Original Article

Large nest micropapillary pattern of lung adenocarcinoma has poorer prognosis than typical floret pattern: analysis of 1,062 resected tumors

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Background: A micropapillary pattern (MP-p) is related to poor prognosis in patients with lung adenocarcinoma (L-ADC). In 2015, the WHO defined the MP-p as "papillary tufts forming florets that lack fibrovascular cores and may appear detached from alveolar walls"; however, the sizes of tumor clusters in air space were not mentioned in this classification.

Methods: We evaluated the MP-p dividing the cluster sizes in the air space by reviewing 1,062 cases of resected L-ADCs. We classified MP-p into two types according to cluster size as follows: typical floret MP-p, tumors with small-to-medium-sized clusters (1–20 tumor cells); and large nest MP-p, tumors with large-sized clusters (>20 tumor cells, large nest). We then recorded the frequency of each type and investigated the association between the MP-p type and clinicopathological factors.

Results: Twenty-nine percent of L-ADCs (n=308) were MP-p-positive. Typical floret MP-p and large nest MP-p were observed in 244 tumors (22.9%) and 64 tumors (6.0%), respectively. Only 7 additional micropapillary ADCs were detected when we reclassified ADCs in addition to large nest MP-p. Tumors with large nest MP-p showed the highest frequency of node metastasis and worse prognosis compared to those with typical floret MP-p and absent (P<0.001). In multivariate analysis, patients with L-ADC with typical floret MP-p and large nest MP-p showed a higher recurrence rate [hazard ratio (HR): 1.762 (type 1 *vs.* absent), HR: 2.450 (type 2 *vs.* absent)].

Conclusions: Large nest MP-p should be included in the original MP-p and recorded separately.

Keywords: Lung adenocarcinoma (L-ADC); micropapillary pattern (MP-p); large nest; prognosis

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Introduction

Lung adenocarcinoma (L-ADC) is the most common histological subtype of primary lung cancer. It exhibits molecular, clinical, radiological, surgical, and pathological heterogeneity (1). The micropapillary pattern (MP-p) of L-ADC was initially reported in 2002 (2) and has been described to have a poor prognostic pattern (3-9). MP adenocarcinoma was proposed as a new histological subtype of L-ADC by the International Association for the Study of Lung Cancer, American Thoracic Society, and the European Respiratory Society in 2011 (10); additionally, the World Health Organization (WHO) renewed its

classification in 2015 (1). The core feature of MP-p is "small papillary tufts with no fibrovascular cores appearing detached from alveolar wall". However, the MP-p criteria fail to define a "large tumor cell nest which does not form a pseudo papillary structure". This flaw in the MP-p definition may cause considerable discrepancies in MP-p diagnosis among different observers (11-13).

In the present study, we divided floating tumor clusters in the air space into two types based on their size and investigated the effect of these types on clinical outcomes by histologically reviewing 1,062 resected L-ADCs.

Methods

Cohort

A retrospective analysis was carried out on patients with L-ADC who underwent complete resection with curative intent at Kyoto University Hospital between 2001 and 2015. Patients who had multiple primary lung cancers, were treated with chemotherapy or radiotherapy before surgery, underwent incomplete resection, or had incomplete follow-up information in the clinical data retrieved from the Thoracic Surgical Database were excluded from the study. The analysis ultimately included 1,062 L-ADCs. The study protocol was approved by the Kyoto University Hospital ethics committee (R1158-1).

Histological evaluation

All resected specimens were fixed in formalin, sectioned, and stained with hematoxylin and eosin according to standard procedures. Small tumors were histologically sampled as one sample. Elastic staining was performed to detect pleural or vessel invasion. All specimens were reviewed by two pathologists (KK and AY) who were blinded to patient information, and all histological parameters were established by consensus after discussion. The average number of tumor specimens reviewed for each case was 3.3 (range, 1–20). According to the 2015 WHO classification (1), each tumor was subjected to comprehensive histological subtyping, and the percentage of each histological component was recorded in 5% increments.

Two MP-p patterns were defined according to cluster size in the air space as follows: small to medium-sized cluster (composed of 1–20 tumor cells), typical floret MP-p (10,14), and large-sized cluster (composed of more than 20 tumor cells), large nest MP-p (*Figure 1*). Before the review, we assessed 100 cases and observed air space tumor clusters of various sizes in individual cases. We found single cells or small clusters (composed of 2–3 tumor cells) showing MP-p-like feature (*Figure 2A*,*B*). Because this feature may be an artifact appearing as tangential cells or small clusters of lepidic or papillary patterns, we classified this structure as absent of Mp-p in this study when a typical floret Mp-p was not identified. Next, we recorded typical floret MP-p when a small-to-medium-sized cluster was observed without a large-sized cluster, and large nest MP-p when a large-sized cluster was observed in the presence or absence of small to medium-sized cluster (*Figure 1*). We also included the stromal MP-p as an additional intra-alveolar floret MP-p (*Figure 2C*,*D*). Moreover, although the feature of large nest MP-p may be considered as an artifact showing pseudostratified tumor cells of acinar or solid patterns, we classified this structure as a new pattern in this study (*Figure 2E*,*F*). Based on the criteria, we assessed the whole specimens and recorded the percentage of MP-p in 5% increments. We then investigated the association between MP-p type and the following clinicopathological factors: sex, age, smoking status, tumor grade, histological subtype, lymphatic/vascular/pleural invasion, spread through air spaces (STAS) according to the 2015 WHO classification (1), and tumor-node-metastasis (TNM) staging according to the 8th TNM classification (15).

