Hypertension Research (2018) 41:165–175
https://doi.org/10.1038/s41440-017-0002-4

ARTICLE

Evaluation of quantitative parameters for distinguishing pheochromocytoma from other adrenal tumors

Youichi Ohno[1] · Masakatsu Sone[1] · Daisuke Taura[1] · Toshinari Yamasaki[2] · Katsutoshi Kojima[1] · Kyoko Honda-Kohmo[1] · Yorihide Fukuda[1] · Koji Matsuo[1] · Toshihito Fujii[1] · Akihiro Yasoda[1] · Osamu Ogawa[2] · Nobuya Inagaki[1]

Received: 10 February 2017 / Revised: 19 July 2017 / Accepted: 23 July 2017 / Published online: 18 January 2018

Abstract

Adrenal tumors are increasingly found incidentally during imaging examinations. It is important to distinguish pheochromocytomas from other adrenal tumors because of the risk of hypertensive crisis. Although catecholamines and their metabolites are generally used to diagnose pheochromocytoma, false-positive test results are common. An effective screening method to distinguish pheochromocytoma from adrenal incidentalomas is needed. We analyzed 297 consecutive patients with adrenal incidentalomas. Our findings included 162 non-functioning tumors, 47 aldosterone-producing adenomas, 26 metastases, 22 cases of subclinical Cushing's syndrome, 21 pheochromocytomas, 12 cases of Cushing's syndrome, and 7 adrenocortical cancers. We checked quantitative parameters such as age, blood, and urine catecholamines and their metabolites, neuron-specific enolase, size and computed tomography (CT) attenuation values. Among catecholamine-related parameters, the sum of urine metanephrine and normetanephrine (urineMNM) levels produced the highest area under the receiver operating characteristic curve regarding discrimination of pheochromocytoma from other lesions. Size and CT attenuation values also differed significantly. However, size was correlated with catecholamine levels. CT attenuation was not correlated with other factors. The optimal thresholds were 19 Hounsfield units (HU) for CT attenuation (sensitivity, 100%; specificity, 60%) and 0.43 mg/24 h for urineMNM (sensitivity, 89%; specificity, 96%). No pheochromocytomas were evident when CT attenuation values were under 19 HU. Even in adrenal tumors with CT attenuation values ≥ 19 HU, when urineMNM was < 0.43 mg/24 h, the frequency of pheochromocytoma was only 4.3%, when urineMNM was ≥ 0.43 mg/24 h, the frequency of pheochromocytoma was 93% and when urineMNM was > 0.77 mg/24 h the frequency of pheochromocytoma was 100%. CT attenuation value and urineMNM represented the most useful combination for diagnosis of pheochromocytoma.

Introduction

Because of advances in medical imaging, incidental findings of adrenal tumors are increasingly common. These tumors are termed adrenal incidentalomas. In previous studies, adrenal incidentalomas have been reported in 0.4–4.4% of patients based on abdominal computed tomography (CT) [1–3]. According to numerous autopsy studies, the frequency of adrenal gland tumors is approximately 6% [4]. Among adrenal incidentalomas, the frequency of pheochromocytoma is nearly 8% [5]. According to research on Measures for Intractable Diseases from the Ministry of Health, Labour and Welfare in Japan, the frequency of pheochromocytoma is 8.5%.

Pheochromocytoma is a challenging disorder because contrast media, drugs (steroid hormones, β-blockers, metoclopramide and others), biopsy and surgery are associated with the risk of hypertensive crises, which pose an immediate threat to life [6, 7]. Therefore, whether an adrenal incidentaloma is a pheochromocytoma or not should be determined. In the present study, we focused on methods that could be used to easily and efficiently exclude pheochromocytomas from other adrenal tumors. We analyzed various quantitative parameters that could be used to

✉ Masakatsu Sone
sonemasa@kuhp.kyoto-u.ac.jp

[1] Department of Diabetes, Endocrinology and Nutrition, Kyoto University Graduate School of Medicine, Kyoto, Japan

[2] Department of Urology, Kyoto University Graduate School of Medicine, Kyoto, Japan

distinguish pheochromocytomas from other adrenal tumors in 297 patients with adrenal incidentalomas.

Materials and methods

Study population

This was a case-control study. A total of 402 consecutive patients with adrenal incidentalomas who were admitted to Kyoto University Hospital in Kyoto City, Japan, from 1 April 2005 to 31 March 2015 were investigated. We excluded those patients with recurrent pheochromocytoma (20), bilateral hyperplasia or multiple adrenal adenomas (59), or neonates (1) and those without endocrinology blood test results (25). As a result, 297 patients were analyzed. These patients had lesions consisting of 21 pheochromocytomas (8 in men) and 276 other types (162 non-functioning adrenal tumors (NFTs), 47 aldosterone-producing adenomas (APAs), 26 metastases, 22 cases of subclinical *Cushing's* syndrome (SCS), 12 cases of *Cushing's* syndrome (CS), and 7 adrenocortical cancers (ACCs)).

Measurements

We compiled the following quantitative parameters at the time of first admission: age, blood adrenaline (bloodA), blood noradrenaline (bloodNA), blood dopamine (bloodD), neuron-specific enolase (NSE), urine adrenaline (urineA), urine noradrenaline (urineNA), urine dopamine (urineD), urine vanillylmandelic acid (urineVMA), urine metanephrine (urineM), urine normetanephrine (urineNM), size and CT attenuation value (Hounsfield units).

Blood samples were collected between 8:00 a.m. and 9:00 a.m. after 30 min of supine rest. BloodA, bloodNA, and bloodD were measured using high-performance liquid chromatography (HPLC) with an HLC-725CAII (Tosoh) using eluent AII, eluent BII, eluent CII, reaction reagent E, and reaction reagent D (LSI Company). A TSKgel Catechol column setII was used. NSE was measured by electrochemiluminescence immunoassay using a Roche Cobas e 602 auto-analyzer with streptavidin magnetic micro particles, biotinylated anti-NSE mouse monoclonal antibody and dichlorotris(2,2′-bipyridyl)ruthenium(II)-labeled anti-NSE mouse monoclonal antibodies.

Twenty-four-hour urine samples were collected in vessels containing 20 ml of 6 N hydrochloric acid. The pH in the vessels was maintained at < 3. UrineA, urineNA, and urineD were measured using HPLC with an HLC-725CAII (Tosoh) with catecholamine reference solution A from LSI Company. A TSKgel Catechol column setII was used. UrineVMA was measured by HPLC coupled with electrochemical detection using a private adjustment reagent from LSI Company. A CAPCELL PAK CR 1:50 4.6 mm × 150 mm column was used. UrineM and urineNM were measured by HPLC using a column-switching trihydroxyindole post-label method with a private adjustment reagent (BML Company). A Hitachi #3013-C 4 φ × 150 mm packed column and a Hitachi #3011-C 4 φ × 35 mm special column were used.

