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Biochemical Diagnosis of Pheochromocytoma: How to Distinguish True- from False-Positive Test Results

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Measurements of plasma normetanephrine and metanephrine provide a highly sensitive test for diagnosis of pheochromocytoma, but false-positive results remain a problem. We therefore assessed medication-associated false-positive results and use of supplementary tests, including plasma normetanephrine responses to clonidine, to distinguish true- from false-positive results. The study included 208 patients with pheochromocytoma and 648 patients in whom pheochromocytoma was excluded. Clonidine-suppression tests were carried out in 48 patients with and 49 patients without the tumor. Tricyclic antidepressants and phenoxybenzamine accounted for 41% of false-positive elevations of plasma normetanephrine and 44–45% those of plasma and urinary norepinephrine. High plasma normetanephrine to norepinephrine or metanephrine to epinephrine ratios were strongly predictive of pheochromocytoma. Lack of decrease and elevated plasma levels of norepinephrine or normetanephrine after clonidine also confirmed pheochromocytoma with high specificity. However, 16 of 48 patients with pheochromocytoma had normal levels or decreases of norepinephrine after clonidine. In contrast, plasma normetanephrine remained elevated in all but 2 patients, indicating more reliable diagnosis using normetanephrine than norepinephrine responses to clonidine. Thus, in patients with suspected pheochromocytoma and positive biochemical results, false-positive elevations due to medications should first be eliminated. Patterns of biochemical test results and responses of plasma normetanephrine to clonidine can then help distinguish true- from false-positive results. (*J Clin Endocrinol Metab* 88: 2656–2666, 2003)

DIAGNOSIS OF PHEOCHROMOCYTOMA depends crucially on biochemical evidence of catecholamine production by the tumor, best achieved using plasma or urinary measurements of normetanephrine and metanephrine, the respective *O*-methylated metabolites of norepinephrine and epinephrine (1). In particular, normal plasma concentrations of free metanephrines (normetanephrine and metanephrine) exclude all but the smallest of pheochromocytomas, whereas normal plasma or urinary catecholamines do not (2–4).

A remaining problem, common to all biochemical tests used for diagnosis of pheochromocytoma, is that a high value for normetanephrine or metanephrine does not necessarily prove the presence of a tumor. False-positive results must be expected when the normal range for a test is set at anything less than the 100% confidence intervals of a reference population. False-positive results usually, however, occur more frequently than expected, probably due to differences in clinical characteristics of reference and patient populations and reduced control over sources of interference and sampling conditions.

In one study, the specificity of plasma free metanephrines, although higher than for plasma or urinary catecholamines or urinary fractionated metanephrines, was as low as 82% in patients with signs and symptoms suggesting pheochromocytoma (4). Another study from an independent center confirmed the high diagnostic sensitivity of plasma-free metanephrines, but noted a specificity of 85% that was less than that for urinary catecholamines and total metanephrines (5). At the usual low prevalence of pheochromocytoma among patients tested for the tumor, such values for specificity mean that false-positive results will greatly exceed true-positive results. This high frequency of false-positive results poses a diagnostic dilemma for clinicians, often requiring extensive and costly follow-up tests, and in many cases instigating unnecessary attempts to localize a suspected tumor. Before imaging studies are undertaken, it is preferable to conclusively establish the presence of a tumor by biochemical testing.

The present study examined medication-associated causes of false-positive results and the use of supplementary biochemical tests to further exclude or confirm pheochromocytoma in patients where initial tests yielded positive, but equivocal results. In particular, the study examined the utility of measurements of plasma normetanephrine responses to clonidine to distinguish positive results due to sympathetic activation from those due to a pheochromocytoma.

Subjects and Methods

Subjects

Patients were tested for pheochromocytoma at the NIH (Bethesda, MD), at St. Radboud University Medical Center (Nijmegen, The Netherlands), or at Sahlgren's University Hospital (Gothenburg, Sweden). Testing was based solely on the presence of signs and symptoms suggestive of pheochromocytoma (*e.g.* therapy-resistant or paroxysmal hy-

pertension and sweating, headache or palpitations) in 401 patients or on the incidental finding of an adrenal mass in 26 patients. In another 409 patients, testing was carried out as part of routine screening for hereditary pheochromocytoma or because of clinical suspicion of a tumor in patients with a family history of pheochromocytoma. Similarly, testing was carried out in a further 20 patients at high risk for pheochromocytoma because of a previous history of sporadic pheochromocytoma.

Pheochromocytoma was confirmed in 208 patients by pathological examination of surgically resected or biopsied tumor tissue or a diagnosis of inoperable metastatic disease by imaging studies. Pheochromocytoma was excluded in 648 patients by one or more of the following criteria: negative abdominal imaging studies, pathological examination of a surgically resected adrenal mass, normal biochemical results, or lack of the tumor on patient follow-up 2 yr or more after initial testing. Studies were approved by the appropriate Institutional Review Boards and all patients gave informed consent.

Biochemical tests

Samples of blood were drawn into 10-ml heparinized tubes via a forearm iv cannula with patients supine for at least 20 min before sampling. Patients were instructed to fast and abstain from caffeinated and decaffeinated beverages overnight before blood sampling. Plasma was analyzed for concentrations of catecholamines and free metanephrines using HPLC with electrochemical detection (6, 7). Twenty-four-hour urine collections were also obtained in most patients and analyzed for catecholamines or fractionated metanephrines by HPLC with electrochemical detection (8). Upper reference limits for biochemical tests were as established previously (2, 4).

Medication-associated false-positive results

All patients tested for pheochromocytoma were instructed to avoid taking acetaminophen for 5 d before blood sampling because of known analytical interference of the drug with assays of plasma free metanephrines (7). Patients did not discontinue other medications but instead were instructed to provide a listing of all medications taken at the time of initial testing. Such listings were obtained from 510 patients in whom the tumor was subsequently excluded according to the criteria outlined above.

Clonidine-suppression testing

Clonidine-suppression tests were carried out in 97 patients. Pheochromocytoma was confirmed pathologically in 48 patients, including 20 with von Hippel-Lindau syndrome and 4 with multiple endocrine neoplasia type 2. These patients were aged 34 ± 16 yr (mean ± SD) and included 24 females and 24 males. The other 49 patients without the tumor were aged 51 ± 14 yr and included 27 females and 22 males. Five had von Hippel-Lindau syndrome, and 44 underwent testing because of signs and symptoms suggesting pheochromocytoma.

