

PLASMA NORMETANEPHRINE AND METANEPHRINE FOR DETECTING PHEOCHROMOCYTOMA IN VON HIPPEL-LINDAU DISEASE AND MULTIPLE ENDOCRINE NEOPLASIA TYPE 2

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ABSTRACT

Background The detection of pheochromocytomas in patients at risk for these tumors, such as patients with von Hippel-Lindau disease or multiple endocrine neoplasia type 2 (MEN-2), is hindered by the inadequate sensitivity of commonly available biochemical tests. In this study we evaluated measurements of plasma normetanephrine and metanephrine for detecting pheochromocytomas in patients with von Hippel-Lindau disease or MEN-2.

Methods We studied 26 patients with von Hippel-Lindau disease and 9 patients with MEN-2 who had histologically verified pheochromocytomas and 50 patients with von Hippel-Lindau disease or MEN-2 who had no radiologic evidence of pheochromocytoma. Von Hippel-Lindau disease and MEN-2 were diagnosed on the basis of germ-line mutations of the appropriate genes. The plasma concentrations of normetanephrine and metanephrine were compared with the plasma concentrations of catecholamines (norepinephrine and epinephrine) and urinary excretion of catecholamines, metanephrines, and vanillylmandelic acid.

Results The sensitivity of measurements of plasma normetanephrine and metanephrine for the detection of tumors was 97 percent, whereas the other biochemical tests had a sensitivity of only 47 to 74 percent. All patients with MEN-2 had high plasma concentrations of metanephrine, whereas the patients with von Hippel-Lindau disease had almost exclusively high plasma concentrations of only normetanephrine. One patient with von Hippel-Lindau disease had a normal plasma normetanephrine concentration; this patient had a very small adrenal tumor (<1 cm). The high sensitivity of measurements of plasma normetanephrine and metanephrine was accompanied by a high level of specificity (96 percent).

Conclusions Measurements of plasma normetanephrine and metanephrine are useful in screening for pheochromocytomas in patients with a familial predisposition to these tumors. (N Engl J Med 1999; 340:1872-9.)

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VON HIPPEL-LINDAU disease and multiple endocrine neoplasia type 2 (MEN-2) are multisystem neoplastic disorders, inherited in an autosomal dominant fashion, that account for most currently identified familial pheochromocytomas.¹ In patients with von Hippel-Lindau disease, family-specific mutations determine the varied clinical manifestations, which — in addition to pheochromocytomas — include retinal angiomas, cerebellar hemangioblastomas, and renal, pancreatic, and epididymal tumors. In addition to pheochromocytomas, patients with MEN-2 are predisposed to have medullary thyroid carcinoma and hyperparathyroidism in the A subtype and mucosal neuromas in the B subtype.

The recommended periodic screening for pheochromocytomas in patients with von Hippel-Lindau disease or MEN-2 is based on biochemical evidence of excessive catecholamine production.¹⁻⁴ However, inadequate sensitivity (resulting in false negative tests) is a problem with available assays of plasma and urinary catecholamines or their metabolites.⁵⁻⁹ This lack of sensitivity is particularly troublesome in patients with von Hippel-Lindau disease or MEN-2, in whom small suspicious masses may be identified by imaging studies but in whom pheochromocytomas may not secrete catecholamines in amounts sufficient to cause an abnormal result on a biochemical test.^{1,10,11}

A promising new biochemical test for detecting pheochromocytomas involves measurements of plasma normetanephrine and metanephrine, the respective *O*-methylated metabolites of norepinephrine and epinephrine.⁹ In our study we compared the sensitivity and specificity of measurements of plasma normetanephrine and metanephrine with those of plasma and urinary catecholamines (norepinephrine and epinephrine), urinary metanephrines (normetanephrine and metanephrine combined), and urinary vanillylmandelic acid for identifying the presence or absence of pheochromocytomas in patients with von Hippel-Lindau disease or MEN-2.

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METHODS

Subjects

We studied 73 patients with von Hippel-Lindau disease and 12 patients with MEN-2. The patients were initially identified on the basis of their medical and family histories, and the diagnosis was confirmed by the identification of germ-line mutations in the von Hippel-Lindau tumor-suppressor gene or the *RET* proto-oncogene. All patients were screened prospectively for the presence of pheochromocytoma by computed tomography and at least two of several biochemical tests. These tests were measurements of normetanephrine, metanephrines, and catecholamines in plasma and of the urinary excretion of catecholamines, metanephrines, and vanillylmandelic acid.

