MAJOR PROGNOSTIC ROLE OF KI67 IN LOCALIZED ADRENOCORTICAL CARCINOMA AFTER COMPLETE RESECTION.

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Major prognostic role of Ki67 in localized adrenocortical carcinoma after complete resection

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Abstract

Background: Recurrence of adrenocortical carcinoma (ACC) even after complete (R0) resection occurs frequently.

Objective: The aim of this study was to identify markers with prognostic value for patients in this clinical setting.

Design, Setting, and Participants: From the German ACC registry 319 patients with ENSAT stage I-III were identified. As an independent validation cohort 250 patients from three European countries were included.

Outcome Measurements and Statistical Analysis: Clinical, histological and immunohistochemical markers were correlated with recurrence-free (RFS) and overall survival (OS).

Results and Limitation: While univariable analysis within the German cohort suggested several factors with potential prognostic power, upon multivariable adjustment only a few including age, tumor size, venous tumor thrombus (VTT), and the proliferation marker Ki67 retained significance. Among these Ki67 provided the single best prognostic value for RFS (HR for recurrence 1.042 per 1% increase; $p<0.0001$) and OS (HR for death 1.051; $p<0.0001$) which was confirmed in the validation cohort. Accordingly, clinical outcome differed significantly between patients with Ki67<10%, 10-19%, and ≥20% (for the German cohort: median RFS: 53.2 *vs.* 31.6 *vs.* 9.4 months; median OS: 180.5 *vs.* 113.5 *vs.* 42.0 months). Using the combined cohort prognostic scores including tumor size, VTT, and Ki67 were established. Although these scores discriminated slightly better between subgroups, there was no clinically meaningful advantage in comparison to Ki67 alone.

Conclusion: This largest study on prognostic markers in localized ACC identified Ki67 as the single most important factor predicting recurrence in patients following R0 resection. Thus, evaluation of Ki67 indices should be introduced as standard grading in all pathology reports of ACC patients.

Introduction

Adrenocortical carcinoma (ACC) is a rare but aggressive tumor entity with overall poor prognosis(1-4). Response to medical treatment is limited and only recently the first randomized trial in patients with advanced disease established doxorubicin, etoposide, cisplatin plus mitotane as first-line cytotoxic therapy(5). However, this trial also demonstrated the limitations of systemic treatment with a median overall survival of only 15 months highlighting the importance of early diagnosis and appropriate initial treatment. Although strategies of surgical resection such as open or laparoscopic approaches are controversial(6-8), surgery is the mainstay of initial ACC therapy and currently provides the only realistic chance for cure of the disease. However, even after complete resection patients with ACC remain at high risk for recurrence.

As a response to this clinical challenge adjuvant treatment with mitotane is frequently recommended(2, 9). Although mitotane has shown significant efficacy in preventing recurrence in this setting(10), it has a wide range of side effects and impacts hormone (11-13) and drug(14) metabolism. An additional adjuvant measure is irradiation of the tumor bed, for which some(15, 16) but not all(17) studies have demonstrated efficacy in preventing local recurrence but not to prolong recurrence-free survival (RFS) or overall survival (OS). Thus, all current treatment concepts have the disadvantages of uncertain efficacy and significant toxicity. Therefore, it would be of major importance to limit these treatments to patients with high risk of recurrence which is highly variable in ACC patients(1, 18-21).

While histopathological scores are in use to differentiate between benign and malignant adrenal neoplasms they have not been investigated for their prognostic value. Recently, a number of molecular markers have been identified that were correlated with clinical outcome(22) and even had predictive value for treatment response(23, 24). However, the majority of applied techniques require fresh frozen tumor material and none of these markers has been evaluated in a large patient cohort. Therefore, we set out to identify prognostic factors from routine diagnostic work-up to provide guidance for adjuvant therapy after radical resection. For this purpose we took advantage of large

cohorts of ACC patients with detailed clinical and histopathological annotations within the European network for the study of adrenal tumors (ENSAT).

