Biochemical Diagnosis of Pheochromocytoma
Which Test Is Best?

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Context Diagnosis of pheochromocytoma depends on biochemical evidence of catecholamine production by the tumor. However, the best test to establish the diagnosis has not been determined.

Objective To determine the biochemical test or combination of tests that provides the best method for diagnosis of pheochromocytoma.

Design, Setting, and Participants Multicenter cohort study of patients tested for pheochromocytoma at 4 referral centers between 1994 and 2001. The analysis included 214 patients in whom the diagnosis of pheochromocytoma was confirmed and 644 patients who were determined to not have the tumor.

Main Outcome Measures Test sensitivity and specificity, receiver operating characteristic curves, and positive and negative predictive values at different pretest prevalences using plasma free metanephrines, plasma catecholamines, urinary catecholamines, urinary total and fractionated metanephrines, and urinary vanillylmandelic acid.

Results Sensitivities of plasma free metanephrines (99% [95% confidence interval {CI}, 96%-100%]) and urinary fractionated metanephrines (97% [95% CI, 92%-99%]) were higher than those for plasma catecholamines (84% [95% CI, 78%-89%]), urinary catecholamines (86% [95% CI, 80%-91%]), urinary total metanephrines (77% [95% CI, 68%-85%]), and urinary vanillylmandelic acid (64% [95% CI, 55%-71%]). Specificity was highest for urinary vanillylmandelic acid (95% [95% CI, 93%-97%]) and urinary total metanephrines (93% [95% CI, 89%-97%]); intermediate for plasma free metanephrines (89% [95% CI, 87%-92%]), urinary catecholamines (88% [95% CI, 85%-91%]), and plasma catecholamines (81% [95% CI, 78%-84%]); and lowest for urinary fractionated metanephrines (69% [95% CI, 64%-72%]). Sensitivity and specificity values at different upper reference limits were highest for plasma free metanephrines using receiver operating characteristic curves. Combining different tests did not improve the diagnostic yield beyond that of a single test of plasma free metanephrines.

Conclusion Plasma free metanephrines provide the best test for excluding or confirming pheochromocytoma and should be the test of first choice for diagnosis of the tumor.

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PHEOCHROMOCYTOMAS ARE chromaffin cell tumors typically arising in the adrenal glands and characterized by excessive production of catecholamines, often leading to increased blood pressure and symptoms of catecholamine excess. If not diagnosed or if left untreated, the excessive secretion of catecholamines by these tumors can have devastating consequences. Thus, although pheochromocytomas are rare tumors, they must be considered in many patients with hypertension, the latter representing up to a quarter of the adult population in Western countries.

The diagnosis of pheochromocytoma depends crucially on demonstration of excessive production of catecholamines.[1,2] This step, however, is fraught with difficulties, in particular false-negative test results. Moreover, due to the low prevalence of pheochromocytoma in the tested population and inadequate specificity of biochemical tests, false-positive results are a common and troublesome occurrence.[3]

The above difficulties in biochemical diagnosis indicate the need for a test that is maximally sensitive and specific to reliably exclude or confirm pheochromocytoma. Previous studies examining the performance of diagnostic tests had small numbers of patients, inappropriate comparison groups used to establish specificity, or limited comparisons of available biochemical tests.[4-16] Thus, the test or combination of tests that provides the best method for diagnosis of pheochromocytoma remains unsettled.

This study examined the diagnostic utility of several biochemical tests in large populations of patients tested for pheochromocytoma because of sugges-

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Table 1. Patient Characteristics

	Pheochromocytoma Confirmed		Pheochromocytoma Excluded	
	Hereditary	Sporadic	Hereditary	Sporadic
No. of patients	76	138	339	305
Sex, No.				
Women	33	72	194	176
Men	43	66	145	129
Age, mean (SD) [range], y	33 (14) [8-63]	47 (15) [17-78]	38 (14) [11-76]	47 (14) [8-77]

tive signs and symptoms or a predisposition to develop the tumor. Biochemical tests included measurements of plasma and urinary catecholamines, urinary fractionated metanephrines, urinary total metanephrines, and urinary vanillylmandelic acid (VMA). These commonly used tests were compared with measurements of plasma concentrations of free metanephrines, normetanephrine, and metanephrine, which is a promising new test for diagnosis of pheochromocytoma.[9,11,14]

METHODS

Study Design and Participants

The study population was selected from a total of 1003 patients tested for pheochromocytoma using plasma free metanephrines. Patients were tested between 1994 and 2001 at 4 referral centers (National Institutes of Health, Bethesda, Md; St Radboud University Medical Center, Nijmegen, the Netherlands; Sahlgren's University Hospital, Göteborg, Sweden; and University of Florence, Florence, Italy). Patients were either tested as part of routine screening for hereditary pheochromocytoma or after referral to 1 of the 4 centers because of a suspicion of pheochromocytoma based on a previous history of the tumor, the finding of an adrenal mass, or more often because of suggestive signs (eg, therapy-resistant or paroxysmal hypertension) and symptoms (eg, sweating, headache, palpitations). Procedures were approved by the intramural research board or hospital ethics committee of the centers in which patients were studied and all patients provided informed consent.