Detection of genetic alterations in various oncogenes

The association between tumors with MP-p and mutations in the epidermal growth factor receptor (*EGFR*), Kirsten rat sarcoma viral oncogene homolog (*KRAS*), human epidermal growth factor receptor 2 (*HER2*), B-raf proto-oncogene serine/threonine protein kinase (*BRAF*), anaplastic lymphoma kinase (*ALK*), and ROS proto-oncogene 1 (*ROS1*) was evaluated according to our previous studies (16-21). Briefly, *EGFR* mutations were evaluated by polymerase chain reaction single-strand conformation polymorphism (PCR-SSCP) before 2009 (18) and the PNA-LNA PCR clamp method after 2010. *HER2* and *BRAF* mutations were also examined by PCR-SSCP (17). *KRAS* mutation was investigated using a modified mutagenic PCR restriction fragment length polymorphism technique (18). *ALK* fusion was detected by reverse transcription PCR and fluorescence *in situ* hybridization (FISH) (16, 20). ROS1 fusion was detected by FISH using ROS1 Dual Color Break Apart Probe (Vysis LSI/Abbott Laboratories, Chicago, IL, USA)

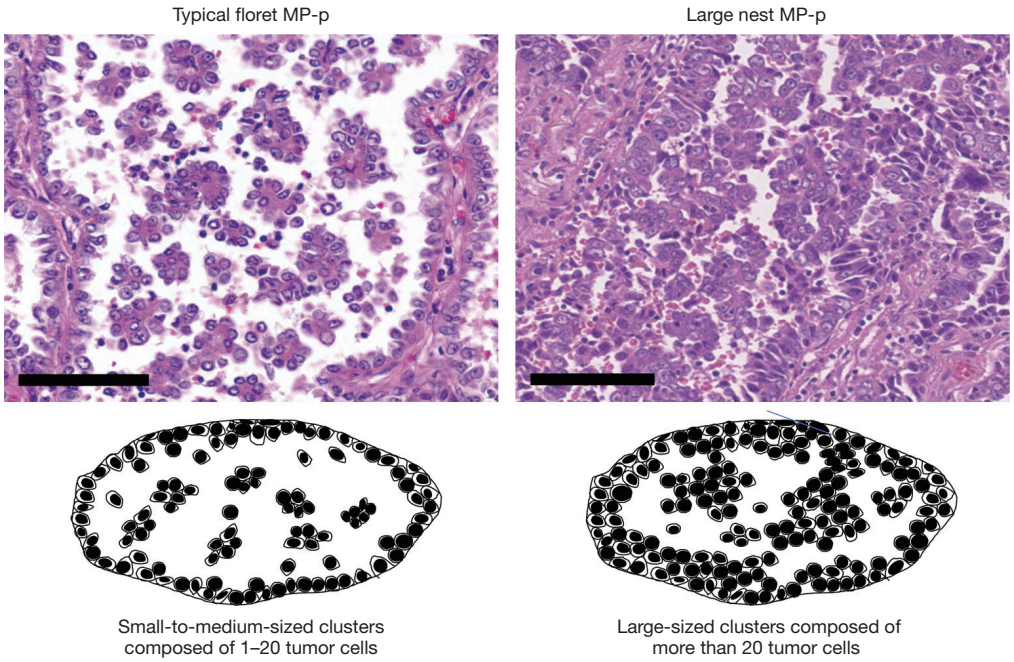

Figure 1 Representative images of micropapillary pattern (MP-p) type and schematic illustrations. MP-p was classified according to cluster size as follows: small-to-medium-sized clusters (composed of 1–20 tumor cells), typical floret MP-p; and large-sized clusters (composed of more than 20 tumor cells), large nest MP-p. We recorded typical floret MP-p when a small-to-medium-sized cluster was observed, without large-sized cluster and large nest MP-p when more than 5% of a large-sized cluster was observed, in the presence or absence of small to medium-sized cluster. Scale bars: 100 μm. MP-p, micropapillary pattern.

according to the manufacturer's instructions (19). Ret proto-oncogene (*RET*) fusion was also detected by 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.

Statistics

The χ^2 and Fisher's exact tests were applied to analyze categorical data. Survival rates were calculated by the Kaplan-Meier method, and differences were analyzed with the log-rank test. Multivariate analysis was performed using Cox's proportional hazards model. Multivariate models were generated to include factors that were significant in univariate analysis. All statistical tests were two-sided at a 5% level of significance. Data analysis was performed using JMP v.13 statistical software package (SAS Institute, Cary, NC, USA). Summary graphs were generated using JMP v.13 statistical software package and Microsoft PowerPoint 2016 (Microsoft Corporation, Redmond, WA, USA).

Results

Clinicopathological characteristics

The clinicopathological characteristics of the study population are shown in *Table 1*. There were 515 (48.5%) male and 547 (51.5%) female patients; the mean age at diagnosis was 66.2±9.8 years (range, 23–88 years); mean tumor size was 23.9±14 mm (range, 3–120 mm); and 561 (52.8%) patients were smokers (221 current smokers, 340 ex-smokers; smoking index =45.7). Most patients underwent lobectomy of one or more lobes (n=766, 72.1%), and the others (n=296, 27.8%) underwent limited resection (segmentectomy or wedge resection). A total of 186 (17.3%) patients died during follow-up, and 241 (22.4%) relapsed. The mean follow-up time at the end-point of analysis was 61.5±35.7 months. The number of patients at each pathologic stage was as follows: stage 0, 20 (1.9%) patients; I, 832 (78.3%) patients; II, 111 (10.5%) patients; and III, 98

