1 New findings on presentation and outcome of patients with adrenocortical cancer:

- 2 results from a national cohort study.
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Keywords

2 Adrenal incidentaloma, gender disease, mitotane, surgery, recurrence, survival.

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Declaration of interests

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ABSTRACT

- 2 Context: Due to the rarity of adrenocortical cancer (ACC), only a few population-based studies are
- 3 available and they reported limited details in the characterization of patients and their treatment.
- 4 **Objective:** To describe in a nation-wide cohort the presentation of ACC patients, treatment strategies
- 5 and potential prognostic factors.
- 6 Methods: Retrospective analysis of 512 ACC patients, diagnosed in twelve referral centers in Italy
- 7 from January 1990 to June 2018.
- 8 Results: ACC diagnosed as incidentalomas accounted for overall 38.1% of cases, with a frequency that
- 9 increases with age and with less aggressive pathological features than symptomatic tumors. Women
- 10 (60.2%) were younger than men, had smaller tumors, which more frequently secreted hormones.
- 11 Surgery was mainly done with an open approach (72%), and after surgical resection, 62.7% of patients
- started adjuvant mitotane therapy. Recurrence after tumor resection occurred in 56.2% of patients.
- 13 In patients with localized disease, cortisol secretion, ENSAT stage III, Ki67% and Weiss score were
- associated with an increased risk of recurrence, while margin-free resection, open surgery and
- adjuvant mitotane treatment were associated with reduced risk. Death occurred in 38.1% of patients
- and recurrence-free survival (RFS) predicted overall survival (OS). In localized disease, age, cortisol
- secretion, Ki67%, ENSAT stage III, and recurrence were associated with increased risk of mortality.
- 18 ACCs presenting as adrenal incidentalomas showed prolonged RFS and OS.
- 19 Conclusions: Our study shows that ACC is a sex-related disease and demonstrates that an incidental
- 20 presentation is associated with a better outcome. Given the correlation between RFS and OS, RFS may
- 21 be used as a surrogate end-point in clinical studies.

INTRODUCTION

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Adrenocortical carcinoma (ACC) is an orphan tumor arising from the adrenal cortex that can have an 2 3 aggressive course (1). Classical estimates of its annual incidence range between 0.7 and 2 cases per million inhabitants (2-5). The rarity of the disease has prevented the acquisition of solid data on its 4 clinical presentation, and it is currently unclear whether either sex- or age-related differences in 5 6 presentation and outcome exist. Patient sex is not usually regarded as an important prognostic factor (6,7), while data on the association of age with outcome are conflicting (6,8-10). Conversely, there is 7 clear evidence that tumor stage, assessed according to the European Network for the Study of 8 Adrenal Tumors (ENSAT) classification, and grade, assessed by the Ki67 count, are important 9 10 determinants of outcome (11-14). Surgery is the first treatment option and international guidelines recommended to offer high-risk patient adjuvant mitotane treatment (1,15). In addition to tumor 11 stage, proliferation activity, and complete tumor resection, studies have identified cortisol excess as 12 another predictor of outcome that was found to be associated with worse prognosis (16-18). This is 13 important considering that manifestations of hormonal excess, mainly due to hypercortisolism, can be 14 15 found in up to 60% of patients at diagnosis (19). In the last decades, an increasing number of ACCs have been discovered serendipitously by imaging tests performed for unrelated conditions (20,21). 16 However, ACCs diagnosed as incidentalomas remain poorly characterized in terms of relative 17 prevalence and outcome. 18 19 Several population-based studies have been carried out in the US (2,4,6,22) and, more recently, one study was conducted in South Korea (23). Conversely, only two population-based studies have been 20 21 carried out in Europe: a retrospective analysis of 99 patients diagnosed between 1970-1984 in 22 Norway (3) and a retrospective study on 359 ACC patients diagnosed between 1993-2010 in the Netherlands (5). In addition, data of 492 patients from the German ACC Registry diagnosed between 23

- 1 1986-2007 have been reported in a paper aiming to assess the prognostic value of the staging system
- 2 for ACC (11).
- 3 We herein present data of a study carried out at tertiary centers for the treatment of adrenal diseases
- 4 in Italy.
- 5 The aims of our study were: i) to describe how patients with ACC presented at diagnosis in terms of
- age- and sex-related differences; ii) to assess how patients with ACC were managed; iii) to identify
- 7 potential predictors of adverse outcome.

