Plasma Metanephrines in the Diagnosis of Pheochromocytoma
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Objective: To examine whether tests for plasma metanephrines, the O-methylated metabolites of catecholamines, offer advantages for diagnosis of a pheochromocytoma over standard tests for plasma catecholamines or urinary metanephrines.

Design: Cross-sectional study.

Setting: 3 clinical specialist centers.

Patients: 52 patients with a pheochromocytoma; 67 normotensive persons and 51 patients with essential hypertension who provided reference values; and 23 patients with secondary hypertension and 50 patients with either heart failure or angina pectoris who served as comparison groups.

Measurements: Plasma concentrations of catecholamines (norepinephrine and epinephrine) and metanephrines (normetanephrine and metanephrine) were measured in all patients. The 24-hour urinary excretion of metanephrines was measured in 46 patients with pheochromocytoma.

Results: Pheochromocytomas were associated with increases in plasma concentrations of metanephrines that were greater and more consistent than those in plasma catecholamine concentrations. No patient with a pheochromocytoma had normal plasma concentrations of both normetanephrine and metanephrine. The sensitivity of these tests was 100% (52 of 52 patients [95% CI, 94% to 100%]), and the negative predictive value of normal plasma concentrations of metanephrines was 100% (162 of 162 patients). Tests for plasma catecholamines yielded eight false-negative results and a sensitivity of 85% (44 of 52 patients [CI, 72% to 93%]). The negative predictive value of normal plasma concentrations of catecholamines was 95% (156 of 164 patients). Tests for urinary metanephrines yielded five false-negative results and a sensitivity of 89% (41 of 46 patients [CI, 76% to 96%]). Because no statistical difference was noted in the number of false-positive results between tests for plasma metanephrines (15%) and tests for plasma catecholamines (18%), the specificities of the two tests did not differ.

Conclusions: Normal plasma concentrations of metanephrines exclude the diagnosis of pheochromocytoma, whereas normal plasma concentrations of catecholamines and normal urinary excretion of metanephrines do not. Tests for plasma metanephrines are more sensitive than tests for plasma catecholamines or urinary metanephrines for the diagnosis of pheochromocytoma.

An understanding of why plasma metanephrines may be particularly useful for diagnosis of pheochromocytoma requires an understanding of catecholamine metabolism. Norepinephrine and epinephrine are first metabolized intraneuronally by deamination to dihydroxyphenylglycol or extraneuronally by O-methylation to the metanephrines (12). Because most dihydroxyphenylglycol is formed from norepinephrine leaking from neuronal stores and little is formed from circulating catecholamines (13, 14), plasma levels of this metabolite are relatively insensitive to the release of catecholamines into the circulation from a pheochromocytoma (6, 15). The formation of most methoxylhydroxynaphetylamine from dihydroxyphenylglycol (14) and the formation of most vanillylmandelic acid from methoxyhydroxynaphetylamine within the liver (16) explains

See editorial comment on pp 150-1.
why a test for vanillylmandelic acid is also a poorer marker for pheochromocytoma than other tests (17). In contrast, preferential metabolism of circulating catecholamines compared with neuronal catecholamines by extra-neuronal pathways (14) suggests that the metaneph- rines—as extraneuronal metabolites—may provide good markers for release of catecholamines from a pheochro- mocytoma. Furthermore, substantial production of meta- nephrines within adrenal tissue (18) suggests that meta- nephrines may be produced within the tumor itself.

In humans, metanephrines are extensively sulfate-con- jugated (18, 19). Assays of metanephrines in urine depend on measurements after deconjugation to free metanephrines (19) so that measurements represent the sum of free and conjugated metabolites (total metanephrines). In contrast, good sensitivity of the assay for plasma metanephrines (20) enables measurements of both free and total metanephrines.

We compared the sensitivity, specificity, and positive and negative predictive values of tests for plasma free and total metanephrines with those of tests for plasma cate- cholamines and urinary total metanephrines. Study par- ticipants included a relatively large sample of patients with pheochromocytoma, patients with essential hyperten- sion or secondary hypertension from causes other than pheochromocytoma, and patients with either heart failure or angina pectoris in whom sympathetically mediated catecholamine release would be expected to be increased.

