Research Article

Recurrence and mortality in adulthood adrenocortical tumors: analysis from the Brazilian National Institute of Cancer experience.

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Abstract

Introduction: While adrenocortical adenomas (ACA) are usually of indolent course, carcinomas (ACC) are very aggressive, extremely rare and harbor a poor prognosis. This study aimed to describe the experience of a high-complexity cancer center in the management of adrenocortical tumors (ACT) and additionally to identify clinical and pathology prognostic factors of recurrence and death in this population.

Methods: Clinical, pathology, demographic, staging, and therapy data from patients with adulthood ACT treated at the Brazilian National Institute of Cancer between 1997 and 2015 were assessed in this retrospective study. Univariate and bivariate analysis were used to study the association of clinical and pathology characteristics with recurrence and mortality. Recurrence and disease-related mortality were the main outcomes.

Results: Twenty-six patients with adrenocortical carcinoma (ACC) and 22 with adrenocortical adenoma (ACA) were included. The median tumor size was 14 cm and 3.1 cm respectively. Complete resection was achieved in only 13 patients (50%) with ACC. Mitotane was the most common adjuvant/palliative therapy (n=15). Recurrence occurred in six ACC patients, after a median time of 1.7 year. Thirteen (54%) patients with ACC died from the disease. Advanced stage ($p<0.001$), metastatic disease ($p=0.004$), incomplete tumor resection ($p=0.04$), and capsular invasion ($p=0.04$) were all associated with increased death risk.

Conclusion: Complete tumor resection remains the only potential curable strategy for ACC patients. Therefore, prompt diagnosis of malignant adrenocortical tumors is essential.

Key words: adrenal tumors, surgery, prognosis, cancer staging.

INTRODUCTION

Adrenocortical carcinomas (ACC) are extremely rare, with a believed incidence of 1 – 2 cases per million individuals (1). Although the incidence of ACC has not significantly changed over last decades, the diagnosis of small non-functioning adenomas has substantially increased, mainly due the increase of availability of imaging techniques as computed tomography and magnetic resonance imaging (2, 3).

Most adrenal incidentalomas are represented by these small benign adenomas with indolent clinical course, meanwhile ACC harbor unfavorable prognosis with up to 30% of patients presenting with metastatic disease (4). For this reason, prompt diagnosis of malignant tumors is essential. Even though, distinguishing adenomas from carcinomas can be often challenging since ACC pathogenesis and identification of reliable clinical and biological prognostic factors are continued areas of uncertainty (4). In this context, pathology scores, immunohistochemical staining markers, genome-risk profiles, and clinical staging systems has emerged in order to optimize management and increase survival chance of ACC patients (5-10).

This study aimed to describe outcomes in patients with ACT treated at a single high-complexity oncology institution between 1997 and 2015, and additionally to identify clinical and pathology prognostic factors for recurrence and mortality in this population.

METHODS

Study design and population

This observational and retrospective study was conducted by the Endocrine Oncology Unit in collaboration with the Division of Pathology (DIPAT) and the Department of Clinical Oncology from the Brazilian National Institute of Cancer – INCA, Rio de Janeiro, Brazil. Cases were identified from the DIPAT laboratory information system, which has been coding all cases since 1997. After filtering for adrenal cortex localization and age $\geq$ 18 years at diagnosis, all cases registered from January 1st, 1997 and March 31st, 2015 were considered eligible. Exclusion criterion was evidence of histological diagnosis other than ACT.

Ethical aspects

This study was approved by INCA´s independent institutional advisory committee in September 24th, 2014 (protocol 33847514.4. 0000.5274) and conducted according to the principles expressed in the Declaration of Helsinki. All involved subjects provided informed consent before study start, so investigators could have access to their medical records and pathology species (slides and formalin-fixed paraffin-embedded tissues).

Procedures

Pathology diagnosis review: In order to confirm the ACT diagnosis, original hematoxylin-eosin stained slides from resected or biopsied cases were initially reviewed by the same two investigators (D.B., P.A.S.F.). After confirmation, the presence of each of the nine following histological features were evaluated and the Weiss-score (11) calculated: nuclear atypia [grade III/IV according to Fuhrman criteria (12)]; increased mitotic rate [more than five mitotic figures in 50 high-power fields (40x objective)]; atypical mitotic figure; eosinophilic ("dark") cytoplasm in at least 75% of tumor cells; diffuse pattern in tumor architecture; microscopic tumor necrosis; unequivocal venous invasion; sinusoidal invasion; and tumor capsule invasion. Patients only submitted to biopsy were excluded from Weiss-score analysis.

Ki-67 immunohistochemistry: Four micron-thick serial sections of formalin-fixed and paraffin-embedded tissues were manually immunostained by applying the NovoLink Polymer Detection System (Leica Biosystems Newcastle Ltd, Newcastle Upon Tyne, UK). For epitope retrieval, a high-temperature (97º C) technique with steamer and Trilogy buffer (Cell mark Inc, Rocklin, USA) was employed. After heating, the slides were allowed to cool down to room temperature during 20 minutes and briefly washed with running water. The sections were incubated in 3% hydrogen peroxide (Novocastra Peroxidase Block, Novocastra Leica Microsystems) to neutralize endogenous peroxidase activity; treated with Novocastra Protein Block to reduce nonspecific binding of primary antibody and polymer; and overnight incubated at 4°C with monoclonal mouse anti-human Ki-67 (clone MIB-1) primary antibodies (Dako, Glostrup, Denmark) diluted at 1:600. At the following day, sections were treated with Novocastra Postprimary Block, containing 10% (v/v) animal serum in tris-buffered saline, to enhance penetration of the subsequent polymer reagent. Poly-HRP anti-mouse/rabbit IgG

