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Pheochromocytoma Catecholamine Phenotypes and Prediction of Tumor Size and Location by Use of Plasma Free Metanephrines

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Background: **Measurements of plasma free metanephrines (normetanephrine and metanephrine) provide a useful test for diagnosis of pheochromocytoma and may provide other information about the nature of these tumors.**

Methods: **We examined relationships of tumor size, location, and catecholamine content with plasma and urinary metanephrines or catecholamines in 275 patients with pheochromocytoma. We then prospectively examined whether measurements of plasma free metanephrines could predict tumor size and location in an additional 16 patients.**

Results: **Relative proportions of epinephrine and norepinephrine in tumor tissue were closely matched by relative increases of plasma or urinary metanephrine and normetanephrine, but not by epinephrine and norepinephrine. Tumor diameter showed strong positive relationships with summed plasma concentrations or urinary outputs of metanephrine and normetanephrine ($r = 0.81$ and 0.77; $P < 0.001$), whereas relationships with plasma or urinary catecholamines were weaker ($r = 0.41$ and 0.44). All tumors in which increases in plasma metanephrine were >15% of the combined increases of normetanephrine and metanephrine either had adrenal locations or appeared to be recurrences of previously resected adrenal tumors. Measurements of plasma free metanephrines predicted tumor diameter to within a mean of 30% of actual diameter, and high plasma concentrations of free metanephrine relative to normetanephrine accurately predicted adrenal locations.**

Conclusions: **Measurements of plasma free metanephrines not only provide information about the likely presence or absence of a pheochromocytoma, but when a tumor is present, can also help predict tumor size and location. This additional information may be useful for clinical decision-making during tumor localization procedures.**

Pheochromocytomas are rare neuroendocrine tumors with highly variable presentation that require consideration in large numbers of patients at considerable cost to healthcare systems. There remains lack of agreement about the most efficient and cost-effective method for diagnosis of the tumor, but as we have reported elsewhere *(1)*, measurements of plasma free metanephrines can now make this process relatively simple. Provided that appropriate reference intervals are used, the high sensitivity of the test means that normal results reliably exclude virtually all catecholamine-producing tumors. Exceptions include microscopic recurrences or small tumors (<1 cm) in usually asymptomatic patients screened because of a hereditary predisposition or a previous tumor. Other exceptions include patients with rare dopamine-producing paragangliomas in which norepinephrine and epinephrine are not synthesized or metabolized to normetanephrine and metanephrine *(2)*. In these patients, additional measurements of plasma methoxytyra-

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mine, the O-methylated metabolite of dopamine, can diagnose the tumor *(3)*.

In cases of positive test results, the extent of increases in plasma free metanephrines can be useful in guiding further diagnostic decision-making. In our experience, increases of plasma free normetanephrine above 2.2 nmol/L (400 ng/L) or of plasma free metanephrine above 1.2 nmol/L (236 ng/L), 3.5- to 4-fold above the upper limits of the adult reference intervals, do not occur in patients without pheochromocytoma (~100% specificity), but occur in ~80% of patients with the tumor *(4)*. In such patients, there is a high likelihood of pheochromocytoma, and the remaining problem is to locate the tumor. In patients with milder increases, pharmacologic, dietary, and other causes of false-positive results should first be eliminated *(4)*. The clonidine suppression test, coupled with measurements of plasma free normetanephrine, then provides a simple pharmacologic method to finally exclude or confirm the tumor.

Despite the above advances, the implementation and interpretation of biochemical tests for diagnosis of pheochromocytoma continue to generate difficulty. We continue to see patients for whom multitudes of unnecessary biochemical tests have been ordered or in whom expensive imaging studies have been carried out to locate a supposed tumor, even after biochemical testing has yielded equivocal or even normal results. We also continue to see occasional patients who have undergone unnecessary surgery because of a suspected, but biochemically unconfirmed, pheochromocytoma.

Although continuing use of unnecessary biochemical tests generates revenue for commercial laboratories, this is not in the best interests of patients or healthcare systems. Of course, this problem is not confined to the laboratory diagnosis of pheochromocytoma; there is a recognized general need for more guidance from the laboratory in the choice, implementation, and interpretation of many laboratory tests *(5)*. Such guidance may include narrative interpretations, which, as described by Smythe and Drew *(6)*, are particularly useful in the diagnosis of pheochromocytoma. In the present study we examined whether measurements of plasma free metanephrines could provide information about the catecholamine phenotype, size, and location of the pheochromocytoma, all of which may be useful for guiding clinical decision-making once the tumor is diagnosed.

Patients and Methods

PATIENTS

The study involved retrospective analysis of data for 275 patients with confirmed pheochromocytoma and prospective examination in an additional 16 patients to determine the ability to predict tumor size and location based on measurements of plasma free metanephrines. The 275 patients in the retrospective analysis included 214 described in a previous report on the biochemical diagnosis of pheochromocytoma *(1)* and 61 additional patients diagnosed with pheochromocytoma at the NIH. The 16 patients in the prospective analysis included 8 at the NIH and 8 at St. Radboud University Medical Center (Nijmegen, The Netherlands). Pheochromocytoma was confirmed based on pathologic examination of tumor tissue or a diagnosis of inoperable metastatic disease indicated by imaging studies. Patients provided informed consent for the studies, which were approved by the intramural review board or hospital ethics committee of the centers where patients were studied.

