

Utility of Plasma Free Metanephrines for Detecting Childhood Pheochromocytoma

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Measurements of plasma free metanephrines, normetanephrine (NMN) and metanephrine (MN), provide a sensitive test for diagnosis of pheochromocytoma in adults but have not been evaluated in children. We therefore established reference ranges for plasma and urinary metanephrines and the catecholamines, norepinephrine (NE) and epinephrine (E), in 86 healthy children (age 5–17). A group of 158 healthy adults (age 18–72) served as a comparison group. Pediatric reference ranges were applied to examine the diagnostic utility of the various tests in 45 children evaluated for pheochromocytoma (age 8–17; 38 with von Hippel-Lindau syndrome), with tumors found on 12 occasions. Upper reference limits for E and MN were higher and those for NE and NMN lower in children than

in adults. Boys had higher plasma levels of E and MN and higher urinary excretion of all four amines than girls. Plasma free metanephrines provided a diagnostic test with values for sensitivity (100%) and specificity (94%) that were equal to or higher than those of other tests. In two children screened for pheochromocytoma on multiple occasions, use of pediatric reference ranges for plasma free metanephrines indicated the tumor a year earlier than indicated using adult reference ranges. The findings indicate that plasma free metanephrines provide a sensitive tool for detection of pheochromocytoma in children. Age appropriate reference ranges should be used and gender differences should be considered. (*J Clin Endocrinol Metab* 87: 1955–1960, 2002)

PHEOCHROMOCYTOMAS ARE CATECHOLAMINE-producing tumors that arise from chromaffin cells of the sympathoadrenal system. Although the peak incidence for pheochromocytoma is in the third and fourth decades, the tumor can develop in children, especially as a feature of the autosomal dominant familial disorders, multiple endocrine neoplasia type 2 (MEN 2) and von Hippel-Lindau syndrome (VHL). In these familial syndromes annual screening for pheochromocytoma is recommended from early childhood (1).

Diagnosis of pheochromocytoma is, however, more problematic in children than in adults (2). The most common associated clinical findings of pheochromocytoma are sustained or paroxysmal hypertension, headache, palpitations, and diaphoresis (3), but many pheochromocytoma—especially those found during screening of patients with MEN 2 or VHL syndrome—are clinically silent (4). When high blood pressure or symptoms are present, these are less likely to be recorded or reported accurately in children than in adults.

Measurements of urinary excretion of catecholamines or metanephrines have long represented the gold standards for biochemical detection of pheochromocytoma (5). However, 24-h urine samples may not always be collected reliably, a problem especially important in children. Correction of urinary outputs of catecholamines or metanephrines for creat-

inine excretion may obviate concerns about completeness of urine collection, but due to dependence of creatinine on diet (6), muscle mass (7), physical activity (8), and diurnal variation (9) such corrections can also further confound interpretation of results. Differences in urinary outputs and plasma concentrations of catecholamines and metanephrines between children and adults represent other potential complicating factors in the diagnosis of childhood pheochromocytoma (10, 11).

Use of computed tomography (CT), magnetic resonance imaging (MRI) and metaiodobenzylguanidine (MIBG) scintigraphy are generally reserved for patients with positive biochemical test results but are also used as part of periodic screening in patients with MEN 2 and VHL syndrome (1, 3, 12). Due to radiation concerns or the need for sedation, these imaging studies are not ideal screening tools in children. Biochemical testing remains essential.

Measurements of plasma free metanephrines, normetanephrine (NMN) and metanephrine (MN), the respective O-methylated metabolites of norepinephrine (NE) and epinephrine (E), represent a recently developed and promising test for diagnosis of pheochromocytoma (3, 13, 14). In the present study, we established the utility of plasma free metanephrines for diagnosis of pheochromocytoma in children prospectively screened for the tumor. Importantly, the study included the establishment of pediatric reference ranges and retrospective analysis of whether these reference ranges might lead to improved diagnosis than achieved using the reference ranges established in adults that were used for prospective screening in our patient population.