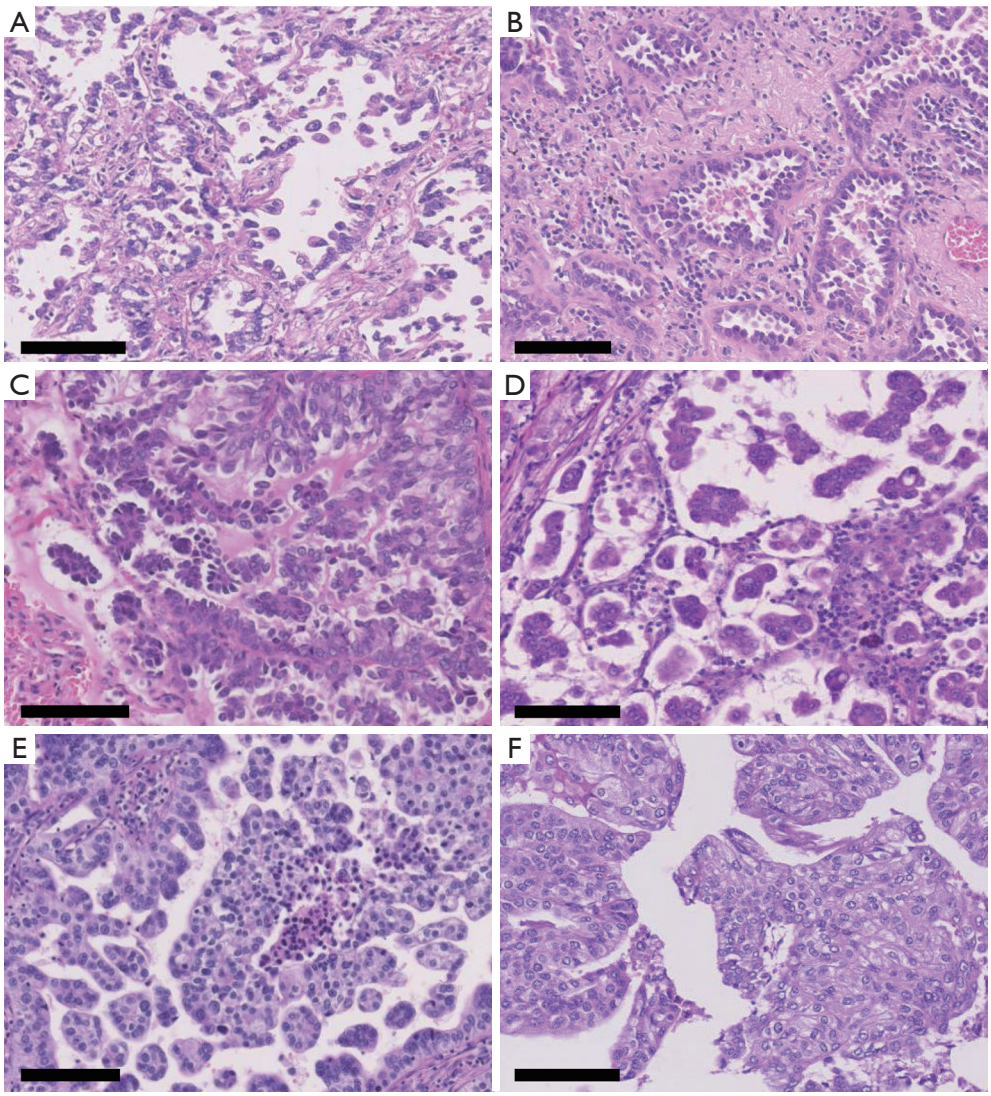

Figure 2 Representative pictures of MP-p. (A) Lepidic growth pattern with floating single tumor cells in the alveolar spaces which is considered as a tangential cut feature. (B) Single tumor cells or small-sized clusters of tumor cells were floating in the airspace in the papillary growth pattern as in (A). (C) Typical floret MP-p: typical floret MP-p showing medium-sized cluster of tumor cells. (D) Typical floret MP-p: medium-sized cluster of tumor cells, which were observed in slit-like spaces, were classified as MP-p (stromal invasive type micropapillary pattern). (E) Large nest MP-p: large-sized clusters consisting of over 20 tumor cells were floating in the gland along with medium-sized clusters. This area was determined as large nest MP-p according to our criteria. (F) Large nest MP-p: only large-sized tumor cell clusters detaching from the alveolar wall were observed in the dilated alveolar space. Some clusters appear to have a desquamative appearance. Scale bars 100 μm. MP-p, micropapillary pattern.

(9.2%) patients.

Correlation between MP-p type and clinicopathological characteristics

Of the 1,062 cases, MP-p was present in 308 tumors (29.0%), predominantly in those with a larger tumor size (P<0.001), exhibiting lymph node metastasis (P<0.001), higher pathological stage (P<0.001), lymphatic invasion (P<0.001), vascular invasion (P<0.001), pleural invasion (P<0.001), and STAS (P<0.001). There was no association between the presence of MP-p and age, sex, or smoking

Table 1 Association between MP-p type and clinicopathological characteristics of the study population

Subject	Overall (n=1,062)	MP-p overall		P
		Present (n=308)	Absent (n=754)	
Sex				0.150
Male	515	160	355	
Female	547	148	399	
Age, years				0.800
Under 65	435	128	307	
Over 66	627	180	447	
Tumor size				<0.001
25 mm or less	703	156	547	
26 mm or over	359	152	207	
Smoking				0.755
Smoker	561	165	396	
Never Smoker	501	143	358	
Procedure				<0.001
Lobectomy or more	766	262	504	
Limited resection	296	46	250	
pN				<0.001
0	904	216	688	
1, 2	158	92	66	
Stage				<0.001
0	20	0	20	
I	833	198	635	
II	111	53	58	
III	98	57	41	
Tumor grade				<0.001
Well diff.	304	24	280	
Moderately diff.	450	163	287	
Poorly diff.	308	121	187	
Lymphatic invasion				<0.001
Positive	101	62	39	
Negative	961	246	715	
Vascular invasion				<0.001
Positive	194	83	111	
Negative	868	225	643	

Table 1 (*continued*)

Table 1 (*continued*)

Subject	Overall (n=1,062)	MP-p overall		P
		Present (n=308)	Absent (n=754)	
Pleural invasion				<0.001
Positive	207	95	112	
Negative	855	213	642	
STAS				<0.001
Positive	385	214	171	
Negative	677	94	583	
2015 WHO classification				<0.001
AIS	20	0	20	
MIA	109	2	107	
Lepidic ADC	120	16	104	
Acinar ADC	118	30	88	
Papillary ADC	459	185	274	
Solid ADC	154	34	120	
Micropapillary ADC	35	35	0	
IMA	42	8	34	
Other ADC*	5	0	5	

*, other ADCs include colloid adenocarcinoma, fetal adenocarcinoma, and enteric adenocarcinoma. MP-p, micropapillary pattern; ADC, adenocarcinoma; AIS, adenocarcinoma *in situ*; diff., differentiated; IMA, invasive mucinous adenocarcinoma; MIA, minimally invasive adenocarcinoma; STAS, spread through air spaces.

status (*Table 1*).

