ORIGINAL ARTICLE

Adrenocortical adenomas with regression and myelolipomatous changes: urinary steroid profiling supports a distinctive benign neoplasm

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Summary

Background Adrenocortical neoplasms are classically divided into adenomas (ACA) and carcinomas (ACC). Heterogeneous appearance and greater size are criteria to suggest malignancy, along with the urinary steroid profile (USP). The presence of regression and myelolipomatous changes in adenomas (ACA-RML) can contribute to confusion with ACC and its USP remains unknown.

Objective To evaluate the features of ACA-RML in comparison with other adrenocortical neoplasms.

Methods We selected consecutive ACA (11), ACA-RML (7) and ACC (13) cases for which USP analysis was performed before surgery and tissue was available for histological evaluation (King’s College Hospital, 2005–2012). Cases were classified according to WHO and Armed Forces Institute of Pathology criteria. USPs were obtained by gas chromatography/mass spectrometry. Total excretion of individual steroids and indices (sums and ratios chosen to reflect steroid metabolic activity) were compared between ACA-RML, ACA and ACC.

Results In comparison with ACA, tumours in ACA-RML were significantly larger (8.5 ± 2.4 vs 3.5 ± 1.0 , $P = 0.002$), presented in older patients and showed relatively higher incidence in males. Mitotic figure counts were significantly lower (0.39 ± 0.04 vs 0.93 ± 0.11 in ACA, $P = 0.001$) and revealed higher frequency of apoptotic cells (100% vs 9% in ACA, $P = 0.001$). The USP of ACA-RML showed no diagnostic features of ACC. No differences from ACA were significant, but

there was a tendency towards lower dehydroepiandrosterone DHA and DHA metabolites.

Conclusions ACA-RML reveals distinctive histological features and lack of USP markers of malignancy. More cases of this rare tumour may confirm differences from ACA in steroid excretion. It is important to recognize ACA-RML because its size and heterogeneous appearance raise the possibility of ACC.

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Introduction

Adrenocortical proliferative lesions represent a heterogeneous group comprising nodular hyperplasias, adenomas (ACA) and carcinomas (ACC).^{1–4} Clues that should alert clinicians to the possibility of malignancy include the following: tumour size >4–5 cm in diameter, imaging features of calcification, blurred margins, an irregular shape, heterogeneous contrast enhancement and distant metastases on computed tomography, together with hormonal overproduction in more than one adrenocortical axis.^{5,6} In contrast, adenomas are smaller, smooth shaped and with homogeneous enhancement.

A minority of adenomas resemble carcinomas in being large, with heterogeneous appearance frequently related to regressive/degenerative changes, which contribute to a more difficult pre-operative diagnosis, especially when the changes are extensive. The criteria used to identify these changes are not clearly defined but include haemorrhage, fibrin deposition, interstitial hyalinization, cystic changes, adipose tissue infiltration and extramedullary hematopoiesis, with or without calcification or ossification.^{7,8} To date, adrenocortical adenomas with extensive regressive changes are extremely rare and are reported as single cases or very short series only.

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The adrenal cortex produces cortisol, corticosterone and C19 steroids. These are metabolized in the liver and the kidney and then excreted in the urine either in the form of free steroids or conjugated with sulphuric or glucuronic acid. The urinary steroid profile (USP) has proved to be very useful in the identification of carcinomas,^{9,10} but findings in adenomas with regression and myelolipomatous changes (ACA-RML) have not been previously reported. Here, we described clinical, histological and USP features of seven cases of this very rare adenoma subtype in comparison with those of conventional adrenocortical adenomas and carcinomas. We aimed to determine if the steroid profile in the ACA-RML group could be securely distinguished from ACC. We further compared this group with ACA and explored whether any differences found might be related to the histological changes.

