Validation of the prognostic role of the "Helsinki Score" in 225 cases of adrenocortical carcinoma

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Summary

Adrenocortical carcinoma patient prognosis is extremely variable and poorly predictable. The newly introduced Helsinki Score is the first so far proposed diagnostic and prognostic system based on the combined evaluation of morphological (mitoses and necrosis) and immunohistochemical (Ki-67) parameters. The aim of the study was to validate the prognostic role of the Helsinki Score for adrenocortical carcinoma characterization. Thus, 225 adrenocortical carcinomas were reclassified using the Weiss Score and the Helsinki Score (3× mitotic count + 5× necrosis + Ki-67 index). At univariate analysis, statistically significant prognostic values were observed at the log-rank test for mitotic count (cut-off values: <6 and ≥55; $P < .0001$), Ki-67 (cut-off values: <20 and ≥50; $P < .0001$), Weiss Score (cut-off values: <5 and ≥8; $P < .0001$), Helsinki Score (cut-off values: <13 and ≥19; $P < .0001$), histological variant (conventional *vs* oncocytic; $P = .009$), necrosis ($P = .001$) and stage ($P = .005$). Cox multivariate analysis using a backward stepwise selection method retained only Helsinki Score and Weiss Score as predictors of poor prognosis ($P < .0001$ and $P = .0005$, respectively). Helsinki Score (with a threshold of 28.5 points; AUC = 0.729, 95% confidence interval [CI] = 0.66-0.79) and Ki-67 (with a threshold of 20.5%; AUC = 0.727, 95% CI = 0.66-0.79) showed the best and equivalent areas under the curve (AUC) predicting disease-related deaths determined using receiver-operating characteristic statistics. In conclusion, the Helsinki Score is a valuable system to predict prognosis in adrenocortical carcinoma, outperforming the currently established prognostic parameters.

Keywords: Adrenocortical carcinoma; Helsinki Score; Ki-67; Prognosis

1. Introduction

Adrenocortical carcinoma (ACC) has a heterogeneous morphology in its classical form and also includes several histological variants (oncocytic, myxoid and sarcomatoid). In addition, the prognosis of ACC patients is extremely variable and poorly predictable, with cases following a rapidly aggressive course along with tumors having an indolent clinical outcome. In the last 25 years, the differential diagnosis between adrenocortical adenoma and carcinoma was defined using different scoring systems, such as the Weiss Score [1,2] or the Van Slooten Index [3], or algorithmic approaches, such as the mitosis method [4] or the Reticulin Algorithm [5,6]. Currently, the Weiss Score is still the most widely employed for diagnostic purposes, but it also possesses a prognostic value, at least at univariate survival analysis, high Weiss Score values being associated with a poor prognosis [7,8].

Recently, a new score, the "Helsinki Score", based on the combined evaluation of morphological parameters (presence of necrosis and a mitotic count >5 per 50 high-power fields) and Ki-67 proliferation index, was demonstrated to accurately predict the metastatic potential of adrenocortical carcinomas [9]. A cut-off value of 8.5 points was able to identify metastatic ACC with an extremely high sensitivity and specificity. In addition, a prognostic role was also identified with two groups of malignant neoplasms having different scores and different survival (score 8.5-17 *vs* >17). The original study included a limited cohort of ACC, as among the 177 adrenocortical tumors analyzed, only 30 had a Weiss Score ≥3, 15 of which (50%) had a Helsinki Score >8.5. In addition, the applicability of the Helsinki Score was not mentioned for ACC morphological variants. These include the oncocytic variant, for which the Weiss Score is not applicable and the specific Lin-Weiss-Bisceglia system is generally used, being based on partially different parameters [10].

Based on the aforementioned, a study was designed on a large series of 225 ACC from two institutions to assess the prognostic role of the Helsinki Score both in conventional and special variants of ACC.

