Subclinical Cushing’s syndrome (SCS) is characterized by subtle autonomous cortisol secretion from an adrenal incidentaloma (AI) without the phenotype of overt Cushing’s syndrome (OCS). This low-grade cortisol excess may worsen cardiovascular (CV) risk factors, increase CV events, and result in a higher mortality rate [1]. However, there is no consensus on how to establish a biochemical diagnosis for SCS. In fact, diagnostic criteria for SCS were highly heterogeneous and varied among previous studies. Although all published guidelines agree with the use of 1-mg dexamethasone suppression test (DST) for hypercortisolism screening, the required cut-off value for pathological DST ranged from 1.8 to 5.0 μg/dL. Additional tests, including low ACTH, loss of the circadian rhythm of cortisol, and low serum dehydroepiandrosterone-sulfate (DHEA-S) levels were considered necessary for the diagnosis of SCS in all studies [2].

As adrenocortical scintigraphy (ACS) uses radiopharmaceuticals specific for the adrenal cortex, uptake patterns are expected to detect the functional statuses of both adrenal glands separately. One study showed that unilateral uptake on ACS and impaired 1-mg DST were independently associated with hormonal progression in patients with AI including SCS [3]. Another recent report demonstrated that positive ACS in patients with AI had better predictability for the development of prolonged post-surgical hypoadrenalism than hormonal work-up for SCS. ACS was also able to predict the
metabolic outcome and identify subjects who could benefit from adrenalectomy, irrespective of the hormonal diagnosis [4].

Thus, it has been suggested that unilateral and/or predominant uptake on the side of adrenal mass is related to autonomous cortisol overproduction from AIs. However, some reports showed that the specificity of this finding was questionable, because increased uptake simply may reflect the presence of enlarged adrenal tissue [5]. There is no information regarding whether increased tracer uptake on the tumor side or decreased tracer uptake on the contralateral (non-tumor) side in ACS is more greatly associated with inappropriate cortisol production. Furthermore, an optimal cut-off value for ACS discrimination between autonomous and non-autonomous adrenocortical adenoma has not been clarified yet.

Therefore, the aim of this study was: (a) to determine whether $^{131}$I-6β-iodomethyl-norcholesterol ($^{131}$I-NP-59) uptake on the adenoma-bearing side or the contralateral side better reflects cortisol production; and (b) to set a cut-off for SCS detection.

### Materials and Methods

#### Subjects

An observational retrospective study was performed of 90 consecutive patients with AI referred to our two hospitals between April 2003 and March 2015 who underwent $^{131}$I-NP-59 scintigraphy and met all of the following criteria. The inclusion criteria were: 1) adrenal masses discovered serendipitously by radiological evaluation in the absence of clinical features suggestive of adrenal diseases, 2) unilateral adrenal mass more than 10 mm in diameter on abdominal computed tomography (CT), 3) abdominal CT and/or magnetic resonance imaging features suggesting benign adrenocortical adenoma; round- or oval-shaped hypodense mass (density on unenhanced CT scan of less than 10 Hounsfield units) with a homogenous pattern and well-defined margin [6], and 4) normal morning serum cortisol levels in repeated testing. Patients with suspected or confirmed adrenomedullary tumors, cysts, and metastatic lesions, and those with primary aldosteronism based on endocrine testing, imaging studies, or histopathological examination of resected tumors were excluded. Patients who were diagnosed pathologically with adrenocortical cancer (ACC) were also excluded because some ACCs are unable to incorporate enough $^{131}$I-NP-59 to be visualized on ACS [7].

Hypertension was defined as the need for drug therapy or systolic blood pressure ≥ 140 mmHg or diastolic pressure ≥ 90 mmHg. Diabetes mellitus (DM) was diagnosed as the need for drug therapy or diabetic pattern on a 75-g oral glucose tolerance test. Hyperlipidemia was defined as the need for drug therapy or low-density lipoprotein cholesterol (LDL-C) levels ≥ 140 mg/dL as calculated using Friedewald’s formula [8] or triglyceride levels ≥ 150 mg/dL [9]. Patients with body mass index (BMI) ≥ 25 kg/m$^2$ were defined as obese. History of nonfatal acute myocardial infarction, percutaneous transluminal coronary angioplasty/surgical bypass for ischemic heart disease, and ischemic/hemorrhagic stroke and arteriosclerosis obliterans were also investigated.