Patients and Methods

Patient selection

Patients diagnosed with ACC between 1979 and 2011 were identified from the German ACC registry that fulfilled the following inclusion criteria: histologically proven ACC, and localized disease (ENSAT stage I-III(18)) after R0 resection. Resection status was judged on the basis of surgical and pathology reports. Furthermore, a minimum follow-up of 12 months was mandatory unless death occurred earlier. Two third of tumor samples of the German cohort were reviewed by the national reference pathologist (W.S.). All items required to calculate the scores suggested by Weiss, van Slooten, and Hough as well as immunohistochemical staining for Mib1 (Ki67) were evaluated. Further clinical information included in the analysis are provided in Table 1+2. Mitotane therapy was defined as adjuvant therapy with mitotane within 3 months following surgery; hormone production was recorded as any biochemically proven adrenocortical hormone excess.

As an independent validation cohort patients from three European countries were identified from the ENSAT ACC registry based on the same inclusion criteria as stated above. Within this cohort a subset of pre-defined clinical parameters such as age, sex, ENSAT stage (tumor size, lymph node status and VTT), endocrine activity of the tumor and adjuvant mitotane therapy as well as Ki67 were analyzed.

Both registries had been approved by the local ethics committees of all partaking centers and all included patients had provided written informed consent.

Outcome definitions and statistical analysis

The pre-specified primary endpoint of the study was RFS, which was defined as the time interval between initial surgery and the date of radiologic evidence of disease relapse, death resulting from

any cause or the date of last follow-up. As a secondary endpoint OS was calculated from the date of first surgery to death from any cause or the last follow-up visit. RFS and OS rates over time from initial surgery were estimated using the Kaplan-Meier method. The significance of the demographic parameters and clinical characteristics for prognosis of RFS and OS was determined by univariable and multivariable Cox regression models. As the lowest category for Ki67 <5% and for tumor size <5cm was set.

As for the diagnostic scores different strategies were applied to take into account limitations of individual residual analyses. Indeed, following an exploratory analysis model construction was based on stepwise, forward and backward selection using p-value criteria in the range between 0.05 and 0.25. Tumor size was explored as a continuous factor and according to cut-off values. Potential cut-off values for tumor size were pre-specified at 5cm, 8cm, 11cm, 15cm, and 20cm. Furthermore, the number of factors included, the score statistic, the contribution to the change of the hazard in the Cox-model as well as differentiation of survival curve estimation by use of the predictor were considered. Martingale residual analysis was performed indicating well fitting with continuous Ki67 and exploring grouping by equidistant increases of 5% or of 10%. Extensive sensitivity analyses with multivariable models including, excluding and exchanging potential factors from the first explored models were performed. Comparisons between groups were conducted applying the log rank test and presenting two-sided p-values. Estimates of median times to event and hazard ratios (HR) are provided with 95% confidence intervals (CI).

Results

Patient cohorts and characteristics

A total of 319 patients from the German registry and additional 250 patients from 7 European centers fulfilled the pre-specified inclusion criteria. Patient characteristics at initial diagnosis are summarized in Table 1. While tumor stage and most of other clinical items were not different between the groups, the percentage of patients on adjuvant mitotane treatment was significantly lower in the German cohort (26.3 *vs*. 64.8%).

German cohort

Univariable analyses for all presumably relevant demographic, clinical, and histopathological parameters were performed (Table 2). None of the clinical parameters showed significant correlation with both RFS and OS. Tumor stage was of prognostic value but the discrimination was not found to be sufficient for clinical guidance on adjuvant therapeutic strategies (Supplementary Figure 1). Within the large set of histological parameters only few were associated with poor clinical outcome (Table 2) and several multi-item scores were not able to predict outcome better than individual parameters. In contrast, the proliferation marker Ki67 was found to be the single most relevant predictor of disease recurrence and survival with an hazard ratio of 1.042 per 1% increase of Ki67 index for recurrence ($p<0.0001$) and 1.051 for death ($p<0.0001$), respectively (Table 2). Along the same line, Ki67 indices of <10%, 10-19%, and ≥20% provided highly significant differences for both RFS ($p<0.0001$) and OS ($p<0.0001$) translating into a median RFS and a median OS of 53.2 [95% CI 37.7; 74.7] and 180.5 [152.9; no upper limit] months for Ki67<10%, 31.6 [21.5; 48.0] and 113.5 [64.4; 153.7] months for Ki67 10-19%, and 9.4 [7.3; 13.1] and 42.0 [33.7; 56.8] months for Ki67 ≥20%, respectively (Figure 1A+C).

