



# Prognostic significance of cribriform adenocarcinoma of the lung: validation analysis of 1,057 Japanese patients with resected lung adenocarcinoma and a review of the literature

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**Background:** Cribriform-predominant adenocarcinoma of the lung (Cribriform-ADC) is a recently described tumor growth pattern. However, its prognostic impact has not been clearly determined. We analyzed the data of a series of 1,057 Japanese patients with resected lung adenocarcinoma to identify the clinical significance of Cribriform-ADC.

**Methods:** Cribriform pattern (Cribriform-p) is defined as invasive back-to-back fused tumor glands with poorly formed glandular spaces or invasive tumor nests comprising tumor cells that produced glandular lumina. We investigated the correlations of Cribriform-p and Cribriform-ADC with clinicopathological factors as well as disease-free survival (DFS) and overall survival (OS).

**Results:** Cribriform-p was present in 217 patients (20.5%) and Cribriform-ADC was determined in 25 patients (2.4%). Cribriform-p was associated with larger tumor size, pleural invasion, vascular invasion, lymphatic invasion, and spreading through air spaces (STAS) (all,  $P < 0.0001$ ). Cribriform-ADC was associated with younger age ( $P = 0.019$ ), vascular invasion ( $P = 0.0025$ ), STAS ( $P < 0.0001$ ), and *ALK* rearrangement ( $P = 0.012$ ). The DFS curve of patients with Cribriform-ADC was identical to that of patients with solid adenocarcinoma; however, the OS curve was located between that of patients with papillary and acinar adenocarcinoma. Of the 10 patients who had tumor recurrences, eight had *EGFR* mutations or *ALK* rearrangement, six of whom achieved relatively long survival (median, 64.6, range, 37.4–113 months) following treatment with tyrosine kinase inhibitors (TKIs). In multivariate analysis, Cribriform-ADC was not an independent prognostic factor of either recurrence or death.

**Conclusions:** Cribriform-ADC is associated with a higher risk of recurrence; however, most patients can be successfully treated with TKIs.

**Keywords:** Lung; adenocarcinoma; cribriform; prognosis

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## Introduction

Lung cancer is the leading cause of cancer-related death worldwide (1,2). Lung adenocarcinoma (L-ADC) is the most common histologic type of lung cancer (3); the latest World Health Organization (WHO) classification for this disease, introduced in 2015 (3), identified five major subtypes according to the most predominant proliferation pattern: lepidic, acinar, papillary, micropapillary, and solid. However, following recent studies, investigators have proposed the cribriform pattern (Cribriform) as a sixth pattern; cribriform-predominant adenocarcinoma (Cribriform-ADC) is reported to be a prognostically unfavorable subtype (4-9). Notably, there were several drawbacks in these studies; the histological definition of Cribriform was ill-defined, the clinical significance of Cribriform in other predominant subtypes was not well-examined, and the prognostic significance of Cribriform-ADC was described inconsistently (4-9).

To address these shortcomings, we performed this validation study to clearly identify the clinical significance of Cribriform and Cribriform-ADC by reviewing the data of 1057 resected L-ADCs.

We present the following article in accordance with the STROBE reporting checklist (<http://dx.doi.org/10.21037/tlcr-20-612>).

## Methods

### Cohort

We retrospectively investigated data of patients with L-ADC who underwent complete curative-intent resection at the Kyoto University Hospital between 2001 and 2015. Patients were excluded from the study if they had multiple primary lung cancers, were treated with chemotherapy or radiotherapy before surgery, underwent incomplete resection, or for whom follow-up information was incomplete based on clinical data retrieved from the Thoracic Surgical Database. The analysis ultimately included 1057 L-ADCs. The requirement for informed consent was waived owing to the retrospective nature of the study and all patients were provided the means to opt out of this study. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013) and the study protocol was approved by the Kyoto University Hospital ethics committee (R1158-2).

