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Pathological evaluation of tumor grade for salivary adenoid cystic carcinoma: A proposal of an objective grading system

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Abstract

Three pathological grading systems advocated by Perzin/Szanto, Spiro, and van Weert are currently used for adenoid cystic carcinoma (AdCC). In these systems, the amount or presence of the solid tumor component in AdCC specimens is an important index. However, the "solid tumor component" has not been well defined. Salivary AdCC cases (N = 195) were collected after a central pathology review. We introduced a novel criterion for solid tumor component, minAmax (minor axis maximum). The

largest solid tumor nest in each AdCC case was histologically screened, the maximum oval fitting the solid nest was estimated, and the length of the minor axis of the oval (minAmax) was measured. The prognostic cutoff for the minAmax was determined using training and validation cohorts. All cases were evaluated for the four grading systems, and their prognostic impact and interobserver variability were examined. The cutoff value for the minAmax was set at 0.20 mm. Multivariate prognostic analyses showed the minAmax and van Weert systems to be independent prognostic tools for overall, disease-free, and distant metastasis-free survival while the Perzin/Szanto and Spiro systems were selected for overall survival but not for disease-free or distant metastasis-free survival. The highest hazard ratio for overall survival (11.9) was obtained with the minAmax system. The reproducibility of the minAmax system (kappa coefficient of 0.81) was scored as very good while those of the other three systems were scored as moderate. In conclusion, the minAmax is a simple, objective, and highly reproducible grading system useful for prognostic stratification for salivary AdCC.

KEYWORDS

adenoid cystic carcinoma, interobserver variability, pathological grading system, prognosis, salivary gland

1 | INTRODUCTION

Adenoid cystic carcinoma (AdCC) is rare but one of the most frequent carcinomas of the salivary gland.[1] While often detected as a small and slow-growing lesion,[2,3] AdCC undergoes frequent distant metastasis in nearly half of patients, most frequently occurring in the lung, followed by the bone and liver.[4,5] Radical surgical resection followed by radiation therapy is the mainstay of treatment for this carcinoma.[4,6]

In clinicopathological studies of AdCC, an association has been recognized between a solid tumor growth pattern and a poor prognosis,[7,8] and the two grading systems described by Perzin/Szanto[9,10] and Spiro[11] have been based on the percentage of solid tumor component (Table 1). The cutoff values employed to predict a worse prognosis based on the amount of the solid tumor component are >30% and >50% according to the Perzin/Szanto and Spiro grading systems, respectively.[1,8-12] According to the current WHO classification for salivary gland tumors, AdCC cases with a solid component constituting more than one third of the tumor may have a worse clinical course.[1] This cutoff may correspond to that for aggressive tumors according to the Perzin/Szanto system. On the other hand, it has been suggested that the presence of any solid tumor component suggests a poor prognosis,[13] and recently van Weert et al studied the usefulness of a novel pathological grading system scoring the mere presence of solid type AdCC in the histological specimen, irrespective of its amount.[14] This system is more objective than the Perzin/Szanto and Spiro systems since it does not require measurement of the amount of solid component for grading AdCC cases. Another advantage of the van Weert system is its low interobserver variability compared with the Perzin/Szanto and Spiro systems.[14]

TABLE 1 Criteria of histopathological grading for adenoid cystic carcinoma

Grading system	Description			Reference
Perzin/Szanto, three-tiered	Grade I, predominantly tubular, no solid	Grade II, predominantly cribriform, solid component < 30%	Grade III, solid component > 30%	9, 10
Spiro, three-tiered	Grade I, mostly tubular or cribriform, occasionally solid	Grade II, mixed with substantial solid (>50%)	Grade III, only solid	11
van Weert, two-tiered	S−, solid component, absent	S+, solid component, present		14
MinAmax, two-tiered	MinAmax ≤ 0.20 mm	MinAmax > 0.20 mm		Present study

Note: Grade I and II in the Perzin/Szanto and grade I in the Spiro grading system are considered low grade in the present study.

The importance of the solid tumor component has been emphasized in all three grading systems. However, it should be noted that the solid tumor component has not been well defined.

In this study, we employed a large number of AdCC cases and tried to objectively define the "solid tumor component" by an easy method of measurement using a standard microscope. We then introduced an alternative grading system, minAmax, and studied its prognostic impact and interobserver concordance with the three previously described grading systems.

2 | CASES AND METHODS

2.1 | Case selection

We retrospectively collected 195 AdCC cases from 15 tertiary hospitals in Japan: Nagoya City University Hospital, Hokkaido University Hospital, the International University of Health and Welfare Mita Hospital, Tokyo Medical University Hospital, Tokai University Hospital, Shizuoka Cancer Center Hospital, Nagoya University Hospital, Fujita Health University Hospital, Aichi-Gakuin University Hospital, Aichi Cancer Center Hospital, Osaka Medical College Hospital, Kobe University Hospital, Ehime University Hospital, Kyushu University Hospital, and Kyushu Cancer Center Hospital. The present study was approved by the Institutional Ethics Review Board of each of the 15 institutions that participated in the study. AdCC cases were registered in order and numbered. The clinical data of patients treated at these institutions were obtained. AdCC with high-grade transformation (or dedifferentiation) refers to the presence of a pleomorphic, mitotically active high-grade carcinoma component arising in an otherwise conventional AdCC of any pattern/grade.[15] The transformed component is typically of a poorly differentiated adenocarcinoma or anaplastic carcinoma negative for myoepithelial markers. AdCC cases with high-grade transformation were not included in this study as the clinical course deviates considerably from the natural course of AdCC.[15] Patients were treated basically according to the NCCN guidelines, and post-operative radiotherapy (PORT) was performed when the surgical margin was positive or equivocal and/or lymph node metastasis was pathologically positive. Concurrent chemotherapy was administered at the surgeon's discretion. To ensure the AdCC diagnosis of the cases included in this study, we performed a central pathology review according to the WHO criteria for the classification of salivary gland tumors as described elsewhere.[1,16] Finally, 195 cases of AdCC were included in this study.

