Daily Cyclic Changes in the Urinary Excretion of 5-Hydroxyindoleacetic Acid in Patients with Carcinoid Tumors

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Background: Vasoactive peptides produced by neuroendocrine tumors can induce characteristic symptoms of the carcinoid syndrome (flushing, diarrhea, and wheezing). To what extent external factors provoke these symptoms and how 5-hydroxyindoleacetic acid (5-HIAA) excretion (the degradation product of serotonin) varies throughout the day remain unknown. In this study, we investigated whether symptoms and daily activity are related to 5-HIAA excretion and whether 24-h urine collection is needed.

Methods: In 26 patients with metastatic carcinoid (14 men and 12 women; median age, 60 years) urine was collected in portions of 4 or 8 h during 2 days. Patients were asked to keep a diary in which they noted symptoms of flushes, consistency of stools, activities, and food intake.

Results: Excretion of 5-HIAA in 24-h urine was increased in 88% of the patients (median, 515 μmol/24 h). Overnight-collected urine appeared the most representative for 24-h collection concentrations (correlation coefficient 0.85). We found no clear correlation between symptoms of the carcinoid syndrome and degree of activity. Watery diarrhea was reported only by patients with strong variations in 5-HIAA excretion. One-half of the patients (n = 16) exhibited a high variability in urinary 5-HIAA excretion throughout the day, with increased concentrations most prominent in morning collections (P = 0.0074) and lower concentrations in the evening (P = 0.0034). In the other patients these curves were flat.

Conclusions: Cyclic changes in patients relate to high variability in 5-HIAA excretion. Overnight collected urine can replace the 24-h urine collection, and strong variations in 5-HIAA excretion seem to be associated with severity of diarrhea.

Carcinoid tumors (well-differentiated neuroendocrine tumors) are derived from the enterochromafin cells and predominantly originate from the gastrointestinal tract and bronchi. Carcinoids are rare and present with distant metastases in 22% (1). These tumors have the ability to secrete vasoactive peptides, especially serotonin, which induce the characteristic symptoms of flushing and diarrhea in patients with liver metastases. Serotonin plays a key role in the development of fibrosis of the tricuspid heart valve (2). Twenty-four-hour concentrations of urinary 5-hydroxyindoleacetic acid (5-HIAA), the degradation product of serotonin, is the cornerstone in diagnosis and follow-up. Although the metabolism of serotonin is regulated by rapid clearance (3–6), urinary 5-HIAA excretion is generally measured in a 24-h collection (7, 8), which is troublesome for the patient and can be unreliable in case of severe diarrhea. It would be beneficial if an urine sample collected during a shorter interval reflects disease activity. It is our clinical impression that symptoms of the carcinoid syndrome are exacerbated by physical efforts, food intake, or starting the day. Information about influences of these factors on hormonal excretion is scarce, and clear-cut correlation between the severity of symptoms and the hormone excretion are not convincing (9–12).

In this study we assessed whether a 4-h urine sample is adequate compared with the traditional 24-h collection and whether cyclic changes in 5-HIAA excretion are...
present. We investigated the relationship between symptoms of the carcinoid syndrome and daily activity on 5-HIAA excretion during 4- and 8-h intervals. In addition, we compared different assays for determination of urinary 5-HIAA excretion. The results of this study might have implications for urine collection instructions and optimum timing of medical treatment during the day.

**Patients and Methods**

**Patients**
Between July 2001 and April 2002, a total of 28 consecutive patients with metastatic well-differentiated neuroendocrine tumors (carcinoid) who visited the outpatient department of The Netherlands Cancer Institute/Antoni van Leeuwenhoek Hospital participated in the study (16 men and 12 women; median age, 60 years; range, 47–74 years). The interval between the diagnosis of metastatic carcinoid and urine collection was 19 months (range, 5–121 months). Two patients were excluded from the analysis because urine collection failed. Written informed consent was obtained from all participants. Advice to avoid serotonin-rich food such as bananas, pineapple, and walnuts 2 days before and during the sampling days was given to all participants. The diagnosis was confirmed by histology in 23 patients (88%) and by fine-needle aspiration in 2 patients, whereas in 1 patient several punctures from a mesenteric tumor failed. The carcinoid syndrome was present in 92% of the patients. Liver metastases were present in all except the patient with the mesenteric tumor. In 10 of 26 patients (38%), the primary tumor was located in the midgut. Despite imaging techniques, the primary tumor remained unknown in one-half of the patients. During the study, the dosage of somatostatin analogs administered in 23 patients was kept stable. Two patients received interferon-α until 2 months before urine collections, and in another two patients embolization of the liver was performed a few months before inclusion in this study (Table 1).