PATIENTS AND METHODS

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For this study, we set up a specific web database and invited the tertiary centers for the care of ACC 2 3 patients in Italy to participate, providing data on all patients with a pathologically confirmed diagnosis of ACC who had been proactively followed at the centers. Patient data were pseudonymized. Care 4 was taken to prevent duplicate entries of patients who had been followed at different centers. The 5 6 twelve participating centers, distributed throughout Italy, are listed in Appendix 1 (24). We retrieved data on patients evaluated from January 1990 to June 2018. Follow up for this study 7 ended on 31 December 2018. Patients included in the ADIUVO study (www.epiclin.it/adiuvo) were 8 excluded. The study was conducted in accordance with the Declaration of Helsinki. The need to obtain 9 10 informed consent from the study participants was waived from the local ethics committees due to the study's retrospective and observational nature. 11 The following patient information was retrieved: sex, age, date of diagnosis, hormone secretion, 12 mode of detection (incidental, hormone excess or local symptoms), tumor size and stage, number of 13 metastatic organs (if any), surgical approach, pathology report, adjuvant mitotane, date of 14 15 recurrence, treatment of advanced disease, date of last follow-up or death. Biochemical confirmation of hormone excess was requested in order to categorize an ACC as secreting. Tumor stage was 16 established according to the ENSAT classification (stage I and II, confined tumor; stage III, positive 17 18 lymph nodes or infiltrating neighboring organs/veins without distant metastases; stage IV, distant metastases) (11). 19 Date of recurrence was defined as the date of appearance of a new lesion confirmed by imaging. 20 Disease recurrence was evaluated using contrast-enhanced computed tomography or magnetic 21 22 resonance imaging at baseline and every 12-18 weeks from the surgery date. Protocols of imaging

surveillance varied slightly across the centers with an interval between imaging tests ranging from 3 1

to 6 months.

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Statistical analysis

4 Categorical data were presented as counts and percentages. Continuous data were presented as 5 6 medians and interquartile ranges (IQR). Differences in categorical variables were analyzed by the Wilcoxon rank-sum test, while differences in continuous variables were analyzed by Pearson's chi-7 squared test. The survival curves were estimated with the Kaplan–Meyer product limit method. 8 Recurrence-free survival (RFS) was calculated from the time of initial diagnosis to the first radiological 9 evidence of recurrence. Overall survival (OS) was calculated from the initial diagnosis date to the date 10 of death from any cause. Patients who did not experience either recurrence or who did not die were 11 censored at the date of the last follow-up visit for the specific survival analysis. To prevent the 12 immortal time bias for patients with early progression or who had died, we calculated RFS and OS 13 estimates from the time point of 3 months, using the landmark method. 14 Cox proportional hazard regression models were fitted to determine prognostic factors on RFS and 15 OS. The following potential prognostic factors for either RFS or OS were investigated in patients with 16 localized disease (stage I-III): patient age and sex, tumor stage, cortisol secretion, other hormonal 17 18 secretion, type of presentation, tumor size, Weiss score and Ki67 at diagnosis, surgical approach, resection (R) status, and adjuvant mitotane treatment; while recurrence was analyzed only for OS. 19 The following potential prognostic factors for OS were investigated in patients with metastatic disease 20 (stage IV) at diagnosis: patient age and sex, tumor size, cortisol secretion, other hormone secretion, 21 22 Weiss score, Ki67, and number of metastatic organs at diagnosis.

- 1 Correlations and 95% CIs between OS and RFS were measured using normal score rank correlation,
- 2 calculated using the iterative multiple imputation approach for analysis of correlations between two
- 3 partially censored failure times (25). Although this approach is semi-parametric since it does not
- 4 require any assumptions about the marginal distributions, it uses a Gaussian dependency structure,
- 5 which may lead to bias due to misspecification. We thus also computed the rank correlation between
- 6 OS and RFS using a non-parametric estimator of Spearman's correlation, based on a nonparametric
- 5 bivariate survival surface estimator (26). Bootstrap resampling was implemented to compute 95% CIs.
- 8 Both methods have their own limitations. Although the non-parametric method does not make
- 9 assumptions about the underlying correlation structure and, therefore, is less prone to bias, the semi-
- parametric method appears more stable than non-parametric estimators, particularly for small
- sample sizes. For these reasons, we reported the results of both methods.
- All reported p values are two-sided. P-values less than 0.05 were considered as statistically significant.
- 13 Statistical analyses were performed with R version 4.0 (R Core Team, USA).

