CLINICAL STUDY

Diagnostic value of various biochemical parameters for the diagnosis of pheochromocytoma in patients with adrenal mass

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Abstract

Objective: Pheochromocytomas are neoplasms generally characterized by the autonomous production of catecholamines. This study compared various biochemical parameters for the diagnosis of adrenal pheochromocytoma in patients with adrenal mass.

Design: One hundred and fifty subjects were studied, including 24 histologically proven pheochromocytomas, 17 aldosterone-secreting and 21 cortisol-secreting adrenal adenomas and 30 nonfunctioning adrenal masses, 16 patients with essential hypertension and 42 healthy normotensive volunteers. Spontaneous blood samples and 24-h urine samples were collected prospectively.

Methods: Plasma and urinary epinephrine and norepinephrine levels were measured by high performance liquid chromatography, whereas plasma and urinary metanephrine and normetanephrine levels were determined by radioimmunoassay (RIA). Putative ratio thresholds were calculated by receiver operating characteristic (ROC) analysis to balance between sensitivity and specificity.

Results: Plasma normetanephrine was found to be the best single parameter with the highest sensitivity (91.7%) and specificity (95.6%) using a threshold of 126 pg/ml. In combination, plasma normetanephrine and metanephrine had a higher sensitivity of 95.8% with lower specificity (79.4%). All other combinations of plasma and/or urinary parameters demonstrated a lower accuracy.

Conclusion: Plasma metanephrines measured by RIA are reliable screening parameters for the diagnosis of pheochromocytoma.

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Introduction

Pheochromocytomas are catecholamine-producing tumors arising from chromaffin cells of the adrenal medulla or extra-adrenal paraganglionic tissue (1). The inappropriate concentration of catecholamines may result in sustained or intermittent severe hypertension. Typical clinical symptoms are headache, sweating and tachycardia. Due to improved magnetic resonance imaging (MRI) or computed tomography (CT) techniques and a frequent use of these tools, adrenal tumors are increasingly recognized. Accurate biochemical parameters are required to discriminate pheochromocytomas from other hormone secreting or inactive adrenal adenomas. An undiagnosed pheochromocytoma might result in life-threatening consequences. Therefore, highly sensitive biochemical assays are essential to avoid false-negative results. The diagnosis of pheochromocytoma is

based on the overproduction of catecholamines. Determinations of plasma and in particular 24-h urinary epinephrine and norepinephrine levels are established diagnostic tools. A handicap is that they may be falsely negative in patients with a biochemically silent or periodically secreting pheochromocytoma (2). On the other hand, they may be falsely elevated in patients with panic disorder or congestive heart failure (2). Metanephrines, which are metabolites of catecholamines, have been suggested as an alternative diagnostic tool (3–5). The aim of this study was to establish thresholds for plasma and urinary catecholamines and metanephrines with optimal sensitivity and specificity for the diagnosis of pheochromocytoma in patients with an adrenal mass. Patients with hereditary syndrome were not considered. Recently, a radioimmunoassay has been developed, which was employed in our study to determine plasma and urinary metanephrine and normetanephrine.

Methods

Subjects

Ninety-two subjects were prospectively included in this study. Inclusion criterion was the presence of an adrenal mass requiring an operation. The patients were grouped into four categories: 24 adrenal pheochromocytomas (PHEO), 17 aldosterone-secreting adenomas (APA), 21 cortisol-secreting adenomas (CPA) and 30 nonfunctioning adrenal masses (NFM; twenty nonfunctioning adenomas (NEA), two cysts, three myelolipoma, one adrenal carcinoma, one ganglioneurinoma, one T-cell-lymphoma, one metastasis of a bronchial carcinoma and one of a hypernephroma). Controls included 16 patients with essential hypertension and 42 healthy normotensive volunteers (pertinent data are given in Table 1). In patients with essential hypertension, a pheochromocytoma was considered unlikely due to the absence of specific clinical symptoms and adrenal tumors in MRI scans. Healthy volunteers presented a normal blood pressure on three different occasions. Therefore, no further procedures were carried out to exclude a pheochromocytoma. Pheochromocytomas were diagnosed preoperatively by endocrine testing, MRI or CT and ^{123}I -meta-iodobenzylguanidine scintigraphy. The diagnoses were postoperatively confirmed by histological and immunohistochemical examinations. All pheochromocytomas were considered benign at the time of diagnosis. Proof of malignancy was the appearance of tumor cells in non-chromaffin tissue or local tumor invasion (6). All pheochromocytomas were considered as sporadic. The family history of the patients investigated did not point to a familial pheochromocytoma. Genetic testing was carried out for MEN2, VHL, and mutations of the succinate dehydrogenase complex, subunit B (SDHB) gene and succinate dehydrogenase complex, subunit D (SDHD) gene. However, we cannot completely exclude a genetic syndrome without family history.