Intra-assay coefficients of variation (CVs) were 0.76–4.08% for bloodA, 0.6–9.34% for bloodNA, 0.99–8.96% for bloodD, < 10% for NSE, 5.14–6.21% for urineA, 2.77–4.09% for urineNA, 4.18–5.32% for urineD, 1.22–1.35% for urineM, 1.38–1.61% for urineNM and 1.22–1.56% for urineVMA. Inter-assay CVs were 2.23% for bloodA, 2.27% for bloodNA, 2.89% for bloodD, < 20% for NSE, 6.35% for urineA, 3.82% for urineNA, 4.46% for urineD, 1.76–2.27% for urineM, 2.07–4.45% for urineNM and 3.2% for urineVMA. NSE levels were checked except in cases of metastasis. This was because metastases are known to exhibit high serum NSE levels.

The CT attenuation value was determined based on non-contrast enhanced CT examination. The tumor axis was measured in a horizontal cross-section using CT or magnetic resonance imaging. The CT attenuation value was measured using a circle or ovoid region of interest (ROI) cursor. Tumor centers were determined using 1-mm or 5-mm slice CT sections. CT attenuation values were checked using the ROI cursor to cover two-thirds of the tumor centers. Areas of calcification were avoided during measurements. Instead, a circle or ovoid ROI was placed below the areas of calcification, and the CT attenuation values were checked as previously described.

Statistical analyses

Stata/SE ver 14 software developed by LightStone® was used for statistical analyses and for plotting the receiver operating characteristic (ROC) curve. Because of skewed distribution, results were expressed as medians and interquartile ranges. We chose parameters that showed statistically significant differences using the Mann–Whitney *U* test. P values of < 0.05 were considered to indicate significant differences. We checked correlations between these parameters using Spearman's rank correlation test and defined statistically significant correlations as those with $R > 0.4$ and P values < 0.05. Odds ratios and 95% confidence intervals were estimated using binary logistic regression analysis. The area under the ROC curve was calculated accordingly. The optimal ROC curve cutoff point was determined using the maximum Youden index. The Youden index was calculated by subtracting 1 from the sum of the sensitivity and specificity.

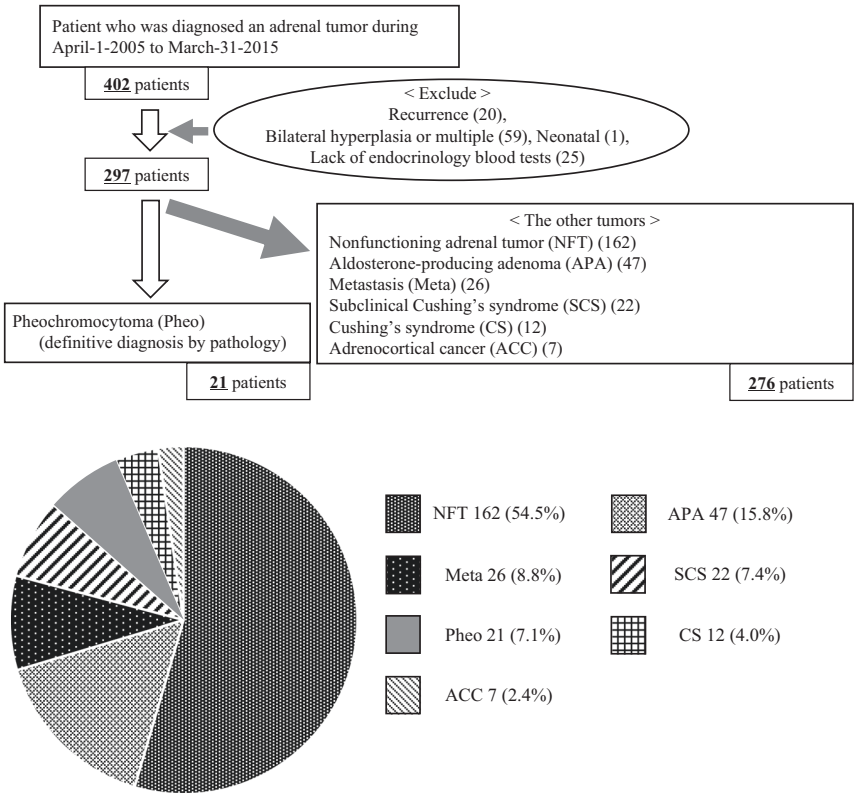

Fig. 1 A total of 402 patients were included in this study. Exclusions: recurrence (20), bilateral hyperplasia or multiple tumors (59), neonates (1), and lack of endocrinology blood tests (25). Finally, 297 patients were enrolled. The clinical characteristics of these patients are as follows: nonfunctioning adrenal tumors (NFTs) (162), aldosterone-producing adenomas (APAs) (47), metastases (Meta) (26), subclinical Cushing's syndrome (SCS) (22), pheochromocytomas (Pheos) (21), Cushing's syndrome (CS) (12), and adrenocortical cancers (ACCs) (7)

Definitions

For the diagnosis of adrenal tumors, primary aldosteronism (PA) was suspected based on the aldosterone-to-renin ratio (ARR; the ratio of plasma aldosterone concentration [PAC] to plasma renin activity [PRA]). PA was screened if ARR was > 200 pg/ml per ng/ml/h. When positive ARR values were detected, captopril-challenge, upright furosemide-loading and/or saline-loading tests were performed to confirm the diagnosis of PA. When the patient tested positive for PA, adrenal venous sampling was conducted to confirm the diagnosis of APA in accordance with Japan Endocrine Society guidelines [8]. The diagnosis of APA in patients who had been operated on was then confirmed pathologically as adrenocortical adenoma and confirmed clinically by subsequent normalization of aldosterone secretion. CS and SCS were diagnosed by high cortisol levels in blood samples collected late at night, the presence of an adrenal lesion, and the lack of suppression following dexamethasone (1 mg and 8 mg) suppression tests [9, 10]. The diagnoses of CS and SCS in patients who had been operated on were then confirmed pathologically as adrenocortical adenoma and confirmed clinically by cessation of autonomous cortisol secretion. Pheochromocytoma was diagnosed clinically by imaging modalities (CT, magnetic resonance imaging, MIBG and fluorodeoxyglucose-positron emission tomography) and laboratory tests (blood and urine catecholamines and their metabolites). All patients with pheochromocytoma underwent surgical excision and confirmation by pathologic examination. ACC was pathologically diagnosed using the Weiss criteria. Metastases were diagnosed by clinical course or pathology.

Results

Of the 402 patients enrolled in this study, 297 were analyzed. Tumors in these patients included 162 NFTs, 47 APAs, 26 metastases, 22 SCSs, 21 pheochromocytomas, 12 CSs, and 7 ACCs. The patients were divided into two groups, one with pheochromocytoma (7.1% of patients) and the other with tumors other than pheochromocytoma (Fig. 1).