Several medications, including diuretics, tricyclic antidepressants, and β blockers, have been described to interfere with norepinephrine responses to the clonidine suppression test (9–11). Additionally, profound hypotensive responses to clonidine can be troublesome in some patients taking certain antihypertensive medications. When indicated, such medications were withdrawn for a period of 1–5 d, depending on the plasma half-life of the particular drug.

All clonidine suppression tests were carried out after an overnight fast with patients supine. A baseline blood sample was drawn into a 10-ml heparinized tube through a forearm venous cannula after at least 20 min of supine rest. Clonidine was then given orally at a dose of 0.3 mg (for a 60–80 kg subject), adjusted for body weight as necessary. A second blood sample was obtained 3 h after administration of clonidine with patients remaining supine.

There are several criteria for what constitute positive or negative results for the clonidine-suppression test. One criterion for a normal response to clonidine is a fall in plasma norepinephrine to within the normal range (12–15). Another is the magnitude of the fall of plasma norepinephrine, with a fall to less than 50% of baseline values considered normal (14–16). Used alone and in combination both criteria have limitations (10, 14, 16–19). In the present study, we examined a combination of the above criteria, and extended the analyses to use of plasma normetanephrine responses to clonidine.

Statistical analysis

Data are presented as means ± SD. Two-sided Student's *t* tests, χ^2 tests, McNemar's test, and ANOVA with Scheffé's test were used to compare results among groups.

Results

Patterns of biochemical test results

Patients with pheochromocytoma showed more consistent elevations of plasma free metanephrines than of catecholamines (Fig. 1). Forty-seven out of 208 (23%) patients with pheochromocytoma had normal plasma concentrations of norepinephrine (<498 ng/liter; <2.95 nmol/liter), compared with only 8 (4%) with normal levels of normetanephrine (<112 ng/liter; <0.61 nmol/liter). Similarly, 142 patients (68%) had normal plasma concentrations of epinephrine (<83 ng/liter; <0.45 nmol/liter), compared with 98 (47%) with normal levels of metanephrine (<61 ng/liter; <0.31 nmol/liter). Among the 47 patients with normal plasma levels of norepinephrine, epinephrine levels were also normal in 36 patients.

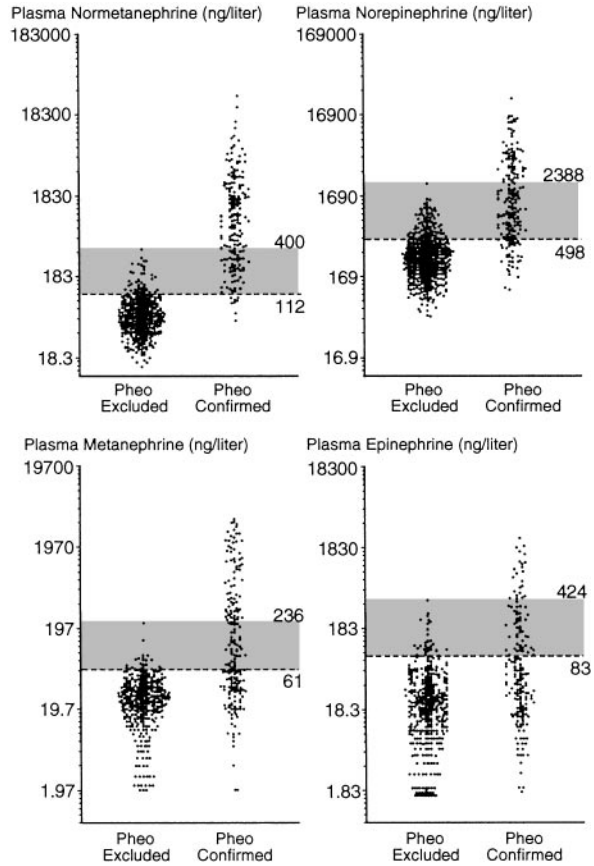

FIG. 1. Plasma concentrations of metanephrines and catecholamines in patients without pheochromocytoma (Pheo Excluded) and with pheochromocytoma (Pheo Confirmed). *Dashed horizontal lines* show the upper reference limits for each test. The *gray areas above the dashed lines* illustrate the range of overlap of false-positive with true-positive results. To convert values to nanomoles per liter, divide by 183 for normetanephrine, 169 for norepinephrine, 197 for metanephrine, and 183 for epinephrine.

Twenty-five of the 36 patients (69%) with pheochromocytoma who had normal plasma levels of norepinephrine and epinephrine were tested because of a hereditary predisposition for pheochromocytoma. Over half of these patients (56%) were both asymptomatic and normotensive. However, 9 patients (36%) reported intermittent symptoms likely due to pheochromocytoma, one of whom had a documented paroxysmal increase in blood pressure recorded after the onset of symptoms. This patient was otherwise normotensive. In the two other patients with familial pheochromocytoma who had documented high blood pressure, hypertension was sustained and appeared unrelated to the tumor. An additional 2 of the 36 patients (6%) with normal plasma catecholamines were tested because of an adrenal incidentaloma. Both these patients were normotensive and asymptomatic. Among the remaining nine patients with normal plasma catecholamines, three were tested because of a previous history of pheochromocytoma and six were tested because of signs and symptoms due to a subsequently confirmed sporadic pheochromocytoma. In one of these patients, hypertension was sustained and in two urinary catecholamines were also normal, but plasma free metanephrines were elevated.

Plasma concentrations of catecholamines and free metanephrines in patients with pheochromocytoma were higher, but showed some overlap with concentrations in patients in whom the tumor was excluded (Fig. 1). The extent of overlap was, however, smaller for plasma free metanephrines than for catecholamines. Only 32% of patients with pheochromocytoma had plasma concentrations of normetanephrine below the highest false-positive level (400 ng/liter; 2.19 nmol/liter) observed in patients without the tumor, compared with 67% for norepinephrine. Similarly, 62% of patients with pheochromocytoma had plasma concentrations of metanephrine below the highest false-positive level (236 ng/liter; 1.20 nmol/liter), compared with 87% for epinephrine.

In contrast to the higher frequency of true-positive results for plasma metanephrines than catecholamines, there was a lower frequency of false-positive results for metanephrines than catecholamines (Fig. 1). There were 104 patients (16%) with false-positive elevations of plasma norepinephrine compared with 55 (8%) with false-positive elevations of normetanephrine, these including 38 (6%) with false-positive elevations of both amines. Other false-positive elevations were observed in 36 patients for plasma epinephrine and 31 for plasma metanephrine, including 16 with false-positive elevations of both amines.

