Importance of Histopathological Grading for Treatment Selection in Malignant Mesothelioma

İlteriş Türk[1] Gokturk Findik[1] Mehmet Çetin[2] Pinar Bicakcioglu[1]
Selim Şakir Erkmen Gülhan[1] Koray Aydoğdu[1] Ülkü Yılmaz[3] Funda Demirağ[4]

[1] Department of Thoracic Surgery, Ankara Atatürk Sanatoryum Eğitim ve Araştırma Hastanesi Ringgold Standard İnstitution, Ankara, Turkey
[2] Department of Thoracic Surgery, Niğde Ömer Halisdemir University Ringgold Standard İnstitution, Niğde Ömer Halisdemir University, Niğde, Turkey
[3] Department of Chest Diseases and Tuberculosis, Ankara Atatürk Sanatoryum Eğitim ve Araştırma Hastanesi Ringgold Standard İnstitution, Ankara, Turkey
[4] Department of Pathology, Ankara Atatürk Sanatoryum Eğitim ve Araştırma Hastanesi Ringgold Standard İnstitution, Ankara, Turkey

Address for correspondence İlteriş Türk, MD, Department of Thoracic Surgery, Ankara Atatürk Sanatoryum Eğitim ve Araştırma Hastanesi Ringgold Standard İnstitution, PınarbaşıMahSanatoryum Cad, Ankara 06290, Turkey (e-mail: turkilteris@gmail.com).

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Abstract

Background Complexities in TNM staging in epithelioid malignant pleural mesothelioma (MPM) may lead to errors in treatment selection, leading to major surgical interventions in patients with low survival expectations.

Methods Sixty-nine stage I epithelioid MPM patients, including 27 patients treated with pleurectomy/decortication (P/D) and multimodal therapy (MMT) (the P/D [MMT] group), and 42 patients treated with chemotherapy or chemoradiotherapy (the CRT group), were included in the study. After an initial evaluation of overall survival, all patients were grouped in terms of histopathological parameters and treatment types, and then, a secondary survival evaluation was performed for the groups.

Results Forty-one patients were male, the mean age was 61.8 years. The median survival time was 26 months in the P/D (MMT) group, and 19.6 months in the CRT group, but the difference was not statistically significant. After grouping according to pathological criteria, a median survival time of 32.4 ± 2.9 months in the P/D (MMT) group and 21.9 ± 3.2 months in the CRT group was obtained among patients with histopathological low-grade tumors. Among patients with high-grade tumors, the median survival time was 18.3 ± 2.6 months in the P/D (MMT) group and 17 ± 4.4 months in the CRT group. Among patients with low-grade tumors, the P/D (MMT) group had longer survival. Median survival times were similar among patients with high-grade tumors.

Conclusion In epithelioid MPM, histopathological grading by video-assisted thoracic surgery pleural biopsy can prove accurate in selecting patients for P/D and MMT.

Keywords
- malignant pleural mesothelioma
- histopathological grading
- nuclear grading system
- pleuretomy/ decortication

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Introduction

Malignant pleural mesothelioma (MPM) is a rare malignancy that originates from mesothelial cells and is associated with asbestos exposure.[1] Whereas macroscopic complete resection accompanied by a multimodal approach with systemic chemotherapy and radiotherapy is preferred in early-stage MPM, most patients undergo chemotherapy since they are diagnosed at an advanced stage.[2] There are no routinely used factors other than clinical data, such as stage, histological type, and performance status, in the selection of patients for multimodal treatment (MMT) that can increase survival in appropriate cases and prevent unnecessary surgeries in unfavorable ones.[3]

TNM staging is crucial in selecting patients for MMT but may be insufficient by itself since the clinical presentation and growth and spread patterns of MPM differ substantively from other solid tumors, and local invasions into organs, such as the pericardium and diaphragm, can in some cases only be detected intraoperatively.[4–7] Therefore, in the past 10 years, various studies have been performed to determine the pathological parameters that can better evaluate the differences in survival time and prognosis compared with the TNM staging, and some alternative grading systems have been proposed.[8,9] In their 2020 study Nicholson et al have recommended that pathologists routinely grade biopsy material from all MPM patients.[10]

The present study aims to evaluate the histopathological grading of biopsy specimens in patients diagnosed with epithelioid MPM by video-assisted thoracic surgery (VATS) pleural biopsy and graded stage I as per the TNM system, regarding its impact on survival and its efficacy in patient selection for pleurectomy/decortication (P/D) and MMT.