Table 1: Demographical, clinical, tumor presentation, histology and treatment modalities from included cases

Clinical characteristic	**N***	**Carcinoma**	**N***	**Adenoma**	**P**
Age, yr Mean (SD) Median (min-max)	26	 45.2 (16.1) 41.3 (22 – 77)	22	 51.5 (13.2) 53.4 (29 – 73)	0.15
Female, n (%)	26	18 (69.2%)	22	16 (72.7%)	0.79
Obesity, n (%)		3 (18.8%)	14	6 (42.9%)	0.24
Diabetes mellitus, n (%)	19	5 (26.3%)	20	2 (10%)	0.23
Hypertension, n (%)	21	14 (66.7%)	21	12 (57.1%)	0.52
Initial clinical presentation, n (%) Incidentaloma Virilization Hypercortisolism Mass effect [a] Incidental finding in malignancy set-up [b]	26	 2 (7.7%) 1 (3.8%) 5 (19.2%) 17 (65.4%) 1 (3.8%)	22	 7 (31.8%) 0 1 (4.5%) 3 (13.6%) 11 (50%)	
Palpable tumor, n (%)	18	9 (50%)	22	1 (4.5%)	**0.002**
Previous malignancy, n (%)	23	2 (8.7%)	22	11 (50%)	**0.002**
Malignancy family history [c], n (%)	21	11 (52.4%)	18	13 (72.2%)	0.32
Time between symptoms and diagnosis, mo Mean (SD) Median (min-max)	20	 10.5 (9.3) 8.2 (0.1 – 29)	15	 6.1 (4.9) 3.9 (0.6 – 13)	0.79
Tumor localization, n (%) Right adrenal Left adrenal	26	 13 (50%) 13 (50%)	22	 10 (45.5%) 12 (54.5%)	0.75
Tumor largest diameter, cm Median (min-max)	23	 14 (3 – 22)	19	 3.1 (2.2 – 7.4)	**< 0.001**
Tumor volume, cm^3 Median (min-max)	17	 540 (50 – 2288)	13	 10.6 (1.6 – 55)	**< 0.001**
Ki-67 labeling index, % Median (min-max)	24	 19 (0 – 90)	19	 0.5 (0 – 8)	**< 0.001**
Distant metastasis, n (%)	26	8 (30.8%)	-	-	**-**
Surgery performed, n (%) Complete resection Macroscopic residual tumor Biopsy	26	 13 (50%) 6 (23.1%) 7 (26.9%)	22	 22 (100%) 0 0	
Adjuvant and palliative therapy, n (%) Mitotane alone Mitotane + Citotoxic chemotherapy Citotoxic chemotherapy alone Radiotherapy	25	 10 (40%) 5 (20%) 0 1 (4%)	22	-	

* Number of subjects in which variable was assessed; [a]Abdominal enlargement and/or abdominal pain; [b] Incidental finding in patients with previous diagnosis of cancer (other than adrenal) during initial staging or post-therapy surveillance; [c] Among first-degree relatives.

reagent (NovoLink Polymer) containing 10% (v/v) animal serum in tris-buffered saline was applied to localize the primary antibody, and the reaction product was visualized by incubation with the substrate/chromogen, 3,3'-diaminobenzidine (DAB) prepared from Novocastra DAB Chromogen and NovoLink DAB Substrate Buffer (Polymer), as a brown precipitate. Finally, the sections were counterstained with hematoxylin (0.02%). Human breast cancer sections were used with each run as a positive control, while negative controls were performed by omitting the application of primary antibody. The Ki-67 labeling index was defined as the percentage of positively stained neoplastic cells among the total number of neoplastic cells.

Clinical data review: Medical records from all patients included were reviewed and a specific questionnaire was completed with demographic information, disease presentation characteristics and data related to therapy and follow-up. The following variables were registered: a) sex; b) age at diagnosis; c) obesity (defined as a body mass index $\geq$ 30 Kg/m^2); d) first-degree relative cancer history; e) initial clinical symptoms; f) time until diagnosis (defined as the interval between initial symptoms and first medical evaluation); g) evidence of endocrine syndrome defined by an unequivocal description of signs or symptoms compatible with autonomous hormone secretion, and/or by evidence of age-sex-specific elevated levels of serum total testosterone, androstenedione or dehydroepiandrosterone sulphate (DHEA-S) or laboratorial evidence of hypercortisolism (impaired dexamethasone low-dose suppression test or elevated late-night salivary cortisol); h) tumor´s largest diameter and fibroid volume (formula = diameter a · diameter b · diameter c · constant 0.523); i) localization (laterality); j) computed tomography or magnetic resonance imaging evidence of para-aortic lymphatic and/or distant metastatic involvement, k) disease´s stage according to the European Network for the Study of Adrenal Tumors (ENSAT) and the Union for International Cancer Control (UICC) staging systems; l) initial treatment; m) adjuvant or palliative therapies; n) performed procedure (complete or incomplete tumor resection, or only biopsy); o) report of tumor spillage during surgery; and p) radiotherapy.

Diseases definitions

ACC was defined as those tumors possessing three or more pathology features according to Weiss-score system (11). Also were considered as ACC patients those harboring tumors considered unresectable but with biopsy confirming an adrenal origin, regardless the presence of distant metastasis. Tumors with Weiss-score of 0-2 were considered as ACA.

