Discriminating Capacity of Indole Markers in the Diagnosis of Carcinoid Tumors

Wim G. Meijer, Ido P. Kema, Marcel Volmer, Pax H.B. Willemse, and Elisabeth G.E. de Vries

Background: We evaluated the discriminating capacity of the indole markers urinary 5-hydroxyindoleacetic acid (5-HIAA), urinary serotonin, and platelet serotonin in the diagnosis of carcinoid tumors.

Methods: Indole markers were measured in 688 patients with suspected carcinoid disease. The initial values of indole markers from patients in whom a carcinoid tumor was confirmed during follow-up (n = 98) were used for ROC analysis. Two groups served as reference populations. The first consisted of 45 healthy individuals (“healthy controls”). The second was a random sample of 40 patients, drawn from the 590 (688 minus 98) patients with carcinoid-like symptoms but without a carcinoid tumor (“clinically suspected patients”).

Results: ROC curve analysis showed platelet serotonin to have the highest discriminating capacity, especially in foregut carcinoids. Cutoff values for platelet serotonin obtained from ROC analysis with healthy controls as reference group (5.4 nmol/10^9 platelets) gave a sensitivity of 74%, specificity of 91%, positive predictive value of 63%, and negative predictive value of 95% when applied to the initial 688 patients. Using the cutoff value with the clinically suspected patients as the reference group (9.3 nmol/10^9 platelets) gave a sensitivity of 63%, specificity of 99%, positive predictive value of 89%, and negative predictive value of 93%. Indole markers were increased in 169 (25%) of 688 patients. In 76 (45%) of these 169 patients, a carcinoid tumor was present. Slight increases of markers were associated with non-carcinoid neuroendocrine tumors, non-neuroendocrine tumors, and disturbed bowel motility.

Conclusions: ROC curve analysis shows that platelet serotonin is the most discriminating indole marker for the diagnosis of carcinoid tumors. Platelet serotonin especially improves the diagnosis of carcinoids producing small amounts of serotonin.

© 2000 American Association for Clinical Chemistry

Carcinoids are neuroendocrine tumors derived from enterochromaffin cells (1). Carcinoid tumors occur in 1.2–2.2 per 100,000 persons in a year. The majority of carcinoids arise in the small intestine and appendix. At presentation, 45% of patients have evidence of metastases. For all carcinoid tumors, regardless of primary localization, the 5-year survival rate is ~50% (2). With recent advances in the palliative treatment of carcinoid tumors, an early diagnosis will improve quality of life and survival (3, 4).

Enterochromaffin cells produce serotonin (5-hydroxytryptamine) as a paracrine hormone (5, 6). Markers such as chromogranins and neuropeptides (7) possess a high sensitivity for neuroendocrine tumors. However, these markers are unable to detect an enhanced serotonin metabolism, which is considered a hallmark of carcinoid tumors. Three indole markers are used in carcinoid disease: urinary 5-hydroxyindoleacetic acid (5-HIAA), urinary serotonin, and platelet serotonin content. Urinary 5-HIAA may be within the reference interval in 30% of carcinoids (8). The predictive value of an increased urinary 5-HIAA is low (9). Moreover, urinary 5-HIAA may increase in normal subjects after consumption of a serotonin-rich diet (10, 11). Urinary serotonin excretion mainly results from decarboxylation of circulating 5-hydroxytryptophan (5-HTP) by the renal tubular cells (12) and can unmask a carcinoid tumor that produces the serotonin precursor 5-HTP predominantly (10, 13, 14). Dietary serotonin only slightly increases the urinary free serotonin excretion (11). Platelets take up serotonin from the circulation by an active transmembrane mechanism.
For the clinical use of these indole markers are presented. Finally, recommendations for the clinical use of these indole markers are presented.

Carcinoid patients

The group of carcinoid patients consisted of patients in whom a histological diagnosis of a carcinoid tumor was made during the study period. Biochemical results of patients after confirmed curative resection of a carcinoid tumor were excluded from the study. In this way, only patients with a carcinoid tumor present at the time of biochemical testing were included. Whether a resection was curative or not was determined with clinical follow-up and octreotide scintigraphy. In this way, two patients were excluded from evaluation. The group contained carcinoid patients, regardless of the outcome of biochemical assessment. Ninety-eight carcinoid patients were included in this group. The 44 patients described in our previous (18) study were included in the present study. The median age was 58 years (range, 23–91); 47 were males, and 51 were females.

