Adjuvant mitotane therapy is beneficial in non-metastatic adrenocortical carcinoma at high risk of recurrence.

Title page

Title: Adjuvant mitotane therapy is beneficial in non-metastatic adrenocortical carcinoma at high risk of recurrence: the experience of the San Luigi Gonzaga Hospital.

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Abstract

Objective. Many patients with adrenocortical carcinoma (ACC) suffer from tumor recurrence despite radical surgery. Evidence on the post-operative use of mitotane is controversial and no predictors of response are available. We aimed to assess whether adjuvant mitotane treatment may prolong survival in patients with non-metastatic ACC following complete resection and whether ACC patients at high risk of recurrence may benefit from treatment.

Design and Methods. We retrospectively reviewed data from 152 non-metastatic ACC patients followed at the San Luigi Gonzaga Hospital: 100 patients were treated with adjuvant mitotane and 52 patients were left untreated following surgery. We assessed a number of potential predictive factors of recurrence and death. Mitotane effect was explored stratifying patients by hormone secretion (yes vs no), staging (stage I-II vs stage III) and Ki67 index.

Results. The control group had a higher risk of recurrence (HR 2.79, 95%CI 1.58-4.91; $p<0.001$) than mitotane group, while overall survival was not significantly different between groups. Hormone secretion, elevated Weiss score and elevated Ki67 index confer a higher risk of both recurrence and death, and stage III ACC of death. Adjuvant mitotane treatment reduced significantly the risk of death in patients with elevated Ki67 index ($p=0.005$), and in patients with stage III ACC ($p=0.02$).

Conclusions. Adjuvant mitotane may prolong recurrence-free survival in radically resected ACC patients with acceptable toxicity and may also prolong overall survival in a subgroup of ACC patients at high risk of recurrence.

Introduction

Adrenocortical carcinoma (ACC) is a rare endocrine neoplasia, with a reported incidence of about two cases per million population per year, which affects more frequently the female sex and has a peak incidence in the middle age (1). ACC is characterized by an overall dismal prognosis and its clinical manifestations are either the consequence of steroid excess or mass effect; however, ACC is increasingly found as an adrenal incidentaloma (2). Surgery is the cornerstone of ACC management and the most effective treatment, while survival is poor when surgical removal of ACC is unfeasible (3). Although radical surgery can be potentially curative, many ACC patients will suffer tumour recurrence following apparently curative resection. Prevention of tumour recurrence is of the utmost importance because recurrence impairs significantly life expectancy and quality of life of affected patients (1),(4). Therefore, adjuvant concepts look sound and the post-operative use of mitotane as an adjuvant therapy following ACC removal has been the most followed approach (5).

Reported outcomes of adjuvant mitotane therapy have been conflicting between studies and, lacking data from randomized controlled trials, evidence is based only on retrospective studies (6),(7) which were often underpowered and did not always include a concomitant control group of untreated patients (5). Previous research from our group showed that adjuvant mitotane was associated with longer recurrence-free survival (RFS) in treated patients with radically resected ACC compared to untreated patients. In that study, confounding by indication was reduced comparing two management strategies applied in different settings and treatment was given according to the centre attitude toward adjuvant mitotane (i.e. all patients of a given centre were treated or not treated) (8). In 2017, we updated the results of our earlier study after nine additional years of follow-up and confirmed that adjuvant mitotane was associated with prolonged RFS (9). Controversy on adjuvant mitotane still lives, however, as demonstrated by a study published in 2016 showing that adjuvant mitotane therapy was associated with

decreased RFS (10). Moreover, the available literature provides scant information on how treatment was delivered (dosing regimen, duration of treatment, circulating levels, etc.) and none of the studies could identify whether outcome of patients at high risk of recurrence is improved with adjuvant mitotane treatment. This information is key considering that adjuvant mitotane is currently recommended in high-risk patients (7).

To contribute to the debate on post-operative adjuvant mitotane therapy, we reviewed retrospectively our experience at the San Luigi Gonzaga Hospital, a tertiary centre for the care of ACC patients in Italy. The main aims of the study were to assess whether adjuvant mitotane treatment may prolong survival in patients with non-metastatic ACC following complete resection and whether ACC patients at high risk of recurrence may benefit from treatment.

Materials and methods

At the San Luigi Gonzaga Hospital, we established in 2001 the Adrenocortical Carcinoma Database with the development of a structured data form to collect comprehensive information of ACC patients managed at our centre. Data were obtained from patient interviews and available medical documentation and were processed by skilled and experienced personnel. In case of missing information, further data were actively requested to medical institutions where patients have been previously managed. For the purpose of this study, we retrieved data of patients who underwent radical surgery from July 2001 to July 2015. Due to the referral pattern of our centre, most patients have been operated on at other institutions (7% of patients had surgery in our centre) and were referred to us after a histologic diagnosis of ACC was secured for considering adjuvant medical therapy. Follow up for this study was closed in May 2017. The institutional ethics committee of our hospital approved the study and all patients provided written informed consent.

Inclusion criteria of the study were: age ≥16 years; pathologically confirmed diagnosis of ACC according to Weiss score (11) (81% of pathological diagnoses were reviewed at our centre); ENSAT stage I-III at diagnosis; complete macroscopic resection, defined as R0, R1 or RX resection on the basis of surgical and pathologic reports; availability of pre-operative and post-operative computed tomography (CT) or magnetic resonance imaging (MRI) scans; complete follow-up information. Exclusion criteria were: incomplete tumour staging, ENSAT stage IV, history of other previous or concomitant malignancies, R2 resection; recurrence or death before the landmark point of three months; incomplete follow-up information, concomitant adjuvant chemotherapy and radiotherapy or both, and patient inclusion in previous studies.