Abbreviations: CI, Confidence interval; CT, computed tomography; E, epinephrine; MEN 2, multiple endocrine neoplasia type 2; MN, metanephrine; MIBG, metaiodobenzylguanidine; MRI, magnetic resonance imaging; NE, norepinephrine; NMN, normetanephrine; VHL, von Hippel-Lindau syndrome.

Materials and Methods

Subjects

Eighty-six healthy normotensive children (age 5–17 yr; 43 boys) and 158 adults (age 18–72; 85 men) constituted the reference populations. The healthy subjects were recruited from the Clinical Research Volunteer Program Office at the National Institutes of Health and underwent thorough medical history and physical examination before testing. The study was approved by the NICHD Institutional Review Board, and written informed consent was obtained from all adult subjects and the parents of participating children. Children gave their assent.

Between 1993 and 2001, 45 children (age 8–17 yr; 26 boys), including 38 patients with VHL syndrome, were prospectively evaluated for pheochromocytoma. Patients with VHL were initially identified on the basis of their family history, and the diagnosis was confirmed clinically or by identification of germ-line mutations in the VHL tumor-suppressor gene.

Most patients were screened for pheochromocytoma on the basis of their hereditary predisposition to the tumor, not because of presenting signs and symptoms. The presence of hypertension or suspicious symptoms (sweatiness, headache, palpitations, anxiety attacks, and/or faintness) led to screening for pheochromocytoma in 3 of the 38 VHL patients and 6 of the 7 children in the sporadic group. One child of the latter group was screened for pheochromocytoma because of suspected medullary thyroid cancer and an underlying multiple endocrine neoplasia syndrome. This suspicion was based on a thyroid nodule and elevated calcitonin levels, which later turned out to be due to an unidentified substance interfering with the calcitonin assay (15).

In addition to biochemical testing, diagnostic evaluation included the use of conventional radiological imaging in all patients, most usually by MRI, but occasionally also by CT. Use of MIBG scintigraphy was reserved for patients in whom there was strong evidence of pheochromocytoma according to the results of conventional radiological techniques or biochemical testing.

Conventional radiological imaging studies provided evidence of an adrenal mass in 13 of the 45 children screened for pheochromocytoma. MIBG scintigraphy was carried out in 14 children, and indicated the presence of pheochromocytoma in 7 of the 10 patients in whom the tumor was subsequently confirmed.

Confirmation of a pheochromocytoma required a histopathological diagnosis after surgery, or in one case, evidence of metastases by imaging studies. Pheochromocytoma was thereby confirmed in 10 children (all with VHL), 2 of whom had separate tumors removed on two occasions one and two years apart, providing a total of 12 separate incidences of pheochromocytoma. One symptomatic child presented with an adrenal tumor that had metastasized to liver and bone at the time of diagnosis. Two additional children with VHL were suspected of harboring pheochromocytoma because of radiological and biochemical evidence but had not been operated at the time of publication. In these two children, pheochromocytoma remained unconfirmed according to our predetermined criteria, and the results of their biochemical tests were not included in analyses of diagnostic test performance.

Exclusion of pheochromocytoma in this study was based on predetermined criteria that were necessarily independent of the biochemical tests examined for diagnostic test performance. Lack of radiological evidence of pheochromocytoma by MRI or CT provided criteria for exclusion of pheochromocytoma in 32 children. In one additional child with both CT and MRI evidence of an adrenal mass, exclusion of pheochromocytoma was based on a subsequent diagnosis of Cushing's disease. In the other children who presented with hypertension or suspicious symptoms, exclusion of pheochromocytoma required a final diagnosis that explained the patients' symptoms.

