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ORIGINAL ARTICLE

Subclinical hypercortisolism and CT appearance in adrenal incidentalomas: a multicenter study from Southern Sweden

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Abstract Evaluation of subclinical hypercortisolism (SH) in patients with adrenal incidentaloma (AI) including its correlation to size, attenuation at unenhanced computed tomography (CT) and unilateral or bilateral adrenal disease. Nine hospitals in Southern Sweden investigated during 2005–2007 consecutively patients with AI with hormonal and CT examinations according a regional protocol. Two hundred and twenty-eight patients with AI with median size 2.0 cm were included. One mg overnight dexamethasone suppression test (DST) was performed in 223 patients and basal P-ACTH measured in 146 patients. SH was defined as cortisol ≥50 nmol/l at DST in combination with basal ACTH <2 pmol/l. In patients with unilateral AI 42% (76/180) had inadequate suppression at DST and 23% (27/115) had SH. The probability for SH and inadequate suppression at DST correlated positively to size and inversely to attenuation at CT. Bilateral AI were found in 43 patients and of these 70% (30/43) had inadequate suppression at DST and 42% (13/31) SH. The patients with SH or inadequate suppression at DST had increased frequency of hypertension which increased further in patients with post-DST cortisol ≥140 nmol/l. The applied criterion for SH is useful for initial evaluation of patients with AI. SH is common in patients with AI, particular in bilateral disease. In patients with unilateral AI the probability for SH correlated positively to size and inversely to attenuation at CT. Furthermore, SH and the post-DST cortisol concentration was associated with hypertension.

Keywords Adrenal incidentaloma · Subclinical Cushing syndrome · Subclinical hypercortisolism · Adrenal CT · Adrenal imaging · Houndsfield units

On behalf of the Southern Sweden network group for diseases in thyroid, parathyroid, adrenal glands and abdominal endocrine tumours.

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Introduction

Adrenal incidentaloma (AI) is defined as a mass in one or both adrenal glands diagnosed at computed tomography (CT), ultrasonography or magnetic resonance imaging performed without suspicion of adrenal disease. AI is most commonly diagnosed with CT and in a recent study AI was found in about 4% of CT examinations in patients with a mean age 60 years, but the frequency varies between 0.2 and 7% depending on the age of the patients [1, 2].

The majority of AI are benign cortical adenomas and a minority are clinical symptomatic endocrine tumours or malignant tumours [3, 4]. Pheochromocytomas and malignant tumours have been found in approximately 5%, respectively, of AI, but a recent study reported much lower

prevalence [4–6]. Cushing's syndrome and primary aldosteronism are also found in a low proportion of patients with AI [3–5]. On the other hand, discrete cortisol secretion with subclinical hypercortisolism (SH) has more recently been reported to be a frequent finding in benign cortical adenomas but the diagnostic criteria for SH are not well established [7–11]. SH as well as overt Cushing's syndrome is linked to increased prevalence of cardiovascular disease risk factors such as hypertension, dyslipidemia and diabetes mellitus [12–14]. Also, decreased bone density and increased numbers of vertebral fractures has been reported in patients with SH [15]. Studies on small numbers of patients with SH have reported beneficial effect of adrenalectomy on these risk factors but the long-term effect on cardiovascular morbidity and mortality has not been clarified [16–18].

Morphological features on CT may be helpful to distinguish adenomas from non-adenomatous lesions, i.e. malignancies and pheochromocytomas. Non-contrast medium-enhanced CT densitometry takes advantage of that most adrenal adenomas are lipid rich and consequently have a low attenuation, whereas malignancies and most pheochromocytomas are low in intracellular fat [19, 20]. The cut-off for attenuation at $\leq$10 Hounsfield units (HU) to exclude malignancy is commonly used since it has a high specificity [21]. However, not all adrenal adenomas are lipid rich and consequently only 70–80% have attenuation $\leq$10 HU [6, 21]. Lesion 4 cm and larger or significant growth may indicate malignancy, while there is considerable overlap between benign and malign lesions regarding shape and homogeneity [21]. Bilateral AI are found in approximately 15% of the patients with AI [4, 5, 22] and in this group of patients the probability for the presence of SH has been reported to be increased [23]. Several studies also have reported that SH is more frequent in larger AI [10, 24] but a possible association between SH and lipid content and thereby to unenhanced CT attenuation has not been examined.

