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Comparison of Diagnostic Accuracy of Urinary Free Metanephrines, Vanillyl Mandelic Acid, and Catecholamines and Plasma Catecholamines for Diagnosis of Pheochromocytoma

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Context: Recent evidence suggests that plasma-free metanephrines provide a highly sensitive test in patients requiring exclusion of pheochromocytoma. The diagnostic efficacy of urinary free metanephrines, however, has not been evaluated.

Objective, Design, Setting, Patients, and Outcome Measures: We compared retrospectively the diagnostic efficacy of 24-h urinary free metanephrines with our currently available measurements of 24-h urinary vanillyl mandelic acid (VMA), urinary catecholamines, and plasma catecholamines in 159 outpatients tested in a tertiary referral center for pheochromocytoma over a 4-yr period.

Results: The sensitivity of urinary free metanephrines was 100% [25 of 25 patients; 95% confidence interval (CI) 86–100%)] compared with the sensitivity of 84% (21 of 25; 95% CI 64–95%) for urinary catecholamines; 72% (18 of 25; 95% CI 51–88%) for urinary VMA; and 76% (16 of 21; 95% CI 53–92%) for plasma catecholamines. The specificity of urinary free metanephrines was 94% (116 of 123; 95% CI 89–98%), compared with the specificity of 99% (127 of 129; 95% CI 96–100%) for urinary catecholamines; 96% (130 of 134; 95% CI 91–98%) for urinary VMA; and 88% (66 of 75; 95% CI 78–94%) for plasma catecholamines. Receiver operating characteristic curves for all test groups were generated. Pairwise comparisons of the area under the receiver operating characteristic curve for urinary free metanephrines with that of each of the other three test groups individually were: 0.993 (95% CI 0.962–0.999) *vs.* 0.919 (95% CI 0.862–0.957, $P = 0.032$) for urine catecholamines; 0.993 (95% CI 0.962–0.999) *vs.* 0.846 (95% CI 0.778–0.900, $P = 0.002$) for urine VMA; and 0.992 (95% CI 0.945–0.998) *vs.* 0.852 (95% CI 0.762–0.918, $P = 0.009$) for plasma catecholamines. Testing with urinary free metanephrines failed to misidentify a single case of pheochromocytoma, compared with four missed cases for urinary catecholamines, seven missed cases for urinary VMA, and five missed cases for plasma catecholamines.

Conclusion: Urinary free metanephrines were superior to urinary VMA, urinary catecholamines, and plasma catecholamines and can provide a valuable test for diagnosis of pheochromocytoma in adults. **(*J Clin Endocrinol Metab* 92: 4602–4608, 2007)**

PHEOCHROMOCYTOMA IS A rare, life-threatening condition. Its accurate diagnosis is important to make because, if detected in time, surgical treatment is usually successful (1–3). The conventional means of detecting pheochromocytoma is by identifying elevations of catecholamines in urine. However, there have been a number of case reports in which these results were not abnormal (4–9).

Measurement of plasma free (unconjugated) metanephrines has been advocated as a highly sensitive test for the detection of pheochromocytoma (10). In addition, it has been proposed that estimation of the free metanephrines (fMNs) in urine specimens would be a useful adjunct to more conventional approaches in the identification of pheochromocytoma, particularly when catecholamines or other metabolites are normal (11). A more recent, independent evaluation of this latter approach has recommended the simultaneous determination of both free catecholamines and fMNs in urine to ensure that abnormalities associated with catecholamine-secreting tumors are detected (12).

A recent comparative evaluation of fMN measurements in plasma specimens, conducted in a tertiary care setting was reported from the Mayo Clinic. They concluded that plasma metanephrines are highly sensitive for the detection of pheochromocytoma but lack specificity when compared with the combination of 24-h urinary total metanephrines and free catecholamines (13).

The purpose of the present retrospective study was to assess the diagnostic efficacy of urinary fMNs for the detection of pheochromocytoma. The value of this test was compared with our currently available measurements of urinary free catecholamines, vanillyl mandelic acid (VMA), and plasma catecholamines.

Subjects and Methods

Using request form information, a retrospective search was conducted at the Biochemistry Department, Crosshouse Hospital, in which all diagnostic urinary assays from patients attending the Western Infirmary and suspected to have the diagnosis of pheochromocytoma were identified over a 4-yr period from September 1999 to March 2003. Selection of patients for inclusion in the study required appropriate testing

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Abbreviations: CI, Confidence interval; CT, computerized tomography; DA, dopamine; EPI, epinephrine; fMN, free metanephrine; fNMN, free normetanephrine; HVA, homovanillic acid; MIBG, metaiodobenzylguanidine; MRI, magnetic resonance imaging; NE, norepinephrine; ROC, receiver-operating characteristic; VMA, vanillyl mandelic acid.