The medians, interquartile ranges and *P* values were calculated using the Mann–Whitney *U* test for quantitative parameters (Table 1; Fig. 2). Diagnostic power for discrimination between pheochromocytoma and other adrenal tumors was analyzed using the area under the ROC curve. The sums of bloodA and bloodNA (bloodANA), urineA and urineNA (urineANA) and urineM and urineNM (urineMNM) each had superior diagnostic power compared to their respective components alone. Therefore, we added bloodANA, urineANA and urineMNM to the parameters for analysis.

Table 1 Quantitative parameters in pheochromocytoma and the others

Parameters	Pheochromocytoma ($n = 21$) Median (Interquartile range)	Others ($n = 276$) Median (Interquartile range)	P value
Age (year)	52 (45–66)	61 (53–69)	0.243
SBP (mmHg)	121 (109–134)	127 (115–139)	0.141
DBP (mmHg)	71 (67–83)	78 (69–85)	0.155
Antihypertensive drugs (%)	85.7	52.9	0.004*
BloodA (pg/ml)	32 (19–187)	21 (13–33)	0.001*
BloodNA (pg/ml)	410 (285–1985)	234 (159–333)	<0.001*
BloodANA (pg/ml)	596 (421–2017)	258 (179–365)	<0.001*
BloodD (pg/ml)	10 (5–16)	9 (5–14)	0.295
UrineA (μg/24 h)	9.7 (8.3–83.2)	6.3 (4.1–12)	0.001*
UrineNA (μg/24 h)	279 (189–928)	106 (77–147)	<0.001*
UrineANA (μg/24 h)	377.1 (213.8–937.1)	113 (83–163)	<0.001*
UrineD (μg/24 h)	862.7 (536.8–1381)	672 (503–892)	0.056
UrineVMA (mg/24 h)	10.1 (5.6–16.3)	3.3 (2.6–4.1)	<0.001*
UrineM (mg/24 h)	0.19 (0.06–1.3)	0.06 (0.04–0.08)	0.001*
UrineNM (mg/24 h)	1.3 (0.57–2.1)	0.13 (0.09–0.18)	<0.001*
UrineMNM (mg/24 h)	2.01 (0.77–4.8)	0.19 (0.13–0.27)	<0.001*
Size (cm)	4.2 (2.7–5.8)	2 (1.5–2.7)	<0.001*
CT attenuation value (HU)	36.5 (31.5–43)	15 (1–29)	<0.001*
NSE (ng/ml)	9.2 (8.6–13.0)	10.1 (8.9–12.1)	0.443

Values represent medians (first interquartile to third interquartile)

BloodA blood adrenaline, *BloodANA* summation of bloodA and bloodNA, *Blood D* blood dopamine, *BloodNA* blood noradrenaline, *CT* computed tomography, *DBP* diastolic blood pressure, *HU* Hounsfield unit, *NSE* neuron specific enolase, *SBP* systolic blood pressure, *UrineA* urine adrenaline, *UrineANA* summation of urineA and urineNA, *Urine D* urine dopamine, *UrineM* urine metanephrine, *UrineMNM* summation of urineM and urineNM, *UrineNA* urine noradrenaline, *UrineNM* urine normetanephrine, *Urine VMA* urine vanillylmandelic acid

* Significant difference (i.e., $P < 0.05$; per Mann–Whitney U test or Pearson's χ^2 test)

Significant differences were observed regarding bloodA, bloodNA, bloodANA, urineA, urineNA, urineANA, urineM, urineNM, urineMNM, urineVMA, tumor size, and CT attenuation values. We evaluated the correlations among these 12 parameters using Spearman's rank correlation coefficient test with respect to pheochromocytoma (Table 2). All parameters except for the CT attenuation value were significantly correlated with each other (correlation coefficient $r > 0.4$; $P < 0.05$). Because the CT attenuation value was independent of other parameters, we proposed it as one of the candidates for distinguishing pheochromocytoma from other tumors. Because the other parameters were correlated with one another, we considered which of them was the best to be combined with the CT attenuation value for diagnosis of pheochromocytoma. We plotted ROC curves for each of the parameters to distinguish pheochromocytoma from the other tumors (Fig. 3). The parameter exhibiting the greatest area under the ROC curve (0.9794) was urineMNM. We therefore considered that urineMNM would be the best parameter to be combined with CT attenuation for the diagnosis of pheochromocytoma.

We performed binary logistic regression analysis using urineMNM and the CT attenuation value. UrineMNM and CT attenuation were both significantly useful for diagnosis of pheochromocytoma. The log odds ratio was 10 for diagnosis using urineMNM and 0.19 for diagnosis using CT attenuation value. The respective 95% confidence intervals ranged from 2.2 to 19 for urineMNM and from 0.017 to 0.36 for CT attenuation value. We calculated the optimal thresholds of these parameters using the ROC curve. These thresholds were determined using the maximum Youden index. The optimal threshold value for urineMNM of 0.43 mg/24 h suggested a sensitivity of 89% and a specificity of 96% with a positive likelihood ratio of 22 and a negative likelihood ratio of 0.11. The optimal CT attenuation value threshold of 19 HU suggested high sensitivity (100%) and low specificity (60%) with a positive likelihood ratio of 2.5 and a negative likelihood ratio of 0. Even cystic pheochromocytoma cases had CT attenuation values of not less