Upon a stepwise and a backward multivariable analysis involving all parameters with a p-value of <0.15 in the univariable analysis plus adjuvant mitotane therapy and taking into account sensitivity

analyses and regression diagnostics, Ki67 remained the factor with the best prognostic power with a hazard ratio of 1.046 per 1% for RFS and 1.061 per 1% for OS (Table 4). When Ki67 was used as a categorical variable the corresponding HR for RFS was 1.94 [1.25-3.03; p=0.0034] for Ki67≥10% and 2.58 [1.71-3.92; p<0.0001] for Ki67≥20% and for OS 3.69 [1.75-7.77; p=0.0006] for Ki67≥10% and 3.59 [1.99-6.48; p<0.0001] for Ki67≥20%. Notably, no other histological parameter retained its relevance when the analysis was adjusted for Ki67. While age, tumor size, and VTT had some association with clinical outcome none of these factors showed a similar prognostic power for both RFS and OS as Ki67.

Of note, adjuvant treatment with mitotane was not significantly associated with clinical outcome in univariable analysis, but after multivariable adjustment a trend for longer RFS was detectable (HR 0.71; p=0.087) and it became a significant factor for OS (HR 0.41; p=0.009, Table 4).

European validation cohort

Following these observations we investigated a defined sub-set of clinical and histopathological parameters within the European validation cohort. Comparable to the German cohort the proliferation marker Ki67 provided the best prognostic value for prediction of RFS and OS, respectively (Table 3 and Figure 1B+D). Following multivariable analysis Ki67 retained highly significant association with RFS and OS with a hazard ratio for recurrence of 1.020 [1.010-1.029] and for overall survival of 1.026 [1.013-1.039] per 1% increase, respectively (Table 4). Similar to the German cohort, age tumor size and the presence of VTT harbored none or only minor prognostic value (Table 4).

Establishment of a prognostic score

In a next step, we aimed at the development of a prognostic score for further clinical guidance in the management of patients with ACC after complete resection. For this purpose, we applied two different models using the pooled data of both cohorts (n=569). Both algorithms included the following 3 risk factors (RF): Ki67, tumor size between 15 and 20 cm and presence of VTT.

In the first, basic model Ki67 was regarded as one RF for Ki67≥10% and as a second RF for Ki67≥20%. Either tumor size of 15-20cm or presence of VTT was accounted as one combined RF. Each of these 3 RF was counted as 1 point in the prognostic score, which resulted in 4 groups (0-3 RF) with different outcome. However, applying this score, estimated RFS and OS of the different risk groups by Kaplan-Meier analysis provided no clinically meaningful separation between the two groups with the highest scores which were therefore combined (Figure 2B, Supplementary Figure 2B). In the next model the same RF were weighted individually according to their prognostic power and specifically for RFS. This second modeling resulted in a slightly improved risk prediction and allowed for differentiation in more subgroups (Figure 2C, Supplementary Figure 2C).

Discussion

This largest study on prognostic factors in localized ACC provides strong evidence that Ki67 index is the most powerful tool of all parameters analyzed in this study to predict recurrence in two independent cohorts of patients after complete surgical resection. Likewise, OS was also strongly associated with the Ki67 index. Following multivariable analysis including age, tumor stage, adjuvant mitotane treatment and all standard histological parameter used in ACC, the Ki67 index retained its outstanding prognostic power. Importantly, these results initially obtained from a German cohort could be validated in an independent European sample. In a next step, we aimed at the establishment of clinical risk scores. Although both applied models including the parameters Ki67, tumor size, and presence of VTT were able to discriminate patient cohorts with different clinical outcome, the added value of these scores in comparison to the use of Ki67 alone was modest. Thus, Ki67 is obviously the best factor to establish a grading system in ACC with Ki67 <10% defining grade 1 tumors, Ki67 10-19% grade 2 and Ki67 ≥20% grade 3 tumors.