The etiology of the remaining adrenal masses was preoperatively established by respective biochemical parameters and MRI or CT. All adrenal masses were subsequently removed and the diagnosis was postoperatively confirmed by histological examinations

as well as by clinical follow-up. At the time of investigation, patients were on various antihypertensive medications including beta-blockers as well as central and peripheral alpha-blockers. Blood samples were drawn in a seated body position with standard venipuncture from a forearm vein. Urine samples were collected in vessels containing HCl. Blood and urine samples were obtained within the same day. All samples were collected prospectively from June 2002 to June 2004 and were analyzed by the same investigator blinded to the clinical condition of the patients. To test the reproducibility of the plasma metanephrine and normetanephrine measurements within individuals, blood samples from nine healthy volunteers (3 male, 6 female) were collected on three consecutive days. Mean age ($\pm$ S.E.M.) was 34.6 ± 3.4 years. The local ethical committee approved the study protocol and all patients gave informed consent.

Assays

Plasma and urinary catecholamines were measured by high-performance liquid chromatography (HPLC; Chromsystems, Munich, Germany). Assay sensitivity for plasma catecholamines was 10 pg/ml each. Assay variability was determined by within-assay coefficients of variation of 5.8% for 218 pg/ml, 5.4% for 54 pg/ml and 6.8% for 47 pg/ml. Assay sensitivity for urinary catecholamines was 1 $\mu\text{g}/24\text{ h}$ each. Assay variability was determined by within-assay coefficients of variation of 3.7% for 48 $\mu\text{g}/24\text{ h}$, 4.2% for 17 $\mu\text{g}/24\text{ h}$ and 4.4% for 167 $\mu\text{g}/24\text{ h}$. Plasma free and urinary metanephrine and normetanephrine were analyzed by RIA (DLD Diagnostika GmbH, Hamburg, Germany). Assay sensitivity was 10 pg/ml for plasma metanephrine and normetanephrine. Assay variability was determined by coefficients of variation of 6.5% for 62 pg/ml and 7.3% for 713 pg/ml for plasma metanephrine and 6.9% for 170 pg/ml and 7.8% for 161 pg/ml for plasma normetanephrine. Between-assay coefficients of variation were 7.8% for 69 pg/ml and 8.3% for 804 pg/ml for plasma metanephrine and 7.7% for 154 pg/ml and 8.9% for 2312 pg/ml for plasma normetanephrine. Assay sensitivity was 11 ng/ml for urinary metanephrine and 26 ng/ml for urinary

Table 1 Clinical characteristics of patients and volunteers. Values are means $\pm$ S.E.M.

Diagnosis	No. of patients	Age (years)	Sex (m/f)	Hypertension	Tumor size (cm)
PHEO	24	41.7 ± 3.2	13/11	92%	3.9 ± 0.5
CPA	21	52.8 ± 3.3	2/19	67%	3.3 ± 0.2
APA	17	53.1 ± 3.6	5/12	100%	1.7 ± 0.2
NFM	30	51.6 ± 2.3	15/15	57%	4.6 ± 0.5
EH	16	43.0 ± 3.6	9/7	100%	na
VOL	42	33.0 ± 1.7	28/14	0%	na
Total	150	43.3 ± 3.5	72/78	na	na

PHEO, pheochromocytoma; CPA, cortisol-secreting adrenal adenoma; APA, aldosterone-secreting adrenal adenoma; NFM, nonfunctioning adrenal mass; EH, essential hypertension; VOL, healthy volunteers; na, not available.

normetanephrine. Assay variability was determined by within-assay coefficients of variation of 7.7% for 53 µg/ml and 4.2% for 201 µg/ml for urinary metanephrine and 11.1% for 248 µg/ml and 7.3% for 954 µg/ml for urinary normetanephrine. Between-assay coefficients of variation were 10.4% for 71 µg/ml and 6.7% for 289 µg/ml for urinary metanephrine and 13.9% for 247 µg/ml and 7.8% for 881 µg/ml for urinary normetanephrine.