This study protocol was approved by the Human Ethical Committee of St. Marianna University School of Medicine (No. 3108) and was performed according to the principles of the Helsinki Declaration.

### Diagnostic method for SCS

A diagnosis of SCS was defined as the presence of an AI, absence of specific clinical features of OCS, normal morning serum cortisol levels on more than 2 different evaluations, the presence of autonomous cortisol secretion confirmed by overnight 1-mg DST (≥ 3.0 μg/dL serum cortisol following the DST), and at least 1 impaired endocrine test including low plasma ACTH levels in the early morning (ACTH < 10 pg/mL at 0800 h) or poor ACTH response to CRH (i.e., peak ACTH < 30 pg/mL or more than 1.5 times greater than baseline), no diurnal changes in serum cortisol levels (serum cortisol level ≥ 5.0 μg/dL at 2400 h), and low serum DHEA-S levels (lower than age- and sex-matched reference levels). This diagnostic method basically followed the criteria (hereinafter referred to as criteria A) set forth by the Intractable Disease Research Grant of the Ministry of Health, Labour, and Welfare (MHLW), Japan [10].

The American Endocrine Society published clinical practice guidelines for Cushing’s syndrome (CS) in 2008 [11] and advocated that the cortisol level threshold after overnight 1-mg DST should be lowered to < 1.8 μg/dL, a level that increases the diagnostic sensitivity to > 95%. Recently Akehi et al. proposed an alternative combination criterion for the diagnosis of SCS: serum cortisol ≥ 1.8 μg/dL after 1-mg DST with
ACTH < 10 pg/mL or serum cortisol levels ≥ 5.0 μg/dL at 2400 h, and they showed it had high sensitivity and specificity for the detection of impaired glucose tolerance (IGT)-DM [12]. Therefore, we decided to use this diagnostic procedure (hereinafter referred as to criteria B) for SCS as well.

**Adrenocortical scintigraphy**

Prior to administering norcholesterol, patients received a saturated solution of potassium iodine to block thyroid iodine. In all cases, $^{131}$I-NP-59 (Fujifilm Pharma Co., Ltd., Tokyo, Japan) was injected intravenously, and ACS was performed 7 days after the injection using a digital single-head gamma camera (Toshiba Medical Systems, Tokyo, Japan) equipped with a high-energy, parallel-hole collimator. Regions of interest (ROIs) were placed over areas corresponding to the adrenal glands and background (caudal site of the adrenal gland) on posterior planar images.

The uptake rate was determined as follows: 37 MBq (1 mCi) of $^{131}$I-NP-59 diluted with 5 mL saline, and the solution was divided into volumes of 0.2 mL ($C_{0.2}$) and 4.8 mL ($C_{4.8}$). While $C_{0.2}$ was saved for the control, $C_{4.8}$ was administered intravenously to each patient. The counts of adrenal ROI ($C_{ADR}$) and its background ROI ($C_{PBG}$) as well as those of the control $C_{0.2}$ ($C_{STD}$) and its background ($C_{STDBG}$) were determined 7 days after injection, using the collimator. The uptake rate of $^{131}$I-NP-59 (%) was determined using the following equation: $100 \times \frac{(C_{ADR} - C_{PBG})}{(C_{STD} - C_{STDBG})} \times \left(\frac{C_{4.8}}{C_{0.2}}\right)^{0.8}$. The ratio of the uptake rate of the affected adrenal gland and to the contralateral gland (laterality ratio) was also calculated. The application of this method in daily practice is feasible, conditional upon the close cooperation of radiologists.