LABORATORY TESTS

Blood samples were collected into heparin-containing tubes by use of a forearm venous cannula, with patients supine for at least 20 min 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. All samples were collected on ice, and plasma was separated and stored at −80 °C before analysis according to recommended procedures *(7)*. Plasma was assayed by HPLC for concentrations of free metanephrines and catecholamines, as described previously *(8, 9)*, with further details and modifications as listed on the Clinical Neurochemistry Laboratory web site (http://www.catecholamine.org/labprocedures). The 24-h urinary outputs of catecholamines and deconjugated (free plus conjugated) fractionated metanephrines for patients seen at the NIH were measured by HPLC or liquid chromatography with tandem mass spectroscopy, under a contract between the NIH Clinical Center and an outside commercial laboratory (Mayo Medical Laboratories, Rochester, MN). HPLC procedures were also used for urinary measurements in patients seen elsewhere, as described previously *(1)*.

Reference intervals for plasma concentrations of free normetanephrine (0.10–0.61 nmol/L), free metanephrine (0.06–0.31 nmol/L), norepinephrine (0.47–2.95 nmol/L), and epinephrine (4–83 0.02–0.45 nmol/L) were established from combined groups of 175 normotensive and 110 hypertensive volunteers, as detailed elsewhere *(10)*. Reference intervals for 24-h urinary outputs of deconjugated normetanephrine (0.70–2.64 μmol/24 h), deconjugated metanephrine (0.22–1.32 μmol/24 h), norepinephrine (0.09–0.47 μmol/24 h), and epinephrine (0–0.11 μmol/24 h) were those provided by Mayo Medical Laboratories.

TUMOR CATECHOLAMINE CONCENTRATIONS, LOCATION, AND SIZE

Samples of tumor tissue were procured from 114 patients, generally within 1 h of surgical resection of tumors. Small 10- to 50-mg tissue samples were immediately frozen for storage at −80 °C before further processing. Weighed samples of tissue were homogenized in 0.5 mL of 0.4 mol/L perchloric acid containing 0.5 mmol/L EDTA. After centrifugation, appropriately diluted samples of

supernatants were assayed for catecholamines by the same HPLC methodology used for plasma analyses.

Adrenal and extraadrenal locations of tumors were determined in 274 patients by use of results of imaging studies and surgical and pathology records. For patients presenting with recurrent or malignant pheochromocytoma, careful attention was made to assess the adrenal or extraadrenal location of primary tumors or presence of multifocal disease. Tumor dimensions from pathology records were available for 214 patients. Care was taken to ensure that dimensions represented those of actual tumors or nodules rather than whole resected masses.

DATA ANALYSIS AND STATISTICS

For initial examination of tumor catecholamine phenotypes, designations of epinephrine-producing (adrenergic) and predominantly norepinephrine-producing (noradrenergic) tumors were based on tissue epinephrine content as a percentage of both epinephrine and norepinephrine, according to previous observations in two groups of patients with differing production of phenylethanolamine-*N*-methyltransferase, the enzyme that converts norepinephrine to epinephrine *(11, 12)*. Absolute increases in plasma or urinary catecholamines or metanephrines above normal were determined as increases above the upper limits of the reference intervals for each analyte. A value of zero was assigned when there was no increase. Percentage increases in plasma or urinary metanephrine relative to combined increases in normetanephrine and metanephrine, or increases in plasma or urinary epinephrine relative to combined increases in norepinephrine and epinephrine, were then estimated for comparisons with relative tumor tissue epinephrine content.

Statistical methods, performed with Statview (SAS Institute Inc.), included linear regression analysis for examination of relationships of relative tumor epinephrine content with relative increases in plasma or urinary metanephrine or epinephrine. Comparisons of test sensitivities in patients with adrenergic and noradrenergic tumors were performed with the χ^2 test. Regression analysis and multiple linear regression analysis were used to examine relationships between mean tumor diameters and plasma concentrations or urinary outputs of metanephrines or catecholamines. Mean tumor diameters were calculated from cubed roots of rectangular volumes. Where there were two or more masses, rectangular volumes were calculated separately for each mass and summed to obtain a total rectangular volume.

Prospective predictions of tumor diameter were calculated by use of the logarithmic relationship of mean tumor diameter with the summed plasma concentrations of normetanephrine and epinephrine. The 95% confidence intervals of this relationship were estimated by regression analysis of calculated upper and lower intervals. These were estimated by use of logarithmically transformed data to generate geometric means ± 2 SD over different intervals of tumor diameter.

Results

TUMOR CATECHOLAMINE PHENOTYPES

Among the samples of tumor tissue taken from the 114 patients with pheochromocytoma, there were 54 in which epinephrine content was >10% of the combined epinephrine and norepinephrine content. In the other 60 tumor samples, epinephrine content was <10% of the combined epinephrine and norepinephrine content. After previous observations *(11, 12)*, the former tumors were designated as epinephrine-producing tumors with an adrenergic phenotype, whereas the latter were designated as predominantly norepinephrine-producing tumors with a noradrenergic phenotype (Table 1).

All patients with adrenergic tumors had increased plasma free metanephrine, and all except one of the patients with noradrenergic tumors had increased plasma free normetanephrine (Table 1). The patients with noradrenergic tumors all had no or negligible increases in plasma free metanephrine amounting to <10% of the combined increases in normetanephrine and metaneph-

Table 1. Biochemical profiles in patients with adrenergic (epinephrine-producing) and noradrenergic (predominantly norepinephrine-producing) tumors.