2. Materials and methods

2.1. Case collection

We retrieved 225 adrenocortical tumors having a Weiss Score ≥3 [1] from the pathology files of two Italian institutions between January 2004 and December 2015, namely the University of Turin at San Luigi Hospital (205 cases) and the University of Padua (20 cases). The majority of these patients were treated at San Luigi Hospital in Orbassano-Turin, which serves as one of the referral centers for ACC in Italy. The study received ethical approval from the local Review Board of San Luigi Hospital. The clinical and pathological features of the whole series are summarized in Table 1.

2.2. Case classification

All available hematoxylin and eosin–stained slides were reviewed by two of us (E.D. and M.V.) having a specific interest in endocrine pathology and classified according to the appropriate diagnostic systems, ie, Weiss Score [1] and Revised Weiss Score [2] for conventional ACC, Lin-Weiss-Bisceglia System [10] for pure oncocytic adrenocortical tumors and Wienecke classification [11] for pediatric tumors. In addition, the Helsinki Score [9] was generated as follows: 3 points for a mitotic count >5/50 high-power fields + 5 points in the presence of necrosis + the absolute value of Ki-67 proliferation index (Fig. 1).

2.3. Immunohistochemistry

From a representative paraffin block for each case, 5-μm-thick serial paraffin sections were processed by means of immunohistochemistry using an antibody against Ki-67 (clone MIB-1, diluted 1:150; Dako-Agilent, Glostrup, Denmark). A biotin-free, dextran chain–based detection system (EnVysion, Dako) and diaminobenzidine as the chromogen were used according to a standard protocol. Ki-67 proliferation index was determined by counting 1000 cells in hot spots and calculated as the percentage of positive neoplastic nuclei. The Ki-67 values of 51 cases belonging to

this data set have already been reported [12].

2.4. Statistical analysis

Nonparametric tests (Wilcoxon rank-sum and Kruskal-Wallis rank-sum) were used to analyze differences between various conditions. Fisher exact test analyzed differences in categorical variables. Spearman ρ coefficient was calculated to assess correlations among various parameters. Survival curves were estimated with the Kaplan-Meier method and were compared with the log-rank test. Cox proportional hazards regression (HR) models were used both to estimate the HRs and for multivariate survival analyses. The ROC curves and the area under the ROC curve (AUC) were used to assess scores' ability to differentiate between "status" (alive *vs* dead). Two cut-off points for mitotic count, Ki-67 proliferation index, Weiss Score and Helsinki Score, which divided cases into three prognostically different groups, were selected both on the basis of the highest statistical significance in the log-rank test and with maximizing the Youden index in the context of the ROC surface. The volume under ROC surface (VUS) was taken as the global measure summarizing the test discrimination power. A significance level of $P < .05$ was used.

3. Results

All scoring systems were well correlated (Table 2). At univariate analysis of overall survival with the Cox model, high values of mitotic count, Ki-67 proliferation index, Weiss Score (both its original and revised form) and Helsinki Score, all used as numerical scores, were strongly associated with a poor prognosis. In addition, other clinical (ENSAT stage and hormonal secretion) and pathological parameters (size of the tumor, presence of necrosis and conventional/myxoid variant) were associated with a shorter overall survival. Cox multivariate analysis using a backward stepwise selection method revealed Helsinki Score and Weiss Score to be the sole predictors of poor prognosis (Table 3).

Specific cut-off points for mitotic count, Ki-67 proliferation index, Weiss Score and

Helsinki Score, which divided cases into three distinct prognostically different groups, were selected on the basis of the highest statistical significance on the log-rank test. The optimal cut-offs were <6 and ≥55 for mitotic count, <20 and ≥50 for Ki-67 proliferation index, <5 and ≥8 for Weiss Score and, finally, <13 and ≥19 for Helsinki Score (Fig. 2). Concerning the Helsinki Score, the cut-offs proposed by Pennanen et al [9] (<8.5 and >17) were also used, however, resulting in a lower performance in term of prognostic stratification.