**Hormone assays**

Blood samples were immediately centrifuged at 4°C and then stored at -80°C until assay. Commercially available assay kits were used for hormone measurements: DHEA-S radioimmunoassay (RIA) kits from Mitsubishi Chemical Co. (Tokyo Japan); ACTH immunoradiometric assay kits from Mitsubishi Chemical Co. (Tokyo Japan); and urinary free cortisol (UFC) RIA kits by TFB (Immunotech, Marseilles, France). In patients who underwent thorough tests before March 2008, levels of serum cortisol were measured using RIA kits (Immunotech, Marseilles, France). In the remaining patients, serum cortisol levels were assayed by using chemiluminescence enzyme immunoassay (CLEIA) kits (Beckman Coulter, CA, USA). A previous study showed an extremely high correlation ($r = 0.99$) between cortisol concentrations from the CLEIA and RIA kits [13].

**Statistical analysis**

The results are shown as mean ± standard error. The Mann-Whitney’s test or $\chi^2$ test was used for inter-group comparisons. Pearson’s correlation coefficient and multiple regression analysis were performed to determine factors contributing to the uptake rate on ACS. Factors that were determined via univariate regression analysis to be significantly correlated with the uptake rate or ratio on ACS were further using multiple regression analysis to identify independent factors associated with the uptake parameters. Because of non-normal distribution of some parameters, natural logarithmic transformation was used in the analysis to satisfy the requirement for normality. Values of $p < 0.05$ were considered statistically significant. Receiver operating characteristic (ROC) analysis was performed to assess possible threshold values for the laterality ratio to detect patients with SCS using the two diagnostic criteria described above. All analyses were performed with Excel Statistics 2012 software (SSRI, Tokyo, Japan) for Windows.

**Results**

Classification according to the criteria A resulted in 68 (75.5%) patients in the non-functioning adenoma (NFA) group and 22 (27.7%) patients in the SCS group. The clinical and laboratory characteristics of the patients in both groups at baseline are given in Table 1. Age, sex, BMI, and the prevalence of obesity, DM, and hyperlipidemia were similar in the SCS and NFA groups. Patients with SCS had larger tumor sizes (24.6 ± 9.8 vs. 19.6 ± 6.3 mm, $p = 0.0219$) and an increased frequency of hypertension (77.0% vs. 47.0%, $p = 0.0141$). The number of CV events in all subjects was 7 (cerebral infarction, n = 4; cerebral hemorrhage, n = 1; myocardial infarction, n = 1; arteriosclerosis obliterans, n = 1). The prevalence of CV events was not significantly different between the SCS and NFA groups (13.6% and 5.9%, respectively, $p = 0.0814$). Serum cortisol levels at 2400 h and serum cortisol levels after 1-mg DST were significantly higher in the SCS group than in the NFA group ($8.9 ± 3.7$ and $4.6 ± 3.0$ μg/dL, respectively, $p < 0.001$; and $7.5 ± 4.3$ and $1.8 ± 0.7$ μg/dL, respectively, $p = 0.0001$).
respectively, \( p < 0.001 \). Plasma ACTH levels at 0800 h was lower in the SCS group than in the NFA group (8.9 ± 3.7 and 4.6 ± 3.0 pg/mL, respectively, \( p < 0.0001 \)). Eighteen (20%, SCS, n = 12; NFA, n = 6) patients underwent unilateral adrenalectomy and 4 (SCS, n = 3; NFA, n = 1) of 18 patients received postoperative glucocorticoid replacement for more than 6 months. Although the patients with NFA who received postoperative glucocorticoid replacement exhibited normal results for all hypothalamus-pituitary-adrenal axis function tests except 1-mg DST, atrophy of the normal adrenal gland was observed in the resected specimen, and her postsurgical urinary free cortisol level was substantially low. Consequently, glucocorticoid replacement was maintained more than 2 years after adrenalectomy.

We also compared the uptake rate on ACS in both groups. On the occasion of this analysis, 12 (SCS, n = 7; NFA, n = 5) patients were excluded because uptake on the contralateral side could not be measured in these patients (the tracer uptake was zero owing to the complete suppression of cortisol production), and hence the laterality ratio of the uptake could not be calculated. Surprisingly, the uptake rate on the affected side in the SCS group was comparable to that in the NFA group (0.36 ± 0.21 and 0.34 ± 0.14%, respectively, \( p = 0.75 \)). In contrast, the uptake rate on the contralateral side was significantly lower (0.09 ± 0.04 and 0.21 ± 0.12%, respectively, \( p = 0.002 \), and the laterality ratio was significantly higher (4.69 ± 1.34 and 1.96 ± 1.49, respectively, \( p < 0.001 \)) in the SCS group than in the NFA group. These results suggest that the uptake not on the affected side, but on the contralateral side, may reflect the cortisol producing ability of tumors.