The carcinoid patients were classified into subgroups according to primary tumor localization (24) as foregut tumors (upper respiratory tract, lungs, esophagus, stomach, and duodenum; n = 22), midgut tumors (jejunum, ileum, appendix, cecum, and the ascending colon; n = 51), and hindgut tumors (transverse and left colon and rectum; n = 8). Biochemical results of patients unable to be classified into one of these groups (n = 17) were not included in subgroup calculations but were used in overall computations.

Clinically suspected patients

The remaining 590 patients, after exclusion of the 98 carcinoid patients, represented a group in which biochemical evaluation was performed because of the clinical suspicion of a carcinoid tumor, based on the presence of symptoms such as diarrhea, flushing, wheezing, and unexplained abdominal pain. These patients had no evidence of a carcinoid tumor during follow-up. Of these 590, we drew a random sample of 40 patients to serve as a reference group for ROC curve computations. The median follow-up in this group was 8.5 years (range, 4–12 years). The median age was 51 years (range, 25–78); 18 were males, and 22 were females.

Healthy controls

Of a population of hospital workers, some of whom were retired, an age- and sex-matched control group was recruited. Disease states affecting indole markers were excluded by taking a medical history. From these individuals, blood and urine were collected, after informed consent was given. This group of healthy controls provided a second reference group for the construction of ROC curves. In this group, the median age was 54 years (range, 44–75); 20 were males, and 25 were females.

Patients with increased indole markers

Of the complete population of 688 patients, including the carcinoid patients and the clinically suspected patients, we identified those patients who had increased indole

Materials and Methods

Patients

In a retrospective study over the period from January 1987 until May 1997 at the University Hospital Groningen, all patients with biochemical evaluation prompted by the clinical suspicion of a carcinoid tumor were identified. During the study period, indole markers were measured in 688 patients. For each patient, the initial results of urinary 5-HIAA, urinary serotonin, and platelet serotonin in carcinoid tumors. On the basis of ROC curves, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of these indole markers were calculated. When we applied the ROC-based cutoff values to the entire population under investigation, patients with increased indole markers were identified. For these patients, the diagnosis and symptoms were identified. Finally, recommendations for the clinical use of these indole markers are presented.

Patients

In a retrospective study over the period from January 1987 until May 1997 at the University Hospital Groningen, all patients with biochemical evaluation prompted by the clinical suspicion of a carcinoid tumor were identified. During the study period, indole markers were measured in 688 patients. For each patient, the initial results of urinary 5-HIAA, urinary serotonin, and platelet serotonin in carcinoid tumors. On the basis of ROC curves, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of these indole markers were calculated. When we applied the ROC-based cutoff values to the entire population under investigation, patients with increased indole markers were identified. For these patients, the diagnosis and symptoms were identified. Finally, recommendations for the clinical use of these indole markers are presented.

Healthy controls

Of a population of hospital workers, some of whom were retired, an age- and sex-matched control group was recruited. Disease states affecting indole markers were excluded by taking a medical history. From these individuals, blood and urine were collected, after informed consent was given. This group of healthy controls provided a second reference group for the construction of ROC curves. In this group, the median age was 54 years (range, 44–75); 20 were males, and 25 were females.

Patients with increased indole markers

Of the complete population of 688 patients, including the carcinoid patients and the clinically suspected patients, we identified those patients who had increased indole
markers. For this, we used selected cutoff values obtained from ROC curve analysis with either the clinically suspected patients or the healthy controls as reference group. Of the patients with increased indole markers, the primary site of the carcinoid tumor or the non-carcinoid diagnosis or symptoms were specified.

**BLOOD AND URINE SAMPLES**
The 24-h urine samples from patients and controls were collected in 2-L brown polypropylene bottles (Sarsted) containing 250 mg each of Na₂S₂O₅ and EDTA as preservatives. Samples were acidified to pH 4 with acetic acid, before freezing. Venous blood samples were collected in 10-mL Vacutainer Tubes (Becton Dickinson) containing 0.12 mL of 0.34 mol/L EDTA solution and were immediately put on ice. Platelet-rich plasma was prepared from whole blood within 1 h after sampling by centrifugation for 30 min at 120 g and 4 °C. Na₂S₂O₅ and EDTA were added as preservatives in final concentrations of ~10 g/L each. Platelet concentrations were measured with a Coulter Counter Model S plus 4 (Coulter Electronics). Samples were stored at −20 °C and analyzed within 1 week after collection.

