Abstract

Nuclear grading system for epithelioid malignant pleural mesothelioma (MPM) has been proposed but it remains uncertain if they could be applied in a biopsy-heavy setting. Using the proposed system, we conducted an independent, external validation study using 563 consecutive cases of epithelioid MPM diagnosed at our institution between 2003 and 2017, of which 87% of patients underwent biopsies only. The median number of sites sampled was 1, with median maximum tissue dimension of 17mm (Biopsy) and 150mm (Resection). The median overall survival (OS) was 14.7 months. The frequencies of Grade I, II and III tumors were 31% (132/563), 52% (292/563) and 17% (94/563). Grade I tumors were associated with the most favorable median OS (24.7 months) followed by grade II (12.7 months) and III (7.2 months). 2-tier nuclear grade separated tumors into low grade (19.3 months) and high grade (8.9 months). In multivariate analysis, 3-tier nuclear grade, 2-tier nuclear grade and mitosis-necrosis score predicted OS independent of age, procedural type, solid-predominant growth pattern, necrosis and atypical mitosis (all $p<0.001$ except 2-tier nuclear grade, $p=0.001$). In the scenario of a single site biopsy with tissue dimension less than or equal to 10mm, none but age ($p=0.002$) were independently predictive. Our data also suggested sampling 3 sites or a maximum tissue dimension of at least 20mm from a single site is optimal for nuclear grade assessment. In conclusion our study confirmed the utility of nuclear grade in epithelioid MPM using a biopsy-heavy cohort provided the tissue sample met minimum dimensional criteria.

Keywords: Mesothelioma, nuclear grade, biopsy

Utility of Nuclear Grading System in Epithelioid Malignant Pleural Mesothelioma in Biopsy-heavy Setting: an External Validation Study of 563 Cases

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Introduction

Malignant pleural mesothelioma (MPM) is a rare pleural malignancy, by far the most common type of mesothelioma affecting approximately 30000 to 40000 patients globally [1] [2] with the majority (74-80%) attributable to asbestos exposure [3]. The prognosis of MPM is poor, with reported overall survival (OS) between 9-12 months in the western world [4] [5]. Even for the minority of patients eligible for surgical resection, OS varies between 10-35 months depending on pathologic stage [6] [7] [8] [9] [10]. Histologic subtype is an established and robust predictor of survival to date, and is used in both routine care and patient stratification in research studies. However literature evidence is mounting with regard to epithelioid MPM representing a spectrum of tumors with variable disease behavior rather than being a homogeneous group. The median OS for pleomorphic epithelioid MPM for instance, was reported to be around 8 months [11] [12] whilst epithelioid MPM with microcystic-predominant growth pattern in a myxoid stroma was associated with median OS of 24 months [12]. There are promising therapeutic options being explored for MPM [13] [14] [15] [16] [17] [18] [19] [20] [21]. Among the ongoing trials, epithelioid MPM represents the majority of enrolled patients.

The search for robust predictors of survival, which allow better stratification of patients and comparison between results from different studies, is limited by several factors. First, there is considerable degree of practice variation in the radiologic staging for MPM [22] [23]. Second, pathologic staging suffers from heavy selection bias as the majority of MPM patients are ineligible for surgery with curative intent. Third, unlike lung cancer, comprehensive multi-omics studies in mesothelioma have only been reported in the last few years [24] [25] [26] [27]. Therefore histopathologic predictors, ideally validated in biopsy setting, remain attractive in pre-treatment evaluation.

A 3-tier nuclear grading system was developed in 2012 using 232 cases of epithelioid MPM (Biopsy: 5%), based on the extent of nuclear atypia and mitotic activity [28]. It was subsequently validated using an international, multi-institutional cohort of 776 cases (Biopsy: 27%) [29]. It demonstrated superior degree of separation between the best- and worst-prognostic groups compared with established predictors such as age and sex in multivariate models without staging. A recent study including 87

epithelioid MPM also validated the nuclear grading system using presumably a high proportion of biopsies [30]. Rosen *et al*. also proposed two alternatives to the 3-tier nuclear grade: a simplified 2-tier nuclear grade based on 3-tier nuclear grade and necrosis, and mitosis-necrosis (M-N) score based on mitotic activity and the presence of necrosis.

The aims of our study are (1) to externally validate the 3-tier nuclear grading system, M-N scoring system and 2-tier nuclear grading system using a large, biopsy-heavy cohort, (2) to evaluate the discriminative power of nuclear grade with regard to grade III versus pleomorphic MPM, and (3) evaluate importance of single versus multiple sites and biopsy size in the value of grading.

Materials and Methods

Study Population

We identified 632 consecutive cases of histologically confirmed epithelioid MPM at our institution between 2003 and 2017 (Royal Brompton And Harefield NHS Foundation Trust, London, United Kingdom) where approximately 70 new cases of MPM are diagnosed and/or resected annually. An institutional mesothelioma database was retrospectively curated and prospectively maintained in conjunction with the National Centre of Mesothelioma Research (NCMR; National Heart & Lung Institute, Imperial College London) (M.F.M., W.O.C.C.). Institutional review board (IRB) approval was obtained for this study. Clinical and histopathologic information were collected from the database, with additional outcome data retrieved from the electronic patient record, National Health Service Spine repository, surgical records and regional thoracic oncology service. We excluded 51 cases classified as pleomorphic epithelioid MPM from the main study but they served as a comparator. A further 18 cases were excluded because they were unavailable for histologic assessment (12), post-mortem diagnoses (2), containing less than 100 viable tumor cells for assessment (2), diagnosis made solely on pleural effusion cell block and a definitive diagnosis was achieved only after extensive multi-disciplinary team discussion (1). Our final study cohort is therefore compiled of 563 cases.

Clinical variables included in this study were age, sex, laterality of disease, date and type of procedure, number of sites sampled, and date of death or last follow-up. Survival time was defined as time (measured in months) between the date of initial procedure from which a definitive diagnosis of MPM was made and the date of death or last follow-up. Information in relation to asbestos exposure, TNM staging, metabolic uptake and chemotherapy/radiotherapy in either neoadjuvant or adjuvant setting were not included in the study as these were incompletely recorded in the electronic patient record. Censor fraction in our study was 23% (131/563).

Microscopy and Imaging

Microscopic assessment was performed using a Nikon Eclipse Ci-L microscope (Nikon Corporation, Japan) with a field area measuring 0.24mm^2 per high power field (HPF, ×400 magnification).

Microscopic images in 300dpi TIF format were taken from representative cases using Nikon Digital Sight DS-L3 camera (Nikon Corporation, Japan), annotated using GNU Image Manipulation Program 2.10.10 (http://www.gimp.org, retrieved on 20.04.2019).