Outcomes

Recurrence and mortality among ACC patients were the main outcomes. Recurrence was defined as evidence of a new local or distant expansive mass in a previously considered free-of-

Table 2: Weiss system score and age according to staging systems

UICC	**Weiss-score Median (min-max)**	**Age, yr Median (min-max)**	**ENSAT**	**Weiss-score Median (min-max)**	**Age, yr Median (min-max)**
I, n=1	4 (4)	45	I, n=1	4 (4)	45
II, n=12	5 (3 – 8)	36 (30 – 66)	II, n=12	5 (3 – 8)	36 (30 – 66)
III, n=2	7 (6, 8)	24 (22, 27)	III, n=5	7 (6 – 8) [b]	27 (22 – 66)
IV, n=11	7 (5 – 9) [a]	60 (21 – 76)	IV, n=8	7.5 (5 – 9) [c]	60 (21 – 76)

UICC: Union for International Cancer Control; ENSAT: European Network for the Study of Adrenal Tumors; [a] n= 5, Weiss-score was not assessed in six cases only submitted to biopsy; [b] n=3, Weiss-score was not assessed in two cases only submitted to biopsy; [c] n=4, Weiss-score was not assessed in four cases only submitted to biopsy.

disease patient. Time until recurrence (defined as the interval between complete tumor resection and image confirmed relapse), disease-specific mortality and overall survival were assessed.

Statistical analysis

Analyses were performed using SPSS version 20.0 for Macintosh (IBM SPSS Statistics, Armonk, NY, USA). In descriptive analysis, categorical variables were expressed as percentages while numerical variables were expressed as mean ± (standard deviation) and/or median (minimum - maximum). The Kolmogorov-Smirnov test was used to evaluate whether numeric variables were normally distributed. The Kruskal-Wallis test was used to compare numerical variables among three or four groups and the Mann–Whitney test was performed for comparison between two groups. Means of normally distributed variables were compared using Student t-test. Chi-square or Fisher exact tests were used to evaluate categorical variables. A p-value < 0.05 was considered statistically significant, except for comparisons among more than two groups, when p-values < 0.017 (3 groups) or < 0.013 (4 groups) were considered significant (Bonferroni post hoc analysis). Kaplan-Meier´s survival analysis from the entire population according to UICC and ENSAT staging systems was assessed. Data was analyzed up to 31st December 2015.

RESULTS

Population characteristics

Forty-eight patients with adrenocortical tumors were included. According to Weiss-score system 22 tumors were defined as ACA [mean score 0.2 ± 0.5; median 0 (0 – 2)] and 26 as ACC [mean score 6.1 ± 1.7; median 6.5 (3 – 9)]. No case of misdiagnosed tumor (adrenal tumor other than adrenocortical) was identified. Median follow up time for survivors patients was of 48 months (1 – 159), with 63 months (7 – 159) for ACA and 39 months (1 – 130) for ACC, p=0.73. Data on endocrine syndrome evidence were available in 9 cases of ACA and in 15 of ACC. Considering valid cases, 33% of ACA and 86.7% of ACC were considered autonomously functional tumors. Length of hospitalization for surgery was significantly higher in ACC than in ACA patients [median 7 days (2 - 87) versus 2 days (0 – 17), p=0.001]. In terms of surgical technique, 17 patients with ACA were submitted to a laparoscopic approach, while it was performed in only two patients with ACC. In both cases the preoperative diagnosis of ACC was unlikely, and tumor size was 7cm and 3cm, respectively. Additional data on clinical and tumor characteristics are summarized in table 1.

Tumor size was significantly larger in ACC (95% confidence interval of the difference 6.5 – 10.8 cm). Weiss-score pathology features could be reviewed in all surgically treated cases (22 adenomas and 20 carcinomas). The UICC and ENSAT staging systems were applied in all ACC cases. No association between age and Weiss-score was found when cases were categorized into staging groups of both systems (p=0.08 and 0.17 respectively) – table 2.

Mitotane was the most common adjuvant/palliative prescribed therapy, either alone or in combination with conventional chemotherapy. Etoposide, cisplatin and doxorubicin scheme was used in five patients and different other schemes in the other two. Only one patient received 45 Gy of palliative external-beam radiotherapy.

Recurrence prognostic factors

No recurrence was observed among patients with ACA. ACC patients only submitted to biopsy (n=7) or with macroscopic residual tumor after initial surgery (n=6) were excluded from recurrence analysis. Recurrence occurred in six ACC patients (46%), after a mean time of 28.2 ± 22.8 months [median 20.7 (3.8 – 70)]. Three cases presented with distant recurrence (two with lung, and one with liver metastasis) and three with locoregional disease. Two patients with local recurrence and one with lung metastasis were submitted to surgery as rescue therapy after a disease-free interval (DFI) of 70; 36 and 20 months respectively. All were considered free from disease after 4.6; 5.0 and 5.6 years after therapy respectively. One patient with multiple lung micrometastasis (DFI: 21 months) was referred to exclusive mitotane palliative therapy, being in use of the drug 1.5 year after recurrence diagnosis. The remaining two patients [one with local recurrence (DFI: 4 months) and other with liver metastasis (DFI: 18 months)] died one and 19 months after recurrence diagnosis. Except for one stage III (UICC and ENSAT), all recurrences occurred in stage II patients. No recurrence was observed among stage I. No demographic, tumor, therapy or pathology related features were related to recurrence risk (tables 3 and 4). Sub analysis from stage II group (n=12) found no significant difference between