Patients with pheochromocytoma who had elevations of plasma metanephrines or catecholamines below the highest respective levels in patients without pheochromocytoma showed different patterns of biochemical results than those observed in patients with false-positive results (Table 1). In contrast to similar plasma levels of catecholamines, concentrations of metanephrines in patients with true-positive results were 7.5- to 9.1-fold higher ($P < 0.001$) than in patients with false-positive results. Thus, ratios of plasma normetanephrine to norepinephrine or of metanephrine to epinephrine were 6.8- to 7.7-fold higher ($P < 0.001$) in patients with true-positive compared with false-positive results.

No patient with a false-positive elevation of normetanephrine or norepinephrine had a normetanephrine to norepinephrine ratio greater than 0.52, and no patient with a false-positive elevation of metanephrine or epinephrine had a metanephrine to epinephrine ratio greater than 4.2 (Fig. 2). In contrast, normetanephrine to norepinephrine ratios were greater than 0.52 in 20 of the 64 (31%) patients with pheochromocytoma who had elevations of normetanephrine in the false-positive range (112–400 ng/liter; 0.61–2.19 nmol/liter); and metanephrine to epinephrine ratios were greater than 4.2 in 6 of 39 (15%) patients with pheochromocytoma who had elevations of metanephrine levels in the false-positive range (61–236 ng/liter; 0.31–1.20 nmol/liter).

TABLE 1. Patterns of biochemical test results in patients with true positive and false positive results

Biochemical measurement	True positive[a]	False positive[b]
Plasma normetanephrine or norepinephrine	(n = 141)	(n = 121)
Normetanephrine (ng/liter)	1112 ± 2330	123 ± 64[c]
Norepinephrine (ng/liter)	929 ± 556	710 ± 311[d]
Plasma metanephrine or epinephrine	(n = 93)	(n = 51)
Metanephrine (ng/liter)	473 ± 663	63 ± 35[c]
Epinephrine (ng/liter)	123 ± 99	128 ± 92

Data are given as means ± SD. To convert values to nanomoles per liter, divide by 183 for normetanephrine, 169 for norepinephrine, 197 for metanephrine, and 183 for epinephrine.

[a] True positive results are restricted to data where either of a pair of results for normetanephrine or norepinephrine or a pair of results for metanephrine or epinephrine are lower than the highest results in patients in whom pheochromocytoma was excluded.

[b] False positive results are restricted to data where either of a pair of results for normetanephrine or norepinephrine or a pair of results for metanephrine or epinephrine are above the upper reference limits for those analytes.

[c] $P < 0.001$, compared with patients with true positive results.

[d] $P < 0.05$, compared with patients with true positive results.

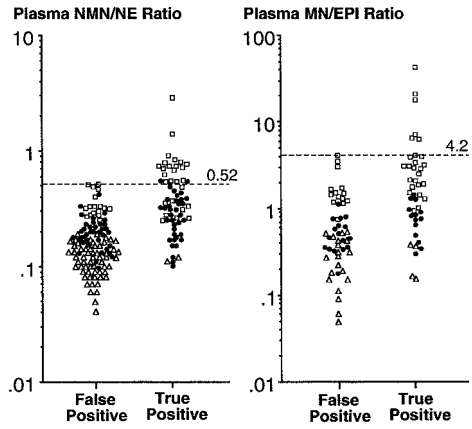

FIG. 2. Ratios of plasma concentrations of normetanephrine to norepinephrine (NMN/NE) and of metanephrine to epinephrine (MN/EPI) in patients with false-positive results and true-positive results within the equivocal range as shown in Fig. 1. Results are shown for three groups of patients: (□) Patients with equivocal elevations of normetanephrine (112–400 ng/liter; 0.61–2.19 nmol/liter) or metanephrine (61–236 ng/liter; 0.31–1.20 nmol/liter) and normal plasma concentrations of catecholamines; (Δ) patients with equivocal elevations of norepinephrine (498–2388 ng/liter; 2.95–14.13 nmol/liter) or epinephrine (83–424 ng/liter; 0.45–2.32 nmol/liter) and normal plasma concentrations of metanephrine; (●) Patients with equivocal elevations of metanephrines and catecholamines. *Dashed horizontal lines* show the highest ratio in patients with false-positive results.

Medication-associated false-positive results

Phenoxybenzamine and tricyclic antidepressants were the medications most frequently associated with false-positive results, together accounting for 41–45% of all elevated plasma levels of normetanephrine and norepinephrine in patients without pheochromocytoma (Table 2). Patients taking these medications had 1.9- to 2.6-fold higher ($P < 0.001$) plasma concentrations or urinary outputs of normetanephrine and norepinephrine and 2.3- to 7.7-fold higher ($P < 0.02$) likelihoods of false-positive results compared with patients taking other drugs or no medications. These influences were restricted to norepinephrine and normetanephrine and not to epinephrine and metanephrine.

Seven patients with elevated plasma concentrations of norepinephrine and normetanephrine on phenoxybenzamine had consistently lower plasma concentrations of norepinephrine ($P = 0.011$) and normetanephrine ($P = 0.015$) when off the drug (Fig. 3). Also, compared with patients taking tricyclics, those taking selective serotonin reuptake blockers had lower plasma concentrations ($P < 0.001$) and lower rates of false-positive results for plasma norepinephrine ($P < 0.001$) and normetanephrine ($P = 0.016$).

In contrast to the influence of phenoxybenzamine, selective α_1-adrenoceptor blocking drugs such as doxazosin, terazosin, and prazosin were not associated with an increased frequency of false-positive results for plasma norepinephrine or normetanephrine. These medications were, however, associated with a 4-fold higher ($P = 0.017$) frequency of false-positive elevations of urinary norepinephrine (7% *vs.* 27%) but were without influence on other urinary analytes.