Patients charts were reviewed and the following information was retrieved for the study: patient's age and sex, date of diagnosis, imaging data, ACC stage, clinical presentation including assessment of hormone secretion, type of surgery, pathology report, adjuvant

111 treatment, date and type of recurrence, treatment of recurrence, last follow up, or death. Date
112 of diagnosis was defined as the date of surgery and conversion to open adrenalectomy was
113 considered as open surgery. Completeness of surgery was established by R status: R0, free
114 resected margins; R1, microscopic involvement of resected margins; RX, not determined, and
115 R2, macroscopic invasion of resected margins. Tumour stage was established according to the
116 ENSAT classification (I-II, confined tumour; III, positive lymph nodes or infiltrating
117 neighbouring organs/veins without distant metastases; IV, distant metastases (12). Biochemical
118 confirmation of hormone excess was requested to categorize an ACC as hormone secreting.
119 Patients were stratified for Ki67 index (Ki67 ≤10% and Ki67 >10%). Date of recurrence was
120 defined as the date of radiological evidence of a new lesion. Recurrence was described as 'local'
121 (involving the adrenal region), 'single distant' (one affected organ), or 'multiple distant' (more
122 than one affected organ). If adjuvant mitotane therapy was instituted, we analysed mitotane
123 levels on treatment, duration of treatment, reasons of treatment discontinuation, and severe
124 toxicity. Mitotane levels were attained by the Lysosafe® service since July 2005 while in the
125 preceding years we got data from in-house measurements, as previously described (13).
126 Duration of treatment was calculated from the date of initiation of therapy until ACC
127 recurrence, or discontinuation of treatment, or end of follow up, whatever occurring first.
128 From a total of 217 ACC patients on database, 152 patients fulfilled inclusion/exclusion criteria
129 and were retrospectively included in the study (Figure 1). Of these, 100 patients underwent
130 adjuvant mitotane therapy after initial surgery (mitotane group) and 52 patients were not treated
131 with any adjuvant treatment following surgery (control group). Patient management included
132 follow-up visits, imaging, hormone assessment, routine laboratory tests and measurement of
133 mitotane level for treated patients, every 3-4 months. Mitotane was given according to a low-
134 dose monitored regimen, as previously detailed (14). Briefly, treatment is started at 1 g daily
135 with further dose increase every 4-7 days up to 8-12 g daily, or the maximum tolerated dose.

Mitotane dose is adjusted to patient tolerability and drug levels that are regularly monitored aiming at plasma concentration of 14-20 mg/L. Severe mitotane related effects leading to hospitalization or treatment discontinuation, either more than three months or permanent, were analysed for the study.

Outcomes

The main aims of our study were to compare recurrence free survival (RFS) and overall survival (OS) in patients who received adjuvant mitotane therapy after complete macroscopic resection with that of patients who did not receive adjuvant therapy and to identify whether outcome of high-risk patients is improved with treatment. RFS was calculated from the time of initial surgery to the first radiological evidence of recurrence. OS was calculated from the date of initial surgery to the date of death. The following potential predictive factors for either RFS or OS were investigated: patient sex and age, tumour stage, hormone secretion, cortisol excess, type of surgery, R status, Weiss score, Ki67 index, adjuvant mitotane, and plasma mitotane levels. Mitotane effect was explored stratifying patients by staging (stage I-II vs stage III), hormone secretion (yes vs no), and Ki67 index.

Statistical analysis

Categorical data are presented as counts and percentages. Continuous data are presented as medians and ranges. Differences in categorical variables were analysed by means of the Chi-Square Test while differences in continuous variables by the two-tailed Mann-Whitney U test. The survival distribution was assessed by the Kaplan-Meier product-limit methods and survival curves were compared by the log-rank test. Patients who did not experience the event (recurrence or death) were censored at the date of the last follow-up visit for the specific survival analysis. Cox proportional hazards regression models were fitted to determine predictive factors on RFS and OS. Proportional hazard assumption was verified by

Schoenfeld's residuals and by the log minus log method. To reduce the inherent bias of patients with early progression or death, all survival analyses were performed with the landmark method. Patients who experienced the event (recurrence or death) before the landmark point at three months were excluded from the analyses. Complete case analysis was used. All reported P values are two-sided. P-values of less than 0.05 were considered as statistically significant. Statistical analyses were done with R Version 3.4.3.

Results

Patients

Baseline characteristics of patients are reported in Table 1. Female sex was mostly affected (61.8%) and median age at diagnosis was 46 years [range, 16-77]. ACC was stage I-II in 75.7% and hormone secreting in 47.4% of cases; cortisol was the most frequently secreted hormone, alone or in combination with other steroid hormones. Secreting ACCs were mostly found in women and in younger patients and had higher Ki67 index (Table 2). Surgery was done as an open approach in 71.6% of cases; 63.5% in stage I-II and 94.6% in stage III ACC. R0 resection was attained in 74.2% of open surgery vs 76.9% of laparoscopy. Median Weiss score was of 6 [3-9] with a median Ki67 of 20% [1%-70%]; 67.2% of ACC had Ki67 >10%. The median follow-up was 51 months, 57 in the mitotane group vs 45 months in the control group. The mitotane and control group were evenly distributed according to patient sex, ACC stage, hormone secretion, type of surgery, and R status. The patients of the mitotane group were younger and showed higher Weiss score and Ki67 index than the control group (Table 3).