The aim of this study was to examine a consecutive series of patients with AI with regard to SH, including its correlation to size, attenuation at unenhanced CT and unilateral or bilateral adrenal disease.

Patients and methods

The study was performed by the South Sweden Network Group for diseases in thyroid, parathyroid, and adrenal glands and abdominal endocrine tumours. Two hundred and twenty-eight patients diagnosed with AI during 3 years (2005–2007) at nine hospitals (Halmstad, Helsingborg, Kristianstad, Landskrona, Lund, Ljungby, Malmö, Växjö and Ystad) covering a catchment area of approximately 1.3 million inhabitants were included. An AI was defined as a lesion in an adrenal gland sized 1 cm or more diagnosed on an imaging examination performed for another indication than suspected disease in the adrenal glands [3, 25]. CT imaging performed as staging or control of known malignancy was not included according to the definition of an AI. The study was approved by the Ethics committee at the Medical faculty, Lund University.

Study protocol

All patients were examined according to a predefined study protocol.

Imaging

Four to 64 multirow detector CT (MDCT) equipments were used. Attenuation (HU) was measured on the CT examination where the AI was first diagnosed if unenhanced CT scans were available, and if the size of the lesion and slice thickness matched so that any partial volume effect could be avoided. Otherwise a dedicated adrenal CT examination was performed to characterise the AI, including imaging without contrast enhancement. Attenuation measurements were performed with a circular or elliptic region of interest placed in the centre of AI and covering not more than half to two-thirds of its diameter to avoid any partial volume effect. The measurements were done by the attending radiologists at time of the examination except for all bilateral AI and a minority of the unilateral lesions where attenuation was not reported, which were analysed by the radiologist participating in the study group. The attending radiologists and the radiologist participating in the study group were not informed about the cortisol secretory status of the AI. The size of an AI was defined as the maximal axial diameter. Due to inhomogeneity of the AI adequate evaluation could not be achieved in three patients. AIs with size <4 cm, attenuation 10 HU or less, internal homogeneity and regular borders were considered as benign [6, 21]. The other AIs were considered indeterminate or malignant and further diagnostics was required to evaluate the risk for malignancy. In most cases, contrast medium-enhanced CT was performed to establish contrast medium washout but these results are not included in this study.

Biochemistry

Evaluation for pheochromcytoma consisted of two 24 h samples of urinary catecholamines and metoxycatecholamines. Primary aldosteronism was evaluated by plasma renin and serum aldosterone and the calculated aldosterone/renin ratio. Plasma ACTH and serum dehydroepiandrosterone sulphate (DHEAS) were measured as markers of the pituitary–adrenal axis. The blood samples for basal values were collected at 8:00 AM and followed by an overnight 1 mg dexamethasone suppression test (DST).

The patients received 1 mg dexamethasone at 11:00 PM and blood samples for serum cortisol were collected the following morning at 8:00 AM.

Criteria for adrenal subclinical hypercortisolism

Inadequate suppression at 1 mg DST was defined as serum cortisol ≥50 nmol/l post-dexamethasone [26]. In addition to an abnormal DST ACTH and DHEAS was measured as complementary criteria for SH. A post-DST serum cortisol ≥50 nmol/l and a basal ACTH <2 pmol/l were used to identify SH. The hormonal tests were performed in the following number of patients: DST ($n = 223$), DHEAS ($n = 216$) and basal ACTH ($n = 146$). Since basal ACTH was available in only 146 patients, a complete evaluation of the frequency of SH according to that criterion could only be fulfilled in this subset of patients.

Possible consequences of an abnormal DST and subclinical hypercortisolism

The medication for the patients was registered and blood pressure measured. Definition for hypertension was treated hypertension or in untreated patients systolic and diastolic blood pressure 140 and 90 mg Hg or above, respectively.