**ANALYTICAL METHODS**
The serotonin contents of platelet-rich plasma and urine were determined by HPLC with fluorometric detection according to the method of Kwarts et al. (25) in the following way. Platelet-rich plasma or urine were mixed with an ammonium acetate buffer, pH 7.5. Samples were subsequently applied to Amberlite CG-50 columns and washed with the ammonium acetate buffer and 0.01 mol/L acetic acid. Elution was done with 1.0 mol/L acetic acid that contained ascorbic acid. Chromatographic analysis was performed by isocratic reversed-phase HPLC with fluorometric detection. Urinary 5-HIAA concentrations were determined in ether extracts by HPLC with fluorometric detection (26). Urinary creatinine concentrations were measured by a picric acid method (Mega analyzer; Merck). Urinary serotonin and 5-HIAA were expressed in μmol/mol and mmol/mol urinary creatinine, respectively (27). Platelet serotonin content, expressed as nmol serotonin/10⁹ platelets, was calculated by dividing the serotonin concentration in platelet-rich plasma by the concentration of platelets in the plasma.

**STATISTICS**
The discriminating capacity of urinary 5-HIAA, urinary serotonin, and platelet serotonin was calculated from
ROC curves (28). The area under the ROC curve (AUC), indicating the capability of a marker to discriminate between normal and abnormal groups, is the so-called “discriminating capacity”, “diagnostic accuracy”, or “performance” of a marker. In the computation of the ROC curves, either the healthy controls or the clinically suspected patients were used as reference groups. The numbers of individuals in both reference groups were not based on a power analysis but were chosen to obtain balanced numbers for comparison. This way the numbers of healthy controls and clinically suspected patients were in the same range as the numbers of patients in the respective subgroups of carcinoid patients (i.e., foregut, midgut, and hindgut carcinoid). To select clinically suspected patients, we identified those patients with all three indole markers measured within a time interval of 3 months and with adequate follow-up available. From this group, we selected random samples based on an exact number of 40 cases using SPSS (SPSS 9.0; SPSS, Inc).

We selected a cutoff value for each marker that produced the highest differential positive rate (sensitivity minus false-positive rate) using either healthy controls (low-level cutoff value) or clinically suspected patients (high-level cutoff value) as reference group. For computation and analysis of ROC curves, we used the software programs ROC 2.1 (University Hospital Groningen) and Clinical Laboratory, Ver. 1.28 (Analyze-It Software, Leeds, United Kingdom). Linear discriminant analysis (29) was used to test whether combinations of indole markers would have a better discriminating ability than a single indole marker.

For comparison of age and sex distribution between groups, respectively, the Student t-test and Pearson’s $\chi^2$ test were used. Group differences between the biochemical values were evaluated using the Kruskal–Wallis test. Whenever differences were found, the individual group differences were tested with the Mann–Whitney $U$-test, using Bonferroni correction. In all calculations, $P < 0.05$ indicated a significant difference.

**Results**

**Indole markers in patients and controls**

During the study period, indole markers were measured in 688 patients. Initial results of urinary 5-HIAA were available in 519, urinary serotonin in 124, and platelet serotonin in 561 patients. In Fig. 1, concentrations of indole markers in carcinoid patients with primary localization in the foregut (Fore), midgut (Mid), and hindgut (Hind) region. See the legend to Fig. 1.
urinary serotonin values were found, compared with the healthy controls. Values of platelet serotonin differed among all three groups.

Fig. 2 represents the distribution of the concentrations of urinary 5-HIAA, urinary serotonin, and platelet serotonin in the carcinoid group, subdivided according to the primary localization.

DISCRIMINATING CAPACITY
In Fig. 3, the relevant ROC curves are depicted. To facilitate comparisons of the markers, the ROC curves obtained from urinary 5-HIAA, urinary serotonin, and platelet serotonin are combined in each plot. Two ROC curves were drawn for the complete carcinoid group, including the 17 patients with unknown primary localiza-

Fig. 3. ROC curves for three indole markers.
(A), all carcinoid patients with healthy controls as reference group. (B), all carcinoid patients with clinically suspected patients as reference group. (C), midgut carcinoid patients with the healthy controls as reference group. (D), foregut carcinoids with the healthy controls as reference group. The calculated AUCs are presented in Table 1.
tion. In the first ROC curve, the healthy controls were used as reference group (Fig. 3A), whereas the second ROC curve was drawn with the clinically suspected patients as reference (Fig. 3B). Separate ROC curves were constructed for midgut (Fig. 3C) and foregut (Fig. 3D) carcinoids. In the latter two ROC curves, the healthy controls were used as reference group. For the hindgut group, it was not possible to construct a reliable ROC curve because of the small number of patients (n = 8).