Table 3: Recurrence and mortality prognostic factors - clinical variables

	N*	Recurrence	No recurrence	P	N*	Non-survivors	Survivors	P
N	13	6	7	-	24	13	11	-
Age, yr Mean (SD)	13	39.5 (12.7)	43.9 (13.6)	0.56	24	47.0 (19.4)	44.7 (11.8)	0.72
Gender, n (%) Female Male	13	5 (83.3%) 1 (16.7%)	4 (57.1%) 3 (42.9%)	0.56	24	9 (69.2%) 4 (36.4%)	7 (63.6%) 4 (30.8%)	1.00
Tumor localization, n (%) Left adrenal Right adrenal	13	3 (50%) 3 (50%)	4 (57.1%) 3 (42.9%)	1.00	24	7 (53.8%) 6 (46.2%)	5 (45.5%) 6 (54.5%)	1.00
Obesity, n (%)	7	2 (100%)	5 (100%)	-	16	2 (22.2%)	1 (14.3%)	1.00
Palpable tumor, n (%)	9	2 (66.7%)	2 (33.3%)	0.52	17	5 (71.4%)	4 (40%)	0.33
Positive family history for cancer, n (%)	11	3 (60%)	3 (50%)	1.00	21	7 (58.3%)	4 (44.4%)	0.67
Para-aortic lymphatic involvement, n (%)	13	0 (0%)	0 (0%)	-	24	2 (15.4%)	0 (0%)	0.48
Distant metastasis, n (%)	-	-	-	-	24	7 (53.8%)	0 (0%)	**0.006**
Liver metastasis, n (%)	-	-	-	-	24	6 (46.2%)	0 (0%)	**0.01**
Lung metastasis, n (%)	-	-	-	-	24	3 (23.1%)	0 (0%)	0.08
Time until diagnosis, mo‡ Mean (SD)	10	2.5 (2.1)	11.2 (7.8)	0.10	18	11.8 (11.2)	9.2 (8.0)	0.58
Incomplete tumor resection, n (%)	-	-	-	-	18	4 (57.1%)	1 (9.1%)	**0.04**
Tumor spillage during surgery, n (%)	5	1 (50%)	0 (0%)	0.40	9	2 (50%)	2 (40%)	1.00
Recurrence, n (%)	-	-	-	-	13	2 (66.7%)	4 (40%)	0.55
Mitotane use, n (%)	13	4 (66.7%)	2 (28.6%)	0.28	23	8 (66.7%)	6 (54.5%)	0.68
Cytotoxic chemotherapy, n (%)	13	1 (16.7%)	0 (0%)	0.46	23	3 (25%)	2 (18.2%)	1.00

* Number of subjects in which variable was assessed; ‡ Time between initial symptoms and diagnosis.

patients who suffered recurrence or did not in terms of age (p=1.00), gender (p=1.00), tumor size, total Weiss-score (and prevalence of each individual feature) (p=1.00), Ki-67 labeling index (p=1.00), tumor's side (p=0.57), and mitotane therapy (p=1.00) – table 5.

Mortality prognostic factors

Data on survival was not assessed in two ACC and three cases of ACA patients because of lost of follow-up. Thirteen ACC patients (54.2%) died from disease during follow up, with a mean survival time

Table 4: Recurrence and mortality prognostic factors – pathology variables

	N*	Recurrence	No recurrence	P	N*	Non-survivors	Survivors	P
Weiss-score Median (min - max)	13	5.5 (3 – 8)	5 (4 – 8)	0.63	18	8 (4 – 9)	5 (3 – 8)	0.10
Pathology feature, n (%) Nuclear grade Fuhman III/IV	13	6 (100%)	7 (100%)	-	18	7 (100%)	11 (100%)	-
Mitotic rate >5/50 HPF[a]		2 (33.3%)	4 (57.1%)	0.59		5 (71.4%)	5 (45.5%)	0.36
Abnormal mitoses		3 (50%)	3 (42.9%)	1.00		5 (71.4%)	4 (36.4%)	0.33
≤25% clear cells		5 (83.3%)	5 (71.4%)	1.00		5 (71.4%)	9 (81.8%)	1.00
>1/3 diffuse architecture		6 (100%)	6 (85.7%)	1.00		6 (85.7%)	11 (100%)	0.39
Necrosis		5 (83.3%)	5 (71.4%)	1.00		7 (100%)	8 (72.7%)	0.24
Vascular invasion		4 (66.7%)	6 (85.7%)	0.56		5 (71.4%)	8 (72.7%)	1.00
Sinusoid invasion		2 (33.3%)	1 (14.3%)	0.56		4 (57.1%)	3 (27.3%)	0.33
Capsular invasion		1 (16.7%)	0 (0%)	0.46		4 (57.1%)	1 (9.1%)	**0.04**
Tumor size, cm Median (min – max)	13	14.2 (6 – 27)	11.5 (5 – 20)	0.73	18	16 (11 – 26)	11.5 (5 – 27)	0.33
Tumor volume, cm^3 Mean (SD)	11	937 (867)	635 (409)	0.49	16	920 (680)	784 (749)	0.71

* Number of subjects in which variable was assessed; [a] HPF: high-power fields

of 23.3 ± 26 months [median 10.8 months (min – max: 2.7 – 90.1)]. Among clinical and pathology features, those significantly associated with mortality risk among carcinomas were: completeness of tumor resection (p=0.04), histological evidence of capsular invasion (p=0.04), and presence of distant metastasis (p=0.006) – tables 3 and 4. Figure 2 describes Kaplan-Meier's survival rate of ACC patients according to UICC and ENSAT staging systems. Among adenomas, only one case of a 1.9 cm adrenal adenoma, accidentally found inside en-bloc clear-cell renal carcinoma resection, died from the malignant disease.