To further determine the prognostic power of the numerical variables to assess scores' ability to differentiate between "status" (ie, different scores and mitotic/proliferation indexes), ROC curves were plotted and the AUC calculated. The best and equivalent AUCs were found for Helsinki Score (with a threshold of 28.5 points; AUC=0.729, 95% confidence interval [CI] = 0.66-0.79) and Ki-67 (with a threshold of 20.5%; AUC=0.727, 95% CI = 0.66-0.79) compared to mitotic count (with a threshold of 15 mitoses/50 HPF; AUC=0.673, 95% CI = 0.603-0.74), Weiss Score Revised (with a threshold of 5; AUC=0.646, 95% CI = 0.573-0.646) and Weiss Score (with a threshold of 7; AUC=0.624, 95% CI = 0.55-0.7) (Fig. 3).

In the present study, purely oncocytic tumors and myxoid neoplasms with Weiss score ≥3 accounted for 10.0% (24/225) and 7.5% (17/225) of the whole series, respectively. After applying the LWB classification for oncocytic tumors, 1 oncocytic case was reclassified as benign, 5 as "uncertain malignant potential" (UMP), while the remaining 18 were confirmed as malignant. The benign case and all 5 UMP cases had a Helsinki Score ranging from 1 to 6, except for one UMP case with a score of 25. Conversely, malignant cases (according to LWB classification) had a Helsinki Score ranging from 3 to 79. When the above proposed Helsinki Score cut-offs (<13 *vs* ≥19) were applied, 15 cases (12 alive with no evidence of disease and 3 alive with disease) were allocated in the group with better prognosis; no cases had a score between 13 and 19, while the remaining 9 cases (5 alive with no evidence, 2 alive with disease and 2 died of disease) belonged to the group associated with a worse prognosis (Fig. 4A). Consistently, patients with Helsinki Score ≥19 had decreased survival compared with those with Helsinki Score <13 (log-rank test, P = .015,

mean survival of 32 months and 74 months for Helsinki Score ≥19 and <13, respectively).

Concerning myxoid tumors, 3 cases (2 without evidence of disease and 1 alive with disease) had a Helsinki Score lower than 13, while 14 (1 without evidence of disease, 8 alive with disease and 6 died of disease) had a Helsinki Score higher than 19 (Fig. 4B). Myxoid cases were too few to perform a further specific survival analysis.

4. Discussion

In the present study, the Helsinki Score emerged as the most relevant system with an impact on prognosis, outperforming the most established prognostic parameters such as clinical stage, mitotic index and Ki-67 proliferation index. In addition, it turned out to be applicable to both conventional and oncocytic variant of ACC.

Since its introduction in 1984 [1], the Weiss Score has obtained the widest acceptance by practicing pathologists worldwide to determine the malignant potential among adrenocortical tumors. Consistently, it has been integrated in several molecular studies [13-16]. Beyond its diagnostic role, the Weiss Score also possesses prognostic significance [7], because high Weiss Score tumors generally display an aggressive clinical behavior [8], although this finding was not confirmed in other papers [17,18]. The newly introduced Helsinki Score [9] is the first so far proposed system based on the combined evaluation of morphological (mitoses and necrosis) and immunohistochemical (Ki-67) parameters. The morphological criteria are among the most reproducible [19] and prognostically relevant [5,20] among those included in the Weiss Score. As a matter of fact, in 1989 Dr Weiss himself proposed a mitotic count–based, two-tier grading system, distinguishing low-grade (<20/50 HPF) from high-grade ACC (>20/50 HPF) [20]. Concerning Ki-67 proliferation index, several groups proved its superiority to mitotic count to prognosticate ACC, although the optimal cut-offs are still to be identified among those so far proposed by our group [12] (<20%, 20%-50% and >50%) and by Beuschlein et al [8] (<10%, 10%-20%). In addition, a recent international reproducibility study involving 14 endocrine pathologists [21] revealed that Ki-67

assessment has a great interobserver variation, which imposes particular limitations to its clinical utility, especially around clinically relevant cut-offs.