2.2 | Tumor grading using the Perzin/Szanto, Spiro, and van Weert systems

According to criteria of the three grading systems, Perzin/Szanto, Spiro, and van Weert (Table 1), our 195 AdCC cases were graded pathologically using all available H&E tumor slides, almost always more than one per case. Grading was performed by two of the authors independently (T. Murase and H. Inagaki). According to the former two systems, AdCC cases were divided into three grades, and following the latter system, the cases were divided into two grades. In case of discordant grading, an agreement was reached.

2.3 | Tumor grading using the minAmax system

The basic idea for grading AdCC tumors with the above three grading systems is to evaluate the presence or percentages of the histological solid tumor component in all the tumor specimens of each case. However, the definition of "solid" has not yet been made clear. To define a solid tumor component objectively, we introduced a novel index for this feature.

All available H&E tumor slides per case were examined under a microscope equipped with a micrometer. Using a 4× objective lens, the observer screened the solid tumor nests in each AdCC case. A solid tumor nest was defined in this study as tissue composed of tumor cells with no recognizable duct lumen or cystic space as determined under low-power magnification. To detect solid tumor nests, mucus staining was useful in some cases (Figure S1A). When solid tumor nests were found histologically, the observer specified the largest one, estimated the maximum oval fitting this solid tumor nest, and measured the length of the minor axis of the oval (Table 1 and Figure 1). We designated this length as minAmax (minor axis maximum). For measuring minAmax, necrotic areas and eosinophilic hyalinized areas were not taken into account (Figure S1B,C). The minAmax was scored independently by two expert pathologists (T. Murase and H. Inagaki), and in the case of a discordant grading, an agreement was reached. Using the first half of the registered cases (a training cohort, #1 to #100), the minAmax cutoff value was determined as giving a statistically superior hazard ratio (HR) for overall survival (OS) for segregation of AdCC patients into two prognostic groups. The cutoff value thus obtained was verified using the second half of the cases (a validation cohort, #101 to #195).

2.4 | Statistical analysis

The OS was defined as the interval between the beginning of treatment and the date of death or last follow-up. Disease-free survival (DFS) and distant metastasis-free survival (DMFS) were defined as the intervals between the beginning of treatment and the date of relapse of any type, and between the beginning of treatment and the date of distant metastasis, respectively. The association between clinicopathological factors and OS, DFS, and DMFS was analyzed by the Kaplan-Meier method and univariate and multivariate Cox proportional hazards models. The HR and 95% confidence interval were calculated, and HRs were used to evaluate the prognostic impact of these factors on survival.

Cohen's kappa test was used to assess the reproducibility of tumor grade evaluation between two pathologists beyond what would be expected by chance.[17] The kappa coefficient varies from 0 to 1.0, and

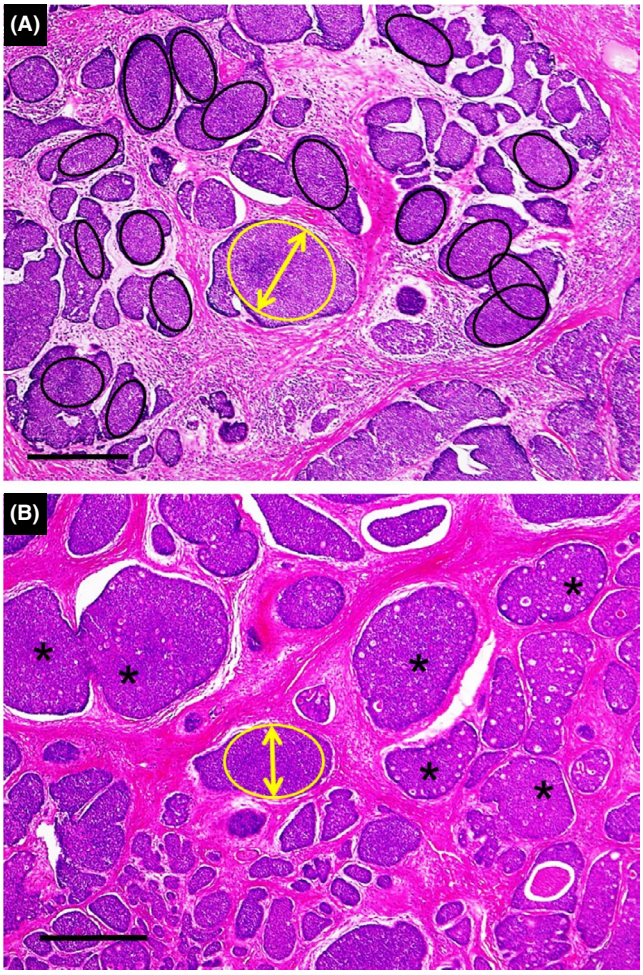

FIGURE 1 Measurement of the minAmax in adenoid cystic carcinoma cases. A, The microscopic image showed several tumor nests (indicated by black ovals). Of these, the nest indicated by the yellow oval was the largest. In this case, the length of the minor axis of this oval, minAmax, was calculated to be 0.48 mm. Bar = 0.5 mm. B, A solid tumor nest of adenoid cystic carcinoma is defined as that in which there is no recognizable duct lumen or cystic space found in a low-power (4×) microscopic field. The tumor nests indicated by asterisks have recognizable duct lumens or cystic spaces, and the one indicated by the yellow oval is used for measurement of minAmax. The minAmax was calculated to be 0.33 mm in this case. Bar = 0.5 mm