Methods

Patients

Fifty-two patients with a histologically proven pheochromocytoma were studied. Thirty patients were studied retrospectively, and 22 were studied before the final diagnosis was made. The pheochromocytoma was benign in 39 patients and malignant in 13. Sixty-seven healthy, normotensive persons and 51 patients with essential hypertension served as a reference group. Blood samples were obtained from 23 patients with secondary hyper- tension (12 patients with renal artery stenosis, 2 with kidney disease, 1 with Cushing disease, 1 with primary hyperaldosteron- ism, and 7 with cyclosporine-induced hypertension) and from 50 patients with either heart failure or angina pectoris. The age, sex, and specialty center where the patients were studied for each of the five groups are shown in Table 1. Except for the few patients who were being treated with phenoxybenzamine, no patients with pheochromocytoma had been receiving medication for at least 2 weeks at the time of blood sampling. No patients with essential hypertension had been receiving medication for at least 2 weeks at the time of blood sampling. Medications taken by the other patient groups included digoxin, calcium channel blockers, diuretics, acetylsalicylic acid, diprydamole, and cyclosporine. Procedures used in our study were approved by the hospital ethics committee or intramural research board of each of the three centers where patients were studied.

Blood and Urine Samples

All patients refrained from ingesting methylxanthine-containing food products and from smoking after midnight on the day before blood sampling. Blood was collected from an indwelling catheter in an antecubital vein after the patients had rested supine for 20 minutes. In 39 patients with heart failure and 15 with secondary hypertension, arterial blood was obtained through an indwelling arm arterial catheter. Blood samples were collected into precooled tubes containing heparin or EGTA and glutathione and were centrifuged within 30 minutes to separate the plasma, which was stored frozen until assayed. All plasma cate- cholamine and urinary metanephrine assays were done within 2 weeks of sample collection. Seven of the 52 pheochromocytoma samples were assayed for plasma metanephrines after being stored at −80°C for more than 2 years (range, 2 to 8 years), whereas the remaining 45 samples were assayed within 2 years of collection (22 samples within 4 weeks). In 46 of the 52 patients with pheochromocytoma, a 24-hour urine collection was obtained, with 30 mL of 6-M hydrochloric acid used as a preservative.

Analytic Methods

Plasma metanephrines were assayed at the National Institutes of Health (NIH) using liquid chromatography with electrochemical detection (20). Concentrations of total metanephrines (the sum of concentrations of free and sulfocjugated metanephrines) were measured after incubation of 0.25 mL of plasma with 0.1 units of sulfatase (Sigma Chemical Company, St. Louis, Missouri) at 37°C for 30 minutes. The detection limits were 0.013 nmol/L for normetanephrine and 0.019 nmol/L for metanephrine. At a plasma normetanephrine concentration of 0.31 nmol/L and a metanephrine concentration of 0.21 nmol/L, the interassay coefficients of variation were 12.2% for normetanephrine and 11.2% for metanephrine. As previously reported (20), the presence of acetylaminoeph in samples of plasma can substantially interfere with measurements of plasma normetanephrine concentra- tions. Therefore, this analgesic must not be used by patients for several days before blood samples are collected. No analytic interference of various other drugs with this assay has been shown (20).

Plasma catecholamines were assayed using liquid chromatography. Electrochemical detection was used for quantification at the NIH (21), and fluorometric detection was used at St. Radboud University Hospital, Nijmegen, the Netherlands (22). At the NIH, the detection limits were 0.006 nmol/L for norepinephrine and 0.010 nmol/L for epinephrine. At a plasma norepinephrine concentration of 2.4 nmol/L and an epinephrine concentra- tion of 0.39 nmol/L, the interassay coefficients of variation were 6.5% for norepinephrine and 11.4% for epinephrine. At St. Radboud University Hospital, the detection limits for norepinephrine and epinephrine were 0.002 nmol/L and 0.003 nmol/L, respectively. At plasma concentrations of 1.02 nmol/L for norepinephrine and 0.15 nmol/L for epinephrine, interassay coefficients of variation were 9.5% for norepinephrine and 7.2% for epinephrine.

Urinary concentrations of metanephrines were measured according to a previously described method (23); the upper reference limit of the normal range for the 24-hour urinary output of metanephrines was 6.8 μmol/d.