Materials and Methods

Study Group

The study included 69 clinical stage I patients diagnosed with epithelioid MPM via VATS pleural biopsy in our clinic between 2016 and 2020. Twenty-seven of the patients had undergone P/D and MMT, and 42 chemotherapy or chemoradiotherapy. Patient data, including age, gender, presenting complaints, tumor location, treatment modality (MMT, chemotherapy, chemoradiotherapy), and survival, were evaluated retrospectively. Local ethics committee approval was obtained for the investigation (No: 2012-KAEK-15/2178).

VATS pleural biopsy involved multiple biopsies containing subpleural adipose tissue taken from at least four different areas, including the diaphragmatic surface, costodiaphragmatic recess, cardiophrenic space, parietal pleura lateral wall, and, if necessary, visceral pleura. Mediastinal pleura, chest wall, diaphragm, pericardium, and visceral pleura were evaluated to select patients for P/D during VATS.

In addition to VATS pleural biopsy, all patients underwent positron emission tomography/computed tomography for assessment of distant metastases and lymph nodes. MMT was administered in patients who accepted surgical treatment, and chemotherapy and prophylactic local radiotherapy were given in those who were considered clinically inoperable and refused surgical treatment, thus forming the two patient groups. Platinum-based pemetrexed was used as chemotherapeutic agent. All patients on curative radiotherapy underwent intensity-modulated radiotherapy.

Pathological Examination

All pathological analyses were performed on large biopsy specimens obtained by VATS. Following the confirmation of epithelioid MPM diagnosis by immunohistochemical evaluation, hematoxylin and eosin stained slides (2–18 slides) representing the tumor were examined for each case. All slides were examined for nuclear atypia, mitotic count, and necrosis. Mitosis counting was done with an Olympus BX53 microscope in 10 high-power fields ($\times$400) on the most mitotically active area. Pathological scoring was performed as per the nuclear grading system criteria for epithelioid MPM proposed by Kadota et al (►**Table 1**).[8]

Adding the necrosis parameter to Kadota et al's nuclear grading system, Rosen et al classified nuclear grade I and nuclear grade II patients without necrosis as "low-grade MPM," and nuclear grade II patients with necrosis and nuclear grade III patients as "high-grade MPM."[9,10]

In our study, we compared the survival times between patients receiving P/D (the P/D [MMT] group) and those on chemo/chemoradiotherapy (the CRT group) in terms of nuclear atypia, mitotic count, nuclear grade, necrosis, and histopathological grade.

Statistical Analysis

IBM SPSS Statistics Standard Concurrent User V 26 (IBM Corp., Armonk, New York, United States) software package was used for the statistical analysis of data. Descriptive statistics were given in number of units (n), percentage (%), and median $\pm$ standard deviation values for survival times. Comparisons between survival times in the groups by categorical variables were made using the log-rank (Mantel–Cox) test in the Kaplan–Meier analysis. The effects of sociodemographic and clinical characteristics of the patients on survival were evaluated with a single-index Cox regression model. A p-value <0.05 was considered statistically significant.

Results

The mean age of the patients was 61.8 years. Forty-one of 69 patients were male (59.4%), and 28/69 patients were female (40.6%). Note that 50.7% of the cases were on the left side ($n=35$), 49.3% were on the right side ($n=34$).

Twenty-seven of 69 patients received P/D (MMT), and 42/69 patients received chemotherapy or chemoradiotherapy. There was no significant difference between the two treatment groups by age ($p=0.078$), gender ($p=0.206$), nuclear atypia grade (0.752), mitotic count (0.165), nuclear grade (0.968), presence of necrosis (0.237), and histopathological grade (0.255). ►**Fig. 1** shows samples of the pathological examinations of patients.