Histopathological Assessment

All cases were diagnosed by at least one specialized pulmonary pathologist (C.B., A.R., J.L.R., A.G.N.) using the current histologic and immunohistochemical criteria [31] [32]. Maximum tissue dimension as a routinely recorded item was taken from the pathology gross descriptions. Histopathologic parameters were assessed and recorded independently, blinded to outcome, by one pathologist. Problematic cases (1.6%) were joint reviewed with a second pathologist. All available hematoxylin & eosin (H&E) sections were reviewed, with an average of 3.9 sections per case (range: 1-47). Quality control parameters were recorded for the presence of crush/cauterization artefacts representing more than 50% of tissue area, and less than 300 (but more than 100) viable tumor cells present. The criteria for nuclear atypia and mitotic activity were taken from earlier studies [26] [27] (Supplementary Table 1, Supplementary Digital Content). Mitosis scores were subsequently assigned: 1 (0-1 per 10 HPF), 2 (2-4 per 10 HPF), 3 (≥5 per 10 HPF). 3-tier nuclear grade was computed by adding nuclear atypia score (1-3) to mitosis score (1-3): Grade I (2-3), grade II (4-5), and grade III (6). Representative images illustrating the relevant nuclear features are shown (Figure 1).

Growth patterns were evaluated using the current diagnostic criteria [32] and a solid pattern was defined as sheets and/or nests of cohesive tumor cells without otherwise discrete architectural patterns. A predominant pattern was defined as the most abundant, frequently but not necessarily representing >50% of the entire tumor. Necrosis was assessed under ×400 magnification and recorded as present or absent. M-N score (0, I, II) was computed by adding the scores based on the presence (1) or absence (0) of necrosis, and a mitotic count of ≥5 (1) or less than 5 (0). 2-tier nuclear grade was computed using necrosis to sub-classify grade I and II tumors: low grade included all grade I and grade II tumors without necrosis, and high grade includes grade II with necrosis and all grade III tumors.

Statistical Analysis

Descriptive statistics were employed to analyze the baseline demographic and clinicopathologic parameters. Fisher exact test was used to evaluate associations between categorical variables. Wilcoxon rank sum test was used for continuous variables. Kruskal-Wallis test was used to evaluate differences on continuous variables by categorical variables. OS was estimated using the Kaplan-Meier method. Exact p values were recorded and $p<0.05$ denotes statistical significance. Multivariate Mantel-Cox regression model was used to evaluate the effect size and statistical significance of each variable which demonstrated $p<0.05$ in univariate analysis. Proportional hazard assumption was confirmed using log-log and residual plots. All statistical analyses were performed using SPSS 24 (IBM Corp., Armonk, NY, USA). Kaplan-Meier curves were generated using GraphPad Prism Version 8 (GraphPad Software, La Jolla California, USA).

Results

Demographic and Clinicopathologic Characteristics

We included 563 patients in our study. The median age was 69.1 years (range 32-91), with 75.1% (423/563) of patients being male. 87.0% (490/563) of patients underwent biopsy only. 11.6% (65/563) of patients underwent limited (PD) or extended pleurectomy and decortication (EPD), 0.9% (5/563) underwent extrapleural pneumonectomy (EPP), and 0.5% (3/563) underwent other procedures. Extensive crush/cauterization artefacts occupying more than 50% of tissue area were present in 3.7% of cases (21/563), and in 7.5% (42/563) there were less than 300 but more than 100 viable tumor cells available for evaluation.

37.8% (213/563) of patients had solid-predominant disease and necrosis was present in 33.7% (190/563). The frequencies of nuclear grade I, II, III tumors were 31.4% (177/563), 51.9% (292/563), and 16.7% (94/563) respectively (Figure 2A). 10.2% (18/177) of grade I and 26.4% (77/292) of grade II tumors were associated with necrosis (Figure 2C). For M-N score, 51.5% (290/563) were assigned score of 0, 27.5% (155/563) score of I, and 17.0% (118/563) score of II (Figure 2B). Using the 2-tier grading system, 65.0% (366/563) of tumors were low grade and 35.0% (197/563) were high grade (Figure 2D). Pleomorphic epithelioid MPM represented 8.3% (51/614) of the epithelioid MPM cohort.

The median OS of our cohort was 14.7 months (95% confidence interval (CI) 12.9-16.4 months). Univariate analysis demonstrated age, type of procedure, solid-predominant growth pattern, and necrosis as predictors of OS (all $p<0.001$) (Table 1) (Supplementary Figure 1, Supplementary Digital Content). Lymphatic and vascular invasion were present in 8.3% (47/563) and 7.5% (42/563) of cases, and were not shown to be statistically significant predictors of survival. Higher incidences of lymphovascular invasion were seen in resected cases (Supplementary Table 2, Supplementary Digital Content).

All Three Grading Systems Predicted Survival in Univariate and Multivariate Analyses

Univariate analysis of nuclear features demonstrated nuclear atypia, mitotic count, and atypical mitosis as predictors of OS (all $p<0.001$) (Table 2) (Supplementary Figure 2, Supplementary Digital Content). Nuclear grade I tumors were associated with the most favorable median OS of 24.7 months, followed by grade II (12.7 months) and III (7.2 months) ($p<0.001$) (Figure 2A). Pleomorphic epithelioid MPM had the worst OS (5.4 months) but not statistically significant compared with grade III tumors ($p=0.208$). They also showed similar mitotic activity ($p=0.547$) (Supplementary Figure 3, Supplementary Digital Content). Adopting the M-N scoring system, a score of 0 was associated with the most favorable median OS (19.8 months) followed by score I (12.0 months) and II (8.4 months) ($p<0.001$) (Figure 2B). Overall our findings are congruent with previous studies.

The presence of necrosis modified the prognosis of nuclear grade I (15.1 months versus 26.1 months, $p=0.053$) and II (11.1 months versus 14.3 months, $p=0.058$) tumors with borderline statistical significance (Figure 2C). 2-tier nuclear grade resulted in satisfactory separation in terms of survival between low grade (19.3 months), high grade (8.9 months) and pleomorphic (5.4 months) MPM ($p<0.001$) (Figure 2D).