**Collection of urine samples**
Urine samples were collected at 4-h intervals during the day (0700–1100, 1100–1500, 15.00–19.00, and 1900–2300) and an 8-h interval (2300–0700) during the night for 2 days. The urine was collected in bottles that contained acetic acid as preservative.

The patients noted symptoms of flushes (none, moderate, severe), abdominal pain (yes or no), consistency of the stools (none, solid, loose, and watery), activities (none/sleeping, sitting, moderate, and severe) and food intake in a diary.

**Laboratory techniques**
Urinary 5-HIAA was measured by three different techniques.

**ELISA.** The ELISA (DRG Instruments) measures 5-HIAA in human urine. This competitive-binding, microtiter-plate assay has intraassay variation of 5% at a mean of 20 pmol/L and 7% at a mean of 200 pmol/L, and interassay variation of 13% at a mean of 22 and 12% at a mean of 141 pmol/L.

**HPLC.** 5-HIAA was analyzed by reversed-phase HPLC with a fluorescence detector (13).

**Spectrophotometry.** The original assay is based on the absorbance at 540 nm of the violet chromophore formed when 5-HIAA reacts with 1-nitroso-2-naphthol in nitrous acid medium (14)). In the present assay, 2-mercaptoethanol is added to the reaction mixture to improve the specificity (15).

**Urinary creatinine**
Urinary creatinine was measured on the Hitachi 917 (Roche Diagnostics) based on the Jaffe method (16).

Urine 5-HIAA excretion is expressed as μmol/24 h (reference values <40 μmol/24 h; excretion rates for 4-
and 8-h intervals are expressed in terms of excretion per 24 h) or μmol/mmol urinary creatinine. If not otherwise specified, the 5-HIAA excretion described in the Results were determined by the ELISA technique.

STATISTICS
The Mann–Whitney test or Fisher exact test was used to compare categorical variables, and the Kruskal–Wallis test was used in case of continuous variables. The Spearman correlation coefficient was used for ordinal variables. To examine the variation of urinary 5-HIAA during the day in relation to the complete 24-h collection, we calculated differences between the excretion rates for 4- and 8-h intervals and the full 24-h excretion from the corresponding day for each patient. The resulting variable was approximately gaussian distributed. The variance and confidence limits were estimated by using Proc Mixed (SAS system for Windows 8.02) to model the repeated measurements for patients over 2 days.

We observed a quadratic relationship for the variation in urinary 5-HIAA excretion rates from the overall 24-h excretion rate for each patient. The sum of the absolute difference in excretion rate for each time interval from the 24-h excretion rate was used as a measure of variation in each patient. The relevant inflection point for the quadratic relationship was found to be a 5-HIAA excretion of ~16.12 μmol/mmol creatinine. From this, the patients with a variation in HIAA excretion <16 μmol/mmol creatinine were divided into a “low-variation” group (10 patients), and the others were placed in a “high-variation” group (16 patients).

Results

LABORATORY VALUES
The measurements of 5-HIAA excretion by the HPLC and spectrophotometric analysis, the ELISA and spectrophotometric analysis, and by the HPLC and ELISA method correlated well (correlation coefficients, 0.98, 0.96, and 0.95, respectively; slopes, 0.96, 0.75, and 0.76 respectively, P < 0.001 for all three comparisons). In one patient, all urinary 5-HIAA values were significantly higher when assayed by the spectrophotometric method.

Increased concentrations of 5-HIAA in 24-h urines were present in 22 of 26 patients on both collecting days (range, 77–1529 μmol/24 h). Four patients had normal 24-h urinary 5-HIAA excretion rates on the first day: two of them had a slightly increased excretion rate in one of the collection intervals (47 and 55 μmol/24 h) and in one patient the excretion rate was slightly increased on the second day (53 μmol/24 h). These finding were consistent across the different assays. No significant difference was found in the urinary 5-HIAA excretion between the two collecting days (P = 0.77; Table 2).