Among mitotane treated patients, 47% of them began adjuvant treatment within one month after surgery, 44% within three months, and the remaining 9% between 4-10 months. In 62.8% of patients the therapeutic range of mitotane concentrations (14-20 mg/L) was maintained in at least 50% of measurements. The patients who maintained the therapeutic range did not show a significant reduction in the risk of recurrence (HR 0.79, [0.41-1.50], p=0.47). Median duration of treatment was 21 months (14 months in patients who developed recurrence vs 45 months in patients without recurrence). Causes of treatment discontinuation were toxicity (n=11), poor compliance (n=7) or concomitant diseases (n=4). Unwanted effects of mitotane therapy mainly affected the gastrointestinal system, and are reported in Table 4.

Outcomes

Recurrence occurred in 62.5% of cases: 56.5% of stage I-II and 81.1% of stage III ACC. The median RFS of the overall cohort was 25 months [4-199]. Recurrence was mostly observed within five years after surgery (93.5%), as showed in Figure 2. Only six patients recurred after five years; they had stage II ACC with a Ki67 ≤10% in three cases and no secretion in four of them. Recurrence occurred in 55 patients of the mitotane group (55%) and in 40 of the control group (76.9%). The median RFS was 36.5 months [4-199] in the mitotane group and 21 months [4-180] in the control group. RFS of the two groups were significantly different when compared with Kaplan Meier survival analysis ($p<0.001$) (Figure 3). Univariate analysis showed that stage, hormone secretion, Weiss score, Ki67 index, and adjuvant mitotane treatment were independent predictors of recurrence (Table 5). Multivariate analysis confirmed hormone secretion, Weiss score, and Ki67 index as predictive factors of recurrence (Table 6). After adjusting for prognostic factors, the control group had a higher risk of recurrence (HR 2.79, 95% CI 1.58-4.91; $p<0.001$) than the mitotane group (Figure 4).

Local recurrence occurred in 20% of cases, single distant recurrence in 42.2%, and multiple distant recurrences in 37.8%. Loco-regional treatments, including surgery or radiofrequency ablation, were used in 57.6% of ACC recurrence, while 31.5% underwent systemic therapy, such as mitotane alone (17.2%) or in combination with chemotherapy (82.8%). Two patients did not receive any treatment, six patients received multiple treatments, and two additional patients received other types of treatment.

Death occurred in 42.1% of cases; 33% of stage I-II and 70.3% of stage III ACC. The median OS of the overall cohort was 57 months [4-231]. Death occurred in 40% patients of the mitotane group and in 46.2% of the control group. The median OS was 57.5 months [8-199] in the mitotane group and 50.5 months [4-231] in the control group. Kaplan Meier analysis did not find a significant difference between groups ($p=0.85$) (Figure 5). Univariate analysis showed

that stage, Weiss score, and Ki67 index were independent predictors of OS (Table 5). Multivariate analysis confirmed stage, hormone secretion, Weiss score, and Ki67 index as predictive factors of OS (Table 6). After adjusting for prognostic factors, OS was not different between the control group and the mitotane group (HR 1.22, 95% CI 0.61-2.42; p=0.57) (Figure 6).

Mitotane treatment reduced significantly the risk of both recurrence and death in patients with elevated Ki67 index and the risk of death in patients with stage III ACC. Mitotane treatment was not associated with any difference in survival between patients with secreting or non-secreting ACC (Table 7).

Discussion

The rarity of ACC has hampered the implementation of large studies, so that only two randomized controlled trials on treatment of advanced ACC are available up to now (15),(16) and no randomized study has been conducted in the adjuvant setting. Therefore, evidence on the efficacy of adjuvant mitotane treatment relies on six retrospective studies that included a concomitant control group of untreated patients to allow a comparative assessment of treatment efficacy (9),(10),(17),(18),(19),(20). The multicentre nature of some studies (9),(10),(20) carries the disadvantage of an inherent heterogeneity in the management of ACC patients and in the modalities of delivering mitotane therapy (i.e. dosing, duration of treatment). Most importantly, no information on the key issue of what characteristics are associated with benefit from adjuvant mitotane treatment can be retrieved from these studies.

In the present study, we report on our experience with adjuvant mitotane treatment at the San Luigi Gonzaga Hospital, a tertiary centre for ACC patients in Italy. None of the patients has been included in previous studies. The major findings of the present study are the observation that adjuvant mitotane may prolong RFS and the identification of subgroups of patients that may particularly benefit from treatment. Moreover, we defined predictive factors for recurrence and death.

As expected, patients treated with adjuvant mitotane had worse prognostic factors (higher Weiss score and Ki67 index); however, we observed a significant increase in the risk of recurrence in the untreated patients, whereas the risk of death was similar between untreated and treated patients. That adjuvant mitotane may increase significantly RFS but not OS has been already reported (9),(18) and an explanation for this apparent discrepancy may be an insufficient number of events to reach statistical levels of significance. In our study, however, adjuvant mitotane was associated with a significant increase in OS in patients with elevated Ki67 index or stage III ACC, characteristics associated with a high risk of recurrence. A recent

meta-analysis of the available studies demonstrated that adjuvant mitotane was linked with significantly better OS despite some heterogeneity between studies attributable to the variable inclusion criteria but was not able to identify predictors of response (7).