a kappa coefficient of 1 indicates a perfect agreement between two observers while a value of 0 indicates no agreement. The strength of agreement was categorized as follows: 0.00 to 0.20, poor; 0.21 to 0.40, fair; 0.41 to 0.60, moderate; 0.61 to 0.80, good; and 0.81 to 1.00, very good.[18] The Perzin/Szanto and Spiro grading systems are three-tiered and the van Weert and MinAmax systems are two-tiered. To equitably compare the prognostic impact and interobserver variability of these four systems, we translated the Perzin/Szanto (grade I/II vs grade III) and Spiro (grade I vs II/III) systems into two-tiered systems according to previous publications.[1,8-12] All statistical analyses were performed using the statistical package JMP® 12 (SAS Institute Inc, Cary, NC, USA). All tests were two-sided, and a *P* value of <.05 was considered statistically significant.

3 | RESULTS

3.1 | Clinicopathological features of AdCC patients

The clinicopathological features of the AdCC patients (N = 195) enrolled in this study are summarized in Table S1. The patients consisted of 73 men and 122 women with a median age of 61 years (range 19-89). The primary tumor sites were the major salivary gland in 147 (75.4%) cases and the minor salivary gland in 48 (24.6%) cases. Ninety-two (47.2%) patients had large (pT3/4) tumors, and 29 (14.9%) patients were positive for cervical lymph node metastasis. Surgical margins were microscopically positive in 94 (48.2%) cases. While 108 patients underwent surgery alone, 87 underwent surgery plus adjuvant radiotherapy and/or chemotherapy. The median follow-up time was 52 months (range 1-263). The OS, DFS, and DMFS rates at 5 years were 90.7%, 51.7%, and 63.0%, and those at 10 years were 81.7%, 34.3%, and 54.5%, respectively. The survival curves for OS, DFS, and DMFS are shown in Figure S2.

3.2 | Cutoff value for the minAmax grading system

In the training cohort (N = 100), the tumors were divided into two groups employing cutoff values for minAmax (Table 1) set at one of 0.10 mm, 0.15 mm, 0.20 mm, or 0.25 mm, and HRs for OS, DFS, and DMFS using the respective cutoff values were examined. As shown in Table S2, the cutoff value of 0.20 mm showed the highest HRs for OS (7.24) among the four cutoff values examined. In the validation cohort (N = 95), the cutoff value of 0.20 mm showed the highest HRs for OS (6.57) among the four (Table S3). Thus, the minAmax of 0.20mm was considered to be the most discriminant cutoff for survival of the AdCC patients. In the subsequent analysis employing total AdCC cohort, the minAmax cutoff value of 0.20 mm showed the highest HRs for OS (6.12), DFS (1.94), and DMFS (2.40) among the four values employed (Table S4). Collectively, we divided our AdCC cases into lower minAmax ≤ 0.20mm (low-grade tumors) and higher minAmax > 0.20mm (high-grade tumors).

3.3 | Prognostic impact of the Perzin/Szanto, Spiro, van Weert, and minAmax grading systems

The univariate prognostic analysis including various clinicopathological factors and the four grading systems was performed for OS, DFS, and DMFS (Table 2). The Perzin/Szanto and Spiro systems were selected as significant prognostic tools for OS but not for DFS or DMFS. The van Weert and MinAmax systems were prognostically useful not only for OS but also for DFS and DMFS. The HRs for OS were similarly high in the Perzin/Szanto, Spiro, and minAmax systems, but the HR for OS in the van Weert system was low. Multivariate prognostic analyses for OS (Table 3), DFS (Table 4), and DMFS (Table 5) were performed using the clinicopathological factors and the respective grading systems. The van Weert and minAmax

systems were selected as independent prognostic tools for all OS, DFS, and DMFS while the Perzin/Szanto and Spiro systems were selected for OS but not for DFS or DMFS. The highest HR for OS was obtained with the minAmax system (HR = 11.9). The Kaplan-Meier survival curves stratified by these four grading systems are shown for OS (Figure 2), DFS (Figure 3), and DMFS (Figure 4).

3.4 | Interobserver variability in the Perzin/Szanto, Spiro, van Weert, and minAmax grading systems

The four grading systems were evaluated for interobserver variability using Cohen's kappa test. The kappa coefficients between the two pathologists were 0.51 ($P < .0001$), 0.51 ($P < .0001$), 0.44 ($P < .0001$), and 0.81 ($P < .0001$) for the Perzin/Szanto, Spiro, van Weert, and minAmax systems, respectively. The reproducibility of the former three systems was scored as moderate and that of the latter as very good.