Table 1 shows the AUC calculated from the ROC curves in Fig. 3. In the ROC curve constructed with all carcinoid patients and the healthy controls as a reference group, no differences between the AUCs of the various markers were found. The difference in the AUCs for platelet serotonin and urinary 5-HIAA did not reach significance (P = 0.08). Analysis of the carcinoid group with the clinically suspected patients as reference group revealed larger AUCs for platelet serotonin vs urinary serotonin (P = 0.05) and for urinary 5-HIAA vs urinary serotonin (P = 0.02). With the healthy controls as a reference group, subgroup calculations were performed. In foregut carcinoids, higher AUCs were found for platelet serotonin vs urinary 5-HIAA (P = 0.03) and in midgut carcinoids for platelet serotonin vs urinary serotonin (P = 0.02). Linear discriminant analysis showed that no possible combination of the indole markers yielded higher AUCs for platelet serotonin vs urinary serotonin (P = 0.05) and for urinary 5-HIAA vs urinary serotonin (P = 0.02). With the healthy controls as a reference group, subgroup calculations were performed. In foregut carcinoids, higher AUCs were found for platelet serotonin vs urinary 5-HIAA (P = 0.03) and in midgut carcinoids for platelet serotonin vs urinary serotonin (P = 0.02). Linear discriminant analysis showed that no possible combination of the indole markers yielded higher AUCs, compared with the single use of platelet serotonin.

**CUTOFF VALUES**

From the ROC curve with all carcinoid patients vs the healthy controls (Fig. 3A), the cutoff values (low-level, Table 1) were calculated as described in the methods for the respective indole markers: urinary 5-HIAA, 2.8 mmol/mol creatinine; urinary serotonin, 55.0 μmol/mol creatinine; and platelet serotonin, 5.4 nmol/10^9 platelets. Use of the clinically suspected patients as reference group (Fig. 3B) produced somewhat higher cutoff values (high-level, Table 1): urinary 5-HIAA, 6.7 mmol/mol creatinine; urinary serotonin, 99.0 μmol/mol creatinine; and platelet serotonin, 9.3 nmol/10^9 platelets.

**TEST CHARACTERISTICS WITH SELECTED CUTOFF VALUES**

The sensitivity, specificity, PPV, and NPV of the indole markers for the presence of a carcinoid tumor are represented in Table 2. In contrast to the discriminating capacity, these test characteristics depend on the chosen cutoff value. In accordance with the outcome of the ROC curve analysis, platelet serotonin showed better test characteristics than did urinary 5-HIAA and urinary serotonin using either the low-level or high-level for cutoff values.

**PATIENTS WITH INCREASED INDOLE MARKERS**

When we applied the low-level cutoff values to all 688 patients in the study, 169 patients had at least one indole marker increased. In 76 (45%) of these patients, a carcinoid tumor was present. In Table 3, the primary site of the carcinoid tumor is specified. The remaining 93 patients had increased indole markers in the absence of a carcinoid tumor. The diagnosis or symptoms of these patients are also shown in the table. Non-carcinoid neuroendocrine tumors, non-neuroendocrine tumors, and disturbances in bowel motility accounted for increased markers in 18, 11, and 17 patients, respectively. The 18 non-carcinoid neuroendocrine malignancies were pancreatic islet cell tumors (n = 6), solitary medullary thyroid cancer (n = 4), pheochromocytoma and medullary thyroid cancer asso-

**Table 1. AUCs of Fig. 3: AUC and selected cut-off values for each marker for all carcinoid patients, subgroups of carcinoid patients, and reference groups.**