To the best of our knowledge, this is the first evidence supporting the concept that high-risk patients should be treated with adjuvant mitotane, as recommended in previous guidelines on the basis of expert opinion (6),(7). In the present study, we were able to identify factors that portend a worse prognosis. Hormone secretion, elevated Weiss score and Ki67 index confer a higher risk of recurrence and death, while stage III predicts shorter OS. ACC stage is generally regarded as the most important prognostic factor (4),(7),(12),(21),(22) and was a strong predictor of death also in our cohort. Evidence that the capability of ACC to secrete hormone is a negative prognostic factor is growing (18),(23),(24) and we confirmed and extended this finding showing that secreting tumours may be more aggressive due to a higher proliferation rate, since they had an elevated Ki67 index compared to non-secreting ACC. This finding is novel and fits well with the observation that hormone secretion is linked to a transcriptome signature typical of aggressive ACC (25). We found that secreting ACCs were more frequent in younger patients and in women, while hormone secretion did not affect efficacy of mitotane treatment, a controversial issue in previous studies (9),(19). The Weiss score has not been uniformly considered as a predictor of long-term outcome (26) while higher Ki67 index have been consistently associated with poor outcome (20),(27) and a cut off at 10% has been proposed to categorize patients for adjuvant treatment (7). This concept is currently tested in the ongoing ADIUVO study, the first randomized controlled trial on post-operative adjuvant mitotane in ACC patients (www.epiclin.it/adiuvo).

In our experience, toxicity associated to adjuvant mitotane was acceptable, even if we should acknowledge the fact that unwanted effects might be underestimated in a retrospective analysis. Severe toxicity was recorded in only 16 patients, and 11 of them were not able to tolerate

chronic mitotane treatment. Management of patients on adjuvant mitotane is complex, since it implies specific experience to adjust supportive therapy, a careful follow-up to cope with patient needs and regular monitoring of drug levels (14). Mitotane monitoring was likely a key in limiting severe neurological toxicity. Our findings support the view that such patients should be managed by expert centres and that a low-dose starting regimen may increase tolerability and compliance. Mitotane is generally viewed as a toxic drug (28) but very few data on how treatment was delivered and on related toxicity are available (8). Despite using a low-dose approach, 62.8% of patients had their mitotane concentrations in the 14-20 mg/L range for at least 50% of measurements. In the only study reporting about mitotane levels in an adjuvant setting, <50% of patients were at target (10). We were not able to confirm a relationship between the target mitotane concentration and survival advantage (29) and this may suggest that lower levels could be efficacious in an adjuvant setting or that a more precise estimate of chronic mitotane exposure is needed.

Strengths of the present study are the accurate characterization and uniform management of the patients, who were cared by the same team of physicians during their disease course. This allowed to capture details of mitotane treatment that were not available in previous studies. We should acknowledge the limits of a retrospective analysis, although we limited bias with landmark analysis and central review of pathological and radiological materials, and the fact that primary surgery was done outside our institution in most patients. However, this pattern of patient referral is typical of tertiary centres (17),(18),(30).

To conclude, our study shows that adjuvant mitotane treatment prolongs survival in non-metastatic ACC patients and is effective in patients at high risk of recurrence. Use of a low-dose mitotane regimen with careful patient follow up accomplishes sustained adherence to therapy.

Declaration of interest

MT has received research grant from HRA Pharma and Novartis, and advisory board honoraria from HRA Pharma. AB has received advisory board honoraria from HRA Pharma. SP has received research grant from HRA Pharma. The other authors have explicitly stated that there are no conflicts of interest in connection with this article.

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Author contribution statement

MT, and AB designed the study, interpreted the results and revised the final version. VB, SP, AP, GR, and MT managed the patients. LS did hormone measurements. AC, VB, and PP collected data and contributed to data interpretation. PP took care of ethic commitments. AC and PB did the statistical analyses. FP did the surgical operations. AV did the central radiologic review. MV did the central pathological review. AC, VB and SP wrote the first draft of the report. All authors made critical revisions of the report.

Acknowledgement

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Figure 1. Study cohort.

Figure 2. Recurrence rate by time of follow up.

Figure 3. Kaplan Meier estimates of recurrence-free survival.

Figure 4. Recurrence-free survival curves adjusted for prognostic factors.

Figure 5. Kaplan Meier estimates of overall survival.

Figure 6. Overall survival curves adjusted for prognostic factors.

Figure 1. Study cohort.

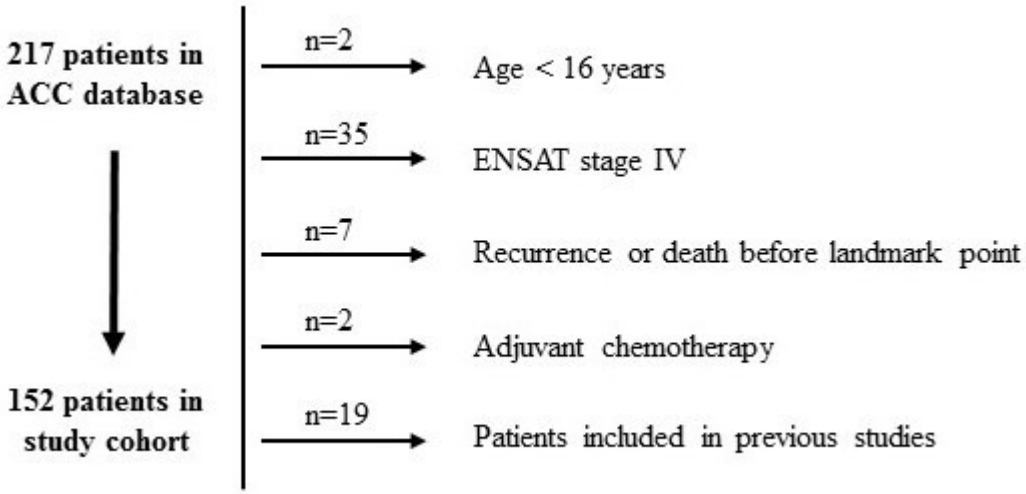

Figure 2. Recurrence rate by time of follow up.