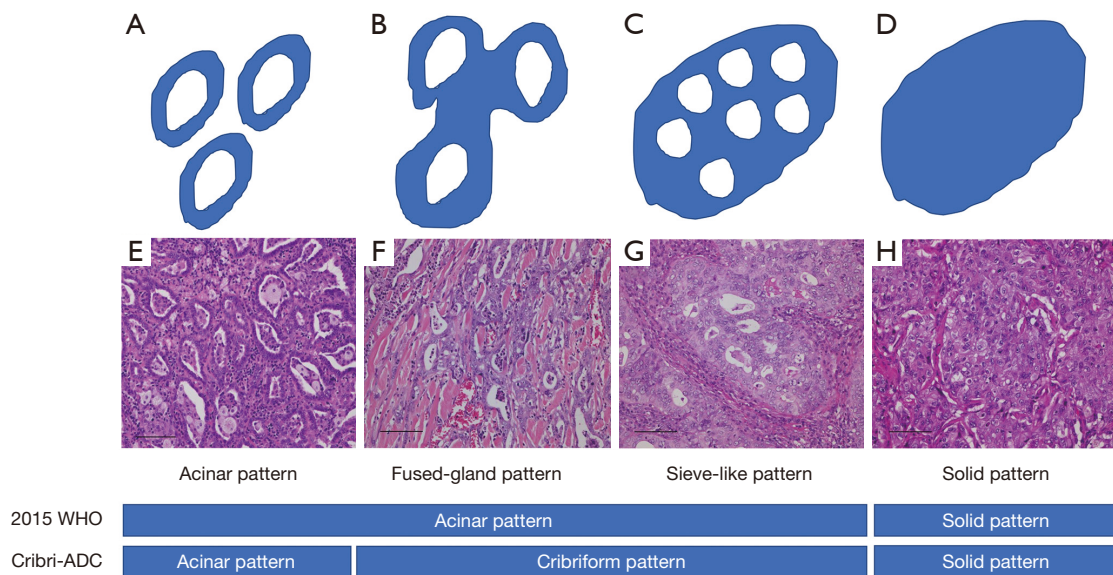
### Histological evaluation

All resected specimens were fixed in formalin, sectioned, and stained with hematoxylin and eosin according to standard procedures. Small-sized tumors were histologically sampled as a whole. Elastic staining was performed to detect pleural or vascular invasion. All of the specimens were reviewed by the authors (NN and AY, both pathologists) who were blinded to the patients' information. The average number of tumor specimens reviewed for each patient was 3.3 (range, 1-20). According to the 2015 WHO classification, each tumor was subjected to comprehensive histological subtyping, and the percentage of each histological component was recorded in 5% increments (3).

In the current WHO classification, cribriform arrangement was considered as a part of acinar pattern, which is defined as round-to-oval-shaped glands with central luminal space surrounded by tumor cells (*Figure 1*); however, 'cribriform arrangement' is not clearly defined (3). Furthermore, previous studies described two qualitatively different Cribriform components; one description is invasive back-to-back fused tumor glands with poorly formed glandular spaces lacking intervening stroma (fused-gland pattern) (*Figure 1B,F*) or invasive nests of tumor cells that produce glandular lumina without solid components (sieve-like pattern) (*Figure 1C,G*) (4,6,8,9). The other description is nests of tumor cells with sieve-like perforations, which are identical to the 'sieve-like pattern' described by the aforementioned researchers (5,7). In some previous studies, the fused-gland pattern was interpreted under a complex glandular pattern (5,7). In our study, we defined Cribriform as fused-gland pattern or sieve-like pattern and separately recorded their extents in 5% increments. Then, we designated tumors as Cribriform-positive when their proportions of fused-gland and/or sieve-like pattern were 5% or greater. Cribriform-ADC was defined as a tumor which predominantly showed fused-gland and/or sieve-like pattern in the individual tumor (*Figure 1*). We then evaluated the clinicopathological significance of Cribriform and Cribriform-ADC and studied their prognostic significance.

### Detection of genetic alterations in various oncogenes

To evaluate the association between Cribriform-exhibiting tumors and gene alterations, such as *EGFR*, *KRAS*,



**Figure 1** Schema and representative histological images of acinar-, cribriform-, and solid pattern-samples stained with hematoxylin and eosin. (A,E) Acinar pattern showing round-to-oval-shaped glands with a central luminal space surrounded by tumor cells; (B,F) cribriform pattern showing invasive back-to-back fused tumor glands with poorly formed glandular spaces lacking intervening stroma (fused-gland pattern); (C,G) cribriform pattern showing invasive nests of tumors cells that produce glandular lumina without solid components (sieve-like pattern); (D,H) solid pattern showing solid nests without lumina. Cribriform adenocarcinoma (Cribriform-ADC) was defined as an area of predominantly cribriform pattern including fused-gland and sieve-like pattern within the individual tumor (Cribriform pattern). “2015 WHO” refers to the 2015 World Health Organization classification. Bars indicate 0.1 mm; (E,F,G,H) hematoxylin and eosin staining.