Biochemical testing

Biochemical testing included measurements of plasma concentrations of free metanephrines (NMN and MN) and catecholamines (NE and E) in all cases and urinary fractionated metanephrines (NMN and MN) and catecholamines in subsets of patients. Plasma NMN, MN, NE, and E were determined by liquid chromatography with electrochemical detection, as previously described (16, 17). The detection limits of the assays were 5–10 fmol/ml. Twenty-four-hour urinary excretion of fractionated metanephrines and catecholamines were determined by HPLC

with electrochemical detection at Mayo Clinic Laboratories (Rochester, MN) and at Quest Diagnostics, Inc. (San Juan, CA), respectively.

All subjects were instructed to abstain from caffeinated foods and drinks for at least 24 h and to avoid acetaminophen, which interferes with the plasma NMN assay (16), for at least 5 d before the blood draw. After an overnight fast (9 h, water permitted), a blood sample was drawn into a prechilled 10 ml heparinized tube through an indwelling iv cannula in the forearm. The subjects rested in the supine position for 20 min after insertion of the cannula before the blood sample was drawn. The blood was immediately spun and the plasma frozen at -70°C until assayed. Concomitant 24-h urine specimens for determination of both fractionated metanephrines and catecholamines were obtained in 73 healthy children. Forty of the 45 children evaluated for pheochromocytoma had concomitant measurements of urinary catecholamines, 27 of whom also had measurements of urinary fractionated metanephrines.

Biochemical testing for pheochromocytoma was carried out prospectively using reference ranges established in adults. Seven patients including six with subsequently pathologically confirmed pheochromocytoma, were tested on two or more occasions over the course of several years. The time of initial diagnosis of pheochromocytoma in these children was set at the time one of more of the biochemical tests yielded a positive test result, according to the adult reference ranges in use at the time, combined with positive imaging studies.

Analysis of data

Upper and lower reference limits for plasma and urinary NMN, MN, NE, and E were determined from the 95% confidence intervals (CI) in separate adult and pediatric reference groups. NMN, MN, NE, and E were normally distributed after logarithmic transformation. Therefore upper and lower reference limits for the plasma and urinary values were calculated from the antilogarithm of the mean \pm 2 sd of the transformed data.

A true positive result for pairs of measurements (NMN and MN, NE and E) in a patient with confirmed pheochromocytoma, or a false positive result in a patient without pheochromocytoma, was defined as values for either or both measurements that were higher than the respective upper reference limit, as determined from the pediatric reference population. A false negative test for pairs of measurements in a patient with pheochromocytoma, or a true negative test in a patient without pheochromocytoma, were defined as values for both measurements that were lower than the respective upper reference limits, as determined from the pediatric reference population.

The specificity of each pair of measurements was determined from the number of true negative results divided by the total number of true negative and false positive results. The sensitivity of each pair of measurements was calculated from the number of true positive results divided by the total number of true positive and false negative results for patients with confirmed pheochromocytoma. In patients with confirmed pheochromocytoma in whom multiple tests were carried out over several years, values for sensitivity were determined at the time of initial diagnosis. Data were then retrospectively analyzed to establish whether use of pediatric reference ranges would have led to an earlier initial diagnosis.

Gender and age-related differences of catecholamine and metanephrine concentrations in the reference populations were assessed by *t* test or Mann-Whitney *U* test as appropriate. For graphical presentation, patient data were normalized using the gender-specific pediatric reference ranges and expressed as percentages of the corresponding upper reference limits. Data from the two patients with suspected but not histologically confirmed pheochromocytoma are shown for comparison but were excluded from analyses of diagnostic sensitivity or specificity.

Results

Upper reference limits for plasma concentrations of E and MN were higher and those for plasma NE and NMN lower in children than in adults (Fig. 1). Similarly, mean plasma concentrations of E and MN in the healthy reference populations were 47 and 22% ($P < 0.01$) higher in children than in adults. In contrast, mean plasma NE and NMN did not differ significantly between pediatric and adult groups.