In the present large series of ACC from two institutions, a statistically significant prognostic value at univariate analysis was observed only for those two morphological parameters of the Weiss Score included in the Helsinki Score (necrosis and mitotic count), for Ki-67 proliferation index, for the Weiss Score and the Helsinki Score itself. Interestingly, at Cox multivariate analysis, only the Weiss Score and the Helsinki Score, and not mitotic count nor Ki-67 index alone, were confirmed as predictors of poor prognosis ($P < .0001$ and $P = .0005$, respectively). However, ROC statistics revealed that the best and equivalent AUC to predict the occurrence of disease-related deaths were those of Helsinki Score (with a threshold of 28.5 points) and Ki-67 (with a threshold of 20.5%). Such a preponderance of the Helsinki Score above the other established prognostic parameters can be easily explained observing that the Helsinki Score is a unique tool that combines the most prognostically relevant morphological parameters of the Weiss Score and Ki-67 proliferation index.

As our series included both conventional and morphological variants of ACC, we could also verify the prognostic value of the Helsinki Score in oncocytic and myxoid ACC cases, although the number of cases in the latter group was not enough for statistical analysis of survival. In fact, applying the newly proposed cut-offs (<13 *vs* ≥19), the Helsinki Score demonstrated a worse prognosis for oncocytic neoplasms having a score ≥19 with a 66% probability of correctly classifying the aggressive cases and to predict the occurrence of disease-related deaths. Regarding the myxoid variant of ACC, whose malignancy risk is potentially underestimated by the Weiss Score, the probability of correctly classifying aggressive cases with the Helsinki Score was even higher (88%).

In conclusion, the Helsinki Score is a valuable system to predict prognosis in ACC, outperforming in our series the currently established prognostic parameters.

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FIGURE LEGENDS

Fig. 1 Representative example of the Helsinki Score application. This case had 25 mitoses/50 HPF (A), diffuse areas of necrosis (B) and a Ki-67 proliferation index of 30%. Consistently the Helsinki Score was 38 (3 points for a mitotic count + 5 points in the presence of necrosis + 30 of Ki-67 proliferation index) (A and B, hematoxylin and eosin, original magnification ×400; C, immunoperoxidase, ×400).

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Fig. 2 Distinct prognostically different groups based on specific cut-off points for mitotic count (A), Ki-67 proliferation index (B), Weiss Score (C) and Helsinki Score (D).

Fig. 3 ROC curves determining the prognostic power of the numerical variables. The best and equivalent AUCs were found for Helsinki Score (A, threshold= 28,5 points; AUC=0.729) and Ki-67 (B, threshold=20.5%; AUC=0.727) compared to mitotic count (C, threshold=15 mitoses/50 HPF; AUC=0.673) and Weiss Score (D, threshold=7; AUC=0.624).

Fig. 4 Classification of oncocytic (A) and myxoid cases (B) according to Weiss Score, Helsinki Score and Lin-Weiss-Bisceglia System (only for oncocytic tumors). Green, not evidence of disease; yellow, alive with disease; red, dead of disease; UMP, uncertain malignant potential.

Table 1 Descriptive clinicopathological features of the whole series of 225 ACC

Parameter	
F/M ratio	131:94 (1.40)
Mean age, y (range)	46 (1-79)
Functional status	
Not functioning	114
Functioning	94
Not known	17
Mean size, cm (range)	11.3 (2-38)
Mean weight, g (range)	429 (8-3100)
ACC variant	
Classical	170
Myxoid	17
Oncocytic (pure/mixed)	38 (24/14)
ENSAT stage	
I	15
II	71
III	62
IV	41
Not known	36
Disease status	
NED	76
AWD	60
DOD	89
Median overall survival, mo (range)	36 (1-246)

Abbreviations: ACC, adrenocortical carcinoma; M, male; F, female; FU, follow up; NED, no evidence of disease; AWD, alive with disease; DOD, died of disease.