DISCUSSION

The current study describes the last 18-year period experience from a single high-complexity oncology center with ACT among adults. Although performed in a single institution, some findings of this study must be acknowledged.

To differ benign from malignant ACT can be often challenging (4, 8). Preoperative clinical and imaging studies features have been extensively described as useful in this context (1, 6, 13). Tumor size is one of the best discriminatory characteristics, as ACC measure on average 10 to 13cm, and lesions with diameter bellow 4cm are very unlikely to be malignant, representing only up to 3% of all cases (14, 15). As expected, in authors' study ACC tumors were significantly larger than ACA. In terms of clinical presentation, in this series 65.4% of ACC patients presented with mass effect, mainly abdominal enlargement and/or pain. This data differs from previous studies that reported that up to 40-70% of ACC cases present with evident biochemically or clinical hormone overproduction (4, 14). However, information regarding hormonal production was unreported in nearly half of the entire cohort, and therefore we believe that collected data might not be representative of the real hormonal status of some included cases. We speculate that, based on the fact that hypertension and diabetes mellitus prevalence was substantially higher among ACC patients in comparison to described in general population (16), endocrine syndromes could not always be easily recognized and subtle hypercortisolism and/or hyperandrogenism might have been misdiagnosed.

Table 5: Stage II patients' characteristics

Patient	Age (yr)	Gender	Tumor size (cm)	Tumor side	Ki-67	Weiss-score	Mitotane	Recurrence	Death
1	35	Female	12.5	Right	1%	8	Yes	Yes	Yes
2	35	Female	18	Right	20%	7	Yes	Yes	No
3	34	Female	6	Left	40%	7	Yes	Yes	No
4	51	Female	19	Right	5%	5	Yes	Yes	No
5	37	Male	6	Left	3%	3	No	Yes	No
6	57	Female	27	Right	40%	5	Yes	Yes	No
7	31	Female	18	Left	NA	4	No	No	Yes
8	58	Male	7	Right	5%	4	No	No	No
9	36	Female	11.5	Left	15%	8	No	No	No
10	67	Female	16	Right	30%	5	Yes	No	No
11	36	Male	8	Left	35%	7	Yes	No	No
12	33	Female	20	Left	1%	5	No	No	No

Interestingly, 81.8% of ACA cases presented as an incidental finding. In 50% of these, the adrenal tumor was incidentally discovered through imaging studies directed to a previous known malignancy work-up. It is noteworthy, that since an adrenal nodule can not be considered an unexpected finding in this context, we considered "incidentaloma" a questionable term to define these lesions (1). The present study was performed in a high-complexity cancer center, therefore half of ACA cases were adrenal lesions in which metastasis was a preoperative potential diagnosis. Finally, the remaining ACA cases with no prior cancer diagnosis, no mass effect complaints and no clear evidence of endocrine syndrome were referred to surgery based mainly on surgeons´ decision, patients choice and/or tumor size which ranged from 2.2 to 7.4 cm.

In the absence of metastatic disease, the pathology diagnosis of ACC is not always clear (4). As clinical implications of isolated pathology features are not fully understood, different scores clustering histological and non-histological features have been described in order to distinguish those cases more likely to evolve with metastatic disease (17-19). Of these, Weiss-score have been preferred because of its high interobserver agreement (20) and simplicity, using well-established pathology parameters, i.e., vascular and capsular invasion, mitotic rate, nuclear atypia, tumor necrosis. In authors' series, Weiss-score was significantly higher among ACC cases. However, no difference was observed among survivors *vs*. non-survivors, or those who suffered recurrence or not. The only isolated histological feature associated with increased mortality risk was capsular invasion. Possibly, this association might be related to an incomplete tumor resection, a traditional poor outcome prognostic factor (4).

Surgery is the only potentially curable strategy for non-metastatic cases of ACC (4, 7, 8). For that, surgeon experience plays a significant role in terms of outcomes. However, only a few part of adrenalectomies performed for ACC are performed in high volume cancer centers (21). In authors' study, more than a half of patients with ACC died from disease after a median time of less than one year. Coincidentally, complete resection was achieved