Methods

Selection and description of participants

All adrenocortical proliferative lesions available at King's College Hospital (Dept Histopathology, 1988–2012) were reviewed, including nodular hyperplasias (44), adenomas (118) and carcinomas (28). From this series, extensive regressive myelolipomatous changes (present in at least one-third of the tumour) were observed in 7 ACA (2005–2012); erythroblasts and red blood cells closely collocated with macrophages containing hemosiderin with or without megakaryocytes were required in the focus to identify the myeloid component. During this period, all consecutive ACA (11), ACA-RML (7) and ACC (13) had urine steroid profiles performed before surgery and histopathology available for evaluation and were selected for this analysis. Myelolipomas in the context of hyperplastic conditions (such as congenital adrenocortical hyperplasia or Cushing's disease) were excluded from this study. Conventional ACA and ACC were used as benign and malignant controls, respectively.

The clinical syndrome was investigated in each case by standard clinical and laboratory approaches, including basal hormone determination, along with stimulation and suppression tests if necessary and imaging methods.^{4,11–13} The bare adrenal weight was also recorded in each case.

Histologic criteria and classification

All surgical specimens were serially sectioned and routinely processed for histological diagnosis and sampled with at least 1 block per cm of maximum lesion diameter. The cases were classified according to the World Health Organization and Armed Forces Institute of Pathology criteria^{12,13}; if there were proven metastases, they were classified as malignant. All haematoxylin and eosin-stained sections were evaluated histologically and graded by two of the authors (both pathologists, FAH and SDC) independently, using criteria previously described.³ In case of grading disagreement, the lesions were discussed during simultaneous inspection before final categorization. Reproducibility data

were not recorded. Standard scores for the classification of these lesions^{4,14,15} were also registered; as the benign group scores low figures (up to 2),^{14,15} no further analysis was performed with these scores in the distinction of ACA-RML from ACA and ACC.

Urinary steroid profiling

Examination of 24-h urinary steroid metabolite excretion was carried out using gas chromatography/mass spectrometry (GC/MS). A detailed description of this methodology has been published.¹⁶ In summary, free and conjugated steroids were extracted from 10 ml urine by solid-phase extraction. Steroid conjugates were enzymatically hydrolysed, reextracted, and chemically derivatized to form methyloxime–trimethylsilyl ethers. GC/MS was carried out on a Clarus 500[®] instrument (Perkin-Elmer, Waltham, MA, USA).

The steroid metabolites quantified in our study are listed in supplemental Table 1 and the calculated sums and ratios in supplemental Table 2. Levels of steroid metabolite excretion were expressed as µg/day. Supplemental Figure 1 shows the metabolic relationships of the studied steroids. Other steroids that have proved to be useful markers of ACC were also compared empirically between groups. These included tetrahydro-11-deoxycortisol (16), pregnene-3,16,20-triols (15), 16a- and 21-hydroxypregnenolone and tetrahydro-11-deoxycorticosterone.

Statistics

Total excretion of individual steroids and steroid indices were compared between ACA-RML, ACA and ACC groups using the Kruskal–Wallis nonparametric test; for significance, values of $P < 0.05$ and pair-wise comparisons were carried out using Dunn's *post hoc* test.

Results

Clinical characteristics of the patient groups are listed in Table 1.

Histological features

ACA-RML. (Figure 1) After removing the adipose tissue, the adrenal glands weighed 291.4 ± 366.3 g. Histologically, the peritumoral gland was atrophic in five cases (71%) and unremarkable in two cases (29%). The tumours showed an alveolar growth pattern in four cases (57%) and a trabecular pattern in 3 (43%). Cytologically, tumours composed of foamy clear cells predominated in this series (5 cases, 71%) and no pigment-containing cells were observed.

Small nonconfluent necrotic foci were present in two cases (29%) and isolated apoptotic cells in all seven cases (100%). No or minimal inflammatory response was found, predominantly lymphocytic (6 cases, 86%). Myxoid stromal reaction was observed in five cases (71%) and no desmoplastic tissue was observed. No capsular, lymphovascular, sinusoidal or

Table 1. Clinical findings of patients with adrenocortical adenoma (ACA), adrenocortical adenoma with regression and myelolipomatous changes (ACA-RML) and adrenocortical carcinomas (ACC)