β-Adrenoceptor blocking drugs, such as atenolol, metoprolol, propranolol, and including the combined α- and β-adrenoceptor blocker, labetolol, were not associated with an increased frequency of false-positive elevations of plasma normetanephrine, norepinephrine or epinephrine, as measured using the assays in this study. However, as a group these medications were associated with 60% (9/15) of all false-positive elevations of plasma metanephrine. Although

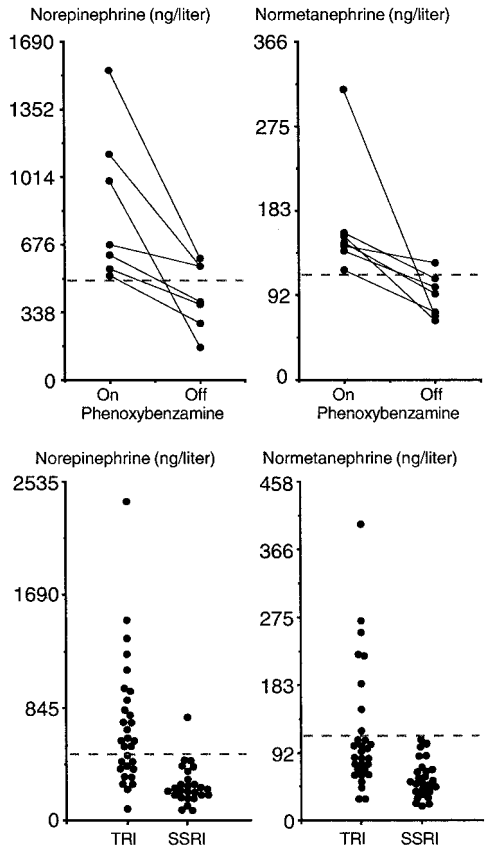

FIG. 3. Effects of phenoxybenzamine and tricyclic antidepressants on plasma concentrations of norepinephrine and normetanephrine. The effects of phenoxybenzamine were studied in seven patients without pheochromocytoma, six studied while on and after discontinuation of the drug and one studied before and after commencement of therapy. The effects of tricyclic antidepressants (TRI) in 30 patients without pheochromocytoma were compared with those of selective serotonin uptake inhibitors (SSRI) in a separate group of 26 patients without the tumor. The *dashed horizontal lines* show the upper reference limits for each test. To convert values to nanomoles per liter, divide by 183 for normetanephrine and 169 for norepinephrine.

TABLE 2. False positive test results in patients taking phenoxybenzamine or tricyclics

Biochemical measurement	Control[a] (n = 338–452)	Phenoxybenzamine (n = 16–28)	Tricyclics (n = 17–30)
Plasma normetanephrine			
Concentration (ng/liter)[b]	62 ± 33	115 ± 66[d]	117 ± 82[d]
False-positive rate[c]	7% (30/452)	46% (13/28)[d]	27% (8/30)[d]
Plasma norepinephrine			
Concentration (ng/liter)[b]	294 ± 163	639 ± 389[d]	710 ± 470[d]
False-positive rate[c]	9% (42/452)	57% (16/28)[d]	60% (18/30)[d]
Urinary normetanephrine			
24-h output (μg/d)[b]	300 ± 179	602 ± 430[d]	783 ± 564[d]
False-positive rate[c]	25% (87/344)	56% (9/16)[e]	71% (12/17)[d]
Urinary norepinephrine			
24-h output (μg/d)[b]	44 ± 32	98 ± 20[d]	91 ± 56[d]
False-positive rate[c]	7% (28/375)	54% (13/24)[d]	45% (9/20)[d]

[a] The control group represents patients in whom pheochromocytoma was excluded and in whom a listing of medications was available that excluded phenoxybenzamine or tricyclic antidepressants.

[b] Plasma concentrations and 24-h urinary outputs are shown as means ± SD. To convert values for plasma concentrations to nanomoles per liter or urinary outputs to μmoles/d, divide by 183 for normetanephrine and by 169 for norepinephrine.

[c] False positive rates are shown as percentage values calculated from the number of false positives divided by the total number of false positive and true negative values shown in *parentheses*.

[d] $P < 0.001$, compared with control.

[e] $P < 0.02$, compared with control.

the frequency of elevated plasma concentrations of metanephrine in patients taking β-adrenoceptor blockers was not high, it was substantially higher ($P < 0.001$) than in patients not taking these drugs (12.5% *vs.* 1.6%).

β-Adrenoceptor blockers were also associated with a 2.6-fold higher ($P = 0.03$) frequency of false-positive elevations of urinary norepinephrine, a 6.5-fold higher ($P = 0.003$) frequency of false-positive elevations of urinary epinephrine, a 2.1-fold higher ($P < 0.001$) frequency of false-positive elevations of urinary normetanephrine, and a 2.2-fold increase ($P = 0.024$) in false-positive elevations of urinary metanephrine. These influences did not appear to be associated with any specific type of β-adrenoceptor blocker.

Calcium channel blockers were associated with a 3-fold higher ($P = 0.003$) frequency of false-positive elevations of plasma norepinephrine, but not plasma normetanephrine, epinephrine, or metanephrine. These drugs were also associated with a 3-fold higher ($P = 0.01$) frequency of false-positive results for urinary norepinephrine, a 5-fold higher ($P = 0.02$) frequency of false-positive elevations of urinary epinephrine but had no effects on frequencies of false-positive results for urinary normetanephrine and metanephrine. In contrast to the above antihypertensives, diuretics, angiotensin-converting enzyme inhibitors, and angiotensin-1 receptor blockers appeared to have little influence on frequencies of false-positive biochemical results for any of the analytes measured in either plasma or urine.

All of 7 patients taking sympathomimetics (*e.g.* pseudoephedrine) had false-positive elevations of urinary normetanephrine or metanephrine compared with only 2 of 10 patients with elevations of plasma normetanephrine or metanephrine. In three patients with highly elevated urinary outputs of metanephrine (4393, 2561, and 1064 μg/d), the cause was traced to buspirone, an anxiolytic agent known to interfere with HPLC analysis of urinary metanephrine (20). There were no effects of this drug on HPLC analysis of plasma metanephrine.