The median follow-up of the study group was 22.5 ± 2.0 months. An evaluation of patients by survival time regardless

Table 1 Kadota et al's nuclear grading system[8]

Histopathological parameter	Score	Description
Nuclear atypia		
Mild	1	Small, uniform nuclei with indeterminate nucleoli
Moderate	2	Variable nuclear shape and variable prominent nucleoli
Severe	3	Bizarre, enlarged nuclei, multinucleation, and macronucleoli in > 5% of tumor cells
Mitotic count		
Low	1	0–1 mitosis on the most mitotically active area
Moderate	2	2–4 mitosis on the most mitotically active area
High	3	5 or more mitosis on the most mitotically active area
	Total score	
	2–3	Nuclear grade I
	4–5	Nuclear grade II
	6	Nuclear grade III

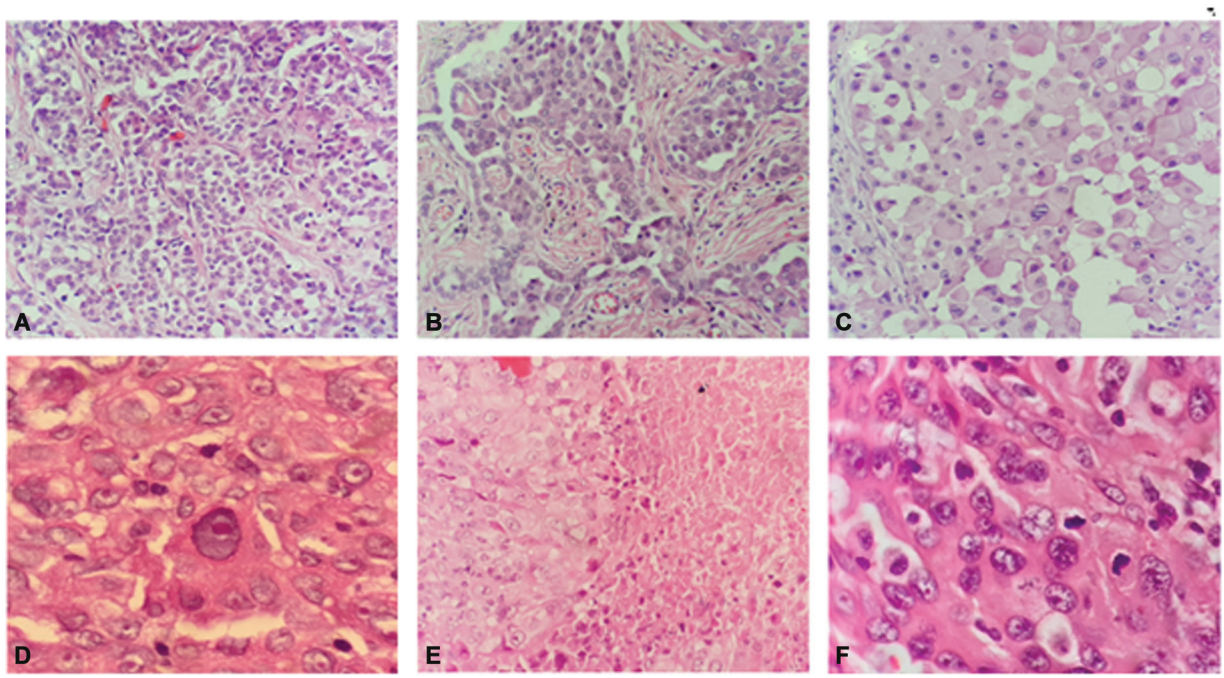

Fig. 1 (**A**) Tumor cells with uniform nuclei, hematoxylin and eosin (HE) ×200 (mild nuclear atypia). (**B**) Cells with indeterminate nucleoli, HE ×400 (mild nuclear atypia). (**C**) Nuclei of various diameter and shape, HE ×400 (moderate nuclear atypia). (**D**) Macronucleolus, HE ×1000 (severe nuclear atypia). (**E**) Areas of necrosis, HE ×400. (F) Mitotic figures, HE ×1000.

of the treatment approach showed that nuclear atypia grade, nuclear grade, and histopathological grade were statistically significant variables (►**Table 2**).