For the purposes of patient selection into the study, the results of biochemical tests could not be used to exclude or confirm pheochromocytoma, since by definition this would bias the analyses of test performance. Therefore, selection of patients for inclusion in the final analyses was based on whether pheochromocytoma could be excluded or confirmed by standard criteria that were necessarily independent of the diagnostic biochemical tests being evaluated.

Confirmation of pheochromocytoma required pathological examination of surgically resected or biopsied tumor tissue or a diagnosis of inoperable malignant pheochromocytoma based on findings of metastatic disease by imaging studies. Exclusion of pheochromocytoma required lack of radiological evidence of a tumor by computed tomography or magnetic resonance imaging, pathological examination of a surgically resected or biopsied adrenal mass, or lack of pheochromocytoma on patient follow-up 2 or more years after initial testing. Using the above criteria, pheochromocytoma was confirmed in 214 patients and excluded in 644 patients, all of whom were included in the final analyses (TABLE 1).

Among the 145 patients who did not fulfill the criteria for exclusion or confirmation of pheochromocytoma, and who were not included in the final analyses, 25 patients had a high likelihood of pheochromocytoma but had not been operated on at the time of analysis. All 25 patients had evidence of a small adrenal mass by imaging studies. All had some biochemical evidence of pheochromocytoma and most had a hereditary predisposition to the tumor but were asymptomatic and normotensive. Pheochromocytomas in the remaining group of 120 patients were unlikely based on findings that did not fulfill the criteria of the study for selection of patients into the final analyses (eg, patients in whom pheochromocytoma was excluded solely on the basis of negative biochemical test results).

The patients with (n=214) and without (n=644) pheochromocytoma who were selected into the final analyses were further divided into 2 subgroups based on whether testing was performed because of a hereditary predisposition for pheochromocytoma or because of clinical suspicion and no hereditary predisposition (Table 1). Among patients with hereditary pheochromocytoma, 48 had the tumor due to von Hippel-Lindau syndrome, 23 due to multiple endocrine neoplasia type 2, and 3 due to neurofibromatosis type 1. The mutation remained undetermined in 2 patients.

Patient Follow-up: Validation of Negative Imaging Studies

To validate the use of negative imaging studies as a criterion for exclusion of pheochromocytoma, patients were followed up if they had been tested more than 1 year previously. Follow-up information confirming lack of pheochromocytoma was obtained in 330 of the 546 patients in whom imaging studies were used to exclude pheochromocytoma (mean follow-up, 2.5 years; range, 1-7.8 years).

Only 1 case of pheochromocytoma was confirmed by follow-up in a patient with a previously negative computed tomographic scan result. This patient was diagnosed with metastatic pheochromocytoma 3½ years after the negative scan and 16½ years after removal of the primary tumor.

Biochemical Tests

Blood samples were collected from all patients using a forearm venous cannula with patients supine for at least 20 minutes before sampling. Patients were instructed to fast and abstain from caffeinated and decaffeinated beverages overnight and to avoid taking acetaminophen for 5 days before blood sampling. Collection of blood for measurements of plasma free metanephrines

was performed prospectively in 819 of the 858 patients included in the final analyses. In the remaining patients, all with pheochromocytoma, measurements of plasma free metanephrines were performed after removal of tumors. Twenty-four hour urine collections were obtained from 790 of the 858 patients included in the final analyses. Urine samples were usually subjected to assays of 2 to 3 different analytes.