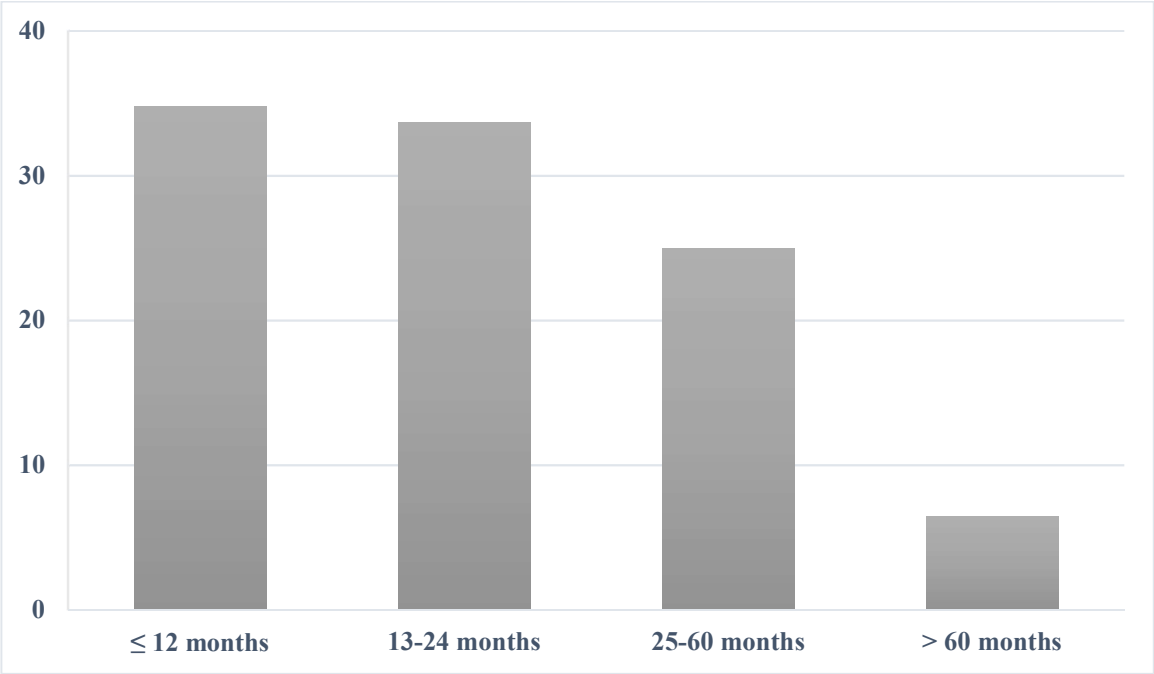

Figure 3. Kaplan Meier estimates of recurrence-free survival.

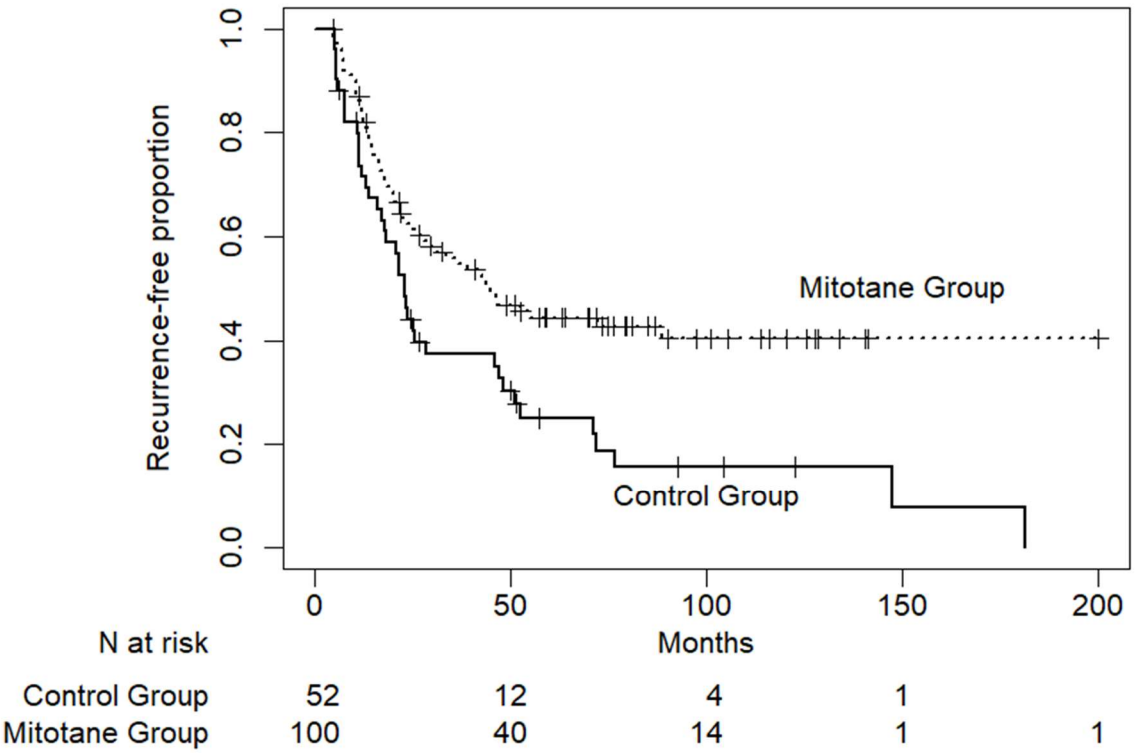

Figure 4. Recurrence-free survival curves adjusted for prognostic factors.