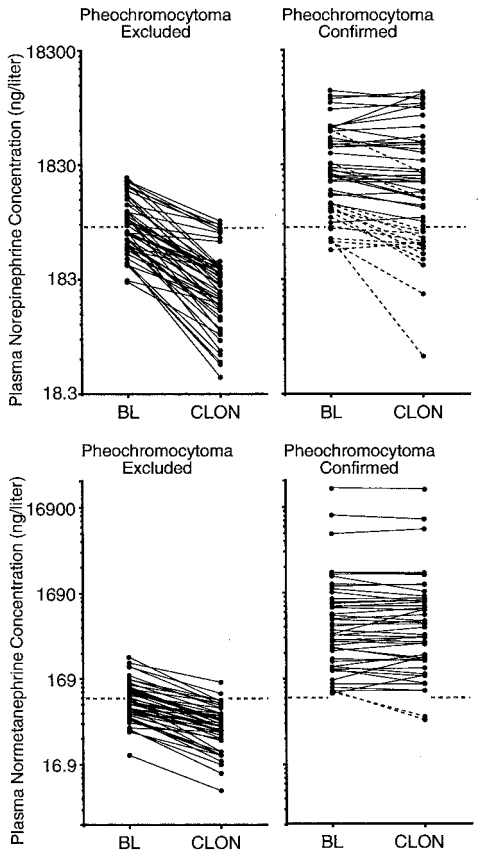

FIG. 4. Plasma concentrations of norepinephrine and normetanephrine before and after clonidine in patients with and without pheochromocytoma. Results in patients with (n = 48) and without (n = 49) pheochromocytoma are shown at baseline (BL) compared with after administration of clonidine (CLON). The *dashed horizontal lines* show the upper reference limits for each test. False-positive test results, either reflecting larger than 50% falls in norepinephrine or 40% falls in normetanephrine, or normal levels of both amines after clonidine are indicated by the *dotted lines*. To convert values to nanomoles per liter, divide by 183 for normetanephrine and 169 for norepinephrine.

Clonidine-suppression testing

Clonidine decreased ($P < 0.001$) plasma norepinephrine and normetanephrine in all patients without pheochromocytoma (Fig. 4). Percent decreases in plasma norepinephrine after clonidine varied from 16–91%, and on average were larger ($P < 0.001$) than those of normetanephrine (65 ± 19% *vs.* 48 ± 15%), which varied from 4–77%. Plasma concentrations of epinephrine showed variable responses to clonidine, but on average were decreased ($P < 0.001$) by 33 ± 51% (Table 3). In contrast, plasma concentrations of metanephrine remained unchanged.

TABLE 3. Plasma concentrations of catecholamines and metanephrines before and after clonidine

	Before clonidine	After clonidine
Pheochromocytoma excluded (n = 49)		
Norepinephrine (ng/liter)	546 ± 309	189 ± 144[a]
Normetanephrine (ng/liter)	112 ± 57	57 ± 29[a]
Epinephrine (ng/liter)	57 ± 31	37 ± 46[a]
Metanephrine (ng/liter)	33 ± 18	33 ± 20
Pheochromocytoma confirmed (n = 48)		
Norepinephrine (ng/liter)	2040 ± 1803	1960 ± 2067
Normetanephrine (ng/liter)	1811 ± 3691	1784 ± 3605
Epinephrine (ng/liter)	88 ± 141	95 ± 179
Metanephrine (ng/liter)	368 ± 888	355 ± 936

Data are shown as means ± SD. To convert values to nanomoles per liter, divide by 183 for normetanephrine, 169 for norepinephrine, 197 for metanephrine, and 183 for epinephrine.

[a] $P < 0.001$, compared with before clonidine.

Most of the 48 patients with pheochromocytoma had little change in plasma norepinephrine or normetanephrine after clonidine (Fig. 4). However, nine patients with pheochromocytoma had falls in norepinephrine of more than 50%, including two with parallel falls of normetanephrine (Figs. 4 and 5). Among these nine patients, two had baseline levels of norepinephrine that were moderately increased (1758 and 3397 ng/liter), 5 had slightly increased norepinephrine levels (575–761 ng/liter) and two had normal baseline levels (< 498 ng/liter). An additional seven patients with pheochromocytoma had normal plasma concentrations of norepinephrine after clonidine, with no or little change from normal baseline levels (n = 5), or a small decrease from slightly elevated baseline levels to normal levels after clonidine (n = 2). All had elevated plasma concentrations of normetanephrine that were unaffected by clonidine.

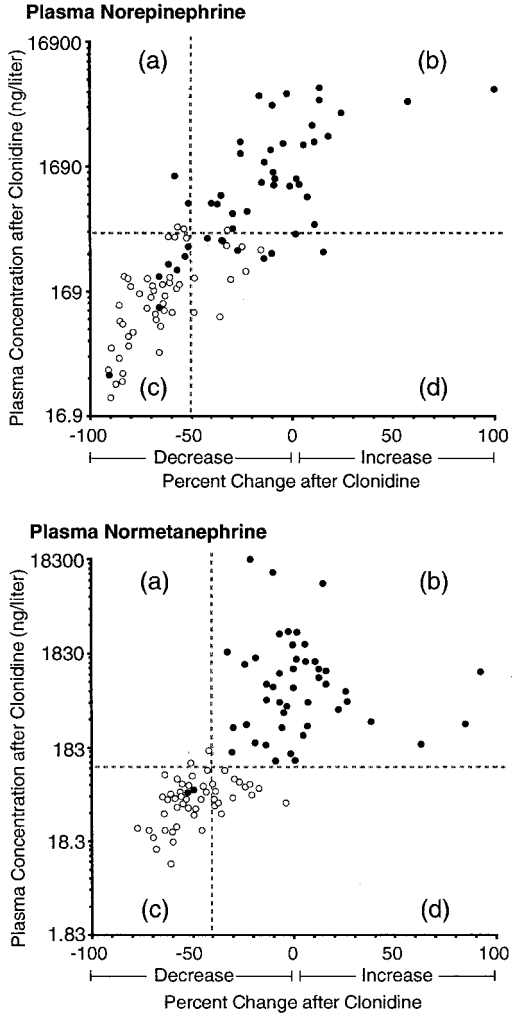

FIG. 5. Scatter plots showing distributions of clonidine-suppression test end-points for plasma norepinephrine and normetanephrine in patients with and without pheochromocytoma. Patients with pheochromocytoma are represented by the *filled circles* and those without pheochromocytoma by the open circles. The *dashed horizontal lines* represent test end-points for plasma concentrations of norepinephrine (498 ng/liter) or normetanephrine (112 ng/liter) after clonidine. The *dashed vertical lines* represent test end-points for percent changes in plasma concentrations of norepinephrine (50% decrease) or normetanephrine (40% decrease) after clonidine. The four quadrants (a–d) bound by the *dashed horizontal and vertical lines* illustrate how differing definitions of positive test results lead to the different test characteristics for the clonidine-suppression test shown in Table 4. To convert values to nanomoles per liter, divide by 183 for normetanephrine and 169 for norepinephrine.