In multivariate analysis, 3-tier nuclear grade, M-N score and 2-tier nuclear grade predicted OS independent of age, type of procedure, necrosis, solid-predominant growth pattern and atypical mitosis (all $p<0.001$ except 2-tier nuclear grade, $p=0.001$). The constituents of nuclear grade, nuclear atypia and mitotic count, were also independent predictors of OS (all $p<0.001$). On the other hand, solid-predominant growth pattern ($p=0.725$) and atypical mitosis ($p=0.640$) did not predict OS independent of age, type of procedure, necrosis, 3-tier nuclear grade and atypical mitosis (for solid-predominant growth pattern, atypical mitosis was used as the covariate) (Table 3). Laterality of disease, although demonstrated borderline statistical significance in univariate analysis ($p=0.053$), was not shown to be an independent predictor in multivariate setting ($p=0.977$). 3-tier nuclear grade created a greater degree of prognostic separation among the three grading systems.

Association between Nuclear Grade and Clinicopathologic Variables

We then evaluated the association between nuclear grade and other clinicopathologic variables. Higher nuclear grades were associated with solid-predominant growth pattern, necrosis, atypical mitosis, lymphatic and vascular invasion (all $p<0.001$) (Table 4). No association was seen with patient age ($p=0.600$), sex ($p=0.092$), or laterality of disease ($p=0.101$). Interestingly higher nuclear grades were more frequently encountered in larger resection specimens ($p=0.003$) albeit the absolute number of cases was small. Furthermore we confirmed the strong association between nuclear grade and M-N score ($p<0.001$) (Supplementary Table 3, Supplementary Digital Content).

Impact of Small Biopsies on the Performance of Grading Systems

In our cohort, the median number of sites sampled was 1 (range 1-20), with median maximum tissue dimensions of 17mm for biopsies (range 2-140mm) and 150mm for resections (range 40-350mm) (Figure 3A). 19.4% of all biopsies (95/490) were taken from a single site with a maximum dimension of less than or equal to 10mm (median: 8mm), of which 10.5% (10/95) were estimated to contain between 100 and 300 viable tumor cells.

All three grading systems independently predicted survival in single site biopsy setting (all $p<0.05$), but with reduced degree of separation (Table 5). In single site and ≤10mm scenario, 3-tier nuclear grade and M-N score did not discriminative survival differences between nuclear grade II versus III ($p=0.468$) (Supplementary Figure 4A, Supplementary Digital Content), and score I versus II respectively ($p=0.175$) (Supplementary Figure 4B, Supplementary Digital Content). Although predictive in univariate analysis ($p<0.001$) (Figure 3B), 2-tier nuclear grade was not shown to be an independent predictor of OS in multivariate setting ($p=0.572$) (Table 6). Age was the only statistically significant predictor in such circumstance ($p=0.002$). We consider this as the minimum tissue requirement for nuclear grade assessment.

Finally, we investigated the relationship between the sampling parameters and the performance of the 2-tier nuclear grade. We incorporated the pleomorphic MPM cohort in this part of analysis. A grade-shift phenomenon was observed, where more high grade diseases were detected as maximum dimension increased ($p=0.017$) (Figure 3C) or more anatomical sites being sampled ($p<0.001$) (Figure 3D). The

relatively small number of cases with 3 or more sampled sites or ≥30mm in maximum dimension in our cohort did not permit more extensive analysis. Using hazard ratio and *p* values derived from multivariate analysis as surrogates, the incremental improvement in the performance plateaued out when the number of sampled sites and maximum tissue dimension reached 3 or 20mm respectively (Supplementary Figure 5, Supplementary Digital Content). We proposed this as the optimal sampling standard based on available evidence.

Discussion

Using a large, biopsy-heavy (87%) cohort we validated the utility of epithelioid MPM grading systems proposed by colleagues [28] [29]. The demographic and clinicopathologic characteristics of our study cohort were similar to theirs, in terms of patient sex, proportion of tumors with solid-predominant growth pattern and necrosis (Supplementary Table 4, Supplementary Digital Content). The mean age of our patient population (69.1 years), although slighter older in comparison, is expected of the UK mesothelioma population (74.8 years) according to the latest UK national cancer audit [5]. The incidences of lymphatic and vascular invasion were lower than earlier report (44% and 23% [11]) and we hypothesize this was due to the limited nature of biopsy materials.

3-tier nuclear grade in our hand generated a similar degree of separation in median OS compared with earlier studies (Supplementary Table 4, Supplementary Digital Content), whilst 2-tier nuclear grade was also shown to be independently predictive of OS. The question therefore arises: which grading system should be adopted in clinical practice and/or patient stratification in future basic and clinical research? We believe each have their strengths and weaknesses. The 3-tier nuclear grade demonstrates superior discriminative power. However inter-observer agreement between grade III tumor and pleomorphic epithelioid MPM could be of concern, with the latter reported to represent 8-15% of the epithelioid subtype [11] [12]. The M-N score eliminates the potential caveat of inter-observer disagreement regarding nuclear atypia; the 2-tier nuclear grade is simpler to implement by segregating patients into two groups instead of three, and allows better separation from pleomorphic epithelioid MPM. Our data add to the evidence base for guideline development in this regard.

There is currently no guidance on the optimal number and size of biopsies available from major guideline committees and international expert consortia, although this is currently a topic of high priority relevant for both pathologists and operators. It is not uncommon based on our findings for the pathology department to receive materials taken from a single anatomical site that measured less than ≤10mm. In this setting we found all grading systems lost predictive value in multivariate analysis. We believe this is not a high hurdle to reach in routine diagnostic practice therefore it should not be

interpreted as the optimal standard but an absolute minimum requirement. In our cohort the performance of the 2-tier nuclear grade started to plateau out with 3 sampled sites or a maximum tissue dimension of 20mm. Below such cut-off values, there was a clear benefit in obtaining more tissue in terms of detecting high grade disease. Such notion was supported by the results from a previous study of 305 cases of biopsy-EPP where more non-epithelioid disease was detected in the resection specimen [33]. However our findings have to be interpreted with caution as there was likely operator bias i.e. the biopsies were not taken in blind fashion. Specimen size is a parameter that could be easily measured or estimated during sampling procedures. We advocate future audits and prospective cohort studies across centers to compare best practice versus a more extensive sampling protocol. These will be crucial to address the correlation between diagnostic adequacy, including but not limited to grading, and the number and size of diagnostic specimens.

Our study has two major weaknesses. Firstly, our study is retrospective in nature, with incomplete data on staging and treatment including chemotherapy. Secondly, we were unable to compare the discriminative power of the grading systems with TNM staging due to incomplete data. We can only infer from results reported by Kadota *et al*. [28] where the difference in OS between Stage I and IV tumors was 7 months hence TNM staging is a weaker prognostic variable than any of the grading systems. In the future we wish to explore the association of nuclear grade with growth patterns [11], cytological variants [34] [35] [36], stromal features [37] [38] and genomic signatures via deep sequencing using a mesothelioma-specific gene panel.