*HER2*, *BRAF*, *ALK*, and *ROS1*, we used the data that we had performed in our previous studies. Briefly, *EGFR* mutations were evaluated with the polymerase chain reaction (PCR) single-strand conformation polymorphism method until 2009 (10) and with the peptide nucleic acid-locked nucleic acid polymerase chain reaction clamp method from 2010 onward. The *HER2* and *BRAF* mutations were also examined using PCR-single-strand conformation polymorphism (11,12). The *KRAS* mutation was investigated using a modified mutagenic PCR restriction fragment length polymorphism technique (10). *ALK* fusion was detected via reverse transcription PCR and fluorescence in situ hybridization (FISH) until 2009 and via immunohistochemistry and FISH from 2010 onward (13,14). *ROS1* fusion was detected via FISH using a *ROS1* Dual Color Break Apart Probe (Vysis LSI/Abbott Laboratories, Chicago, IL, USA) according to the manufacturer’s instructions (15). *RET* fusion was also detected via FISH using the Kreatech *RET* (10q11) Break FISH probe (Leica Biosystems, Wetzlar, Germany) and *RET* Split Dual Color FISH probe (GSP Lab., Tokyo, Japan) according to the manufacturer’s instructions (15).

**Statistics**

The  $\chi^2$  and Fisher’s exact tests were used to analyze categorical data. Survival rates were calculated using the Kaplan-Meier method, and differences in overall survival (OS) and disease-free survival (DFS) were analyzed using the log-rank test. Univariate and multivariate analyses were performed using the Cox’s proportional hazards model. A P value <0.05 was considered statistically significant. Data analysis and graph-plotting were performed using the JMP statistical software package version 12 (SAS Institute, Cary, NC, USA).

**Results**

**Patient and tumor characteristics**

The clinicopathological characteristics of the study population are shown in *Table 1*; there were 514 (48.6%) men and 543 (51.4%) women with a mean age of 66.2±9.8 years at diagnosis. The mean tumor size was 23.9±14.4 mm; and 52.9% of the patients were smokers (including current and former smokers). Moreover, 2.0% of

**Table 1** Association between cribriform pattern and clinicopathological features

Variable	Total	Cribriform pattern			Cribriform-ADC		
		Present	Absent	P value	Yes	No	P value
N	1,057	217	840		25	1,032	
Sex							
Male	514 (48.6%)	129	385	0.0003	15	499	0.25
Female	543 (51.4%)	88	455		10	533	
Age (mean 66.2; range, 23–88)							
<65 years	433 (41.0%)	100	333	0.087	16	417	0.019
≥65 years	624 (59.0%)	117	507		9	615	
Smoking							
Ever	559 (52.9%)	139	420	0.0002	17	542	0.13
Never	498 (47.1%)	78	420		8	490	
Stage							
0	21 (2.0%)	0	21	<0.0001	0	21	0.12
I	829 (78.4%)	130	699		16	813	
II	109 (10.3%)	38	71		3	106	
III	98 (9.3%)	49	49		6	92	
Tumor size (mean 23.9; range, 3–120)							
>30 mm	255 (24.1%)	80	175	<0.0001	4	251	0.48 <sup>†</sup>
≤30 mm	802 (75.9%)	137	665		21	781	
Pleural inv.							
Absent	853 (80.7%)	148	705	<0.0001	23	830	0.15 <sup>†</sup>
Present	204 (19.3%)	69	135		2	202	
Vascular inv.							
Absent	865 (81.8%)	130	735	<0.0001	14	851	0.0025 <sup>†</sup>
Present	192 (18.2%)	87	105		11	181	
Lymphatic inv.							
Absent	958 (90.6%)	177	781	<0.0001	20	938	0.11 <sup>†</sup>
Present	99 (9.4%)	40	59		5	94	
STAS							
Absent	673 (63.7%)	90	583	<0.0001	6	667	<0.0001 <sup>†</sup>
Present	384 (36.3%)	127	257		19	365	