Plasma was analyzed, using high-performance liquid chromatography (HPLC), for concentrations of catecholamines, norepinephrine and epinephrine, and free metanephrines, normetanephrine, and metanephrine.[17-19] Metanephrines in urine were analyzed as fractionated normetanephrine and metanephrine by HPLC or as total metanephrines by spectrophotometry.[10,20] Urinary VMA was measured spectrophotometrically and urinary catecholamines by HPLC.[21,22] Upper reference limits for biochemical tests are provided as established by the principal laboratories responsible for each test (TABLE 2).[11]

All 858 assays of plasma free metanephrines and 91% of the 855 assays of plasma catecholamines were performed at the National Institutes of Health. Seventy-five percent of the 557 assays of urinary fractionated metanephrines were performed by Quest Diagnostics (Collegeville, Pa). Seventy-two percent of the 710 assays of urinary catecholamines, 74% of the 297 assays of urinary total metanephrines, and 79% of the 616 assays of urinary VMA were performed by Mayo Medical Laboratories (Rochester, Minn). The remaining assays were performed at different laboratories depending on the center where patients were evaluated.

Data Analysis

For biochemical tests involving pairs of measurements (eg, normetanephrine and metanephrine or norepinephrine and epinephrine in plasma or urine), a false-negative result in a patient with pheochromocytoma or a true-negative result in a patient without pheochromocytoma was defined as a value for each measurement lower than the upper reference limit. A true-positive result for pairs of measurements in a patient with pheochromocytoma or a false-positive result in a patient without pheochromocytoma was defined as a value for either or both measurements equal to or higher than the appropriate upper reference limit.

Sensitivity was calculated from the percentage of true-positive over the total of true-positive plus false-negative test results in patients with pheochromocytoma. Specificity was calculated from the percentage of true-negative over the total of true-negative plus false-positive test results in patients without pheochromocytoma. Differences in sensitivity and specificity were examined using the McNemar test and are illustrated using 95% confidence intervals (CIs).

For each analyte, a receiver operating characteristic (ROC) curve was constructed from the relationship between true-positive and false-positive results (ie, sensitivity vs 1 – specificity) at different upper reference limits for each analyte.[23] As summary measures of the diagnostic utility of each test, independent of upper reference limits, areas under ROC curves were calculated and differences among tests examined according to the method by Hanley and McNeil.[24]

Negative predictive values were calculated from the percentage of true-negative over the total of true-negative plus false-negative test results. Positive predictive values were calculated from the percentage of true-positive over the total of true-positive plus false-positive results. Positive and negative predictive values of each test were calculated at different prevalences of pheochromocytoma to establish posttest probabilities of pheochromocytoma at different pretest probabilities of the tumor.

RESULTS

Biochemical Test Results

Relative to patients in whom pheochromocytoma was excluded, median plasma concentrations of free metanephrines were increased by 7-fold in patients with hereditary pheochromocytoma and 21-

Table 2. Plasma Concentrations of Catecholamines and Metanephrines and Urinary Outputs of Catecholamines, Metanephrines, and Vanillylmandelic Acid

	Upper Reference Limit	Pheochromocytoma Confirmed*		Pheochromocytoma Excluded*	
		Hereditary	Sporadic	Hereditary	Sporadic
Plasma, nmol/L					
Free metanephrines					
Normetanephrine	0.6	2.2 (0.3-69)	6.3 (0.4-173)	0.3 (0.1-1.6)	0.3 (0.1-2.2)
Metanephrine	0.3	0.2 (0-15)	0.6 (0-383)	0.1 (0-0.5)	0.2 (0-1.2)
Catecholamines					
Norepinephrine	2.9	4.8 (1-80)	10.6 (0.7-1360)	1.4 (0.4-8.1)	2.1 (0.3-29)
Epinephrine	0.5	0.2 (0-4.8)	0.4 (0-1111)	0.1 (0-1.3)	0.1 (0-18)
Urine, µmol/d					
Fractionated metanephrines†					
Normetanephrine					
Women	1.7	5.2 (1.8-33)	17.7 (0.9-191)	1.2 (0.1-8.2)	2.1 (0.4-10)
Men	3.0				
Metanephrine					
Women	0.7	0.5 (0.1-28)	1.1 (0.1-466)	0.4 (0.1-2.8)	0.5 (0.1-22)
Men	1.2				
Catecholamines					
Norepinephrine	0.5	1 (0.1-5.4)	1.7 (0.1-31)	0.2 (0-1)	0.3 (0.1-5.6)
Epinephrine	0.1	0 (0-2.1)	0.1 (0-7)	0 (0-0.1)	0 (0-0.4)
Total metanephrines‡	6	8 (2-260)	21 (3-234)	3 (1-12)	3 (1-16)
Vanillylmandelic acid	40	37 (11-330)	83 (13-777)	17 (3-56)	23 (2-81)

*Values are expressed as median (range).
†Measured by high-performance liquid chromatography after acid deconjugation.
‡Measured together as a single analyte by spectrophotometry after acid deconjugation.