In conclusion we have validated the 3-tier nuclear grading system for epithelioid MPM and the derived 2-tier grading system based on nuclear features and necrosis using a large, biopsy-heavy cohort. In addition we propose a minimum sampling standard for accurate evaluation of nuclear grade.

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Figure Legends

Figure 1 Nuclear features of epithelioid malignant pleural mesothelioma (H&E stain; original magnification, ×400). **(A)** Nuclear grade I tumor with trabecular growth pattern. **(B)** Tumor cells show features of mild nuclear atypia: small, uniform nuclei with fine chromatin pattern, and no nucleoli are seen. **(C)** Nuclear grade II tumor with predominantly tubulo-papillary growth pattern. **(D)** Tumor cells show features of moderate nuclear atypia: enlarged and moderately pleomorphic nuclei with small but conspicuous nucleoli. **(E)** Nuclear grade III tumor with predominantly solid growth pattern. **(F)** Tumor cells show features of severe nuclear atypia: large and pleomorphic nuclei with prominent nucleoli and occasionally multiple nucleoli. Tumor giant cells are seen. A minor transitional component is also present. Scale bar = 100μm.

Figure 2 Overall survival by 3-tier nuclear grade, mitosis-necrosis score, 3-tier nuclear grade and necrosis, and 2-tier nuclear grade. **(A)** Nuclear grade I tumors showed the most favorable survival followed by grade II and III. **(B)** Mitosis-Necrosis score 0 tumors showed the most favorable survival followed by score I and II. **(C)** The presence of necrosis had an adverse prognostic impact on the survival of patients with nuclear grade I and II tumors. **(D)** Low grade tumors showed more favorable survival than high grade tumors.

Figure 3 Utility of nuclear grade in small biopsy setting. **(A)** The distribution of maximum tissue dimension between biopsies and resections (p<0.001, Kruskal-Wallis test). **(B)** 2-tier nuclear grade, **(C)** high grade diseases were detected at higher frequencies with increasing maximum tissue dimension **(D)** high grade diseases were detected at higher frequencies with more sites being sampled.

Legends for Supplementary Digital Content

Supplementary Table 1 Assessment criteria for nuclear atypia

Supplementary Table 2 Detection of lymphatic and vascular invasion by type of procedure

Supplementary Table 3 Association between nuclear grade and mitosis-necrosis score. N, necrosis; (+), present; (-), absent.

Supplementary Table 4 Comparison of demographic and clinicopathologic characteristics with previous studies. RBHT, Royal Brompton And Harefield NHS Foundation Trust; M, male; F, female; PD, pleurectomy and decortication; EPD, extended pleurectomy and decortication; EPP, extrapleural pneumonectomy; H&E, hematoxylin & eosin; OS, overall survival.

Supplementary Figure 1 Overall survival by clinicopathologic variables**.** In univariate analysis, **(A)** age≤65 years, **(B)** surgical resection, **(C)** non solid-predominant growth pattern and **(D)** absence of necrosis were favorable prognostic variables (all p<0.001). CI, confidence interval; OS, overall survival.

Supplementary Figure 2 Overall survival by nuclear features. **(A)** Nuclear atypia score of 1 was associated with the most favorable prognosis followed by score 2 and 3. **(B)** Mitotic count of 0-1 per 10 HPF was associated with the most favorable prognosis followed by 2-4 and ≥5. **(C)** The absence of atypical mitosis was associated with more favorable prognosis (all p<0.001). CI, confidence interval; HPF, high power field; OS, overall survival.

Supplementary Figure 3 Mitotic activity by nuclear grade. No significant difference was found comparing the mitotic activity between nuclear grade III and pleomorphic malignant pleural mesothelioma (p=0.547, Kruskal-Wallis test).

Supplementary Figure 4 3-tier nuclear grade and mitosis-necrosis score in single site, ≤10mm setting. **(A)** 3-tier nuclear grade discriminates survival difference between grade I and II/III, but not between

grade II and III. **(B)** M-N score discriminates survival difference between score 0 and I/II, but not between grade I and II. CI, confidence interval; OS, overall survival.

Supplementary Figure 5 Association between hazard ratio/*p* values (2-tier nuclear grade) and maximum tissue dimension /number of sites sampled. **(A)** Hazard ratio reached plateau with maximum tissue dimension of 20mm. **(B)** Hazard ratio reached plateau with three or more sites being sampled. Error bar denotes 95% confidence interval and the dotted orange line denotes p=0.05.

Supplementary Digital Content.pdf

Table 1 Univariate analysis in predicting overall survival by clinicopathologic factors

Variable	Patients (%)	Median OS (months)	*p*
All Patients	563 (100.0)	14.7	-
Age (Years)			
≤65	193 (34.3)	18.2	*<0.001*
>65	370 (65.7)	12.7	
Sex			
Male	423 (75.1)	14.7	*0.469*
Female	140 (24.9)	14.7	
Laterality			
Left	227 (40.3)	15.6	*0.053*
Right	331 (58.8)	13.8	*(Left vs Right)*
Not Documented	5 (0.9)	-	
Procedure			
Biopsy	490 (87.0)	13.2	
PD or EPD	65 (11.6)	25.3	*<0.001*
EPP	5 (0.9)	28.4	*(Biopsy vs Resection)*
Other Procedures	3 (0.5)	32.9	
Solid-predominant Growth Pattern			
Yes	213 (37.8)	10.5	*<0.001*
No	350 (62.2)	18.0	
Necrosis			
Present	190 (33.7)	9.3	*<0.001*
Absent	373 (66.3)	18.5	
Lymphatic Invasion			
Present	47 (8.3)	16.8	*0.169*
Absent	516 (91.7)	14.6	
Vascular Invasion			
Present	42 (7.5)	15.6	*0.303*
Absent	521 (92.5)	14.6	

EPP, extrapleural pneumonectomy; EPD, extended pleurectomy and decortication; PD, pleurectomy and decortication; OS, overall survival.