Statistical evaluation

Results are expressed as median and range. GraphPad Prism 3.0 (GraphPad Software Inc., San Diego, CA, USA) was used for statistical and receiver operating characteristic (ROC) analysis. Thresholds were established by ROC analysis. The Kruskal-Wallis test, followed by Dunn's multiple comparison test, were performed where appropriate. Sensitivity was calculated by a positive test result of patients with a pheochromocytoma divided by the total number of these patients. Specificity was obtained by a negative test result of patients with a surgically removed adrenal mass divided by the total number of these patients. ROC analyses included only patients with a surgically removed adrenal mass. Patients with essential hypertension and normotensive healthy volunteers were not considered. Corresponding 95% confidence intervals as well as positive and negative likelihood ratios for the sensitivity and specificity are provided. A very high positive likelihood ratio (LR+) is defined by a value > 10, high LR+ is suggested by values between 5 and 10, weak LR+ is defined by values between 2 and 5 and very weak LR+ is defined by values between 1 and 2. A very high negative likelihood ratio (LR-) is reflected by values < 0.1, high LR- is defined by values between 0.1 and 0.2, weak LR- is demonstrated by values between 0.2 and 0.5 and very weak LR- is suggested by values between 0.5 and 1. When considering respective pairs of parameters (e.g. plasma metanephrine and normetanephrine), a true-positive test result was defined as patients with pheochromocytoma and at least one parameter over the threshold. A true-negative test result was defined as patients without pheochromocytoma and both parameters below the threshold. A false-negative test result was defined as patients with pheochromocytoma and normal measurements of both parameters. A false-positive test result was defined as patients without pheochromocytoma and at least one elevated parameter. Analysis of correlations among tumor size and plasma and urinary parameters was carried out by the Spearman test. The reproducibility of the plasma metanephrine measurements was analyzed using the coefficient of variation. The results are expressed as means ± S.E.M.

Results

Plasma catecholamines

Samples from twenty patients with pheochromocytoma and 62 control subjects were available for analysis. Epinephrine was 10.0 pg/ml (10–1780 pg/ml) (median and range) in patients with pheochromocytoma (Table 2, Fig. 1), which was not significantly different to the other subgroups. ROC analysis revealed a threshold of > 24.5 pg/ml (0.134 nmol/l) (sensitivity 40.0%, specificity 88.7%) for the diagnosis of pheochromocytoma using this parameter (Table 3, Fig. 2). LR+ and LR- were weak and very weak respectively (Table 3). Norepinephrine was 378.5 pg/ml (10–10 000 pg/ml) in patients with pheochromocytoma (Table 2). It was significantly higher compared with patients with APA and hypertensive patients, but not significantly higher compared with any other subgroup (Table 2). ROC analysis provided a threshold of > 219.5 pg/ml (1.299 nmol/l) (sensitivity 60.0%, specificity 72.6%) for the diagnosis of pheochromocytoma (Table 3, Fig. 2). LR+ and LR- revealed a weak and very weak significance respectively (Table 3). Both parameters showed high specificity, but due to the low sensitivity a large number of patients with pheochromocytoma remained falsely undiagnosed (Fig. 1). When considering the combination of epinephrine and norepinephrine for the diagnosis of pheochromocytoma, the sensitivity increased to 70.0% with decreased specificity of 69.4% (Table 3).

Urinary catecholamines

Samples from fifteen patients with pheochromocytoma and 53 control subjects were available for analysis. Epinephrine was 7.4 µg/24 h (1–469 µg/24 h) in patients with pheochromocytoma (Table 2). It was significantly higher than in patients with cortisol-secreting or non-functioning adenomas (Table 2). ROC analysis suggested a threshold of 2.5 µg/24 h (0.014 µmol/24 h) (sensitivity 73.3%, specificity 64.2%) (Table 3, Fig. 2). LR+ and LR- reflected a rather weak significance. Norepinephrine was 112.6 µg/24 h (29.6–1000 µg/24 h) in patients with pheochromocytoma, which was significantly higher than in all other subgroups except for patients with aldosterone-producing adenomas (Table 2). ROC analysis revealed an optimal threshold of 68.1 µg/24 h (0.403 µmol/24 h) (sensitivity 73.3%, specificity 84.9%) (Table 3, Fig. 2). Using this threshold, four pheochromocytomas remained undiagnosed (Fig. 1). LR+ and LR- reflected a rather weak significance. A high clinical sensitivity > 95% decreased the specificity to 35.9% (Table 4). Combined use of urinary catecholamines increased the sensitivity to 93.3%, but decreased the specificity to 58.5% in relation to the single parameters (Table 3).