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indications, complete specimen collection, and case note availability. Indications for testing included: patients presenting with hypertension and/or symptoms that were suggestive of pheochromocytoma; incidental mass on imaging; previous history of tumor; positive family history of tumor; and individuals with familial syndromes associated with higher risk of pheochromocytoma. The number of consecutive requests during the time period was 179. Twenty requested measurements were excluded for a variety of clinical reasons. In seven cases the physician had requested testing for circulating 5-hydroxyindoleacetic acid levels in known or suspected cases of carcinoid syndrome (these tests are measured concomitantly in our assay) in which pheochromocytoma was not one of the considered diagnoses. There was one case in which there was an incomplete urine collection (volume 200 ml), which was not subsequently repeated. There were two instances in which case records had been destroyed and 10 instances in which case records were lost or unobtainable. The remaining 159 patients formed the basis of the study. Each of the patients' case records were obtained and reviewed.

Medications taken by the patient group at the time of testing included aspirin, diuretics, calcium antagonists, angiotensin-converting enzyme inhibitors, angiotensin II antagonists, digoxin, isosorbide mononitrate, metformin, and T_4. The criteria for positivity were either histologic confirmation or, in the case of metastatic disease without histology, radiological evidence of metastatic disease with a positive metaiodobenzylguanidine (MIBG) scan. In cases in which histopathological confirmation was available in positive cases, an estimate of size was determined by multiplying together the quoted length, breadth, and height of resected tumor tissue to produce an index of volume (milliliters). The criteria for negativity were negative imaging of abdomen and chest by cross-sectional computerized tomography with contrast (CT) or magnetic resonance (MRI) ± MIBG scintigraphy; alternative diagnosis; and in all cases, no occurrence of pheochromocytoma for at least 2 yr after the diagnosis was rejected. A minimum of 2 yr of clinical follow-up was available for each patient in whom the diagnosis of pheochromocytoma was rejected. This follow-up was carried out by the referring physician in each instance and consisted, principally, of clinical evaluation supplemented, if indicated in the referring institution, by repeat negative biochemical measurement by our laboratory.

Biochemical assays

Urine (24 h) was collected into opaque polyethylene bottles containing 10 ml concentrated hydrochloric acid preservative. On receipt in the laboratory, collections were checked for adequate acidity (pH < 4), volume recorded, and a 20-ml aliquot obtained. The accuracy of collection was assessed by measuring creatinine output (11). Each urine sample was analyzed at the biochemistry department, Crosshouse Hospital for urinary VMA, homovanillic acid (HVA), norepinephrine (NE), epinephrine (EPI), dopamine (DA), free normetanephrine (fNMN), and fMN. VMA and HVA were measured using an automated HPLC kit method (Bio-Rad Laboratories, Hemel Hempstead, UK) on Gilson equipment that included the ASPEC automated sample preparation and injection unit (Gilson Medical Electronics Inc., Middleton, WI) and an ESA Coulochem II coulometric detector (11). Urinary free catecholamines and free metanephrines were analyzed simultaneously, without prior sample acid deconjugation, by automated HPLC using the Gilson ASTED system (11). Plasma NE and EPI were measured by the HPLC-electrochemical detection technique of Goldstein *et al.* (14). For urine measurements, interassay coefficients of variation were determined by replicate analysis of a commercial quality control material, Lyphochek II (Bio-Rad Laboratories). Typical coefficients of variation were (mean in *parentheses*): VMA, 4.5% (76 μmol/liter, 15.05 mg); HVA, 6.0% (76 μmol/liter, 13.83 mg); NE, 8.6% (1281 nmol/liter, 216.49 μg); EPI, 8.4% (274 nmol/liter, 50.12 μg); DA, 5.0% (3329 nmol/liter, 509.34 μg); fNMN, 7.4% (4839 nmol/liter, 8090.38 μg); and fMN, 6.2% (1757 nmol/liter, 68.88 μg).