	Adrenergic tumors (n = 54)	Noradrenergic tumors (n = 60)
Tumor EPI[a] content,[b] % of EPI + NE	49.3 (11.0–90.2)	1.3 (0.0–9.8)
Increase in plasma MN,[c] % of MN + NMN	55.7 (8.1–100)	0.0 (0.0–9.8)
Increase in plasma EPI,[c] % of EPI + NE	31.8 (0.0–100)	0.0 (0.0–1.3)
Increase in urinary MN,[c] % of MN + NMN	63.0 (7.5–100)	0.0 (0.0–9.8)
Increase in urinary EPI,[c] % of EPI + NE	47.3 (0.0–100)	0.0 (0.0–2.0)
Tumor markers,[d] % positive results (n)		
Plasma MN	100 (54/54)	20 (12/60)
Urinary MN	97 (34/35)	9 (3/33)
Plasma EPI	65 (35/54)	7 (4/60)
Urinary EPI	69 (31/45)	6 (3/51)
Plasma NMN	89 (48/54)	98 (59/60)
Urinary NMN	89 (31/35)	97 (32/33)
Plasma NE	54 (29/54)	87 (52/60)
Urinary NE	60 (27/45)	80 (41/51)

[a] EPI, epinephrine; NE, norepinephrine; MN, metanephrine; NMN, normetanephrine.

[b] Tumor epinephrine content was determined as the percentage of total epinephrine and norepinephrine and is shown as the median value with ranges in parentheses.

[c] Increases in plasma or urinary metanephrine or epinephrine were determined as increases above the upper reference limits, expressed as a percentage increase relative to combined increases in both metanephrine and normetanephrine or in epinephrine and norepinephrine, and are shown as median values with ranges in parentheses.

[d] Values for tumor markers indicate the percentage of positive results, as indicated in parentheses, from the number of results where values were increased above the upper reference limits divided by the total number of results.

rine. In contrast, increases in plasma free metanephrine in patients with adrenergic tumors were >10% of the combined increases in metanephrine and normetanephrine in all except one patient, who had an 8% increase. Among these patients, there was a strong positive relationship ($r = 0.82$; $P < 0.001$) between the tumor tissue epinephrine content and increases in plasma free metanephrine relative to combined increases in metanephrine and normetanephrine (Fig. 1).

The above findings for plasma free metanephrines differed markedly from those for plasma catecholamines: only 65% of patients with adrenergic tumors had increases in plasma epinephrine and 87% of patients with noradrenergic tumors had increases of norepinephrine (Table 1). In addition, the relationship between tissue epinephrine content (as a percentage) and relative increases in plasma epinephrine was weaker than that with relative increases in plasma metanephrine (Fig. 1).

Differences similar to the above for plasma markers of pheochromocytoma were observed for urinary markers. Ninety-seven percent of patients with adrenergic tumors had increased urinary metanephrine compared with only 71% with increased urinary epinephrine, and 97% of patients with noradrenergic tumors had increased urinary normetanephrine compared with only 77% with increased urinary norepinephrine (Table 1). Among patients with adrenergic tumors, the relationship of tumor tissue epinephrine content with increases in urinary metanephrine was stronger than that with urinary epinephrine ($r = 0.84$ vs $r = 0.60$).

TUMOR LOCATION

Among the 274 patients for whom there were records of tumor locations, 186 patients had tumors with adrenal locations, 35 patients had recurrences (mainly malignancies) that appeared to have developed from previously resected adrenal tumors, and 53 patients had extraadrenal primary tumors or recurrences of previously resected extraadrenal tumors (Fig. 2). Ninety-two of the 186 (49%) patients with adrenal tumors had increases in plasma free metanephrine that were >10% of the combined increases in normetanephrine and metanephrine, indicating an epinephrine-producing (adrenergic) biochemical phenotype for tumors in these patients. The other 94 (51%) patients had no or negligible increases in metanephrine relative to normetanephrine, indicating a predominantly norepinephrine-producing (noradrenergic) biochemical phenotype. This compared with 12 (34%) recurrences of adrenal tumors with an adrenergic phenotype and 23 (66%) with a noradrenergic phenotype.

In contrast to the above patterns of catecholamine phenotypes in patients with adrenal tumors or recurrences, all except 1 of the 53 patients with extraadrenal tumors (98%) had patterns of increases in plasma free metanephrines that indicated a noradrenergic phenotype. The single patient with the adrenergic extraadrenal tumor was an unusual case. Imaging studies suggested a large

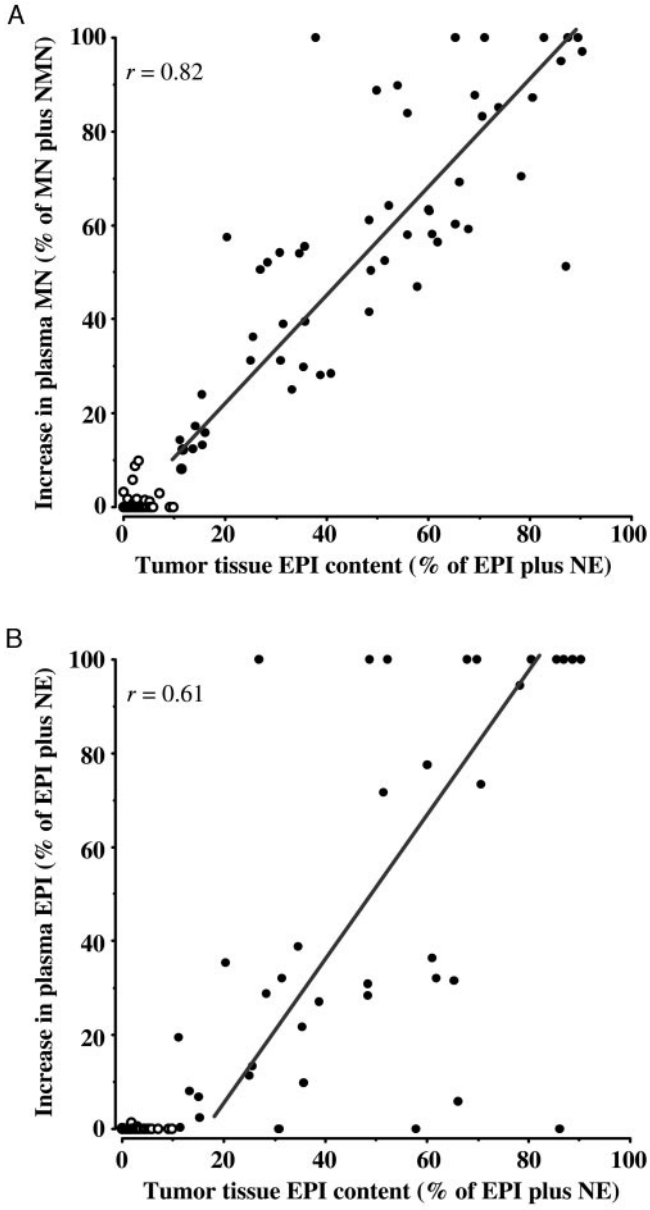

Fig. 1. Relationships of tumor tissue epinephrine content with increases in plasma free metanephrine (*A*) or epinephrine (*B*).