Table 2 Plasma and urinary catecholamines and metanephrines of the respective subgroups are given as median and range (min–max).

	Plasma				Urine			
	E (pg/ml)	NE (pg/ml)	MN (pg/ml)	NMN (pg/ml)	E (μg/day)	NE (μg/day)	MN (μg/day)	NMN (μg/day)
PHEO	10.0 (10–1780)	378.5 (10–10000)	103.5 (10–1971)	296.0 (46–8500)	7.4 (1–469.0)	112.6 (29.6–1000.0)	246 (30–5040)	1040.0 (224–4466)
CPA	10.0 (10–68)	104.0 (10–924)	21.0 (10–263)	54.0 (23–163)**	1.5 (1–6.6)*	32.2 (1.0–83.0)**	74.5 (37–116)*	269.2 (92–813)*
APA	10 (10–21)	27.0 (10–591)*	19.0 (10–73)*	53.0 (27–146)**	2.1 (1–6.4)	45.8 (5.0–94.6)	67.0 (28–188)*	163.0 (29–516)**
NFM	10.0 (10–33)	98.5 (10–856)	15.5 (10–104)**	57.5 (15–106)**	1.0 (1–100.0)**	32.2 (6.3–128.7)**	50.0 (13–300)**	141.0 (38–596)**
EH	10.0 (10–10)	10.0 (10–399)**	22.5 (10–125)	38.0 (17–108)**	4.7 (1–13.7)	42.8 (1.0–84.9)*	85.5 (14–266)	200.0 (26–396)**
VOL	30.5 (10–118)	292.5 (10–604)	16.5 (10–51)**	30.5 (14–88)**	6.2 (1–13.3)	33.8 (17.7–63.4)**	70.2 (11–130)**	193.2 (48–747)**

To convert values in pg/ml to nanomoles per liter and μg/day to micromoles per day, divide by 183 for epinephrine and normetanephrine, 169 for norepinephrine and 197 for metanephrine. The asterisks demonstrate parameters with significantly lower median and range than pheochromocytomas (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). PHEO, pheochromocytoma; CPA, cortisol-secreting adrenal adenoma; APA, aldosterone-secreting adrenal adenoma; NFM, nonfunctioning adrenal mass; EH, essential hypertension; VOL, healthy volunteers; E, epinephrine; NE, norepinephrine; MN, metanephrine; NMN, normetanephrine.

Urinary metanephrines

Samples from fifteen patients with pheochromocytoma and 52 control subjects were available for analysis. Metanephrine was 246 μg/24 h (30–5040 μg/24 h) in patients with pheochromocytoma (Table 2). It was significantly higher compared with patients with APA, CPA, NFM and healthy volunteers (Table 2). ROC analysis revealed a threshold of 110.7 μg/24 h (0.561 μmol/24 h) for the diagnosis of pheochromocytoma (sensitivity 80.0%, specificity 82.7%) (Table 3, Fig. 2). LR + and LR – revealed a rather weak significance. A sensitivity >95% revealed a specificity of 9.6% (Table 4). Normetanephrine was 1040.0 μg/24 h (224–4466 μg/24 h) in patients with pheochromocytoma, which was significantly higher than in all other subgroups (Table 2). ROC analysis provided a threshold of 436.5 μg/24 h (2.383 μmol/24 h) with high sensitivity (93.3%) and specificity (86.5%) for the diagnosis of pheochromocytoma (Table 3, Fig. 2). Only one pheochromocytoma remained below the threshold (Fig. 1). LR + and LR – demonstrated a high and very high significance respectively. A sensitivity >95% decreased the specificity to 59.6% (Table 4). When considering the combination of urinary metanephrine and normetanephrine for the diagnosis of pheochromocytoma, a similar sensitivity of 93.3%, but a lower specificity of 75.0% in relation to the single parameters was revealed (Table 3).