Analysis of measurements

The upper reference limit for each of the urinary analytes were: VMA (<35 μmol per 24 h, < 6.93 mg per 24 h); HVA (<40 μmol per 24 h, < 7.28 mg per 24 h); NE (<900 nmol per 24 h, 152.1 μg per 24 h); EPI (<230 nmol per 24 h, 42.09 μg per 24 h); DA (<3300 nmol per 24 h, 504.9 μg per 24 h); fNMN (<650 nmol per 24 h, 119.6 μg per 24 h); and fMN (< 350 nmol per 24 h, 69.3 μg per 24 h) (11, 15–17). The upper reference limit values for plasma measurements were: NE (<4.0 nmol/liter, 676.8 ng/liter) and EPI (<0.4 nmol/liter, 73.28 ng/liter) (14). Results at or above these values were considered to be positive. Normal reference ranges for the catecholamines (15, 16) and VMA and HVA were based on published data from 50 hypertensive patients being investigated for pheochromocytoma but not subsequently found to have the disease (17). Because the values for urinary NE and EPI showed a positive skew, the results were normalized by logarithmic transformation, and the upper reference limits were calculated as mean plus 2 SD of the log-transformed data (15). For fNMN and fMN, provisional upper reference limits were determined in a separate study by analysis of 24-h urine specimens from 230 adult patients (130 women, 100 men, median age 50 yr, range 18–86 yr) who were being investigated for possible pheochromocytoma but not subsequently found to have the disease. Because both urinary fNMN and fMN also exhibit a positive skew, upper reference limits were determined nonparametrically and represent 97.5 percentiles (11).

Statistical analysis

Sensitivities and specificities of tests in plasma and urine specimens, using the definitions of positivity as described, were calculated for each of four test groups: plasma catecholamines = plasma NE and EPI; urine VMA; urine catecholamines = urinary NE and EPI; urine free metanephrines = urinary fNMN and fMN. Where a grouping contained two analytes (*e.g.* fNMN and fMN, or NE and EPI), elevation in either analyte was deemed to be positive (18, 19). Only when both analytes were below their respective URLs was the grouping considered to be negative. Where more than one specimen was available for the same patient, collected at time of diagnosis, the mean value for that analyte was taken. Comparison of receiver-operating characteristic (ROC) curves were performed for each test grouping only on patients for whom all plasma and urinary results were available, and for biochemical tests involving pairs of measurements, a false-negative result in a patient with pheochromocytoma or a true negative result in a patient without pheochromocytoma was defined as a value for each measurement lower than the upper reference limit. A true positive result for pairs of measurements in a patient with pheochromocytoma or a false-positive result in a patient without pheochromocytoma was defined as a value for either or both measurements equal to or higher than the appropriate upper reference limit in accordance with the procedure described by Lenders *et al.* (18, 19). ROC curve analysis was performed using the MedCalc software package (version 7.2; MedCalc Software, Mariakerke, Belgium), which uses calculation of the area under the curve and 95% confidence intervals by the technique described by Hanley and McNeil (20). Statistical significance of the difference between the areas under two or more ROC curves for different test groups was calculated by the method of Hanley and McNeil (21).

In positive cases, the association between preoperative test group findings and eventual tumor size was assessed by Spearman rank correlation. Association between tumor location and malignancy was examined by Fisher's exact test. Other relationships (*e.g.* between DA or HVA and malignancy) were assessed using the Mann-Whitney test for unpaired data, and quoted *P* values were two sided.

Results

The study group consisted of 159 patients (81 male, 78 female) of mean age 41 yr including 25 patients subsequently proven to have pheochromocytoma. Histological confirmation was available in 20 of these 25 patients. The other five cases, each with inoperable metastatic pheochromocytoma, were confirmed by both CT/MRI imaging and a positive MIBG scan.

In the 134 patients deemed negative for pheochromocytoma, none have subsequently been identified as harboring a pheochromocytoma for at least 2 yr after the diagnosis was rejected. The alternative diagnoses in this group included: essential hypertension (65), adrenal adenoma (seven), adrenal carcinoma (two), renal carcinoma (two), carotid body

TABLE 1. The biochemical values, tumor size, tumor location, and malignancy status for patients with pheochromocytoma