	ACA (n = 11)	ACA-RML (n = 7)	ACC (n = 13)
Age at presentation (years), Av $\pm$ SD (Range)	54.3 $\pm$ 12.35 (38–70)	70.14 $\pm$ 8.84 (57–79)	51.7 $\pm$ 16.2 (26–76)
Sex	F – 9 (82%)	F – 3 (43%)	F – 9 (69%)
Female (%), male (%)	M – 2 (18%)	M – 4 (57%)	M – 4 (31%)
Size of adrenal nodule (cm), Av $\pm$ SD (Range)	3.5 $\pm$ 1.0 (2.5–6)	8.5 $\pm$ 2.4 (5.5–13)	13.9 $\pm$ 4.8 (7.0–25.0)
Endocrine syndrome at presentation			
Cushing's	9	5	3
Conn's	2	0	0
Mixed (androgen, glucocorticoid)	0	0	3
Mass only	0	2	9
Weiss score * Median (Range)	1 (0–2)	1 (0–2)	6 (3–9)

*The Weiss system scores the presence or absence of nine histopathological features (Weiss score range 0–9); scores under 3 are indicative of a benign adrenal tumour, scores of 3 and above are indicative of malignancy¹⁴. As Weiss scores are pure diagnostic markers and provided no additional prognostic information, no further investigation was carried out with them^{14,15}.

perineural invasion was identified. Haemorrhage and stromal hyalinization with myelolipomatous changes as inclusion diagnostic criteria were present in at least 1/3 of the neoplasm in all seven cases.

Nuclear features disclosed low grade (6 cases, 86%), prominent anisokaryosis (6 cases, 86%), mild pleomorphism (5 cases, 71%), fine chromatin distribution (5 cases, 71%), inconspicuous nucleoli (6 cases, 86%), and low nucleus–cytoplasm ratio (4 cases, 57%). Nuclear pseudoinclusions were observed in 2 cases (29%) and multinucleated cells in 3 cases (43%). The mitotic figure (MF) counting was 0.39 ± 0.04 MF/50 HPF with no atypical mitoses.

Aca. After removing the adipose tissue, the adrenal glands weighed 47.9 ± 92.6 g. Histologically, the peritumoral gland was atrophic in nine cases (82%) and unremarkable in two cases

(18%). The tumours showed an alveolar growth pattern in four cases (36%) and a trabecular pattern in 4 (36%), and were nodular in 3 (28%). Cytologically, tumours composed of foamy clear cells predominated in this series (7 cases, 64%) and two cases (18%) had pigment-containing cells.

Small nonconfluent necrotic foci were present in one case (9%) and occasional isolated apoptotic cells in one case (9%). No or minimal inflammatory response was found, predominantly lymphocytic (10 cases, 91%). A myxoid stromal reaction was observed in two cases (18%) and no desmoplastic tissue was observed. No capsular, lymphovascular, sinusoidal or perineural invasion was identified.

Nuclear features disclosed low grade (9 cases, 82%), prominent anisokaryosis (10 cases, 91%), mild pleomorphism (8 cases, 73%), fine chromatin distribution (7 cases, 64%), inconspicuous nucleoli (9 cases, 82%), and low nucleus–cytoplasm ratio

Fig. 1 Pathologic features of ACA-RML. This ACA subtype revealed haemorrhagic and fibrinous foci irregularly distributed, along with areas of adipose tissue (a). These findings were confirmed histologically by the presence of trabecular-nested cortical tumour cells, along with interstitial mature adipose tissue and myeloid elements (b, HE 100x), hyalinized eosinophilic material (c, HE 100x) and erythroblasts (d, HE 200x).

(6 cases, 55%). Nuclear pseudoinclusions were observed in three cases (27%) and multinucleated cells in five cases (45%). The MF counting was 0.93 ± 0.11 MF/50 HPF with no atypical mitoses.

Acc. After removing the adipose tissue, the adrenal glands weighed 470.0 ± 590.8 g. Microscopically, the peritumoral gland was atrophic in 1 case (8%), hyperplastic in 1 (8%) and unremarkable in 11 cases (84%). The tumours displayed diffuse growth patterns in eight cases (61%), insular in 3 (23%), nodular in 1 (8%) and trabecular in 1 (8%). Cytologically, all tumours predominantly revealed eosinophilic cells. Cytoplasmic pigment was seen in one case (8%).