Furthermore, we evaluated the relationship between the degree of autonomous cortisol secretion from the tumor determined by serum cortisol after 1-mg DST and uptake on each side of the adrenal glands on ACS (Fig. 1). As expected, there was no correlation between uptake on the affected side and serum cortisol after 1-mg DST (\( r = 0.042 \), \( p = 0.714 \)). On the other hand, a significant inverse correlation between uptake on the contralateral side and serum cortisol after 1-mg DST was observed (\( r = -0.327 \), \( p = 0.004 \)). The laterality ratio of the uptake was also associated with serum cortisol after 1-mg DST (\( r = 0.378 \), \( p = 0.001 \)).

To determine the effect of tumor size on the uptake rate on ACS, we analyzed the correlation between tumor diameter and the uptake of each adrenal gland on ACS. As shown in Fig. 2, the uptake on the affected side did not correlate with tumor diameter (\( r = 0.16 \), \( p = 0.161 \)). By contrast, an inverse correlation was observed between tumor size and uptake on the contralateral side (\( r = -0.331 \), \( p = 0.003 \)). A significant correlation also was observed between the uptake ratio and tumor diameter (\( r = 0.419 \), \( p < 0.001 \)).
Fig. 1  Correlation of serum cortisol levels after 1-mg DST with uptake rates on the affected (A) and contralateral (B) sides and laterality ratio (C) on ACS images
At the time of this analysis, 12 (SCS, n = 7; NFA, n = 5) patients were excluded because tracer uptake on the contralateral side in these patients was zero because of the complete suppression of cortisol production, and, consequently, the laterality ratio of uptake could not be calculated. Because of non-normal distribution of serum cortisol levels after 1-mg DST, natural logarithmic transformation was used in the analysis to satisfy the requirement for normality. ACS, adrenocortical scintigraphy; SCS, subclinical Cushing’s syndrome; NFA, non-functioning adenoma; DST, dexamethasone suppression test.

Fig. 2  Correlation of tumor size with uptake rates on the affected (A) and contralateral (B) sides and laterality ratio (C) on ACS images
At the time of this analysis, 12 (SCS, n = 7; NFA, n = 5) patients were excluded because tracer uptake on the contralateral side in these patients was zero because of the complete suppression of cortisol production, and, consequently, the laterality ratio of uptake could not be calculated. Because of non-normal distribution of tumor size, natural logarithmic transformation was used in the analysis to satisfy the requirement for normality. ACS, adrenocortical scintigraphy; SCS, subclinical Cushing’s syndrome; NFA, non-functioning adenoma.
Next, we performed univariate and multivariate regression analyses to identify clinical parameters related to uptake on the contralateral side and the laterality ratio on ACS. The results of the univariate regression analysis showed that uptake on the contralateral side was significantly associated with serum cortisol levels after 1-mg DST, 0800 h plasma ACTH levels and tumor size (Table 2), whereas laterality ratio was associated with serum cortisol levels after 1-mg DST and at 2400 h, plasma ACTH at 0800 h and tumor size (Table 3). Upon multivariate regression analysis, both uptake rate on the contralateral side and laterality ratio were found to be associated with cortisol level after 1-mg DST and tumor size (Tables 2 and 3).

At the end of this study, we performed an ROC analysis to determine the best cut-off values for the laterality ratio for the diagnosis of SCS (Fig. 3). The cut-off value to detect patients with SCS according to criteria A was 3.07 (sensitivity, 66.7%; specificity, 85.7%; and area under the curve [AUC], 0.712), and that for criteria B was 2.49 (sensitivity, 69.2%; specificity, 76.9%; and AUC, 0.740).