Plasma metanephrines

Samples from all twenty-four patients with pheochromocytoma and 68 control subjects were available for analysis. All patients satisfying the inclusion criterion underwent this index test. Metanephrine was 103.5 pg/ml (10–1971 pg/ml) in patients with pheochromocytoma (Table 2). It was significantly higher compared with the remaining subgroups except for patients with CPA and hypertensive patients (Table 2). ROC analysis revealed a threshold of 38 pg/ml (0.193 nmol/l) (sensitivity 70.8%, specificity 79.4%) for the diagnosis of pheochromocytoma (Table 3, Fig. 2). LR + and LR – revealed a weak significance. Seven patients with pheochromocytoma were falsely classified as negative (Fig. 1). Of these, six patients demonstrated elevated plasma normetanephrine levels. Falsely elevated measurements were detected in 20 patients. Three of these also had elevated normetanephrine levels. Normetanephrine was 296 pg/ml (46–8500 pg/ml) in patients with pheochromocytoma, which was significantly higher compared with all other groups (Table 2). ROC analysis suggested an optimal threshold of 125.5 pg/ml (0.686 nmol/l) with very high sensitivity (91.7%) and specificity (95.6%) (Table 3, Fig. 2). The positive and negative likelihood ratios demonstrated very high diagnostic significance. Only two patients with pheochromocytoma remained

Figure 1 (A) Plasma and (B) urinary epinephrine and norepinephrine, (C) urinary and (D) plasma metanephrine and normetanephrine in patients with pheochromocytoma (Pheo), adrenal mass (Tu), essential hypertension (EH) and healthy volunteers (C). The horizontal bar demonstrates the threshold for the diagnosis of pheochromocytoma calculated by ROC analysis.

Table 3 Optimal thresholds for the single parameters and respective pairs determined by ROC analysis.

	Threshold	Sens (%) (95% CI)	False-neg. (n)	Spec (%) (95% CI)	False-pos. (n)	LR+	LR –	AUC (95% CI)
Plasma (pg/ml)								
E	24.5	40.0 (19.12–63.95)	12	88.7 (78.11–95.34)	7	3.54	0.70	0.64 (0.48–0.79)
NE	219.5	60.0 (36.05–80.88)	8	72.6 (59.77–83.15)	17	2.19	0.59	0.73 (0.60–0.86)
E + NE	24.5/219.5	70.0	6	69.4	19	1.65	0.51	na
MN	38.0	70.8 (48.91–87.38)	7	79.4 (67.88–88.26)	14	3.44	0.29	0.76 (0.62–0.89)
NMN	125.5	91.7 (73.00–99.97)	2	95.6 (87.64–99.08)	3	20.78	0.08	0.96 (0.90–1.01)
MN + NMN	38.0/125.5	95.8	1	79.4	14	4.82	0.05	na
Urine (µg/d)								
E	2.5	73.3 (44.99–92.21)	4	64.2 (49.80–76.86)	19	2.05	0.39	0.78 (0.65–0.92)
NE	68.1	73.3 (44.90–92.21)	4	84.9 (72.41–93.25)	8	4.86	0.32	0.87 (0.76–0.97)
E + NE	2.5/68.1	93.3	1	58.5	22	2.16	0.11	na
MN	110.7	80.0 (51.91–95.67)	3	82.7 (69.67–91.77)	9	4.62	0.30	0.86 (0.74–0.99)
NMN	436.5	93.3 (68.05–99.83)	1	86.5 (74.21–94.41)	7	6.93	0.07	0.96 (0.89–1.01)
MN + NMN	110.7/436.0	93.3	1	75.0	13	3.55	0.08	na

To convert values in pg/ml to nanomoles per liter and µg/day to micromoles per day, divide by 183 for epinephrine and normetanephrine, 169 for norepinephrine and 197 for metanephrine. The positive and negative likelihood ratios (LR) of plasma normetanephrine reflect very high diagnostic evidence. E, epinephrine; NE, norepinephrine; MN, metanephrine; NMN, normetanephrine; Sens, sensitivity; neg., negative; Spec, specificity; pos., positive; LR+, positive likelihood ratio; LR –, negative likelihood ratio; CI, confidence interval; AUC, area under the curve; na, not available.