Apart from symptoms, signs, or biochemical evidence of a pheochromocytoma, the decision to perform surgery required radiologic evidence of a tumor. Confirmation of the presence of a pheochromocytoma and consequent inclusion in the study required a pathological diagnosis of pheochromocytoma. The presence of pheochromocytomas was confirmed in 26 patients with von Hippel-Lindau disease and 9 patients with MEN-2 (8 with MEN-2A and 1 with MEN-2B) (Table 1). Three of the patients with von Hippel-Lindau disease had pheochromocytomas removed on two separate occasions, one to two years apart. Thirteen patients had bilateral adrenal tumors. Two other patients had previously had bilateral adrenal tumors removed and had recurrent extra-adrenal or adrenal tumors. Four patients had metastases.

Nine patients presented with sustained hypertension (systolic

blood pressure of >140 mm Hg or diastolic blood pressure of >90 mm Hg). Five other patients — four with MEN-2 and one with von Hippel-Lindau disease — had documented periods of intermittent hypertension. All patients with hypertension and an additional four patients with normal blood pressure also reported symptoms of pheochromocytoma (e.g., headache, palpitations, or sweatiness).

Forty-seven patients with von Hippel-Lindau disease and three with MEN-2A had no radiologic evidence of pheochromocytoma; we included them in the study as a comparison group for the patients with pheochromocytoma (Table 1). A third group of 125 normal subjects and 53 patients with hypertension served as a reference population for establishing the 95 percent confidence intervals for assays of plasma catecholamines, normetanephrine, and metanephrine. The study was approved by the appropriate institutional review boards, and all subjects provided written informed consent.

Biochemical Assays

Samples of blood were drawn into 10-ml heparinized tubes through an intravenous cannula in the forearm. The subjects rested in a supine position for 15 minutes after insertion of the cannula before the blood sample was collected. They were instructed to avoid acetaminophen, which interferes with the plasma normetanephrine assay,¹² for at least five days before blood sampling. Plasma was stored at -70°C before all assays, which were usually carried out within one month. Urine specimens were assayed within two weeks after collection. Plasma norepinephrine, epi-

TABLE 1. DEMOGRAPHIC CHARACTERISTICS AND BIOCHEMICAL VALUES IN THE REFERENCE GROUPS, PATIENTS WITH VON HIPPEL-LINDAU DISEASE OR MULTIPLE ENDOCRINE NEOPLASIA TYPE 2 WITHOUT PHEOCHROMOCYTOMA, AND PATIENTS WITH VON HIPPEL-LINDAU DISEASE OR MULTIPLE ENDOCRINE NEOPLASIA TYPE 2 WITH PHEOCHROMOCYTOMA.*

CHARACTERISTIC	PHEOCHROMOCYTOMA ABSENT		PHEOCHROMOCYTOMA PRESENT	
	REFERENCE GROUP (N=178)	PATIENTS WITH VHL DISEASE OR MEN-2 AND NORMAL CT SCANS (N=50)	PATIENTS WITH VHL DISEASE (N=26)	PATIENTS WITH MEN-2 (N=9)
Age (yr)				
Mean	38±13	38±14	33±15	40±11
Range	18-72	11-61	11-61	25-53
Sex (M/F)	100/78	23/27	16/10	4/5
		mean (95% CI)	mean (range)	
Biochemical measures				
Plasma normetanephrine (pg/ml)	45 (18-112)	59 (24-150)	401 (52-2217)	1109 (99-9366)
Plasma metanephrine (pg/ml)	27 (12-61)	24 (0-56)	30 (8-103)	877 (270-2886)
Plasma norepinephrine (pg/ml)	200 (80-498)	274 (95-780)	938 (260-2799)	723 (260-2315)
Plasma epinephrine (pg/ml)	18 (4-83)	15 (2-144)	22 (4-146)	151 (27-879)
Urinary norepinephrine (µg/day)	38 (15-80)†	34 (12-95)	163 (40-870)	137 (10-1109)
Urinary epinephrine (µg/day)	9 (0-20)†	3 (0-15)	5 (1-17)	72 (14-298)
Urinary metanephrines (mg/day)	(0-1.2)†	0.5 (0-1.1)	1.3 (0.3-5.2)	5.8 (0.5-51.2)
Urinary vanillylmandelic acid (mg/day)	(0-7.9)†	3.5 (0.6-6.3)	6.8 (2.2-21.3)	19.9 (3.6-65.0)