4 | DISCUSSION

In this study, we introduced a novel grading system, minAmax, and analyzed its prognostic impact on a large AdCC cohort whose diagnoses of AdCC were validated by a central pathology review. The minAmax is an objective criterion for solid tumor components, and the minAmax system was shown to be useful as a prognostic tool for OS, DFS, and DMFS. In addition, we found that the reproducibility of the minAmax system was very good, with a kappa coefficient of 0.81. The minAmax was defined as the length of the minor axis

TABLE 2 Unvariate prognostic analysis in adenoid cystic carcinoma patients

			OS			DFS			DMFS		
Variable		N	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
Age (years)	<60	89	1.00			1.00			1.00		
	>60	106	1.64	0.70-3.84	.252	1.20	0.78-1.84	.412	0.98	0.59-1.63	.951
Sex	Female	122	1.00			1.00			1.00		
	Male	73	1.54	0.64-3.68	.339	1.49	0.97-2.30	.0746	1.34	0.81-2.24	.262
Site	Minor	48	1.00			1.00			1.00		
	Major	147	0.81	0.30-2.24	.695	0.78	0.48-1.26	.315	1	0.55-1.82	.998
pT	T1/T2	103	1.00			1.00			1.00		
	T3/T4	92	3.26	1.27-8.36	.0087	2.08	1.35-3.21	.0008	2.94	1.72-5.03	<.0001
pN	pN0	166	1.00			1.00			1.00		
	pN1-3	29	6.67	2.73-16.3	.0001	3.05	1.85-5.03	<.0001	3.83	2.21-6.62	<.0001
Surgical margin	Negative/close	101	1.00			1.00			1.00		
	Positive	94	5.28	1.77-15.7	.0006	2.56	1.64-4.00	<.0001	2.54	1.49-4.31	.0004
PNI	Absent	96	1.00			1.00			1.00		
	Present	99	1.43	0.62-3.28	.394	1.56	1.01-2.41	.0413	1.41	0.85-2.34	.182
PORT	Not performed	108	1.00			1.00			1.00		
	Performed	87	1.15	0.49-2.70	.756	1.12	0.74-1.73	.582	2.17	1.30-3.63	.0028
Perzin/Szanto	I/II	168	1.00			1.00			1.00		
	III	27	7.26	2.71-19.4	.0004	1.54	0.81-2.92	.207	1.48	0.70-3.13	.327
Spiro	I	173	1.00			1.00			1.00		
	II/III	22	6.54	2.37-18.1	.0014	1.64	0.84-3.20	.169	1.47	0.67-3.25	.362
van Weert	S−	135	1.00			1.00			1.00		
	S+	60	3.86	1.67-8.90	.0020	2.09	1.32-3.29	.0024	2.26	1.34-3.83	.0034
MinAmax (mm)	<0.20	147	1.00			1.00			1.00		
	>0.20	48	6.12	2.61-14.4	<.0001	1.94	1.19-3.14	.0110	2.4	1.40-4.12	.0027

Note: Univariate Cox hazard model.

Abbreviations: CI, confidence interval; DFS, disease-free survival; DMFS, distant metastasis-free survival; HR, hazard ratio; OS, overall survival; PNI, perineural invasion; PORT, postoperative radiation therapy.

TABLE 3 Mutivariate prognostic analysis for overall survival

Variable		N	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
Age (years)	<60	89	1.00			1.00			1.00			1.00		
	≥60	106	1.41	0.52-3.81	.493	1.52	0.57-4.00	.402	1.75	0.68-4.50	.245	1.47	0.58-3.81	.413
Sex	Female	122	1.00			1.00			1.00			1.00		
	Male	73	0.71	0.25-2.06	.528	0.81	0.29-2.32	.702	0.74	0.27-2.03	.558	0.58	0.20-1.68	.308
Site	Minor	48	1.00			1.00			1.00			1.00		
	Major	147	0.86	0.29-2.56	.789	0.9	0.31-2.65	.851	0.77	0.26-2.30	.648	0.94	0.32-2.79	.914
pT	pT1/2	103	1.00			1.00			1.00			1.00		
	pT3/4	92	1.7	0.58-5.02	.328	1.62	0.55-4.80	.374	2.04	0.67-6.26	.198	1.76	0.55-5.71	.336
pN	pN0	166	1.00			1.00			1.00			1.00		
	pN1-3	29	5.99	1.85-19.4	.0032	5.6	1.77-17.7	.0039	6.72	2.01-22.5	.0020	10.1	2.82-36.0	.0003
Surgical margin	Negative/close	101	1.00			1.00			1.00			1.00		
	Positive	94	5.33	1.54-18.4	.0040	5.13	1.51-17.5	.0045	3.78	1.12-12.8	.0208	6.33	1.66-24.1	.0028
PNI	Absent	96	1.00			1.00			1.00			1.00		
	Present	99	0.6	0.22-1.67	.327	0.66	0.24-1.81	.420	0.51	0.17-1.49	.214	0.35	0.12-1.04	.0572
PORT	Not performed	108	1.00			1.00			1.00			1.00		
	Performed	87	0.64	0.23-1.75	.382	0.71	0.25-1.97	.505	0.85	0.32-2.27	.747	0.52	0.19-1.41	.2
Perzin/Szanto	I/II	168	1.00											
	III	27	8.7	2.65-28.6	.0006									
Spiro	I	173				1.00								
	II/III	22				6.97	2.10-23.1	.0030						
van Weert	S−	135							1.00					
	S+	60							5.53	1.92-15.9	.0012			
MinAmax (mm)	<0.20	147										1.00		
	>0.20	48										11.9	4.12-34.3	<.0001

Note: Multivariate Cox hazard model.