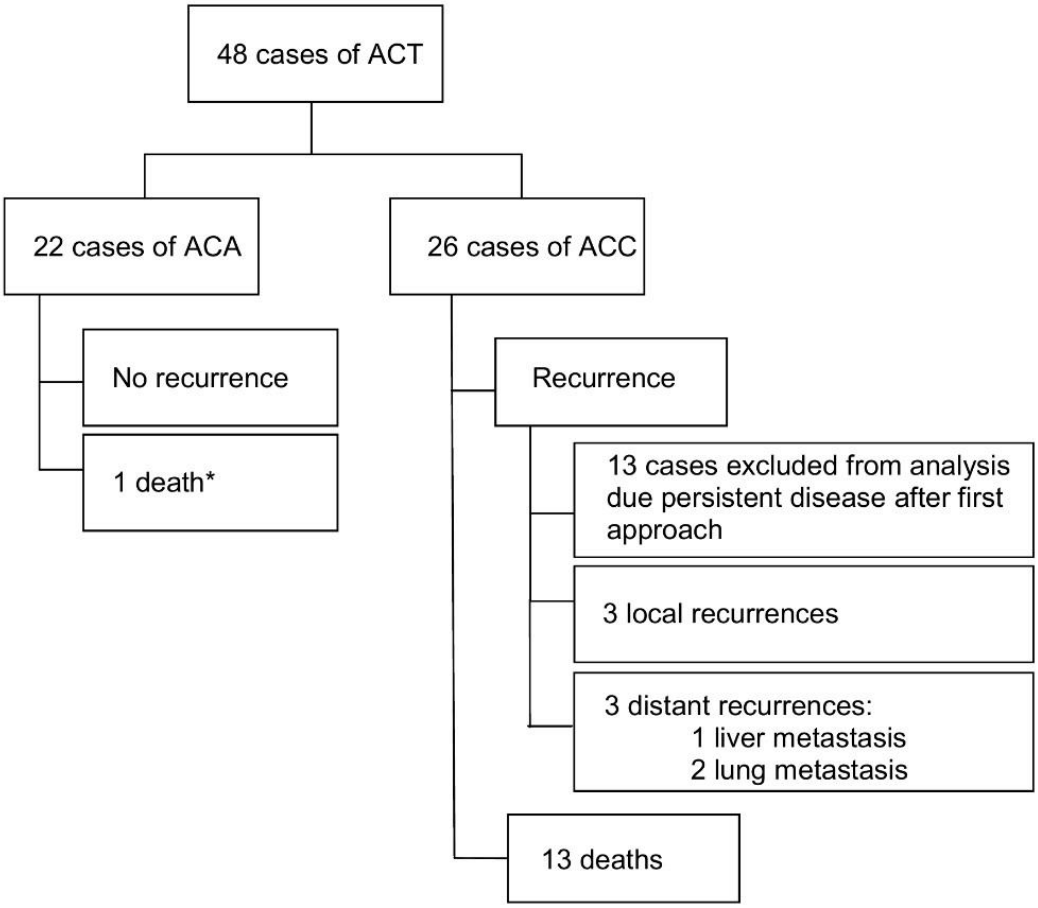

Figure 1: Flow-chart describing patients' enrollment and main outcomes – recurrence and mortality. ACT: adrenocortical tumors; ACA: adrenocortical adenomas; *death not related to the adrenal tumor in a patient with renal carcinoma.

only in half of ACC patients. The long period until diagnosis of almost one year might have led to large and invasive tumors. Notably, extension of resection was the only clinical prognostic factor significantly related to mortality risk, reinforcing that the initial surgical procedure is the best and probably only intervention that can lead to long-term remission. Due to relatively small number of subjects within groups, and also because of lack of reliable information in medical records, the impact of controversial ACC surgery-related aspects such as paraortic lymphadenectomy and intraoperative tumor spillage could not be assessed. Also, the relation between surgery technique and oncological outcomes could not be evaluated since distribution of employed techniques was not uniform among groups: most ACC cases had an open access approach and ACA a laparoscopic one. However, we believe that this fact was responsible for the significantly longer post-operative hospitalization in patients with ACC in comparison to those with ACA. No other therapy was related to improved recurrence and mortality outcomes in this study. Moreover, mitotane therapy and conventional chemotherapy was more common among non-survivors and those who suffered recurrence. This can be attributable to the fact that those patients with more advanced disease were more likely to be referred to adjuvant or palliative therapies.

The consensus of surgery for recurrent disease is to be determined. Specialists agree that factors as extent of disease and progression rate should guide decision, which ultimately must be individualized (8). After a DFI of 70; 36 and 20 months each, three from the six recurrence cases in authors' cohort were referred to a second surgery, with apparent significant improve in survival. These data are in accordance with previous findings from Fassnacht *et al*. (8) who described better second surgery outcomes in patients with a DFI higher than