**Table 2** Results of univariate (upper) and multivariate (lower) regression analyses of factors related to uptake on the contralateral side on ACS images

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Regression coefficient</th>
<th>Correlation coefficient</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortisol levels after 1-mg DST</td>
<td>-1.6495</td>
<td>0.3268</td>
<td>0.0035</td>
</tr>
<tr>
<td>2400 h cortisol</td>
<td>-0.0242</td>
<td>0.1127</td>
<td>0.3258</td>
</tr>
<tr>
<td>0800 h ACTH</td>
<td>0.0355</td>
<td>0.2539</td>
<td>0.0249</td>
</tr>
<tr>
<td>Tumor size</td>
<td>-0.9482</td>
<td>0.3308</td>
<td>0.0031</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Regression coefficient</th>
<th>Standard error</th>
<th>Decision coefficient</th>
<th>F value</th>
<th>t value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortisol level after 1-mg DST</td>
<td>-0.2566</td>
<td>0.0216</td>
<td>0.0686</td>
<td>5.528</td>
<td>-2.351</td>
<td>0.0213</td>
</tr>
<tr>
<td>Tumor size</td>
<td>-0.2622</td>
<td>0.0381</td>
<td>0.0715</td>
<td>5.769</td>
<td>-2.402</td>
<td>0.0188</td>
</tr>
</tbody>
</table>

Upon multivariate regression analysis, cortisol levels after 1-mg DST and tumor size were found to be significant parameters for uptake on the contralateral side. Because of non-normal distribution of these parameters, natural logarithmic transformation was used in the analysis to satisfy the requirement for normality. ACS, adrenocortical scintigraphy; DST, dexamethasone suppression test.

**Table 3** Results of univariate (upper) and multivariate (lower) regression analyses of factors related to laterality ratio on ACS images

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Regression coefficient</th>
<th>Correlation coefficient</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortisol levels after 1-mg DST</td>
<td>1.0966</td>
<td>0.3778</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2400 h cortisol</td>
<td>0.9115</td>
<td>0.2897</td>
<td>0.0101</td>
</tr>
<tr>
<td>0800 h ACTH</td>
<td>-0.6062</td>
<td>0.2958</td>
<td>0.0085</td>
</tr>
<tr>
<td>Tumor size</td>
<td>2.1417</td>
<td>0.4189</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Regression coefficient</th>
<th>Standard error</th>
<th>Decision coefficient</th>
<th>F value</th>
<th>t value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortisol level after 1-mg DST</td>
<td>0.2862</td>
<td>0.3001</td>
<td>0.0922</td>
<td>7.619</td>
<td>2.761</td>
<td>0.0073</td>
</tr>
<tr>
<td>Tumor size</td>
<td>0.3423</td>
<td>0.5301</td>
<td>0.1269</td>
<td>10.903</td>
<td>3.302</td>
<td>0.0015</td>
</tr>
</tbody>
</table>

Upon multivariate regression analysis, cortisol levels after 1-mg DST and tumor size were found to be significant parameters related to laterality ratio. Because of non-normal distribution of these parameters, natural logarithmic transformation was used in the analysis to satisfy the requirement for normality. ACS, adrenocortical scintigraphy; DST, dexamethasone suppression test.
Contralateral suppression on ACS

in patients with adrenal masses to identify cortisol-producing adenomas including SCS, but these studies have been performed on relatively small numbers of subjects [16-18]. To date, no study has examined which adrenal uptake rate on ACS (the tumor or non-tumor side) reflects the cortisol production ability of the adenoma.

To clarify the problem, we attempted to investigate the relationship between the accumulation rate of $^{131}$I-NP-59 in each adrenal gland determined using a quantitative method and hormonal autonomy estimated by serum cortisol concentration after 1-mg DST. In this study, we observed a weak but significant correlation between uptake not on the tumor side, but on the contralateral side, and the degree of cortisol production. Furthermore, as opposed to the healthy side, the uptake rate on the affected side in the SCS group was comparable to that in the NFA group. These results clearly indicate that contralateral adrenal suppression on ACS is good evidence of subclinical cortisol overproduction.