ENSAT stage III has been defined by the presence of positive lymph nodes, tumor infiltration in surrounding tissue or the presence of VTT, while stage IV is restricted to patients with distant metastasis(18). This system which had been independently validated(25) performs well over the whole spectrum of ACC patients to predict overall prognosis. However, as we demonstrate herein within the pre-selected patient group with localized disease following complete surgical resection the ENSAT staging system seems to be of limited relevance. The reason for this lack of prognostic power is most likely due to the fact that stage III usually comes with a relatively high risk of incomplete resection. In fact, according to a German(26) and an U.S. series(20) this accounts for about 10% of patients with localized ACC. However, these cases as well as those with uncertain resection status were excluded from our analysis, because the high risk for recurrence in these patients is obvious.

Another initially surprising result is the fact that the outcome of patients with a tumor size >20 cm was better than of those with smaller tumors. However, as described above, this series is a highly selected cohort and very large tumors that are still resectable and not metastasized at the time of surgery might indeed represent a subgroup of tumors with specific biological behavior.

To appreciate the quite variable outcome even in the well defined subgroup of completely resected patients, additional parameters with prognostic value need to be taken into consideration. Recently, inclusion of tumor grading on the basis of mitotic counts has been proposed to improve the prediction of prognosis(27). Accordingly, in our two independent cohorts, quantification of Ki67 as a well defined marker of cellular proliferation provided additional prognostic information with relevant clinical impact. This is in good agreement with earlier studies which had suggested Ki67 as a marker with prognostic value in ACC patients(28-31). While these series were small and the overall results therefore not consistent, based on the current results, we strongly suggest tumor grading based on three categories of a Ki67 index <10%, 10-19% and above 20%.

Ki67 index in our analysis proved to be superior to different histological scores such as those proposed by Weiss, van Slooten or Hough that are currently in clinical use for the differential diagnosis of adrenal tumors. The reason for this finding probably relates to the fact that a number of sub-items required for these scores such as atypical mitoses, abnormal nucleoli and nuclear atypia had no prognostic potential or were even associated with a trend towards better outcome. This does not question the overall applicability of the scores to discern between benign adrenal adenomas and ACC for which purpose they had been originally proposed. However, the findings highlight the limitation of the scores as prognostic tools for this particular group of patients and fuels speculation whether these sub-items are of particular importance for the scores. Along the same line, some pathologists have argued for a simplified Weiss score that bases on the more reliable criteria only(32, 33).

The effect of mitotane on clinical outcome was surprisingly different between the German cohort and the validation cohort. Due to the retrospective nature of our study we can only speculate about the underlying reasons for this difference. However, the fact that in the German series only 84 out of 319 patients (26%) were treated with mitotane in comparison to 65% in the validation series already point towards a general difference in therapeutic policies. One explanation for this discrepancy is probably related to the time interval of patient inclusion. Two third of the German patients were diagnosed before 2007, when the land-mark study on the adjuvant usage of mitotane had been published(10), whereas the non-German cohorts were recruited mostly after that time period. However, this observation could also be interpreted as another hint that not all patients will benefit from mitotane treatment and prospective trials are required to provide reliable answers.

In variance to recent studies in the current cohort no relevant adverse effect of steroid excess on RFS and OS was found. One of the differences that are present in the current publications refer to the cohorts. While in a recent manuscript by Berruti and colleagues (34) some overlap with our study is present adding patients from North America might have contributed to the observed differences. In fact, in two studies from single US centers (35, 36) both find Cushing's syndrome as a marker of poor prognosis. In addition – and probably more importantly - while in our study hormone excess was defined by biochemical means, in the published studies this was judged on a clinical basis.

Our study has obvious limitations as a result of its non-randomized design, in which multiple factors may have led to different treatment decisions in individual patients. These limitations are shared by other studies that have investigated prognostic factors in ACC patients (for details see Suppl. Table 1). Furthermore, variability of Ki67 index evaluation at different clinical centers is to be expected.

This refers to pre-analytic variations, usage of different antibodies and staining reagents as well as quantification underestimating tumor heterogeneity.