Pediatric reference ranges for plasma and urinary metanephrines and catecholamines differed according to gender (Table 1). Boys had 45% ($P < 0.05$) and 30% ($P < 0.01$) higher

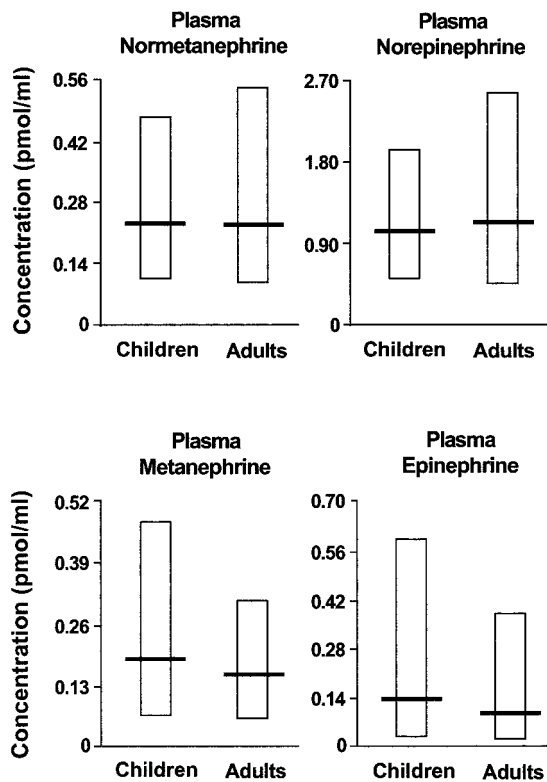


FIG. 1. Comparison of pediatric (n = 86, 43 boys) and adult (n = 158, 85 men) reference ranges for plasma free metanephrines (normetanephrine and metanephrine) and catecholamines (norepinephrine and epinephrine). Horizontal line, Median; box, 95% CI as calculated from the logarithmically transformed data. Children had higher ($P < 0.01$) plasma concentrations of epinephrine and metanephrine than adults, whereas plasma norepinephrine and normetanephrine levels did not differ significantly between age groups.

TABLE 1. Demographic characteristics and biochemical values in healthy boys and girls and in pediatric patients with or without pheochromocytoma

Characteristic	Reference groups		Patients	
	Boys (n = 43)	Girls (n = 43)	Tumor confirmed (n = 12)	Tumor excluded (n = 33)
Age (Y)				
Mean \pm SD	12.6–3.3	12.2–3.2	12.4–2.7	14.7–2.3
Range	5.3–17.9	7.3–17.9	8.1–16.9	9.5–17.2
Gender (M/F)			8/4	20/13
Biochemical measures	Median (95% CI)		Median (range)	
Plasma normetanephrine (pmol/ml)	0.26 (0.11–0.53)	0.21 (0.11–0.42)	3.19 (1.41–9.06)	0.25 (0.08–0.56)
Plasma metanephrine (pmol/ml)	0.20 (0.08–0.52)	0.15 (0.06–0.37)	0.17 (0.01–0.24)	0.17 (0.06–0.30)
Plasma norepinephrine (pmol/ml)	1.04 (0.53–2.02)	1.03 (0.57–1.91)	8.60 (1.84–33.39)	1.07 (0.38–4.88)
Plasma epinephrine (pmol/ml)	0.16 (0.05–0.59)	0.11 (0.03–0.46)	0.10 (0.03–0.43)	0.09 (0.01–0.40)
Urinary normetanephrine (μ mol/24 h)	0.98 (0.49–1.78) ^a	0.69 (0.35–1.56) ^b	6.69 (4.84–23.49) ^c	0.92 (0.44–2.61) ^c
Urinary metanephrine (μ mol/24 h)	0.52 (0.24–1.01) ^a	0.32 (0.15–0.85) ^b	0.30 (0.22–0.53) ^c	0.47 (0.16–2.19) ^e
Urinary norepinephrine (μ mol/24 h)	0.15 (0.08–0.29) ^a	0.12 (0.06–0.25) ^b	1.48 (0.58–5.56) ^d	0.15 (0.04–0.82) ^f
Urinary epinephrine (μ mol/24 h)	0.02 (0.01–0.06) ^a	0.01 (0–0.04) ^b	0.03 (0–0.05) ^d	0.02 (0–0.11) ^f

^a n = 34.