Table 2 Reciprocal correlations among proliferation indices and scoring systems

Parameter	Mitotic count	Helsinki score	Weiss score
Helsinki Score	$r = 0.667$ $P < .0001$	–	–
Weiss Score	$r = 0.624$ $P < .0001$	$r = 0.513$ $P < .0001$	–
Weiss Score revised	$r = 0.642$ $P < .0001$	$r = 0.573$ $P < .0001$	$r = 0.782$ $P < .0001$

Table 3 Univariate and multivariate Cox regression analyses of overall survival

Variable	Univariate analysis		*P*
	HR	95% CI	
Sex (F *vs* M)	0.89	0.58-1.38	.61
Age	1.01	0.99-1.02	.435
Hormonal secretion[a]	1.63	1.06-2.56	.023
Size	1.05	1.01-1.10	.031
Weight	1.00	1.00-1.01	.067
ENSAT stage			
I *vs* II	3.26	0.77-13.79	.107
I *vs* III	3.79	0.90-15.87	.068
I *vs* IV	7.48	1.78-31.43	.005
Presence of necrosis[b]	3.61	1.57-8.30	.001
Mitotic count	1.02	1.01-1.02	<.0001
Ki-67	1.02	1.01-1.03	<.0001
ACC variant			
Conventional *vs* myxoid	0.96	0.45-2.39	.924
Conventional *vs* oncocytic	6.57	0.04-0.63	.009
Weiss Score [c]	1.50	1.29-1.74	<.0001
Weiss Score Revised	1.52	1.29-1.79	<.0001
Helsinki Score [c]	1.05	1.02-1.03	<.0001

NOTE. The HR is for each additional year of age, cm of size, g of weight, mitosis, percentage point of Ki-67, unit of Weiss Score, Weiss Score Revised and Helsinki Score.
Abbreviations: HR, hazard ratio; CI, confidence interval.
[a] Nonsecreting ACC was the reference category.
[b] Absence of necrosis was the reference category.
[c] Parameters significant at multivariate survival analysis: Helsinki Score, HR 1.019 (95% CI 1.010-1.028), $P < .0001$; Weiss Score, HR 1.332 (95% CI 1.132-1.566), $P = .0005$.