Table 1. Patient Characteristics*  

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normotension</th>
<th>Essential Hypertension</th>
<th>Secondary Hypertension</th>
<th>Heart Failure or Angina Pectoris</th>
<th>Pheochromocytoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients, n</td>
<td>67</td>
<td>51</td>
<td>23</td>
<td>50</td>
<td>52</td>
</tr>
<tr>
<td>Mean age ± SD (range), y</td>
<td>39 ± 12 (20–72)</td>
<td>43 ± 13 (16–69)</td>
<td>48 ± 14 (20–69)</td>
<td>57 ± 11 (34–81)</td>
<td>39 ± 13 (11–71)</td>
</tr>
<tr>
<td>Male/female, n/n</td>
<td>40/27</td>
<td>24/27</td>
<td>14/9</td>
<td>42/8</td>
<td>28/24</td>
</tr>
<tr>
<td>Study center (NIH/SR/SH), n/n/n</td>
<td>36/31/0</td>
<td>15/00</td>
<td>17/15</td>
<td>13/7/0</td>
<td>41/11/0</td>
</tr>
</tbody>
</table>

* NIH = National Institutes of Health; SH = Sahlgrens Hospital; SR = St. Radboud University Hospital.
Data Analysis

Because plasma concentrations of catecholamines and metanephrines were not normally distributed, only medians and ranges are presented for these concentrations. Differences in plasma concentrations of metanephrines and catecholamines among patients with pheochromocytoma and other groups were tested using the Kruskal-Wallis test. We assessed relations among variables using the Spearman rank correlation coefficient.

Normal distributions of plasma concentrations of catecholamines and metanephrines were obtained after logarithmic transformation of the data. Thus, upper reference limits, defined as the 97.5th percentile, were determined after logarithmic transformation of individual values for the combined data from normotensive persons and those with essential hypertension (118 persons). The 97.5th percentiles were calculated from the antilogarithm of the mean plus 2 standard deviations of the transformed data. A false-negative result of a test for plasma metanephrines in a patient with pheochromocytoma was defined as plasma concentrations of both normetanephrines and metanephrines that were below their respective upper reference limits. Similarly, a false-negative result of a test for plasma catecholamines was defined as plasma concentrations of norepinephrine and epinephrine that were below their respective upper reference limits. A false-positive result of a test for plasma metanephrines that was equal to or above the upper reference limits. We calculated the sensitivity and specificity (with 95% CIs), pretest and post-test probabilities, and positive and negative predictive values for each analyte (24).

Differences in tumor-associated elevations in plasma catecholamine concentrations and free and total metanephrine concentrations were assessed from the fold-increases in plasma concentrations of compounds in patients with pheochromocytoma that were greater than median values in the normotension and essential hypertension reference group. We computed mean \( \pm \) SE fold-increases after logarithmic transformation of individual fold-increases. We estimated differences among fold-increases by analysis of variance; post hoc tests were done with the Schef"e F-test.

Receiver-operating characteristic curves were constructed from the relation between the rates of true-positive and false-positive results (that is, sensitivity compared with 1 minus the specificity) for diagnosis of pheochromocytoma that are based on different upper reference limits for each analyte (25). These curves enabled us to compare the sensitivity and specificity of tests for plasma metanephrines for diagnosing pheochromocytoma with those of tests for plasma catecholamines, as a function of different upper reference limits for each analyte. The areas under the receiver-operating characteristic curves for plasma catecholamines and metanephrines were calculated as summary measures of the diagnostic power that were independent of upper reference limits. We calculated the difference between the two areas and tested them according to the method of Hanley and McNeil (26).

Results

Plasma Concentrations of Catecholamines and Free and Total Metanephrines

Plasma concentrations of free normetanephrine and metanephrine in the normotension and hypertension reference groups were not normally distributed until the data were logarithmically transformed (Figure 1). Ranges of plasma concentrations of normetanephrine and metanephrine were wider and the values were considerably higher \((P < 0.001)\) in patients with pheochromocytoma than in any other patient group (Table 2).

In each group, plasma concentrations of total metanephrines were much higher than concentrations of free metanephrines; only a small proportion (<7%) of the normetanephrine or metanephrine in plasma was in the free form (Table 2). Like the free metanephrines, ranges of plasma concentrations of total metanephrines were much wider and the values much higher \((P < 0.001)\) in patients with pheochromocytoma than in any other patient group. Similarly, ranges of plasma concentrations of norepinephrine and epinephrine were wider in patients with pheochromocytoma than in other groups, but only norepinephrine concentrations were consistently higher \((P < 0.001)\) in patients with pheochromocytoma than in other groups.