URINE SAMPLES OVER THE DAY
A significant correlation was found for the absolute excretion of urinary 5-HIAA (μmol/24 h) compared with the 5-HIAA excretion per mmol creatinine excretion (P < 0.0001; correlation coefficient, 0.92). We expressed the urinary 5-HIAA excretion as μmol/mmol creatinine, the overnight interval produced the best estimate compared with the 24-h excretion (correlation coefficient, 0.81). We observed a quadratic relationship between the variation for each patient and the 24-h collection value. This variation was approximated by the sum of the absolute difference from the interval values and the 24-h excretion values. We divided our patients into a low-variation group (10 patients) and a high-variation group (16 patients). In the low-variation group, we could not detect peaks in 5-HIAA excretion during the day. In the high-variation patients, the excretion rate for 5-HIAA was significantly lower in the evening between 1900 and 2300

| Table 2. Urinary 5-HIAA excretion in the collection intervals, as measured by the ELISA. |
|---------------------------------|-----------------|-----------------|
| Median (range) urinary 5-HIAA excretion | Day 1 | Day 2 |
| Interval 1 (0700–1100) | | |
| Excretion, μmol/24 h | 192 (17–1505) | 172 (6–1886) |
| Excretion, μmol/mmol urinary creatinine | 18.4 (1.3–137.6) | 19.5 (1.1–166.4) |
| Interval 2 (1100–1500) | | |
| Excretion, μmol/24 h | 222 (10–1747) | 203 (11–1563) |
| Excretion, μmol/mmol urinary creatinine | 19.7 (1.8–126.8) | 16.3 (1.5–133.4) |
| Interval 3 (1500–1900) | | |
| Excretion, μmol/24 h | 256 (11–2210) | 245 (22–1458) |
| Excretion, μmol/mmol urinary creatinine | 21.9 (1.4–127.9) | 23.4 (1.7–114.8) |
| Interval 4 (1900–2300) | | |
| Excretion, μmol/24 h | 214 (13–1597) | 161 (21–1558) |
| Excretion, μmol/mmol urinary creatinine | 17.1 (1.6–120.2) | 15.2 (1.3–127.6) |
| Interval 5 (2300–0700) | | |
| Excretion, μmol/24 h | 206 (16–1566) | 206 (14–1370) |
| Excretion, μmol/mmol urinary creatinine | 21.6 (1.4–122.8) | 16.5 (1.0–125.7) |
| Interval 6 (24-h collection interval, 0700–0700) | | |
| Excretion, μmol/24 h | 225 (19–1511) | 230 (16–1529) |
| Excretion, μmol/mmol urinary creatinine | 18.8 (1.6–126) | 17.1 (1.3–131.1) |
(P = 0.0034) compared with the 24-h excretion and significantly higher in the morning between 0700 and 1100 (P = 0.0074; Fig. 1). In the patient group with high variability, the overnight interval remained the most representative sample for the 24-h excretion (correlation coefficient, 0.85).

**Urine samples in relation to symptoms and daily activity**

Although not significant, median urinary 5-HIAA excretion was lower during all collection intervals in which no stools were produced (158 μmol/24 h) compared with the intervals during which stools were produced (335 μmol/24 h). There was a trend between the rates of hormonal excretion and the consistency of the stools: no stools, 5-HIAA = 158 μmol/24 h; solid/loose stools, 5-HIAA = 332 μmol/24 h; watery stools, 5-HIAA = 790 μmol/24 h. However, given the small number of patients with watery stools (n = 5), this physiologically reasonable trend might have arisen by chance. We observed a trend in which 4 of 5 (80%) patients with at least one watery stool were categorized into the high-variation group compared with 12 of 21 (57%) with no watery stools. Abdominal pain as a symptom of increased bowel movement was present in 19 of 26 patients and reported in 29 of 260 intervals. No relationship between pain and 5-HIAA excretion in these intervals could be detected (P = 0.488). Flushes were reported by 20 of 26 patients. Although not significant, median urinary 5-HIAA excretion was lower during the 75 of 260 collection intervals with flushes (132 μmol/24 h) compared with those without (234 μmol/24 h). We found no relationship between physical activity and changes in 5-HIAA excretion: 220 μmol/24 h vs 174 μmol/24 h in the intervals with and without activity (P = 0.766). The degree of exercise was also not related to 5-HIAA excretion (P = 0.446). Hormonal excretion was not measurably related to food intake.

A delayed release of hormonal activity could not be detected when we compared 5-HIAA excretion rates in the subsequent time intervals. Correction of the 5-HIAA concentrations by creatinine in the urine samples did not change these outcomes.

**Discussion**

Production of vasoactive peptides, especially serotonin, by carcinoid tumors is related to symptoms of the carcinoid syndrome and development of carcinoid-related heart disease (2, 17). The 24-h urinary concentration of
5-HIAA, the degradation product of serotonin, is used for diagnosis and follow-up of carcinoid patients. Several methods are available to measure 5-HIAA excretion. In this study, three different assays gave highly correlated results. In one patient, all urine samples showed significantly higher 5-HIAA values in the spectrophotometric method. This patient used naproxen (nonsteroidal antiinflammatory drug), which may interact with this colorimetric method (18).