To clarify the problem, we attempted to investigate the relationship between the accumulation rate of $^{131}$I-NP-59 in each adrenal gland determined using a quantitative method and hormonal autonomy estimated by serum cortisol concentration after 1-mg DST. In this study, we observed a weak but significant correlation between uptake not on the tumor side, but on the contralateral side, and the degree of cortisol production. Furthermore, as opposed to the healthy side, the uptake rate on the affected side in the SCS group was comparable to that in the NFA group. These results clearly indicate that contralateral adrenal suppression on ACS is good evidence of subclinical cortisol overproduction.

Discussion

SCS is a condition characterized by autonomous, albeit subtle hypercortisolism without the typical signs and symptoms of OCS. No clear consensus has been reached on which combination of biochemical tests achieves the highest diagnostic accuracy. ACS is thought by some, but not all, investigators to be the most sensitive method to detect the endocrinological autonomy of incidental adrenocortical tumors. However, in most previous studies, patients with AI were classified qualitatively as having an exclusive, prevalent, symmetric, and reduced or absent uptake base on ACS [4, 5]. Exclusive as well as prevalent uptake were considered concordant patterns, but we sometimes face difficulties determining the implications of the clinical significance of the latter finding.

Barzon et al. described the relationship between endocrine data and radiocholesterol uptake, indicating that the presence of both concomitantly increased the tracer uptake of the affected adrenal gland, and contralateral adrenal suppression was suggestive of a hyperfunctional adrenal adenoma [14]. On the other hand, Osella et al. questioned the specificity of this finding because increased uptake simply may reflect the presence of enlarged adrenal tissue [15]. Several reports quantified $^{131}$I-NP-59 adrenal uptake in patients with adrenal masses to identify cortisol-producing adenomas including SCS, but these studies have been performed on relatively small numbers of subjects [16-18]. To date, no study has examined which adrenal uptake rate on ACS (the tumor or non-tumor side) reflects the cortisol production ability of the adenoma.

Fig. 3 The receiver operating characteristic curve for laterality ratio on ACS images for the detection of SCS according to criteria A (A) and B (B)

The best cut-off value, sensitivity, specificity, and area under the curve for the detection of SCS according to criteria A were 3.07, 0.667, 0.857, and 0.712, respectively, and those for the detection of SCS according to criteria B were 2.449, 0.692, 0.769, and 0.740, respectively. ACS, adrenocortical scintigraphy; SCS, subclinical Cushing’s syndrome.
Based on our results, the laterality ratio of $^{131}$I-NP-59 uptake was a better index for cortisol production from adenomas than tracer uptake on the non-tumor side. Hence, we determined factors related to the index using a multivariate regression analysis. Tumor size and cortisol levels after 1-mg DST were selected as significant and independent determinants of the laterality ratio. Moreover, there was a significant correlation between these 2 factors, suggesting that tumor size is another important factor for both the functional statuses of adrenal tumors and $^{131}$I-NP-59 imaging patterns in this study.

Yoh et al. indicated there was a statistically significant correlation between tumor size and $^{131}$I-NP-59 uptake in a functional adenoma group using a conventional planar method. However, the study included patients with aldosterone-producing adenoma [16]. Imperiale et al. proposed a new index that takes tracer accumulations on both the adenoma and contralateral sides into account on semi-quantitative tomographic ACS [17]. They suggested that the possibility of quantifying contralateral uptake provides useful information about normal adrenal parenchymal status and hence indirectly describes the functional autonomy of the adenoma. However, this report did not examine the correlation between $^{131}$I-NP-59 uptake and cortisol production from the tumor at all. Donadio et al. also investigated the role of quantitative ACS in the diagnosis of subtle hypercortisolism (SH) in 41 patients with AI [18]. They calculated the difference and ratio between the uptakes of the affected and healthy adrenal glands on ACS and concluded that the 2 parameters can be useful to exclude the presence of subtle cortisol excess in these patients. Nevertheless, in our study, the difference in tracer uptake in both adrenal glands was not associated with hormonal state (data not shown). The inconsistency in observations may result from the different diagnostic criteria for SCS and/or the numbers of patients with discordant patterns on ACS.