Abbreviations: CI, confidence interval; DFS, disease-free survival; DMFS, distant metastasis-free survival; HR, hazard ratio; OS, overall survival; PNI, perineural invasion; PORT, postoperative radiation therapy.

TABLE 4 Mutivariate prognostic analysis for disease-free survival

		N	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
Age (years)	<60	89	1.00			1.00			1.00			1.00		
	≥60	106	1.09	0.69-1.73	.717	1.10	0.69-1.74	.697	1.16	0.73-1.84	.525	1.12	0.71-1.78	.622
Sex	Female	122	1.00			1.00			1.00			1.00		
	Male	73	1.45	0.91-2.33	.124	1.46	0.92-2.33	.111	1.31	0.82-2.09	.261	1.33	0.83-2.12	.233
Site	Minor	48	1.00			1.00			1.00			1.00		
	Major	147	0.96	0.58-1.58	.868	0.96	0.58-1.58	.877	0.9	0.55-1.49	.686	0.92	0.56-1.51	.740
pT	pT1/2	103	1.00			1.00			1.00			1.00		
	pT3/4	92	1.54	0.94-2.55	.0893	1.52	0.92-2.52	.101	1.67	1.01-2.78	.0468	1.63	0.98-2.72	.059
pN	pN0	166	1.00			1.00			1.00			1.00		
	pN1-3	29	2.5	1.39-4.47	.0031	2.48	1.38-4.45	.0023	2.59	1.43-4.69	.0025	2.55	1.42-4.57	.0017
Surgical margin	Negative/close	101	1.00			1.00			1.00			1.00		
	Positive	94	2.31	1.39-3.86	.0012	2.32	1.39-3.87	.0013	2.25	1.35-3.75	.0016	2.39	1.42-4.01	.0010
PNI	Absent	96	1.00			1.00			1.00			1.00		
	Present	99	1.13	0.70-1.83	.607	1.14	0.71-1.84	.588	1.01	0.62-1.64	.975	1.06	0.66-1.71	.802
PORT	Not performed	108	1.00			1.00			1.00			1.00		
	Performed	87	0.69	0.42-1.15	.151	0.7	0.42-1.17	.175	0.71	0.43-1.16	.167	0.65	0.39-1.07	.090
Perzin/Szanto	I/II	168	1.00											
	III	27	1.35	0.69-2.65	.392									
Spiro	I	173				1.00								
	II/III	22				1.42	0.71-2.84	.325						
van Weert	S−	135							1.00					
	S+	60							2.24	1.37-3.67	.0019			
MinAmax (mm)	<0.2	147										1.00		
	>0.2	48										2.16	1.29-3.61	.0036

Note: Multivariate Cox hazard model.

Abbreviations: CI, confidence interval; DFS, disease-free survival; DMFS, distant metastasis-free survival; HR, hazard ratio; OS, overall survival; PNI, perineural invasion; PORT, postoperative radiation therapy.

TABLE 5 Mutivariate prognostic analysis for distant metastasis-free survival

Variable		N	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
Age (years)	<60	89	1.00			1.00			1.00			1.00		
	≥60	106	1.01	0.59-1.73	.975	1.01	0.59-1.74	.963	1.05	0.61-1.79	.866	1.05	0.61-1.79	.873
Sex	Female	122	1.00			1.00			1.00			1.00		
	Male	73	1.53	0.87-2.68	.140	1.54	0.88-2.69	.131	1.26	0.72-2.22	.418	1.3	0.74-2.28	.369
Tumor site	Minor	48	1.00			1.00			1.00			1.00		
	Major	147	1.13	0.61-2.09	.691	1.14	0.62-2.10	.678	1.02	0.55-1.89	.939	1.05	0.57-1.93	.885
pT	T1/T2	103	1.00			1.00			1.00			1.00		
	T3/T4	92	1.83	0.99-3.37	.051	1.82	0.99-3.37	.053	1.96	1.06-3.64	.031	1.89	1.02-3.52	.0410
pN	pN0	166	1.00			1.00			1.00			1.00		
	pN1-3	29	2.98	1.56-5.68	.0014	2.98	1.56-5.68	.0014	3.07	1.59-5.93	.0012	2.93	1.53-5.62	.0017
Surgical margin	Negative/close	101	1.00			1.00			1.00			1.00		
	Positive	94	1.5	0.80-2.81	.198	1.51	0.81-2.81	.195	1.5	0.81-2.78	.192	1.54	0.83-2.87	.169
PNI	Absent	96	1.00			1.00			1.00			1.00		
	Present	99	0.94	0.53-1.67	.838	0.95	0.53-1.68	.852	0.79	0.44-1.41	.425	0.84	0.47-1.49	.548
PORT	Not performed	108	1.00			1.00			1.00			1.00		
	Performed	87	1.56	0.86-2.84	.144	1.56	0.85-2.86	.145	1.66	0.93-2.97	.085	1.52	0.84-2.75	.161
Perzin/Szanto	I/II	168	1.00											
	III	27	1.14	0.52-2.53	.742									
Spiro	I	173				1.00								
	II/III	22				1.11	0.48-2.56	.812						
van Weert	S−	135							1.00					
	S+	60							2.48	1.39-4.43	.0029			
MinAmax (mm)	<0.2	147										1.00		
	>0.2	48										2.29	1.28-4.10	.0073

Note: Multivariate Cox hazard model.