Case no.	Age (yr) and sex	VMA (μmol per 24 h) (<35)	HVA (μmol per 24 h) (<40)	fNMN (nmol per 24 h) (<650)	fMN (nmol per 24 h) (<350)	NE (nmol per 24 h) (<900)	EPI (nmol per 24 h) (<230)	DA (nmol per 24 h) (<3300)	Plasma NE (nmol/liter) (<4.0)	Plasma EPI (nmol/liter) (<0.4)	Tumor size index (ml)	Tumor location
1	33 F	34	19	1,560[a]	96	1,482[a]	25	2,106	1.9	0.1	80	E
2	40 F	56[a]	36	10,492[a]	1,549[a]	1,335[a]	384[a]	1,564	8.7[a]	0.2	163.9	I
3	66 F	36[a]	17	763[a]	187	765	25	1,090	7[a]	0.75[a]	42	E
4	62 F	40[a]	34	1,399[a]	64	1,082[a]	25	1,780	4.9[a]	0.17	n/a	E
5	66 M	28	24	148	800[a]	302	220	n/a	n/a	22.5	I	
6	36 M	79[a]	21	5,520[a]	5,281[a]	1,772[a]	1,066[a]	1,267	6.3[a]	0.47[a]	48.1	I
7	30 F	642[a]	111[a]	60,258[a]	16,751[a]	11,472[a]	3,172[a]	61,832*	n/a	n/a	1,088.5	E
8	47 F	28	14	797[a]	781[a]	1,065[a]	400[a]	1,231	9.8[a]	0.75[a]	12	I
9	78 F	156[a]	35	7,713[a]	35,456[a]	10,444[a]	20,112[a]	5,702*	7.1[a]	3.2[a]	218.4	I
10	35 F	83[a]	22	4,931[a]	110	13,317[a]	202	1,270	2	0.19	23.1	I
11	29 F	156[a]	40[a]	7,178[a]	561[a]	5,734[a]	61	1,901	34.3[a]	0.12	n/a	E
12	69 F	50[a]	170[a]	1,387[a]	845[a]	1,207[a]	214	5,516*	13.9[a]	0.2	n/a	E
13	21 F	50[a]	18	1,989[a]	364[a]	3,273[a]	34	1,837	16.2[a]	0.1	27	E
14	35 M	1,189[a]	188[a]	88,435[a]	344	62,362[a]	175	7,601[a]	28.6[a]	0.18	n/a	E
15	43 M	60[a]	25	1,238[a]	50	2,024[a]	25	2,620	3.1	0.1	13.1	I
16	44 M	23	25	50	1,466[a]	148	25	1,418	3.9	0.1	16.9	I
17	59 F	211[a]	19	7,455[a]	224	6,991[a]	25	984	44.6[a]	0.15	63	E
18	31 F	141[a]	27	12,375[a]	80	11,396[a]	136	1,152	34[a]	0.17	72	I
19	49 M	43[a]	22	750[a]	50	1,439[a]	25	1,675	7.3[a]	0.1	0.24	I
20	56 F	285[a]	49[a]	19,936[a]	50	29,172[a]	33	2,979	32.6[a]	0.11	n/a	I
21	41 M	18	17	1,174[a]	240	169	25	1,662	1.9	0.1	900	I
22	71 F	229[a]	28	7,924[a]	5,476[a]	9,937[a]	4,153[a]	1,283	56.9[a]	29.1[a]	51.2	I
23	27 F	33	16	1,959[a]	100	2,328[a]	43	1,357	n/a	n/a	10.3	I
24	54 M	94[a]	105[a]	3,171[a]	50	3,828[a]	25	605	n/a	n/a	n/a	E
25	70 F	31	19	371	916[a]	356	645[a]	1,266	3.9	4.2[a]	n/a	I

Normal ranges and conversion factors are given in *parentheses*. To convert SI units to mass units, the conversion factors are: VMA (micromoles per 24 h) × 0.198 = milligrams per 24 h; HVA (micromoles per 24 h) × 0.182 = milligrams per 24 h; fNMN (nanomoles per 24 h) × 0.184 = micrograms per 24 h; fMN (nanomoles per 24 h) × 0.198 = micrograms per 24 h; NE (nanomoles per 24 h) × 0.169 = micrograms per 24 h; EPI (nanomoles per 24 h) × 0.183 = micrograms per 24 h; DA (nanomoles per 24 h) × 0.153 = micrograms per 24 h; plasma NE (nanomoles per liter) × 169.2 = nanograms per liter (or picograms per milliliter); plasma EPI (nanomoles per liter) × 183.2 = nanograms per liter (or picograms per milliliter). M, Male, F, female; E, extraadrenal; I, intraadrenal; n/a, not available.

[a] Abnormal results.

tumor (two), Cushing's disease (one), polycystic ovary disease (one), CT's adenoma (one), adrenal leiomyosarcoma (one), menopause (one), primary hyperparathyroidism (two), medullary thyroid carcinoma (one), and antidepressant drug therapy (two). In the remaining 46 patients in the negative group, seven were undergoing routine follow-up because of a previous history of resected pheochromocytoma, one had neurofibromatosis, and one other had familial hyperparathyroidism. A satisfactory alternative diagnosis was not available for the remaining 37 patients in the negative group. These were patients being investigated for relevant symptoms including hyperhidrosis, flushing, spells, headache, or palpitations. Of these 37 patients, 17 had a negative CT or MRI scan.