*Two groups of patients without pheochromocytoma were compared with patients with pheochromocytoma: a reference group of patients with or without hypertension, for establishing the 95 percent confidence intervals (CIs) and the upper reference limits for plasma normetanephrine, metanephrine, norepinephrine, and epinephrine, and a group of patients with multiple endocrine neoplasia type 2 (MEN-2) or von Hippel-Lindau (VHL) disease but no pheochromocytoma (as evidenced by a normal computed tomographic scan of the abdomen), for establishing specificity. The values for patients with pheochromocytoma were determined when the tumors were first identified by computed tomography. For data that were not distributed normally, the mean values are geometric means. The 95 percent confidence intervals were calculated from the antilogarithm of the mean ± 2 SD of the transformed data. To convert values for plasma measurements to picomoles per liter, multiply by 5.46 for normetanephrine, 5.08 for metanephrine, 5.91 for norepinephrine, and 5.46 for epinephrine. To convert values for urinary catecholamines and metabolites to nanomoles per day, multiply by 5.91 for norepinephrine, 5.46 for epinephrine, 5263 for metanephrines, and 5050 for vanillylmandelic acid.

†The values were established by the outside laboratory that measured urinary catecholamines and metabolites in reference groups that were different from the groups used to establish values for biochemical measures in plasma. Mean values for urinary metanephrines and vanillylmandelic acid were not available.

nephrine, normetanephrine, and metanephrine were measured by liquid chromatography with electrochemical detection, as described elsewhere.^{12,13} Twenty-four-hour urinary excretion of norepinephrine, epinephrine, metanephrines (normetanephrine and metanephrine combined), and vanillylmandelic acid was measured at a commercial laboratory by liquid chromatography with electrochemical detection (for catecholamines)¹⁴ or by spectrofluorometry (for metanephrines and vanillylmandelic acid).

Analysis of Data

We determined upper reference limits for plasma normetanephrine, metanephrine, norepinephrine, and epinephrine from the 95 percent confidence intervals in the reference group of 178 subjects (Table 1). The plasma concentrations of normetanephrine, metanephrine, and catecholamines in the 178 subjects were normally distributed after logarithmic transformation. Thus, upper reference limits were calculated from the antilogarithm of the mean +2 SD of the transformed data. The upper reference limits for urinary catecholamines, metanephrines, and vanillylmandelic acid were established by the outside laboratory that carried out the tests.

A true positive result for pairs of measurements (plasma normetanephrine and metanephrine, plasma norepinephrine and epinephrine, and urinary norepinephrine and epinephrine) 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 that was equal to or higher than the respective upper reference limit. A false negative test for pairs of measurements in a patient with pheochromocytoma, or a true negative test in a patient without pheochromocytoma, was defined as values for both measurements that were lower than the respective upper reference limits.

The sensitivity of each biochemical test was estimated from the percentage of true positive results among the total of the true positive and false negative results for patients with pheochromocytoma. The specificity of each biochemical test was estimated from the percentage of true negative results among the total of the true negative and false positive results for patients without pheochromocytoma. The negative predictive value was estimated from the percentage of true negative results among the total of the true negative and false negative results. The positive predictive value was estimated from the percentage of true positive results among the total of the true positive and false positive tests.

Statistical Analysis

Differences in sensitivity or specificity between measurements of plasma metanephrines and the other biochemical tests used for the diagnosis of pheochromocytoma were assessed with use of McNemar's test.¹⁵ Differences in the extent of the increase above the upper reference limit among the biochemical tests were compared by analysis of variance with Scheffé's post hoc test. The relations between tumor mass and biochemical-test results were examined by simple and multiple linear regression analyses. Analysis of variance and linear regression analyses were carried out on log-transformed data.

RESULTS

Biochemical Studies

Of the 35 patients with pheochromocytoma, all but 2 patients, one with von Hippel–Lindau disease and another with MEN-2, had high plasma concentrations of normetanephrine (Fig. 1). The three patients with von Hippel–Lindau disease who had tumors removed on two separate occasions had high plasma normetanephrine concentrations on both occasions. Two patients with von Hippel–Lindau disease and all patients with MEN-2 who had phe-

ochromocytoma also had high plasma concentrations of metanephrine; thus, both plasma normetanephrine and metanephrine were normal in only 1 of the 38 tests in the 35 patients with familial pheochromocytoma.

In contrast, plasma norepinephrine concentrations were normal in eight patients with von Hippel–Lindau disease and pheochromocytoma and four patients with MEN-2 and pheochromocytoma (Fig. 1). Plasma epinephrine concentrations were normal in all except one patient with von Hippel–Lindau disease and high in only six of the nine patients with MEN-2, one of whom had a normal plasma norepinephrine concentration. Ten patients, including seven with normal plasma concentrations of catecholamines, also had normal urinary excretion of both norepinephrine and epinephrine. Urinary excretion of metanephrines was normal in 13 of 37 tests in 34 patients, and the excretion of vanillylmandelic acid was normal in 18 of 34 tests in 31 patients.