In the P/D (MMT) group, the 2-year survival rate was 64.5% and 3-year 13.6%; in the CRT group, the 2-year survival rate was 29.8% and 3-year was 15.9%. The median survival time (95% confidence interval) was 26 (20.3–31.7) months in the P/D (MMT) group and 19.6 (16.8–22.3) months in the CRT group, and the difference was not statistically significant (log-rank = 2.736; $p = 0.098$) (►**Fig. 2A**). An evaluation of the impact of nuclear atypia grade, mitotic count, nuclear grade, presence of necrosis, and histopathological grade on survival between the P/D (MMT) group and the CRT group revealed a significant difference only for low-grade patients (32.4 ± 2.9 vs. 21.9 ± 3.2 months, respectively; $p = 0.041$). No significant difference was obtained for the other variables (►**Table 3**). ►**Fig. 2B** shows the survival curves of low-grade tumors by treatment groups.

Discussion

Our survival analysis in terms of histopathological grade showed that the median survival time difference of 10.5 months in favor of the P/D (MMT) group for low-grade

Table 2 Survival analysis by pathological variables independent of groups ($n = 69$)

	HR (95% confidence interval)	*p*
Nuclear atypia grade		
Mild (ref)	1	
Moderate	1.361 (0.709–2.613)	0.354
Severe	3.204 (1.487–6.901)	**0.003**
Mitotic count		
Low (ref)	1	
Moderate	0.431 (0.178–1.046)	0.063
High	0.563 (0.279–1.133)	0.108
Nuclear grade		
I	1	
II	1.990 (1.014–3.905)	**0.045**
III	6.689 (2.602–17.196)	< **0.001**
Necrosis		
None (ref)	1	
Present	1.565 (0.855–2.865)	0.146
Histopathological grade		
Low (ref)	1	
High	1.961 (1.095–3.510)	**0.023**

Abbreviation: HR, hazard ratio. $n = 69$.

tumors was statistically significant. There was no significant difference in survival times between the treatment groups for high-grade tumors. This indicates that patients determined as low-grade according to VATS biopsy material can be selected for P/D and MMT (►**Fig. 3**).

In patients with epithelioid diffuse MPM, clinical stage is the current primary prognostic factor. Many authors described the prognostic importance of histologic subtyping. Kadota et al suggested that the pleomorphic subtype is a predictor of aggressive behavior in epithelioid MPM with no survival difference from biphasic or sarcomatoid.[11] The difficulty of selecting a treatment approach with TNM staging in MPM prompted Kadota et al to propose a pathologically convenient new grading system published in 2012. In their study on 232 patients previously diagnosed with epithelioid MPM, they reported that nuclear atypia and mitotic count in pathology specimens were predictive of survival and, hence, defined a nuclear grading system using the two parameters. The authors stated that the new system was more accurate than TNM staging and other tools in estimating survival times for grades and could also help in predicting tumor recurrence times. In their study, they indicated a median survival time of 28 months in the nuclear grade I patient group, 14 months in the nuclear grade II group, and 5 months in the nuclear grade III group. However, the investigators had obtained MPM paraffin blocks by different methods such as P/D, extrapleural pneumonectomy, and VATS pleural biopsy.[8] Mlika et al also noted a significant relationship between nuclear atypia and survival time in their smaller-scale study.[12] In the present study, we determined that nuclear atypia grade and nuclear grade significantly affect survival time regardless of treatment type.