The 25 patients with pheochromocytoma comprised 17 females of mean (range) age 49 (21–78) yr, and eight males mean (range) aged 46 (35–66) yr. The biochemical values, tumor location, and malignancy status are shown in Table 1. The tumor was intraadrenal in 15 and extraadrenal in 10. Eight of the tumors were malignant. Of the eight malignant pheochromocytomas, seven arose from an extraadrenal location (Fisher's exact test, $P = 0.0017$). In addition, malignancy was also associated with significantly higher urinary output of DA (median = 2542 nmol per 24 h, 388.93 μg per 24 h *vs.* 1357 nmol per 24 h, 207.62 μg per 24 h; Mann-Whitney test, $P = 0.023$) and HVA (median = 77 μmol per 24 h, 14.01 mg per 24 h *vs.* 22 μmol per 24 h, 4 mg per 24 h; Mann-Whitney test, $P = 0.0014$). In intraadrenal tumors, although urine results tended to be higher than those with extraadrenal tumors for EPI (median = 202 nmol per 24 h, 36.97 μg per 24 h *vs.* 30 nmol per 24 h, 5.49 μg per 24 h) and fMN (median = 781 nmol per 24 h, 143.7 μg per 24 h *vs.* 284 nmol per 24 h, 52.26 μg per 24 h), this did not achieve statistical significance.

An estimate of tumor size was calculated from the histopathology reports of 18 patients. Using Spearman rank correlation, fNMN+fMN correlated positively with tumor volume ($r_s = 0.63$, $P = 0.009$). Urinary (NE+EPI), VMA, and plasma (NE+EPI) did not show a correlation with tumor volume.

Among those with pheochromocytoma (n = 25), plasma NE and EPI results were available for 21 patients. Results for the other three biochemical test groups were obtainable for all 25 patients. For those 134 patients in the study group without pheochromocytoma, results for 75, 134, 129, and 123 were obtainable for plasma catecholamines, urine VMA, urine catecholamines, and urine fMNs, respectively. Summary data for the urinary outputs of the various analytes for both groups of patients with and without pheochromocytoma are shown in Table 2. The incomplete data set for plasma catecholamines was due to failure to request measurement: for urine catecholamines and urinary fMNs, the missing results were due to assay interference with paracetamol. Thus, a complete data set was available only for urinary VMA. The diagnostic efficacy of the various test groups, using all of the available data, is given in Table 3. The sensitivity of urinary fMNs was 100% [25 of 25 patients; 95% confidence interval (CI) 86–100%], compared with the sensitivity of 84% (21 of 25; 95% CI 64–95%) for urinary catecholamines, 72% (18 of 25; 95% CI 51–88%) for urinary VMA, and 76% (16 of 21; 95% CI 53–92%) for plasma catecholamines. The specificity of urinary fMNs was 94% (116

TABLE 2. Summary data of the urinary outputs of the various analytes for both groups of patients with and without pheochromocytoma

Analyte	Cases without pheochromocytoma			Cases with pheochromocytoma		
	Median	Range	n	Median	Range	n
VMA (μmol per 24 h)	21	4–54	134	56	18–1,189	25
NE (nmol per 24 h)	275	25–867	129	2,024	148–62,362	25
EPI (nmol per 24 h)	25	12–314	129	61	25–20,112	25
fNMN (nmol per 24 h)	213	38–857	123	1,989	50–88,435	25
fMN (nmol per 24 h)	84	8–972	123	344	50–35,456	25
Plasma NE (nmol/liter)	1.7	0.1–10.8	75	7.3	1.9–56.9	21
Plasma EPI (nmol/liter)	0.1	0.1–2.3	75	0.17	0.1–29.1	21

To convert SI units to mass units, the conversion factors are: VMA (micromoles per 24 h) × 0.198 = milligrams per 24 h; fNMN (nanomoles per 24 h) × 0.184 = micrograms per 24 h; fMN (nanomoles per 24 h) × 0.198 = micrograms per 24 h; plasma NE (nanomoles per liter) × 169.2 = nanograms per liter (or picograms per milliliter); plasma EPI (nanomoles per liter) × 183.2 = nanograms per liter (or picograms per milliliter).

of 123; 95% CI 89–98%), compared with the specificity of 99% (127 of 129; 95% CI 96–100%) for urinary catecholamines, 96% (130 of 134; 95% CI 91–98%) for urinary VMA, and 88% (66 of 75; 95% CI 78–94%) for plasma catecholamines. In addition, the data given in Table 3 also summarize the published diagnostic sensitivities and specificities for the four test groups and those of other similar studies including the findings for fMNs in plasma (18, 22–24). Urinary fMNs showed no false negative results in the current series, and hence, the sensitivity of this test group was the highest at 100%. However, there were seven false-positives for this test group among the available 123 patients deemed negative for pheochromocytoma producing a specificity of 94.3%.