It is well known that conventional planar $^{131}$I-NP-59 ACS demonstrates low sensitivity in the detection of small lesions, especially when physiological gallbladder and bowel radiotracer accumulation is superimposed. To improve the spatial resolution, some researchers introduced single-photon emission computed tomography (SPECT), as described above [17]. Indeed, it yielded correct information from concordant and discordant uptake in 30 of 43 (70%) subjects in this study with adrenal masses less than 2 cm. The frequency was significantly lower than that in patients with AIs of 2 cm or greater (43 of 47 patients, 91%; $p = 0.0098$), showing that conventional planar ACS imaging is less sensitive to assay the functional autonomy of smaller lesions, even though the tracer uptake is quantified. SPECT imaging is thought to be more accurate than the imaging modality that we used, but the procedure is not always available.

Some authors performed ACS under dexamethasone suppression, and others calculated the average of the adrenal gland uptake on different days [18, 19]. These methods seem to be more accurate, but are somewhat cumbersome and not realistic in daily practice. We analyzed adrenal gland tracer activity under no suppression by dexamethasone 7 days following $^{131}$I-NP-59 tracer injection as the standard imaging timing in Japan, and conclude that our protocol is easy, simple, and practical.

It is important to determine certain criteria for ACS to identify SCS in daily clinical practice. Yoh et al. proposed a cut-off value of 0.23%, which was defined from the 95% confidence interval from non-affected adrenal glands [16]. However, the value of the tracer uptake rate may vary according to differences in the instruments used and image acquisition conditions at each institute; thus, we attempted to determine an optimal cut-off value for the laterality ratio by ROC analysis. The best cut-off value for SCS diagnosis according to criteria A was 3.07 (sensitivity, 66.7%; specificity, 85.7%; and AUC, 0.712), and that according to criteria B was 2.49 (sensitivity, 69.2%; specificity, 76.9%; and AUC, 0.740). The specificity was higher than the sensitivity, suggesting that an additional ACS examination may extract a cortisol-producing adenomas with higher autonomy among AIs. In fact, when patients were classified by whether the laterality ratio was above or below the 2 cut-off points, UFC, 0800 h, 2400 h, and post-dexamethasone serum cortisol levels were also higher in the former group, and were significantly higher than in the latter group.

Furthermore, binomial logistic regression analysis identified laterality ratio exceeding the cut-off points on ACS and BMI as independent predictors associated with the presence of hypertension (data not shown). Hypertension is one of the most frequent CV risk factors in patients with SCS. A recent systematic review showed a greater beneficial effect of surgery on blood pressure compared with conservative management [20, 21]. From this point of view, ACS seems to be a useful
diagnostic tool to select hypertensive cases curable by adrenalectomy among SCS patients.

The main limitations of this study are the relatively small number of patients and the retrospective and cross-sectional analyses. However, we used strict enrollment criteria that excluded all other adrenal disorders, including ACC and primary aldosteronism. Beyond that, to our knowledge, this study is the largest to date to evaluate the usefulness of quantitative ACS in unilateral adrenal adenomas. Another limitation of the present study is that ACS is not available in North America and is limited in clinical use.

In conclusion, we clearly demonstrate that only contralateral adrenal suppression on ACS provides good evidence for subclinical cortisol overproduction in unilateral adrenal adenoma. The laterality ratio of $^{131}$I-NP-59 uptake was a better index for detecting autonomous cortisol-producing adenomas. Tumor size was another important factor for both the functional statuses of adrenal tumors and $^{131}$I-NP-59 imaging patterns in this study. An additional ACS examination is still a useful tool because it may select cortisol-producing adenomas with higher autonomy and hypertensive cases curable by adrenalectomy among SCS patients.

Acknowledgement

This study was supported by the Health and Labor Science Research Grant, Research on Intractable Diseases, Research Committee on Disorder of Adrenal Hormones from the MHLW, Japan (ID: 16769897).

Disclosure Statement

None of the authors have any potential conflicts of interest associated with this research.

References