Table 2 and *Figure 3* show the correlations between clinicopathological factors and detailed MP-p types. Typical floret MP-p was observed in 244 tumors (22.9% of all tumors), whereas large nest MP-p was observed in 64 tumors (6.0% of all tumors). The range of percentage of typical floret MP-p and large nest Mp-p were 5–95% with a mean of 18.3 [standard deviation (SD) 17.9] and 5–80% with a mean of 19.1 (SD 14.9), respectively. We found that lymph node metastasis was most frequently associated with large nest MP-p (45.3%), followed by typical floret MP-p (25.8%) and absent of MP-p (8.7%; *Figure 3A*). In subclass analysis with tumors with typical floret MP-p and large nest MP-p, lymph node metastasis was significantly observed in tumors with large nest MP-p in contrast to tumors with typical floret MP-p (P=0.003). Lymphatic, vascular, and pleural invasion were more frequent in tumors with typical floret MP-p and large nest MP-p compared to those without MP-p, whereas no significant difference was observed between tumors with typical floret MP-p and those with large nest MP-p (*Figure 3B,C,D*). STAS was most frequently detected in tumors with large nest MP-p (75.0%), followed by tumors with typical floret MP-p (68.0%) and tumors without MP-p (22.6%) (P<0.001; *Figure 3E*). Large nest MP-p was frequently present in advanced stage tumors, whereas MP-p were rarely observed in stage I tumors (P<0.001; *Figure 3F*).

Figure 4 shows the incidence of MP-p type by adenocarcinoma subtype. MP-p was frequently observed in tumors of papillary adenocarcinoma (40.3%) except for micropapillary adenocarcinoma, followed by acinar adenocarcinoma (25.4%) and solid adenocarcinoma (22.7%). We reclassified the tumors by including large nest MP-p in the classical MP-p. The number of cases of micropapillary ADC was increased by 7 (tumor incidence: 3.95%) compared to the initial number of cases (tumor incidence: 3.29%). The number of cases of lepidic ADC, papillary ADC, and solid ADC was decreased by 1, 4, and

Table 2 Association between MP-p type and clinicopathological characteristics of the study population

Subject	Overall (n=1,062)	MP-p detail			P
		Absent (n=754)	Typical floret (n=244)	Large nest (n=64)	
Sex					0.234
Male	515	355	130	30	
Female	547	399	114	34	
Age, years					0.736
Under 65	435	307	104	24	
Over 66	627	447	140	40	
Tumor size					<0.001
25 mm or less	703	547	122	34	
26 mm or over	359	207	122	30	
Smoking					0.775
Smoker	561	396	133	32	
Never Smoker	501	358	111	32	
Procedure					<0.001
Lobectomy or more	766	504	208	54	
Limited resection	296	250	36	10	
pN					<0.001
0	904	688	181	35	
1, 2	158	66	63	29	
Stage					<0.001
0	20	20	0	0	
I	833	635	167	31	
II	111	58	35	18	
III	98	41	42	15	
Tumor grade					<0.001
Well diff.	304	280	21	3	
Moderately diff.	450	287	135	28	
Poorly diff.	308	187	88	33	
Lymphatic invasion					<0.001
Positive	101	39	46	16	
Negative	961	715	198	48	
Vascular invasion					<0.001
Positive	194	111	60	23	
Negative	868	643	184	41	

Table 2 (*continued*)

Table 2 (*continued*)

Subject	Overall (n=1,062)	MP-p detail			P
		Absent (n=754)	Typical floret (n=244)	Large nest (n=64)	
Pleural invasion					<0.001
Positive	207	112	76	19	
Negative	855	642	168	45	
STAS					<0.001
Positive	385	171	166	48	
Negative	677	583	78	16	
2015 WHO classification					<0.001
AIS	20	20	0	0	
MIA	109	107	2	0	
Lepidic ADC	120	104	15	1	
Acinar ADC	118	88	23	7	
Papillary ADC	459	274	150	35	
Solid ADC	154	120	22	12	
Micropapillary ADC	35	0	29	6	
IMA	42	34	5	3	
Other ADC*	5	5	0	0	

*, other ADCs include colloid adenocarcinoma, fetal adenocarcinoma, and enteric adenocarcinoma. MP-p, micropapillary pattern; ADC, adenocarcinoma; AIS, adenocarcinoma *in situ*; diff., differentiated; IMA, invasive mucinous adenocarcinoma; MIA, minimally invasive adenocarcinoma; STAS, spread through air spaces.

2, respectively (*Figure 5A,B*). The 3- and 5-year disease-free survival rates of patients with reclassified MP ADC were 31.6% and 20.8%, respectively, which did not differ from those of patients with original MP ADC (33.7% and 20.6%, respectively) (*Figure 5C,D*).

Association between presence of MP-p and alterations in oncogenes

In the 1,062 L-ADC specimens, we observed mutations in *EGFR* (240/495, 48.5%), *KRAS* (26/221, 11.8%), *HER2* (6/145, 4.1%), and *BRAF* (3/228, 1.3%) as well as fusions in *ALK* (17/394, 4.3%), *ROS1* (2/236, 0.8%), and *RET* (3/262, 1.1%). We found a correlation between MP-p-positive tumors and *ALK* fusion (P=0.009, Fisher's exact test), but not between MP-p-positive tumors and *EGFR* (P=0.672), *KRAS* (P=0.125), *HER2* (P=0.081), or *BRAF* (P=0.331) mutation and *ROS1* (P=0.439) or *RET* (P=0.279) fusion (*Table 3*).

MP-p type and patient outcome

We investigated the association between survival and MP-p status in cases of invasive adenocarcinoma except for invasive mucinous adenocarcinoma and other variants (n=886). We found that patients with MP-p-positive tumors had worse prognosis than those with MP-p-negative tumors both in terms of disease-free survival (DFS) and overall survival (OS) (5-year DFS rate, 56.8%, P<0.001; 5-year OS rate, 76.0%; P<0.001; *Figure 6A,B*). Patients with large nest MP-p tumors had the worst prognosis (5-year DFS rate, 39.7%), followed by those with typical floret MP-p tumors (5-year DFS rate, 60.2%), whereas those without MP-p had better prognosis (5-year DFS rate, 82.6%) (*Figure 6C*). Patients with large nest MP-p tumors had the worst prognosis (5-year OS rate, 66.8%) followed by those with typical floret MP-p tumors (5-year OS rate, 78.3%), whereas those without MP-p had better prognosis (5-year OS rate, 87.7%) (*Figure 6D*).