1 **RESULTS**

- 2 Patient characteristics at clinical presentation
- 3 A total of 512 patients were included in the study, and their characteristics at diagnosis are shown in
- 4 **Table 1**.
- 5 The median age was 48 (37-59) years and most patients had stage II ACC (51%), while only 21%
- showed stage IV ACC, mainly with one (41.6%) or two (30.7%) metastatic organs. Only one patient
- 7 had a familial history of ACC, associated with TP53 mutation. Median follow-up was 30 (IQR 10-62)
- 8 months.
- 9 Women were younger than men, and their ACCs were smaller, and more frequently steroid-secreting
- 10 (**Table 1**). In both sexes, the most common steroid secretion was cortisol (alone or in combination
- with other hormones), followed in women by androgen secretion. ACC presented as an adrenal
- incidentaloma in 38.1% of cases, ranging from less than 20% in patients under 30 to more than 50% in
- patients over 70 (Figure 1). ACCs presenting as adrenal incidentalomas were smaller, of early stage,
- less frequently secreting, and with less aggressive pathological features (**Table 1**).

First-line treatment

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- 17 Localized disease (380 patients with stage I-III ACC)
- Surgery was the first treatment in all evaluable cases (missing data in 9 patients), with a
- 19 predominance of open surgery (72%). The surgical approach differed by stage (p<0.01), with more
- 20 laparoscopic surgery in stage I (62%) and more open surgery in stages II and III (71% and 90%,
- 21 respectively) (Table 2).
- 22 After radical surgical resection, 226 out of 360 (62.7%) patients (missing data in 20 cases) received
- 23 adjuvant therapy with mitotane. Patients who were treated with mitotane presented with larger

- 1 (p=0.01) and more frequently symptomatic (p<0.04) tumors, together with more aggressive
- 2 pathological features, such as higher Weiss score (p<0.01) and higher Ki-67 (p<0.01), than patients
- 3 who underwent surveillance only (**Table 2**). Moreover, mitotane treatment increased with stage
- 4 (p<0.01): 45% of stage I, 62% of stage II and 73% of stage III ACCs underwent adjuvant mitotane.

- 6 Metastatic disease (101 patients with stage IV ACC)
- 7 Surgery was performed in 71 patients (70.3%), which was mainly open (45 out of 71, 63.3%). Medical
- 8 treatment was initiated in 89 patients (88.1%), in most cases (76.4%) with a combination of mitotane
- 9 and chemotherapy, while mitotane alone was administered in 13 patients (14.6%) and chemotherapy
- alone in 8 patients (9%). Age differed between the patients who received different types of treatment
- (mitotane and chemotherapy median age 42.5, IQR 34-53.25 years; mitotane alone 68, IQR 64-72
- years; chemotherapy alone 58, IQR 56-68 years; p<0.001). No other significant statistical difference
- 13 among these groups was found.

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Outcomes

- 16 Recurrence free survival
- 17 The median follow-up for RFS was 15 (IQR 5-41) months. Excluding the 8 patients with macroscopic
- residual tumor after surgery (R2), recurrence occurred in 209 out of 372 patients (56.2%). There was
- an inverse relationship between the duration of RFS and tumor stage (Figure 2A). RFS at five years
- 20 was 33% (95%Cl, 28-39%), with 59% (95%Cl, 43-80) for stage I ACC, 36% (95%Cl, 29-44) for stage II
- and 16% (95%CI, 9-27) for stage III.
- The median follow-up for RFS for patients still alive at the study closure was 18 (IQR 6-48.5) months.
- 23 ACCs presenting as adrenal incidentalomas showed prolonged RFS (Figure 2B).