Accuracy of Tests for Plasma Metanephrines

The upper reference limits were 0.66 nmol/L for plasma normetanephrine and 0.30 nmol/L for metanephrine. Only 1 of the 52 patients with pheochromocytoma had a plasma concentration of normetanephrine within the normal range (Figure 2, top), that is, a false-negative result.
However, this patient (patient 42; Table 3) also had an elevated plasma metanephrine concentration. Thus, when both metabolites were considered in the diagnosis rather than plasma normetanephrine alone, the number of false-negative results was reduced from 1 to 0, yielding a sensitivity and negative predictive value of 100% (Table 4). In 29 of the 191 patients (15%) without pheochromocytoma, a test for plasma metanephrine or metanephrine yielded a false-positive result (Figure 2). Six of these 29 false-positive results were obtained in patients with renal artery stenosis or renal failure, and 16 were obtained in patients with heart failure.

Accuracy of Tests for Plasma Catecholamines

The upper reference limits were 3.00 nmol/L for nor-epinephrine and 0.54 nmol/L for epinephrine. In contrast to the one patient with pheochromocytoma and a false-negative result of the plasma normetanephrine test, 10 patients had false-negative plasma norepinephrine test results (Figure 2, top). Of these 10 patients, 2 had elevated plasma epinephrine concentrations (patients 32 and 34; Table 3); thus, the number of false-negative results was reduced from 10 to 8 when the diagnosis was based on plasma concentrations of both norepinephrine and epinephrine rather than on norepinephrine concentrations alone. This resulted in a sensitivity of 85% (Table 4). Tests of plasma catecholamines yielded false-positive results in 35 (18%) of the 191 patients without pheochromocytoma (Figure 2). In 19 of these 35 patients, results of tests for plasma metanephrines were also false-positive. Seven of these 35 false-positive results were obtained in patients with renal artery stenosis or renal failure, and 21 were obtained in patients with heart failure.

Accuracy of Tests for Urinary Metanephrines

Twenty-four-hour urine specimens were obtained from 46 of the 52 patients with pheochromocytoma and were not obtained from patients in any other group. The median urinary excretion rate of metanephrines in these patients was 24.2 μmol/d (range, 2.1 to 242 μmol/d). Use of an upper reference limit of 6.8 μmol/d for the urinary excretion of metanephrines in normotensive persons (23) yielded false-negative results in 5 of the 46 patients and a sensitivity of 89% (95% CI, 76% to 96%); all 5 patients had increased plasma concentrations of metanephrines, but only 3 had increased plasma concentrations of catecholamines. Creatinine excretion among the 5 patients with normal urinary excretion of metanephrine was within the normal range (1 to 2.5 g/d). Use of an upper reference limit of 9.5 μmol/d in hypertensive patients (4)
yielded 10 false-negative results and a sensitivity of 78% (CI, 64% to 89%).

Accuracy of Tests for Plasma Metanephrines Compared with Tests for Catecholamines

Tumor-associated elevations in plasma normetanephrine concentrations were 153% greater than those in plasma norepinephrine and 64% greater than those in plasma concentrations of total normetanephrine (Figure 3, top). Tumor-associated elevations in plasma metanephrine concentrations were 70% greater than those in epinephrine concentrations but did not differ from those in total metanephrine concentrations (Figure 3, bottom). Increases in plasma concentrations of total normetanephrine were 54% greater than increases in plasma concentrations of norepinephrine, whereas increases in total metanephrine concentrations were 46% greater than increases in epinephrine concentrations.

Among the 11 patients with pheochromocytoma and equivocal results of tests for normetanephrine or norepinephrine (Table 3), 17 positive results were obtained for tests for metanephrines (normetanephrine, 10 results; metanephrine, 7 results); only 4 positive results were obtained for tests for catecholamines (norepinephrine, 1 result; epinephrine, 3 results). In 7 of these 11 patients, plasma normetanephrine or metanephrine concentrations were elevated more than three times the upper reference limits for metanephrines; no patients had elevations in plasma norepinephrine or epinephrine concentrations greater than three times the respective upper reference limits.