Tumor epinephrine (*EPI*) content is expressed as a percentage of the combined content of epinephrine and norepinephrine (*NE*); increases in plasma free metanephrine (*MN*) are expressed as a percentage of the combined increases in metanephrine and normetanephrine; and increases in plasma epinephrine are expressed as a percentage of the combined increases in epinephrine and norepinephrine. Data for adrenergic tumors (●) and noradrenergic tumors (○) are shown separately. Regression lines are shown only for relationships for adrenergic tumors.

right adrenal tumor. The right adrenal was removed but was found to be normal. The patient then underwent further surgery to remove a nearby extraadrenal tumor. It is possible that this tumor may have been connected to the previously resected adrenal gland, but this is uncertain; therefore, the tumor was designated as an extraadrenal paraganglioma. In addition, although plasma free metanephrine was abnormally increased [2.6 nmol/L (516

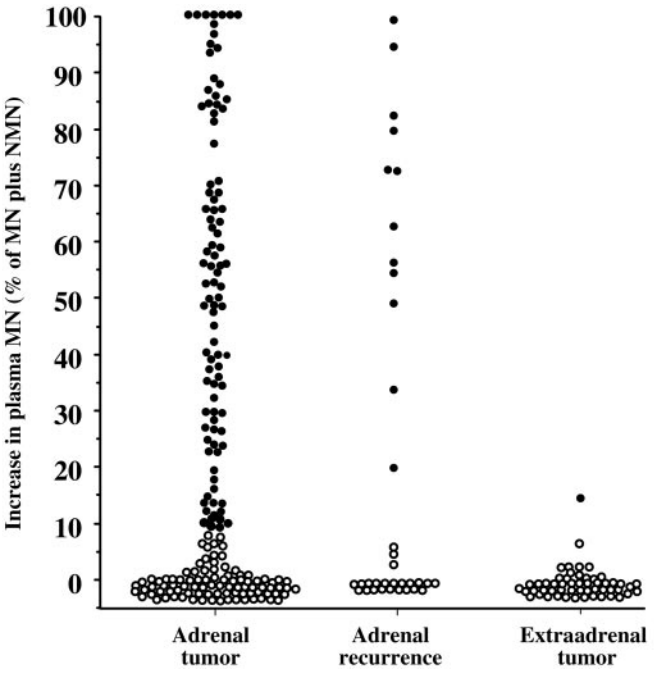

Fig. 2. Distributions of adrenergic and noradrenergic tumors according to location.

The predominantly norepinephrine-producing noradrenergic tumors (○) and epinephrine-producing adrenergic tumors (●) are distinguished by increases in plasma free metanephrine, expressed as a percentage of the combined increases in metanephrine (*MN*) and normetanephrine (*NMN*). The recurrent adrenal tumors are recurrences or malignancies arising from primary adrenal tumors.

ng/L)], the plasma concentration of normetanephrine was more strongly increased [13.7 nmol/L (2513 ng/L)]. Thus, the increase in plasma free metanephrine above the upper reference limit in this patient was only 15% of the combined increases in metanephrine and normetanephrine.

TUMOR SIZE

Among the 214 patients for whom dimensions of resected tumors were recorded, 96 had tumors that were designated as having an epinephrine-producing (adrenergic) biochemical phenotype, and 118 had tumors that were designated as having a predominantly norepinephrine-producing (noradrenergic) biochemical phenotype. There was a strong positive logarithmic relationship ($r = 0.81$; $P < 0.001$) between tumor diameter and summed plasma concentrations of free normetanephrine and metanephrine that did not show any differences among patients with adrenergic and noradrenergic tumors (Fig. 3A). There was also a similarly strong positive relationship ($r = 0.77$; $P < 0.001$) between tumor diameter and summed 24-h urinary outputs of normetanephrine and metanephrine, again with no differences among patients with adrenergic and noradrenergic tumors (Fig. 3B).

In contrast to the strong relationships of tumor diameter with plasma free or urinary deconjugated metanephrines, the relationships of tumor diameter with plasma or urinary catecholamines, although significantly positive ($P < 0.001$), were weaker ($r = 0.41$ for plasma catecholamines; $r = 0.44$ for urinary catecholamines) and showed considerable scatter (Fig. 3, C and D). Multiple linear regression analysis indicated different relationships for adrenergic and noradrenergic tumors. Patients with noradrenergic tumors had larger ($P < 0.001$) increases in plasma and urinary catecholamines relative to tumor diameter than patients with adrenergic tumors. Correlation coefficients for relationships of tumor diameter with plasma catecholamines ($r = 0.41$) and urinary catecholamines ($r = 0.44$) were improved after independent examination of relationships for patients with noradrenergic tumors ($r = 0.52$ for plasma catecholamines; $r = 0.54$ for urinary catecholamines) and adrenergic tumors ($r = 0.47$ for plasma catecholamines; $r = 0.46$ for urinary catecholamines).

