Diagnostic Efficacy of Unconjugated Plasma Metanephrines for the Detection of Pheochromocytoma

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Background: Recently, measurement of plasma metanephrines was suggested to improve the detection of pheochromocytoma compared with the other common biochemical tests.

Objective: To examine the diagnostic precision of measurements of plasma metanephrines, plasma catecholamines, and urinary catecholamines and to assess their variability.

Methods: Plasma metanephrine as well as plasma and urinary catecholamine concentrations were measured by high-performance liquid chromatography with electrochemical detection. Before surgery, responses of plasma metanephrines and catecholamines to change of posture were determined. Intraoperatively, metanephrines and catecholamines were measured before skin incision, during maximal mechanical tumor manipulation, and repetitively after the tumor was separated from the circulation. Patients were reexamined 1 and 3 months after surgery. Patients with pheochromocytoma (n=17) and with histologically proved other adrenal tumors (n=14) were studied before, during, and after surgery.

Results: Measurement of plasma metanephrines and plasma and urinary catecholamines provided 100% and 82% sensitivity, respectively, for the detection of pheochromocytoma (P<.001). Levels of plasma catecholamines but not metanephrines increased in response to change of posture (norepinephrine, P=.03; epinephrine, P=.07) and intraoperative stress (norepinephrine, P=.002; epinephrine, P=.009).

Conclusions: Plasma metanephrines offer improved efficacy for the diagnosis of pheochromocytoma. Less variability in response to external factors may favor plasma metanephrines in the screening for this disease.

Arch Intern Med. 2000;160:2957-2963

D IAGNOSIS OF pheochromocytoma still poses considerable problems, as almost half of patients initially have no or only paroxysmal hypertension or may even be asymptomatic. Even the classic symptom triad of bilateral diffuse headache, sweating, and palpitations provides diagnostic accuracy of only 6%. Autopsy studies show that the disease is not recognized in 20% to 75% of patients during life but represents the cause of death in half of them. Proper diagnosis of pheochromocytoma by highly sensitive biochemical tests therefore remains of paramount importance.

Simple biochemical screening, largely independent of external influences such as posture or stress, in general should improve the diagnostic sensitivity. Improved accuracy in the diagnosis of pheochromocytoma has been suggested with the use of determination of plasma metanephrines (metanephrine and normetanephrine), the O-methylated extraneuronal metabolites of catecholamines, by high-performance liquid chromatography with electrochemical detection. Recently, an extended report by Eisenhofer et al favored plasma metanephrines in particular for oligosymptomatic, and even asymptomatic subjects at high risk for pheochromocytoma, such as patients with von Hippel-Lindau disease or family members of patients with multiple endocrine neoplasia type 2 (MEN 2). However, the superiority of plasma metanephrines has not yet been supported by others.

The aims of this study were (1) to compare the diagnostic efficacy of plasma catecholamines and metanephrines, (2) to investigate the suggested independence of plasma metanephrines of large increases in plasma catecholamine concentrations in patients with pheochromocytoma compared with those with histologically proved other adrenal tumors, and (3) to observe the intraoperative time course of metanephrine and catecholamine levels in patients with pheochromocytoma.
PATIENTS AND METHODS

PATIENTS

We studied 17 patients with pheochromocytoma and 14 patients with histologically verified other adrenal tumors (Table 1). In 3 patients the diagnosis of MEN 2A was proved by identification of germline mutations of the RET proto-oncogene. Fourteen patients with pheochromocytoma (all but 1 with sporadic disease) had sustained hypertension (systolic blood pressure of >140 mm Hg or diastolic blood pressure of >90 mm Hg). Two other patients (1 with sporadic pheochromocytoma and 1 with MEN 2A) had documented periods of intermittent hypertension. All patients with hypertension and an additional patient with normal blood pressure (with the familial disease) also reported symptoms of pheochromocytoma (eg, headache, palpitations, or excessive sweating). Patients with histologically confirmed other adrenal diseases served as a reference group (Table 1).