<sup>†</sup>, Fisher's exact test. Cribriform pattern; Cribriform-ADC, cribriform predominant adenocarcinoma; STAS, spread through air spaces; inv., invasion; MP, micropapillary.

patients had stage 0 disease, 78.4% had stage I, 10.3% had stage II, and 9.3% had stage III. The proportion of stage I patients in our cohort was much higher and the proportion of smokers in our cohort was lower than that in the European cohort (6). However, the proportions were comparable to the other data from Japan or Asian countries (8,9). One hundred seventy-nine patients (16.9%) died during follow-up and 231 (21.9%) relapsed. The mean follow-up time at the end-point of analysis was  $61.6 \pm 35.7$  months.

#### ***Frequency of tumors with Cribri-p/Cribri-ADC and their associated clinicopathological features***

Cribri-p was present in 217 of 1,057 patients (20.5%), and the median proportion of this component was 20.3% (range, 5–90%) (Figure S1A). The median proportion of tumor with the fused-gland and sieve-like patterns were 5.5% (range, 5–30%) and 17.9% (range, 5–90%), respectively (Figure S1B,C). Tumors with only fused-gland patterns and those with both fused-gland and sieve-like patterns were observed in 54 (5.1%) and 163 (15.4%) patients, respectively (Table S1). In the analysis of detailed cribriform pattern, tumor with sieve-like pattern was frequently observed in tumors with solid pattern ( $P < 0.0001$ ). There were 25 patients with Cribri-ADC (2.4%) after adjusting for the number of invasive adenocarcinoma subtypes based on the current WHO classification (Figure S2A,B).

The relationships between Cribri-p and certain clinicopathological features are listed in Table 1. Cribri-p was observed more frequently in men ( $P = 0.0003$ ) and smokers ( $P = 0.0002$ ). It was most frequent in patients with stage III disease (49, 50.0%) followed by stage II (71, 34.9%), whereas it was not frequently associated with stage I (130 patients, 15.7%). There was no statistically significant relationship between the presence of Cribri-p and age. Cribri-p was also associated with a larger tumor size ( $P < 0.0001$ ), pleural invasion ( $P < 0.0001$ ), vascular invasion ( $P < 0.0001$ ), lymphatic invasion ( $P < 0.0001$ ), and spread through air spaces (STAS) ( $P < 0.0001$ ). Cribri-p was also most frequently observed in solid adenocarcinomas (51.3%) followed by adjusted acinar adenocarcinomas (32.7%), whereas it was rare in lepidic adenocarcinomas (2.8%) (Figure S2C).

Cribri-ADC was frequently observed in younger patients ( $P = 0.019$ ) and was also associated with vascular invasion ( $P = 0.0025$ ), solid pattern ( $P < 0.0001$ ), and STAS ( $P < 0.0001$ ) (Table 1). However, Cribri-ADC was not associated with sex, smoking status, stage, tumor size, or the presence of pleural

invasion.

#### ***Associations between Cribri-p/Cribri-ADC and gene alteration***

Among the 1057 L-ADCs, 497, 221, 145, 228, 393, 262, and 236 tumors were assessed for *EGFR*, *KRAS*, *HER2*, *BRAF* mutations, and *ALK*, *RET*, and *ROS1* fusions, respectively. Cribri-p was significantly associated with wild-type *EGFR*, *KRAS* mutations, and *ALK* rearrangement ( $P = 0.0041$ ,  $P = 0.023$ , and  $P = 0.0002$ , respectively) but was not significantly associated with *HER2* mutations, *BRAF* mutations, *RET* rearrangement, or *ROS1* rearrangement (Table 2). Cribri-ADC was only associated with tumors exhibiting *ALK* rearrangement ( $P = 0.012$ ).