Receiver-operating characteristic curves, which show the relation between rates of true-positive and false-positive results at different decision thresholds (that is, at different upper reference limits of plasma concentrations of metanephrines and catecholamines), confirmed the superiority of tests for plasma metanephrines over tests for plasma catecholamines for the diagnosis of pheochromocytoma, regardless of the reference limits used to define an abnormal test result (Figure 4, top). The area under the curve for plasma metanephrines (0.977 ± 0.015) was greater than that for plasma catecholamines (0.917 ± 0.027) (*P = 0.03).

The relation between pretest and post-test probabilities—estimated from the sensitivity and specificity values listed in Table 4—show that as the prevalence rate (that is, the pretest probabilities) increases, the post-test probabilities similarly increase for diagnoses that are based on plasma concentrations of metanephrines and catecholamines (Figure 4, bottom). The negative predictive value of tests for plasma metanephrines for the diagnosis of pheochromocytoma remained constant at 100% for all prevalence rates, whereas the negative predictive value of

Table 3. Neurochemical Characteristics of 11 Patients with Pheochromocytoma and Normal (False-Negative) Plasma Concentrations of Norepinephrine or Normetanephrine

<table>
<thead>
<tr>
<th>Patient</th>
<th>Clinical Syndrome</th>
<th>Concentrations of Norepinephrine or Normetanephrine</th>
<th>Metanephrines</th>
<th>Catecholamines</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Norepinephrine</td>
<td>Epinephrine</td>
<td>Normetanephrine</td>
</tr>
<tr>
<td>14</td>
<td>Sporadic</td>
<td>2.80</td>
<td>0.20</td>
<td>1.03†</td>
</tr>
<tr>
<td>21</td>
<td>Sporadic</td>
<td>2.90</td>
<td>0.36</td>
<td>3.39†</td>
</tr>
<tr>
<td>27</td>
<td>Sporadic</td>
<td>0.69</td>
<td>0.53</td>
<td>1.98†</td>
</tr>
<tr>
<td>32</td>
<td>Sporadic</td>
<td>2.93</td>
<td>0.93†</td>
<td>4.38†</td>
</tr>
<tr>
<td>34</td>
<td>Sporadic</td>
<td>1.73</td>
<td>1.26†</td>
<td>17.19†</td>
</tr>
<tr>
<td>38</td>
<td>Sporadic</td>
<td>2.42</td>
<td>0.17</td>
<td>9.44†</td>
</tr>
<tr>
<td>42</td>
<td>Cushing</td>
<td>4.21†</td>
<td>1.61†</td>
<td>0.48</td>
</tr>
<tr>
<td>43</td>
<td>von Hippel-Lindau</td>
<td>2.08</td>
<td>0.03</td>
<td>1.69†</td>
</tr>
<tr>
<td>48</td>
<td>Multiple endocrine neoplasia</td>
<td>1.83</td>
<td>0.21</td>
<td>4.10†</td>
</tr>
<tr>
<td>49</td>
<td>Multiple endocrine neoplasia</td>
<td>1.74</td>
<td>0.32</td>
<td>1.28‡</td>
</tr>
<tr>
<td>50</td>
<td>von Hippel-Lindau</td>
<td>2.43</td>
<td>0.04</td>
<td>1.71*</td>
</tr>
<tr>
<td></td>
<td>Upper reference limit</td>
<td>3.00</td>
<td>0.54</td>
<td>0.66</td>
</tr>
</tbody>
</table>

* Denotes a concentration above the upper reference limits.
† Denotes a concentration more than threefold above the upper reference limits.

Table 4. Characteristics of Tests for Plasma Metanephrines and Catecholamines

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
<th>Positive Predictive Value</th>
<th>Negative Predictive Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metanephrines*</td>
<td>100 (52/52) (94 to 100)</td>
<td>85 (162/191) (79 to 90)</td>
<td>64 (52/81)</td>
<td>100 (162/162)</td>
</tr>
<tr>
<td>Total metanephrines</td>
<td>98 (42/43) (68 to 100)</td>
<td>82 (112/137) (74 to 88)</td>
<td>63 (42/67)</td>
<td>99 (112/113)</td>
</tr>
<tr>
<td>Catecholamines</td>
<td>85 (44/52) (72 to 93)</td>
<td>82 (156/191) (75 to 87)</td>
<td>56 (44/79)</td>
<td>95 (156/164)</td>
</tr>
</tbody>
</table>