Among the 16 patients with pheochromocytoma who had normal suppression or normal plasma concentrations of norepinephrine after clonidine, the tumor was sporadic in 8 patients, secondary to von Hippel-Lindau syndrome in 7 patients, and due to multiple endocrine neoplasia type 2 in 1 patient. In the 2 patients with pheochromocytoma and normal suppression of plasma normetanephrine, the tumor was sporadic in 1 patient and secondary to von Hippel-Lindau syndrome in the other.

Because clonidine-induced falls in plasma normetanephrine were less than those of norepinephrine, we defined a positive response of plasma normetanephrine to clonidine as a fall of less than 40% (compared with 50% for norepinephrine). These and other criteria of positive test results—values after clonidine that remained above the upper reference limits—were used to examine distributions of positive and negative test results as functions of the different defining criteria (Fig. 5). These distributions were then used to compare the diagnostic utility of plasma norepinephrine and normetanephrine as end-point markers of the clonidine-suppression test according to four different definitions of a positive result (Table 4).

Use of plasma normetanephrine as an end-point marker of the clonidine-suppression test provided higher sensitivities than use of norepinephrine at all definitions of a positive result (Table 4). The difference in sensitivity was particularly significant (96% for normetanephrine *vs.* 67% for norepinephrine, $P < 0.001$) when a positive result was defined as a lack of clonidine-induced suppression combined with a plasma concentration after clonidine remaining above the upper reference limit. This definition of a positive result also yielded high specificities (98–100%) for use of both normetanephrine and norepinephrine as end-point markers. With more relaxed definitions of a positive result, specificities decreased, test sensitivity for norepinephrine increased, but that for normetanephrine remained unchanged.

Similar diagnostic sensitivities and specificities were obtained when patients with von Hippel Lindau syndrome or multiple endocrine neoplasia type 2 were excluded from analysis. For this restricted group of patients who were tested because of suspicion of sporadic pheochromocytoma, diagnostic sensitivities were 96% for responses of plasma normetanephrine and 67% for those of norepinephrine. Respective specificities were 100% and 98%.

Values for diagnostic sensitivity were, however, lower when data were restricted to patients with (n = 15) and without (n = 20) pheochromocytoma who had baseline values for plasma normetanephrine in the equivocal range (112–400 ng/liter; 0.61–2.19 nmol/liter). For these patients, the diagnostic sensitivities were 87% for responses of plasma normetanephrine and only 33% for those of norepinephrine. Respective specificities were 100% and 95%.

TABLE 4. Clonidine-suppression test characteristics

	Norepinephrine	Normetanephrine
Positive result (elevated plasma concentration after clonidine and lack of suppression)[a]		
Sensitivity	67 (32/48)	96 (46/48)[e]
Specificity	98 (48/49)	100 (49/49)
Positive result (elevated plasma concentration after clonidine)[b]		
Sensitivity	71 (34/48)	96 (46/48)[e]
Specificity	94 (46/49)	96 (47/49)
Positive result (lack of suppression)[c]		
Sensitivity	81 (39/48)	96 (46/48)[f]
Specificity	82 (40/49)	71 (35/49)
Positive result (elevated plasma concentration after clonidine or lack of suppression)[d]		
Sensitivity	85 (41/48)	96 (46/48)
Specificity	78 (38/49)	67 (33/49)

Results are percentages (numbers/total number).
[a] Positive results are defined as values falling in quadrants b and negative results those falling in quadrants a, c, or d of Fig. 5.
[b] Positive results are defined as values falling in quadrants a or b and negative results those falling in quadrants c or d of Fig. 5.
[c] Positive results are defined as values falling in quadrants b or d and negative results those falling in quadrants a or c of Fig. 5.
[d] Positive results are defined as values falling in quadrants a, b, or d and negative results those falling in quadrants c of Fig. 5
[e] $P < 0.001$, compared with sensitivity for norepinephrine responses.
[f] $P < 0.02$, compared with sensitivity for norepinephrine responses.

Discussion

This study establishes new and improved procedures for confirming or excluding pheochromocytoma in patients with positive but equivocal biochemical test results after initial testing for the tumor (Fig. 6). More specifically, we identify medications that are commonly associated with false-positive results and show how differences in plasma free metanephrines and catecholamines can be used to distinguish false-positive from true-positive results. We further show that measuring plasma-free normetanephrine, in conjunction with the clonidine-suppression test, improves diagnosis of pheochromocytoma in difficult cases where preliminary testing yields equivocal results.

When is follow-up testing necessary?

Follow-up testing should depend primarily on findings of positive results of initial biochemical tests, which should include measurements of plasma-free metanephrines, or if unavailable, measurements of urinary fractionated metanephrines as the next best test. These recommendations are based on the high diagnostic sensitivity of measurements of plasma free or urinary fractionated metanephrines and findings of normal catecholamines and elevated metanephrines in about 20% of patients with pheochromocytoma, particularly those screened because of a hereditary syndrome or an adrenal incidentaloma (2–5, 21). Such patients are usually normotensive and asymptomatic. However, as we describe here and others have reported elsewhere (22–24), there are also occasional sporadic cases of pheochromocytoma where plasma and urinary catecholamines may be normal, despite signs and symptoms of the tumor.

Normal plasma and urinary catecholamines therefore do not exclude pheochromocytoma, even when testing is done because of hypertension and symptoms suggestive of the tumor. In contrast, levels of normetanephrine, metanephrine, or both metabolites are increased in almost all patients with pheochromocytoma. Exceptions include patients with small or microscopic tumors (<1 cm) that produce only small amounts of catecholamines, who may be encountered during screening because of a hereditary predisposition or a previous history for pheochromocytoma (2, 4). In such patients, follow-up testing at a later date remains mandatory and likely will lead to a positive diagnosis as the disease progresses. Other more rare exceptions include patients with pheochromocytomas that do not produce norepinephrine or epinephrine (5). Normal plasma levels of normetanephrine and metanephrine otherwise exclude pheochromocytoma,