Demirag et al associated the presence of necrosis with poor prognosis in MPM.[13] In a multicenter study including 776 MPM patients published in 2018, Rosen et al proposed a new histopathological grading system to better estimate overall survival times by including necrosis in the nuclear grading system. The study indicated a significant correlation between survival and nuclear grade, necrosis status, growth pattern, nuclear atypia, and mitotic count. The authors reported a median survival time of 29 months for nuclear grade I patients without necrosis, 16 months each for nuclear grade I with necrosis and nuclear grade II without necrosis, 10 months for nuclear grade II with necrosis, and 8 months for nuclear grade III. However, the type of surgery was not randomized in that study, and biopsy materials obtained by different methods were used, as in the study of Kadota et al. Besides, in the multicenter study of Rosen et al, the patients' staging data were lacking, so those who underwent biopsy could have been at more advanced stages than patients with surgical resection.[9] In contrast, in our study, we formed an isolated group consisting of only grade I patients within a certain date range so that our comparison of survival times by histopathological grade would not be affected by changes in survival time depending on the TNM stage. The necrosis parameter alone was not predictive of survival, but the

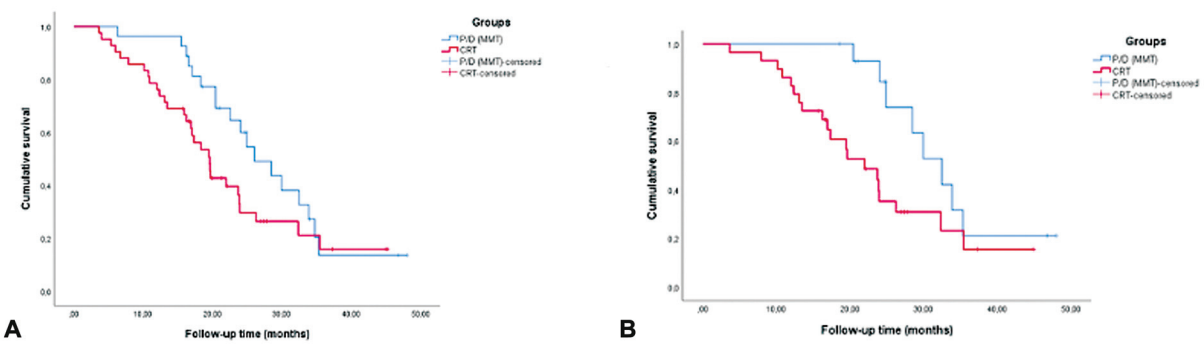

Fig. 2 (**A**) Survival curves of treatment groups. (**B**) Survival curves of low-grade tumors by treatment groups.

Table 3 Comparison and evaluation of survival rates in treatment groups by histological characteristics and pathological findings

	P/D (MMT)			CRT			*p*
	n = 27	%	Survival (mo) median (95% CI)	*n* = 42	%	Survival (mo) median (95% CI)	
Nuclear atypia							
Mild	13	48.2	33.8 (23.7–44.0)	24	57.1	21.9 (16.3–27.6)	0.085
Moderate	9	33.3	24.9 (19.0–30.7)	11	26.2	18.3 (9.1–27.5)	0.540
Severe	5	18.5	16.5 (16.0–17.0)	7	16.7	15.9 (3.7–28.2)	0.551
Mitotic count		–					
Low (0–1)	6	22.2	33.8 (30.8–36.8)	3	7.1	23.7 (17.1–30.2)	0.106
Moderate (2–4)	5	18.5	29.9 (25.0–34.8)	12	28.5	21.9 (4.1–39.7)	0.446
High (> 5)	16	59.3	20.4 (15.9–24.9)	27	64.4	18.3 (14.6–22.0)	0.398
Nuclear grade							
I	9	33.3	33.8 (23.8–36.0)	13	31	23.7 (12.2–35.1)	0.259
II	14	51.9	26.0 (17.7–34.2)	22	52.4	18.3 (13.9–22.6)	0.219
III	4	14.8	16.2 (9.5-22.9)	7	16.6	15.9 (6.1-21.9)	0.852
Necrosis							
None	10	37.0	20.4 (14.4–26.4)	10	23.8	17.0 (5.5–27.5)	0.435
Present	17	63.0	29.9 (23.8–36.0)	32	76.2	21.9 (14.4–29.4)	0.084
Histopathological grade							
Low	15	55.6	32.4 (26.6–38.3)	29	69.0	21.9 (15.5–28.4)	**0.041**
High	12	44.4	18.3 (13.0–23.5)	13	31.0	17.0 (8.3–25.7)	0.586

Abbreviations: CI, confidence interval; CRT, chemoradiotherapy; MMT, multimodal therapy; P/D, pleurectomy/decortication.