Complete data sets of plasma and urinary measurements were available for 90 patients, including 21 with pheochromocytoma, and only these were used for comparison of ROC curves for all four test groups simultaneously. The ROC curves for all four test groups taken together are shown in Fig. 1. In that instance, the values for area under the ROC curve were 0.852 (95% CI 0.762–0.918) for plasma catecholamines, 0.856 (95% CI 0.766–0.921) for urine VMA, 0.929 (95% CI 0.854–0.972) for urine catecholamines, and 0.992 (95% CI 0.945–0.998) for urine fMNs. Pairwise comparison of these areas showed that the urinary fMNs produced an area under the ROC curve, which was significantly greater than either plasma catecholamines ($P = 0.009$) or urinary VMA ($P = 0.008$). Although, in this instance, comparison of the area under the ROC curve for fMNs with that of the urinary catecholamines failed to achieve statistical significance, there were no instances among all 25 patients with pheochromocytoma in which either urinary EPI was elevated with normal fMN or NE elevated with normal fNMN (see Table 1). Using all of the available data, ROC curves for urinary fMNs were also compared with that of each of the other three test groups individually. In each individual comparison, urinary fMNs produced a value for the area under the ROC curve, which was significantly higher than plasma catecholamines ($P = 0.009$), urine VMA ($P = 0.002$), and urine catecholamines ($P = 0.032$). The results of these paired ROC curves are given in Fig. 2, and the findings are summarized in Table 4.

Discussion

The optimum approach to the biochemical confirmation of pheochromocytoma remains debatable. Recommendations are based on individual institutional experience but have traditionally advocated concurrent measurement of urine and/or plasma catecholamines. There have been numerous reports of pheochromocytoma with normal catecholamines, suggesting that this practice is insufficiently sensitive to rule out the diagnosis (4–7). The realization that pheochromocytoma could coexist with normal catecholamines has led to a search to find analytes that complement or perhaps replace catecholamines.

Evidence suggests that metanephrines, the O-methylated metabolites of catecholamines, may be a better test than catecholamines. Historically, metanephrines have been measured by spectrophotometry as urinary total metanephrines, *i.e.* (conjugated + free) normetanephrine + (conjugated + free metanephrine), all as a single entity. This has been largely replaced by HPLC, which allows the measurement of urinary or plasma fractionated metanephrines, *i.e.* (conjugated + free) normetanephrine and (conjugated + free) metanephrine separately (25, 26). Metanephrines are deaminated by monoamine oxidase, terminating in the production of VMA or alternatively undergo sulfate conjugation by a monoamine preferring sulfotransferase (26). This enzyme has not been found in adrenal medullary chromaffin cells but in the gastrointestinal tract. Accordingly, the gut is thought

TABLE 3. Diagnostic sensitivities and specificities for the various test groups in the present study and those of recently published similar studies

Test groups	Sensitivities					Specificities				
	Tormey and Fitzgerald, 1995 (22)	Raber *et al.*, 2000 (23)	Lenders *et al.*, 2002 (18)	Kudva *et al.*, 2003 (24)	This study	Tormey *et al.*, 1995 (22)	Raber *et al.*, 2000 (23)	Lenders *et al.*, 2002 (18)	Kudva *et al.*, 2003 (24)	This study
Plasma NE and EPI	n/a	82% (14/17)	84% (187/212)	n/a	76% (16/21)	n/a	100% (14/14)	81% (523/643)	n/a	88% (66/75)
Urine VMA	60%	n/a	64% (96/151)	n/a	72% (18/25)	96%	n/a	95% (442/465)	n/a	96% (130/134)
Urine NE and EPI	82%	82% (14/17)	86% (151/175)	71% (104/147)	84% (21/25)	88%	94% (13/14)	88% (471/535)	99% (777/781)	99% (127/129)
Urine fNMN and fMN	n/a	n/a	n/a	n/a	100% (25/25)	n/a	n/a	n/a	n/a	94% (116/123)
Plasma fNMN and fMN	n/a	100% (17/17)	99% (211/214)	96% (23/24)	n/a	n/a	100% (14/14)	89% (471/535)	85% (198/234)	n/a

True to total positive ratio given in *parentheses.* n/a, Not available.

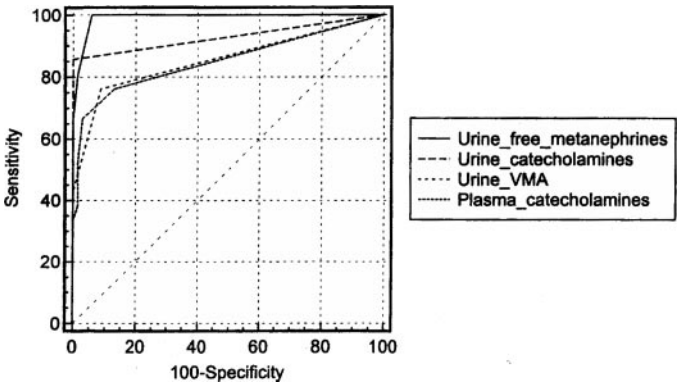

FIG. 1. Comparison of ROC curves for the four test groups for 90 patients (including 21 with pheochromocytoma) who exhibited complete data sets. AUC values included: plasma catecholamines, 0.852 (95% CI 0.762–0.918); urine VMA, 0.856 (95% CI 0.766–0.921); urine catecholamines, 0.929 (95% CI 0.8540.972); and urine fMNs, 0.992 (95% CI 0.945–0.998). AUC, Area under the ROC curve.

to be the primary source of conjugated metanephrines and therefore the measurement of conjugated metanephrines may be less relevant in the diagnosis of pheochromocytoma.