Assays

Serum cortisol was analysed by one-step competitive immunoassay (Roche Diagnostics, Mannheim, Germany, reference range 8:00 AM 200–800 nmol/l). Coefficient of variation was 4.3% at 130 nmol/l. Plasma ACTH was analysed by two-step immunometric sandwich assay and during the study three different kits were used (1. Nichols Institute Diagnostics, CA, USA, 2. Immulite DPC, CA, USA and 3. Roche Diagnostics, Mannheim, Germany). ACTH was analysed with kit 1, 2 and 3 in 22, 17 and 107 patients, respectively. The reference ranges at 8.00 AM were 2–10, <10 and 1–13 pmol/l and coefficients of variation were 9% at 8 pmol/l, 10% at 5.3 pmol/l and 5.4% at 1.1 pmol/l for the three kits, respectively. Serum DHEAS was analysed by competitive immunoassay (Immulite 2000, Siemens, USA, reference range females 1.0–11.7 μmol/l and males 2.2–15.2 μmol/l). Coefficients of variation was 12.6% at 6.4 μmol/l. Serum aldosterone was analysed by competitive radioimmunoassay (reference range 110–860 pmol/l) and plasma renin by one-step radioimmunometric sandwich assay (reference range 5–30 mIE/l). Urinary metoxycatecholamines were measured by HPLC and electrochemical detection (reference range urinary metoxyadrenalin and metoxynoradrenalin 20–200 and 80–360 μmol/mol creatinine, respectively). Urinary catecholamines was analysed by HPLC with fluorimetric detection (reference range urinary adrenalin and noradrenalin < 73 and 130–440 nmol/24 h, respectively).

Operation indications

The radiological indications for operation were size 4 cm and larger, radiological suspicion of malignancy. The hormonal indications for operation were Conn's disease, overt clinical hypercortisolism with Cushing's syndrome and pheochromocytomas, both symptomatic and clinically silent. Relative indications for operation were size between 3 and 4 cm and SH.

Statistics

Statistical analysis was calculated by PASW statistics 18 (SPSS, Inc., Chicago, IL, USA). Results are expressed as median and interquartile range. Comparison between groups was calculated with χ^2 and linear-by-linear association test for categorical data and Mann–Whitney and Kruskal–Wallis for continuous data. Correlation within groups was calculated by multivariable logistic or linear regression analysis where appropriate. A p value <0.05 was considered statically significant.

Results

Two hundred and twenty-eight patients (58% females) with AI and a median age of 65 (range 31–84) years were included. Sixty-eight % had hypertension. Bilateral AI was found in 43 patients (19%).

Dexamethasone test, ACTH, DHEAS and arterial hypertension

An inadequate suppression of cortisol at DST was found in 48%, basal DHEAS was suppressed below the reference range for respective gender in 45% and basal ACTH was below 2 pmol/l in 34% of the analysed samples. The patients where basal ACTH analyses not was available had smaller AI compared to patients with ACTH analysis performed (median 2.0 vs 2.2 cm, $p = 0.031$ and 1.9 vs 2.6 cm, $p = 0.006$ for unilateral and bilateral AI, respectively).

In Table 1, the patients, where DST was performed ($n = 223$), are divided into four quartiles according to cortisol post-DST. The available results of basal ACTH, DHEAS and hypertension are shown for each quartile.

Possible associations to metabolic disturbances

Information about all the medication was recorded from 202 of the patients. Table 2 shows the frequency of

Table 1 The patients, where DST was performed ($n = 223$), are divided into four quartiles according cortisol post-DST

	n	*Q*1 (cortisol at DST 11–32 nmol/l)	*Q*2 (cortisol at DST 33–47 nmol/l)	*Q*3 (cortisol at DST 48–71 nmol/l)	*Q*4 (cortisol at DST 72–684 nmol/l)	*p*
n	223	57	55	56	55	
ACTH (pmol/l)	146	4.0 (3.3; 6.7)	3.4 (2.0; 4.8)	2.4 (1.9; 3.7)	1.4 (0.9; 2.9)	0.000
ACTH <2 pmol/l (number and % of patients)	50	1 (3%)	8 (24%)	15 (42%)	26 (62%)	0.000 (vs ACTH ≥2 pmol/l)
ACTH 2 to <3 pmol/l (number and % of patients)	23	5 (15%)	6 (18%)	6 (17%)	6 (15%)	0.073 (vs ACTH ≥3 pmol/l)
ACTH >3 pmol/l (number and % of patients)	73	28 (82%)	20 (59%)	15 (42%)	10 (24%)	
DHEAS suppressed (number and % of patients)	212	19 (34%)	24 (46%)	18 (34%)	39 (68%)	0.003
Hypertension, all ages (number and % of patients)	218	29 (54%)	39 (72%)	41 (73%)	42 (78%)	0.009
Hypertension in patients younger than 70 years (number and % of patients)	154	20 (49%)	26 (65%)	27 (68%)	26 (79%)	0.009

The available results of basal ACTH, DHEAS and hypertension are shown for each quartile