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Reference group</th>
<th>Marker*</th>
<th>AUC</th>
<th>95% CI of AUC</th>
<th>Selected cutoff value b</th>
</tr>
</thead>
<tbody>
<tr>
<td>All carcinoid patients</td>
<td>Healthy controls</td>
<td>Urinary 5-HIAA</td>
<td>0.79</td>
<td>0.71–0.86</td>
<td>2.8</td>
</tr>
<tr>
<td>n = 98</td>
<td>n = 45</td>
<td>Urinary serotonin</td>
<td>0.84</td>
<td>0.77–0.92</td>
<td>55.0</td>
</tr>
<tr>
<td>All carcinoid patients</td>
<td>Clinically suspected patients</td>
<td>Urinary 5-HIAA</td>
<td>0.77</td>
<td>0.69–0.85</td>
<td>6.7</td>
</tr>
<tr>
<td>n = 98</td>
<td>n = 40</td>
<td>Urinary serotonin</td>
<td>0.64</td>
<td>0.49–0.79</td>
<td>99.0</td>
</tr>
<tr>
<td>Foregut carcinoid</td>
<td>Healthy controls</td>
<td>Urinary 5-HIAA</td>
<td>0.55</td>
<td>0.37–0.72</td>
<td></td>
</tr>
<tr>
<td>n = 22</td>
<td>n = 45</td>
<td>Urinary serotonin</td>
<td>0.71</td>
<td>0.52–0.90</td>
<td></td>
</tr>
<tr>
<td>Midgut carcinoid</td>
<td>Healthy controls</td>
<td>Urinary 5-HIAA</td>
<td>0.89</td>
<td>0.81–0.96</td>
<td></td>
</tr>
<tr>
<td>n = 51</td>
<td>n = 45</td>
<td>Urinary serotonin</td>
<td>0.84</td>
<td>0.75–0.94</td>
<td></td>
</tr>
<tr>
<td>Hindgut carcinoid</td>
<td>Healthy controls</td>
<td>Urinary 5-HIAA</td>
<td>0.55</td>
<td>0.30–0.81</td>
<td></td>
</tr>
<tr>
<td>n = 8</td>
<td>n = 45</td>
<td>Urinary serotonin</td>
<td>0.83</td>
<td>0.69–0.97</td>
<td></td>
</tr>
</tbody>
</table>

* Cutoff values of urinary 5-HIAA expressed in mmol/mol creatinine, urinary serotonin in μmol/mol creatinine, and platelet serotonin in nmol/10^9 platelets.

b Selected cutoff values were calculated with the healthy controls (low-level) or clinically suspected patients (high-level) as reference group.

P = 0.02.

a P = 0.05.

b P = 0.03.
associated with multiple endocrine neoplasia IIa syndrome (n = 3), solitary pheochromocytoma (n = 1), hepatocellular carcinoma with neuroendocrine differentiation (n = 1), and a neuroendocrine laryngeal tumor (n = 1). In two patients, the primary site of the neuroendocrine tumor remained unidentified. The 11 non-neuroendocrine malignancies were adenocarcinomas arising from the digestive tract (n = 8), breast (n = 1), and ovary (n = 1), and a hepatocellular carcinoma (n = 1). Three of 17 patients with disturbances in bowel motility presented with constipation. In 14 patients, diarrhea was attributable to irritable bowel syndrome (n = 4), microscopic colitis (n = 3), inflammatory bowel disease (n = 2), laxative abuse (n = 1), or pancreatic insufficiency (n = 1). In three patients, no causative mechanism for the diarrhea was found. In the remaining 47 patients, no definite diagnosis explaining the increased markers was established.

Application of high-level cutoff values identified 81 patients with increased markers; 64 (79%) of these patients had a carcinoid tumor (Table 3). Of 17 non-carcinoid patients with increased markers on high-level, 7 had neuroendocrine tumors, and 4 had non-neuroendocrine tumors. Only six patients with nonmalignant disorders had markers above high-level cutoff values. One of these patients had inflammatory bowel disease; in the remaining patients, no definite diagnosis was made.

**Discussion**

ROC curve analysis shows that, of the indole markers examined, platelet serotonin is the most discriminating marker for the detection of a carcinoid tumor. Because in clinical practice a marker should differentiate carcinoid patients from patients with carcinoid-like symptoms, an additional ROC analysis with the clinically suspected patients as reference group was performed. In this analysis, platelet serotonin remained the most discriminating marker for a carcinoid tumor. We reincluded the clinically suspected patients in the calculation of the classical test characteristics. Theoretically, this leads to a bias caused by overfitting. This effect, however, is considered small, because only 40 patients were reincluded in a total of 590 patients.

Because midgut carcinoids represent >50% of the carcinoid patients in our study group, differences between subgroups of carcinoid tumors could remain unnoticed. Therefore, an analysis based on the primary localization of the carcinoid tumor was performed.