Despite these limitations the high number of patients as well as the inclusion of an independent validation cohort underscores the overall robustness of the reported findings. The results could have immediate impact on the clinical decision for or against adjuvant treatment options: even after complete resection patients with high Ki67 index have a high likelihood to suffer from recurrent disease, thereby calling for a more aggressive therapeutic course. In contrast, patients with a low Ki67 index are likely to have a less favorable risk/benefit ratio of adjuvant treatment considering its substantial toxicity. Whether or not mitotane is the appropriate treatment particularly for tumors with high proliferation rate remains open. At least part of this question will be answered by the ongoing ADIUVO trial (mitotane vs. observation in low grade tumors after R0 resection; www.adiuvo-trial.org)

Conclusions

In conclusion, in this study analyzing multiple potential prognostic markers in two independent cohorts of 568 patients with completely resected ACC Ki67 emerged as the single most important factor predicting recurrence and should be part of any pathology report of ACC to provide tumor grade. This finding will further guide the management of patients with this rare disease.

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Table 1: Baseline characteristics of the two patient cohorts

	n	German cohort (n=319)	n	Validation cohort (n=250)
Age	319	46.3 (0.4-83.6)	250	46.7 (9.1-83.0)
Sex	319	207 females, 112 males	250	162 females, 88 males
Median tumor size (cm)	318	10.0 (2.3-40.0)	248	11.0 (2.0-30.0)
ENSAT stage (18)	319	I: 27 (8.5%) II: 202 (63.3%) III: 90 (28.2%)	245	I: 22 (9.0%) II: 156 (63.7%) III: 67 (27.3%)
Median Ki67 index (%)	223	10 (1-60)	239	10 (0-82)
Adjuvant mitotane therapy	319	84 (26.3%)	219	142 (64.8%)
Adjuvant radiation	313	30 (9.6%)	250	7 (2.8%)
History of recurrence	319	206 (64.6%)	250	135 (78.4%)
Median follow-up of alive patients (months)	205	43.7	162	69.8
Death from any cause	319	114 (35.7%)	250	88 (35.2%)
Death from ACC	319	100 (31.3%)	250	65 (26.0%)

Table 2: Univariable analysis (Cox regression) of the German cohort (n=319)

			RFS			OS		
Factor [unit]	**N of 319**	**N+**	**events**	**hazard ratio**	**p-value**	**events**	**hazard ratio**	**p-value**
Age [years]	319		218	1.010 per year	0.0132	114	1.008 per year	0.1330
Tumor size [cm]	318		218			114		
≥5		293		1.276	0.3366		1.550	0.2332
≥8		234		1.590	0.0047		1.270	0.2853
≥11		151		1.377	0.0189		1.153	0.4493
≥15		68		1.477	0.0150		1.491	0.0652
≥20		18		0.819	0.5217		0.595	0.3087
Tumor size 8-20 cm	318	216	218	1.589	0.0025	114	1.381	0.1296
Tumor size 11-20 cm	318	133	218	1.450	0.0066	114	1.268	0.2081
Tumor size 15-20cm	318	50	218	1.777	0.0009	114	1.900	0.0048
Infiltration in surrounding tissue	266	58	179	1.236	0.2239	101	1.468	0.0823
Invasion in adjacent organ	271	11	183	1.601	0.0080	105	1.289	0.5838
Lymph node positivity	250	20	171	2.173	0.0020	97	1.846	0.0569
Presence of venous tumor	306	25	210	1.441	0.1215	112	1.742	0.0409

thrombus in renal vein or vena cava								
ENSAT stage	319		218			114		
II				1.257	0.3885		1.290	0.5002
II or III				1.780	0.0373		2.155	0.0449
Glucocorticoid secretion	184	101	125	1.115	0.5512	65	0.715	0.1889
Adjuvant mitotane	319	84	218	0.855	0.3402	114	0.650	0.1038
Ki67 [%]	223		122			69		
≥5		184		2.616	0.0002		4.417	0.0015
≥10		139		2.743	<0.0001		5.322	<0.0001
≥15		82		2.810	<0.0001		4.955	<0.0001
≥20		69		3.526	<0.0001		5.595	<0.0001
≥25		37		3.050	<0.0001		4.320	<0.0001
Ki67 [%]	223		143	1.042 per 1%	<0.0001	69	1.051 per 1%	<0.0001
Weiss Score ≥5[#]	199	143	138	1.435	0.0638	70	1.155	0.5810
Mitotic count >5/50HPF	220	154	149	1.647	0.0088	77	1.357	0.2410
Nuclear atypia	238	193	162	0.675	0.0461	86	0.703	0.1877
Atypical mitoses	227	60	154	0.927	0.6819	80	0.897	0.6760
Clear cells <25 %	201	183	139	2.825	0.0049	70	1.650	0.2822
Diffuse architecture	209	169	141	1.648	0.0297	73	0.834	0.5157