DIAGNOSTIC SENSITIVITIES ACCORDING TO CATECHOLAMINE PHENOTYPE

Among all 275 patients with pheochromocytoma, 109 patients had tumors that were designated with an epinephrine-producing (adrenergic) biochemical phenotype, and 166 had tumors that were designated with a predominantly norepinephrine-producing (noradrenergic) biochemical phenotype. In all but three patients, these designations were based on relative increases above the reference limits of free metanephrine and normetanephrine. In those three patients, who had normal plasma concentrations of free normetanephrine and metanephrine, designation of a noradrenergic biochemical phenotype was based on measurements of tumor tissue catecholamines or subsequent measurements of increased plasma concentrations of normetanephrine with further development of malignant disease.

For patients with adrenergic tumors, the diagnostic sensitivities of both plasma free and urinary deconjugated fractionated metanephrines were higher ($P < 0.002$) than the sensitivities of plasma and urinary catecholamines (Table 2). These differences were less pronounced in patients with noradrenergic tumors, in whom only measurements of plasma free metanephrines offered improved sensitivity over plasma and urinary catecholamines. Measurements of plasma catecholamines provided higher ($P < 0.04$) diagnostic sensitivity in patients with noradrenergic tumors than in those with adrenergic tumors.

PROSPECTIVE PREDICTION OF TUMOR LOCATION AND SIZE

Among the 16 patients with pheochromocytoma in the prospective analysis, 5 had increases in plasma free metanephrine of >10% of the combined increases of normetanephrine and metanephrine, indicating an adrenergic catecholamine phenotype (Table 3). This correctly indicated an adrenal location for all five of the tumors in these patients.

The relationship of plasma concentrations of free meta-

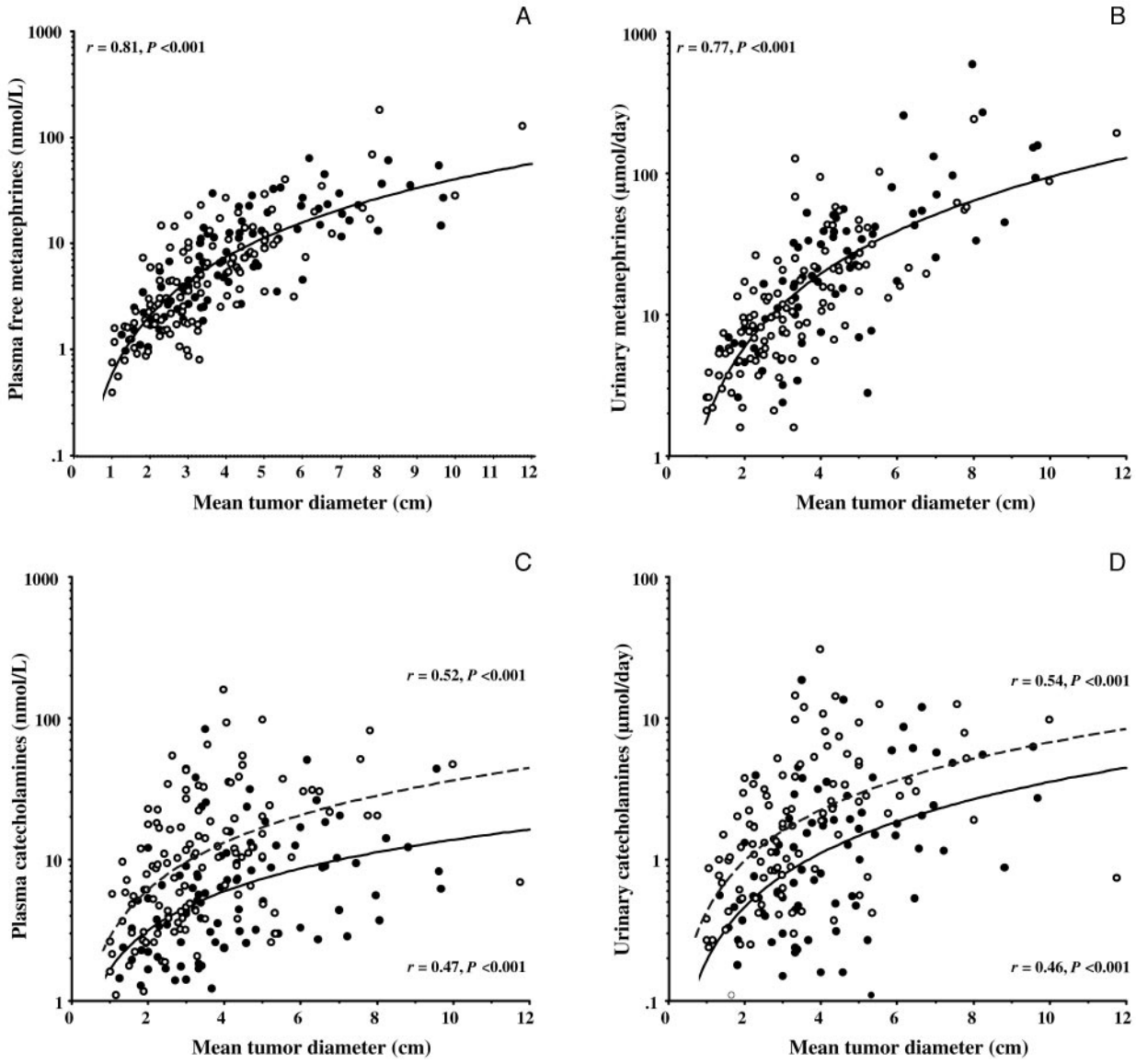

Fig. 3. Relationships of tumor diameter with plasma concentrations of free metanephrines (*A*) or catecholamines (*C*) and urinary output of deconjugated metanephrines (*B*) or catecholamines (*D*).