- 1 At multivariate analysis, stage III, cortisol secretion, Weiss score and Ki67 were associated with an
- 2 increased risk of recurrence, while margin-free resection (R0), open surgery and adjuvant mitotane
- 3 treatment were associated with prolonged RFS (**Table 3, Figure 3**).
- 5 Overall survival

- 6 The median follow-up for OS was 30 (10-62.25) months. Death occurred in 195 out of 512 (38.1%)
- 7 patients. OS progressively decreased with advanced stage (Figure 4A). OS at five years was 54%
- 8 (95%CI, 49-60%), with 71% for stage I ACC (95%CI, 55-92), 68% (95%CI, 61-75) for stage II, 41%
- 9 (95%CI, 30-56) for stage III, and 13% (95%CI, 6-28) for stage IV.
- 10 The median follow-up for OS for patients still alive at the study closure was 34 (IQR 13.75-69.25)
- months. ACCs presenting as adrenal incidentalomas tumor showed prolonged OS (Figure 4B). In
- patients with localized disease, multivariate analysis showed that age at diagnosis, stage III, cortisol
- secretion, Ki67 and the event of recurrence were associated with increased mortality (**Table 3**).
- 14 The ρ correlation between OS and RFS was 0.82 (95%CI, 0.74 0.87), using the iterative multiple
- imputation approach, and 0.70 (0.59 0.78), using a non-parametric estimator of Spearman's
- 16 correlation (Figure 5).
- 17 At multivariate analysis, in patients with metastatic disease, only cortisol secretion (HR 2.05; 95%CI,
- 18 1.06-3.98, p<0.05) and number of metastatic organs (HR 1.77; 95%CI, 1.26-2.50, p<0.001) were
- associated with increased mortality (Supplemental Table 1) (27).

DISCUSSION

- 2 The availability of a large cohort of well-characterized patients with ACC enabled us to retrieve
- 3 interesting data on clinical presentation, prognostication, and outcome of this rare tumor.
- 4 A slight predominance of women was found, confirming previous reports (4-6,9,22,23). After further
- 5 investigations, women were found to be younger than men and harbored smaller tumors that were
- 6 more frequently steroid-secreting.
- 7 Given the increasingly higher number of adrenal incidentalomas detected in every-day practice [26,
- 8 27], we focused on the incidental presentation of ACC which in our study was more frequent (38.1%)
- 9 than reported in a few previous studies (approximately 18%) (9,23). In our cohort, incidental
- 10 presentation of ACC increased with age, thus paralleling the age distribution of adrenal
- incidentalomas. However, a notable number of incidental ACCs were found even among younger
- patients. It is currently not known whether the serendipitous discovery of an adrenal tumor offers the
- opportunity for an early diagnosis, since the issue of ACCs presenting as adrenal incidentalomas has
- been almost neglected. Importantly, in our study ACCs presenting as adrenal incidentalomas were
- diagnosed at an early stage, with a smaller size and less aggressive pathological features than
- symptomatic tumors. Patients with incidental ACCs were thus found to have prolonged RFS and OS
- 17 compared to patients with symptomatic tumors. In multivariable analysis, the effect of incidental
- presentation was no longer evident since it was linked to favorable parameters (stage, pathological
- 19 features) which are the main drivers of prognosis. From a clinical viewpoint, however, the present
- 20 findings show that the serendipitous detection of an ACC provides the opportunity for an early
- 21 diagnosis, thus underlining the importance of confidently excluding the possibility of ACC in all
- 22 patients with an adrenal incidentaloma, and to offer prompt surgery to patients with suspicious
- 23 tumors (28).

- 1 Previous population-based studies on ACC patients have already demonstrated that ENSAT stage and
- tumor grade at diagnosis are significantly associated with survival (5,6), which was confirmed by our
- 3 findings.
- 4 We carried out a study of the management of ACC in Italy. Although open surgery was most
- 5 frequently performed in patients with localized disease (72%), laparoscopic surgery was preferred in
- 6 smaller tumors and stage I ACC. This is considered reasonable according to international guidelines
- 7 (1,15), however, the role of laparoscopic surgery in the management of ACC remains controversial. In
- 8 the present study, open surgery was associated with a slightly beneficial effect on the risk of
- 9 recurrence, despite that this approach was used in patients with worse prognostic factors. This
- reinforces the idea that open surgery is the treatment standard (29,30), at least for patients with
- locally advanced disease (31), to minimize the risk of peritoneal carcinomatosis (32).
- 12 Although it is biologically plausible that disease recurrence portends a worse prognosis, this is the first
- study to formally demonstrate the significant impact of an ACC recurrence on survival. We also
- showed a correlation between RFS and OS, thus suggesting that RFS is an adequate surrogate for OS
- also for patients with ACC, as reported for other tumors (33-35). The demonstration that ACC
- recurrence is a strong predictor of death underscores the importance of developing adjuvant
- 17 concepts to prevent disease recurrence. Uncertainty remains regarding the value of adjuvant
- mitotane despite international guidelines recommending its use after surgery in patients at high risk
- of recurrence (1,15). Therefore, the finding that adjuvant mitotane treatment is linked to a reduced
- 20 recurrence risk, despite the worst characteristics of treated patients (larger and symptomatic tumors,
- 21 higher Weiss scores and Ki-67% compared to the group on surveillance only), supports mitotane use
- 22 in an adjuvant setting. Follow-up is likely immature to demonstrate an effect of adjuvant mitotane on
- 23 overall survival in non-metastatic ACC. Interestingly, this is the first time that adjuvant mitotane