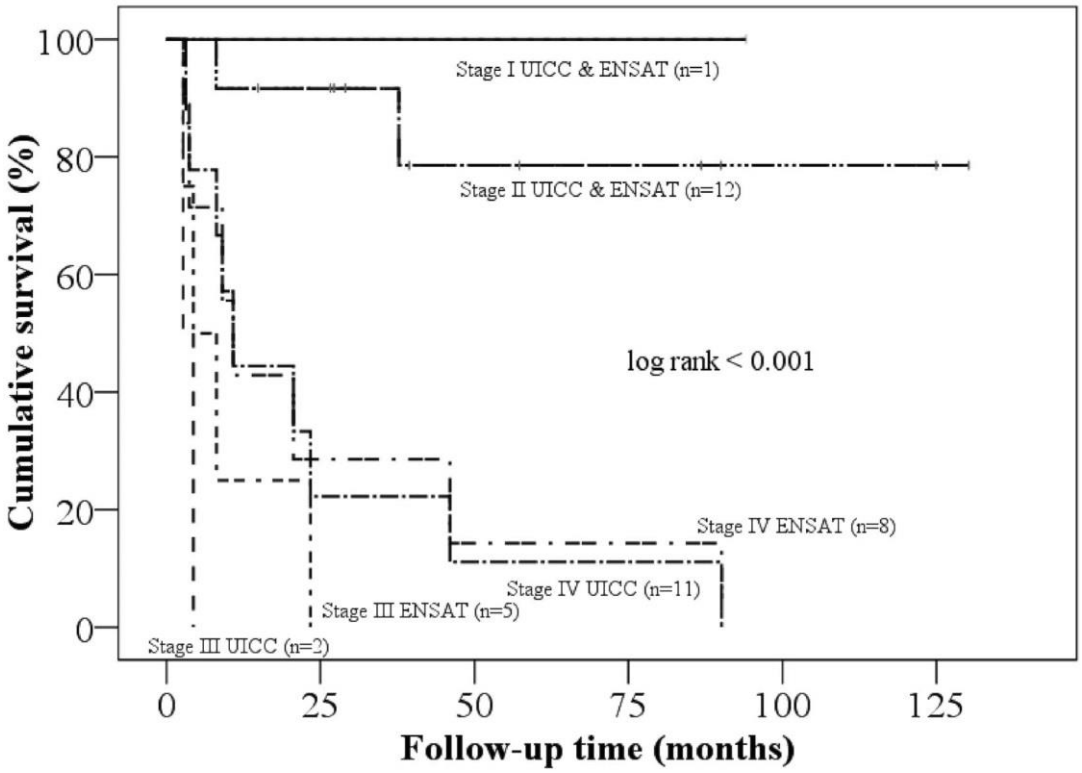

Figure 2: Kaplan-Meier´s survival analysis from the carcinoma population according to the Union for International Cancer Control (UICC) and the European Network for the Study of Adrenal Tumors (ENSAT).

12 months. Similar data were described by Bellatone *et al.* (22) who reported a median survival of 74 months in patients who underwent complete second resections.

Nearly 30% of authors' ACC cases presented with distant metastasis, which was deeply related to mortality. Other series have reported similar rates, with up to 30% of cases considered not potential curable at diagnosis (4, 23). In terms of prognostication, both UICC and ENSAT staging systems were equally effective, with better overall survival among stage I and II patients. However, the reduced sample and the fact that not all patients achieved sufficient long-term surveillance must be acknowledged. Although low stage patients tend be better survival rates, no difference in survival between stage I and II patients have been reported (24, 25). Therefore, different additional clinical or pathology features have emerged in order to distinguish those patients who are more favorable to poor outcomes within this population. Asare *et al.* (26) described additional power in mortality prediction when age was incorporated to the UICC staging system. In a large study from Beuschlein *et al.* (5), Ki-67 labeling index was the major prognostic factor in stage I-III patients after tumor's complete resection. In the present study, five of the six recurrences occurred in stage II patients. This fact led to a sub analysis of this group, with no evidence of association between recurrence risk and age, gender, tumor size, Weiss-score (total score and each individual feature), Ki-67 labeling index, tumor's side, or mitotane therapy (table 5).

Although this study has limitations, we consider that as a rare disease, the number of subjects included was sufficient to provide a reliable analysis in a multiethnic previous unexplored population. Furthermore, data from pathology features of all cases in this study were reassessed by the same adrenal disease expert observers. The most important limitation of this work is its retrospective design. Missing data have limited the statistical power in analysis of some variables and made others impossible. Finally, because of relative

small number of events within groups in addition to the large number of variables, multivariate analysis would be of limited statistic power, and thus, could not be performed.

CONCLUSION

ACC are very aggressive tumors with poor prognosis in a significant proportion of patients as we observed that 54% of study's patients died from disease. Larger studies are needed to determine recurrence and death prognostic factors in order to guide ideal therapy and the most appropriate surveillance. Finally, prompt diagnosis is essential since a complete tumor resection remains the only potential curable strategy.

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ABBREVIATIONS:

ACA: adrenocortical adenoma
ACC: adrenocortical carcinoma
ACT: adrenocortical tumor
DAB: 3,3'-diaminobenzidine
DFI: disease-free interval
DHEA-S: dehydroepiandrosterone sulphate
DIPAT: Division of Pathology
ENSAT: European Network for the Study of Adrenal Tumors
HPF: high-power fields
INCA: Brazilian National Institute of Cancer
UICC: Union for International Cancer Control

REFERENCES

1. Allolio B, Fassnacht M. Clinical review: Adrenocortical carcinoma: clinical update. J Clin Endocrinol Metab. 2006 Jun;91(6):2027-37. DOI: 10.1210/jc.2005-2639.
2. Mansmann G, Lau J, Balk E, Rothberg M, Miyachi Y, Bornstein SR. The clinically inapparent adrenal mass: update in diagnosis and management. Endocr Rev. 2004 Apr;25(2):309-40. DOI: 10.1210/er.2002-0031.
3. Mantero F, Terzolo M, Arnaldi G, Osella G, Masini AM, Ali A, et al. A survey on adrenal incidentaloma in Italy. Study Group on Adrenal Tumors of the Italian Society of Endocrinology. J Clin Endocrinol Metab. 2000 Feb;85(2):637-44. DOI: 10.1210/jcem.85.2.6372.
4. Fassnacht M, Allolio B. Clinical management of adrenocortical carcinoma. Best Pract Res Clin Endocrinol Metab. 2009 Apr;23(2):273-89. DOI: 10.1016/j.beem.2008.10.008.
5. Beuschlein F, Weigel J, Saeger W, Kroiss M, Wild V, Daffara F, et al. Major prognostic role of Ki67 in localized adrenocortical carcinoma after complete resection. J Clin Endocrinol Metab. 2015 Mar;100(3):841-9. DOI: 10.1210/jc.2014-3182.
6. Blake MA, Kalra MK, Sweeney AT, Lucey BC, Maher MM, Sahani DV, et al. Distinguishing benign from malignant adrenal masses: multi-detector row CT protocol with 10-minute delay. Radiology. 2006 Feb;238(2):578-85. DOI: 10.1148/radiol.2382041514.
7. Bourdeau I, MacKenzie-Feder J, Lacroix A. Recent advances in adrenocortical carcinoma in adults. Curr Opin Endocrinol Diabetes Obes. 2013 Jun;20(3):192-7. DOI: 10.1097/MED.0b013e3283602274.
8. Fassnacht M, Kroiss M, Allolio B. Update in adrenocortical carcinoma. J Clin Endocrinol Metab. 2013 Dec;98(12):4551-64. DOI: 10.1210/jc.2013-3020.
9. Gicquel C, Bertagna X, Gaston V, Coste J, Louvel A, Baudin E, et al. Molecular markers and long-term recurrences in a large cohort of patients with sporadic adrenocortical tumors. Cancer Res. 2001 Sep 15;61(18):6762-7.
10. Kerkhofs TM, Verhoeven RH, Van der Zwan JM, Dieleman J, Kerstens MN, Links TP, et al. Adrenocortical carcinoma: a population-based study on incidence and survival in the Netherlands since 1993. Eur J Cancer. 2013 Jul;49(11):2579-86. DOI: 10.1016/j.ejca.2013.02.034.
11. Weiss LM, Medeiros LJ, Vickery AL, Jr. Pathologic features of prognostic significance in adrenocortical carcinoma. Am J Surg Pathol. 1989 Mar;13(3):202-6.
12. Fuhrman SA, Lasky LC, Limas C. Prognostic significance of morphologic parameters in renal cell carcinoma. Am J Surg Pathol. 1982 Oct;6(7):655-63.
13. Elsayes KM, Mukundan G, Narra VR, Lewis JS, Jr., Shirkhoda A, Farooki A, et al. Adrenal masses: mr imaging features with pathologic correlation. Radiographics. 2004 Oct;24 Suppl 1:S73-86. DOI: 10.1148/rg.24si045514.