In foregut carcinoid patients, platelet serotonin has a higher discriminating capacity compared with urinary 5-HIAA. In fact, because the AUC was 0.55, urinary 5-HIAA did not contribute to the diagnosis of foregut carcinoids. This finding is in accordance with Janson et al. (7), describing a poor sensitivity of urinary 5-HIAA in 39 foregut carcinoids. In earlier studies (10, 13, 30, 31), an overproduction of 5-HTP in foregut carcinoids was reported, tentatively resulting from a deficiency of aromatic amino acid decarboxylase in the tumor. Renal decarboxylation of 5-HTP makes urinary serotonin excretion a possible marker in foregut carcinoids. Our study, however, shows that platelet serotonin is at least equivalent to urinary serotonin in the diagnosis of foregut carcinoids.

In midgut carcinoids, all three indole markers possessed a high discriminating capacity, although platelet serotonin was superior to urinary serotonin. This reflects the high rate of serotonin secretion found in midgut carcinoids. The remarkably high urinary excretion of serotonin in midgut carcinoids probably results from serotonin clearance, rather than 5-HTP synthesis. Nevertheless, 5-HTP coproduction may occur in midgut carcinoids as described by Feldman (14), who found increased

### Table 2. Test characteristics of indole markers for carcinoid tumors with application of low-level and high-level cutoff values to the total population.

<table>
<thead>
<tr>
<th>Marker*</th>
<th>Patients tested</th>
<th>Cutoff value</th>
<th>Sensitivity, %</th>
<th>Specificity, %</th>
<th>PPV, %</th>
<th>NPV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinary 5-HIAA</td>
<td>519</td>
<td>2.8</td>
<td>68</td>
<td>89</td>
<td>58</td>
<td>93</td>
</tr>
<tr>
<td>Urinary serotonin</td>
<td>124</td>
<td>55</td>
<td>64</td>
<td>68</td>
<td>70</td>
<td>62</td>
</tr>
<tr>
<td>Platelet serotonin</td>
<td>561</td>
<td>5.4</td>
<td>74</td>
<td>91</td>
<td>63</td>
<td>95</td>
</tr>
</tbody>
</table>

*Urinary 5-HIAA expressed in mmol/mol creatinine, urinary serotonin in µmol/mol creatinine, and platelet serotonin in nmol/10⁹ platelets.

### Table 3. Patients with increased indole markers: Distribution of diagnosis and symptoms.

<table>
<thead>
<tr>
<th>Diagnosis/symptoms</th>
<th>Increased indole markers*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low-level</td>
</tr>
<tr>
<td>Carcinoid</td>
<td></td>
</tr>
<tr>
<td>Foregut</td>
<td>10</td>
</tr>
<tr>
<td>Midgut</td>
<td>46</td>
</tr>
<tr>
<td>Hindgut</td>
<td>4</td>
</tr>
<tr>
<td>Unknown primary site</td>
<td>16</td>
</tr>
<tr>
<td>Total</td>
<td>76</td>
</tr>
<tr>
<td>Non-carcinoid malignancies</td>
<td></td>
</tr>
<tr>
<td>Non-neuroendocrine tumor</td>
<td>18</td>
</tr>
<tr>
<td>Non-NE malignancy</td>
<td>11</td>
</tr>
<tr>
<td>Total</td>
<td>29</td>
</tr>
<tr>
<td>Nonmalignant disorders</td>
<td></td>
</tr>
<tr>
<td>Bowel motility disorder</td>
<td>17</td>
</tr>
<tr>
<td>Flushing</td>
<td>6</td>
</tr>
<tr>
<td>Wheezing</td>
<td>12</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>9</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>20</td>
</tr>
<tr>
<td>Total</td>
<td>64</td>
</tr>
<tr>
<td>Total</td>
<td>169</td>
</tr>
</tbody>
</table>

*Increase of at least one indole marker above cutoff value, with low-level (high-level) cutoff values: urinary 5-HIAA, 2.8 (6.7) mmol/mol creatinine; urinary serotonin, 55.0 (99.0) µmol/mol creatinine; and platelet serotonin, 5.4 (9.3) nmol/10⁹ platelets.

*NE, neuroendocrine.
urinary 5-HTP excretion in 81% of 27 carcinoid patients with increased urinary 5-HIAA excretion.