below the threshold (Fig. 1). Of these, one patient presented with elevated plasma metanephrine levels. While none of the patients with essential hypertension or NFA and none of the healthy volunteers demonstrated elevated normetanephrine levels, three patients with CPA or APA had slightly increased levels. A sensitivity >95% revealed a specificity of 77.9% (Table 4.). Considering the combination of plasma metanephrine and normetanephrine for the diagnosis of pheochromocytoma demonstrated a higher sensitivity of 95.8%, but a lower specificity of 79.4% (Table 3). Only one patient remained with normal plasma metanephrines. Four patients with CPA, two patients with APA, eight patients with NFA, one patient with hypertension and five healthy volunteers were falsely classified as positive. One of the CPA patients had a sevenfold higher plasma metanephrine level, while the remaining subjects demonstrated slightly elevated plasma metanephrines. The histological investigation postoperatively unequivocally confirmed a cortical adenoma in all patients with adrenal adenoma. No obvious drug interference was noticed. However, it cannot be ruled out in our investigation. Blood samples from nine healthy volunteers were collected on three consecutive days. The mean coefficient of variation was $12.3 \pm 1.6\%$ for metanephrine and $14.0 \pm 2.0\%$ for normetanephrine. Both demonstrated a high reproducibility for the plasma metanephrine measurements.

Correlation among tumor size and plasma and urinary parameters

A weak positive correlation was found between the tumor size of pheochromocytomas and plasma metanephrine ($r = 0.56$, $P \leq 0.01$) and urinary normetanephrine ($r = 0.53$, $P \leq 0.05$) (data not shown).

Discussion

This study compared catecholamines and metanephrines in plasma and urine for the diagnosis of pheochromocytoma in patients with adrenal tumors. For the first time, results are presented for plasma and urinary metanephrines measured by radioimmunoassay. ROC analysis revealed plasma normetanephrine as the best single parameter for the diagnosis of pheochromocytoma.

An increasing number of patients present with incidentally discovered adrenal masses. Therefore, reliable and simple parameters are required to exclude hormonally active tumors in these patients. Our study put special emphasis on patients with adrenal mass by including 68 adrenal tumors of various etiologies as controls. This group may not be truly representative of more typical patients tested for pheochromocytoma because of hypertensive signs and symptoms. Similarly, Raber *et al.* studied patients with pheochromocytoma and compared them with 14 patients with other adrenal tumors (7). In that study, normal values for plasma catecholamines and metanephrines were adapted from previous studies (8) and samples were measured by a chromatographic method. The authors demonstrated a sensitivity and specificity of 100% each for plasma normetanephrine.

In our study, twenty-four patients with histologically proven pheochromocytoma were included. Patients with surgically removed adrenal tumors of other etiologies served as control groups. Furthermore, hypertensive patients and normotensive, healthy volunteers were included, but were not considered for determination of thresholds. ROC analysis was employed to provide thresholds with an optimal balance between sensitivity and specificity. However, optimal thresholds by ROC analysis may demonstrate a low sensitivity,

Figure 2 ROC analysis curves for each of the eight parameters.

Table 4 Thresholds with high clinical sensitivity (>95%) and specificity (>95%) determined by ROC analysis.

Threshold (Sens > 95%)		Spec (%) (95% CI)	LR+	LR -	Threshold (Spec > 95%)	Sens (%) (95% CI)	LR+	LR -
Plasma (pg/ml)								
E	na	na	na	na	56.0	30.0 (11.89–54.28)	6.2	0.74
NE	na	na	na	na	674.5	35.0 (15.39–59.22)	7.23	0.68
MN	na	na	na	na	92.0	54.2 (32.82–74.45)	12.28	0.48
NMN	86.0	77.9 (66.24–87.10)	4.34	0.05	125.5	91.7 (73.00–98.97)	20.78	0.09
Urine (µg/d)								
E	na	na	na	na	7.0	53.3 (26.59–78.73)	14.13	0.49
NE	27.9	35.9 (23.14–50.20)	1.56	0	101.5	60.0 (32.29–83.66)	15.90	0.42
MN	29.5	9.6 (3.20–21.03)	1.11	0	191.0	60.0 (32.29–83.66)	15.60	0.42
NMN	221.5	59.6 (45.10–72.99)	2.48	0	633.5	80.0 (51.91–95.67)	20.80	0.21