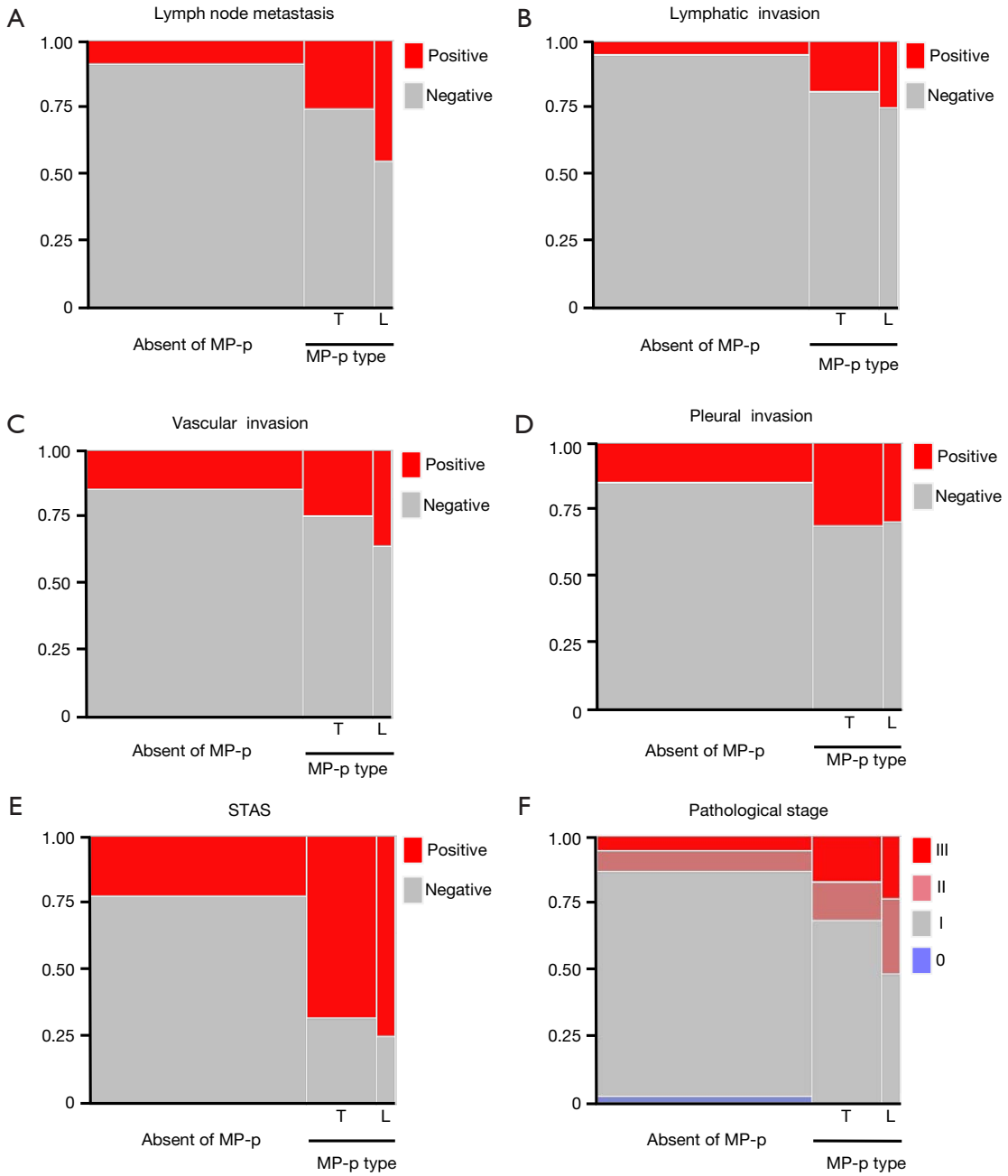

Figure 3 Correlations between MP-p type and clinicopathological factors. (A) Lymph node metastasis; (B) lymphatic invasion; (C) vascular invasion; (D) pleural invasion; (E) spread through air spaces (STAS); (F) pathological stage. These are visualized figures of *Table 2*. MP-p, micropapillary pattern; STAS, spread through air spaces; T, typical floret type; L, large nest type.

Table 4 shows the results of the uni- and multivariate analyses of the clinicopathological factors examined in this study. Based on the results of univariate analysis, we performed multivariate analyses using the Cox proportional hazards model and found that age, smoking status, stage, vascular invasion, and MP-p were independently associated with recurrence risk [typical floret MP-p *vs.* MP-p absent, hazard ratio (HR): 1.762, 95% confidence interval (CI), 1.287–2.413; large nest MP-p *vs.* MP-p absent, HR: 2.450, 95% CI, 1.587–3.784, P<0.0001]. Moreover, age, smoking

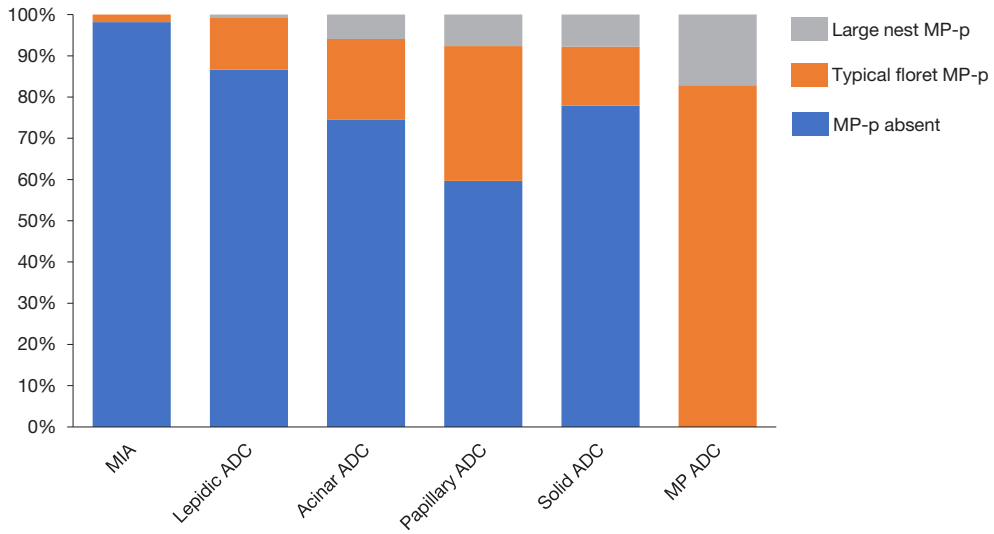

Figure 4 Incidence of MP-p type of each subtype excluding invasive mucinous adenocarcinoma, colloid adenocarcinoma, and fetal adenocarcinoma. MIA, minimally invasive adenocarcinoma; ADC, adenocarcinoma; MP-p, micropapillary pattern.