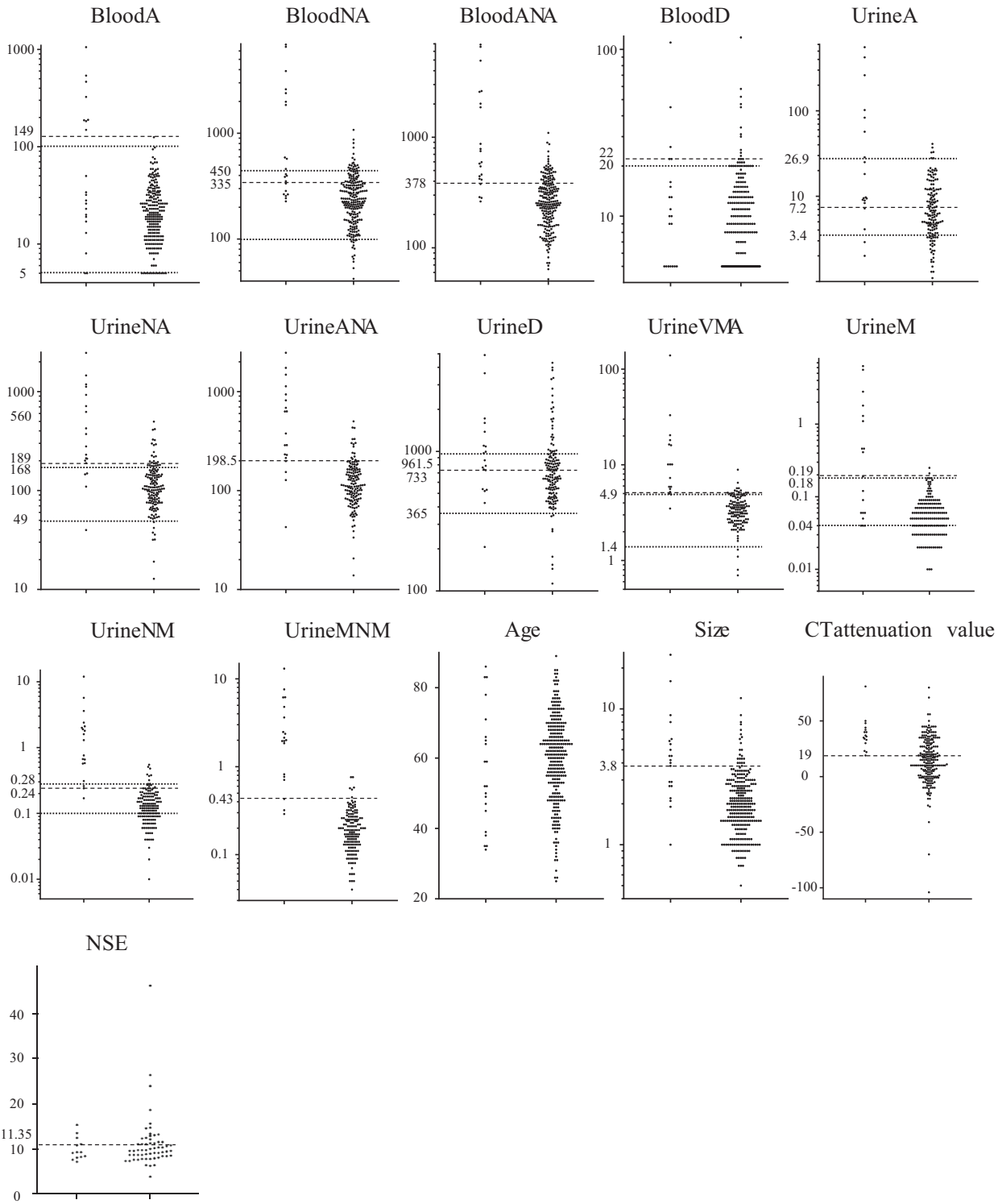

Fig. 2 Frequency distribution of parameters between pheochromocytoma (left points) and other tumors (right points). The dotted line indicates the upper and lower limits of normal values. The dashed line indicates the threshold for diagnosing pheochromocytoma calculated using the receiver operating characteristic (ROC) curve in Fig. 3. The optimal cutoff point of the ROC curve was determined using the maximum Youden index. *Abbreviations*: bloodA: blood adrenaline, bloodANA: sum of bloodA and bloodNA levels, blood D: blood dopamine, bloodNA: blood noradrenaline, CT: computed tomography, NSE: neuron specific enolase, urineA: urine adrenaline, urineANA: sum of urineA and urineNA levels, urine D: urine dopamine, urineM: urine metanephrine, urineMNM: sum of urineM and urineNM levels, urineNA: urine noradrenaline, urineNM: urine normetanephrine, urine VMA: urine vanillylmandelic acid

Table 2 Correlations of quantitative parameters in pheochromocytoma

Parameters	Blood A	Blood NA	Blood ANA	Urine A	Urine NA	Urine ANA	Urine M	Urine NM	Urine MNM	Urine VMA	CT attenuation value	Size
BloodA	1*	–	–	–	–	–	–	–	–	–	–	–
BloodNA	−0.37	1*	–	–	–	–	–	–	–	–	–	–
BloodANA	−0.07	0.86*	1*	–	–	–	–	–	–	–	–	–
UrineA	0.94*	−0.19	0.08	1*	–	–	–	–	–	–	–	–
UrineNA	−0.34	0.68*	0.70*	−0.09	1*	–	–	–	–	–	–	–
UrineANA	0.08	0.48	0.72*	0.31	0.85*	1*	–	–	–	–	–	–
UrineM	0.96*	−0.44	−0.15	0.89*	−0.43	−0.06	1*	–	–	–	–	–
UrineNM	−0.27	0.78*	0.49	−0.06	0.39	0.18	−0.30	1*	–	–	–	–
UrineMNM	0.59	0.35	0.51	0.76*	0.08	0.37	0.55	0.49	1*	–	–	–
UrineVMA	0.53	0.27	0.40	0.65*	0.07	0.35	0.47	0.38	0.87*	1*	–	–
CT attenuation value	−0.57	0.21	0.17	−0.56	0.33	0.13	−0.57	−0.17	−0.49	−0.47	1*	–
Size	0.44	0.33	0.39	0.50	0.05	0.11	0.51	0.46	0.72*	0.71*	−0.53	1*

BloodA blood adrenaline, *BloodANA* summation of bloodA and bloodNA, *BloodNA* blood noradrenaline, *CT* computed tomography, *NSE* neuron specific enolase, *UrineA* urine adrenaline, *UrineANA* summation of urineA and urineNA, *UrineM* urine metanephrine, *UrineMNM* summation of urineM and urineNM, *UrineNA* urine noradrenaline, *UrineNM* urine normetanephrine, *Urine VMA* urine vanillylmandelic acid

* Significant correlation (correlation coefficient $r > 0.4$ and $P < 0.05$; Spearman's rank correlation coefficients)

than 19 HU. After adrenalectomy, histologic proof of a cyst was obtained from 8 pheochromocytoma patients. Six of the 8 patients underwent non-contrast enhanced CT examination with resulting CT attenuation values of 19, 22, 23, 34, 40, and 44 HU (median 28.5 HU).

Figure 4 shows a scatter plot of urineMNM and CT attenuation values as well as the flowchart used to diagnose pheochromocytoma. When the CT attenuation value for an adrenal incidentaloma was < 19 HU, a pheochromocytoma was unlikely. Even adrenal tumors with CT attenuation values ≥ 19 HU and urineMNM < 0.43 mg/24 h were unlikely to be pheochromocytomas (the frequency was approximately 4.3%). In this population, we found only two patients with pheochromocytoma—a 45-year-old woman and a 64-year-old man. Their respective clinicopathological factors included tumor sizes of 2.2 cm and 2.9 cm, CT attenuation values of 50 HU and 33 HU, urineA values of 2.9 μg/24 h and 8.3 μg/24 h, urineNA values of 39.9 μg/24 h and 145.8 μg/24 h, urineD values of 206.9 μg/24 h and 971.2 μg/24 h, urineM values of 0.06 mg/24 h and 0.12 mg/24 h, urineNM values of 0.26 mg/24 h and 0.17 mg/24 h, urineMNM values of 0.32 mg/24 h and 0.29 mg/24 h, bloodA values of 26 pg/ml and 20 pg/ml, bloodNA values of 352 pg/ml and 243 pg/ml, blood values of 11 pg/ml and 5 pg/ml, urineVMA values of no data and 5.9 mg/24 h and NSE values of 8.14 ng/ml and 14.16 ng/ml. The female patient exhibited no symptoms, and the male patient only had slightly higher than normal urineVMA and NSE levels. However, with urineMNM values ≥ 0.43 mg/24 h and CT attenuation ≥ 19 HU, the tumor was very likely to be a pheochromocytoma (frequency in this case: ~93%). With urineMNM values > 0.77 mg/24 h, the tumor probably would be a pheochromocytoma (frequency: 100%).