The sensitivity of measurements of plasma normetanephrine and metanephrine for the diagnosis of pheochromocytoma was 97 percent, a sensitivity significantly higher than that for plasma norepinephrine and epinephrine ($P=0.002$), urinary norepinephrine and epinephrine ($P=0.004$), urinary metanephrines ($P<0.001$), and urinary vanillylmandelic acid ($P<0.001$) (Table 2). The high sensitivity of plasma normetanephrine and metanephrine was accompanied by high specificity (96 percent).

In all patients with MEN-2 or von Hippel–Lindau disease who had pheochromocytomas, the plasma concentrations of normetanephrine were increased by an average of 348 percent above the upper reference limit, a considerably larger relative increase ($P<0.001$) than those in plasma concentrations of norepinephrine (78 percent) and in urinary excretion of norepinephrine (95 percent), metanephrines (55 percent), and vanillylmandelic acid (8 percent) (Fig. 1). In the patients with MEN-2 who had pheochromocytomas, the relative increase in the plasma concentrations of metanephrine above the upper reference limit (1337 percent) was also much larger ($P<0.001$) than the increases in epinephrine in plasma (82 percent) and urine (258 percent) (Fig. 1 and Table 1).

The size of the tumor correlated strongly and positively with the plasma concentration of normetanephrine ($r=0.84$, $P<0.001$) and the urinary excretion of metanephrines ($r=0.78$, $P<0.001$) and vanillylmandelic acid ($r=0.81$, $P<0.001$); it correlated more weakly with the plasma norepinephrine concentration ($r=0.52$, $P=0.001$) (Fig. 2). There were also weak, but significant, positive relations between tumor size and the plasma concentrations of epinephrine ($r=0.44$, $P=0.007$) and metanephrine ($r=0.51$, $P=0.001$) and the urinary excretion of norepinephrine ($r=0.51$, $P=0.001$) and epinephrine ($r=0.54$, $P<0.001$) (data not shown).