#### ***Association between Cribri-p/Cribri-ADC and prognosis***

Cribri-p was associated with significantly higher risks of death and recurrence in patients with all stages (relative risk 2.5; 95% confidence interval 1.9–3.2,  $P < 0.0001$ ) and in those with stage I (relative risk 2.1; 95% confidence interval 1.3–3.3,  $P = 0.0044$ ), but not in those with stages II or III (Table S2). Next, we investigated the prognostic significance of Cribri-p in the acinar-, papillary-, solid-, and micropapillary-ADC. Regarding papillary adenocarcinoma, patients with Cribri-p had significantly worse prognoses than did those without (death:  $P = 0.0096$ , recurrence:  $P = 0.0016$ ) (Table S2). As for micropapillary adenocarcinoma, patients with Cribri-p had significantly higher risks of recurrence than did those without ( $P = 0.024$ ) (Table S2). There were no statistically significant differences in terms of the risk of death and recurrence between patients with acinar and solid adenocarcinoma who had Cribri-p and those who did not. We used factors that were found to be significant in the univariate analysis (data not shown) for the multivariate analysis; Cribri-p was found to not be an independent prognostic factor, whereas a micropapillary pattern was an independent predictor of recurrence while a solid pattern was an independent predictor of both recurrence and death (Table S3, Trial 1).

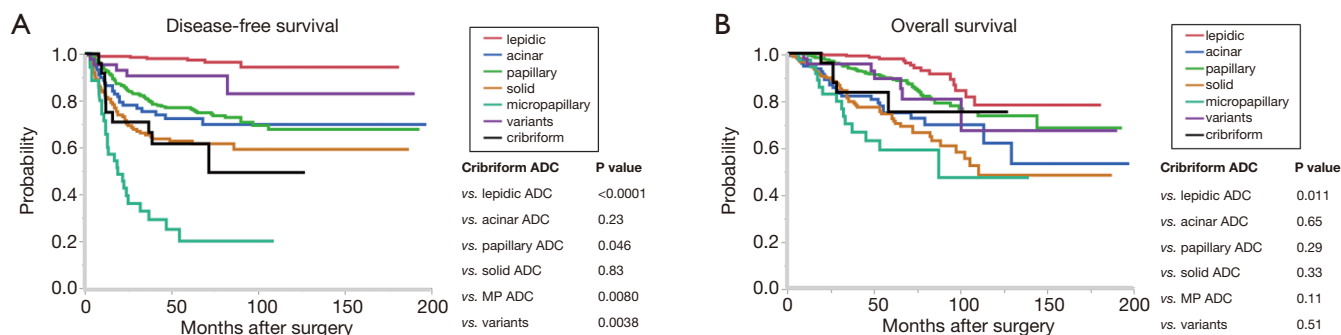
L-ADC was reclassified into seven groups based on the predominant pattern: lepidic, acinar, papillary, solid, micropapillary, variant, and cribriform (Figure S2B). The DFS curves of patients with Cribri-ADC were identical to those of patients with solid adenocarcinoma; furthermore, the DFS of patients with Cribri-ADC was shorter than that of patients with lepidic adenocarcinoma and papillary



**Table 2** Association between cribriform pattern and gene alterations

Gene alteration	No. of patients	Cribri-p			Cribrri-ADC		
		Absent	Present	P value	No	Yes	P value
<b>EGFR</b>							
Wild-type	253	186	67	0.0041	247	6	0.95 <sup>†</sup>
Mutant	244	205	39		238	6	
<b>KRAS</b>							
Wild-type	195	153	42	0.023 <sup>†</sup>	191	4	0.47 <sup>†</sup>
Mutant	26	15	11		25	1	
<b>ALK</b>							
Wild-type	376	298	78	0.0002 <sup>†</sup>	367	9	0.012 <sup>†</sup>
Rearranged	17	6	11		14	3	
<b>HER2</b>							
Wild-type	139	100	39	1.00 <sup>†</sup>	135	4	1.00 <sup>†</sup>
Mutant	6	5	1		6	0	
<b>BRAF</b>							
Wild-type	225	165	60	0.57 <sup>†</sup>	219	6	1.00 <sup>†</sup>
Mutant	3	3	0		3	0	
<b>RET</b>							
Wild-type	259	194	65	1.00 <sup>†</sup>	253	6	1.00 <sup>†</sup>
Rearranged	3	2	1		3	0	
<b>ROS1</b>							
Wild-type	234	174	60	0.46 <sup>†</sup>	229	5	1.00 <sup>†</sup>
Rearranged	2	1	1		2	0	

<sup>†</sup>, Fisher's exact test. Cribri-p, cribriform pattern; Cribrri-ADC, cribriform predominant adenocarcinoma.