Data expressed as median or percentage. Significance levels were calculated by Kruskal–Wallis and linear-by-linear association tests for continuous and categorical data, respectively. The group of patients below 70 years had a median age of 61 years

Table 2 Clinical characteristics of the patients depending on the outcome of 1 mg dexamethasone suppression test and whether they had subclinical hypercortisolism or not

	Suppression on DST ($n = 117$)	Non-suppression on DST ($n = 106$)	*p* value	No subclinical hypercortisolism ($n = 106$)	Subclinical hypercortisolism ($n = 40$)	*p* value
Age (years)	64 (58; 70)	66 (59; 73)	0.076	65 (59; 72)	65(59; 70)	0.568
Systolic blood pressure (mmHg)	140 (128; 150)	140 (130; 150)	0.798	140 (130; 150)	140 (130; 152)	0.552
Diastolic blood pressure (mmHg)	80 (75; 85)	80 (72; 85)	0.445	80 (70; 85)	80 (70; 85)	0.913
Patients with hypertension (*n* and %)	72 (64%)	80 (76%)	0.045	68 (65%)	33 (83%)	0.044
Number of antihypertensive drugs (*n*)	0.83	1.26	0.008	0.87	1.38	0.020
Patients with antidiabetic medication (*n* and %)	8 (8%)	10 (11%)	0.427	8 (8%)	4 (11%)	0.642
Patients with lipid lowering medication (*n* and %)	14 (14%)	24 (25%)	0.056	17 (18%)	11 (30%)	0.120

Results for continuous data are expressed as median and within in parenthesis interquartile range. The number of medications for hypertension as mean. Results for binary data are expressed as percentage

hypertension and medication for hypertension, dyslipidemia and diabetes in relation to outcome of DST ($n = 117 + 106$) and in relation the absence or presence of SH ($n = 106 + 40$). Hypertension was more frequent among those with an abnormal DST and also among those in the subgroup with SH. No impact was observed on the frequency of use of lipid lowering or antidiabetic drugs.

The frequency of hypertension and use of antihypertensive drugs among patients evaluated for SH and found to fulfil or not fulfil the criteria used are shown in Table 3. Moreover, these data were specified for those patients younger than 70 years, and also with regard to the degree of cortisol suppression (50–140 nmol/l and ≥140 nmol/l) at the DST. Thereby, a significant impact of an autonomous secretion of cortisol on the presence of hypertension was observed, specifically among those younger than 70.

Unilateral incidentalomas

Table 4 shows the clinical characteristics, CT findings and hormonal data on the 185 patients with unilateral AI. An abnormal DST was observed in 42% of the patients with unilateral AI. Among those evaluated with the criteria for SH, 23% were diagnosed with SH.

Size and attenuation

Figure 1 shows the distribution of the size of the AI. The median size was 2.0 cm (range 1.0–7.0 cm) and the AI was 4 cm or larger in 10 patients (5%).

Figure 2 shows the distribution of the CT attenuation. The median attenuation on unenhanced CT was 4.0 HU (range −80 to +50 HU). High attenuation (>10 HU) was found in 44 of 183 patients (24%). In 27% of the patients, CT characteristics indicated indeterminate or malignant AI according to the protocol. There was no correlation between size and attenuation of AI at CT. Age did not correlate to size or attenuation at CT.

Figure 3 shows 115 patients with unilateral AI divided into nine groups with different maximal axial diameters (<1.8, 1.8–2.5 and >2.5 cm) and attenuation values in HU at CT (<1 HU, 1–10 HU and >10 HU). The probability for SH was high, 45%, in larger AI with lower attenuation at CT and low in smaller AI with higher attenuation at CT. Logistic regression showed that the probability for SH correlated positively to group of size (OR 2.059, $p = 0.025$) of the AI and inversely to group of attenuation at CT (OR 0.358, $p = 0.003$). Furthermore, the probability for non-adequate suppression on DST also correlated positively to size (OR 1.925, $p = 0.000$) of the AI and inversely to attenuation at CT (OR 0.653, $p = 0.020$).

Hormonal abnormalities and malignancy

Two patients with unilateral AI were diagnosed with non-symptomatic pheochromocytomas, one had primary aldosteronism and one adrenal metastasis. The patient with an adrenal metastasis was found to have disseminated disease and was not operated.

Bilateral incidentalomas

Table 4 shows the clinical characteristics, CT findings and hormonal data of the 43 patients with bilateral AI. An abnormal DST was observed in 70% of the patients with bilateral AI. Among those evaluated with the criteria for SH, 42% were diagnosed with SH.