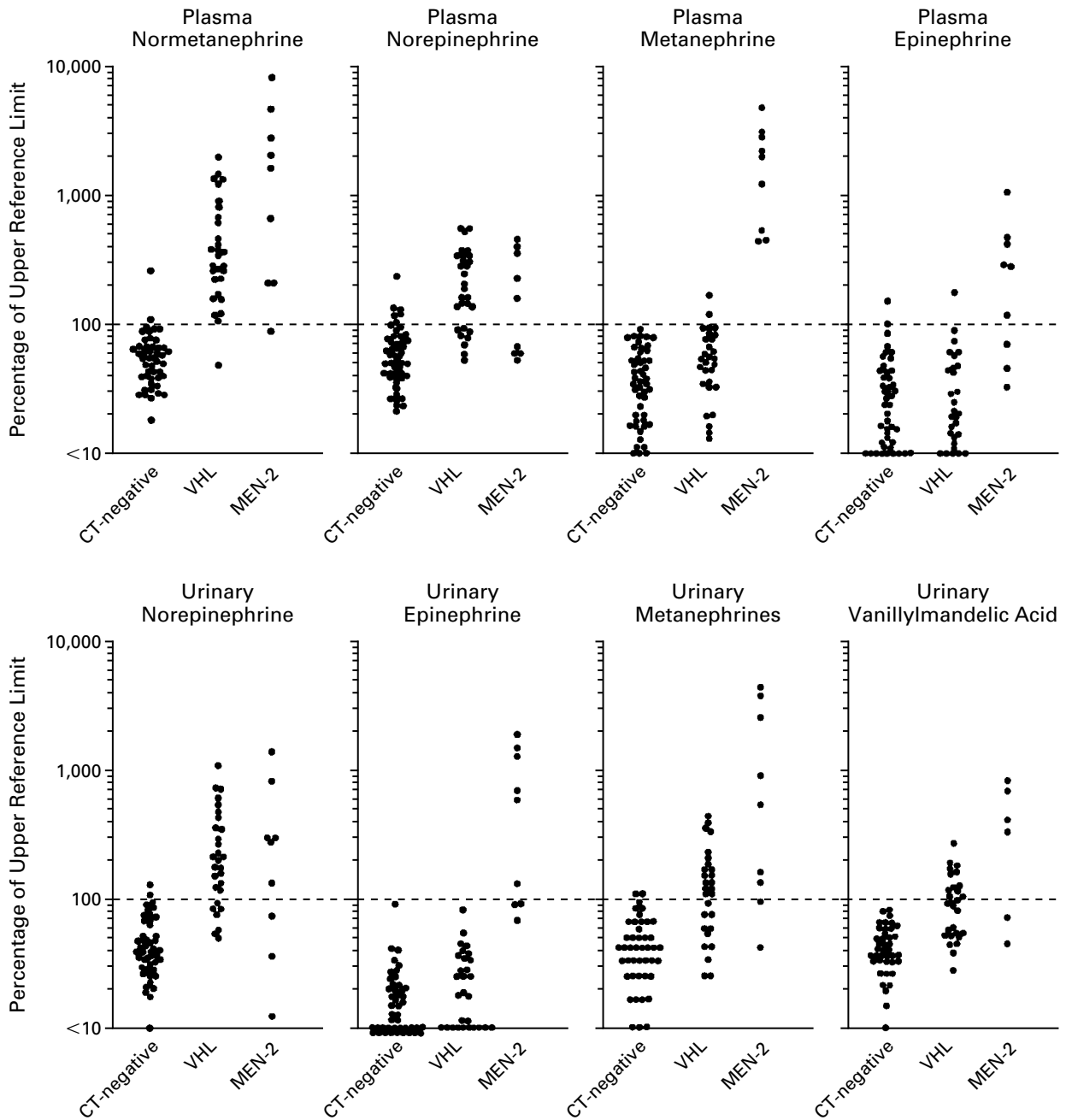


Figure 1. Plasma Concentrations of Normetanephrine, Norepinephrine, Metanephrine, and Epinephrine (Upper Panels) and Urinary Excretion of Norepinephrine, Epinephrine, Metanephrines, and Vanillylmandelic Acid (Lower Panels).

The values are expressed as percentages of the upper reference limit for each test. Data on individual patients are shown for three groups of patients with von Hippel-Lindau disease and multiple endocrine neoplasia type 2 (MEN-2), as follows: patients with von Hippel-Lindau disease or MEN-2 in whom a pheochromocytoma was ruled out on the basis of normal computed tomography (CT-negative), patients with von Hippel-Lindau disease who had histologically verified pheochromocytomas (VHL), and patients with MEN-2 who had histologically verified pheochromocytomas (MEN-2). The values for patients with pheochromocytoma were determined when the tumors were first identified by computed tomography. The dotted horizontal line represents the upper reference limit for each test. The scales are logarithmic.

TABLE 2. CHARACTERISTICS OF BIOCHEMICAL TESTS FOR THE DETECTION OF PHEOCHROMOCYTOMA IN PATIENTS WITH VON HIPPEL-LINDAU DISEASE OR MULTIPLE ENDOCRINE NEOPLASIA TYPE 2.

BIOCHEMICAL TEST	SENSITIVITY	SPECIFICITY	NEGATIVE	POSITIVE
			PREDICTIVE	PREDICTIVE
			VALUE	VALUE
			percent (number/total number)	
Plasma normetanephrine and metanephrine	97 (37/38)	96 (48/50)	98 (48/49)	95 (37/39)
Plasma norepinephrine and epinephrine	71 (27/38)	86 (43/50)	80 (43/54)	79 (27/34)
Urinary norepinephrine and epinephrine	74 (28/38)	96 (45/47)	82 (45/55)	93 (28/30)
Urinary metanephrines	65 (24/37)	95 (42/44)	76 (42/55)	92 (24/26)
Urinary vanillylmandelic acid	47 (16/34)	100 (41/41)	69 (41/59)	100 (16/16)

Despite similar increases in plasma and urinary norepinephrine, the increases in plasma epinephrine and metanephrine and urinary epinephrine and metanephrines were considerably larger ($P < 0.001$) in patients with MEN-2 than in patients with von Hippel-Lindau disease (Fig. 1 and Table 1). The patients with pheochromocytoma who had high blood pressure or symptoms had significantly larger tumors and higher plasma concentrations of normetanephrine, metanephrine, and catecholamines than the patients who were normotensive or had no symptoms.

Case Studies

The results of three or more of the several biochemical tests were normal in 10 of the 35 patients with pheochromocytomas (Table 3). Several of these patients are described below.

Patient 9, a 28-year-old woman with normal blood pressure, reported episodes of facial flushing during the five years she was monitored. She was otherwise asymptomatic until the year preceding surgery, when she had increasingly frequent headache, lightheadedness, and tachycardia. She had normal plasma catecholamine concentrations at rest or after the administration of glucagon on four separate occasions. The results of measurements of the urinary excretion of catecholamines, metanephrines, and vanillylmandelic acid were normal on most of the seven occasions. In contrast, plasma normetanephrine concentrations were consistently high and increased progressively during the five-year follow-up period. Bilateral adrenal pheochromocytomas (each 3 cm in diameter) were removed about five years after they were first detected by computed tomography.