Abbreviations: CI, confidence interval; DFS, disease-free survival; DMFS, distant metastasis-free survival; HR, hazard ratio; OS, overall survival; PNI, perineural invasion; PORT, postoperative radiation therapy.

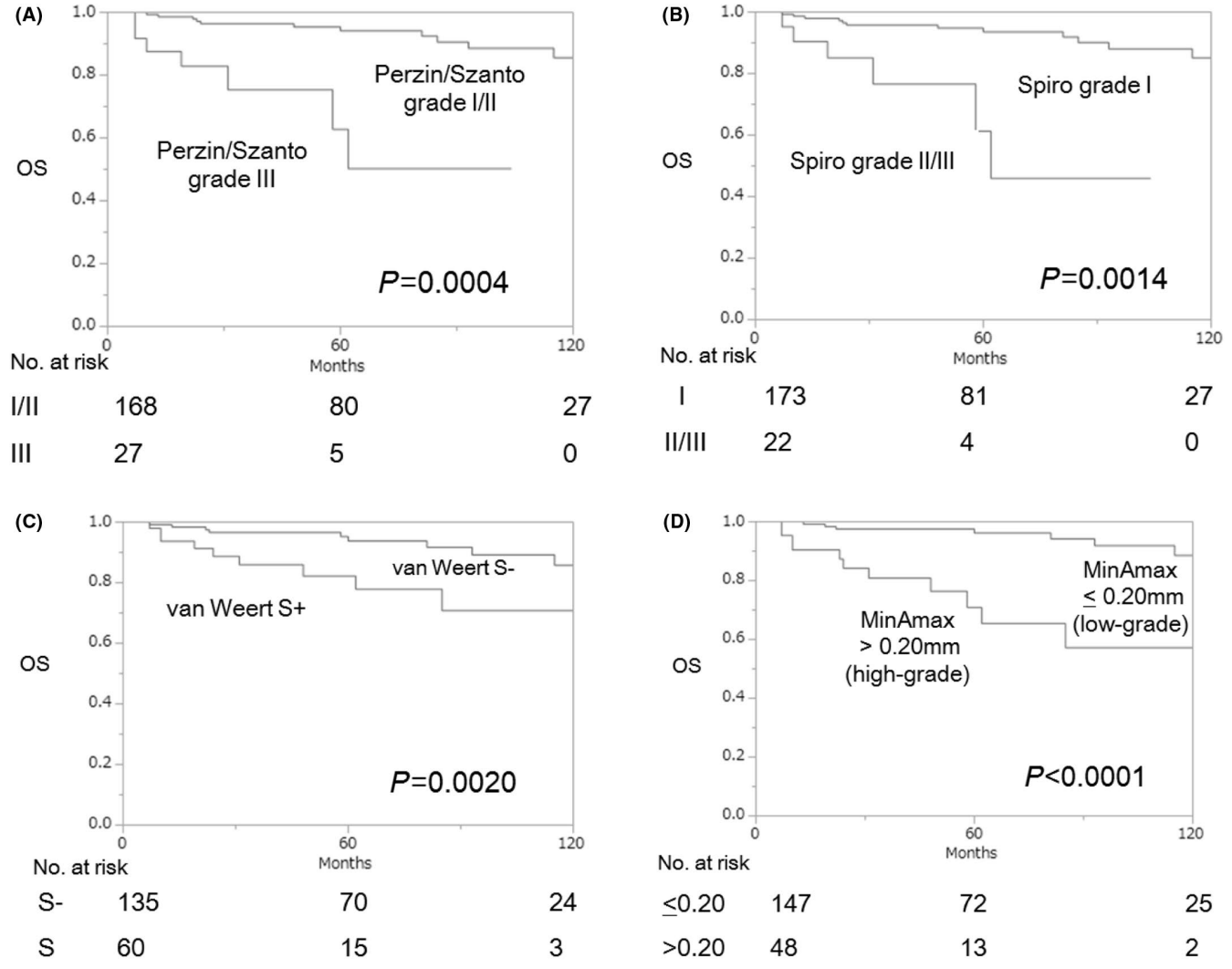

FIGURE 2 Overall survival (OS) for four grading systems (univariate Cox hazard model). Kaplan-Meier analysis stratified for the Perzin/Szanto (A), Spiro (B), van Weert (C), and minAmax (D) systems

of the maximum estimated oval fitting the largest solid tumor nest in each AdCC case. To quantify the amount of a solid area (two-dimensional index) using a length (one-dimensional index), the length of the minor axis of an oval should be measured. The length of the major axis should not be measured since the length of the major axis can sometimes be very long even in a small solid tumor component of a low-grade AdCC case, as illustrated in Figure S3. It should be noted that tumor cell features including nuclear atypia, mitotic figures, and necrosis were not taken into account in the minAmax system. To measure the minAmax correctly, a large tumor area needs to be observed, and for this reason a small tumor biopsy specimen may not be appropriate. The minAmax system was developed for conventional AdCC cases, not for high-grade transformation cases.