- therapy has been assessed in a population-based study, while previous population-based studies did
- 2 not capture data on adjuvant medical therapy (5,6) or show aggregate data including different
- 3 adjuvant treatments (mitotane, radiotherapy and chemotherapy) as a single variable (22,23). In our
- 4 study, adjuvant mitotane treatment was used extensively probably because a seminal paper on this
- 5 treatment was published in 2007 by Italian and German centers (36).
- 6 In patients with metastatic presentation, multimodal treatment was the most common strategy, as
- 7 international guidelines recommend (1,15). Although recent papers seem to demonstrate that
- 8 cytoreductive surgery of the primary tumor in metastatic patients is associated with prolonged
- 9 survival (6,37), we found no evidence of this, however the number of patients with stage IV at
- diagnosis was rather limited. We should acknowledge that patients with advanced ACC may be
- underrepresented in our study, because most of the referral centers in Italy are endocrine
- departments and such patients may be referred to oncological departments. Due to the long time
- frame of data capture, which applies to all population-based studies, the technical changes in imaging
- and surgical techniques represent another limitation, together with the retrospective nature of the
- 15 analysis.
- 16 The strengths of our analysis are the large sample size, the availability of detailed clinical annotation
- and complete follow-up information, plus the use of similar protocols for diagnosis, surveillance and
- 18 treatment across the different centers.

CONCLUSIONS

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- 2 In a large and well-characterized cohort of ACC patients, our study shows that ACC is a sex-related
- disease, and demonstrates that both tumor characteristics (tumor stage and size, hormonal secretion,
- 4 Ki67%, Weiss score) and treatment strategies (open surgery, adjuvant mitotane) have an impact on
- 5 clinical outcome. We demonstrate for the first time that incidental ACC may have a better prognosis
- 6 than symptomatic tumors and that disease recurrence is a strong predictor of mortality. These
- 7 findings highlight the value of surgery and adjuvant mitotane to prevent recurrence and improve
- 8 patient outcome. Open surgery appears to be the most effective way of removing ACC.
- 9 Finally, the present study provides information on the management of patients with ACC in Italy,
- which could be used as a benchmark for future studies.

Data Availability Statement

- 14 Some or all datasets generated during and/or analyzed during the current study are not publicly
- available but are available from the corresponding author on reasonable request.

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Table 1. Baseline features of patients and comparison between sexes or mode of detection*.