^b n = 39.

^c n = 5.

^d n = 10.

^e n = 22.

^f n = 30.

plasma concentrations of E and MN, but plasma NE and NMN levels did not differ significantly. Urinary excretion of E and MN was 48 and 63% ($P < 0.01$) higher in boys than in girls. Additionally, urinary excretion of NE and NMN was 24 and 41% ($P < 0.05$) higher in boys than in girls. The differences in urinary excretion remained significant when the results were corrected for body surface area.

All 14 separate incidences in the 12 children of confirmed or suspected pheochromocytoma were associated with elevated plasma concentrations of NMN, and 12 cases involved elevated plasma concentrations of NE (Table 1 and Fig. 2). In contrast, plasma concentrations of E and MN were within the normal range in all patients (Table 1 and Fig. 2). All patients with confirmed or suspected pheochromocytoma, in whom concomitant urine measurements were available, showed elevated urinary NMN and/or NE values. One of these patients also had mildly elevated urinary E levels (Table 1).

Pheochromocytoma was excluded in 33 of the 45 children who were evaluated (including 26 VHL patients all of whom were asymptomatic). Final diagnoses in the six symptomatic patients of the spontaneous group included anxiety/panic disorder, Cushing's syndrome, and autonomic instability. Two children had borderline elevated plasma NMN levels, and 3 others had elevated plasma NE levels, whereas none had elevated plasma MN or E concentrations (Table 1 and Fig. 2). Of the 30 patients in whom pheochromocytoma was ruled out and urine measurements were available, urinary NMN was elevated in 1 out of 22, NE in 4 out of 30 and E in 2 out of 30 cases (Tables 1 and 2). The 9 children with false positive plasma and/or urine results included 6 patients with VHL, 2 of whom had negative imaging studies and biochemical tests up to 3 yr later. The remaining 4 VHL patients had not returned for follow-up evaluations at the time of study analysis.

Based on the gender-specific cutoff limits for healthy children, both sensitivity and specificity for the diagnosis of pheochromocytoma were similar or higher for measurements of