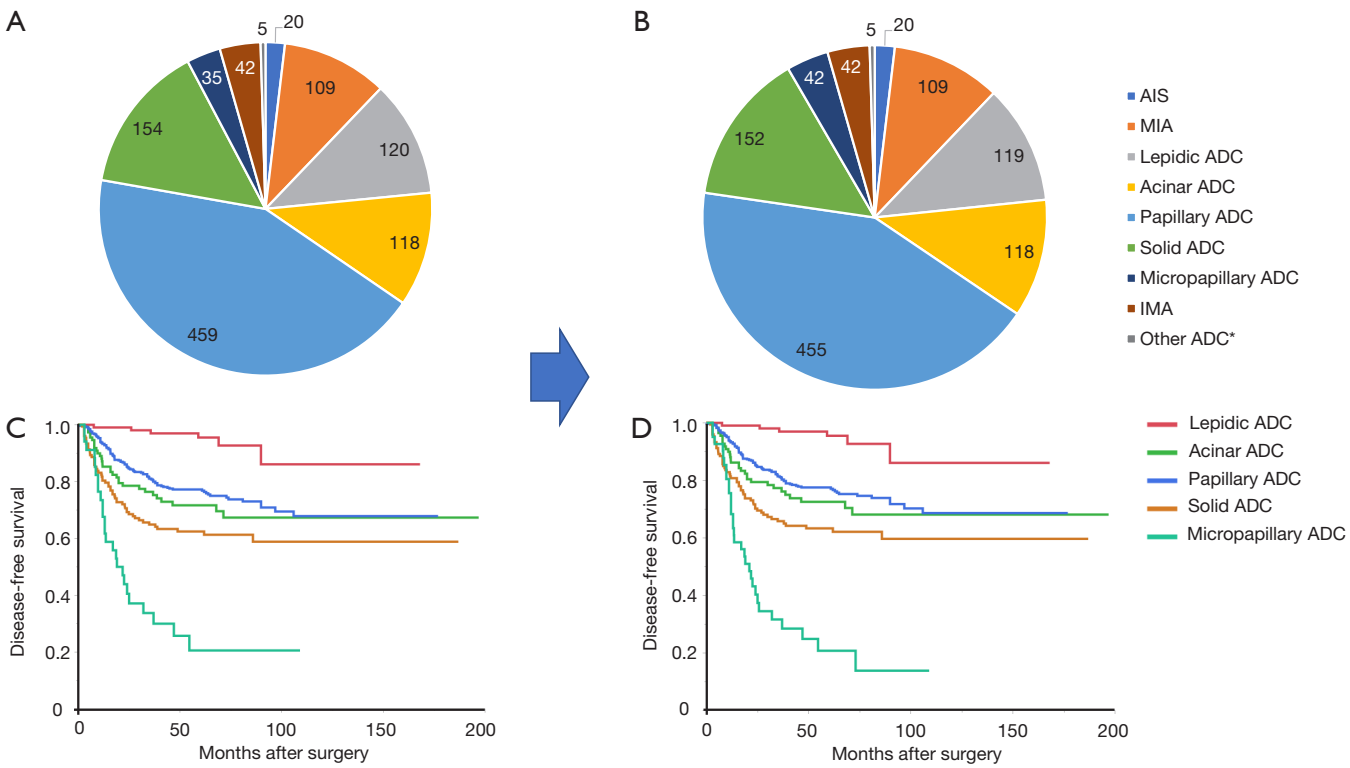

Figure 5 Reclassification of subtype adjusted by the addition of MP-p type. (A) Incidence of subtype based on the classical type MP-p; (B) incidence of subtype adjusted by the addition of large nest MP-p; (C) 5-year DFS curves of patients with original WHO classification (n=886); (D) 5-year DFS curves of patients with reclassified WHO classification (n=886). *, other ADC includes colloid adenocarcinoma and fetal adenocarcinoma. AIS, adenocarcinoma *in situ*; MIA, minimally invasive adenocarcinoma; ADC, adenocarcinoma; IMA, invasive mucinous adenocarcinoma.

Table 3 Correlations between MP-p and genetic alterations

Genetic alterations	N	MP-p		P
		Present	Absent	
EGFR mut.	495			0.672
Mutated		70	170	
Wild		70	185	
KRAS mut.	221			0.125
Mutated		3	23	
Wild		49	146	
HER2 mut.	145			0.081
Mutated		3	3	
Wild		28	111	
BRAF mut.	228			0.331
Mutated		0	3	
Wild		54	171	
ALK fusion	394			0.009*
Fusion		10	7	
Wild		98	279	
ROS1 fusion	236			0.439
Fusion		0	2	
Wild		54	180	
RET fusion	262			0.279
Fusion		0	3	
Wild		73	186	

*, Fisher's exact test. mut., mutation; MP-p, micropapillary pattern.

status, stage, and lymphatic invasion were independent prognostic factors of worse OS; however, MP-p was not a significant independent prognostic factor for OS (P=0.629), although it was significant in univariate analysis (P=0.0002).

Discussion

In this study, we demonstrated that MP-p is present in 29.0% of L-ADCs, with typical floret MP-p as the most common. We found that MP-p is an independent predictor of a worse clinical outcome for recurrent disease in patients with resected L-ADC and is associated with aggressive tumor characteristics such as large tumor size, advanced pathological stage, lymph node metastasis, pleural invasion, lymphovascular invasion, and STAS. The frequency and prognosis of reclassified micropapillary ADC slightly differed from those of the original micropapillary ADC. Further, our results showed that patients with large nest MP-p experienced recurrence more frequently than those with typical floret MP-p and without MP-p. These findings highlight the prognostic value of classifying MP-p type according to cluster size of the air space.