Characteristics	Entire cohort	Women N= 308	Men N= 204	p value	Incidental N= 181	Hormone excess N= 208	Local symptoms N= 86	p value
Sex, N (%)								
Valid cases	512				181	208	86	< 0.01
Male	204 (39.8%)				82 (45.3%)	66 (31.7%)	42 (48.8%)	
Female	308 (60.2%)				99 (54.7%)	142 (68.3%)	44 (51.2%)	
Age, year								
Valid cases	512	308	204	0.02	181	208	86	< 0.01
Median (IQR)	48 (37-59)	47 (35-58)	50 (39-59)		51 (40-61)	47 (32-58)	49 (39-60)	
Tumor stage, N (%)							7	
Valid cases	481	287	194	0.86	173	201	84	0.01
Stage I	40 (8.2%)	25 (8.7%)	15 (7.7%)		20 (11.5%)	13 (6.5%)	3 (3.6%)	
Stage II	247 (51.4%)	146 (50.9%)	101 (52.1%)		97 (56.1%)	97 (48.3%)	40 (47.6%)	
Stage III	93 (19.4%)	53 (18.4%)	40 (20.6%)		33 (19.1%)	35 (17.4%)	20 (23.8%)	
Stage IV	101 (21.0%)	63 (22.0%)	38 (19.6%)		23 (13.3%)	56 (27.9%)	21 (25.0%)	
Tumor size, cm		/	,		()	/	//	
Valid cases	470	277	193	0.03	167	196	83	< 0.01
Median (IQR)	10 (7–13)	9.5 (7-12.7)	10 (7-14)	0.00	9 (6-12)	10 (7-13)	12 (9-18)	10101
Hormone secretion, N (%)	10 (7 10)).5 (F 12.7)	10 (7 11)		(0 12)	10 (7 10)	12 (7 10)	
Valid cases	480	289	191	<0.01	175	208	79	< 0.01
No	219 (45.6%)	114 (39.5%)	105 (55.0%)	VO.01	129 (73.7%)	0 (0%)	72 (100%)	<0.01
Yes	261 (54.4%)	175 (60.5%)	86 (45.0%)		46 (26.3%)	208 (100%)	7 (0.0%)	
Cortisol <u>+</u> other hormones	198 (75.9%)	123 (70.3%)	75 (87.2%)		38 (82.6%)	156 (75.0%)	4 (57.1%)	
Androgen (in women)	45 (17.2%)	45 (25.7%)	0 (0.0%)		6 (13.0%)	37 (17.8%)	2 (28.6%)	
Estrogen (in men)	7 (2.7%)	0 (0.0%)	7 (8.1%)		1 (2.2%)	6 (2.9%)	0 (0.0%)	
Mineralocorticoid	11 (4.2%)	7 (4.0%)	4 (4.7%)		1 (2.2%)	9 (4.3%)	1 (14.3%)	
Incidentaloma, N (%)	11 (4.2 /0)	7 (1.070)	Ŧ (Ŧ.7 70)		1 (2.270)	7 (1.070)	1 (11.070)	
Valid cases	475	285	190	0.06				
Yuna cases Yes	181 (38.1%)	99 (34.7%)	82 (43.2%)	0.00				
No	294 (61.9%)	186 (65.3%)	108 (56.8%)					
	294 (61.9 %)	160 (65.5 %)	100 (30.0 %)					
Weiss score	204	107	447	0.50	100	111	40	.0.01
Valid cases	284	167	117	0.52	108	111	49	<0.01
Median (IQR)	6 (5–7)	6 (5-7)	6 (5-8)		5 (4-7)	6 (5-8)	6 (5-8)	
Ki67								
Valid cases	310	183	127	0.25	121	116	58	< 0.01
Median (IQR)	16 (8–30)	20 (8-30)	15 (6-30)		11 (5-20)	20 (10-30)	20 (10-40)	
2 * Mode of detec					•			
symptoms were co	ounted in the c			_	_	N = number of p	patients. The bold	
4		indicates	statistically si	gnificant	values.			
Υ ,								
Y								

^{*} Mode of detection was known in 475 patients. Patients who were symptomatic for both hormone excess and local $symptoms \ were \ counted \ in \ the \ category \ "hormone \ excess". \ IQR = interquartile \ range. \ N = number \ of \ patients. \ The \ bold$ indicates statistically significant values.

1 Table 2. Breakdown of characteristics of patient with localized disease, by surgical or adjuvant approaches.

Chamastonistics	Open surgery Laparoscopy		p	Mitotane	Surveillance only	р
Characteristics	N= 267	N= 104	value	N= 226	N= 134	value
Age , year						
Valid cases	267	104	0.12	226	134	0.65
Median (IQR)	48 (36.2-58.0)	49.5 (36.8-59.3)		48 (36-58)	49 (37-60)	
Sex, N (%)						
Valid cases	267	104	0.53	226	134	0.95
Male	116 (43.4%)	36 (34.6%)		92 (40.7%)	55 (41.0%)	
Female	151 (56.6%)	68 (65.4%)		134 (59.3%)	79 (59.0%)	
Tumor stage, N (%)						
Valid cases	267	104	< 0.01	226	134	< 0.01
Stage I	15 (5.6%)	24 (23.1%)		18 (8.0%)	22 (16.4%)	
Stage II	171 (64.1%)	71 (68.3%)		145 (64.1%)	89 (66.4%)	
Stage III	81 (30.3%)	9 (8.6%)		63 (27.9%)	23 (17.2%)	
Tumor size, cm						
Valid cases	256	103	< 0.01	220	127	0.01
Median (IQR)	10 (7.8-14)	6 (5-8)		10 (7-14)	8 (6-12)	
Hormone secretion, N (%)		-				
Valid cases	254	100	0.66	216	127	0.04
Yes	131 (51.6%)	49 (49.0%)		120 (55.6%)	56 (44.1%)	
No	123 (48.4%)	51(51.0%)	1	96 (44.4%)	71 (55.9%)	
Incidentalomas, N (%)						
Valid cases	250	100	0.09	214	126	0.04
Yes	98 (39.2%)	49 (49.0%)		81 (37.9%)	62 (49.2%)	
No	152 (60.8%)	51 (51.0%)		133 (62.1%)	64 (50.8%)	
Weiss score						
Valid cases	169	76	0.14	161	82	< 0.01
Median (IQR)	6 (5-7)	6 (5-8)		6 (5-8)	5 (4-7)	
Ki67						
Valid cases	184	79	0.02	172	90	< 0.01
Median (IQR)	18 (8-30)	12 (5-20)		20 (10-31)	10 (5-18)	
≤10%	60 (32.6%)	37 (46.8%)		47 (27.3%)	50 (55.6%)	
>10%	124 (67.4%)	42 (53.2%)		125 (72.7%)	40 (44.4%)	
Resection status, N (%)						
Valid cases	257	99	0.07	219	127	0.70
R0	166 (64.6%)	74 (74.8%)		153 (69.9%)	83 (65.3%)	
R1/RX	86 (33.5%)	22 (22.2%)		61 (27.8%)	42 (33.1%)	
R2	5 (1.9)	3 (3.0%)		5 (2.3%)	2 (1.6%)	