We sub-analyzed only the cases in which diagnoses were confirmed by pathology and improvement of clinical symptoms and laboratory data after adrenalectomy. These cases included 21 pheochromocytomas and 75 other tumor types (41 APAs, 11 CSs, 8 SCSs, 6 NFTs, 3 metastases, and 6 ACCs). Cases in which tumors other than pheochromocytoma were surgically removed and pathologically confirmed are shown as closed squares in Fig. 4. The median urineMNM value in these cases was 0.16 mg/24 h (interquartile range, 0.11–0.22 mg/24 h), and the median CT attenuation value was 13 HU (interquartile range, 3–28 HU). In these two groups, we calculated optimal thresholds for these parameters using ROC curves. These thresholds were determined using the maximum Youden index. The optimal threshold for urineMNM of 0.29 mg/24 h suggested a sensitivity of 100% and a specificity of 92%, with a positive likelihood ratio of 12 and a negative likelihood ratio of 0. The optimal CT attenuation value threshold of 19 HU suggested very high sensitivity (100%) and low specificity (63%), with a positive likelihood ratio of 2.7 and a negative likelihood ratio of 0.

Discussion

In previous studies, blood and urine catecholamine levels (especially metanephrine and normetanephrine) were useful in diagnosing pheochromocytoma [11–14]. It was reported

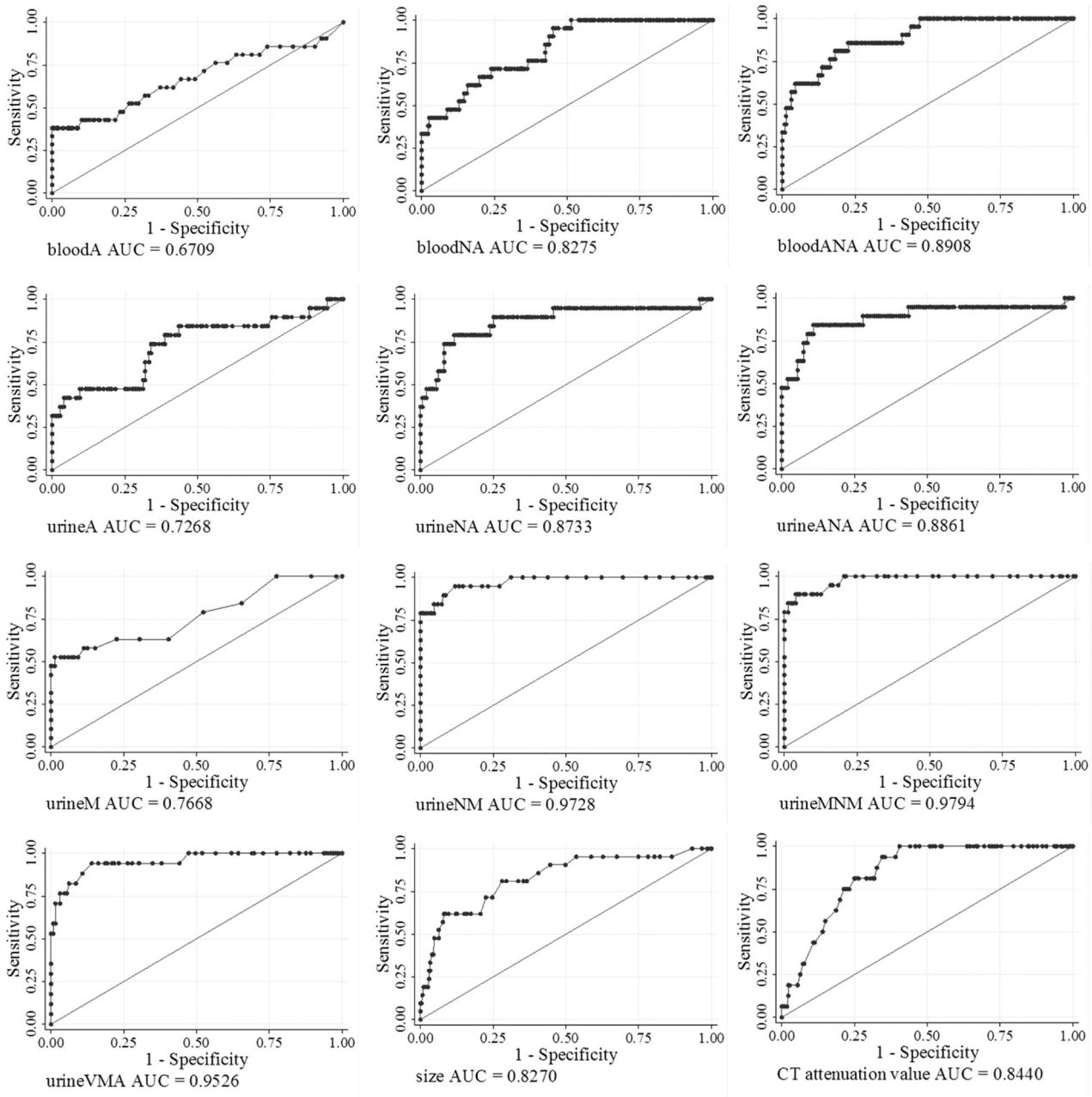

Fig. 3 Receiver operating characteristics (ROC) curves for parameters used to distinguish pheochromocytoma and other tumor types. *Abbreviations*: BloodA: blood adrenaline, bloodANA: sum of bloodA and bloodNA levels, bloodNA: blood noradrenaline, CT: computed tomography, HU: Hounsfield unit, urineA: urine adrenaline, urineANA: sum of urineA and urineNA levels, urineM: urine metanephrine, urineMNM: sum of urineM and urineNM levels, urineNA: urine noradrenaline, urineNM: urine normetanephrine, urine VMA: urine vanillylmandelic acid

that combined urine metanephrine and normetanephrine (urineMNM) levels were useful in the diagnosis of pheochromocytoma, with threshold values between 0.5 and 1 mg/24 h [12, 15, 16]. Additionally, in this study, urineMNM had the highest area under the ROC curve for diagnosis of pheochromocytoma relative to other catecholamine-related parameters. In this study, the optimal screening threshold was 0.43 mg/24 h, and the diagnostic threshold was 0.77 mg/24 h (Figs. 2–4). Tumor size on CT and the CT attenuation values were also useful. Tumor size has been particularly useful because pheochromocytomas tend to be larger than other benign adrenal tumors [17–22]. Adrenal tumor size is an important parameter for distinguishing between malignant and benign lesions. Furthermore, in our study, size differed significantly between pheochromocytomas and other adrenal lesions. We