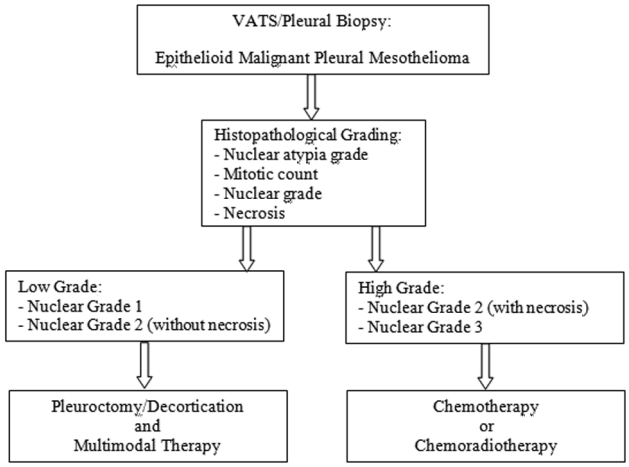

Fig. 3 Recommendation of treatment algorithm according to histopathological grading in malignant pleural mesothelioma.

histopathological grade, a combination of nuclear grade and necrosis parameters, was a significant predictor of survival time.

In their study, Nicholson et al stated that histopathological grading can be used to distinguish aggressive tumors and to select patients for a treatment involving MMT and surgery. Therefore, they recommended that pathologists routinely grade biopsy material from all MPM patients. Drawing on the grading system proposed by Rosen et al, they histopathologically classified nuclear grade I and nuclear grade II tumors without necrosis as "low-grade tumors," and nuclear grade II with necrosis and nuclear grade III tumors as "high-grade tumors." The authors indicated that this classification could prove beneficial in estimating survival.[9,10] Elsewhere, Habougit et al analyzed histopathological criteria, mostly based on VATS pleural biopsy specimens, in 116 MPM patients and found a significant correlation between survival and mitotic count, atypical mitoses, nuclear atypia, nucleolar prominence, and necrosis status. But the authors emphasized that the results were only valid for epithelioid MPM. The aim of their study was to determine the patient group that would actually benefit from surgery.[14] ►**Table 4** summarizes the published literature on histological parameters in MPM.

In contrast with the literature, the present study investigated the usability of histopathological grading criteria in patient selection for different treatment types by comparing epithelioid MPM patients who underwent surgery and those who did not. The median survival times were different between the groups regarding nuclear atypia and nuclear grade, but no significant difference in survival could be obtained, which may be due to the small number of patients and the presence of patients still alive in both groups.

Pleural biopsy should ideally involve multiple materials containing subpleural adipose tissue taken from at least three different areas without fibrosis. Thus, the depth of tumor invasion can be inferred in the pathological

Table 4 Published literature on histological parameters in malignant pleural mesothelioma

Study	Center	Number of patients and subtype	Diagnostic procedure	TNM staging	Significant histopathological parameters for survival
Kadota et al 2012	Single-center	232 epithelioid	P/D, EPP, Bx, other	Stage I: 14 Stage II: 54 Stage III: 130 Stage IV: 34	Nuclear atypia, mitotic count, nuclear grade
Habougit et al 2017	Single-center	116 (77 epithelioid)	%96.55 Bx %3.45 P/D, EPP, autopsy	N/A	Nuclear atypia, mitotic count, necrosis
Rosen et al 2018	Multicenter	776 epithelioid	P/D, EPP, Bx, other	N/A	Nuclear atypia, mitotic count, nuclear grade, necrosis, mitosis-necrosis score, histopathological grade
Mlika et al 2019	Single-center	30 (17 epithelioid)	Cytology, FNAB, thoracoscopy, thoracotomy	Stage I: 12 Stage II: 3 Stage III: 14 Stage IV: 1	Nuclear atypia
Present study 2022	Single-center	69 epithelioid	Only VATS Bx	Only Stage I: 69	Nuclear atypia, nuclear grade, histopathological grade