Patient 20, a 15-year-old girl without hypertension or symptoms, had normal values for plasma catecholamines and urinary metanephrines and vanillylmandelic acid. The only initial evidence of a pheochromocytoma was provided by computed tomography, which showed a 2-cm mass in one adre-

nal gland; by the slightly high urinary excretion of norepinephrine (20 percent above the upper reference limit); and by a high plasma normetanephrine concentration (280 percent above the upper reference limit). One year later, all biochemical tests were strongly positive. An adrenal pheochromocytoma, 2 by 3 cm, was removed.

Patient 21, a 25-year-old woman without hypertension, was followed for nearly four years. She reported occasional headaches associated with anxiety and sweatiness, but her plasma concentrations of catecholamines and urinary excretion of catecholamines, metanephrines, and vanillylmandelic acid were normal on three separate occasions. The patient's plasma normetanephrine concentrations, however, were high on all occasions and increased with time. On initial examination, computed tomography revealed a normal right adrenal gland and some enlargement of the left adrenal gland. Nearly four years later, the left adrenal gland remained unchanged, but a small mass was noted in the right adrenal gland. Two small bilateral pheochromocytomas (1 by 1.5 cm and 2 by 2.5 cm) were subsequently removed at surgery.

DISCUSSION

In our study, plasma concentrations of normetanephrine or metanephrine were high on 97 percent of the 38 occasions when pheochromocytomas were found in 35 patients, a finding that indicates a sensitivity considerably superior to that of all other biochemical tests. This high sensitivity agrees with our previous findings in 52 patients with mainly sporadic pheochromocytoma, in which no patients had normal plasma concentrations of both metanephrine and normetanephrine.⁹ The patient with pheochromocytoma and normal plasma concentrations of normetanephrine and metanephrine (Patient 26, described in Table 3) represents the only case of a normal test result in more than 120 patients with pheochromocytoma who have been studied (including unpub-