Limited by the sensitivity of the assay, a sensitivity >95% was not available for plasma catecholamines and metanephrines and urinary epinephrine. E, epinephrine; NE, norepinephrine; MN, metanephrine; NMN, normetanephrine; Sens, sensitivity; Spec, specificity; LR +, positive likelihood ratio; LR -, negative likelihood ratio; CI, confidence interval; na, not available.

which is clinically not acceptable. A high sensitivity may be more important in the diagnosis of a potentially lethal condition such as pheochromocytoma. However, use of a high clinical sensitivity ($\geq 95\%$) reflecting a reduced number of false-negative test results may be limited by very low specificity as demonstrated for some parameters in our study. The study was designed to analyze catecholamines and their metabolites in an outpatient setting. Therefore, samples were drawn by standard venipuncture, which may result in an increase in catecholamines and their metabolites.

This study revealed that plasma normetanephrine is the best single parameter. Using a threshold of 125.5 pg/ml, only two patients were falsely negative. One of these presented preoperatively without clinical symptoms. Plasma catecholamines as well as plasma metanephrine were also negative. Urinary parameters were not available. Only the histological examination revealed a small (1 cm) pheochromocytoma in combination with an adenoma of the adrenal cortex. For the other patient, all biochemical markers were increased, except for normetanephrine in plasma.

When considering two respective parameters, plasma metanephrine and normetanephrine were the best biochemical combination compared with the other tests. Previous studies provided similar findings, with sensitivities between 97% and 100% and specificities between 85% and 96% using various thresholds (8, 9). However, most of these studies did not compare all four single parameters. An explanation for the potential superiority of metanephrines compared with catecholamines was provided by a recent study (10). Pheochromocytomas contain high amounts of catechol-O-methyltransferase (COMT), an enzyme that metabolizes catecholamines into free metanephrines. Rapid metabolism of catecholamines into metanephrines within the tumor may result in a continued high secretion of the metabolites, but an episodic and low secretion of the parent amines.

This study, for the first time, measured plasma metanephrines by radioimmunoassay, while previous studies

employed chromatographic procedures (8). This might require different thresholds. A comparison of HPLC measurement of the same samples for plasma metanephrines is clearly of interest. This could not be applied in this study due to the small volume of the remaining samples. Whereas our threshold of 125.5 pg/ml for normetanephrine is quite similar to those of the previous studies using HPLC (range: 112–165 pg/ml), a threshold of 38 pg/ml for metanephrine is rather low compared with thresholds between 59 pg/ml and 98 pg/ml in previous studies.

Patients included in this study were on different beta-blockers as well as on different central and peripheral alpha-blockers. Eisenhofer *et al.* demonstrated the possible influence of these drugs on the levels of catecholamines and their metabolites (11). Non-selective alpha-blockers may be associated with higher plasma and urinary levels of norepinephrine and normetanephrine, but not epinephrine and metanephrine. Beta-blockers, to a much lesser extent, may be responsible for elevated levels of plasma metanephrines and urinary catecholamines and metanephrines.

In our study, urinary fractionated metanephrines demonstrated a slightly lower sensitivity and specificity than their plasma counterparts. Urinary metanephrines largely reflect sulfate-conjugated metabolites, which are formed in gastrointestinal tissue. Thus, they are not related only to the pheochromocytoma, which may explain the reduced accuracy compared with plasma metanephrines (12, 13). Previous studies relied mostly on the measurement of urinary total metanephrines. In contrast to a separate determination of metanephrine and normetanephrine in urine, total metanephrines describe the combined measurement of metanephrine and normetanephrine in a single assay. Urinary total metanephrines demonstrated sensitivities between 65% and 89% and specificities between 89 and 95% (8, 11, 14, 15). Determination of fractionated metanephrines measured by HPLC in previous studies resulted in higher sensitivities, from 88 to 97%, but impaired

specificities, from 69 to 85% (14, 15). The thresholds established may not be applicable to pheochromocytomas due to genetically determined syndromes. Biochemical parameters were demonstrated to be higher in sporadic than in hereditary pheochromocytomas (14). Furthermore, in patients with pheochromocytoma and von-Hippel-Lindau syndrome only plasma normetanephrine concentrations were above normal (16). Our study included only patients with sporadic pheochromocytomas.

In conclusion, determination of plasma free metanephrine and normetanephrine, as measured by RIA, is a reliable test for the diagnosis of pheochromocytoma, especially in patients with adrenal tumors of unknown etiology.

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