Table 2 Univariate analysis in predicting overall survival by nuclear features

Variable	Patients (%)	Median OS (months)	*p*
Nuclear Atypia			
Score 1 (Mild)	71 (12.6)	24.3	*Reference*
Score 2 (Moderate)	378 (67.1)	15.5	*0.002*
Score 3 (Severe)	114 (20.3)	7.8	*<0.001*
			(3 vs 2: <0.001)
Mitotic Count			
Score 1 (0-1)	178 (31.6)	23.7	*Reference*
Score 2 (2-4)	182 (32.3)	12.8	*<0.001*
Score 3 (≥5)	203 (36.1)	10.0	*<0.001*
			(3 vs 2: 0.002)
Atypical Mitosis			
Yes	398 (70.7)	12.0	*<0.001*
No	165 (29.3)	20.3	
Nuclear Grade			
I	177 (31.4)	24.7	*Reference*
II	292 (51.9)	12.7	*<0.001*
III	94 (16.7)	7.2	*<0.001*
			(III vs II: <0.001)
Mitosis-Necrosis Score			
Score 0	290 (51.5)	19.8	*Reference*
Score I	155 (27.5)	12.0	*<0.001*
Score II	118 (21.0)	8.4	*<0.001*
			(II vs I: 0.003)
2-Tier Nuclear Grade			
Low Grade	366 (65.0)	19.3	*Reference*
Grade I	159 (28.2)	26.1	-
Grade I with Necrosis	18 (3.2)	15.1	-
Grade II	189 (33.6)	14.3	-
High Grade	197 (35.0)	8.9	*<0.001*
Grade II with Necrosis	103 (18.3)	11.1	-
Grade III	94 (16.7)	7.2	-

OS, overall survival.

Table 3 Multivariate analysis in predicting overall survival

Variable	Hazard Ratio	95% CI	*p*
Age			
>65 vs ≤65 years	1.46	1.19-1.81	*<0.001*
Procedure			
Resection vs Biopsy only	0.30	0.21-0.43	*<0.001*
Predominant Growth Pattern			
Solid vs Non-solid	1.03	0.83-1.27	*0.799*
Necrosis			
Present vs Absent	1.72	1.35-2.20	*<0.001*
Nuclear Atypia			
Score 2 vs 1	1.43	1.04-1.95	*0.027*
Score 3 vs 1	2.29	1.56-3.38	*<0.001*
Mitotic Count			
Score 2 vs 1	2.19	1.55-3.09	*<0.001*
Score 3 vs 1	3.00	2.07-4.34	*<0.001*
Atypical Mitosis			
Present vs Absent	0.95	0.71-1.27	*0.713*
Nuclear Grade			
II vs I	2.56	1.87-3.49	*<0.001*
III vs I	3.77	2.52-5.63	*<0.001*
Mitosis-Necrosis Score			
I vs 0	1.65	1.22-2.23	*0.001*
II vs 0	2.38	1.49-3.78	*<0.001*
2-Tier Nuclear Grade			
High Grade vs Low Grade	2.02	1.33-3.07	*0.001*

CI, confidence interval.

Table 4 Distribution of clinicopathologic variables by nuclear grade

Variable	All Patients	Grade I	Grade II	Grade III	*p*
All Patients (%)	563 (100.0)	177 (31.4)	292 (51.9)	94 (16.7)	-
Age (years)					
Median	70	71	69.5	70	*0.600*
Range	32-91	41-88	37-90	32-91	
Sex (%)					
Male	423 (75.1)	137 (77.4)	209 (71.6)	77 (81.9)	*0.092*
Female	140 (24.9)	40 (22.6)	83 (28.4)	17 (18.1)	
Laterality (%)					
Left	227 (40.3)	80 (45.7)	117 (40.3)	30 (32.3)	*0.101*
Right	331 (58.8)	95 (54.3)	173 (59.7)	63 (67.7)	
Not Documented	5 (0.9)	-	-	-	-
Procedure (%)					
Biopsy	490 (87.0)	169 (95.5)	242 (82.9)	79 (84.0)	*0.003*
PD or EPD	65 (11.6)	7 (4.0)	43 (14.7)	15 (16.0)	
EPP	5 (0.9)	1 (0.5)	4 (1.4)	0 (0.0)	
Other Procedures	3 (0.5)	0 (0.0)	3 (1.0)	0 (0.0)	
Solid-predominant Growth Pattern (%)					
Yes	213 (37.8)	32 (18.1)	120 (41.1)	61 (64.9)	*<0.001*
No	350 (62.2)	145 (81.9)	172 (58.9)	33 (35.1)	
Necrosis (%)					
Present	189 (33.6)	18 (10.2)	103 (35.3)	69 (73.4)	*<0.001*
Absent	374 (66.4)	159 (89.8)	189 (64.7)	25 (26.6)	
Lymphatic Invasion (%)					
Present	47 (8.3)	1 (0.6)	30 (10.3)	16 (17.0)	*<0.001*
Absent	516 (91.7)	176 (99.4)	262 (89.7)	78 (83.0)	
Vascular Invasion (%)					
Present	42 (7.5)	3 (1.7)	24 (8.2)	15 (16.0)	*<0.001*
Absent	521 (92.5)	174 (98.3)	268 (91.8)	79 (84.0)	
Atypical Mitosis (%)					
Present	398 (70.7)	41 (23.2)	265 (90.8)	92 (97.9)	*<0.001*
Absent	165 (29.3)	136 (76.8)	27 (9.2)	2 (2.1)	

EPP, extrapleural pneumonectomy; EPD, extended pleurectomy and decortication; PD, pleurectomy and decortication

Table 5 Multivariate analysis in predicting overall survival (single site biopsy)

Variable	Hazard Ratio	95% CI	*p*
Age			
>65 vs ≤65 years	1.31	0.97-1.77	*0.074*
Predominant Growth Pattern			
Solid vs Non-solid	1.17	0.88-1.55	*0.290*
Necrosis			
Present vs Absent	2.13	1.51-3.01	*<0.001*
Nuclear Atypia			
Score 2 vs 1	1.55	1.01-2.40	*0.046*
Score 3 vs 1	2.23	1.31-3.81	*0.003*
Mitotic Count			
Score 2 vs 1	2.09	1.31-3.32	*0.002*
Score 3 vs 1	2.80	1.67-4.67	*<0.001*
Atypical Mitosis			
Present vs Absent	0.83	0.57-1.21	*0.333*
Nuclear Grade			
II vs I	2.26	1.52-3.37	*<0.001*
III vs I	3.04	1.77-5.20	*<0.001*
Mitosis-Necrosis Score			
I vs 0	2.04	1.36-3.04	*0.001*
II vs 0	2.08	1.10-3.95	*0.025*
2-Tier Nuclear Grade			
High Grade vs Low Grade	1.87	1.11-3.14	*0.019*

CI, confidence interval.