The small number (n = 8) of patients with hindgut carcinoids in our study precludes reliable conclusions in this subgroup. In a study by Koura et al. (32), urinary 5-HIAA was increased in 4 of 22 patients with rectal carcinoids. These four patients had disseminated disease with liver metastases. In agreement with other authors (20, 22), this finding illustrates that hindgut carcinoids usually secrete limited amounts of serotonin. Therefore, the indole markers make a minor contribution to the diagnosis of hindgut carcinoids, except for patients with advanced disease.

As argued previously (21), the high discriminating capacity of platelet serotonin can be explained by the metabolism of serotonin and estimations on pool size of the respective compartments involved. Platelet serotonin content follows saturation kinetics, whereas urinary 5-HIAA represents a metabolic end-compartment with unlimited capacity. This makes urinary 5-HIAA a relevant marker for follow-up of disease activity in carcinoids with grossly enhanced serotonin production. Upper limits of normal for urinary 5-HIAA (10, 33, 34) and platelet serotonin (35) mentioned in the literature are intermediate between the low-level and high-level cutoff value determined in the present study.

Test characteristics (sensitivity, specificity, PPV, and NPV) obviously depend on the selected cutoff value (either low-level or high-level, Table 2). A higher sensitivity and NPV are found when using the low-level cutoff values than with high-level cutoff values. Specificity and PPV were lower with low-level cutoff values compared with high-level cutoff values. The high PPV for urinary serotonin with both low-level and high-level cutoff values was remarkable. This could be the result of a selection bias caused by measurement of urinary serotonin. This test was ordered more frequently in patients in whom increased urine 5-HIAA excretion or platelet serotonin content was found than in patients with normal urinary 5-HIAA excretion or platelet serotonin content. The choice for either low-level or high-level cutoff values depends on the clinical situation. To exclude a carcinoid tumor, the low-level cutoff values are preferred; to confirm the presence of a carcinoid tumor, the high-level cutoff values are indicated. Applying low-level cutoff values decreases the specificity of the indole markers. We therefore evaluated the diagnoses and symptomatology of patients with increase of at least one of the indole markers (Table 3). Evidence of increased serotonin production was found in non-carcinoid neuroendocrine tumors. This is in agreement with other reports describing increased urinary 5-HIAA excretion in non-carcinoid neuroendocrine tumors (9, 10, 34). Non-carcinoid neuroendocrine tumors share metabolic characteristics with carcinoid tumors. In the present study, an enhanced serotonin metabolism was also observed in patients with gastrointestinal motility disorders, presenting with diarrhea or constipation without a carcinoid tumor. Here, serotonin is probably synthesized in above-physiological amounts because of its action as a neurotransmitter in the intestinal tract. This is supported by earlier observations of increased indole markers in patients with coeliac disease (36, 37) and irritable bowel disease (38). Fig. 1 and Table 3 show that only moderately increased indole markers are found in non-carcinoid patients. In the clinically suspected patients, platelet serotonin never exceeded a value of ~10 nmol/10^9 platelets.

Urinary 5-HIAA excretion has a lower discriminating capacity for carcinoid tumors compared with platelet serotonin and is therefore a less reliable marker in the diagnosis of carcinoid tumors. Nevertheless, urinary 5-HIAA is of relevance in the follow-up of carcinoids with a high serotonin secretion rate.

Urinary serotonin makes no additional contribution to platelet serotonin in the detection of carcinoid tumors. Even in foregut carcinoids, which occasionally produce 5-HTP instead of serotonin, there was no advantage of urinary serotonin over platelet serotonin.

Platelet serotonin is the most discriminating indole marker for the detection of carcinoid tumors. The high sensitivity of platelet serotonin is of clinical importance, in particular in carcinoids with a low serotonin production rate. Such low serotonin production is found in foregut and hindgut carcinoids and in midgut carcinoids with a small tumor volume. This establishes platelet serotonin as a reliable marker for the early diagnosis of carcinoids and in the detection of residual tumor after operation. The amount of platelet serotonin is not affected by factors such as stress, position, and diurnal variation, and moreover, the assay can be performed with relatively simple HPLC equipment (39, 40). Developments in chromatographic analyses have moved the analysis of indoles away from the point at which they were considered complicated, cumbersome, and sensitive to interference to a position where they have become accessible to clinical chemical laboratories that are equipped with modern chromatographic facilities. We therefore recommend the use of platelet serotonin as the primary indole marker for the diagnosis of carcinoid tumors.

References