Table 3 The frequency of hypertension and the number of medications for hypertension is shown both for patients in all ages and for patients younger than 70 years

	No subclinical hypercortisolism	Subclinical hypercortisolism Cortisol at DST 50–139 nmol/l	Subclinical hypercortisolism Cortisol at DST ≥140 nmol/l	*p* value
Patients with hypertension, all ages (*n* and %)	68 (65%)	24 (80%)	9 (90%)	0.038
Number of antihypertensive drugs among patients in all ages (mean value)	0.87	1.34	1.50	0.047
Patients younger than 70 years with hypertension (*n* and %)	39 (57%)	19 (79%)	6 (100%)	0.008
Number of antihypertensive drugs among patients younger than 70 years (mean value)	0.54	1.26	1.67	0.003

The patients with SH are subgrouped into those with post-DST cortisol 50–139 nmol/l and ≥140 nmol/l in order to grade the magnitude of cortisol hypersecretion by the AI

Binary data are calculated with linear-by-linear association test and ordinal data (number of medications for hypertension) by Kruskal–Wallis test. The group of patients below 70 years had a median age of 61 years

Table 4 Clinical characteristics of patients with unilateral and bilateral adrenal incidentalomas

	Unilateral incidentaloma ($n = 185$)	Bilateral incidentaloma ($n = 43$)	p value
Age (years)	64 (58; 71)	67 (58; 72)	0.387
Gender (female %)	57	60	0.705
Size (in bilateral AI the larger AI, cm)	2.0 (1.5; 3.0)	2.4 (1.8; 3.0)	0.143
Size (in bilateral AI the smaller AI, cm)	NA	1.5 (1.2; 2.1)	0.000
Attenuation (in bilateral AI the larger AI, HU)	4.0 (−3.0; 10)	0.0 (−4.7; 6.3)	0.080
Attenuation (in bilateral AI the smaller AI, HU)	NA	−1.5 (−8.0; 5.3)	0.011
Cortisol at DST (nmol/l)	44 (32; 66)	68 (46; 93)	0.000
Cortisol at DST ≥50 nmol/l (% and n)	42 (76)	70 (30)	0.001
P-ACTH (pmol/l)	3.2 (1.9; 5.1)	2.0 (1.3; 4.3)	0.070
P-ACTH (<2 pmol/l; % and n)	30 (35)	48 (15)	0.062
Cortisol at DST ≥50 and basal ACTH <2 (% and n)	23 (27)	42 (13)	0.041
Cortisol at DST ≥140 and basal ACTH <2 (% and n)	4 (5)	16 (5)	0.021

NA not applicable

Results for continuous data are expressed as median and interquartile range in parenthesis. Results for binary data are expressed as percentage. p values refer to differences between unilateral and bilateral AI

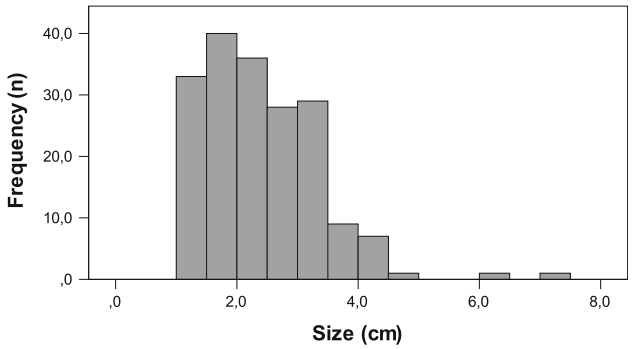

Fig. 1 The maximal axial diameter of unilateral adrenal incidentalomas

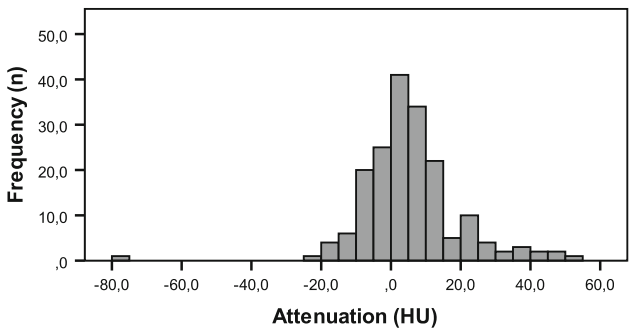

Fig. 2 The attenuation in Hounsfield units (HU) at computed tomography of unilateral adrenal incidentalomas