Table 6 Multivariate analysis in predicting overall survival (single site biopsy, maximum dimension ≤10mm)

Variable	Hazard Ratio	95% CI	*p*
Age			
>65 vs ≤65 years	2.30	1.34-3.94	*0.002*
Predominant Growth Pattern			
Solid vs Non-solid	1.34	0.80-2.25	*0.267*
Necrosis			
Present vs Absent	1.55	0.67-3.55	*0.303*
Nuclear Atypia			
Score 2 vs 1	1.62	0.80-3.27	*0.182*
Score 3 vs 1	2.99	1.23-7.27	*0.015*
Atypical Mitosis			
Present vs Absent	1.31	0.76-2.26	*0.339*
2-Tier Nuclear Grade			
High Grade vs Low Grade	1.27	0.55-2.92	*0.572*

CI, confidence interval.

Figure 1

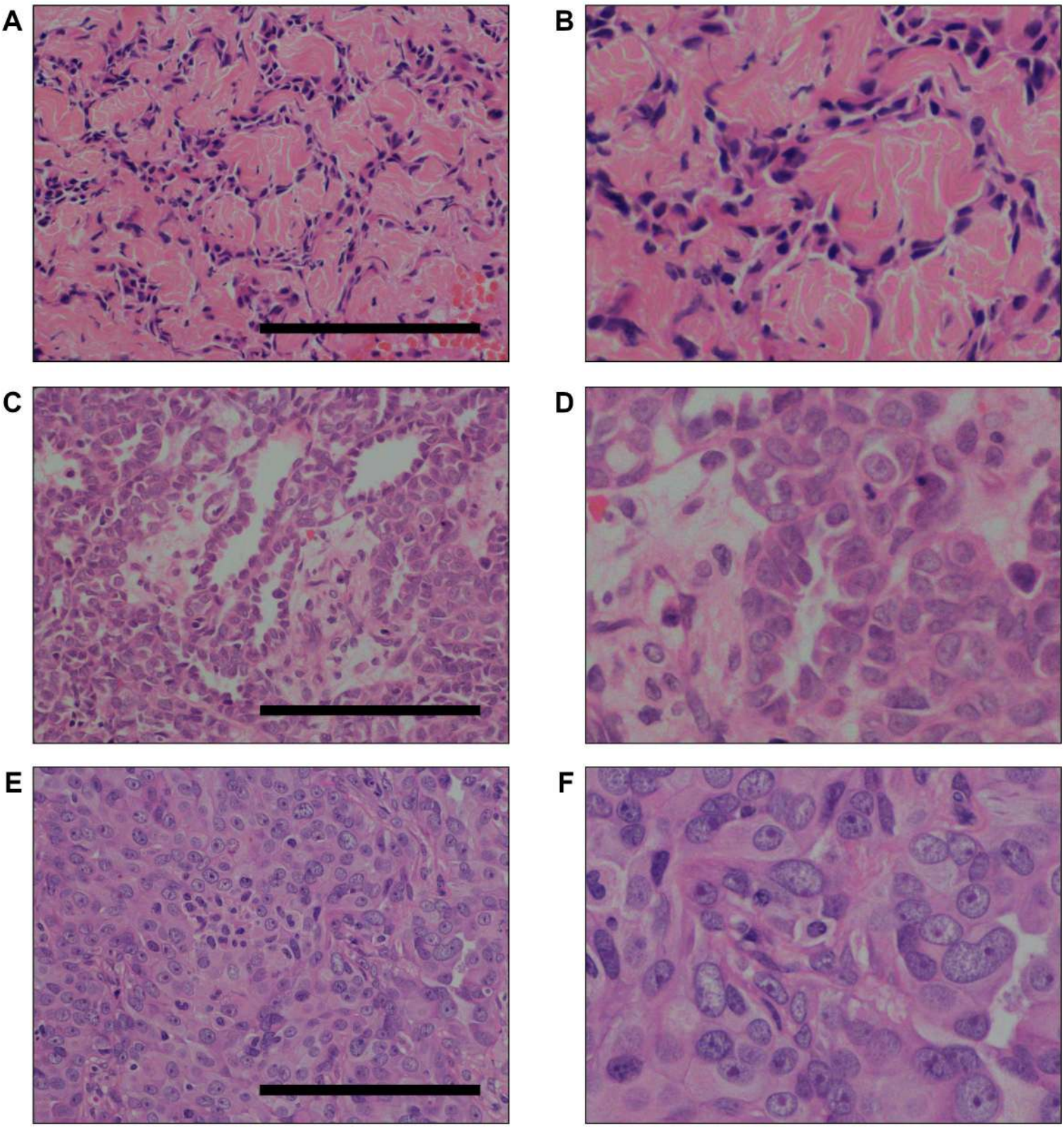


Figure 2

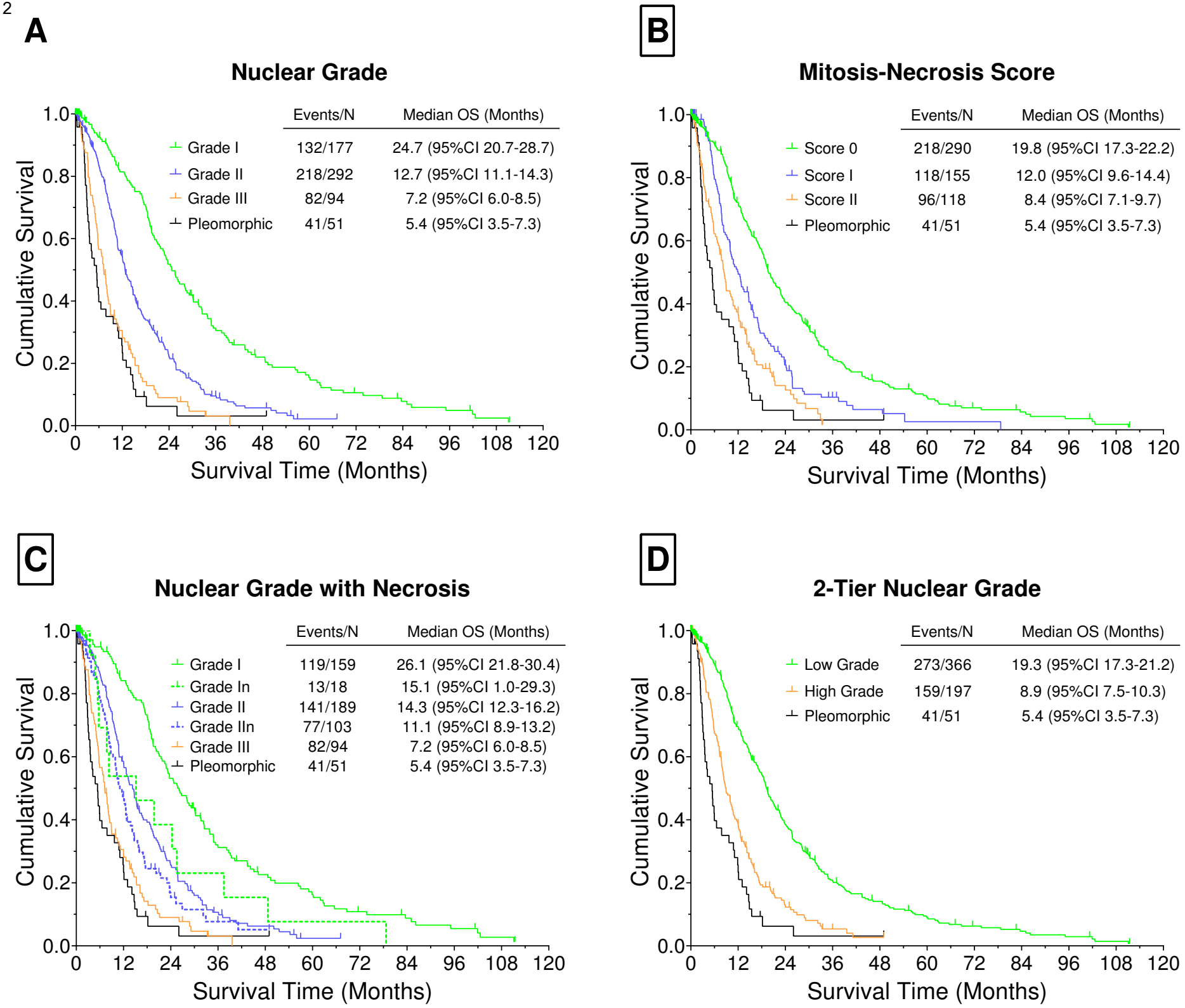
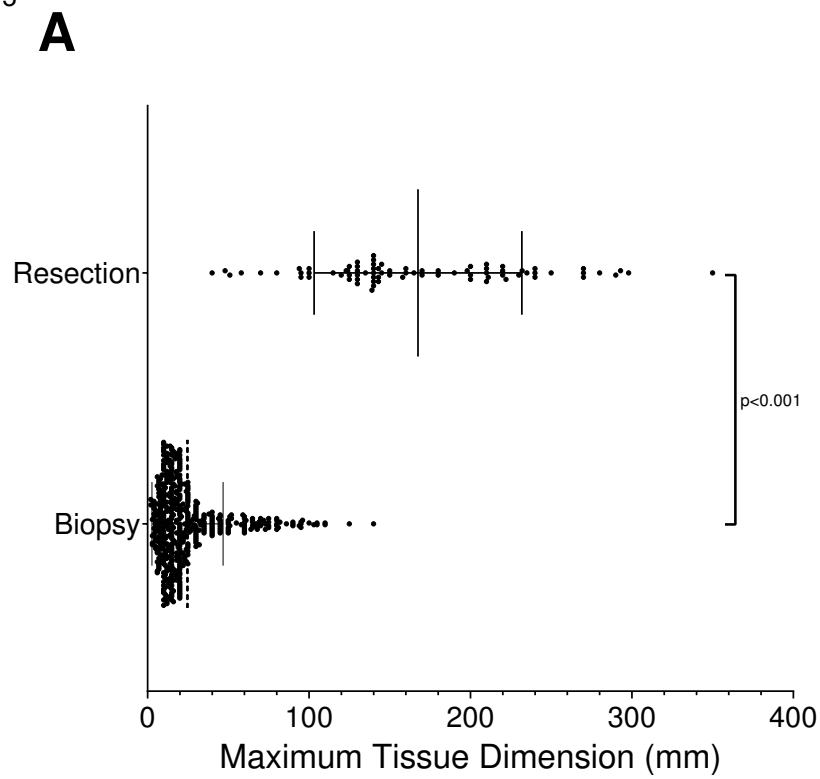
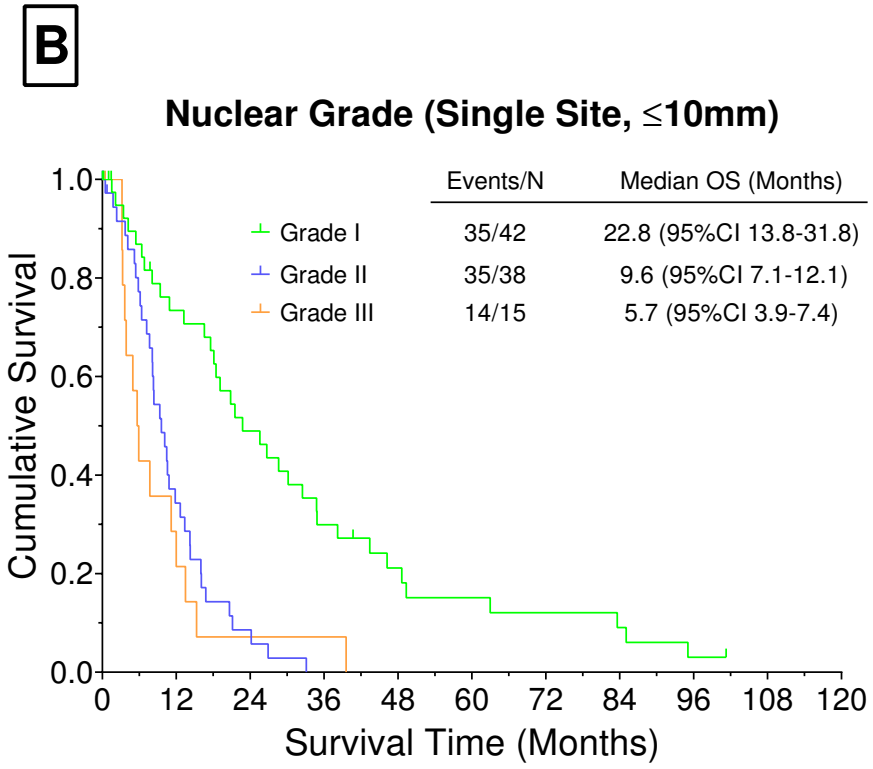
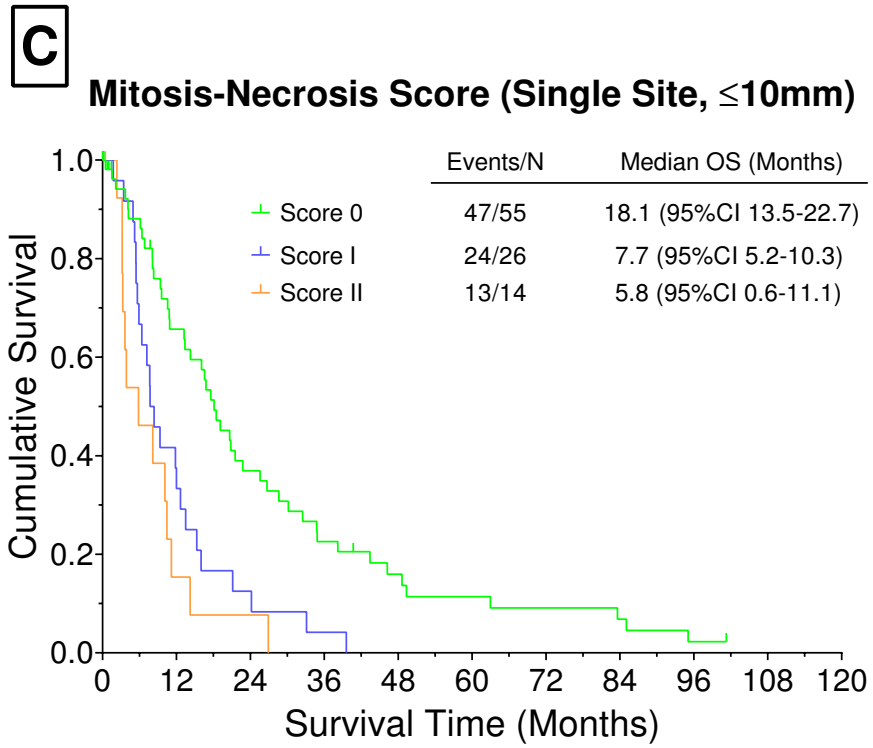
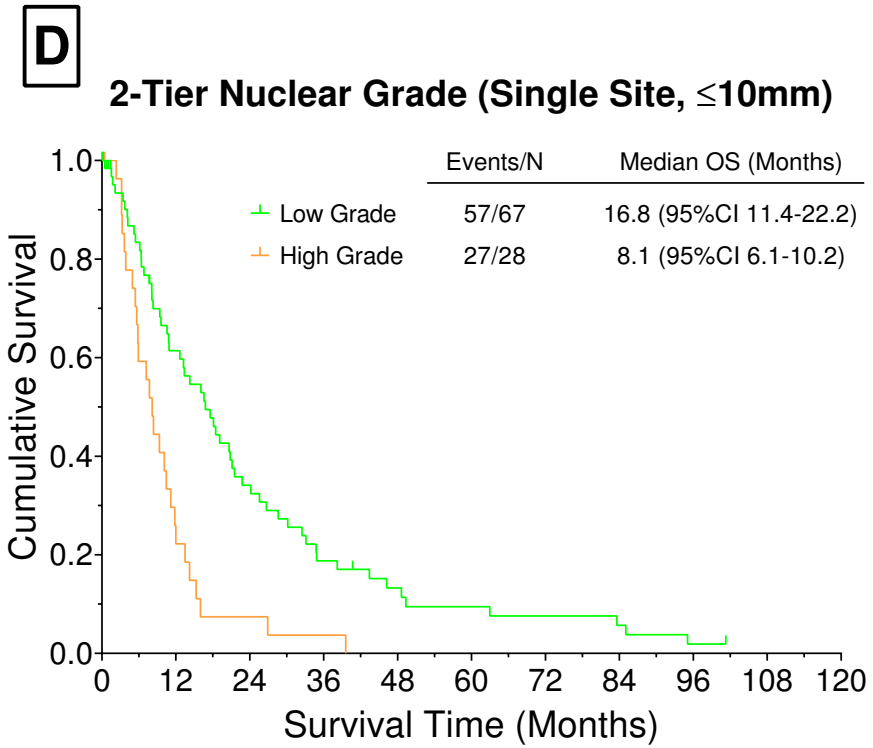