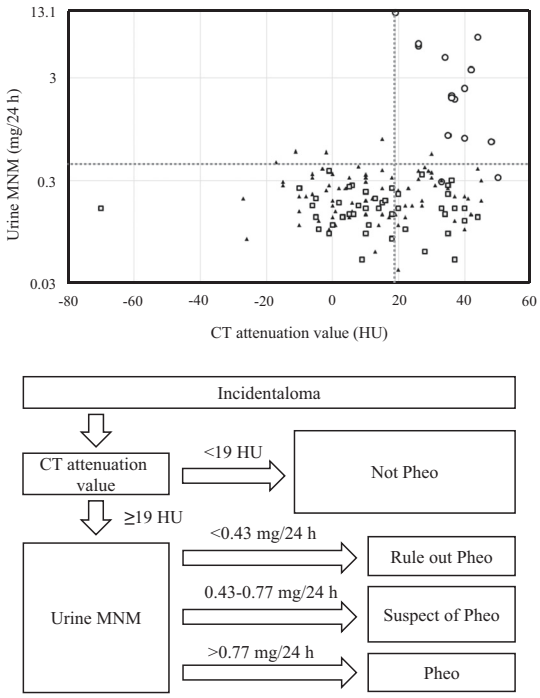

Fig. 4 Scatter plot of urineMNM and CT attenuation value and the flowchart used for diagnosis of pheochromocytoma. Open circles represent patients with pheochromocytomas. Closed triangles represent patients with other adrenal tumors who were not diagnosed by pathology. Closed squares represent patients with other adrenal tumors who underwent surgery and pathological confirmation. Patients with missing CT attenuation or urineMNM data are not represented in the figure. The dotted lines represent the maximum Youden index values (urineMNM, 0.43 mg/24 h; CT attenuation value, 19 HU). The optimal urineMNM threshold of 0.43 mg/24 h suggested that it was sensitive (89%) and specific (96%). The positive likelihood ratio was 22 and the negative likelihood ratio was 0.11. The optimal CT attenuation value threshold of 19 HU suggested high sensitivity (100%) and low specificity (60%). The positive likelihood ratio was 2.5 and the negative likelihood ratio was 0. *Abbreviations*: CT: computed tomography, HU: Hounsfield unit, Pheo: pheochromocytoma, urineMNM: sum of urine metanephrine and normetanephrine

calculated the optimal cutoff point of the ROC curve for size using the maximum Youden index (Fig. 3). The optimal threshold value for size was 3.8 cm, which implied low sensitivity (61.9%) and high specificity (91.9%). At this size, the positive likelihood ratio was 7.6, and the likelihood of pheochromocytoma was high for adrenal tumors ≥ 3.8 cm. However, as the tumor enlarged, the risk of other malignant lesions such as adrenocortical carcinomas and metastases also increased [23]. Therefore, urineMNM would have to remain as part of the differential diagnosis for pheochromocytoma. On the other hand, because the negative likelihood ratio was 0.42, small size alone was not sufficient to exclude pheochromocytoma. Several studies have reported that size and catecholamine level combined were useful diagnostic factors [24, 25]. However, our study revealed that catecholamine level and size were strongly correlated with each other. Our study is the first to report the combination of CT attenuation value and urineMNM as the most effective marker for initial screening of pheochromocytoma among adrenal incidentalomas.

CT attenuation value has also been used to exclude malignant lesions (pheochromocytoma, ACC and metastases). European clinical practice guidelines recommend 10 HU as the cutoff value to differentiate benign adenoma and malignant lesions [26]. In our study, the optimal cutoff CT attenuation value for diagnosing pheochromocytoma was 19 HU, although this was specifically calculated for differentiating pheochromocytoma from other lesions. The difference in cutoff values apparently reflects the different objectives. Although the European guidelines use HU ≤ 10 to separate benign and malignant lesions, they also note that approximately 30% of benign adenomas have attenuation values of > 10 HU, whereas 7% of adrenal metastases have tumor densities ≤ 10 HU, thus indicating some overlap [26]. In the meta-analysis reported by Boland et al. which was a reference for the European guideline, the CT attenuation value cutoff for distinguishing between malignant and benign adrenal lesions was widely a distributed range of 0–18 HU [27]. However, the study of Hamrahian et al. which focused on pheochromocytoma, reported that of 61 pheochromocytoma patients, none had an HU lower than 22 [21]. Furthermore, the cutoff attenuation value for pheochromocytoma recommended by UpToDate® is > 20 HU [28]. We therefore consider the result in our study to be consistent with previous reports. However, the sensitivity of the cutoff threshold of 19 HU might not reach 100% when tested in a large-scale study. A large-scale multi-center prospective analysis of adrenal incidentalomas is needed to validate the appropriate CT attenuation value cutoff to precisely exclude pheochromocytoma. Pheochromocytoma has a higher CT attenuation value than other adrenal tumors because the adrenal cortex and benign adrenal gland tumors contain intracytoplasmic lipids [16]. Even cystic pheochromocytoma cases had CT attenuation values of not less than 19 HU, probably because cystic pheochromocytoma formation might be due to intralesional hemorrhage, necrosis and subsequent cyst formation [29]. Additionally, we revealed that there was no correlation between CT attenuation values and any parameters concerning catecholamines. Our results showed that urineMNM and CT attenuation values represented the best combination of parameters.

With the widespread use of imaging examinations, adrenal tumors are increasingly found incidentally. Substantial time and expense are needed to check catecholamines and their metabolites in every adrenal incidentaloma. In comparison, measurement of CT attenuation values is very easy and inexpensive, especially if the adrenal

incidentaloma was first detected by CT scanning. The CT attenuation value could serve as the initial discriminator, facilitating selection of patients who should undergo urineMNM measurement (Fig. 4). This criterion could reduce medical expenses and incorrect diagnoses.

Our criterion led to false-negative diagnoses of two patients with urineMNM < 0.43 mg/24 h and CT attenuation values ≥ 19 HU, with no other abnormal quantitative parameters. However, one of these patients presented with hypertensive episodes. The other had no symptoms but had a history of lung and bladder cancers. Use of T2-weighted and diffusion-weighted MRIs showed abnormal hyperintense signals on each adrenal tumor. High ^{123}I MIBG uptake was also revealed by SPECT/CT scans in MIBG scintigraphy. After surgery, these specimens were pathologically confirmed as pheochromocytomas. Therefore, when patients with high CT attenuation values and low catecholamine levels have characteristic symptoms such as hypertensive spells or need precise differential diagnoses, they should be checked using other modalities such as MRI and ^{123}I MIBG scintigraphy despite the low prevalence of pheochromocytoma in this population.