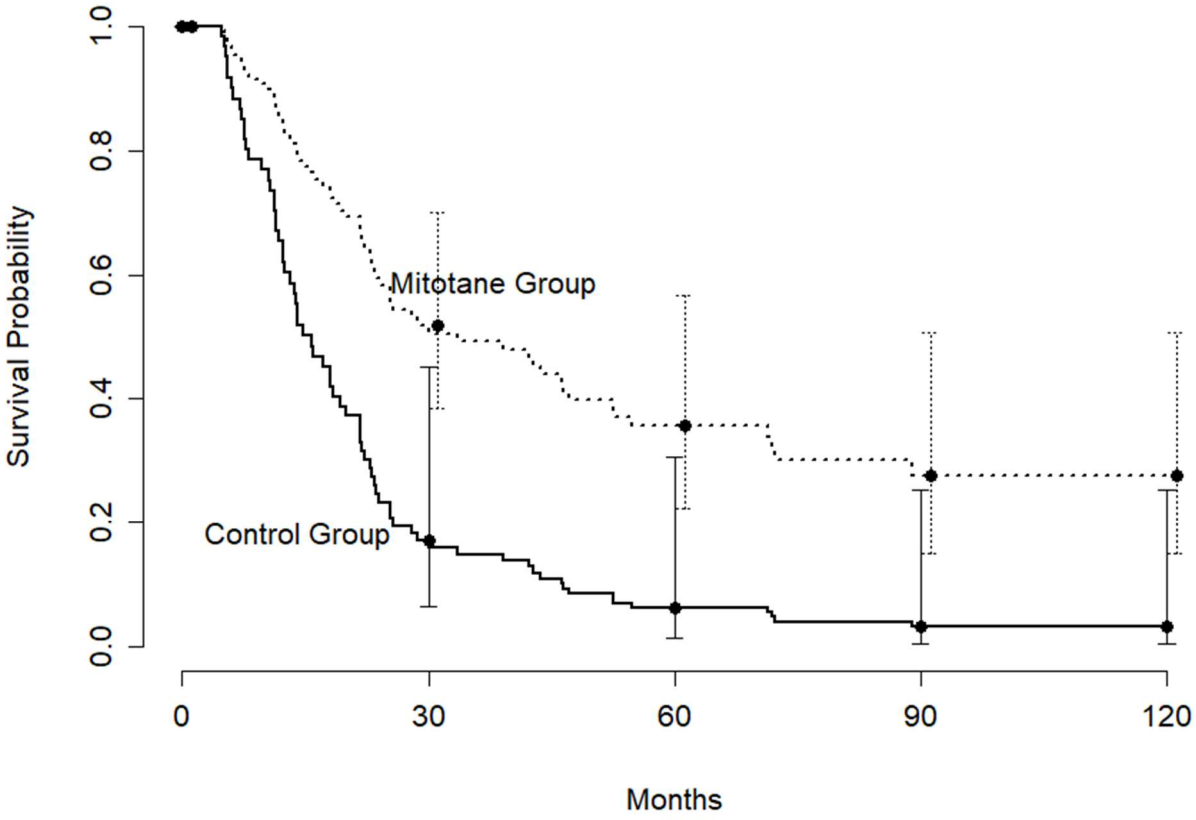

Figure 5. Kaplan Meier estimates of overall survival.

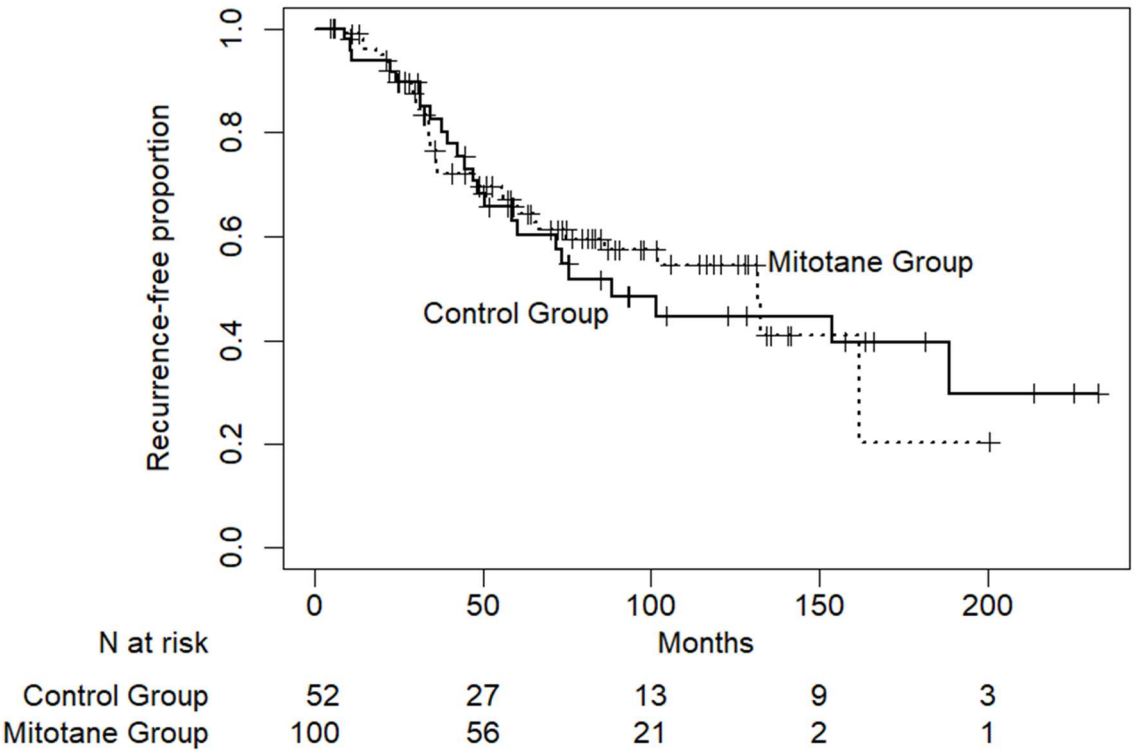

Figure 6. Overall survival curves adjusted for prognostic factors.