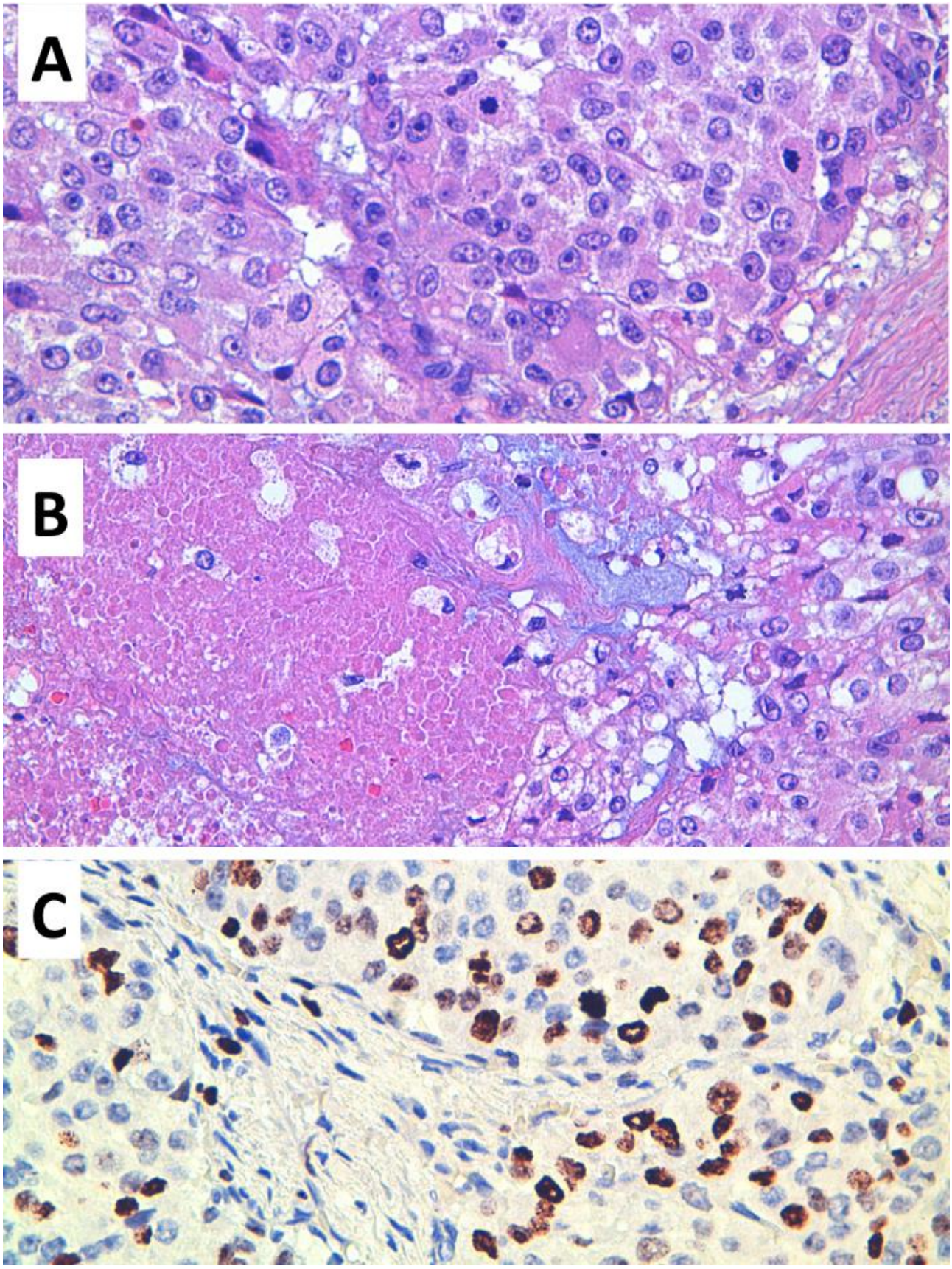

Figure 1

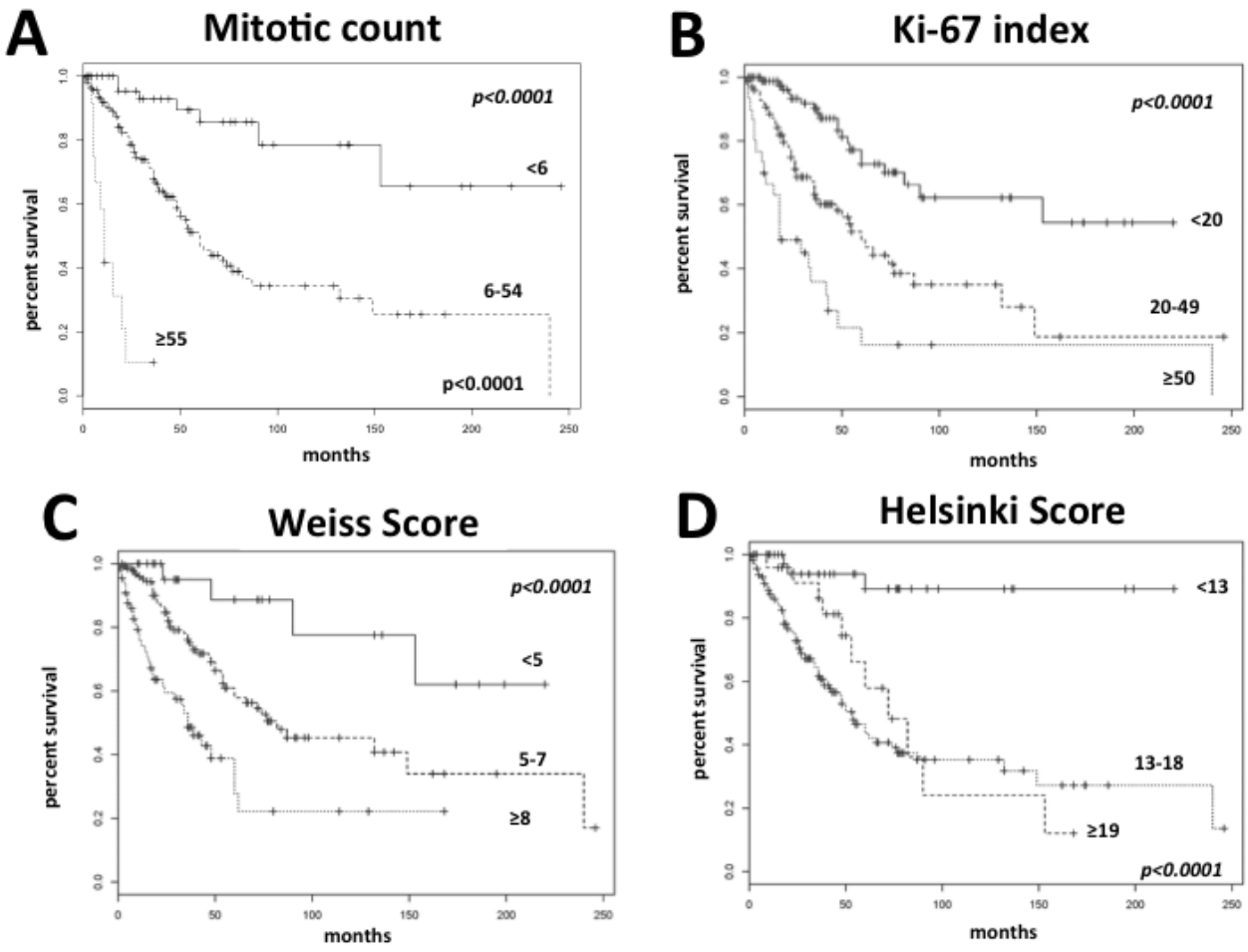

Figure 2

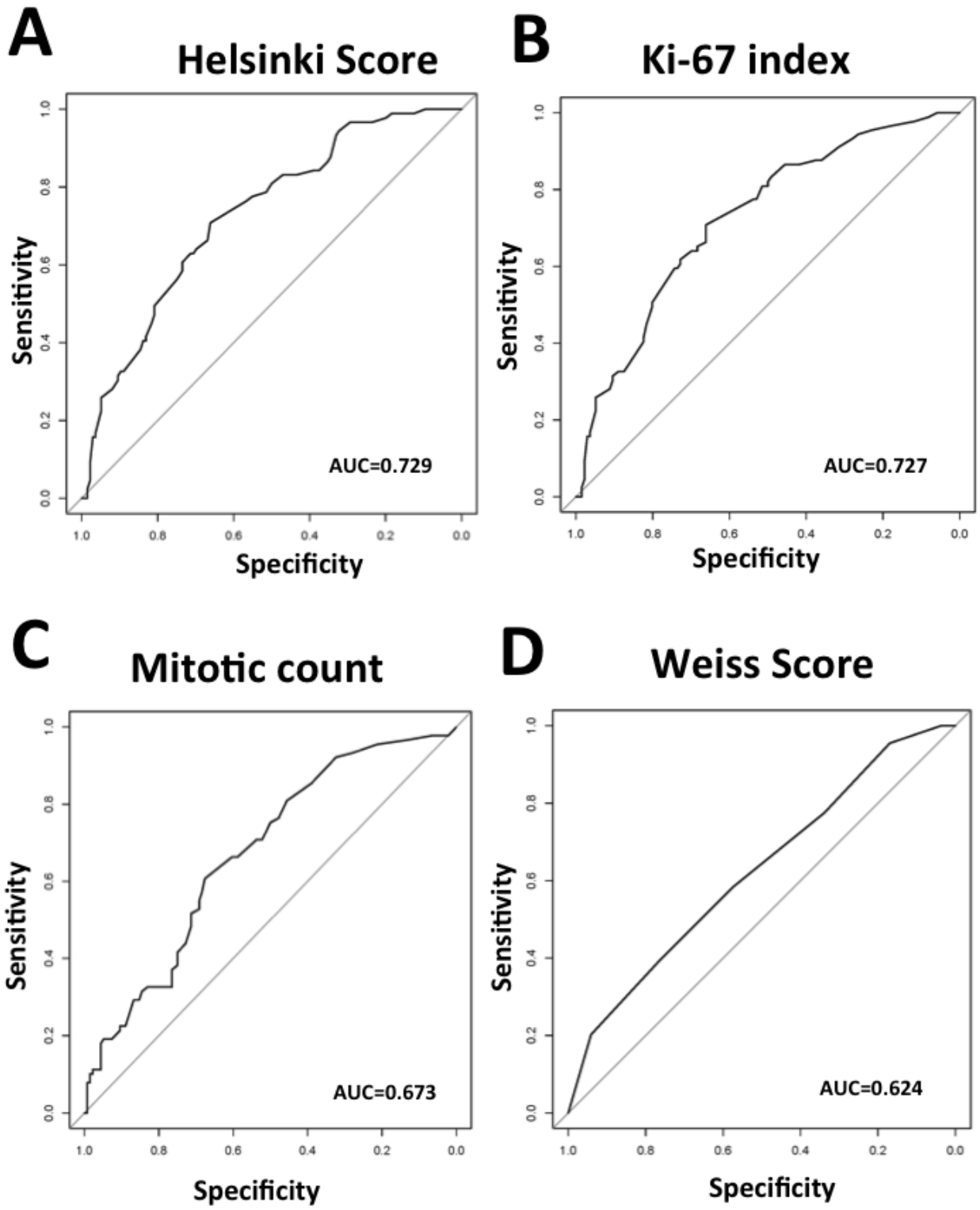

Figure 3

A Oncocytic variant

case	HELSINKI SCORE	LWB CLASSIFICATION
# 1	<13	UMP
# 2	<13	UMP
# 3	<13	malignant
# 4	<13	benign
# 5	<13	malignant
# 6	<13	malignant
# 7	<13	UMP
# 8	<13	malignant
# 9	<13	UMP
# 10	<13	malignant
# 11	<13	malignant
# 12	<13	malignant
# 13	>19	malignant
# 14	>19	malignant
# 15	>19	malignant
# 16	>19	malignant
# 17	>19	malignant
# 18	<13	malignant
# 19	<13	malignant
# 20	<13	malignant
# 21	>19	UMP
# 22	>19	malignant
# 23	>19	malignant
# 24	>19	malignant

B Myxoid variant

case	HELSINKI SCORE	WEISS SCORE
# 1	<13	5
# 2	<13	4
# 3	>19	7
# 4	<13	4
# 5	>19	5
# 6	>19	6
# 7	>19	9
# 8	>19	6
# 9	>19	8
# 10	>19	8
# 11	>19	5
# 12	>19	6
# 13	>19	8
# 14	>19	9
# 15	>19	9
# 16	>19	5
# 17	>19	5

Figure 4

Validation of the prognostic role of the “Helsinki score” in 225 cases of adrenocortical carcinoma

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- The “Helsinki score” is based on morphological (mitoses and necrosis) and IHC (Ki-67) parameters.
- This study validated the prognostic value of Helsinki score for adrenocortical carcinoma.
- Helsinki Score and Weiss Score were predictors of poor prognosis on multivariate analysis.
- Helsinki Score and Ki-67 showed best and equivalent AUC predicting disease-related deaths.
- The Helsinki Score outperforms current prognostic parameters in adrenocortical carcinoma.