Venous invasion	213	91	147	1.758	0.0007	75	1.669	0.0286
Sinusoidal invasion	210	123	146	1.271	0.1594	74	0.988	0.9574
Capsular invasion	271	142	182	1.343	0.0489	99	1.252	0.2694
Necrosis	234	189	159	1.633	0.0226	83	1.830	0.0626
Hough score≥3.23[#]	189	96	134	1.390	0.0001	68	1.065	0.5869
Vascular invasion	273	176	187	1.462	0.0152	98	1.297	0.2289
Fibrous bands	204	121	140	1.270	0.1696	69	1.275	0.372
van Slooten score[#]	189	96	134	1.027	0.0576	68	1.002	0.9181
Mitotic count >2/10HPF	220	110	149	1.828	0.0003	77	1.383	0.1603
Nuclear hyperchromasia	231	157	159	0.876	0.4347	83	0.820	0.3891
Abnormal nucleoli	223	92	155	0.804	0.1803	81	0.980	0.9275

[#] the cutoff for these scores were set as the median.

N+, number of patients that fulfilled the given criterion.

Table 3: Univariable analysis (Cox regression) of the validation cohort (n=250)

	RFS					OS				
Factor [unit]	**N of 250**	**N +**	**events**	**hazard ratio**	**p-value**	**N of 250**	**N +**	**events**	**hazard ratio**	**p-value**
Age [years]	247		145	1.007 per year	0.2917	249		87	0.996 per year	0.6151
Tumor size [cm]	245		144			247		86		
≥5		230		1.344	0.4803		232		0.576	0.2348
≥8		195		2.034	0.0051		197		1.305	0.3790
≥11		131		1.772	0.0012		133		1.408	0.1287
≥15		64		1.245	0.2268		66		1.344	0.1907
≥20		21		0.984	0.9540		23		0.849	0.6426
Tumor size 8-20 cm	245	174	144	1.658	0.0111	247	174	86	1.302	0.2863
Tumor size 11-20 cm	245	110	144	1.760	0.0008	247	110	86	1.483	0.0690
Tumor size 15-20cm	245	43	144	1.361	0.1356	247	43	86	1.621	0.0512
Infiltration in surrounding tissue	195	41	124	1.100	0.6534	195	41	69	0.982	0.9500
Lymph node positivity	164	9	100	1.060	0.8923	164	9	54	2.027	0.1058
Presence of venous tumor thrombus in renal vein or	195	23	124	2.207	0.0012	195	23	69	2.024	0.0237

vena cava										
ENSAT stage	242		141			244		84		
II				1.342	0.4291				0.540	0.1356
II or III				2.675	0.0099				1.389	0.4268
Ki67 [%]	236		137			238		84		
≥5		151		2.715	<0.0001		151		1.925	0.0070
≥10		126		2.734	<0.0001		126		2.164	0.0009
≥15		88		3.015	<0.0001		88		2.835	<0.0001
≥20		74		2.751	<0.0001		74		2.866	<0.0001
≥25		47		2.667	<0.0001		47		2.355	0.0005
Ki67 [%]	236		137	1.024 per 1%	<0.0001	238		84	1.023 per 1%	<0.0001
adjuvant mitotane	218	142	133	1.095	0.6209	218	142	77	1.054	0.8226

N+, number of patients that fulfilled the given criterion.