In the present series of 26 patients, rates of 5-HIAA excretion determined in overnight urine samples were representative of 24-h excretion. Excretion in all other intervals appeared to be less related to the 24-h excretion rate. This might be explained by induction of hormonal release by physical activity or food intake, but this hypothesis could not be confirmed in our study. Another reason might be the longer interval (8-h vs 4-h) during the night, which was 30% of the total sample.

Only slightly increased 5-HIAA excretion could be detected in the shorter interval samples for the four patients with a normal excretion in the 24-h urine sample. This supports the idea that repeated 24-h urine sampling does not add additional information. For follow-up and monitoring of treatment, an overnight sample should be sufficient.

In this study, we found a high correlation between absolute 5-HIAA excretion rates and 5-HIAA excretion corrected for creatinine excretion. However, correction for creatinine excretion increased the reliability of urine sampling by lowering the variance among patients and reducing the effect of collection interval duration. The latter could reduce the number of erratic results attributable to inaccurate collection interval by the patient.

Our patients could be divided into a group with low variability in urinary 5-HIAA excretion vs a group of patients with highly variable hormonal excretion. In the patient group with highly variable 5-HIAA excretion (n = 16), we found the highest excretion rates in the morning with an opposite finding in the urine samples collected in the evening. This is in accord with our clinical impression that symptoms of carcinoid patients are the most prominent at the start of the day. There are no published reports about cyclic changes in serotonin metabolism in carcinoid patients, although Pietraszek et al. (19) reported fluctuations in the concentrations of serotonin and its metabolites in blood during the day in healthy controls and depressed patients. In healthy adults, we found no clear diurnal variations in urinary 5-HIAA excretion based on two 12-h urine samples (20). In line with our results, Singh et al. (21) reported cyclic changes in excretion of creatinine and 5-HIAA during the day, with the highest 5-HIAA values in the morning.

Although symptoms of the carcinoid syndrome seem to be exacerbated by physical exertion, food intake, or starting of the day, information about this subject is scarce. The correlation between hormonal excretion and symptoms of the carcinoid syndrome is not clear. Plasma concentrations of serotonin sampled from the venous drainage areas of 10 carcinoid patients with symptoms of flushing were found to be increased (9). Provocation of flushes by food intake was described in a study by Richter et al. (11), but no correlation between intensity of the flush and serotonin concentrations was found. In a report by Oberg et al. (10), a decrease in 24-h urinary 5-HIAA excretion after octreotide did not lead to a change in flushes and diarrhea in 20 carcinoid patients. Because the degradation of serotonin to 5-HIAA is regulated by rapid clearance mechanisms (3–5), we measured 5-HIAA excretion in intervals of 4- and 8-h in an attempt to detect a relationship between symptoms of the carcinoid syndrome and transient changes in hormonal excretion, but we found no such relationship. Contrary to our expectations, the rates of 5-HIAA excretion were higher in the intervals without flushes than in those with flushes. This might be explained by the idea that hormones other than serotonin (e.g., tachykinines) play a role in the pathogenesis of flushing. A search of the literature for a correlation between serotonin production and symptoms of flushes failed to find earlier reports on this subject (11, 22–24). 5-HIAA excretion was found to be the highest in the collection intervals in which stools were produced and tended to be correlated with the consistency of the stools, but the number of observations may have been too small for significance. Furthermore, watery diarrhea was reported only by patients with a strong variation in 5-HIAA excretion. Thus, rapid changes in hormonal concentrations may play a more important role in the development of diarrhea than the hormonal excretion itself.

The use of somatostatin analogs could have influenced the results of our study. However, the medication consisted of slow-release preparations with no changes during the collection intervals. Although stabilization of hormonal activity could mask cyclic changes, we did find variable hormonal excretion during the day. A more pronounced pattern could possibly have been detected if no octreotide analogs were used.

In conclusion, we could not detect a definite relationship between the severity of symptoms of the carcinoid syndrome or the degree of physical activity and 5-HIAA excretion in 4- and 8-h urine samples. However, strong variations in 5-HIAA excretion are likely to be associated with the severity of diarrhea. Cyclic changes in urinary 5-HIAA excretion with peaking in the morning and lower excretion rates in the afternoon were found in those patients with highly variable 5-HIAA excretion. Hormonal excretion in an overnight-collection urine sample appeared highly correlated to 24-h excretion rates and could be used to monitor carcinoid patients.

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