* "Metanephrines" refers to free normetanephrine and metanephrine. "Total metanephrines" refers to sulfone conjugated and free normetanephrine and metanephrine. The positive predictive value is calculated from patients with both a false-negative plasma test result and a false-negative total metanephrine test result. The specificity of catecholamines was calculated from patients with both a false-negative plasma norepinephrine test result and a false-negative epinephrine test result. The positive predictive value of tests for plasma metanephrines for the diagnosis of pheochromocytoma remained constant at 100% for all prevalence rates, whereas the negative predictive value of
In terms of positive and negative predictive values, measurement of plasma concentrations of total norepinephrine and metanephrine provided no advantage over measurement of free metanephrine concentrations (Table 4). However, measurements of plasma concentrations of total metanephrines provided greater sensitivity for the diagnosis of pheochromocytoma than measurements of plasma concentrations of catecholamines.

Patient and Tumor Characteristics in Relation to Neurochemical Indices

Four patients with pheochromocytoma had von Hippel-Lindau disease, and six had multiple endocrine neoplasia. Two of the patients with von Hippel-Lindau disease had normal plasma concentrations of catecholamines but elevated plasma normetanephrine concentrations (patients 43 and 50; Table 3). One of these patients was asymptomatic; initial testing was done after an adrenal mass was noted during computed tomography for an unrelated condition. In this patient, an elevated plasma normetanephrine concentration provided the only other indication for a tumor; results of all other neurochemical tests (those for plasma catecholamines, urinary metanephrines, clonidine suppression, and glucagon stimulation) were negative. Because of these negative results and for personal reasons, the patient did not have surgery until 11 months later. At this time, she became symptomatic and had elevated plasma concentrations of catecholamines and urinary metanephrines in addition to consistently elevated plasma concentrations of metanephrines. All patients with multiple endocrine neoplasia were symptomatic, but two had normal plasma concentrations of catecholamines (patients 48 and 49; Table 3). Both these patients had grossly elevated plasma concentrations of metanephrines.

In only one patient (patient 42; Table 3) did plasma concentrations of metanephrines provide a tumor marker that was inferior to that provided by plasma catecholamine concentrations. This patient was unusual, presenting with Cushing disease secondary to an adrenocorticotropic-secreting pheochromocytoma.

Thirty-two pheochromocytomas were located in the adrenal glands, and 19 were located at extra-adrenal sites. Patients with the adrenal tumors had higher plasma concentrations of metanephrine than patients with extra-adrenal tumors (0.61 nmol/L compared with 0.27 nmol/L; \(P = 0.03\)). Similarly, plasma concentrations of epinephrine were higher in patients with adrenal tumors than in those with extra-adrenal tumors (0.34 nmol/L compared with 0.14 nmol/L; \(P = 0.01\)). In contrast, plasma concentrations of norepinephrine were higher in patients with extra-adrenal tumors (22.3 nmol/L compared with 8.40 nmol/L; \(P = 0.009\)). Plasma normetanephrine concentrations did not differ among patients with adrenal and extra-adrenal tumors (5.83 nmol/L compared with 5.44 nmol/L; \(P = 0.30\)).

We found strong positive relations between the size of the tumor and plasma concentrations of normetanephrine (\(r = 0.61; P < 0.001\)), plasma concentrations of metanephrine (\(r = 0.45; P = 0.007\)), and urinary excretion of metanephrines (\(r = 0.64; P < 0.001\)). No association was seen between tumor size and plasma concentrations of norepinephrine (\(r = 0.12; P = 0.48\)) or epinephrine (\(r = 0.14; P = 0.42\)).

Discussion

Because a pheochromocytoma secretes catecholamines directly into the circulation, any of several means to detect the tumor by assays of plasma or urinary catecholamines and their metabolites should theoretically prove adequate. However, the occurrence of false-nega-

![Figure 3. Differences among fold-increases in plasma concentrations of norepinephrine (NE), normetanephrine (NMN), and total normetanephrine (top) and differences among fold-increases in plasma epinephrine (EPI), metanephrine (MN), and total metanephrine (bottom) in patients with pheochromocytoma. Fold-increases were estimated from ratios of plasma concentrations of each compound in patients with pheochromocytoma to the respective median value for each compound in the reference group. Results represent mean ± SE values determined from logarithmically transformed values. * = significant difference (\(P < 0.05\)).](image-url)
The relation between pretest probability (that is, prevalence) and post-test probability shows the effect of prevalence of pheochromocytoma and plasma catecholamines.