Data for plasma or urinary metanephrines and catecholamines are shown on a logarithmic scale and represent summed plasma concentrations or urinary output of normetanephrine and metanephrine (*A* and *B*) or norepinephrine and epinephrine (*C* and *D*). Data for adrenergic tumors (●) and noradrenergic tumors (○) are shown separately. Multiple linear regression analysis indicated significantly different ($P < 0.001$) relationships of tumor diameter with plasma or urinary catecholamines for patients with noradrenergic (*dashed lines*) and adrenergic tumors (*solid lines*).

nephrines with tumor diameters among the 16 patients in the prospective analysis showed a distribution that fell within the 95% confidence intervals of the relationship for the 214 patients in the retrospective analysis (Fig. 4A). Predictions of most likely tumor diameters and intervals for minimum and maximum diameters, estimated by use of the regression equations in Fig. 4A, ranged from 0.8 to >10 cm (Table 3). Measured diameters of tumors from all 16 patients fell within the ranges estimated by use of regression equations for the 95% confidence intervals of the relationship between tumor diameter and plasma free metanephrines for the 214 patients in the retrospective analysis.

Predictions of tumor diameters for the 16 patients in the prospective analysis were, on average, 19% higher than measured diameters and ranged from 47% smaller than to 83% larger than measured diameters (Table 3). The mean positive or negative difference of predicted from measured diameter was 30% (range, 3–83%). Predicted tumor diameters showed a positive relationship ($r = 0.87$; $P < 0.001$) with measured diameters (Fig. 4B).

Discussion

The measurement of plasma free metanephrines is a relatively new test for diagnosis of pheochromocytoma that in some laboratories is becoming the test of choice by

Table 2. Diagnostic test sensitivities in patients with epinephrine-producing vs predominantly norepinephrine-producing pheochromocytomas.

	Tumor catecholamine phenotype, % (n)		
Biochemical test	**Epinephrine**	**Norepinephrine**	***P*[a]**
Plasma free metanephrines	100 (109/109)	98 (163/166)	0.41
Plasma catecholamines	75 (82/109)[b,c]	86 (143/166)[b]	<0.04
Urinary fractionated metanephrines	97 (66/68)	94 (87/93)	0.52
Urinary catecholamines	78 (73/93)[b,c]	85 (121/142)[b]	0.71

[a] *P* values for differences between epinephrine- and norepinephrine-producing tumors determined by χ^2 test.

[b] Significantly ($P < 0.001$) lower sensitivity compared with that for plasma metanephrines (χ^2 test).

[c] Significantly ($P < 0.002$) lower sensitivity compared with that for urinary metanephrines (χ^2 test).

ordering physicians *(13)*. Provided the test is implemented correctly and results are interpreted and followed up appropriately, it can now be a relatively simple matter to exclude or confirm the tumor *(1, 4)*. The effectiveness of this process may be best ensured by recommendations on testing and assistance to clinicians with interpretation of test results from the clinical laboratory. Here we show how measurements of plasma free metanephrines can be used to provide information about the catecholamine phenotype, size, and location of a suspected tumor.

Pheochromocytomas are highly heterogeneous tumors with diverse phenotypes. Although the tumors are characterized by production of catecholamines, the nature of this production can vary considerably, accounting in part for variable clinical presentations *(14)*. As shown here, and in agreement with the findings of Kimura et al. *(15)*, approximately one half of adrenal tumors produce nearly exclusively norepinephrine and the other half a variable mixture of epinephrine and norepinephrine. Among hereditary tumors, the pattern of catecholamine production can depend on the underlying mutation, with those from patients with von Hippel–Lindau syndrome all producing predominantly norepinephrine and those from patients with multiple endocrine neoplasia type 2 all producing a mixture of epinephrine and norepinephrine *(11, 16)*. The former noradrenergic tumors and the latter adrenergic tumors have distinct patterns of gene expression that are retained even when there is no clear hereditary basis *(12)*. These distinct patterns of gene expression indicate different pathways of tumorigenesis, possibly involving development of tumors from different populations of chromaffin cells with different susceptibilities to the effects of a particular mutation. Identifying the catecholamine phenotype of a pheochromocytoma may therefore not only be useful for pointing to a possible underlying mutation, but also for understanding the cellular origins of the tumor and pathways of tumorigenesis.

The catecholamine phenotype of a pheochromocytoma is most accurately determined by measurements of catecholamine concentrations or production of biosynthetic enzymes in resected tumor tissue. Biopsies are dangerous; therefore, determination of phenotypes before surgical resection must rely on standard laboratory tests. As shown here, measurements of plasma or urinary cat-

Table 3. Prospective prediction of tumor size and location in 16 patients with pheochromocytomas.

	Plasma metanephrines					
Patient	**NMN,[a] nmol/L**	**MN, nmol/L**	**ΔMN,[b] %**	**Tumor location[c]**	**Measured tumor diameter, cm**	**Predicted tumor diameter,[d] cm**
1	41.20	1.22	2	A	6.6	10.3 (4.7 to >10)
2	2.37	0.37	3	A	2.0	2.3 (1.2–4.9)
3	5.27	0.93	12	A	2.7	3.6 (1.8–8.0)
4	10.19	0.13	0	E	3.3	4.7 (2.3 to >10)
5	1.91	0.17	0	A	3.8	2.0 (1.1–4.2)
6	7.91	29.07	80	A	5.2	9.5 (4.4 to >10)
7	3.10	0.42	4	A	1.7	2.6 (1.4–5.7)
8	1.06	0.18	0	A	1.3	1.5 (0.8–3.1)
9	1.31	0.07	0	A	1.7	1.6 (0.9–3.3)
10	0.46	2.97	100	A	3.0	2.6 (1.4–5.6)
11	2.08	0.09	0	E	1.5	2.0 (1.1–4.3)
12	8.42	5.69	41	A	4.9	5.6 (2.8 to >10)
13	2.10	3.32	67	A	4.1	3.3 (1.7–7.4)
14	4.13	0.12	0	E	2.7	2.9 (1.5–6.4)
15	6.46	0.14	0	A	2.7	3.7 (1.9–8.3)
16	3.70	0.46	5	A	3.0	2.9 (1.5–6.3)

[a] NMN, normetanephrine; MN, metanephrine.