**Figure 2** Association between adenocarcinoma (ADC) subtype and disease-free survival (DFS) and overall survival (OS). (A) The DFS of patients with cribriform ADC is shorter than that of patients with lepidic ADC ( $P<0.0001$ ) and those with papillary ADC ( $P=0.046$ ), while the survival curve of patients with cribriform ADC was similar to that of patients with solid ADC; (B) the OS of patients with cribriform ADC was relatively favorable compared to that of patients with solid and micropapillary adenocarcinoma. MP, micropapillary.

**Table 3** Status after initial resection of cribriform adenocarcinoma in patients with recurrence

No.	Status	Stage (8th)	Last follow-up (months)	Recurrent months after surgery	Recurrence or metastasis status	EGFR mutations	ALK rearrangement	Tx-TKIs	Tx-Chemo	Tx-Rad
1	AWD	3A (T1bN2M0)	113	71.5	CNS, lung	NA	Yes	Yes	Yes	Yes
2	DOD	3A (T1bN2M0)	26	8	CNS	NA	NA	No	Yes	Yes
3	DOD	2B (T1bN1M0)	58	11.5	CNS	Yes	NA	Yes	Yes	Yes
4	DOD	1B (T2aN0M0)	19	9.6	Mediastinal LN, pleura, adrenal gland	NA	NA	No	Yes	Yes
5	DOD	1A2 (T1bN0M0)	26	11.8	CNS	Yes	NA	Yes	No	No
6	AWD	2B (T1bN1M0)	62.5	36.9	Mediastinal and axillary LN	Yes	No	Yes	Yes	No
7	AWD	3A (T1bN2M0)	52	12.1	Mediastinal LN, CNS	Yes	No	Yes	No	No
8	AWD	3B (T3N2M0)	37.4	12	Intraabdominal LN	No	Yes	Yes	No	Yes
9	AWD	3A (T1bN2M0)	52	52	CNS	No	Yes	Yes	No	Yes
10	AWD	3A (T1cN2M0)	44	38.8	Mediastinal LN, lung, bone	Yes	No	Yes	Yes	Yes

AWD, alive with disease; DOD, dead of disease; CNS, central nervous system; LN, lymph node; NA, not available; Tx-TKIs, treatment with tyrosine kinase inhibitors; Tx-Chemo, platinum-base chemotherapy; Tx-Rad, radiation therapy.

adenocarcinoma, and was longer than that of patients with micropapillary adenocarcinoma ( $P < 0.0001$ ,  $P = 0.046$ , and  $P = 0.0080$ , respectively) (Figure 2A). The OS curve of patients with Cribriform-ADC was located between that of patients with papillary and acinar adenocarcinoma (Figure 2B). In multivariate analysis, Cribriform-ADC was not an independent prognostic factor of either recurrence or death, whereas age ( $>65$  vs.  $\leq 65$  years), stage (II vs. I and III vs. I), lymphatic invasion, and pleural invasion were independent predictors of both recurrence and death (Table S3, Trial 2). Table 3 shows the statuses of 10 patients with Cribriform-ADC with recurrence after resection. EGFR mutations or ALK rearrangements were detected in all six patients who were alive with disease, those of whom achieved long survival (median, 64.6 months; range, 37.4–113 months) following treatment with tyrosine kinase inhibitors (TKIs) like gefitinib, erlotinib, or crizotinib. Among the four deceased patients, EGFR mutations were detected in two; these patients were also treated with TKIs and died 58 and 26 months after initial resection, respectively.

As for detail of Cribriform-p and prognosis, the 5-year DFS rate of patients exhibiting tumors with only the fused-gland pattern tended to be lower than that of patients having tumors without Cribriform-p but higher than that of patients having tumors with sieve-like pattern, although

these differences were not significant ( $P = 0.10$  and  $P = 0.053$ , respectively) (Figure S3A). Moreover, the five-year OS rate of patients with only fused-gland pattern tumors were significantly higher than that of those with sieve-like pattern tumors ( $P = 0.037$ ), although there was no significant difference in OS between those with fused-gland pattern tumors and those without Cribriform-p tumors ( $P = 0.364$ ) (Figure S3B).