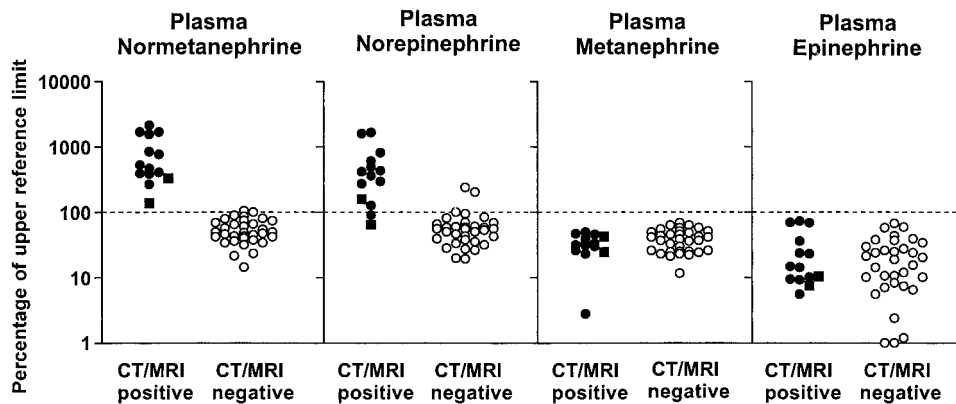


FIG. 2. Plasma concentrations of free metanephrines (normetanephrine and metanephrine) and catecholamines (norepinephrine and epinephrine) in pediatric patients evaluated for pheochromocytoma ($n = 45$). Symbols indicate individual patients. Data were normalized using the gender-specific pediatric reference ranges and expressed as percentage of the corresponding upper reference limit. Pheochromocytoma confirmed (●) histologically or by presence of metastases. Pheochromocytoma suspected (■) based on positive biochemical test and positive CT and/or MRI. Pheochromocytoma excluded (○) on the basis of negative imaging studies or surgical pathology. Horizontal dotted lines represent upper reference limit for each test.

TABLE 2. Characteristics of biochemical tests for the detection of pheochromocytoma in children

Biochemical test	Sensitivity Percentage (number/total number)	Specificity Percentage (number/total number)
Plasma normetanephrine and metanephrine	100 (12/12)	94 (31/33)
Plasma norepinephrine and epinephrine	92 (11/12)	91 (30/33)
Urinary normetanephrine and metanephrine	100 (5/5)	95 (21/22)
Urinary norepinephrine and epinephrine	100 (10/10)	83 (25/30)

plasma metanephrines than for plasma catecholamines, urinary metanephrines, or urinary catecholamines (Table 2). In the patients with pheochromocytoma, the extent of elevations (mean \pm SD) above the upper reference limits were $831 \pm 659\%$ for plasma NMN, $498 \pm 519\%$ for plasma NE, $528 \pm 448\%$ for urinary NMN and $676 \pm 615\%$ for urinary NE.

Six children with confirmed and one child with suspected pheochromocytoma were evaluated more than once over several years at our institute. Plasma concentrations of NMN and NE in these patients showed near continuous increases in plasma NE and more pronounced and consistent increases in plasma NMN with time (Fig. 3). Two children (siblings) developed rapidly growing tumors, both with a concomitant sharp rise in plasma NMN.

When the newly established pediatric reference ranges were applied, two patients with pheochromocytoma were retrospectively found to have had elevated plasma NMN concentrations (one also with elevated plasma NE levels) more than 1 yr before the initial diagnosis was made according to reference ranges in place at the time in adults (Fig. 3).

Discussion

Our study provides unique data on pediatric reference ranges for plasma concentrations of free metanephrines and

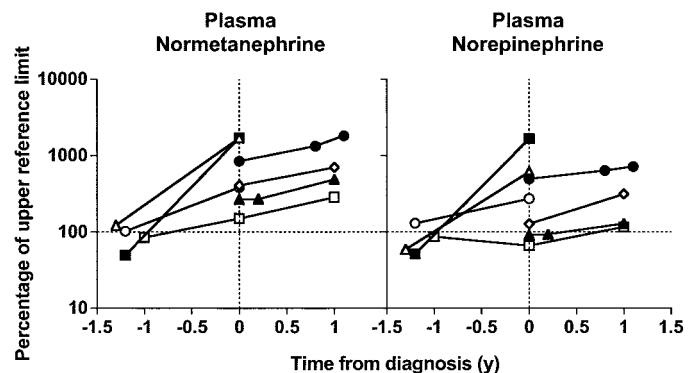


FIG. 3. Time-dependent changes in plasma concentrations of NMN and NE in 6 children with confirmed pheochromocytoma and one child with suspected (□) pheochromocytoma. The time-line (x-axis) is drawn with respect to the date pheochromocytoma was first diagnosed based on upper reference limits available at the time that had been established in adults, combined with positive imaging studies. Data were normalized using the gender-specific pediatric reference ranges and expressed as percentage of the corresponding upper reference limit determined from the newly established pediatric reference population. Data include the two children in whom use of pediatric reference ranges for plasma free normetanephrine indicated the tumor a year earlier than indicated using adult reference ranges (○ △). Patient with metastatic pheochromocytoma (●); siblings with rapidly growing tumors (■ △); horizontal dotted lines represent upper reference limit for each test.

catecholamines and their use in diagnosis of childhood pheochromocytoma. The study shows that measurement of plasma free metanephrines provides a sensitive test for diagnosis of childhood pheochromocytoma, but that age- and gender-appropriate reference ranges should be used to ensure accurate and timely detection of these tumors.