Although the current WHO classification briefly references the micropapillary histological subtype (1), considerable inter-observer variability is common in the identification of MP-p (11-13). Thunnissen *et al.* reported that the concordance rate of the micropapillary subtype was lower (62%) than those of the other subtypes (92–100%) (13); the authors noted that only 12% of participant pathologists in the study identified MP-p as a single pattern. This may

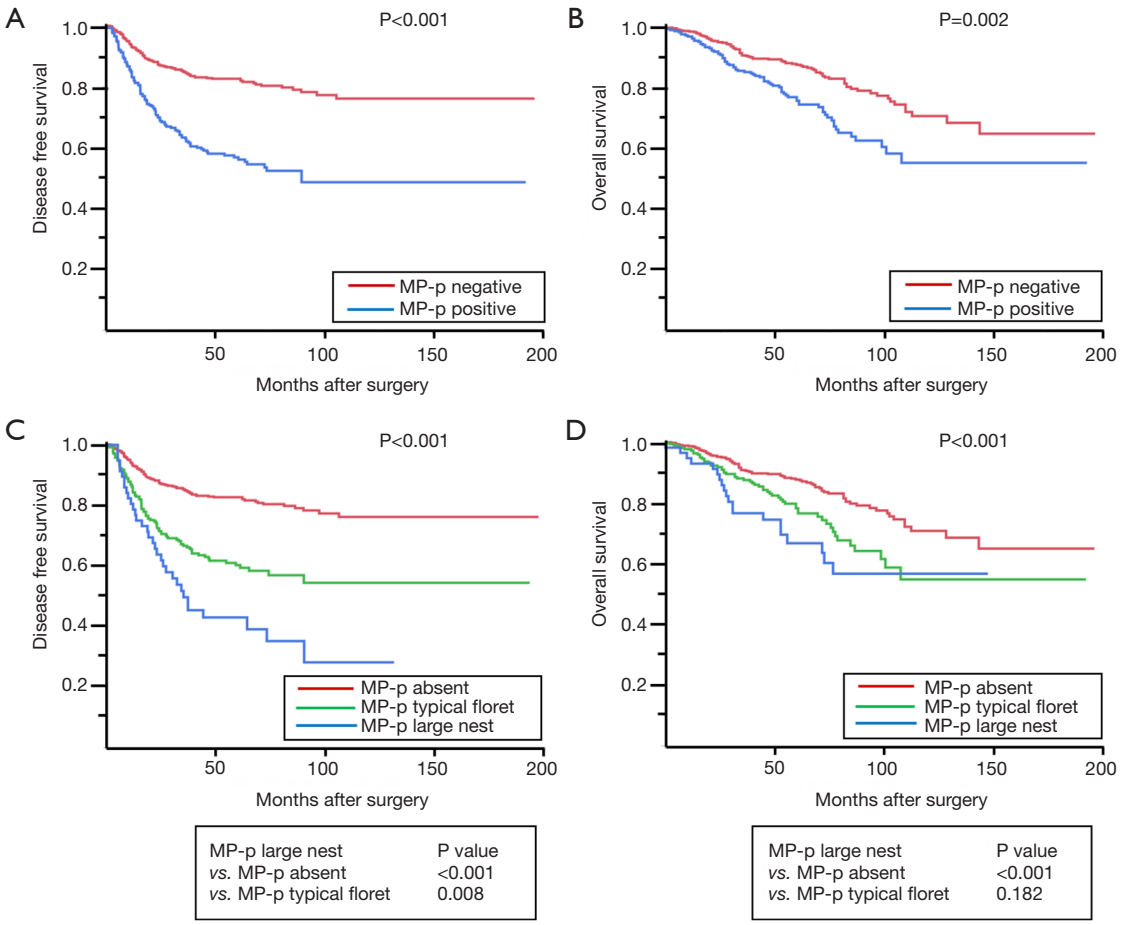

Figure 6 Survival of patients with invasive adenocarcinoma excluding invasive mucinous adenocarcinoma and other variants according to the presence of MP-p (n=886). (A,B) DFS and OS curves illustrating poorer prognosis for patients with MP-p positive than for those with MP-p negative tumors (P<0.001 and P=0.002, respectively); (C,D) DFS and OS curves for each MP-p type. MP-p, micropapillary pattern.

be because of flaws in the MP-p definition, which prompted the current study.

In routine clinical practice, single cells and small clusters (composed of 2–3 tumor cells) floating within tumor glands (*Figure 2A,B*), resembling a MP-p, are often observed and confuse pathologists. This pattern does not represent a typical MP-p, as it is not mentioned in the WHO classification. In Thunnissen's report, we found some interesting images showing tiny tumor cell clusters mixed with the other growth patterns (Figure 3g and 3j in ref. 12) (12). Warth *et al.* also reported that this pattern was particularly challenging for distinguishing between papillary structures and MP growth (Figure 2c in ref. 11) (11). However, this pattern type has never been studied to determine its clinical significance. In the current study, we explored the prognostic significance and found DFS and OS curves between tumors with single cells and small clusters and without MP-p were not separated (data not shown). This may be because the feature is an artifact showing tangential cells or small peel-off clusters of lepidic or papillary patterns. Thus, single cells and small clusters should not be considered as a part of MP-p.