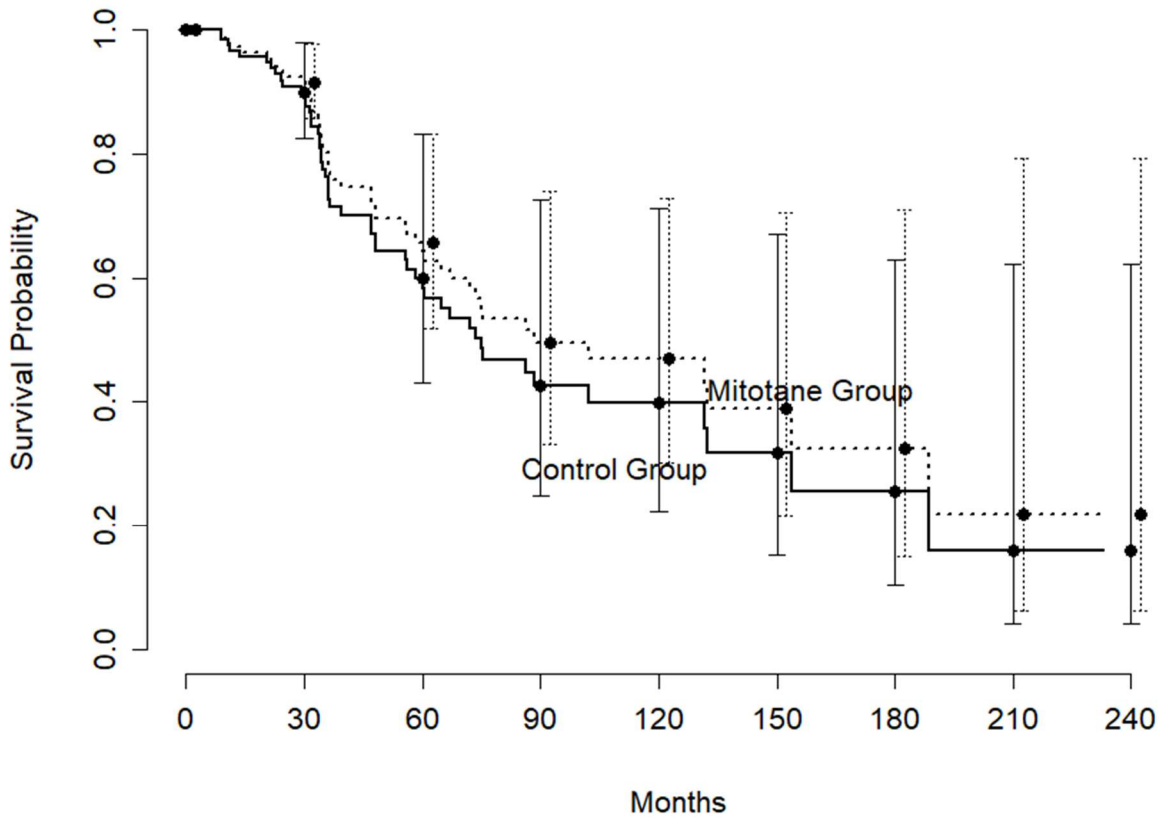

Table 1. Baseline features of patients.

Characteristics	
Sex – *N*. (%)	
Male	58 (38.2)
Female	94 (61.8)
Age at diagnosis – yr	
Median [range]	46 [16-77]
Tumour stage – *N*. (%)	
Stage I-II	115 (75.7)
Stage III	37 (24.3)
Hormone secretion – *N*. (%)	
None	69 (45.4)
Cortisol	29 (19.1)
Cortisol + other steroids	22 (14.5)
Androgens	10 (6.6)
Aldosterone	4 (2.6)
Other hormones	7 (4.6)
No hormone work up	11 (7.2)
Type of surgery – *N*. (%)	
Open adrenalectomy	101 (71.6)
Laparoscopic adrenalectomy	40 (28.4)
R status – *N*. (%)	
R0	111 (75.0)
RX	34 (23.0)
R1	3 (2.0)
Tumor size – cm	
Median [range]	11 [3-25]
Weiss score	
Median [range]	6 [3-9]
Ki67 index – %	
Median [range]	20 [1-70]
Ki67 ≤10% – *N*. (%)	44 (32.8)
Ki67 >10% – *N*. (%)	90 (67.2)
Adjuvant treatment – *N*. (%)	
Mitotane	100 (65.8)
No treatment	52 (34.2)

Table 2. Baseline features of patients stratified by hormone secretion.