Table 3. Sensitivities and Specificities of Biochemical Tests for Diagnosis of Hereditary and Sporadic Pheochromocytoma*

	Sensitivity, %†		Specificity, %‡	
	Hereditary	Sporadic	Hereditary	Sporadic
Plasma				
Free metanephrines	97 (74/76)	99 (137/138)	96 (326/339)	82 (249/305)
Catecholamines	69 (52/75)	92 (126/137)	89 (303/339)	72 (220/304)
Urine				
Fractionated metanephrines	96 (26/27)	97 (76/78)	82 (237/288)	45 (73/164)
Catecholamines	79 (54/68)	91 (97/107)	96 (312/324)	75 (159/211)
Total metanephrines	60 (27/45)	88 (61/69)	97 (91/94)	89 (79/89)
Vanillylmandelic acid	46 (30/65)	77 (66/86)	99 (310/312)	86 (132/153)

*The reference limits used to calculate sensitivity and specificity are presented in Table 2.
†For free plasma metanephrines or urinary fractionated metanephrines, sensitivity was calculated from patients with pheochromocytoma and false-negative test results for both normetanephrine and metanephrine. For plasma and urine catecholamines, sensitivity was calculated from patients with both false-negative test results for nonrepinephrine and epinephrine. Numbers in parentheses indicate true positive over true positive plus false-negative.
‡For free plasma metanephrines or urinary fractionated metanephrines, specificity was calculated from patients without pheochromocytoma and with false-positive test results for either normetanephrine or metanephrine. For plasma and urine catecholamines, specificity was calculated from patients without pheochromocytoma and with false-positive test results for either nonrepinephrine or epinephrine. Numbers in parentheses indicate true negative over true negative plus false-positive.

fold in patients with sporadic pheochromocytoma (Table 2). These increases were consistently larger than those of plasma norepinephrine (3-fold and 5-fold increases), urinary norepinephrine (5-fold and 6-fold increases), urinary fractionated normetanephrine (4-fold and 8-fold increases), urinary total metanephrines (3-fold and 7-fold increases), and urinary VMA (2-fold and 4-fold increases). Increases in all analytes were larger in patients with sporadic rather than hereditary pheochromocytoma.

Test Sensitivity

The sensitivities of diagnostic tests for detection of hereditary or sporadic pheochromocytoma ranged from a low of 46% (95% CI, 34%-59%) for use of urinary VMA in the detection of hereditary pheochromocytoma to a high of 99% (95% CI, 96%-100%) for use of plasma free metanephrines in the detection of sporadic pheochromocytoma (TABLE 3).

Among all patients with pheochromocytoma, sensitivities were the highest for measurements of plasma free metanephrines at 99% (95% CI, 96%-100%), followed closely by urinary fractionated metanephrines at 97% (95% CI, 92%-99%). Sensitivities of both the above tests considerably ($P<.001$) exceeded those for urinary catecholamines at 86% (95% CI, 80%-91%), plasma catecholamines at 84% (95% CI, 78%-89%), urinary total metanephrines at 77% (95% CI, 68%-85%), and urinary VMA at 64% (95% CI, 55%-71%).

The above variations in sensitivities of diagnostic tests showed similar patterns in patients with hereditary and sporadic pheochromocytoma (Table 3). Plasma free metanephrines and urinary fractionated metanephrines offered the highest sensitivities. Plasma and urinary catecholamines had intermediate sensitivities. Urinary total metanephrines and VMA consistently showed the lowest sensitivities in both groups of patients. The sensitivities of all tests were higher for detection of sporadic pheochromocytoma than for detection of hereditary pheochromocytoma.

Test Specificity

Specificities of biochemical tests ranged widely from 45% (95% CI, 36%-51%) for urinary fractionated metanephrines in patients tested for sporadic pheochromocytoma to 99% (95% CI, 98%-100%) for urinary VMA in patients tested for hereditary pheochromocytoma (Table 3).

Among all patients tested for pheochromocytoma, the highest specificities were 95% (95% CI, 93%-97%) for tests of urinary VMA and 93% (95% CI, 89%-97%) for tests of urinary total metanephrines. Specificities were intermediate for tests of plasma free metanephrines at 89% (95% CI, 87%-92%), urinary catecholamines at 88% (95% CI, 85%-91%), and plasma catecholamines at 81% (95% CI, 78%-84%), and lower than those of all other tests ($P<.001$) for urinary fractionated metanephrines at 69% (95% CI, 64%-72%).

In contrast to sensitivities, specificities of all tests were higher in patients tested for hereditary pheochromocytoma than for sporadic pheochromocytoma (Table 3). In both groups, urinary VMA and total metanephrines offered the highest specificities and urinary fractionated metanephrines the lowest specificities.