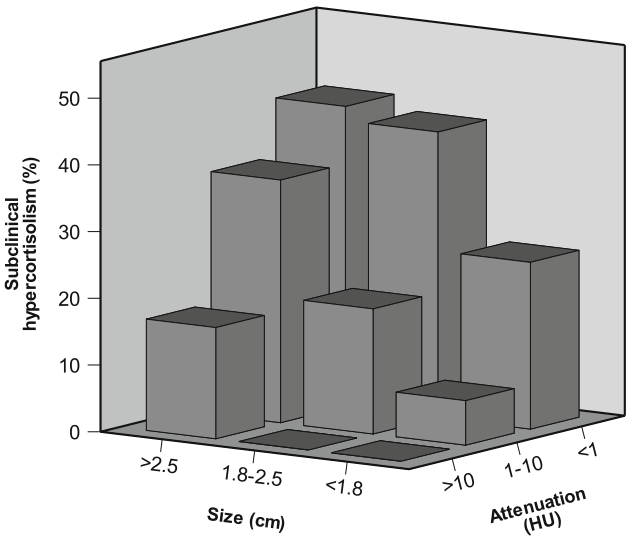

Fig. 3 The frequency of subclinical hypercortisolism in unilateral adrenal incidentalomas. The lesions are divided into nine groups with different maximal axial diameters (<1.8, 1.8–2.5 and >2.5 cm) and attenuation values in Hounsfield units (HU) at computed tomography (<1 HU, 1–10 HU and >10 HU). The frequency of subclinical hypercortisolism (SH) is expressed as percentage. SH was defined as inadequate suppression on DST (serum cortisol ≥50 nmol/l) in combination with basal plasma ACTH <2 pmol/l. Multivariate logistic regression showed that the probability for SH correlated to size of the AI ($p = 0.025$) and inversely to attenuation at CT ($p = 0.003$)

Size and attenuation

The median size of the larger of the AI was 2.4 (range 1.0–5.0) cm and five AI (12%) were 4 cm or larger. The median attenuation of the larger of the bilateral AI was 0.0 HU (range −17 to +33 HU) and in 17% the attenuation was >10 HU. The smaller of the bilateral AI had lower attenuation compared to unilateral AI −1.5 HU (range −18 to +33 HU, $p = 0.011$). The attenuation at CT of the AI on the right- and left-side correlated ($R^2 = 0.49$, $p = 0.000$) as well as the size ($R^2 = 0.14$, $p = 0.012$).

Table 4 shows that the median S-cortisol concentration after DST was higher in patients with bilateral compared to unilateral AI. Patients with bilateral AI had a higher frequency of pathological DST and SH, 70 and 42%, respectively, as compared to unilateral AI, 42 and 23%, respectively.

Logistic regression in patients with bilateral AI showed that probability for SH correlated positively to the size ($p = 0.049$) but was independent of the attenuation at CT.

Hormonal abnormalities and malignancy

None of the patients with bilateral AIs had pheochromocytoma, primary aldosteronism or malignant lesions.

Surgery

Sixteen of the patients underwent laparoscopic robotic-assisted adrenalectomy. In the three patients with bilateral AI unilateral adrenalectomy was performed. Table 5 shows the clinical indication for adrenalectomy and the histological diagnosis.

Discussions

In this study, we could demonstrate a high frequency of abnormal cortisol suppression after an overnight 1 mg DST among patients with AI, and in a subset of patients, also of SH which in patients with unilateral AI was positively related to size and inversely to CT attenuation. In patients with bilateral AI, abnormal DST and SH was even more prevalent. In this group, SH was positively correlated to the size of the larger AI, but not to its attenuation on CT.

Table 5 Indications and histological diagnoses in 16 patients undergoing adrenalectomy

Indication for operation	*n*	Histological diagnosis
Unilateral AI		
Subclinical hypercortisolism	4	Adrenal cortical adenoma 3
		Nodular hyperplasia 1
Suspected pheochromocytoma	4	Pheochromocytoma 2
		Adrenal cortical adenoma 2
Suspected malignancy	4	Adrenal cortical adenoma 2
		Nodular hyperplasia 1
		Myelolipoma 1
Primary aldosteronism	1	Nodular hyperplasia 1
Bilateral AI		
Subclinical hypercortisolism	3	Adrenal cortical adenoma 2
		Macronodular hyperplasia 1