[b] Increases in plasma free metanephrine (ΔMN) are shown as a percentage of combined increases in plasma free metanephrine and normetanephrine.

[c] Tumor locations are shown as adrenal (A) or extraadrenal (E).

[d] Predicted tumor diameters were estimated by use of the regression equations in Fig. 4A and are shown as most likely diameters with ranges of possible diameters in parentheses.

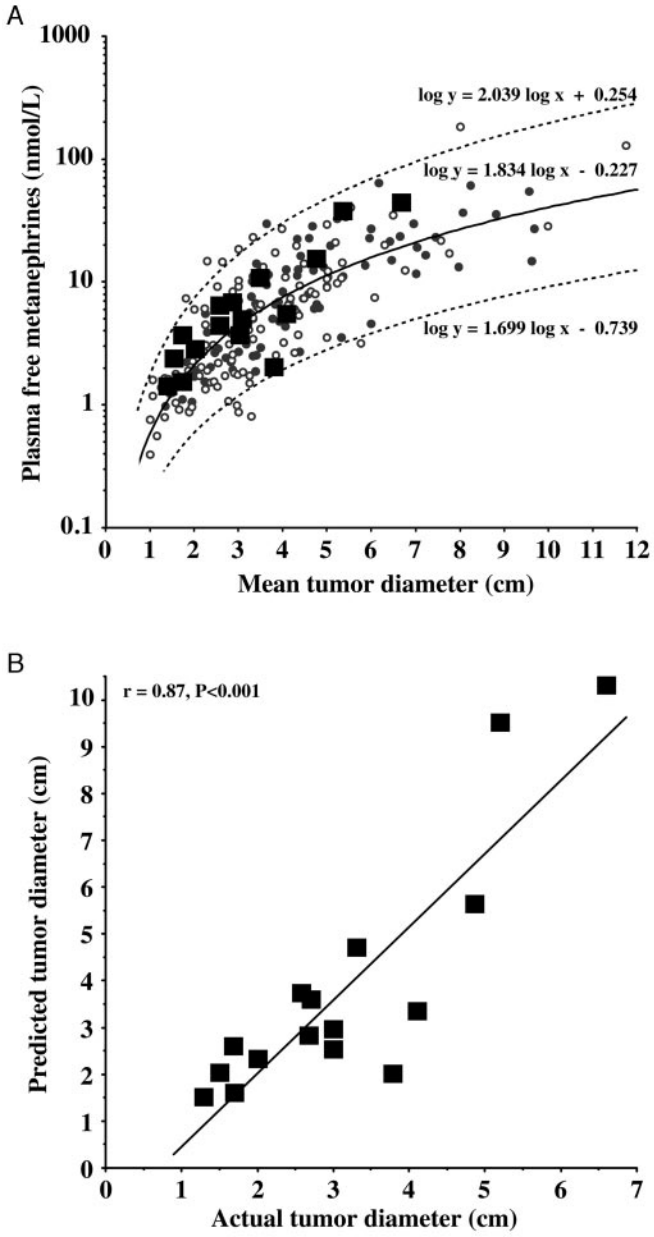

Fig. 4. Prediction of tumor diameter based on plasma concentrations of free metanephrines.

Panel A includes the data shown in Fig. 3A, but also includes data for the 16 patients with pheochromocytomas in the prospective analysis (■), along with the 95% confidence intervals (*dotted regression lines*) of the relationship and regression equations for relationships. Predictions of tumor diameter in *panel B* and in Table 3 were estimated by use of the regression equations for the relationships.

echolamines are not only relatively insensitive markers for diagnosis of pheochromocytoma, but also fail to accurately identify the catecholamine phenotype of many tumors. In contrast, the catecholamine biochemical phenotype is more reliably indicated by measurements of plasma free metanephrines, although this is with some degree of error in a small proportion of tumors with significant but relatively low epinephrine content. Nevertheless, an increase in free metanephrine larger than 10% of the summed increases of normetanephrine and metanephrine seems useful for identifying most adrenergic tumors, whereas no increase or an increase in plasma free metanephrine <5% of the combined increases in normetanephrine and metanephrine appears to reliably indicate noradrenergic tumors.

Previous observations have indicated that extraadrenal pheochromocytomas secrete predominantly or exclusively norepinephrine, whereas epinephrine secretion is usually confined to adrenal tumors *(17)*. These observations are supported in the present study, in which measurements of plasma free metanephrine and normetanephrine were used to more accurately indicate the catecholamine phenotype. Although some extraadrenal pheochromocytomas produced significant quantities of free metanephrine and had an adrenergic phenotype, almost without exception these were recurrences from previously resected adrenal tumors. The single exception in our series was an extraadrenal tumor that might have arisen from a previously resected adrenal gland in close proximity to the resected tumor. This tumor was nevertheless designated with an adrenergic extraadrenal phenotype. Although exclusive increases in plasma free normetanephrine cannot be used to indicate adrenal or extraadrenal locations of a pheochromocytoma, our data indicate that increases in plasma free metanephrine >10% of the combined increases in metanephrine and normetanephrine can be used to indicate an adrenal location or recurrence of an adrenal tumor.