Tumour cell necrosis was frequently observed (9 cases, 69%), with confluent (6 cases, 46%) or perithelial patterns (3 cases, 23%). In addition, apoptotic cells were present in 10 cases (77%), but associated with lymphocytic satellitosis in only four cases (31%). The inflammatory response was minimal (10 cases, 77%), predominantly lymphocytic (6 cases, 55%). Stromal reactions were observed in 10 cases (77%), desmoplastic in 9 (69%) and myxoid in 1 (8%). Local staging showed extracapsular extension in seven cases (54%), blood vessel invasion (capsular in 6 cases, 43.8% and extracapsular in 2, 15%). Only one case (8%) showed perineural invasion.

Nuclear features displayed high grade (12 cases, 92%), prominent anisokaryosis (13 cases, 100.0%), severe pleomorphism (12 cases, 92%), coarse chromatin (10 cases, 77%), inconspicuous nucleoli (10 cases, 77%) and low nucleus–cytoplasm ratio (9 cases, 69%). Nuclear pseudoinclusions were present in three cases (23%) and multinucleated cells in 4 (31%). The MF counting was 17.38 ± 0.57 MF/50 HPF with atypical mitoses in nine cases (69%, indexing 1.95 ± 2.94 /50 HPF).

Urinary steroid profiling

The USP of ACA-RML revealed no unusual patterns and the cases clustered with ACA and not with ACC. When compared with ACA (regardless of the inclusion or not of ACA with

Conn's syndrome), no statistically significant differences for any individual steroid or index could be demonstrated. However, there appeared to be a trend for ACA-RML towards decrease in dehydroepiandrosterone (DHA) and DHA metabolites (Fig. 2) whether graphed as individual steroids, expressed as a total of DHA and DHA metabolites or as a ratio related to total cortisol metabolites (Fig. 3). 16α -Hydroxy DHA showed a greater difference than DHA, suggesting a decrease in 16α -hydroxylation activity in ACA-RML. However, a ratio of 16α -hydroxylated vs. non- 16α -hydroxylated DHA metabolites was not different between ACA-RML and ACA.

Urinary steroid profiling analysis revealed statistically significant differences between both ACA groups (i.e.: ACA and ACA-RML) and the ACC group. ACC patients had higher excretion of numerous steroids, especially metabolites of steroid pathway intermediates. Those with the greatest differences are shown in Fig. 4, while the differences for DHA and DHA metabolites were also all significant (Fig. 3).

Discussion

ACA-RML represents a distinctive benign adrenocortical neoplasm for which the USP findings cluster with ACA. Although both the variability and rarity of this condition preclude firmer conclusions, the USP suggests an impaired DHA metabolic pathway with relatively preserved cortisol biosynthesis. Both age and ACTH (adrenocorticotroph hormone) suppression can contribute to this finding. Secretion of adrenal steroids, especially DHA and its sulphate, declines with age.^{10,17} Given that the ACA-RML group is older than the ACA group (Table 1), it is possible that the decrease in total C19 steroid sulphate (C19SS) metabolites and the increased cortisol metabolites (FM)/C19SS ratio are age related. However, sample numbers were too few to be able to apply procedures such as stepwise regression analysis to examine the influence of age. No glucocorticoid suppression testing was undertaken in the ACA-RML group, so the contribution of the tumour tissue to steroid secretion cannot be estimated. Likewise any

Fig. 2 C19SS metabolic pathway.