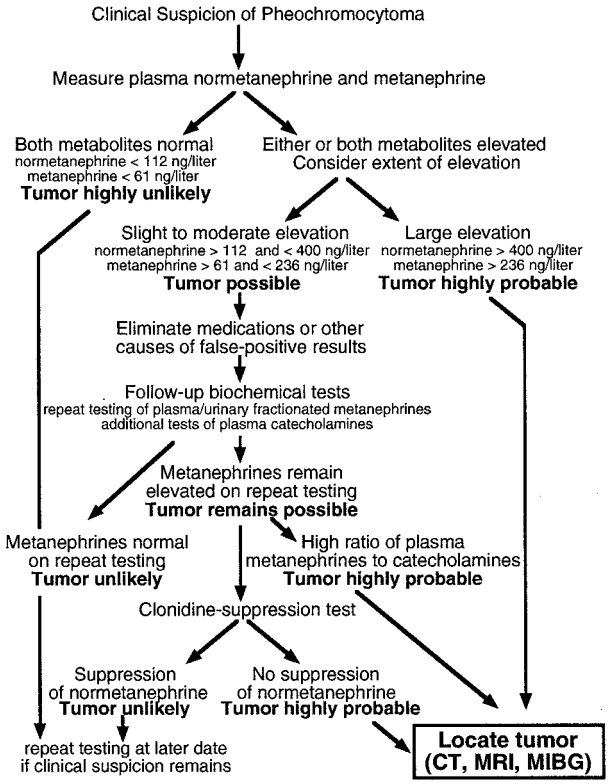

FIG. 6. Algorithm for biochemical diagnosis of pheochromocytoma. To convert values to nanomoles per liter, divide by 183 for normetanephrine, and 197 for metanephrine.

so that no immediate further testing for the tumor should usually be necessary.

Although problems of false-negative results in the diagnosis of pheochromocytoma are virtually eliminated by measurements of plasma free metanephrines, false-positive results remain a commonly encountered and potentially time-consuming and expensive problem for follow-up. The nature of this problem is illustrated in this study by the substantial overlap of biochemical results in patients with and without pheochromocytoma. For all biochemical tests there is a gray area where positive results in patients with the tumor are difficult to distinguish from positive results in patients without the tumor.

As shown here, the gray area for measurements of plasma free metanephrines extends from the upper reference limits of normal to 400 ng/liter (2.19 nmol/liter) for normetanephrine and to 236 ng/liter (1.2 nmol/liter) for metanephrine. In another independent study, the highest false-positive result was about 467 ng/liter (2.55 nmol/liter) for normetanephrine and about 158 ng/liter (0.8 nmol/liter) for metanephrine (5). In our experience, close to 80% of patients with pheochromocytoma have plasma concentrations of normetanephrine, metanephrine or both above these equivocal ranges (4). In such patients, the probability of pheochromocytoma is so high that the immediate task is to locate the tumor. In the remaining patients with elevated levels that fall within the equivocal ranges, additional tests are required to confirm or exclude the tumor.

Medication-associated false-positive results

Before additional biochemical tests are implemented, some consideration should be given to possible causes of false-positive results, including accompanying medical conditions, medications, inappropriate sampling conditions, and diet. In our study, the latter two causes of false-positive results were minimized by adherence to an overnight fast before blood sampling, which was carried out with patients resting supine.

Biochemical testing for pheochromocytoma should ideally be carried out after discontinuation of medications known to elevate levels of catecholamines and their metabolites or interfere directly with biochemical analyses. Patient safety considerations often, however, make this impractical. It is also often difficult to identify which medications interfere with a given test, particularly as new tests and drugs are developed.

Among our patients, phenoxybenzamine and tricyclic antidepressants accounted for up to 45% of false-positive elevations of plasma or urinary norepinephrine and normetanephrine. Tricyclic antidepressants are established causes of false-positive results (25), whereas phenoxybenzamine has not until now been recognized as an important problem.

The high rates of false-positive results in patients taking tricyclic antidepressants are probably due to the primary actions of these agents to inhibit monoamine reuptake (26, 27). These drugs also profoundly suppress release of norepinephrine from sympathetic nerves so that plasma concentrations of norepinephrine are usually decreased and not increased after acute administration (26). This influence, however, lessens after chronic administration (27). The primary action of these drugs to block monoamine reuptake then predominates resulting in increased escape of norepinephrine from sympathetic nerve terminals into the bloodstream. These complex actions may be responsible for the blood pressure disturbances that can accompany use of tricyclic antidepressants (28), and which may contribute to suspicion of pheochromocytoma. As we show here, selective serotonin reuptake inhibitors are not a significant cause of false-positive results and may provide an alternative medication when biochemical testing for pheochromocytoma is necessary.

Phenoxybenzamine is a nonspecific α-adrenoceptor blocker commonly used to treat patients with pheochromocytoma. Presumably, the drug elevates norepinephrine and normetanephrine by attenuating α_2-adrenoceptor-mediated feedback inhibition of norepinephrine release, possibly combined with reflexive sympathetic activation. Our findings of high rates of false-positive results associated with phenoxybenzamine lead us to suggest that this drug be avoided until biochemical testing is complete. As advocated elsewhere (15, 29, 30), alternative medications for blood pressure control include calcium channel blockers and selective α_1-adrenoceptor blockers such as doxazosin. As we show here, false-positive elevations of plasma metanephrines and catecholamines with selective α_1-adrenoceptor blockers are not a problem and with calcium channel blockers appear restricted to norepinephrine, an effect most likely due to the reflexive sympathetic activation occurring with the short-acting agents (31).

Most other antihypertensive medications were not significant sources of false-positive elevations of plasma metanephrines or catecholamines. However, β-adrenoceptor blockers were associated with 60% of all false-positive elevations of plasma metanephrine. Nevertheless, the false-positive rate was not high (12.5%) and does not appear to justify withdrawing these medications unless an equivocal result has been obtained and repeat testing is necessary.

Patterns of biochemical test results

After the potential confounding influence of medications or other causes of false-positive results have been eliminated, some consideration should be given to the choice of additional biochemical tests most appropriate for more firmly establishing or refuting the diagnosis of pheochromocytoma. When initial testing yields an elevated plasma metanephrine or normetanephrine, this may be corroborated by a similar pattern of results after additional measurements of urinary normetanephrine and metanephrine. Conversely, when initial testing yields a positive result for measurements of urinary normetanephrine and metanephrine, additional measurements of plasma free metanephrines are useful.

As we show here, patterns of increases in plasma-free metanephrines and catecholamines can provide additional information for confirming pheochromocytoma in patients where initial tests of plasma free metanephrines are positive but are insufficiently elevated for a firm diagnosis. More specifically, patients with pheochromocytoma usually have larger relative increases in metanephrines than catecholamines, whereas pa-

tients with false-positive results due to sympathoadrenal activation usually have larger increases in catecholamines than metanephrines.