The most important aspect of this study was that we developed an objective definition of a "solid tumor component" by measuring the minAmax value. The minAmax could be quickly and easily determined using a standard microscope equipped with conventional micrometers. A cutoff value of 0.20 mm for the minAmax proved to be useful for predicting the survival of AdCC patients. In the multivariate prognostic analysis for OS, the minAmax showed the highest HR, followed by the Perzin/Szanto, Spiro, and van Weert systems. In the multivariate prognostic analysis for DFS and DMFS, the minAmax and van Weert systems, but not the Perzin/Szanto and Spiro systems, were selected as independent prognostic systems with moderately high HRs ranging from 2.16 to 2.48. These findings suggest that minAmax has several advantages as a prognostic tool compared to the other three grading systems.

When we first tried to apply the van Weert system to our daily pathological practice, we encountered difficulty in grading since the definition of a solid tumor component remained ambiguous. This experience lead us to introduce an objective criterion for this feature. The basic idea of the minAmax system was the same as that of the van Weert system in that the presence of a solid tumor component is an important prognostic indicator.[13,14] The lower inter-observer variability of the van Weert system compared to those of the Perzin/Szanto and Spiro systems has been emphasized by the authors.[14] However, probably owing to the ambiguity in defining a solid tumor

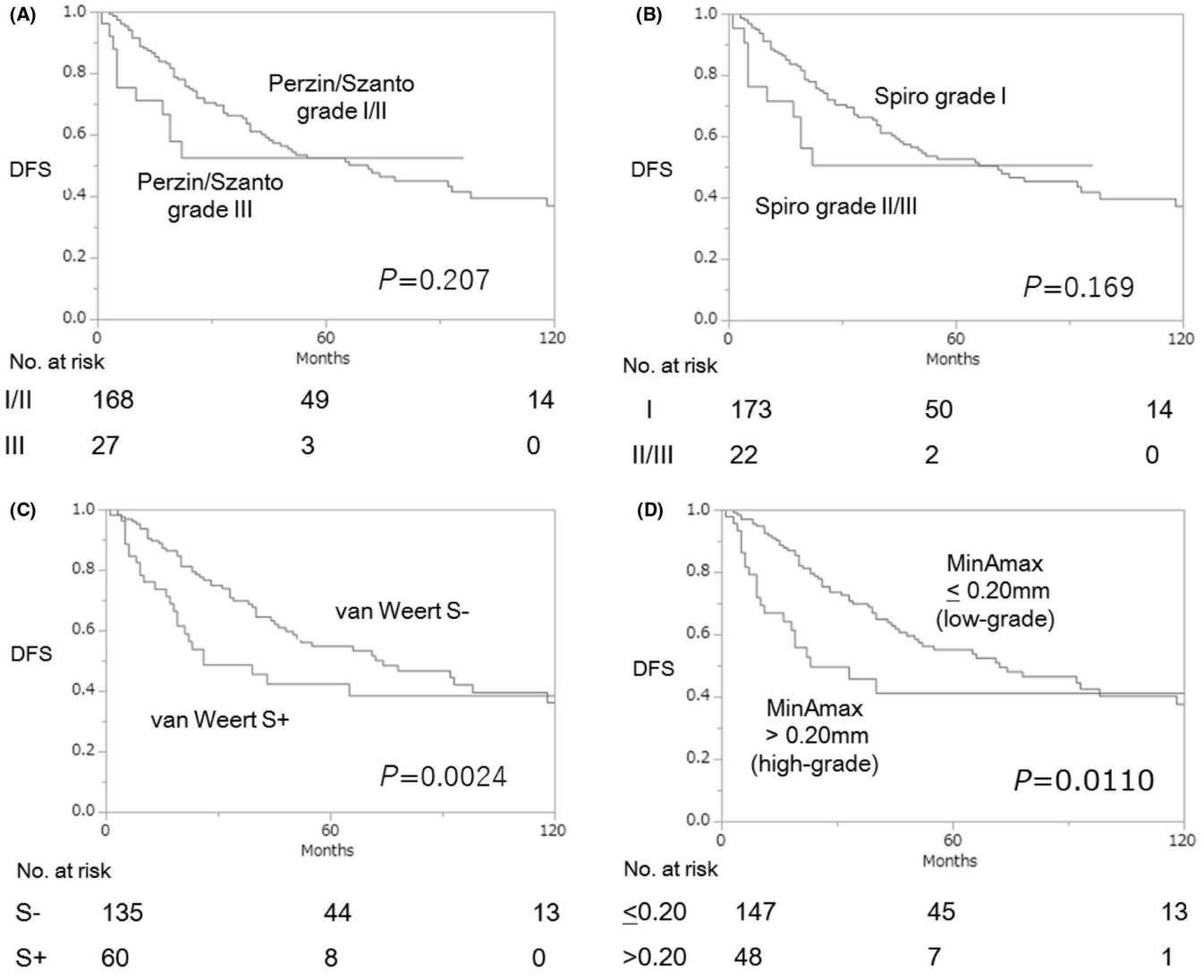

FIGURE 3 Disease-free survival (DFS) for four grading systems (univariate Cox hazard model). Kaplan-Meier analysis stratified for the Perzin/Szanto (A), Spiro (B), van Weert (C), and minAmax (D) systems

component, we failed to obtain high reproducibility in the van Weert system (kappa coefficient of 0.44), which was similar to those of the Perzin/Szanto and Spiro systems (kappa coefficient of 0.51 in both systems). On the other hand, with a clear definition of this tumor feature, a very good concordance with a kappa coefficient of 0.81 was achieved with the minAmax system. The low interobserver variability of the minAmax system is another advantage over the other three grading systems.