BIOCHEMICAL ASSAYS

Three 24-hour urine samples (of which the highest concentrations are given) for the determination of normetanephrine and epinephrine were obtained before surgery, and one 24-hour urine collection was obtained at 1 and at 3 months after surgery. Blood samples for the determination of normetanephrine, metanephrine, norepinephrine, and epinephrine in plasma were drawn into 10-mL heparinized tubes through an intravenous cannula in the forearm. Patients rested in the supine position for 20 minutes before blood sampling. Additional blood samples were subsequently collected after 10 minutes in the upright position in all patients. Blood pressure and heart rate were measured repeatedly. None of the patients was taking acetaminophen, which can interfere with the plasma normetanephrine assay.6 Intraoperatively, blood was obtained at baseline (after intubation, before skin incision) and during maximal mechanical tumor manipulation in 15 patients. Blood samples were also drawn from a central venous cannula immediately before and repeatedly after clipping of the adrenal vein to follow the time course of plasma metanephrine as low as 82 pmol/L. Recovery was estimated to range from 90% to 105% of the internal standard. The interassay coefficients of variation were 5% (normetanephrine) and 8% (metanephrine) at physiologic plasma hormone concentrations ±1 SD (normetanephrine, 430±30 pmol/L; metanephrine, 150±10 pmol/L). Upper limits of the normal range were adapted from Lenders et al10 to allow comparison of results (normetanephrine, 660 pmol/L; metanephrine, 300 pmol/L; norepinephrine, 3000 pmol/L; and epinephrine, 340 pmol/L).

Plasma catecholamines were analyzed by reverse-phase high-performance liquid chromatography7 with the use of plasma catecholamine extraction tubes (ESA) for the isolation procedure. Interassay and intra-assay coefficients of variance were less than 5% for both compounds.

Urine samples were collected in plastic flasks containing 10 mL of 25% hydrochloric acid. After extraction by ion-exchange columns and separation by high-performance liquid chromatography, catecholamines were measured by electrochemical detection (Pharmacia KB, Uppsala, Sweden) by means of kits (Chromsystem, Munich, Germany).

DATA ANALYSIS

The 3 tests involved the determination of 2 compounds (plasma normetanephrine and metanephrine, plasma norepinephrine and epinephrine, and urinary norepinephrine and epinephrine). A positive or negative result was defined as normal or elevated values, respectively, for both substances in the pair. Preoperative values were obtained before the institution of ß-blockade in 16 patients. Two patients (1 with life-threatening paroxysms of hypertension and 1 with sustained hypertension >250/140 mm Hg) had been taking this medication for 3 and 10 days before the first blood sample was drawn. Diagnostic procedures were evaluated by sensitivity, specificity, and positive and negative predictive values.10

STATISTICAL ANALYSIS

Comparisons of sensitivity or specificity between plasma metanephrines and other biochemical tests were performed with the use of the McNemar test.11 Median increases of plasma metanephrine and catecholamine concentrations during the upright position test and the maximum intraoperative stress, respectively, were analyzed by the Wilcoxon rank sum test. The differences in the extent of increase above the upper reference limit of normal among the biochemical tests (both preoperatively and during surgery) were compared by analysis of variance with Scheffé post hoc test. The correlation between tumor mass and biochemical test results was described by the Spearman rank correlation.

RESULTS

DIAGNOSTIC EFFICACY

All patients with pheochromocytoma were separated from all patients with other adrenal tumors by determination of plasma normetanephrine level (Figure 1). Plasma metanephrine testing was falsely negative in 5 of the 17 patients with pheochromocytoma. However, these 5 patients (4 with adrenal tumors and 1 with an extra-adrenal sporadic tumor) had elevated normetanephrine concentrations. Thus, measurement of plasma normeta-
nephrine with or without metanephrine was positive in all patients with pheochromocytoma. In contrast, plasma norepinephrine level was normal in 4 patients with pheochromocytoma (2 with sporadic and 2 with familial adrenal tumors). Plasma epinephrine level was normal in 9 of 17 patients with pheochromocytoma (2 with familial adrenal tumors, 6 with sporadic adrenal tumors, and 1 with a sporadic extra-adrenal tumor). Three of these patients had elevated plasma norepinephrine concentrations. Both plasma norepinephrine and epinephrine levels were therefore normal in 3 of 17 patients with pheochromocytoma (Figure 1).