It has previously been suggested that differences in norepinephrine and epinephrine secretion between adrenal and extraadrenal tumors are attributable to the proximity of the former tumors to adrenal cortical steroids, which induce production of phenylethanolamine-*N*-methyltransferase, the enzyme that converts norepinephrine to epinephrine *(18)*. Our observation that recurrent adrenal tumors can retain the ability to produce significant quantities of epinephrine indicates that continual proximity of tumor cells to adrenal steroids is not necessary for maintaining the adrenergic phenotype. This has practical implications during routine annual screening of patients for recurrent tumors, where patterns of increases of metanephrine and normetanephrine in such recurrences might be expected to follow the catecholamine phenotype of the original tumor.

Poor or no relationships of pheochromocytoma tumor mass or volume with urinary outputs or plasma concentrations of catecholamines and improved relationships with catecholamine metabolites are well documented *(16, 19–21)*. These differences reflect variable and intermittent secretion of catecholamines by tumors, compared with continuous production of free metanephrines within tumor cells by O-methylation of catecholamines leaking from storage vesicles, a process that is independent of catecholamine release *(22)*. Here we show that variable catecholamine release by tumors is in part attributable to

differences in adrenergic and noradrenergic phenotypes of tumors. Relative to tumor size, noradrenergic tumors secrete more catecholamines than adrenergic tumors. Similar differences have been observed in adrenal medullary chromaffin cells, where cells that produce and store mainly norepinephrine secrete catecholamines more actively than those that produce epinephrine *(23)*. Presumably our findings of higher sensitivities of measurements of catecholamines for diagnosis of noradrenergic than adrenergic tumors reflect underlying differences in catecholamine release.

The strong positive relationship between tumor diameter and summed plasma concentrations of free normetanephrine and metanephrine observed here indicate that these measurements might be useful for predicting tumor size. Indeed, based on this relationship, we were able to prospectively predict the diameters of tumors to within a mean of 30% of the actual diameter measured in resected tissue. Moreover, we were also able to accurately predict adrenal locations for tumors that produced relatively high concentrations of plasma free metanephrine compared with normetanephrine. Such predictions may provide useful supporting confirmatory information during subsequent imaging procedures for tumor localization. Conversely, during biochemical testing for an adrenal incidentaloma, the likelihood that the mass might be a pheochromocytoma can be indicated not only by increases in plasma free metanephrines, but by the nature of these increases and how they fall within the range predicted by the size of the mass. A limitation is the fairly wide range of concentrations possible for a given tumor size.

How might the above information derived from measurements of plasma free metanephrines best be translated for use in the diagnostic decision-making process? As reviewed elsewhere *(5, 24)*, clinicians cannot be expected to appropriately implement and accurately interpret all of the ever-increasing plethora of complex and new laboratory tests available to their patients. There is a growing need for laboratory medicine to become better integrated in the diagnostic decision-making process, and more specifically for laboratory directors to provide guidance with testing procedures and interpretation and follow-up of test results *(25–27)*.

The laboratory diagnosis of pheochromocytoma is one example where such guidance in diagnostic decision-making may be particularly valuable, because of the rarity of the tumor amid the large numbers of patients tested and the high frequency of largely unnecessary costly tests and imaging procedures used to track down suspected tumors. Indeed, others have recognized this need and have developed computerized systems for providing, along with laboratory results, useful narrative interpretations to assist clinicians with follow-up *(6)*. It seems that such systems could easily be adapted for newer and improved laboratory tests, such as measurements of plasma free metanephrines, and used on a wider scale to provide more valuable information to clinicians and patients than is possible with numerical results alone. As outlined in the examples in Table 4, such information could include statements about the likelihood of a tumor and recommendations for further testing, based on previous studies and published algorithms *(1, 4)*. Documentation of the catecholamine phenotype and predictions about size and location of the tumor, as further outlined here, although unlikely to have any major impact on the choice of imaging procedures may be useful for coregistration with the information obtained during these tumor localization procedures.

Table 4. Examples of narrative interpretations for four patients from the prospective analysis (see Table 3).

Patient	Narrative interpretation
3	The marked increase in normetanephrine provides strong evidence for a catecholamine-producing tumor. The associated moderate increase in metanephrine amounting to 12% of the combined increase of both metabolites indicates an epinephrine-producing tumor with an adrenal location (or alternatively, a recurrence of a previously resected adrenal tumor). The tumor has a likely diameter of 3.6 cm (range, 1.8–8.0 cm). Imaging studies are recommended with particular attention focused on the adrenals.
4	The marked increase in normetanephrine provides strong evidence for a catecholamine-producing tumor. The normal metanephrine indicates a norepinephrine-producing adrenal or extraadrenal tumor. The tumor has a likely diameter of 4.7 cm (range, 2.3–10.0 cm). Imaging studies are recommended with consideration of an extraadrenal tumor if no adrenal mass is evident.
9	The mild increase in normetanephrine in the presence of a normal metanephrine does not provide sufficient evidence to firmly indicate a catecholamine-producing tumor. Certain medications (e.g., tricyclic antidepressants, phenoxybenzamine), clinical conditions, or inappropriate sampling conditions can cause increases in normetanephrine of this magnitude. Follow-up confirmatory biochemical testing is recommended. A clonidine suppression test with measurements of plasma normetanephrine and norepinephrine can be useful if results remain positive. If a tumor is present, it could have either an adrenal or extraadrenal location with a most likely diameter of 1.6 cm (range, 0.9–3.3 cm).
10	The marked increase in metanephrine in the presence of a normal normetanephrine provides strong evidence for an epinephrine-producing tumor with an adrenal location (or alternatively, a recurrence of a previously resected adrenal tumor). The tumor has a likely diameter of 2.6 cm (range, 1.4–5.6 cm). Imaging studies are recommended with particular attention focused on the adrenals.

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