In accordance with these criteria, we excluded recurrent cases. Twelve patients with recurrent disease underwent plane CT scanning and their 24-hour urine metanephrine and normetanephrine levels were assessed. Their median CT attenuation value was 41 HU, with a range of 26–78 HU. While none of the 12 had CT attenuation values below 19 HU, their urineMNM levels tended to be low compared with the initial pheochromocytoma group (median urineMNM: 1.48 mg/24 h; range: 0.23–6.1 mg/24 h). Pheochromocytoma was ruled out in one patient whose urineMNM level was 0.23 mg/24 h but was suspected in 2 other patients (0.68 and 0.74 mg/24 h, respectively). These results may reflect the strong correlation between tumor size and urineMNM, as the recurrences were found in patients with smaller tumors (median: 2.5 cm; range: 1.1–3.4 cm). The results might also reflect malignancy. Malignant pheochromocytomas might produce lower catecholamine levels because of poor differentiation. However, we did not have enough cases to validate appropriate urineMNM and CT attenuation cutoff values for diagnosing recurrent pheochromocytoma.

In the present study, we could not use plasma-free metanephrine and normetanephrine as parameters because they are not covered by medical insurance in Japan. The sensitivity and specificity of plasma free metanephrine and normetanephrine are reported to be superior or similar to urineMNM [11, 14]. Thus, we propose that it may also be useful to combine plasma-free metanephrine and normetanephrine with CT attenuation values in the diagnosis of pheochromocytoma. Values for 24-h urineMNM have been reported to be similar to those for single-voided urine samples corrected by creatinine [30, 31]. Therefore, it is possible that single-voided urine samples may be an acceptable substitute for 24 h urine collection.

In a previous study, the prevalence of adrenal adenomas was reported to increase with age [4]. It has been reported that patients with pheochromocytomas are younger than patients with other adrenal tumors [24]. In our study, patients with adrenal incidentalomas tended to be older than patients with pheochromocytomas. However, there was no significant difference.

NSE, a neuronal form of the glycolytic enzyme enolase, is localized in neuroendocrine tumors (pheochromocytomas, medullary thyroid carcinomas, small cell lung cancers, neuroblastomas and others) [32, 33]. Serum NSE levels were high because of cytolysis in these tumors. Therefore, the presence of metastasis is associated with high serum NSE levels [34]. It has been reported that patients with non-metastatic pheochromocytomas do not show high serum NSE levels relative to normal adults [35] This could be a reason why serum NSE levels were not significantly different between pheochromocytomas and other adrenal tumors in our study.

Our study has several limitations. Although all patients diagnosed as having pheochromocytoma underwent surgery and pathological diagnosis, some patients with other types of adrenal tumors did not undergo surgery and were not pathologically diagnosed. Therefore, we cannot deny the possibility that some pheochromocytomas were misdiagnosed as other types of tumors. It is difficult for clinicians to justify biopsies or operations on all patients with adrenal tumors (especially NFTs). We sub-analyzed only the cases in which diagnoses were confirmed by pathology. The results of operated cases were similar to the results including all patients with adrenal tumors.

Our study was retrospective study and performed in a single center, leading to possible patient selection bias. A multi-center prospective analysis of adrenal incidentalomas is needed to validate appropriate cut-off values for urineMNM and CT attenuation in diagnosing pheochromocytoma.

Conclusions

Our study revealed that the CT attenuation value and urineMNM were independent factors and presented a convenient and useful combination of parameters to distinguish pheochromocytomas from other adrenal tumors. In patients with suspected pheochromocytoma based on the cutoff values of these parameters, further examinations using MRI and MIBG scintigraphy are recommended.