14. Abiven G, Coste J, Groussin L, Anract P, Tissier F, Legmann P, et al. Clinical and biological features in the prognosis of adrenocortical cancer: poor outcome of cortisol-secreting tumors in a series of 202 consecutive patients. J Clin Endocrinol Metab. 2006 Jul;91(7):2650-5. DOI: 10.1210/jc.2005-2730.
15. Sturgeon C, Shen WT, Clark OH, Duh QY, Kebebew E. Risk assessment in 457 adrenal cortical carcinomas: how much does tumor size predict the likelihood of malignancy? J Am Coll Surg. 2006 Mar;202(3):423-30. DOI: 10.1016/j.jamcollsurg.2005.11.005.
16. Schmidt MI, Duncan BB, Hoffmann JF, Moura L, Malta DC, Carvalho RM. Prevalence of diabetes and hypertension based on self-reported morbidity survey, Brazil, 2006. Rev Saude Publica. 2009 Nov;43 Suppl 2:74-82. DOI: S0034-89102009000900010 [pii].
17. Hough AJ, Hollifield JW, Page DL, Hartmann WH. Prognostic factors in adrenal cortical tumors. A mathematical analysis of clinical and morphologic data. Am J Clin Pathol. 1979 Sep;72(3):390-9.
18. van Slooten H, Schaberg A, Smeenk D, Moolenaar AJ. Morphologic characteristics of benign and malignant adrenocortical tumors. Cancer. 1985 Feb 15;55(4):766-73.
19. Weiss LM. Comparative histologic study of 43 metastasizing and nonmetastasizing adrenocortical tumors. Am J Surg Pathol. 1984 Mar;8(3):163-9.
20. Aubert S, Wacrenier A, Leroy X, Devos P, Carnaille B, Proye C, et al. Weiss system revisited: a clinicopathologic and immunohistochemical study of 49 adrenocortical tumors. Am J Surg Pathol. 2002 Dec;26(12):1612-9.
21. Bilimoria KY, Shen WT, Elaraj D, Bentrem DJ, Winchester DJ, Kebebew E, et al. Adrenocortical carcinoma in the United States: treatment utilization and prognostic factors. Cancer. 2008 Dec 1;113(11):3130-6. DOI: 10.1002/cncr.23886.
22. Bellantone R, Ferrante A, Boscherini M, Lombardi CP, Crucitti P, Crucitti F, et al. Role of reoperation in recurrence of adrenal cortical carcinoma: results from 188 cases collected in the Italian National Registry for Adrenal Cortical Carcinoma. Surgery. 1997 Dec;122(6):1212-8.
23. Wooten MD, King DK. Adrenal cortical carcinoma. Epidemiology and treatment with mitotane and a review of the literature. Cancer. 1993 Dec 1;72(11):3145-55.
24. Fassnacht M, Johanssen S, Quinkler M, Bucsky P, Willenberg HS, Beuschlein F, et al. Limited prognostic value of the 2004 International Union Against Cancer staging classification for adrenocortical carcinoma: proposal for a Revised TNM Classification. Cancer. 2009 Jan 15;115(2):243-50. DOI: 10.1002/cncr.24030.
25. Lughezzani G, Sun M, Perrotte P, Jeldres C, Alasker A, Isbarn H, et al. The European Network for the Study of Adrenal Tumors staging system is prognostically superior to the international union against cancer-staging system: a North American validation. Eur J Cancer. 2010 Mar;46(4):713-9. DOI: 10.1016/j.ejca.2009.12.007.
26. Asare EA, Wang TS, Winchester DP, Mallin K, Kebebew E, Sturgeon C. A novel staging system for adrenocortical carcinoma better predicts survival in patients with stage I/II disease. Surgery. 2014 Dec;156(6):1378-85; discussion 85-6. DOI: 10.1016/j.surg.2014.08.018.