The above differences are partly due to the substantial amounts of free metanephrines formed continuously within pheochromocytoma tumor cells and released into the circulation independently of variations in release of the parent catecholamines (32). Another factor is the large contribution of the adrenal medulla to normal circulating levels of metanephrine (91%) and normetanephrine (23%), a contribution that is again independent of catecholamine release (33). Thus, during sympathoadrenal activation, increases in plasma-free metanephrine are negligible and increases of normetanephrine are smaller than those of the respective parent amines (34).

The above explains why, contrary to usual considerations (35), a patient with an elevated plasma normetanephrine or metanephrine, but normal or slightly elevated norepinephrine or epinephrine is more likely to have a pheochromocytoma than a patient with a highly elevated norepinephrine or epinephrine and slightly elevated normetanephrine or metanephrine. As we show here, a plasma normetanephrine to norepinephrine ratio above 0.52 or a metanephrine to epinephrine ratio above 4.2 can provide confirmatory evidence of pheochromocytoma in up to 30% of patients where increases in plasma metanephrines are insufficient to conclusively prove the tumor. However, because some tumors secrete relatively large amounts of catecholamines compared with metanephrines, use of the reverse pattern to exclude pheochromocytoma is unreliable. Other tests, such as the clonidine-suppression test, remain essential.

Clonidine-suppression testing

The clonidine-suppression test was introduced by Bravo *et al.* (12) to address the problem of how to distinguish patients with pheochromocytoma from those with false-positive biochemical results after initial testing for the tumor. By activating α_2-adrenoceptors in the brain and on sympathetic nerve endings, clonidine suppresses norepinephrine release by sympathetic nerves. Decreases in elevated plasma norepinephrine concentrations after clonidine therefore suggest sympathetic activation, whereas a lack of decrease suggests pheochromocytoma.

The normal suppression of plasma norepinephrine after clonidine in many of our patients with pheochromocytoma, but particularly those with mildly elevated or normal baseline levels, agrees with several other reports documenting similar limitations of the clonidine-suppression test (13, 14, 36–38). Presumably, normal suppression occurs because much of the norepinephrine is derived from sympathetic nerves and remains responsive to clonidine. The clonidine-suppression test is therefore recommended for patients with plasma catecholamine levels over 1000 ng/liter (5.9 nmol/liter), with a normal response defined as a fall to within the normal range (39).

The above recommendation makes it problematic to use the clonidine-suppression test in patients with normal or only mildly elevated plasma norepinephrine levels. This is particularly troublesome because such patients represent those in whom it is most difficult to conclusively diagnose pheochromocytoma (18, 36). To overcome this limitation, another criterion for a normal response has been a fall in plasma norepinephrine after clonidine of more than 50% (14, 16). Although this allows identification of additional patients with pheochromocytoma who have normal or mildly increased norepinephrine levels, the trade-off, as shown here and elsewhere (10, 14, 16, 19), is increased numbers of false-positive results and reduced diagnostic specificity. Also, the gain in diagnostic sensitivity is offset by false-negative results in patients with episodically secreting pheochromocytomas, who can show apparent suppression due to sampling after clonidine on a downward swing in norepinephrine release by a tumor.

The above limitations led us to consider whether the clonidine-suppression test might be improved by measurements of plasma normetanephrine. Because pheochromocytomas cause larger, more consistent, and less episodic increases of plasma normetanephrine than of norepinephrine (3, 32, 40), we hypothesized that the metabolite would provide a better end-point marker for the clonidine-suppression test than the parent amine, but that this would depend on the normal responsiveness of the metabolite to clonidine. Because over 90% of circulating metanephrine is normally derived from metabolism of epinephrine within adrenal chromaffin cells, a process that is independent of epinephrine release (33, 34), we did not expect plasma levels of metanephrine to decrease after clonidine. However, because about 76% of plasma normetanephrine is derived from norepinephrine released by sympathetic nerves (33, 34), we did expect this metabolite to respond to clonidine in patients without pheochromocytoma.

Our expectations were confirmed by absent decreases in plasma metanephrine and consistent decreases in normetanephrine after clonidine in patients without pheochromocytoma. Thus, similar to the findings for norepinephrine, lack of decrease of plasma normetanephrine combined with a high plasma level after clonidine establishes a high probability of pheochromocytoma. More importantly, plasma normetanephrine concentrations did not fall and remained elevated after clonidine in 96% of patients with pheochromocytoma compared with only 67% for norepinephrine. Thus, normetanephrine responses to clonidine confirmed over 40% more pheochromocytomas and enabled more reliable exclusion of the tumor than did use of norepinephrine responses.

A remaining minor limitation is that responses of plasma metanephrine to clonidine cannot be used to distinguish true- from false-positive results for this metabolite. Clonidine-induced changes of epinephrine also offer limited help (17, 41). However, our experience shows that normetanephrine is the metabolite that is invariably increased and for which confirmation of pheochromocytoma is most important. Nevertheless, rather than replacing measurements of catecholamines, we recommend that measurements of plasma normetanephrine be included to optimize performance and extend the range of the test to patients where plasma norepinephrine is insufficiently elevated for reliable use.

Conclusions

The present findings suggest a new and improved strategy for biochemical diagnosis of pheochromocytoma (Fig. 6). As described elsewhere (4), findings of plasma levels of normetanephrine less than 112 ng/liter (0.61 nmol/liter) and of metanephrine less than 61 ng/liter (0.31 nmol/liter) virtually excludes pheochromocytoma so that no immediate further testing for the tumor should be necessary. With plasma concentrations of normetanephrine above 400 ng/liter (2.19 nmol/liter) or of metanephrine above 236 ng/liter (1.20 nmol/liter), the probability of pheochromocytoma is so high that the immediate task is to locate the tumor. The key problem until now has been conclusive diagnosis in patients with plasma concentrations of normetanephrine or metanephrine between the above levels, where numbers of false-positive results can exceed those of true-positive results.

The present study illustrates that consideration of medications, particularly phenoxybenzamine, tricyclic antidepressants, and β-adrenoceptor blockers can be useful in identifying many patients with false-positive results. Additional appropriately selected biochemical tests and patterns of test results can provide further information for firmly establishing a diagnosis. Conclusive diagnosis of pheochromocytoma in most of the remaining patients can then be achieved by coupling the clonidine-suppression test with measurements of plasma normetanephrine, in addition to standard measurements of norepinephrine. This considerably extends the range of the test to patients in whom plasma norepinephrine is not sufficiently elevated for reliable diagnosis. The above strategy should minimize delay in diagnosis of pheochromocytoma and avoid expensive, time-consuming, and imperfect imaging studies in patients who do not have the tumor.

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