In five of the operated patients with unilateral AI the maximal axial diameter was 4 cm or above. The size at CT of the AIs in the patient with bilateral AI diagnosed as macronodular hyperplasia was 4 and 5 cm

Difficulties in establishing a generally accepted criterion for SH in patients with AI are caused by several reasons. Most likely, there is a continuum from normal cortisol secretion to overt Cushing's syndrome. DST is used as a test for SH, however, different S-cortisol cut-off levels from 50 to 140 nmol/l has been applied, resulting in various frequency of SH and differences in sensitivity and specificity. The cut-off level for cortisol 50 nmol/l is commonly used as a screening test to exclude hypercortisolism, as well as to diagnose SH, while a higher cut-off level at 140 nmol/l is more specific for the Cushing's syndrome diagnose [26]. To improve accuracy in the diagnosis of SH, ACTH, 24 h urinary cortisol, DHEAS, midnight saliva—and serum—cortisol have all been suggested as complementary diagnostic measures [8, 27, 28].

Low, nearly suppressed ACTH, i.e. below 10 ng/l (2.2 pmol/l), has been used as a complementary criterion for SH [11, 17, 23, 27]. In our study, the frequency of ACTH below 2 pmol/l increased stepwise through the quartiles of suppressed cortisol at DST and only one of the 34 patients who suppressed cortisol to $\leq$32 nmol/l had basal ACTH $<$2 pmol/l. Further, the present data signalises that also some of those patients who suppressed cortisol to between 33 and 47 nmol/l (second quartile) had an impact on the pituitary–adrenal axis, probably due to subtle cortisol hypersecretion from an adrenal adenoma. As an additional indication on the inverse continuum between basal ACTH and post-DST cortisol, patients with ACTH between 2 and 3 pmol/l was an indeterminate group compared to those with ACTH above 3 pmol/l.

Since only 42 and 62% our patients, who had cortisol at DST between 47 and 71 nmol/l and $\geq$72 nmol/l (third and fourth quartile), respectively, had ACTH below 2 pmol/l, a single basal ACTH seems to have relative low sensitivity for detecting SH. On the other hand, our DHEAS analysis did not at all separate between the different DST cortisol suppression quartiles. This is not surprising, since DHEAS in our study was only standardised against gender but not against age. Altogether, these data illustrate the progressive disturbance of cortisol secretion in AI patients and support a role for basal ACTH as a complementary tool for the SH diagnose in addition to the DST. Thereby, the criteria selected to diagnose SH in this study, seems justified and should be useful for initial evaluation of patients with AI since it is relatively uncomplicated to perform. An easy to perform initial evaluation might reduce the risk that not all patients with AI are evaluated against SH [29].

In this study, despite the small size of the AI, no less than 42% of unilateral AI had inadequate suppression of cortisol at DST and as much as 23% had SH diagnosed by our criteria. We found no study as have evaluated DST

with a cut-off for cortisol at 50 nmol/l in consecutive patients with AI. In one study, 42 of 83 (52%) non-consecutive patients with AI were found to have cortisol 50 nmol/l or above at DST [30]. In recent studies in patients with unilateral AI using a combination of criteria the frequency of SH varied between 10 and 40%. The highest frequency (56.6%) was reported in a study using 48 h DST with a cut-off level for cortisol at 30 nmol/l which was mean +2 SD found in healthy controls [9]. Thus, the frequency of SH on 23% in this study is similar compared to the previous studies.

We found that the probability for SH correlated positively to the size of the AI and inversely to the attenuation of a unilateral AI on unenhanced CT. The correlation between SH and size is in accordance with previous studies [10, 24] but the relation to attenuation is a new finding. The large and lipid rich adenoma (>2.5 cm, ≤10 HU and/or ≥1.8 cm, <1 HU) had SH in 35–45% of the cases studied. Conversely, the small adenomas with high attenuation values (>10 HU) had a low risk. However, in AI with attenuation values >10 HU, the prevalence of SH was about 15% if the AI was larger than 2.5 cm which demonstrates that also lipid poor adenomas may produce cortisol [19]. The size of our study does, however, not allow us to define a cut-off level for size and attenuation when SH does not need to be suspected.

In this study, 19% of the patients had bilateral AI and this is a larger proportion than in most previous studies with a frequency of 6–17% [4, 5, 22, 31]. The larger of the bilateral AI was similar in size compared with the unilateral AIs. In former studies, bilateral AI has been reported to be larger as well as smaller compared to unilateral lesions, a variance difficult to explain [22, 23].