Characteristics	Non-secreting ACC (*n*=69)	Secreting ACC (*n*=72)	*p*-value
Sex – *N*. (%)			
Male	33 (47.8)	20 (27.8)	0.022
Female	36 (52.2)	52 (72.2)	
Age at diagnosis – yr			
Median [range]	47.0 [16-70]	41.5 [21-77]	0.014
Tumour stage – *N*. (%)			
Stage I-II	51 (73.9)	55 (76.4)	0.885
Stage III	18 (26.1)	17 (23.6)	
Type of surgery – *N*. (%)			
Open adrenalectomy	44 (65.7)	49 (74.2)	0.374
Laparoscopic adrenalectomy	23 (34.3)	17 (25.8)	
R status – *N*. (%)			
R0	52 (76.5)	52 (75.4)	0.850
RX	15 (22.0)	15 (21.7)	
R1	1 (1.5)	2 (2.9)	
Weiss score – Median [range]	6 [3-9]	6 [3-9]	0.162
Ki67 index – %			
Median [range]	14 [1-70]	20 [2-70]	0.025

Table 3. Baseline features of patients stratified by adjuvant treatment.

Characteristics	**Mitotane group** (*n*=100)	**Control group** (*n*=52)	***p*-value**
Sex – *N*. (%)			
Male	37 (37.0)	21 (40.4)	0.817
Female	63 (63.0)	31 (59.6)	
Age at diagnosis – yr			
Median [range]	45.0 [16-77]	49.5 [18-70]	0.015
Tumour stage – *N*. (%)			
Stage I-II	77 (77.0)	38 (73.1)	0.580
Stage III	23 (23.0)	14 (26.9)	
Hormone secretion – *N*. (%)			
Non-secreting tumor	41 (41.0)	28 (53.8)	0.154
Secreting tumor	52 (52.0)	20 (38.5)	
No hormone work up	7 (7.0)	4 (7.7)	
Type of surgery – *N*. (%)			
Open adrenalectomy	67 (72.0)	34 (70.8)	0.467
Laparoscopic adrenalectomy	26 (28.0)	14 (29.2)	
R status – *N*. (%)			
R0	71 (74.0)	40 (76.9)	0.435
RX	22 (22.9)	12 (23.1)	
R1	3 (3.1)		
Weiss score – Median [range]	6 [3-9]	5 [3-9]	0.014
Ki67 index – %			
Median [range]	20 [1-70]	11 [1-70]	0.001

Table 4. Adverse events of mitotane therapy.

Type of toxicity – *N.* (%)	
Gastrointestinal	6 (37.5)
Hepatic	3 (18.8)
Haematological	1 (6.3)
Neurological	3 (18.7)
Other	3 (18.7)

Table 5. Univariate analyses of predictive factors of RFS and OS.

Factor	RFS			OS		
	HR	**95% CI**	***p*-value**	**HR**	**95% CI**	***p*-value**
Sex[1]	0.93	0.62-1.40	0.74	0.81	0.49-1.35	0.42
Age at diagnosis	0.98	0.70-1.39	0.93	0.97	0.63-1.50	0.90
Tumour stage[2]	2.21	1.43-3.43	<0.001	2.41	1.46-3.99	<0.001
Hormone secretion[3]	1.82	1.14-2.86	0.01	1.67	0.94-2.94	0.08
Cortisol secretion[4]	1.15	0.62-2.13	0.66	1.01	0.51-2.04	0.97
Type of surgery[5]	0.73	0.45-1.17	0.19	0.72	0.39-1.33	0.29
R status[6]	1.06	0.67-1.68	0.82	0.72	0.39-1.31	0.28
Weiss score	1.75	1.33-2.29	<0.001	1.75	1.28-2.39	<0.001
Ki67 index	1.57	1.21-2.03	<0.001	1.80	1.33-2.43	<0.001
Mitotane treatment	1			1		
No treatment	1.81	1.20-2.73	0.005	1.05	0.63-1.76	0.85

Reference for categorical variables: [1]Male sex, [2]ACC stage III, [3]Secreting tumours, [4]Cortisol secretion, [5]Laparoscopic surgery, [6]RX-R1.

Reference for continuous variables: HR was calculated comparing interquartile ranges.

Table 6. Multivariate analyses of predictive factors of RFS and OS.

Factor	RFS			OS		
	HR	**95% CI**	***p*-value**	**HR**	**95% CI**	***p*-value**
Sex[1]	0.93	0.57-1.52	0.77	0.80	0.44-1.46	0.47
Age at diagnosis	1.20	0.80-1.81	0.37	1	0.60-1.65	0.98
Tumour stage[2]	1.41	0.78-2.54	0.25	2.26	1.20-4.25	0.01
Hormone secretion[3]	2.78	1.61-4.76	<0.001	2.08	1.11-4.00	0.02
Weiss score	1.69	1.24-2.32	0.001	1.47	1.02-2.12	0.04
Ki67 index	1.71	1.23-2.38	0.001	1.73	1.16-2.60	0.008
Mitotane treatment	1			1		
No treatment	2.79	1.58-4.91	<0.001	1.22	0.61-2.42	0.57

Reference for categorical variables: [1]Male sex, [2]Stage III, [3]Secreting tumours.

Reference for continuous variables: HR was calculated comparing interquartile ranges.

Table 7. Risk of recurrence and death in patients treated with adjuvant mitotane.

ACC characteristics	RFS			OS		
	HR	**95%CI**	***p*-value**	**HR**	**95%CI**	***p*-value**
Stage III	0.96	0.32-2.92	0.95	0.21	0.06-0.78	0.02
Hormone secretion	0.58	0.21-1.62	0.30	2.53	0.69-9.37	0.16
Ki67 index[1]	0.97	0.94-0.99	0.02	0.94	0.90-0.98	0.005

[1] HR of Ki67 index per 1% increase.