Predictive values for given test results of plasma metanephrines and catecholamines are tabulated below:

<table>
<thead>
<tr>
<th>Pretest probability of a pheochromocytoma (bottom)</th>
<th>Catecholamines</th>
<th>Metanephrines</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Norepinephrine</td>
<td>Epinephrine</td>
</tr>
<tr>
<td></td>
<td>Normetanephrine</td>
<td>Metanephrine</td>
</tr>
<tr>
<td>1.0 SD</td>
<td>1.93</td>
<td>0.24</td>
</tr>
<tr>
<td>1.5 SD</td>
<td>2.41</td>
<td>0.36</td>
</tr>
<tr>
<td>2.0 SD</td>
<td>3.00</td>
<td>0.54</td>
</tr>
<tr>
<td>2.5 SD</td>
<td>3.75</td>
<td>0.79</td>
</tr>
<tr>
<td>3.0 SD</td>
<td>4.66</td>
<td>1.18</td>
</tr>
<tr>
<td>3.5 SD</td>
<td>5.85</td>
<td>1.76</td>
</tr>
</tbody>
</table>

The relation between pretest probability (that is, prevalence) and post-test probability shows the effect of prevalence of pheochromocytoma on positive (upper curve) and negative (lower curve) predictive values for given test results of plasma metanephrines (○) and plasma catecholamines (●).

Because no test can be completely sensitive, one may ask what our findings of 100% sensitivity and 100% negative predictive value for tests for plasma metanephrines actually mean and in which clinical settings they would apply. In our study, 100% sensitivity resulted from the consideration of normotensive persons and those with essential hypertension as a single reference group. When we considered only persons with essential hypertension as the reference group, one false-negative result occurred, and the sensitivity decreased to 98% (CI, 90% to 100%). However, as shown by the receiver-operating characteristic curves, the sensitivity of tests for metanephrines for the diagnosis of pheochromocytoma remained higher than that of tests for catecholamines, regardless of changes in the upper reference limits. For a disease such as pheochromocytoma, in which a missed diagnosis could have life-threatening consequences for a patient, the diagnostic test should exclude false-negative results as efficiently as possible. The results show that measurements of plasma concentrations of metanephrines provide a method of excluding the presence of a tumor that is superior to measurements of plasma catecholamines.

Although pheochromocytoma may be excluded by normal plasma concentrations of metanephrines, an abnormal test result does not always positively confirm the presence of a tumor. We found elevated plasma concentrations of metanephrines in a few patients with secondary hypertension or cardiac failure. Elevated concentrations of total metanephrines have also been reported in patients with renal failure (27). When plasma concentrations of metanephrines are increased in the presence of either heart failure or kidney disease, additional diagnostic techniques, including imaging studies, are necessary.

Positive and negative predictive values depend on the prevalence of a disease. The predictive values reported here correspond to a 21% prevalence rate of pheochromocytoma—a much higher rate than that in the overall population of patients with hypertension and probably higher than the rate in the hypertensive patients referred to most specialist centers. The negative predictive value of normal plasma concentrations of metanephrines was superior to that of normal plasma catecholamines regardless of prevalence rates (Figure 3, bottom), a disparity that increased with increasing prevalence. Even at the highest prevalence rates, normal plasma concentrations of metanephrines almost exclude diagnosis of pheochromocytoma. This means that in a general practice setting or in patients in whom pheochromocytoma is strongly suspected (for example, patients at a referral center who have hypertension, headache, and adrenal mass), normal plasma concentrations of metanephrines may exclude the diagnosis of pheochromocytoma.
toma, whereas normal plasma concentrations of catecholamines may not.

The additional sensitivity that tests for plasma metanephrines provide over tests for catecholamines for the detection of a pheochromocytoma may be particularly relevant for persons at increased risk for the tumor because of a family history of multiple endocrine neoplasia or von Hippel-Lindau disease. This is shown by the two patients with multiple endocrine neoplasia and the two patients with von Hippel-Lindau disease in whom plasma concentrations of metanephrines, not catecholamines, provided evidence for a tumor. In one of the latter patients, elevated plasma concentrations of metanephrines provided the initial diagnosis 11 months before any presenting symptom. Although this finding is promising, the helpfulness of plasma concentrations of metanephrines in screening asymptomatic persons with hereditary endocrine syndromes remains to be established by studies with larger samples of such patients.