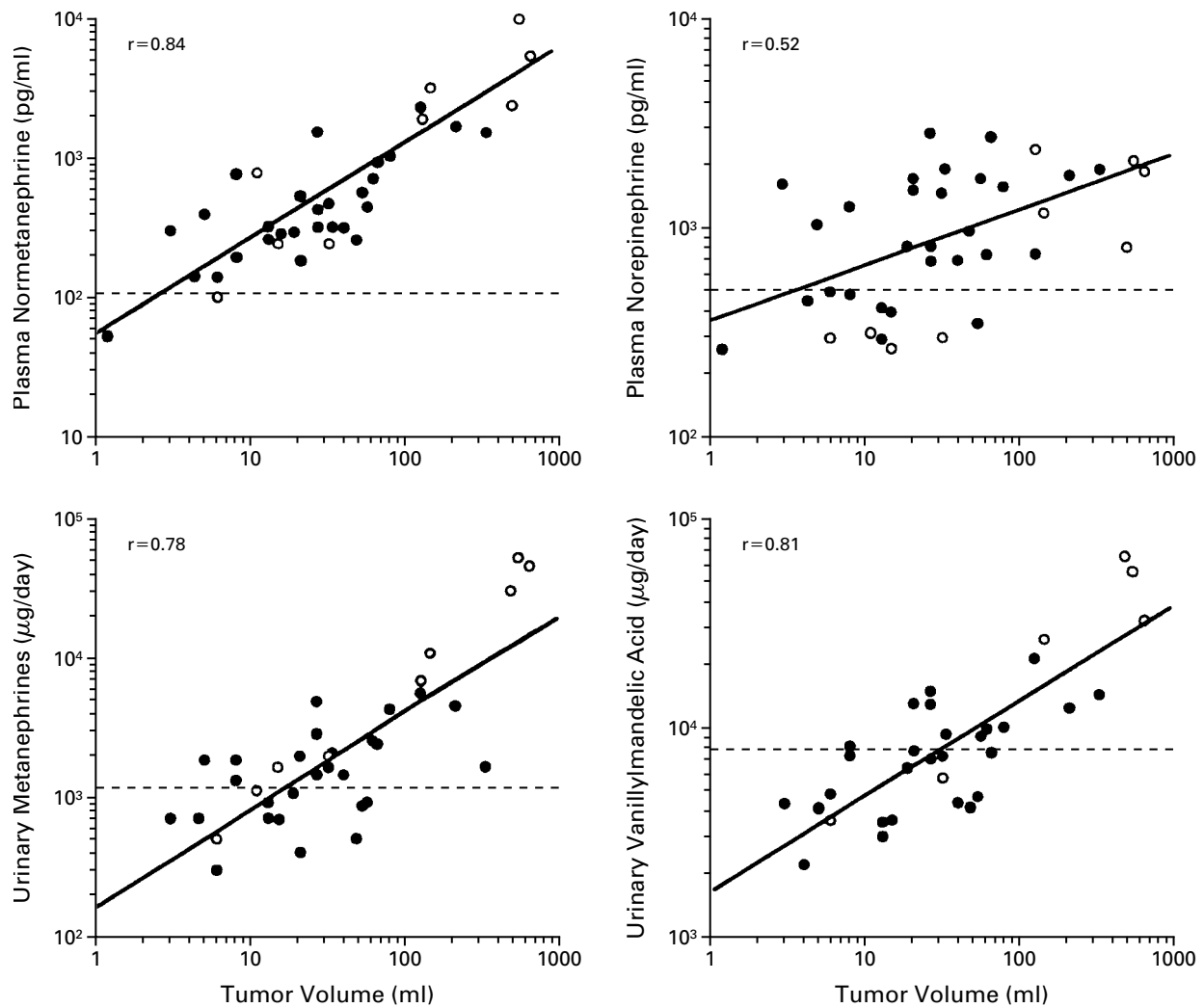


Figure 2. Relations of Tumor Volume to Plasma Concentrations of Normetanephrine and Norepinephrine and Urinary Excretion of Metanephrines and Vanillylmandelic Acid.

The patients with von Hippel-Lindau disease are represented by the solid circles, and the patients with MEN-2 by the open circles. The scales are logarithmic. To convert values for plasma measurements to picomoles per liter, multiply by 5.46 for normetanephrine and 5.91 for norepinephrine. To convert values for urinary catecholamines and metabolites to nanomoles per day, multiply by 5.26 for metanephrines and 5.05 for vanillylmandelic acid.

lished observations in patients with sporadic pheochromocytomas). The tumor in this asymptomatic patient was positively identified only after the patient underwent surgery for a renal carcinoma on the same side on which computed tomography had revealed a 1-cm adrenal mass.

The large amounts of membrane-bound catechol-O-methyltransferase in chromaffin cells¹⁶ are the reason for the high sensitivity of plasma normetanephrine and metanephrine in detecting pheochromocytomas. The membrane-bound enzyme has much higher affinity for catecholamines than does the soluble enzyme present in other tissues; thus, the adre-

nal glands constitute the single largest source of metanephrine and normetanephrine, contributing more than 90 percent of metanephrine and 24 to 40 percent of normetanephrine in plasma.¹⁷ In contrast, only 7 percent of plasma norepinephrine is derived from the adrenal glands; the remaining 93 percent is derived from sympathetic nerves.¹⁷ To increase plasma norepinephrine concentrations from normal (50 percent of the upper reference limit) to the upper reference limit would therefore require a pheochromocytoma to secrete 14.3 times as much norepinephrine as is normally secreted by the adrenal glands (7 percent of the total norepinephrine in plasma). In

TABLE 3. BIOCHEMICAL VALUES IN PATIENTS WITH PHEOCHROMOCYTOMA AND NEGATIVE RESULTS ON THREE OR MORE OF THE FIVE BIOCHEMICAL TESTS.*

PATIENT NO.	DISEASE	PLASMA CONCENTRATION				URINARY EXCRETION			
		NMN	MN	NE	E	NE	E	M	VMA
		pg/ml				μg/day		mg/day	
9	VHL	132†	12	346	4	46	5	0.3	5.0
10	VHL	249†	32	949†	17	40	1	0.5	4.1
19	VHL	117†	20	388	62	63	4	0.5	3.6
20	VHL	313†	36	411	8	98†	5	0.9	3.5
21	VHL	251†	12	289	5	57	7	0.7	3.0
23	VHL	136†	22	488	15	80	9	0.3	4.8
24	VHL	162†	28	475	28	71	2	0.7	2.2
26	VHL	52	21	260	12	43	4	Not done	Not done
32	MEN-2	234†	760†	294	59	10	11	1.9†	5.7
35	MEN-2	99	329†	295	99†	29	18	0.5	3.6
Upper reference limit		112	61	498	83	80	20	1.2	7.9