In contrast, large-sized clusters comprising over 20 tumor cells within the tumor nest or air space are often observed in poorly differentiated L-ADC; however, the clinical significance of this pattern has not been determined. These cases may represent a spectrum of MP-p because they are often admixed in the same tumor and appear to arise from tumor cells that have detached from the wall of tumor glands or papillae. In this study, we classified this pattern as large nest MP-p and evaluated its clinical significance. Tumors with large nest MP-p were rare (6%)

Table 4 Univariate and multivariate analyses of the clinicopathological factors

Variables	Disease-free survival						Overall survival					
	Uni-variative			Multi-variative			Uni-variative			Multi-variative		
	HR	95% CI	P	HR	95% CI	P	HR	95% CI	P	HR	95% CI	P
Age (>66 *vs.* ≤65 years)	1.360	1.036–1.798	0.026	1.496	1.131–1.994	0.005	1.630	1.182–2.278	0.003	1.835	1.325–2.622	0.0003
Smoking (smoker *vs.* never)	1.322	1.015–1.728	0.038	1.328	1.015–1.744	0.038	1.688	1.234–2.329	0.001	1.701	1.238–2.357	0.0001
Stage			<0.0001			<0.0001			<0.0001			<0.0001
1	1			1			1			1		
2	4.054	2.914–5.641		2.663	1.870–3.794		3.670	2.517–5.351		2.761	1.838–4.146	
3	7.923	5.819–10.786		4.235	2.928–6.125		6.029	4.194–8.667		4.195	2.714–6.482	
Pleural invasion (present *vs.* absent)	2.929	2.240–3.815	<0.0001	1.342	0.996–1.808	0.052	2.797	2.053–3.796	<0.0001	1.358	0.963–1.914	0.0807
Lymphatic invasion (present *vs.* absent)	3.735	2.746–5.007	<0.0001	1.371	0.975–1.928	0.069	3.286	2.296–4.612	<0.0001	1.588	1.063–2.372	0.0237
Vascular invasion (present *vs.* absent)	3.135	2.393–4.088	<0.0001	1.678	1.244–2.261	0.0007	2.548	1.855–3.473	<0.0001	1.318	0.927–1.874	0.1229
STAS (present *vs.* absent)	2.157	1.658–2.816	<0.0001	1.129	0.838–1.521	0.422	1.848	1.362–2.518	<0.0001	1.165	0.817–1.662	0.3981
MP-p (absent)	1		<0.0001	1		<0.0001	1		0.0002	1		0.6297
Typical floret MP-p	2.252	1.900–3.357		1.762	1.287–2.413		1.717	1.223–2.391		1.074	0.729–1.581	
Large nest MP-p	4.254	2.828–6.235		2.450	1.587–3.784		2.436	1.459–3.869		1.297	0.763–2.206	

STAS, spread through air spaces; MP-p, micropapillary pattern; HR, hazard ratio; CI; confidence interval.

but strongly influenced the risk of recurrence and death. Further, patients with large nest MP-p tumors had a worse prognosis than those with typical floret MP-p tumor. These findings indicate that large nest MP-p should be categorized as MP-p and separately from typical floret MP-p.

STAS has recently been recognized as an invasive pattern of lung cancer. It is a prognostic factor in patients who have undergone limited resection (22,23). In 2015, in the WHO classification of lung tumor fascicles, STAS was defined as "micropapillary clusters, solid nests, or single cells extending beyond the edge of the tumor into air spaces" (1). The classification is conceptually similar to our extended MP-p concepts, as it also focuses on floating tumor cell clusters of variable size, although STAS is proposed to occur outside the tumor mass. In our study, STAS was most frequently observed in tumors with large nest MP-p, followed by those with typical floret MP-p, and was rarely observed in tumors without MP-p. Thus, larger clusters presumably spread beyond the edge of the tumor mass. Interestingly, both STAS and MP-p were risk factors for recurrence according to univariate analysis; however, STAS was eliminated as an independent risk factor in multivariate analysis. This indicates that although they are closely correlated, MP-p is a stronger risk factor for recurrence than STAS and may have prognostic value. To clarify this hypothesis, additional studies examining the association between the size of floating cell clusters inside (MP-p) and those outside (STAS) of the mass are needed.

The filigree pattern is a newly proposed addition to the morphological spectrum of micropapillary ADCs with poor prognosis (24). This pattern is defined as tumor cells growing in delicate lace-like narrow stacks of cells (at least three stacked nuclei) without fibrovascular cores, with visible attachments to alveolar walls. No significant differences in prognosis were observed between the filigree (reclassified from papillary, acinar, and solid predominant adenocarcinoma) and classical type MP-predominant groups. In the current study, we did not evaluate this distinct subtype of MP-p. Because the prognostic significance of the filigree pattern in adenocarcinoma has not been extensively studied, additional studies are needed to validate these definitions along with large nest MP-p.

There were some limitations to this study. First, it may be challenging to accurately distinguish large nest MP-p from typical floret MP-p; however, there were a few disagreements among the observers regarding the identification and assessment of MP-p. This may be because we had a "consensus" track with 100 cases; future studies are required to validate these findings. Second, some researchers recently reported that STAS, as well as detached tumor cells inside the tumor area, were identified as a sampling artifact caused by gross pathology preparation (25). Although we did not examine this point in detail, tumors with discohesive or easy-detaching character should be recorded even if they are generated from artifacts. Further multiple institute analyses are required. Third, when samples are not sufficiently fixed in formalin solution, tumor cells may appear to be floating in the stroma (a so-called fixation artifact). In this study, we did not consider that such an artifact constituted a feature of MP-p. Moreover, previous studies of MP adenocarcinoma of the lung have not evaluated this possibility, which should be further examined. Fourth, in mucinous type tumors, particularly invasive mucinous adenocarcinoma, floating tumor nests are often observed in the extracellular mucin. In this study, we did not consider such floating nests as a feature of MP-p because the frequency of invasive mucinous adenocarcinoma was low (3.8% in the current study); however, further examination of the significance of floating nests in the mucinous tumor are needed.

Conclusions

We identified only 7 more micropapillary ADC cases when we reclassified ADCs in addition to large nest MP-p; however, a lack of significant prognostic differences between classical and reclassified micropapillary ADC was observed. These findings support the expansion of morphological criteria for micropapillary ADC to include large nest MP-p. This expansion may help to achieve a good concordance in the recognition of the MP-p. Further, the present study demonstrated that MP-p is an independent factor for predicting the recurrence and poor prognosis that may follow resection of L-ADC. We also showed that tumors with large nest MP-p were related to the highest recurrence rate compared to tumors without MP-p and those with typical floret MP-p. We consider that MP-p should be separately recorded according to their size regardless of the tumor subtype.

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tlcr-19-731). The authors have no conflicts of interest to declare.

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 study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by the Ethics Committee of Kyoto University Hospital (approval number: R1158-1). This article is a retrospective analysis, so informed consent is waived.

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