False-Negative Plasma Free Metanephrines

Only 2 of the 76 patients with hereditary pheochromocytoma and 1 of the 138 patients with sporadic pheochromocytoma had normal levels of plasma free metanephrines. Both hereditary cases were in patients who were normotensive and asymptomatic and had no other biochemical evidence of the tumor. Both were patients with von Hippel-Lindau syndrome and had single adrenal tumors of less than 1 cm in diameter, which were identified and removed coincidentally during surgery for renal carcinoma.

The single false-negative result for plasma free metanephrines in patients with sporadic pheochromocytoma involved a patient tested for possible tumor recurrence 13 years after the removal of a large extra-adrenal pheochromocytoma. Computed tomography and all biochemical tests yielded negative results. The patient was subsequently diagnosed with metastatic pheochromocytoma 3½ years later. Since there was no evidence for a hereditary basis for the patient's disease, it was presumed that the malignancy developed secondary to remaining disease that went undetected for more than 16 years after the original tumor was removed. Thus, despite the considerable time between biochemical testing and final diagnosis, all tests performed were designated as providing false-negative results.

ROC Curves

Integrated comparison of sensitivity and specificity using ROC curves showed that the diagnostic power of plasma free metanephrines was superior to that of all other tests (FIGURE 1). The areas under the ROC curves for plasma catecholamines (0.927), urinary catecholamines (0.931), urinary total metanephrines (0.919), and urinary VMA (0.896) were all significantly lower ($P<.001$) than the area for plasma free metanephrines (0.985). Although closer, the area under the ROC curve for urinary fractionated metanephrines (0.960) was also lower ($P=.01$) than that for plasma free metanephrines (0.985).

Areas under the ROC curves were only marginally improved when tests of urinary fractionated metanephrines were combined with those for urinary catecholamines (0.965) or plasma catecholamines (0.969) or when tests of urinary total metanephrines and catecholamines were combined (0.949) (Figure 1). Thus, combining tests for different analytes did not improve diagnostic efficacy beyond that of a single test of plasma free metanephrines.

True-positive rates (ie, test sensitivity) at higher upper reference limits when false-positive rates were zero (ie, when test specificity equaled 100%) were higher for plasma free metanephrines than for other tests (Figure 1). None of the 644 patients without pheochromocytoma had a plasma concentration of normetanephrine above 2.19 nmol/L or of metanephrine above 1.20 nmol/L, whereas 79% of patients with pheochromocytoma had plasma concentrations of normetanephrine or metanephrine above these levels (TABLE 4).

Positive and Negative Predictive Values

Negative predictive values of tests of plasma and urinary metanephrines at different prevalences of pheochromocytoma showed that negative test results for plasma free and urinary fractionated metanephrines provided the highest probabilities for excluding pheochromocytoma at all pretest prevalences of the tumor (FIGURE 2). However, the posttest probability of a pheochromocytoma from a positive test result for plasma free metanephrines, although similar to that for urinary total metanephrines, was consistently higher than that from a positive test result for urinary fractionated metanephrines at all pretest prevalences of the tumor.

COMMENT

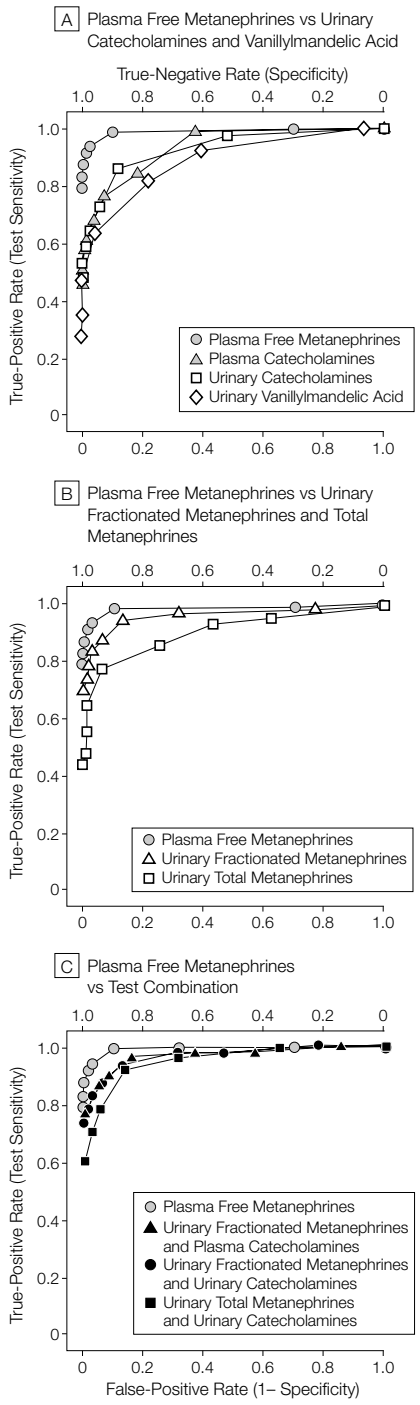
The present examination of biochemical tests used in the diagnosis of pheochromocytoma provides several advances over previous studies. First, this study comprehensively compared measurements of plasma free metanephrines with all other commonly available biochemical tests used to diagnose excess catecholamine production. Second, these comparisons were made in large populations of patients with and without pheochromocytoma, who were tested for the tumor because of clinically appropriate predisposing conditions or suspicious symptoms and signs. Finally, standard criteria that were independent of the biochemical tests being compared were used to assign patients into groups with and without the tumor.