In patients with pheochromocytoma, not only is the urinary output of the metanephrines found to be increased, but also there is a disproportionate increase in the unconjugated moiety, particularly for normetanephrine (27–29). Evidence from a report by Eisenhofer (30) has established that in pheochromocytoma the fMNs are produced within the tumor cells continuously and independently of catecholamine release. In the measurement of total urinary fractionated metanephrines, however, samples undergo a deconjugation step with acid hydrolysis to liberate the conjugated fraction and therefore represent different metabolites from endogenous fMNs. An alternative approach is to measure plasma or urinary free metanephrine directly. Plasma fMNs are highly sensitive in detection of pheochromocytoma (13, 18, 24). There are, however, no reports of the validity of urinary fMNs for the detection of pheochromocytoma.

In the present study, we used a HPLC-electrical detection (11) technique to measure urinary free norepinephrine, epinephrine, normetanephrine, and metanephrine independently of their conjugated forms and without the need for a deconjugation step (31, 32). A recent report from our laboratory suggested that the measurement of urinary fMNs may be potentially valuable by describing a number of examples of patients with pheochromocytoma who exhibited normal urinary catecholamines but elevated urinary fMNs (11). This present study is the first formal assessment of the diagnostic efficacy of this novel test.

This study demonstrates that the measurement of urinary fMNs are a highly sensitive and specific test that appears to be superior to urinary VMA, urinary catecholamines, or plasma catecholamines for the diagnosis of pheochromocytoma. The measurement of urinary fMNs failed to misidentify a single case of pheochromocytoma, providing a sensitivity of 100%, compared with four missed cases for urinary catecholamines, seven missed cases for urinary VMA, and five missed cases for plasma catecholamines. There were only seven false-positive cases from the remaining 123 patients tested for urinary fMNs. Furthermore, in keeping with similar evidence for plasma fMNs (30), there was a significant correlation between elevations in urinary fMNs and tumor volume. When examining the evidence available for plasma fMNs (Table 3), fMNs measured in urine are at least as effective in the diagnosis of pheochromocytoma. It is important, however, to remember the limitations of comparing the efficacy of different diagnostic tests performed in different laboratories in an unmatched patient population.