IQR = interquartile range. N = number of patients. The bold indicates statistically significant values.

Table 3. Multivariate analysis of prognostic factors for RFS and OS in patients with localized disease.

*	Recurr	ence free surviv	Overall survival (OS)			
Factor	HR	95% CI	p Value	HR	95% CI	<i>p</i> Value
Age at diagnosis	1.01*	1.00-1.03	0.082	1.04*	1.01-1.07	0.003
Male sex	0.78	0.50 - 1.23	0.30	0.69	0.39-1.24	0.213
ENSAT Tumor stage ¹	2.18	1.54-3.11	< 0.001	2.06	1.27-3.36	0.004
Tumor size	1.04^{*}	0.99 - 1.09	0.103	1.01*	0.97-1.06	0.546
Cortisol secretion ²	2.17	1.50-3.12	< 0.001	2.15	1.34-3.46	0.002
Other hormone secretion ³	1.19	0.71-1.97	0.509	1.40	0.71-2.77	0.330
Incidental presentation ⁴	0.73	0.45 - 1.20	0.214	1.03	0.55-1.93	0.927
R status ⁵	0.56	0.36-0.88	0.013	0.92	0.48-1.79	0.811
Weiss score	1.26*	1.08-1.47	0.003	1.05*	0.88 - 1.26	0.590
Ki67	1.03*	1.01-1.04	< 0.001	1.02*	1.01-1.04	0.004
Open surgery ⁶	0.67	0.46-1.00	0.047	0.94	0.47 - 1.89	0.865
Adjuvant Mitotane Therapy ⁷	0.55	0.34 - 0.88	0.012		-	-
History of recurrence	-	-	-	11.09	2.65-46.43	0.001

*Per unit increase. Reference categories: ¹ ENSAT Stage III *versus* I-II; ² cortisol-secreting tumors *versus* not-secreting tumors; the category "cortisol secretion" includes tumors secreting cortisol with or without other steroids; ³ other hormone-secreting tumors *versus* not-secreting tumors; the category "other steroid secretion" includes secretion of any steroid except cortisol; ⁴incidental tumors *versus* symptomatic tumors; ⁵ R0 versus R1-Rx; ⁶ open *versus* laparoscopic surgery; ⁷ adjuvant mitotane *versus* surveillance only. HR = Hazard Ratio. Bold indicates statistically significant values.

1 Figure legends

- 2 **Figure 1.** Patients with ACC presenting as incidentaloma by age.
- 3 Figure 2. Kaplan Meier curves for recurrence free survival according to tumor stage (A) and mode of
- 4 detection (B).
- 5 Figure 3. Kaplan Meier curves for recurrence free survival of patients who underwent adjuvant
- 6 mitotane treatment or surveillance only.
- 7 Figure 4. Kaplan Meier curves for overall survival according to tumor stage (A) and mode of detection
- 8 (B).

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- 9 Figure 5. Correlation between recurrence free survival (RFS) and overall survival (OS) calculated using
- the iterative multiple imputation approach (A) or a non-parametric estimator of Spearman's
- 11 correlation (B).