Interestingly, our patients with bilateral AI had a higher frequency of pathological DST and tended to more often fulfil the criteria for SH, 70 and 42%, respectively, as compared to unilateral AI, 42 and 23%, respectively. The frequency of SH correlated to size but not to attenuation of the larger of the bilateral AI. This is in accordance with a recent study as demonstrated that SH and aberrant cortisol response correlates to size in bilateral AI [32]. We found a strong correlation between the attenuation of the lesions in bilateral AI which gives support to the hypothesis of a different pathogenesis in bilateral AI compared to unilateral AI as have been suggested [33].

SH might have negative effects on metabolism and bone density [12–15]. In this study, the patients medication was recorded and blood pressure measured so we could examine the correlation to hypertension and also indirectly to some of the other metabolic complications. We found that arterial hypertension was more frequent in patients with SH, supporting a role of the increased cortisol secretion for blood pressure. The increase in frequency of hypertension was more marked when the oldest quartile of patients, that had a high frequency of hypertension independent of SH, was excluded. Surprisingly, a higher frequency of hypertension was seen in patients who suppressed cortisol between 33 and 46 nmol/l (second quartile) compared to the first quartile (Table 1). Furthermore, it seemed that the patients with cortisol post-DST ≥140 nmol/l had an even stronger influence on blood pressure compared to patients with cortisol post-DST 50–139 nmol/l. This might indicate that even very subtle cortisol secretion may have an effect on blood pressure and that the effect is progressive with increasing post-DST cortisol concentration. We found no impact on the use of medication for diabetes among patients with SH but the number of patients was small. There was a non-significant tendency to increased use of lipid lowering drugs in the patients with SH. The indication for adrenalectomy for SH is not defined but some experts recommend that it should be offered to patients with SH and complications as cannot be adequately controlled by medication [26]. Thereby, the level of cortisol at DST could be a maker for improvement after adrenalectomy.

The low occurrence of pheocromocytoma and malignant adrenal tumours among the present series of AI is in accordance with a recent study in 973 patients with AI with a mean size on 2.0 cm reporting no malignancies and 0.3% pheochromocytomas [6]. In this study, only 7% of the patients with AI underwent adrenalectomy, which is less compared with earlier studies [4, 5]. One reason could be that AIs were smaller and only 5% of unilateral AI was 4 cm or larger. In addition, 9 of the 15 patients with large AI did not undergo adrenalectomy for clinical reasons. The use of emerging diagnostic methods to exclude malignancy such as positron emission tomography might also contribute to the low frequency of adrenalectomy.

This study has some limitations. The multicenter setting increases the risk that not all patients with AI were included. The CT examinations were analysed in routine care but according to a defined protocol guaranteeing consistent evaluation. ACTH was analysed with different methods but the majority with the method with the best precision. Basal ACTH data was available in only 64% of the patients and these patients had larger adenomas. A potential bias of this and an incomplete registration might be an overestimation of the frequency of SH among AI, since SH is less frequent in smaller AI, which also can be anticipated to more easily be overlooked. However, since overall the AI were small in size, the result should to be representative, as supported by the finding of a SH frequency in accordance with previous studies. The specificity and sensitivity for the criteria for SH used is not known which could lead to both under and over diagnosing of SH. The present broad analysis of the relation between ACTH and the DST lends support to the

criteria used for the diagnosis of SH. Furthermore, the absence of SH in the patients with small high attenuating unilateral AI supports good specificity.

In summary, according to this new series of AI's using MDCT scanners, these tumours are mostly small in size and in the vast majority benign. The criterion for SH applied in this study should be useful for initial evaluation of patients with AI. SH is common in patients with AI and even more often present in patients with bilateral AI. In patients with unilateral AI the probability for SH correlated positively to size of the AI, and inversely to its attenuation at CT. Hypertension was more often present in patients with SH compared to AI patients with normal cortisol secretion. Furthermore, the impact of SH on the blood pressure seems to correlate with the post-DST cortisol concentration, which should be considered in the decision to perform further investigations. We hypothesise that, in unilateral AI, also the size and the attenuation at CT could be taken into account. So far, however, further studies are needed to explore whether the appearance at CT might be an important factor also for the development of complications associated to SH, beyond its relation to post-DST cortisol concentrations as observed in this study.

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Conflict of interest No conflict of interests to declare.

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