Table 1. Demographic and Clinical Characteristics of Patients With Pheochromocytoma and With Histologically Verified Other Adrenal Tumors

<table>
<thead>
<tr>
<th></th>
<th>Pheochromocytoma (n = 17)</th>
<th>Other Adrenal Tumors (n = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, No. F:M</td>
<td>14:3</td>
<td>10:4</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>Sporadic pheochromocytoma (n = 14)</td>
<td>Hormonally inactive (n = 6)</td>
</tr>
<tr>
<td></td>
<td>MEN 2A (n = 3)</td>
<td>Conn syndrome (n = 5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cushing disease (n = 2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cushing adrenal adenoma (n = 1)</td>
</tr>
<tr>
<td>Age, mean (SD), y</td>
<td>46 (5)</td>
<td>44 (3)</td>
</tr>
<tr>
<td>Hypertension, No./Total No. (%)</td>
<td>14/17 (82)</td>
<td>7/13 (54)</td>
</tr>
<tr>
<td>Diameter on CT, mean (SD), cm</td>
<td>5.5 (2.5)</td>
<td>5.0 (3.0)</td>
</tr>
<tr>
<td>MIBG scan abnormal</td>
<td>15/17</td>
<td>ND</td>
</tr>
<tr>
<td>Surgery, No.</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Endoscopic</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Open</td>
<td>0</td>
</tr>
</tbody>
</table>

*MEN indicates multiple endocrine neoplasia; CT, computed tomography; MIBG, iodine 131\(^{131}\text{I}\) meta-iodobenzyl guanidine; and ND, not done.

Figure 1. Preoperative concentrations, expressed as percentages of the upper reference limit (dashed line), for plasma metanephrines, plasma catecholamines, and urinary excretion of catecholamines. Data are presented for individual patients with pheochromocytoma (PHEO) and histologically verified other adrenal tumors (OAT).
Similarly, urinary norepinephrine concentration was normal in 5 patients with pheochromocytoma (3 with sporadic and 2 with familial adrenal tumors). One of these patients (with sporadic adrenal disease) had elevated urinary epinephrine concentration. Seven patients with pheochromocytoma (1 with a familial tumor, 5 with sporadic adrenal tumors, and 1 with a sporadic extra-adrenal tumor) had urinary epinephrine concentrations within the normal range. Four of them, however, had elevated urinary norepinephrine excretion. Overall, urinary norepinephrine and epinephrine levels were within the normal range in 3 of 17 patients with pheochromocytoma (Figure 1).

In patients with pheochromocytoma, plasma normetanephrine concentrations were increased up to approximately 1000% above the upper reference limit (Figure 1), which was not larger (P = .77) than the increase in plasma norepinephrine level but considerably higher (P < .001) than that in plasma metanephrine (600%) and epinephrine (400%) concentrations, and in the urinary excretion of norepinephrine (600%) and epinephrine (550%).

**POSTURE DEPENDENCE**

Mean plasma norepinephrine concentration increased (P < .03) in all patients with pheochromocytoma by 300% in response to change to the upright position (Figure 2). The median increase was 19858 pmol/L (range, 1478-35460 pmol/L), whereas plasma normetanephrine concentrations did not change significantly. Plasma epineph-

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**Table 2. Diagnostic Efficacy of Biochemical Tests for the Detection of Pheochromocytoma**