Sensitivity, Specificity, and ROC Curves

The present study confirms the findings of several other reports that measurements of plasma free metanephrines or urinary fractionated metanephrines offer higher sensitivity for diagnosis of pheochromocytoma than measurements of plasma or urinary catecholamines or of urinary total metanephrines or VMA.[8,9,11,14,16] Our comparisons further establish that among all tests, including urinary fractionated metanephrines, measurements of plasma free metanephrines provide the best test for excluding or confirming pheochromocytoma.

Since measurements of urinary fractionated metanephrines and plasma free metanephrines offer similarly high sensitivity, a negative result for either test is equally effective for excluding pheochromocytoma. However, because urinary fractionated metanephrines have low specificity, tests of plasma free

Figure 1. Receiver Operating Characteristic Curves



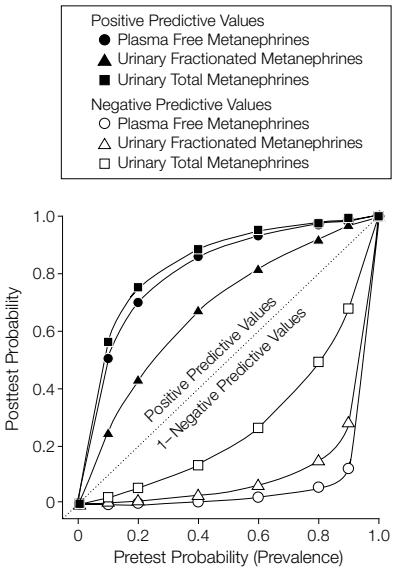
Relationships between rates of true-positive test results (ie, sensitivity) and rates of false-positive test results (1 – specificity) were calculated at different upper reference limits for each of the tests. At higher upper reference limits, rates of true-positive test results decrease (ie, sensitivity decreases), whereas rates of false-positive test results increase (ie, specificity increases).

Table 4. Plasma Concentrations, Specificity, and Sensitivity of Plasma Free Metanephrines at Established Upper Reference Limits Compared With Reference Limits Adjusted to Provide a Zero False-Positive Rate

Upper Reference Limits	Plasma Level, nmol/L	Test Specificity, %	Test Sensitivity, %
Established			
Normetanephrine	0.61	89	99
Metanephrine	0.31		
Adjusted*			
Normetanephrine	2.19	100	79
Metanephrine	1.20		

*Plasma concentrations and test sensitivity adjusted to provide a zero false-positive rate were established from receiver operating characteristic curves in Figure 1.

Figure 2. Relationships Between Pretest and Posttest Probability



A positive or a negative result for tests of plasma or urinary metanephrines changes the respective probabilities of having or not having pheochromocytoma in relationship to different pretest probabilities (prevalences) of the tumor. The dotted line represents the relationship expected between pretest and posttest probability if a test had no diagnostic value. Relationships illustrated by the filled symbols show the probabilities of having a pheochromocytoma based on positive (abnormal) test results, whereas the open symbols show the probabilities of not having a pheochromocytoma based on negative (normal) test results.

metanephrines exclude pheochromocytoma in many more patients without the tumor than do tests of urinary fractionated metanephrines.

The above considerations illustrate the importance of ROC curves for comparing different tests. At equivalent levels of sensitivity, the specificity of plasma free metanephrines is higher than that of other tests. At equivalent levels of specificity, the sensitivity of plasma free metanephrines is also higher than that of other tests, including urinary fractionated metanephrines.