Interpretation of data in the few previous reports on plasma catecholamines in healthy children was hampered by small numbers of subjects, use of hospitalized patients, or lack of standardized test conditions (18–20). In our study of a large number of carefully evaluated normal children, blood samples were collected under conditions controlled to avoid

influences associated with the stress of venipuncture and physical activity. The data show that plasma concentrations of the adrenomedullary hormone E and its metabolite MN were higher in boys than in girls, a finding in accordance with published data in adults (21, 22). Furthermore, the data show higher plasma concentrations and associated upper reference limits of both E and MN in children than in adults. Estrogen has been shown to suppress epinephrine secretion *in vitro* (23) and *in vivo* (24, 25). Such effects of E2 on adrenomedullary function and differences in E2 levels among males and females and with advancing age might therefore contribute to the observed gender and age-associated differences in plasma E and MN.

Although plasma concentrations of NE and its metabolite NMN did not differ significantly between the pediatric and adult reference populations, reference ranges were wider in adults than in children, presumably reflecting greater variability in sympathetic outflow in adults than in children. Adiposity is associated with increased sympathetic outflow (26). Increased prevalence of obesity in adults (27, 28) may therefore partially explain the observed high variability of plasma NE and NMN levels in the adult population.

The data indicate that for diagnostic purposes, normal ranges for plasma catecholamines and metanephrines observed in adults are not applicable to children. Upper reference limits for E and MN were higher and those for NE and NMN were lower in the pediatric than in the adult reference population.

The importance of using age-appropriate reference ranges for detection of childhood pheochromocytoma is illustrated by the finding that two children with pheochromocytoma had normal plasma concentrations of NMN using the reference ranges established for adults, but elevated plasma levels using the reference ranges established for children. Because pediatric reference ranges were not available at the time that these two children were first evaluated, diagnosis of the tumor in both cases was delayed by a year or more, at which time plasma levels of NMN were more dramatically increased.

As demonstrated by the two patients with rapidly developing tumors, early diagnosis of pheochromocytoma may be particularly important in children. The finding of inoperable and terminal metastatic disease at the time of initial diagnosis in one of the three symptomatic cases of childhood pheochromocytoma further illustrates the importance of early diagnosis and the need for periodic screening in children with a familial history of the tumor. Such periodic screening should be carried out regardless of the presence of signs or symptoms.

Based on the age-appropriate and gender-specific upper reference limits, values of sensitivity and specificity for measurements of plasma free metanephrines to detect pheochromocytoma were higher than those for measurements of plasma catecholamines. Plasma free metanephrines were elevated in all 12 confirmed and in 2 highly suspicious cases of pheochromocytoma, whereas measurements of plasma catecholamines missed one confirmed and one suspected case of pheochromocytoma.

Although the sensitivity and specificity of tests of urinary fractionated metanephrines and the sensitivity of tests of

urinary catecholamines appeared equally high as those for tests of plasma free metanephrines, these comparisons were limited by the incomplete nature of the 24 h urine collections in our patient population. Regardless of the above limitations, use of plasma free metanephrines to diagnose childhood pheochromocytoma has the advantage over 24-h urine tests that concerns about completeness of sample collections can be eliminated. Although the required blood sample may be more time consuming for clinical staff to collect than a urine sample, the trade-off is a test result that can be more comfortably relied on by the clinician responsible for the child.

In summary, plasma-free metanephrines provide a valuable test for diagnosis of pheochromocytoma in children. Age-appropriate reference ranges should be used and gender differences should be considered.

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