Fig. 3 Comparison of patient groups: C19 steroid sulphates metabolites: -ACA vs ACC: * $P < 0.05$, ** $P < 0.01$. -ACA-RML vs ACC: ** $P < 0.01$, *** $P < 0.001$. ▲, ▲▲, ▲▲▲: Not significant for the comparison ACA vs ACA-RML. Only asterisks comparison were significant, all remaining (triangles) were found not significant.

contribution of tumour tissue to further metabolism of steroids arising in normal adrenocortical tissue cannot be gauged. In our series, the majority of the ACA and ACA-RML had ACTH-independent Cushing's syndrome. As DHA is secreted from the normal cortex and the abnormal secretion is usually along a single pathway (glucocorticoid, mineral cor-

ticoid or androgen), low DHA and DHA metabolites levels may reflect ACTH suppression; however, ACTH independent mechanisms must also be considered from secretory adrenal neoplasms. Conversely in the ACC, it is common for multiple steroidogenic pathways to be upregulated independently of ACTH, hence the different profile. However, the values are

Fig. 4 Comparison of patient groups: steroid precursor metabolites: -ACA vs ACC: *** $P < 0.01$, *** $P < 0.001$. -ACA-RML vs ACC: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. ▲, ▲▲, ▲▲▲: Not significant for the comparison ACA vs ACA-RML. Only asterisks comparison were significant, all remaining (triangles) were found not significant.

much lower for ACA-RML and they cannot be explained by ACTH suppression only. Low secretion in DHA and metabolites in ACA-RML have a multifactorial origin. It cannot be argued that all DHA is from the normal cortex and that it will be due to ACTH suppression because Cushing's syndrome was not universally identified and cortical atrophy was not present in all cases: two patients presented with adrenal mass only. In addition, any regression analysis of this very rare subtype of ACA (with a reduced number of cases) will not result in any statistically significant differences. Considering the proportionally big size of these neoplasms, the ischaemic changes associated with the mass effect (stromal hyalinization, oedema) will be a major contributor to this low secretion, especially in combination with age,^{17,18} incomplete ACTH intratumoral functional activity¹⁹ and oxidative effect of mononuclear bone marrow cells.^{20–22}

ACA is a proliferative lesion with low cellular turnover, along with heterogeneous kinetic and clonal profiles.^{23–25} The mitotic figure counting was significantly lower in ACA-RML than in ACA and revealed a higher frequency of apoptotic cells. This kinetic pattern corresponds with the right tail of regressive monoclonal ACA (low proliferation and high apoptosis), which frequently shows interstitial haemorrhage and myelolipomatous changes.^{23,24,26} These added interstitial features give rise to lesions greater in size and with heterogeneous appearance.⁸ ACA-RML tumours were of significantly larger size than ACA, presented in older patients and showed a similar incidence in males and females, unlike the common epidemiology in ACA.

USP profiles of ACA-RML do not show any of the ACC markers previously reported, such as increased steroid precursor metabolites (pregnenediol and pregnenetriol) or increased DHA metabolites.²⁷ The evaluation of USP in adrenocortical neoplasms includes the identification of abnormal metabolites and/or the presence of a heterogeneous background associated with partial enzymatic deficiencies; the former findings being more accurate predictors of a malignant diagnosis. The pathological heterogeneity contributes to this complexity, but no single marker or value on its own is pathognomonic of this disease. Although USP will be more widely used in the future, considering the low ACC incidence and its heterogeneity with several patterns, it is more likely that accurate interpretations would be more reliable from specialized centres.

Although no significant differences between the ACA-RML and the ACA groups were found, there is an apparent decrease in the C19SS metabolic pathway in the group of adenomas with regressive changes. ACA-RML typically presented in older patients (as compared to both ACA and ACC), with large adrenal masses that reveal some degree of radiological heterogeneity. These features lead to investigate a potentially malignant condition, for which the urine steroid profile can be valuable. Our results probed the absence of positive malignant markers to support a benign lesion. Like with other diagnostic tests, it cannot be expected 100% specificity and/or sensitivity and that is our experience with the USP for the diagnosis of ACC. For that reason, observation only would be advised with caution as we can be dealing with the odd case, and the surgical intervention must

be individually evaluated and, if necessary, a less radical excision should always be considered. Although the absence of markers will not definitely exclude a malignant lesion, a less radical surgical intervention can be planned in advance; this is a more desirable option for elderly patients who are the most frequently affected by this tumour type.

