heart disease. *Am J Cardiol* 2011; **107**(8): 1221-6.
26. Dobson R, Valle JW, Burgess MI, Poston GJ, Cuthbertson DJ. Variation in Cardiac Screening and Management of Carcinoid Heart Disease in the UK and Republic of Ireland. *Clin Oncol (R Coll Radiol)* 2015; **27**(12): 741-6.
27. Korse CM, Taal BG, de Groot CA, Bakker RH, Bonfrer JM. Chromogranin-A and N-terminal pro-brain natriuretic peptide: an excellent pair of biomarkers for diagnostics in patients with neuroendocrine tumor. *J Clin Oncol* 2009; **27**(26): 4293-9.
28. Gedde-Dahl M, Thiis-Evensen E, Tjolsen AM, Mordal KS, Vatn M, Bergestuen DS. Comparison of 24-h and overnight samples of urinary 5-hydroxyindoleacetic acid in patients with intestinal neuroendocrine tumors. *Endocr Connect* 2013; **2**(1): 50-4.
29. Zuetenhorst JM, Korse CM, Bonfrer JM, Peter E, Lamers CB, Taal BG. Daily cyclic changes in the urinary excretion of 5-hydroxyindoleacetic acid in patients with carcinoid tumors. *Clin Chem* 2004; **50**(9): 1634-9.