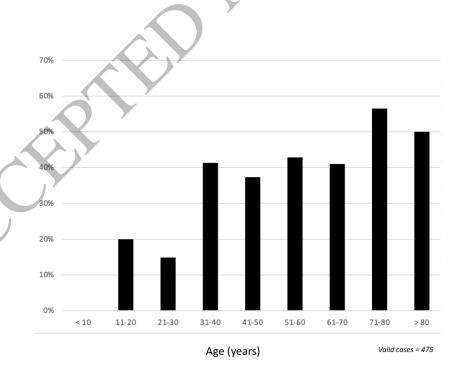


Figure 1 170x96 mm (.06 x DPI)

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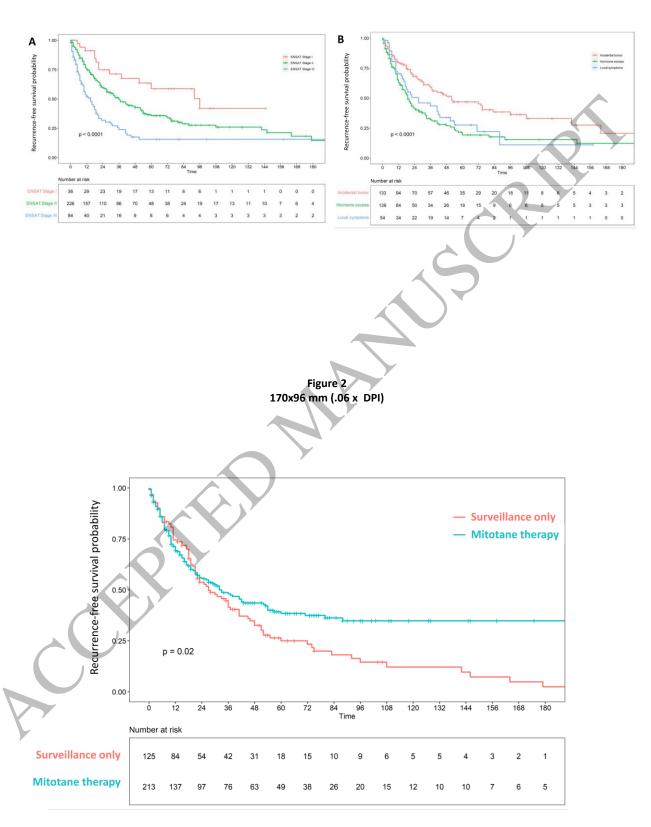
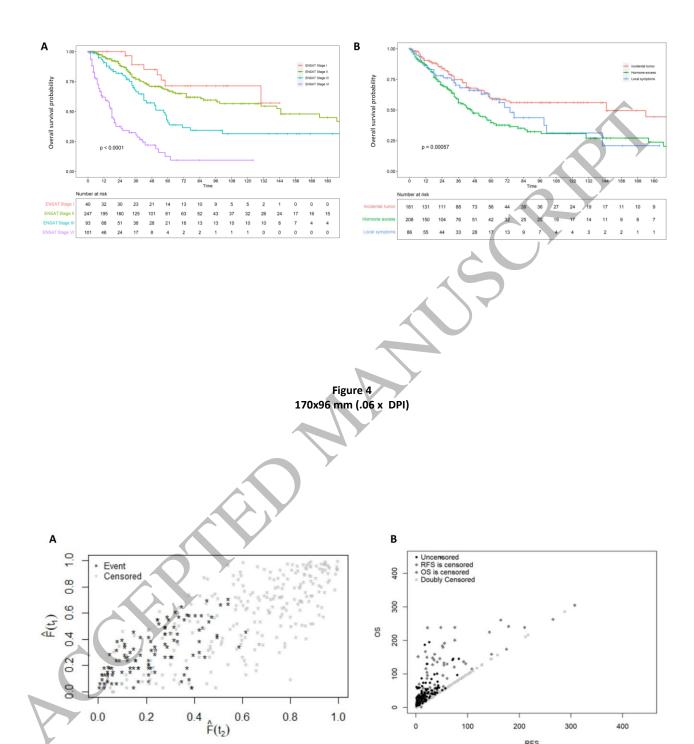


Figure 3 170x96 mm (.06 x DPI)



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Figure 5 170x96 mm (.06 x DPI)

RFS