Table 4: Multivariable analysis (Cox regression) of the most relevant factors for recurrence-free and overall survival*

	Recurrence-free survival								
		German cohort				**Validation cohort**			
Factor [unit]	**N+**	**hazard ratio**	**95% CI**	**p-value**		**N+**	**hazard ratio**	**95% CI**	**p-value**
Age [years]		1.013	1.004-1.022	**0.0054**			1.001	0.986-1.016	0.8961
Tumor size 15 – 20 cm	34	1.601	1.033-2.480	**0.0354**		38	1.369	0.876-2.138	0.1678
Presence of venous tumor thrombus in renal vein or vena cava	15	1.327	0.724-2.432	0.3599		23	1.828	1.111-3.008	**0.0176**
Ki67 [%]		1.046	1.033-1.059	**<0.0001**			1.020	1.010-1.029	**<0.0001**
Adjuvant mitotane	63	0.705	0.473-1.052	0.0867		117	0.966	0.654-1.426	0.817
	Overall survival								
		German cohort				**Validation cohort**			
Factor [unit]	**N+**	**hazard ratio**	**95% CI**	**p-value**		**N+**	**hazard ratio**	**95% CI**	**p-value**
Age [years]		1.014	1.001-1.028	**0.0314**			0.990	0.970-1.010	0.3325
Tumor size 15 – 20 cm	34	1.192	0.632-2.,251	0.5872		38	1.830	1.070-3.128	**0.0273**

Presence of venous tumor thrombus in renal vein or vena cava	15	2.141	1.075-4.265	**0.0303**		23	1.438	0.762-2.712	0.2622
Ki67 [%]		1.061	1.044-1.079	**<0.0001**			1.026	1.013-1.039	**<0.0001**
Adjuvant mitotane	63	0.410	0.211-0.797	**0.0086**		117	0.804	0.482-1.343	0.4053

* the analyses include only patients for whom all parameters were available (n=214 for the German cohort, of whom 139 experienced recurrence and 114 died and n= 181 with 114 recurrences and 65 deaths for the validation cohort)

N+, number of patients that fulfilled the given criterion.

Supplemental Table 1: Overview on recently published cohorts of patients with adrenal cancer

Study	Cohort size	Description	Remarks
Terzolo 2007 (10)	177	Patients after radical surgery with or without adjuvant mitotane therapy	Partial overlap of patient cohorts. Study includes patients also with uncertain restriction status
Berruti 2014 (34)	524	Patients after R0 resection investigated for the prognostic value of clinically overt Cushing's syndrome for OS and RFS	Partial overlap of patient cohorts. Study with the primary aim to assess cortisol excess as a prognostic factor
Else 2014 (36)	391	Prognostic value present for cortisol excess, tumor stage, tumor grade (on the basis of mitotic count lower/higher 20 per 50 HPF)	No overlap of patient cohorts. Cohort including patients of all stages irrespective of resection status
Ayala-Ramirez 2014 (35)	330	Prognostic value for RFS present for surgical margins, and disease stage	No overlap of patient cohorts. Cohort including patients of all stages irrespective of resection status

Figure 1: Kaplan-Meier analysis of Ki67 index on recurrence free survival (A+B) and overall survival (C+D) of the German cohort (A+C) and the validation cohort (B+D), respectively.

Figure 2: Kaplan-Meier analyses for recurrence free survival on the complete cohort with Ki67 only (≥10% and ≥20%, A), and based on a basic risk score (Ki67 10-20%, 1 point; Ki67 >20%, 2 points; tumor size 15-20 cm or presence of venous tumor thrombus, 1 point, B), and weighted risk score (Ki67 per 1%, presence of venous tumor thrombus or tumor size 15-20cm, C), respectively.

Supplemental Figure 1: Kaplan-Meier analysis of ENSAT stage on recurrence free survival (A+B) and overall survival (C+D) for the German (A+C) and the validation cohort (B+D), respectively.

Supplemental Figure 2: Kaplan-Meier analyses for overall survival on the complete cohort with Ki67 only (≥10% and ≥20%, A), and based on a basic risk score (Ki67 10-20%, 1 point; Ki67 >20%, 2 points; tumor size 15-20 cm or presence of venous tumor thrombus, 1 point, B) and weighted risk score (Ki67≥10%, Ki67≥20%, presence of venous tumor thrombus or tumor size 15-20cm, C), respectively.

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