Multiple Biochemical Tests

To minimize the risk of missing a patient with pheochromocytoma, clinicians often use multiple biochemical tests during the initial diagnostic workup of patients with suspected tumors. Although this may increase sensitivity, it is at the cost of decreased specificity. Thus, tests involving pairs of measurements, such as fractionated catecholamines or metanephrines, have lower specificity and higher sensitivity than tests involving single measurements, such as urinary VMA or total metanephrines (Table 3).[8] As shown by ROC curves, the diagnostic utility of tests of plasma free metanephrines remains superior to that of other tests even when the latter are used in combination.

The above considerations lead us to recommend against use of multiple biochemical tests to exclude pheochromocytoma in favor of a single test of plasma free metanephrines. In patients with negative test results for plasma free metanephrines, indiscriminate use of extra tests is unlikely to improve diagnostic efficacy. If multiple biochemical tests have been run, the decision to exclude pheochromocytoma should be based on whether plasma free metanephrines show a negative test result, regardless of whether other test results are positive or negative.

Differences in Test Performance Explained

Why do plasma free metanephrines provide the best test to diagnose pheochromocytoma? First, plasma free metanephrines are produced continuously by metabolism of catecholamines within pheochromocytoma tumor cells.[25-27] This contrasts with episodic secretion of catecholamines. Second, sympathoadrenal excitation causes large increases in catecholamine release, whereas plasma free metanephrines remain relatively unaffected.[14,25,27,28] Third, VMA and the total and fractionated metanephrines measured in urine are different metabolites from the free metanephrines measured in plasma, and are produced in different parts of the body by metabolic processes not directly related to the tumor itself.[28-30] Urinary total and fractionated metanephrines are measured after a deconjugation step and largely reflect levels of conjugated metanephrines that are produced outside of tumor tissue. Similarly, VMA is produced mainly in the liver.

Sporadic vs Hereditary Pheochromocytoma

The lower sensitivity and higher specificity of biochemical tests for hereditary compared with sporadic pheochromocytoma reflect different reasons for testing in the 2 groups.[31] Routine screening for pheochromocytoma in patients with a hereditary predisposition to the tumor often leads to detection of small tumors that release catecholamines in amounts that are insufficient to produce signs or symptoms of the tumor. In contrast, sporadic pheochromocytoma is typically suspected because of signs and symptoms of catecholamine excess, produced by larger more easily detected tumors than found by routine screening in hereditary pheochromocytoma. Moreover, patients tested for sporadic pheochromocytoma who do not have the tumor are often symptomatic of some condition associated with sympathoadrenal activation, leading to relatively high numbers of false-positive results.

The consistently lower specificities of biochemical tests in patients tested for sporadic rather than for hereditary pheochromocytoma may also reflect referral of patients in the former group with previously determined positive

biochemical tests. Thus, specificities of biochemical tests for detection of sporadic pheochromocytoma in the present study are likely to be lower than in unselected populations tested by commercial laboratories, but should reflect those expected in populations tested at referral centers.

Apart from patients at risk for hereditary pheochromocytoma, patients with previously resected tumors are another at-risk group who should be tested periodically for the tumor regardless of signs and symptoms. The importance of this group is illustrated by the single patient who tested negative for pheochromocytoma by all tests 3½ years before metastatic disease was finally diagnosed and 16 years after removal of the primary tumor. The sensitivity of plasma free metanephrines is not always sufficient for detection of microscopic recurrent or metastatic disease or small tumors (<1 cm) in patients with hereditary pheochromocytoma.

Pretest and Posttest Probabilities

Typically fewer than 1% of hypertensive patients tested for pheochromocytoma have the tumor. In some patient groups, such as those with hypertension and an adrenal mass, the pretest probability of a pheochromocytoma may be higher. The probability that a negative test result excludes pheochromocytoma or that a positive test result confirms the tumor depends in part on the pretest probability of the disease. These posttest probabilities therefore require calculation of positive and negative predictive values at different pretest probabilities (prevalences) of the tumor.