## Discussion

We found that 25 of our patients (2.4%) had Cribriform-ADC, and that this pattern was associated with male sex, lymphovascular invasion, and STAS. Patients with Cribriform-ADC had relatively worse DFS rates than did those with acinar adenocarcinoma but had similar rates to those of patients with solid-ADC. The OS curve of patients with Cribriform-ADC was located between that of patients with papillary and acinar adenocarcinoma. Most patients with recurrent tumors harbored druggable gene alterations and achieved relatively long-term survival following treatment with TKIs. Our results indicate that Cribriform-ADC is the unfavorable lung adenocarcinoma subgroup in terms of prognosis, but that it can be controlled with the appropriate TKIs.

A summary of previous large-scale studies that investigated Cribriform-ADC is shown in *Table 4*. In six previous studies (4-9), the frequency of Cribriform-ADC was found to be 4.2–12%. The DFS of patients with Cribriform-ADC tended to be worse than that of patients with acinar L-ADC but comparable to that of patients with solid ADC in five of the studies (4,5,7-9). Our current study demonstrated that patients with Cribriform-ADC had DFS rates that were relatively worse than those with acinar L-ADC but comparable to those with solid-ADC; thus, our results are consistent with those from previous studies (4-9). Based on the evidence that Cribriform-ADC tends to recur, we agree with the notion that Cribriform-ADC should be considered a new tumor subtype derived from acinar-ADC.

With respect to OS, three previous studies (7-9) demonstrated that patients with Cribriform-ADC have relatively poor outcomes compared to patients with solid ADC. Warth *et al.* reported that the OS rates of patients with Cribriform-ADC was in between those of patients with acinar ADC and solid ADC (6); our data were consistent with theirs. They suggested that the natural course of Cribriform-ADC is aggressive but that these tumors might respond better to chemotherapy administered after relapse than would tumors of the other patterns. In the current study, eight of 10 patients who experienced recurrence harbored *EGFR* mutations or *ALK* rearrangements (data were not available for the other two), and six were alive with disease following treatment with TKIs.

In our study, Cribriform-p was observed in 20.1% of L-ADCs and was associated with several clinicopathologic factors; patients of all stages, stage I, and papillary adenocarcinoma subtype with Cribriform-p had significantly higher risks of death or recurrence than did those without. Regarding the significance of Cribriform-p as a minor component, Kadota *et al.* reported that the ‘recurrence-free probability’ among patients with predominantly acinar tumors exhibiting  $\geq 10\%$  Cribriform-p was significantly lower than that among patients with tumors exhibiting  $< 10\%$  Cribriform-p (4). Moreover, they also demonstrated that the presence of Cribriform-p did not correlate with the risk of recurrence among patients with papillary or solid predominant tumors. In our study, we found that the DFS and OS rates of patients with papillary-predominant adenocarcinomas who had  $\geq 5\%$  Cribriform-p were significantly lower, and that their recurrence rates were significantly higher than those of patients with  $< 5\%$  Cribriform-p. In contrast, there were no significant differences in the risks of death and recurrence between patients

with acinar and solid adenocarcinomas with  $\geq 5\%$  versus those with  $< 5\%$  Cribriform-p. This discrepancy may be owing to the differences in the thresholds used to determine the presence of Cribriform-p. However, we recommend that the presence of Cribriform-p be reported as a potential prognostic variable even if other predominant subtypes are noted.

Regarding the association between Cribriform-p and gene alterations, earlier studies found that tumors with *ALK* rearrangements tended to exhibit Cribriform-p (6,7,16,17), while others found no such association (8,9). As such, a clear link between these two occurrences has not been established. In our study, the presence of Cribriform-p and Cribriform-ADC was associated with *ALK* rearrangement. The discrepancy between the studies may be caused by differences in the thresholds and/or methodologies used for identifying gene alteration; hence, the relationship between gene alterations and Cribriform-p ought to be investigated using uniform methods such as next-generation sequencing.