<table>
<thead>
<tr>
<th>Biochemical Test</th>
<th>No./Total No. (%)</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Negative Predictive Value</th>
<th>Positive Predictive Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma normetanephrine and metanephrine</td>
<td>17/17 (100)</td>
<td>14/14 (100)</td>
<td>14/14 (100)</td>
<td>17/17 (100)</td>
<td></td>
</tr>
<tr>
<td>Plasma norepinephrine and epinephrine</td>
<td>14/17 (82)</td>
<td>14/14 (100)</td>
<td>14/17 (82)</td>
<td>14/17 (82)</td>
<td></td>
</tr>
<tr>
<td>Urinary norepinephrine and epinephrine</td>
<td>14/17 (82)</td>
<td>13/14 (94)</td>
<td>16/17 (94)</td>
<td>14/17 (82)</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 2.** Plasma metanephrines and catecholamines in the recumbent and upright positions in patients with pheochromocytoma (PHEO) and those with other adrenal tumors (OAT). Individual absolute concentrations are given. Median values are represented by full horizontal lines. Blood samples were drawn after 20 minutes in the recumbent position and subsequently after 10 minutes in the upright position from an indwelling forearm catheter. To convert values for plasma measurements to picomoles per liter, multiply by 5.46 for normetanephrine, 5.91 for norepinephrine, 5.08 for metanephrine, and 5.46 for epinephrine.
rine displayed a trend ($P = .07$) to rise (median increase, 546 pmol/L; range, 27-8190 pmol/L) in 14 patients. Four patients (who had adrenal pheochromocytoma with normal plasma epinephrine levels) had identical or lower plasma epinephrine concentrations in the upright compared with the supine position. Plasma metanephrine concentrations were not significantly different.

Similarly, plasma norepinephrine level was increased ($P = .02$) in the upright posture, while plasma concentrations of epinephrine ($P = .09$), normetanephrine, and metanephrine were not significantly higher in patients with other adrenal tumors (Figure 2).

**INTRAOPERATIVE STRESS DEPENDENCE**

Plasma norepinephrine concentrations rose ($P = .002$) markedly during surgery (Figure 3) in all patients with pheochromocytoma (median increase, 164000 pmol/L; range, 6800-2240000 pmol/L) when measured during maximal mechanical tumor manipulation and compared with that before skin incision. Plasma normetanephrine level did not change significantly (median increase, 7370 pmol/L; range, 300-22600 pmol/L). Plasma epinephrine level increased ($P = .009$) markedly in all patients (median increase, 24730 pmol/L; range, 440-371280 pmol/L), while plasma metanephrine level did not. Again, the extent of increase above the upper reference limit of normal in response to intraoperative mechanical stress was higher ($P < .001$) for plasma catecholamines than for plasma metanephrines. Similar results were obtained in patients with other adrenal tumors (Figure 3).

The size of the tumor in patients with pheochromocytoma but not in those with other adrenal tumors correlated positively with the preoperative plasma concentrations of normetanephrine ($r = 0.70$; $P < .001$) and metanephrine ($r = 0.62$; $P < .001$) but not with plasma norepinephrine ($r = 0.18$; $P = .86$) or epinephrine ($r = 0.28$; $P = .68$).

**INTRAOPERATIVE TIME COURSE OF PLASMA METANEPRHINES AND CATECHOLAMINES**

Plasma concentrations of catecholamines and metanephrines were studied in 6 patients during unilateral laparoscopic adrenal surgery for sporadic ($n = 5$) and familial ($n = 1$) pheochromocytoma (Figure 4). A transient increase in catecholamine level and, to a lesser extent, in metanephrine level was seen after clipping of the adrenal vein and before a fall in the respective plasma concentrations.

**POSTOPERATIVE EVALUATION**

Fourteen (88%) of 16 patients displayed normal biochemical results in all tests at 3 months after surgery (median values, 290 pmol/L for normetanephrine, 76 pmol/L for metanephrine, 1890 pmol/L for norepinephrine, and 87 pmol/L for epinephrine). One patient with MEN 2A displayed continuously increasing plasma metanephrine concentrations at 1 month (290 pmol/L), 3 months (533 pmol/L), and 6 months (645 pmol/L) after unilateral adrenal surgery despite a negative postoperative iodine 131 [$^{131}$I] meta-iodobenzyl guanidine (MIBG) scan.
Another patient with the familial disease had increased plasma metanephrine level (498 pmol/L), borderline plasma normetanephrine level (628 pmol/L), and elevated urinary epinephrine excretion (41 µg/24 h). This patient remained asymptomatic and refused further diagnostic and therapeutic procedures.