In conclusion, ACA-RML reveals distinctive histological features, along with a regressive kinetics profile that may co-segregate with decreased DHA synthesis. Importantly, they lack USP markers of malignancy, so that clear distinction from ACC is possible without surgical intervention. These conditions are radiologically similar, so USP findings can be critical in determining treatment strategy.

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Conflict of interest

The authors of this manuscript have no competing financial interests. They have no commercial associations that might create a conflict of interest in connection with the submitted manuscript.

The protocol used in the study was approved by the Hospital Research Board and Ethical Committees and complied with their requirements.

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Supporting Information

Additional supporting information may be found in the online version of this article at the publisher's website.

SUPPLEMENTARY TABLES

Table S1. Steroid metabolites quantitated and metabolic pathways

Trivial name
1. Androsterone
2. Etiocholanolone
3. Dehydroepiandrosterone
4. 17 α -Androstenediol
5. 17 β -Androstenediol
6. 16 α -Hydroxydehydroepiandrosterone
7. Androstenetriol
8. 11-Oxoetiocholanolone
9. 11-Hydroxyandrosterone
10. 11-Hydroxyetiocholanolone
11. Pregnanediol
12. Pregnanetriol
13. Pregnenediol
14. Pregnenetriol
15. 17 α -Hydroxypregnanolone
16. 11-Oxo-pregnanetriol
17. Tetrahydro-11-dehydrocorticosterone
18. Tetrahydrocorticosterone

19. Allo-tetrahydrocorticosterone
20. Tetrahydrocortisone
21. Tetrahydrocortisol
22. Allo-tetrahydrocortisol
23. Cortisol
24. 6 β -Hydroxycortisol
25. α -Cortolone
26. β -Cortolone
27. β -Cortol
28. α -Cortol

Table S2. Calculated sums and ratios.

Ratio	Abbreviation	Formula
Androgen metabolites	AM	1+2+3
Cortisol metabolites	FM	20+21+22+25+26+27+28
Corticosterone metabolites	BM	17+18+19
Cortisone metabolites	EM	20+25+26
C19 Steroid sulphates	C19SS	3+4+5+6+7
11-Hydroxyandrosterone/ Cortisol metabolites	11A/FM	(9)
		(20+21+22+25+26+27+28)
11-Hydroxyetiocholanolone/ Cortisol metabolites	11Ae/FM	(10)
		(20+21+22+25+26+27+28)
11-Hydroxyandrostenedione metabolites/Cortisol metabolites	11As/FM	(8+9+10)
		(20+21+22+25+26+27+28)
5 α reduced/ 5 β reduced Androstenedione metabolites	A/Ae	(1)
		(2)
5 α reduced/ 5 β reduced 11-Hydroxyandrostenedione metabolites	11A/11Ae	(9)
		(10)
11-oxo/ 11-hydroxy	11oxo/11OH	(8)

Androstenedione metabolites		(9+10)
Non 16-hydroxylated DHA metabolites/ 16-hydroxylated DHA metabolites	Non/ 16OHDHAM	(3+4+5)
		(6+7)
20α reduced/ 20β reduced Cortisol metabolites	20α/20β	(25+28)
		(26+27)
20-oxo Cortisol metabolites/ 20-hydroxy Cortisol metabolites	20oxo/20OH	(20+21+22)
		(25+26+27+28)
5α reduced/ 5β reduced Cortisol metabolites	aTHF/THF	(22)
		(21)
Tetrahydrocortisone/ Tetrahydrocortisols	THE/THFs	(20)
		(21+22)
17-Hydroxyprogesterone metabolites/Cortisol metabolites	17PM/FM	(12+15)
		(20+21+22+25+ 26+27+28)
Cortisol metabolites/ Androgen metabolites	FM/AM	(20+21+22+25+ 26+27+28)
		(1+2+3)
Cortisol metabolites/ C19 steroid sulphates	FM/C19SS	(20+21+22+25+ 26+27+28)
		(3+4+5+6+7)
Cortisone metabolites/ Cortisol metabolites	EM/FM	(20+25+26)
		(21+22+27+28)
Cortisol metabolites/ Corticosterone metabolites	FM/BM	(20+21+22+25+ 26+27+28)
		(17+18+19)