A limitation of our study is the lack of a reference group of patients with a panic disorder syndrome; a diagnosis of pheochromocytoma in these patients must sometimes be excluded (28). Stress-induced elevations of plasma catecholamines in these patients may present a diagnostic challenge. Because mild mental stress causes little change in plasma concentrations of metanephrines despite significant increases in plasma catecholamine concentrations (18), measurements of plasma concentrations of metanephrines may be particularly useful for excluding pheochromocytoma in patients with a panic disorder.

Greater and more consistent tumor-associated increases in plasma concentrations of metanephrines than in catecholamine concentrations explain the better sensitivity of the test for the former for diagnosing pheochromocytoma. Intravenous infusion of catecholamines results in increases in plasma concentrations of metanephrines that are less than 6% of those of the precursor amines (18).

Thus, metabolism of catecholamines after they are released by a tumor into the circulation is not responsible for the greater and more consistent increases in plasma concentrations of metanephrines compared with those in catecholamine concentrations in patients with a pheochromocytoma. The production of 90% of plasma metanephrine as much as 40% of plasma normetanephrine from metabolism of catecholamines within the adrenal glands (18, 29) suggests that metanephrines are produced within the tumor itself. This conclusion is supported by observations of high tumor-tissue concentrations of metanephrines (30, 31) and high plasma normetanephrine concentrations in the venous effluent of pheochromocytomas (32). The conclusion is also supported by our findings reported here and elsewhere (33) that tumor size is a determinant of metabolite production but not of catecholamine release. Thus, even when pheochromocytomas are quiescent and are not releasing catecholamines, they appear to be actively metabolizing catecholamines to metanephrines.

Our results confirm the high sensitivity and specificity of tests for plasma total metanephrines for the diagnosis of pheochromocytoma that has been shown previously (34); however, only concentrations of total metanephrines were considered in that study. We found that measurement of total (unconjugated and conjugated) metanephrines had no advantage over measurement of free (unconjugated) metanephrines. Rather, the presence of a tumor causes relatively larger increases in free normetanephrine concentrations than in total normetanephrine concentrations. It is the free, not the conjugated, metanephrines that are produced within chromaffin tissue (unpublished observations). Thus, although plasma concentrations of total metanephrines are technically easier to measure than plasma concentrations of free metanephrines, the latter yield superior results.

Consistent with findings in previous studies (2, 11), measurement of urinary metanephrines yielded false-negative results in a few patients. Why would a test for urinary metanephrines be less sensitive than a test for plasma concentrations of the same compounds? One explanation is that a small percentage of patients in any large-scale study would be expected to provide an incomplete urine collection; this would yield false-negative results. However, the normal creatinine excretion in the patients with false-negative results of tests for urinary metanephrines rules out this explanation. Another possible explanation is that the assay technique used (23) is a colorimetric method. An assay for urinary metanephrines that uses the high-performance liquid chromatography technique might be superior to the colorimetric technique. Finally, individual differences in the renal conversion of metanephrines to metoxyhydroxyphenylglycol and vanillylmandelic acid might be responsible for some of the false-negative results.

Plasma catecholamines were assayed at two centers, whereas plasma metanephrines were assayed at one center. The involvement of different laboratories in our study could have resulted in wider distributions and higher upper reference limits for plasma catecholamines than might have been obtained had measurements been done in one laboratory. This in turn could have resulted in more false-negative results for plasma catecholamines determined in two laboratories than would have occurred in one. However, separate analysis of the data for the two centers indicated a 14.6% rate of false-negative results for catecholamines assayed at St. Radboud University Hospital compared with 18.2% for those assayed at NIH. In addition, the reference limits of 3.00 nmol/L for norepinephrine and 0.54 nmol/L for epinephrine obtained in our study were substantially lower than those of other studies (4, 6, 10). Because many of the patients with equivocal catecholamine test results had plasma concentrations of catecholamines well below the upper reference limits, a substantial reduction in these limits would be required to influence the results.

In conclusion, normal plasma concentrations of metanephrines exclude a diagnosis of pheochromocytoma, and normal plasma catecholamines or urinary metanephrines do not. Tests for plasma metanephrines are more sensitive than tests for plasma catecholamines or urinary metanephrines for the diagnosis of pheochromocytoma.

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References