**COMMENT**

Plasma concentrations of unconjugated normetanephrine and metanephrine showed superior sensitivity ($P < 0.01$) for the diagnosis of pheochromocytoma compared with plasma and urinary catecholamines. While plasma and urinary catecholamines were increased in only 83% of patients, plasma metanephrines were elevated in all patients. This is in keeping with previous findings in sporadic pheochromocytoma and, more recently, MEN 2 and von Hippel–Lindau disease. In particular, all patients with pheochromocytoma in our study could be identified by determination of plasma normetanephrine alone. Thus, one patient with a small pheochromocytoma and normal plasma normetanephrine concentrations described by Eisenhofer et al continued to represent the only false-negative normetanephrine result reported so far in histologically confirmed pheochromocytoma.

The present study also found that plasma metanephrine concentrations are largely unsusceptible to external influences such as change of posture (Figure 2) or the mechanical stress imposed by either endoscopic or open surgery (Figure 3). In contrast, large increases in plasma catecholamine levels were observed in response to these procedures, findings that were observed equally in patients with pheochromocytoma and those with histologically documented other adrenal tumors. This is in keeping with results obtained by others in humans (by means of glucagon stimulation or insulin hypoglycemia tests) that show plasma metanephrine levels to be insensitive to short-term increase of plasma catecholamine levels. This independence of external influences indicates that determination of plasma metanephrine levels will not require time-consuming standardization of blood sampling necessary for correct determination of plasma catecholamine levels and will be therefore more suitable as a screening test.

Tumor volume was highly correlated with plasma metanephrine concentrations but not with plasma catecholamine levels, supporting evidence that metanephrine...
rines are produced by catechol-O-methyltransferase within tumors. This is in keeping with previous findings in sporadic pheochromocytoma.

While the adrenal gland constitutes the single largest source of metanephrine and normetanephrine, contributing more than 90% of metanephrine and up to 40% of normetanephrine in plasma, only approximately 7% of plasma norepinephrine is derived from the adrenal glands, with the remainder being derived from sympathetic nerves. About 3.5 times more norepinephrine than normetanephrine that is normally secreted by the adrenal glands would therefore have to be produced by an adrenal pheochromocytoma to increase the respective plasma concentrations to above the upper limit of normal, which has been suggested to explain the higher sensitivity of plasma normetanephrine for detection of pheochromocytoma.

Plasma norepinephrine concentrations, on the other hand, do not directly reflect sympathetic neural activity despite the fact that sympathetic nerves contribute by approximately 93% to plasma norepinephrine. Sympathetic neural activity, ie, the body's adaptation to "flight-or-fight" situations (including increased heart rate, blood pressure, or sweating), is accomplished by only a small portion of the neuronally produced transmitter. Most secreted norepinephrine undergoes neuronal reuptake, either into the axon itself or into dendrites of nearby cells in an autocrine and paracrine fashion to modify overall sympathetic activity. Alternatively, norepinephrine can escape from nerve cells into plasma. The spillover of norepinephrine from the neural cleft, the reuptake into the nerve terminal, and the local metabolism of norepinephrine may differ considerably from one stress situation to another, explaining the lack of correlation between plasma norepinephrine concentrations and clinical features such as blood pressure.

Plasma catecholamine levels are therefore, by nature, imprecise markers of a disease such as pheochromocytoma.

Intraoperatively, plasma catecholamines and metabolites would be expected to rapidly decline after maximal mechanical tumor manipulation. Repetitive blood sampling, however, showed a transient increase of both metanephrine and catecholamine concentrations, even after clipping of the adrenal vein, and a slow decline thereafter (Figure 3). Release of catecholamines and metabolites from as yet undefined compartments could explain this phenomenon after the tumor is separated from blood circulation. In addition, the slow decline in the subsequent period suggests continuous production and/or release from storage sites (eg, platelets, endothelium) of plasma catecholamines and metabolites after clipping of the adrenal vein, not compatible with half-lives of 1 to 2 minutes (catecholamines) and 3 to 5 minutes (metanephrines) reported in clearance studies of these compounds.

In conclusion, determination of plasma metanephrine and, in particular, normetanephrine levels offers improved efficacy for the detection of pheochromocytoma. Less variability in response to external factors may favor determination of metanephrine levels in screening for and postoperative control of the disease.

Accepted for publication April 28, 2000.

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