It is also notable that, in the present study, malignant

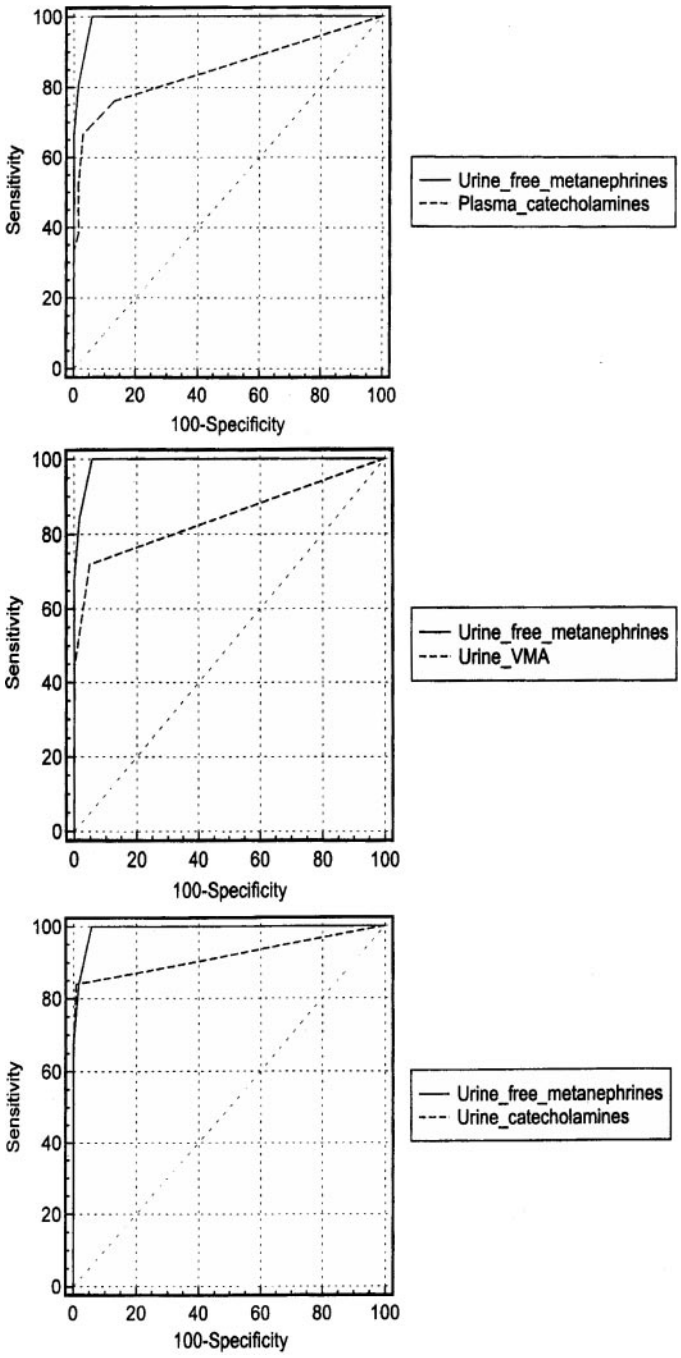

FIG. 2. Individual comparison of ROC curves using all available data: urine fMNs *vs.* plasma catecholamines (A), urine VMA (B), and urine catecholamines (C) (see Table 4).

TABLE 4. Summary of AUC values for test group ROC curves, each compared individually with that of the urine fMNs (see Fig. 2)

Test group comparison	Patients with pheochromocytoma	Patients without pheochromocytoma	AUC (95% CI)	*P* value
Urinary fMNs *vs.* plasma catecholamines	21	69	0.992 (0.945–0.998) *vs.* 0.852 (0.762–0.918)	0.009
Urinary fMNs *vs.* urinary VMA	25	123	0.993 (0.962–0.999) *vs.* 0.846 (0.778–0.990)	0.002
Urinary fMNs *vs.* urinary catecholamines	25	123	0.993 (0.962–0.999) *vs.* 0.919 (0.862–0.957)	0.032

AUC, Area under the ROC curve.

tumors were most often located at extraadrenal sites. This observation has been made previously in a study of 86 patients with pheochromocytoma (33). Furthermore, in keeping with previous reports, malignancy was often associated with elevations in urinary dopamine and its metabolite HVA (34).

The strengths of this study include the avoidance of selection bias, maintained by consecutive patient recruitment over a 4-yr period. Furthermore, strict inclusion criteria for testing ensured that the diagnostic estimates of specificity were clinically relevant to the patient groups tested. However, there are several limitations to our study. We performed a retrospective study of limited sample size. The study was conducted in only one center, making it difficult to generalize our results. Moreover, the interpretation of tests and final diagnosis was not blinded. A key feature to studies involving diagnostic tests is that the disease must be excluded by methods other than the diagnostic tests being compared. The gold standard definition of a true negative result would require histological confirmation of the absence of pheochromocytoma. Clearly this is not possible. Our approach to the definition of a true negative was similar to that used by Lenders *et al.* (18). None of the 134 patients in whom the diagnosis was rejected was found to have a pheochromocytoma in the subsequent 2 yr and in some cases up to 5.5 yr. Of the those deemed true negatives, 97 had an alternative diagnosis. Of the remaining 37 patients, 17 had negative imaging in the form of a CT or MRI scan. Although in the Mayo clinic study by Sawka *et al.* (13), exclusion was based on an alternative diagnosis, our experience and that of others (18) are that finding an alternative diagnosis in this patient population is not always possible.

It is also important to discuss the implications of the incomplete data set. VMA was the only diagnostic test to have a complete data set (134 of 134). This is due to the assay's low susceptibility to drug interference. In contrast, five of urinary catecholamine assays (4%) and 11 of urinary FMNs assays (8%) were uninterpretable due to assay interference, which we subsequently identified as a consequence of paracetamol. We have reported that approximately one fourth of specimens received from throughout Scotland had a level of paracetamol, which could result in spurious urinary catecholamine and urinary fMN results. In this analysis urinary fMNs were the most affected (35). The level of drug interference is considerably less in this study group, but this may reflect the higher standard of specimen collection in a tertiary referral center. This evidence therefore raises questions over the reliability of HPLC techniques. The implementation of alternative analytical strategies to avoid drug interference, such as mass spectrometry, is a likely improvement but would have serious cost implications.

Conclusion

This study demonstrates that urinary fMNs were superior to urinary VMA, urinary catecholamines, and plasma catecholamines and can provide a valuable test for diagnosis of pheochromocytoma in adults. A large prospective study is now required to confirm the findings of this paper.

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