*Three of the five tests involved measurements of two substances (plasma normetanephrine and metanephrine, plasma norepinephrine and epinephrine, and urinary norepinephrine and epinephrine). For these three tests, a negative result was defined as normal values for both substances in the pair. The values were determined when the tumors were first identified by computed tomography. To convert values for plasma measurements to picomoles per liter, multiply by 5.46 for normetanephrine, 5.08 for metanephrine, 5.91 for norepinephrine, and 5.46 for epinephrine. To convert values for urinary catecholamines and metabolites to nanomoles per day, multiply by 5.91 for norepinephrine, 5.46 for epinephrine, 5263 for metanephrines, and 5050 for vanillylmandelic acid. NMN denotes normetanephrine, MN metanephrine, NE norepinephrine, E epinephrine, M metanephrines, VMA vanillylmandelic acid, VHL von Hippel-Lindau disease, and MEN-2 multiple endocrine neoplasia type 2.

†This value is above the upper limit of the normal reference value.

contrast, with a 24 percent contribution by the adrenal glands to plasma normetanephrine, only an increase by a factor of 4.2 would be necessary to raise plasma normetanephrine concentrations to the upper reference limit. These estimates provide one explanation for the fact that plasma normetanephrine has higher sensitivity than does norepinephrine for detecting pheochromocytoma.

This explanation assumes that catecholamines are metabolized and released by pheochromocytoma cells in a fashion similar to that of normal adrenal chromaffin cells. The available evidence indicates that metabolism is similar,¹⁶ but other evidence suggests that catecholamine secretion is not. In particular, despite consistently high plasma concentrations of normetanephrine or metanephrine, some patients with pheochromocytoma have normal plasma concentrations of catecholamines or have high concentrations only during paroxysmal attacks.¹⁶ The silent or intermittently secreting tumors in these patients are therefore continually metabolizing catecholamines to metanephrines, without consistently secreting the parent amines into the circulation. This process provides another explanation for the superior sensitivity of measurements of plasma normetanephrine and metanephrine to those of plasma and urinary catecholamines.

The low sensitivity of measurements of urinary vanillylmandelic acid for the diagnosis of pheochromocytoma is explained by findings that less than 20 percent of the vanillylmandelic acid derives from hepatic metabolism of circulating catecholamines and metanephrines and more than 80 percent from deaminated catecholamine metabolites.¹⁸ The latter are derived mainly from norepinephrine in sympathetic neurons.¹⁹ Thus, to increase urinary excretion of vanillylmandelic acid above the upper reference limit requires large increases in plasma catecholamines, normetanephrine, or metanephrine. Consequently, as shown here and in other studies,^{20,21} urinary excretion of vanillylmandelic acid has poor sensitivity for identifying pheochromocytomas.

We measured urinary metanephrines as the sum of both normetanephrine and metanephrine. Better sensitivity can be obtained by using fractionated liquid chromatographic measurements of normetanephrine and metanephrine.^{22,23} Urinary measurements of metanephrines are, however, routinely determined after the sulfate-conjugated metanephrines are hydrolyzed to the free metanephrines. Because sulfate-conjugated metanephrines constitute more than 95 percent of both free and conjugated metanephrines,²⁴ measurements of urinary metanephrines reflect different

catecholamine metabolites than measurements of free metanephrines. The free metanephrines are produced largely in chromaffin tissue by catechol-*O*-methyltransferase,¹⁶ whereas the sulfate-conjugated metanephrines are produced by monoamine-preferring sulfotransferase, an enzyme concentrated in the gut.²⁵ It is therefore unlikely that fractionated measurements of urinary metanephrines would have higher sensitivity than the measurements of plasma normetanephrine and metanephrine in the free form in the present study.

The consistently high plasma concentrations of metanephrine in patients with MEN-2 and pheochromocytoma and the almost universal elevation of only plasma normetanephrine in patients with von Hippel-Lindau disease and pheochromocytoma indicate a noradrenergic tumor phenotype in von Hippel-Lindau disease, as compared with an adrenergic phenotype in MEN-2. The latter finding is consistent with the high incidence of epinephrine-producing pheochromocytomas in patients with MEN-2²⁶⁻²⁸ and also shows that a high plasma metanephrine value is more sensitive than a high plasma or urinary epinephrine value for detecting such tumors.

In summary, the measurement of plasma normetanephrine and metanephrine is a highly sensitive test for detecting pheochromocytoma in patients with a familial predisposition to these tumors. As more patients are identified with familial disorders associated with pheochromocytoma, more are being identified as having such tumors at an earlier age.¹ In these and other patients in whom the tumors are small, standard biochemical tests often yield false negative results.^{1,10,20} The superior sensitivity of measurements of plasma normetanephrine and metanephrine should help overcome this limitation.

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