One of the controversies regarding management of AdCC patients is whether the grading of AdCC cases has clinical significance.[8] In some studies, grading AdCC cases was useful as a prognostic factor[14,19-21] and in other studies, the usefulness was not evident.[8,22-24] In addition, it is not unusual that the AdCC grade has not been incorporated as a factor in the prognostic analysis.[25-29] This discrepancy is difficult to account for but some explanations can be offered. One is that as AdCC is a slow-growing malignancy,[2,3] a prognostic difference is difficult to distinguish in a small cohort with a short follow-up period. Another possible explanation is due to the ambiguous definition of a solid tumor component, which is the basis of all known grading systems.[9-11,14] In this study employing a large AdCC cohort and an objective grading system, we showed that grading AdCC cases was important for estimation of the survival of AdCC patients.

In conclusion, the minAmax system provides a simple and objective means of assessing the solid tumor component in AdCC, and it can be easily employed using a standard microscope equipped with a micrometer. The minAmax can also be measured simply using the diameter of the microscopic field as a guide. In practice, AdCC cases with a high minAmax are easily distinguished from those with a low minAmax without precisely measuring minAmax in most AdCC cases. Further studies are warranted to clarify the utility of the minAmax in clinical settings.

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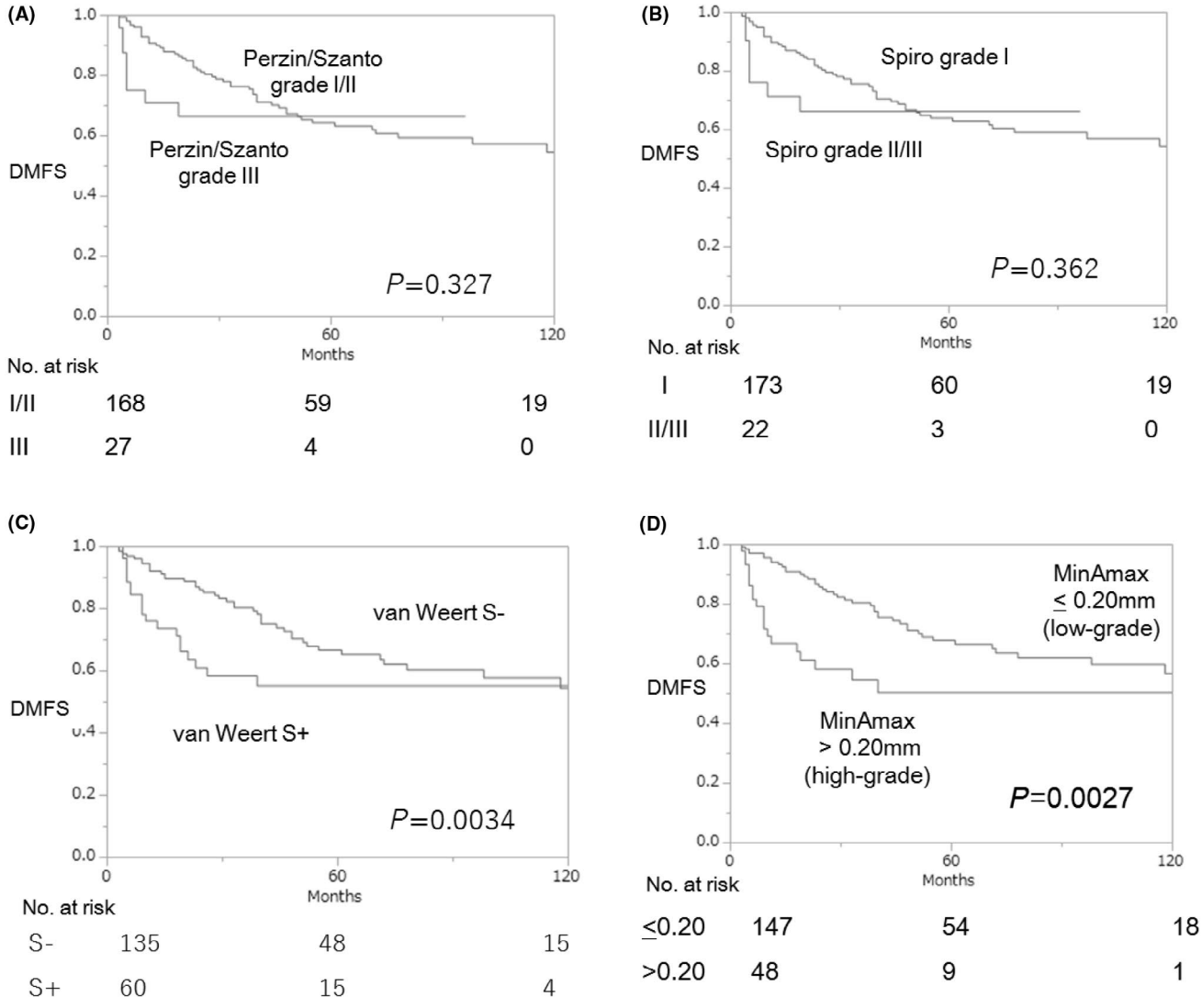

FIGURE 4 Distant metastasis-free survival (DMFS) for four grading systems (univariate Cox hazard model). Kaplan-Meier analysis stratified for the Perzin/Szanto (A), Spiro (B), van Weert (C), and minAmax (D) systems

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CONFLICT OF INTEREST

None to declare.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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