Acknowledgements The authors gratefully acknowledge the support of the nursing, laboratory, and secretarial staff in our department, as well as the medical staff in the Departments of Urology, Radiology, and Pathology at Kyoto University Hospital. This study was also supported by a grant from Ministry of Health, Labor, and Welfare, Japan (Nanbyo-Ippan-046).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Prinz RA, Brooks MH, Churchill R, Graner JL, Lawrence AM, Paloyan E, Sparagana M. Incidental asymptomatic adrenal masses detected by computed tomographic scanning. Is operation required? Jama. 1982;248:701–4.
2. Bovio S, Cataldi A, Reimondo G, Sperone P, Novello S, Berruti A, Borasio P, Fava C, Dogliotti L, Scagliotti GV, Angeli A, Terzolo M. Prevalence of adrenal incidentaloma in a contemporary computerized tomography series. J Endocrinol Invest. 2006;29:298–302.
3. Herrera MF, Grant CS, van Heerden JA, Sheedy PF, Ilstrup DM. Incidentally discovered adrenal tumors: an institutional perspective. Surgery. 1991;110:1014–21.
4. Young WF Jr. Clinical practice. The incidentally discovered adrenal mass. N Engl J Med. 2007;356:601–10.
5. Nawar R, Aron D. Adrenal incidentalomas—a continuing management dilemma. Endocr Relat Cancer. 2005;12:585–98.
6. Wu LT, Dicpinigaitis P, Bruckner H, Manger W, Averbuch S. Hypertensive crises induced by treatment of malignant pheochromocytoma with a combination of cyclophosphamide, vincristine, and dacarbazine. Med Pediatr Oncol. 1994;22:389–92.
7. Vilela-Martin JF, Vaz-de-Melo RO, Kuniyoshi CH, Abdo AN, Yugar-Toledo JC. Hypertensive crisis: clinical-epidemiological profile. Hypertens Res. 2011;34:367–71.
8. Nishikawa T, Omura M, Satoh F, Shibata H, Takahashi K, Tamura N, Tanabe A. Task force committee on primary aldosteronism, The Japan endocrine society. Guidelines for the diagnosis and treatment of primary aldosteronism--the Japan Endocrine Society 2009. Endocr J. 2011;58:711–21.
9. Nawata HDH, Suda T, Takayanagi R. Adrenal preclinical Cushing's syndrome, Annual report of the Ministry of Health and Welfar "Disorder of Adrenal Hormones" Research Committee. Japan 1995;223-6.
10. Nieman LK, Biller BM, Findling JW, Newell-Price J, Savage MO, Stewart PM, Montori VM. The diagnosis of Cushing's syndrome: an endocrine society clinical practice guideline. J Clin Endocrinol Metab. 2008;93:1526–40.
11. Kim HJ, Lee JI, Cho YY, Lee SY, Kim JH, Jung BC, Kim SW, Chung JH, Min YK, Lee MS, Lee MK, Kim JH. Diagnostic accuracy of plasma free metanephrines in a seated position compared with 24-hour urinary metanephrines in the investigation of pheochromocytoma. Endocr J. 2015;62:243–50.
12. d'Herbomez M, Forzy G, Bauters C, Tierny C, Pigny P, Carnaille B, Pattou F, Wemeau JL, Rouaix N. An analysis of the biochemical diagnosis of 66 pheochromocytomas. Eur J Endocrinol. 2007;156:569–75.
13. Unger N, Pitt C, Schmidt IL, Walz MK, Schmid KW, Philipp T, Mann K, Petersenn S. Diagnostic value of various biochemical parameters for the diagnosis of pheochromocytoma in patients with adrenal mass. Eur J Endocrinol. 2006;154:409–17.
14. Lenders JW, Pacak K, Walther MM, Linehan WM, Mannelli M, Friberg P, Keiser HR, Goldstein DS, Eisenhofer G. Biochemical diagnosis of pheochromocytoma: which test is best? Jama. 2002;287:1427–34.
15. Ito Y, Obara T, Okamoto T, Kanbe M, Tanaka R, Iihara M, Okamoto J, Yamazaki K, Jibiki K. Efficacy of single-voided urine metanephrine and normetanephrine assay for diagnosing pheochromocytoma. World J Surg. 1998;22:684–8.
16. Bilek R, Zelinka T, Vlcek P, Duskova J, Michalsky D, Novak K, Bestak J, Widimsky J Jr.. Deconjugated urinary metanephrine, normetanephrine and 3-methoxytyramine in laboratory diagnosis of pheochromocytoma and paraganglioma. Physiol Res. 2015;64: S313–22.
17. Schalin-Jantti C, Raade M, Hamalainen E, Sane T. A 5-year prospective follow-up study of lipid-rich adrenal incidentalomas: no tumor growth or development of hormonal hypersecretion. Endocrinol Metab. 2015;30:481–7.
18. Al-Thani H, El-Menyar A, Al-Sulaiti M, ElGohary H, Al-Malki A, Asim M, Tabeb A. Adrenal mass in patients who underwent abdominal computed tomography examination. N Am J Med Sci. 2015;7:212–9.
19. Caoili EM, Korobkin M, Francis IR, Cohan RH, Platt JF, Dunnick NR, Raghupathi KI. Adrenal masses: characterization with combined unenhanced and delayed enhanced CT. Radiology. 2002;222:629–33.
20. Lee JH, Kim EK, Hong AR, Roh E, Bae JH, Kim JH, Shin CS, Kim SY, Kim SW. Radiographic characteristics of adrenal masses in oncologic patients. Endocrinol Metab. 2016;31:147–52.
21. Hamrahian AH, Ioachimescu AG, Remer EM, Motta-Ramirez G, Bogabathina H, Levin HS, Reddy S, Gill IS, Siperstein A, Bravo EL. Clinical utility of noncontrast computed tomography attenuation value (Hounsfield units) to differentiate adrenal adenomas/hyperplasias from nonadenomas: Cleveland clinic experience. J Clin Endocrinol Metab. 2005;90:871–7.
22. Grumbach MM, Biller BM, Braunstein GD, Campbell KK, Carney JA, Godley PA, Harris EL, Lee JK, Oertel YC, Posner MC, Schlechte JA, Wieand HS. Management of the clinically inapparent adrenal mass incidentaloma. Ann Intern Med. 2003;138:424–9.
23. Cawood TJ, Hunt PJ, O'Shea D, Cole D, Soule S. Recommended evaluation of adrenal incidentalomas is costly, has high false-positive rates and confers a risk of fatal cancer that is similar to the risk of the adrenal lesion becoming malignant; time for a rethink? Eur J Endocrinol. 2009;161:513–27.
24. Carr JC, Spanheimer PM, Rajput M, Dahdaleh FS, Lal G, Weigel RJ, Sugg SL, Liao J, Howe JR. Discriminating pheochromocytomas from other adrenal lesions: the dilemma of elevated catecholamines. Ann Surg Oncol. 2013;20:3855–61.
25. Balog B, Toke J, Rona K, Szucs N, Igaz P, Pusztai P, Sarman B, Glaz E, Kiss R, Patocs A, Racz K, Toth M. [Analysis of laboratory data of 155 patients with pheochromocytoma-paraganglioma syndrome diagnosed during the past 20 years]. Orv Hetil. 2015;156:626–35.
26. Fassnacht M, Arlt W, Bancos I, Dralle H, Newell-Price J, Sahdev A, Tabarin A, Terzolo M, Tsagarakis S, Dekkers OM. Management of adrenal incidentalomas: European society of endocrinology clinical practice guideline in collaboration with the European network for the study of adrenal tumors. Eur J Endocrinol. 2016;175:G1–34.
27. Boland GW, Lee MJ, Gazelle GS, Halpern EF, McNicholas MM, Mueller PR. Characterization of adrenal masses using unenhanced CT: an analysis of the CT literature. AJR Am J Roentgenol. 1998;171:201–4.
28. William F Young J, Electron Kebebew. The adrenal incidentaloma. https://www.uptodate.com/contents/the-adrenal-incidenta

loma?source=search_result&search=adrenal%20incidentaloma&selectedTitle=1~21. Accessed 7 April 2017.
29. Andreoni C, Krebs RK, Bruna PC, Goldman SM, Kater CE, Alves MT, Ortiz V. Cystic phaeochromocytoma is a distinctive subgroup with special clinical, imaging and histological features that might mislead the diagnosis. BJU Int. 2008;101:345–50.
30. Korobkin M, Brodeur FJ, Yutzy GG, Francis IR, Quint LE, Dunnick NR, Kazerooni EA. Differentiation of adrenal adenomas from nonadenomas using CT attenuation values. AJR Am J Roentgenol. 1996;166:531–6.
31. Kaplan NM, Kramer NJ, Holland OB, Sheps SG, Gomez-Sanchez C. Single-voided urine metanephrine assays in screening for pheochromocytoma. Arch Intern Med. 1977;137:190–3.
32. Tapia FJ, Polak JM, Barbosa AJ, Bloom SR, Marangos PJ, Dermody C, Pearse AG. Neuron-specific enolase is produced by neuroendocrine tumours. Lancet. 1981;1:808–11.
33. Mori S, Okura T, Kitami Y, Takata Y, Nakamura M, Watanabe S, Iwata T, Hiwada K. A case of metastatic extra-adrenal pheochromocytoma 12 years after surgery. Hypertens Res. 2002;25:141–4.
34. Carney DN, Marangos PJ, Ihde DC, Bunn PA Jr., Cohen MH, Minna JD, Gazdar AF. Serum neuron-specific enolase: a marker for disease extent and response to therapy of small-cell lung cancer. Lancet. 1982;1:583–5.
35. Oishi S, Sato T. Elevated serum neuron-specific enolase in patients with malignant pheochromocytoma. Cancer. 1988;61:1167–70.