There were some limitations in this study, the most notable of which was the inter-observer agreement. Although we subclassified Cribriform-p from acinar-pattern tumors after careful discussion, we noted some difficulty in distinguishing simple acinar glands from fused-glands because most were continuously present in the same regions. However, we consider that we can overcome interobserver disagreement by training to recognize the pattern carefully because our concordance rate was not very low when analyzing the selected 100 cases (data not shown). In contrast, if the Cribriform-ADC is recognized as the sixth histological subtype of L-ADC, it will potentially be more difficult to reach interobserver agreement than it would be with the current scheme. Deep learning techniques may be promising to overcome this difficulty (18,19).

In addition, we also consider classification by tumor grading to be more optimal than determining the tumor subtype for predicting the prognosis (20). While some grading systems have been proposed, additional multi-center validation studies are needed. A second limitation was that some investigators set their Cribriform-p cut-off values at 5% and others at 10%; we set ours at 5% because the percentage of each histological component is recorded in 5% increments per the 2015 WHO classification recommendations (3). As such, Cribriform-p should be more strictly identified because the presence of other subtypes with Cribriform-p appear to be associated with relatively poorer prognoses; this also requires future validation.



**Table 4** Previous studies that investigated the prognostic significance of the cribriform pattern in lung adenocarcinoma

Author (Ref.), year/country	Patients' cohort	Cribriform				Cribriform-ADC			
		Frequency	Prognosis	Genetic alterations	Frequency	Genetic alterations	DFS	OS	
Kadota <i>et al.</i> (4), 2014/USA	1,038 (stage I)	262 (25.2%)	Acinar ADCs with Cribriform were worse than those without Cribriform	NA	46 (4.4%)	Not correlated with EGFR & KRAS mut. & ALK fusions	Comparable to solid ADC	NA	
Moreira <i>et al.</i> (5), 2014/USA	249 (stage I)	98 (39.4%)	NA	Not correlated with EGFR & KRAS mut.	30 (12.0%)	NA	Comparable to solid ADC	NA	
Warth <i>et al.</i> (6), 2015/Germany	674	193 (28.6%)	NA	Correlated with EGFR wild-type, ROS1 & ALK fusions	28 (4.2%)	Correlated with KRAS mut., not correlated with ALK fusions	Worse than solid ADC	Ranged between acinar and solid ADC	
Kuang <i>et al.</i> (7), 2018/China	356	156 (43.8%)	NA	Correlated with HER2 mut. & ALK fusions	NA	NA	Comparable to solid ADC	Comparable to solid ADC	
Qu <i>et al.</i> (9), 2019/China	395	130 (32.9%)	NA	Not correlated with EGFR & KRAS mut. & ALK fusions	32 (8.1%)	NA	Comparable to solid ADC	Comparable to solid ADC	
Kadota <i>et al.</i> (8), 2019/Japan	735	131 (17.7%)	NA	Not correlated with ALK fusions	54 (7.3%)	NA	Comparable to solid ADC	Comparable to solid ADC	
Current study/ Japan	1,057	218 (20.6%)	Papillary or MP ADCs with Cribriform were worse than those without Cribriform	Correlated with EGFR & KRAS wild-type, & ALK fusions	26 (2.5%)	Correlated with ALK fusions	Comparable to solid ADC	Comparable to intermeditated grade ADC	

Ref., reference number; Cribriform, cribriform pattern; Cribriform-ADC, cribriform-predominant adenocarcinoma; ADC, adenocarcinoma; NA, not available; mut., mutations.

## Conclusions

Our evaluation of the clinical significance of Cribriform-ADC revealed that this pattern portends a poor prognosis with respect to recurrence, which is consistent with data from previous studies. However, most patients with recurrent tumors harbor druggable genetic alterations, such as *EGFR* mutations and *ALK* rearrangements, and are therefore responsive to TKIs. Given these results, we propose that TKIs should be added to the postoperative adjuvant therapy regimens administered to such patients. Furthermore, our findings suggest that Cribriform-p should be recognized as a poor prognostic factor even when it is not a major tumor component.

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*Ethical Statement:* The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The requirement for informed consent was waived owing to the retrospective nature of the study and all patients were provided the means to opt out of this study. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013) and the study protocol was approved by the Kyoto University Hospital ethics committee (R1158-2).

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