As shown in Figure 2, a negative test result for plasma free metanephrines or urinary fractionated metanephrines provides a high probability of excluding pheochromocytoma at all clinically relevant pretest probabilities of the tumor. In contrast, at the typically low prevalences of pheochromocytoma, the likelihood of the tumor after a single positive test remains low even for tests with up to 95% specificity. A routine practice to further increase or decrease the likelihood of pheochromocytoma involves use of additional biochemical tests.[32]

In patients with positive results for an initial test of plasma free metanephrines, extra tests can be useful, but judging the likelihood of a pheochromocytoma should first take into account results of ROC curves. In particular, at higher upper reference limits, in which test specificity is 100%, plasma concentrations of free normetanephrine higher than 2.19 nmol/L or of free metanephrine higher than 1.20 nmol/L unequivocally confirm a pheochromocytoma in 79% of patients with the tumor (Table 4), a higher proportion than for other tests (Figure 1). The probability of pheochromocytoma in these patients is so high that further biochemical tests to confirm the tumor may be unnecessary. In the remaining patients, in whom increased levels are insufficient to unequivocally confirm a tumor, additional judiciously selected follow-up tests are appropriate, with additional attention focused on possible causes of false-positive test results.[32-35]

False-Positive Test Results

Because of the low prevalence of pheochromocytoma in the patient groups usually tested for the tumor, false-positive results can be expected to outnumber true-positive results for all biochemical tests, including plasma free metanephrines. There are 3 potential sources of false-positive test results: diet, drugs, and stressors.

Caffeic acid, a catechol found in coffee (including decaffeinated coffee), and its derivative dihydrocaffeic acid are dietary substances known to interfere with assays of plasma catecholamines.[36] Moreover, both catechols are excellent substrates for catechol *O*-methyltransferase, the enzyme that converts catecholamines to metanephrines, and this easily could affect plasma levels of metanephrines. There are many other unidentified dietary constituents that can influence the results of HPLC assays. The simplest way to avoid these sources of false-positive results is by drawing blood samples in patients who have fasted.

Acetaminophen is the only direct source of interference with assays of plasma free metanephrines that we have identified to date.[19] However, caffeine and nicotine both increase plasma levels of catecholamines and should also be avoided. In our series, treatment with tricyclic antidepressants or phenoxybenzamine (dibenzyline) were major causes of false-positive test results for norepinephrine and its metabolites, presumably due to presynaptic actions on sympathetic nerves. Phenoxybenzamine, a nonselective α-adrenoceptor blocker commonly used to treat patients with pheochromocytoma, can be particularly troublesome.

Although plasma levels of free metanephrines are less sensitive to changes in sympathoadrenal activity than are levels of the parent amines, these metabolites are nevertheless influenced by many of the same stimuli and drugs that influence plasma catecholamines.[25-28] Upright posture and emotional stress are well-known to stimulate release of catecholamines from sympathetic nerves and the adrenal medulla. To minimize the possibility of false-positive test results, we collected blood samples for plasma free metanephrines under the same conditions used for collection of samples for measurements of plasma catecholamines. Blood samples were drawn with patients in the supine position, through an in-dwelling intravenous catheter, and after an overnight fast.

Study Limitations

The major strength of this study—all patients were examined because of clinical suspicion of pheochromocytoma—was associated with the limitation that exclusion of pheochromocytoma required use of methods other than the biochemical tests normally used in clinical practice. Although computed tomography and magnetic resonance imaging offer high sensitivity for detecting adrenal tumors, sensitivity decreases for detecting extra-adrenal disease. We therefore followed up patients for an average of 2.5 years to further exclude pheochromocytoma in patients with negative imaging. Only 1 patient in whom pheo-

chromocytoma was initially excluded by imaging studies was subsequently found to have the disease at follow-up. It remains possible, however, that other patients in whom pheochromocytoma was excluded according to the criteria of our study may have had undiagnosed disease. Even so and unless these numbers were large, it is unlikely that incorrect categorization of these patients would make a significant difference to the results and conclusions of the study.

A related potential limitation of the study was the need to omit from the analyses 145 patients who did not meet the research criteria for exclusion or confirmation of pheochromocytoma. Separate analyses of how inclusion of the data from these patients would affect test performance revealed little influence on the results and conclusions of the study.

Another potential limitation of the study involved the multicenter nature of patient recruitment and subsequent measurements of urinary analytes by different laboratories compared with the single laboratory used for plasma free metanephrines. However, separate analysis of data derived from single laboratories revealed no obvious influences. Thus, rather than a limitation, the multicenter nature of the study is a strength establishing that many of the findings (eg, low specificity of urinary fractionated metanephrines, low sensitivity of urinary VMA) were independent of the laboratory where tests were run.

Conclusions

Plasma free metanephrines constitute the best test for excluding or confirming pheochromocytoma and should be the test of first choice for diagnosis of the tumor. A negative test result virtually excludes pheochromocytoma. In such patients, representing more than 80% of those tested, no immediate further tests for the tumor are necessary. Furthermore, in about 80% of patients with pheochromocytoma, the magnitude of increase in plasma free metanephrines is so large that the tumor can be confirmed with close to 100% probability. In these patients, the immediate task is to locate the tumor.

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