A
Nuclear Grade
Events/N
Median OS (Months)
Grade I 132/177 24.7 (95%CI 20.7-28.7)
Grade II 218/292 12.7 (95%CI 11.1-14.3)
Grade III 82/94 7.2 (95%CI 6.0-8.5)
Pleomorphic 41/51 5.4 (95%CI 3.5-7.3)
Cumulative Survival
Survival Time (Months)
B
Mitosis-Necrosis Score
Events/N
Median OS (Months)
Score 0 218/290 19.8 (95%CI 17.3-22.2)
Score I 118/155 12.0 (95%CI 9.6-14.4)
Score II 96/118 8.4 (95%CI 7.1-9.7)
Pleomorphic 41/51 5.4 (95%CI 3.5-7.3)
Cumulative Survival
Survival Time (Months)
C
Nuclear Grade with Necrosis
Events/N
Median OS (Months)
Grade I 119/159 26.1 (95%CI 21.8-30.4)
Grade In 13/18 15.1 (95%CI 1.0-29.3)
Grade II 141/189 14.3 (95%CI 12.3-16.2)
Grade IIn 77/103 11.1 (95%CI 8.9-13.2)
Grade III 82/94 7.2 (95%CI 6.0-8.5)
Pleomorphic 41/51 5.4 (95%CI 3.5-7.3)
Cumulative Survival
Survival Time (Months)
D
2-Tier Nuclear Grade
Events/N
Median OS (Months)
Low Grade 273/366 19.3 (95%CI 17.3-21.2)
High Grade 159/197 8.9 (95%CI 7.5-10.3)
Pleomorphic 41/51 5.4 (95%CI 3.5-7.3)
Cumulative Survival
Survival Time (Months)

Figure 3


A
Resection
Biopsy
p<0.001
0
100
200
300
400
Maximum Tissue Dimension (mm)


B
Nuclear Grade (Single Site, ≤10mm)
Events/N
Median OS (Months)
Grade I
35/42
22.8 (95%CI 13.8-31.8)
Grade II
35/38
9.6 (95%CI 7.1-12.1)
Grade III
14/15
5.7 (95%CI 3.9-7.4)
Cumulative Survival
Survival Time (Months)


C
Mitosis-Necrosis Score (Single Site, ≤10mm)
Events/N
Median OS (Months)
Score 0
47/55
18.1 (95%CI 13.5-22.7)
Score I
24/26
7.7 (95%CI 5.2-10.3)
Score II
13/14
5.8 (95%CI 0.6-11.1)
Cumulative Survival
Survival Time (Months)


D
2-Tier Nuclear Grade (Single Site, ≤10mm)
Events/N
Median OS (Months)
Low Grade
57/67
16.8 (95%CI 11.4-22.2)
High Grade
27/28
8.1 (95%CI 6.1-10.2)
Cumulative Survival
Survival Time (Months)