Abbreviations: Bx, biopsy; EPP, extrapleural pneumonectomy; FNAB, fine-needle aspiration biopsy; N/A, not available; P/D, pleurectomy/decortication; VATS, video-assisted thoracic surgery.

examination.[10] A large amount of tumor tissue is required for accurate diagnosis and histological subtyping of MPM.[15] VATS is a preferred method thanks to its visual advantage and safety, as well as the fact that it allows adequate tissue biopsy by dissection of all layers of the parietal pleura.[16] In MPM, the European Respiratory Society and European Society of Thoracic Surgeons guidelines particularly recommend pleural biopsy by VATS, whose predictive value is reported to be as high as 99.7%, to obtain multiple and deep tissue biopsies.[5] Besides its diagnostic accuracy, VATS allows for wide observation of the intrapleural space, enabling the performance of staging procedures in patients, the identification of potentially resectable tumors, and the selection of patients for surgical modalities.[17] In our clinic, a diagnostic VATS approach is used in all patients, along with preoperative imaging, to accurately detect the "T" component of TNM staging and differentiate potentially resectable tumors. Patients who turned out to be T4 were excluded from this study.

Published literature had established the effect of histopathological parameters on survival. Our study is valuable since it is the first to compare these parameters between MPM patients who underwent surgery and those who did not. The fact that we included only stage I epithelioid MPM patients within a certain date range in the survival analysis is the reason for our limited study population on the one hand and a unique aspect of our study on the other.

In conclusion, histopathological grading with VATS pleural biopsy should be performed in all cases of suspected MPM. Patients thus determined as "low-grade" can obtain the highest benefit from P/D and MMT in terms of survival. Future studies with larger series of patients will potentially confirm our conclusions.

Conflict of Interest
None declared.

References

1 Robinson BW, Musk AW, Lake RA. Malignant mesothelioma. Lancet 2005;366(9483):397–408
2 Nelson DB, Rice DC, Niu J, et al. Predictors of trimodality therapy and trends in therapy for malignant pleural mesothelioma. Eur J Cardiothorac Surg 2018;53(05):960–966
3 Bueno R, Opitz IIASLC Mesothelioma Taskforce. Surgery in malignant pleural mesothelioma. J Thorac Oncol 2018;13(11): 1638–1654
4 Opitz I, Weder W. Pleural mesothelioma: is the surgeon still there? Ann Oncol 2018;29(08):1710–1717
5 Rusch VW, Chansky K, Kindler HL, et al; IASLC Staging and Prognostic Factors Committee, advisory boards, and participating institutions. The IASLC Mesothelioma Staging Project: proposals for the M descriptors and for revision of the TNM stage groupings in the forthcoming (eighth) edition of the TNM classification for mesothelioma. J Thorac Oncol 2016;11(12):2112–2119
6 Nowak AK, Chansky K, Rice DC, et al; Staging and Prognostic Factors Committee, Advisory Boards and Participating Institutions. The IASLC Mesothelioma Staging Project: Proposals for Revisions of the T Descriptors in the Forthcoming Eighth Edition of the TNM Classification for Pleural Mesothelioma. J Thorac Oncol 2016;11(12):2089–2099
7 Hashimoto M, Takeuchi J, Takuwa T, et al. Pleural thickness after neoadjuvant chemotherapy is a prognostic factor in malignant pleural mesothelioma. J Thorac Cardiovasc Surg 2019;157(01): 404–413
8 Kadota K, Suzuki K, Colovos C, et al. A nuclear grading system is a strong predictor of survival in epitheloid diffuse malignant pleural mesothelioma. Mod Pathol 2012;25(02):260–271
9 Rosen LE, Karrison T, Ananthanarayanan V, et al. Nuclear grade and necrosis predict prognosis in malignant epithelioid pleural mesothelioma: a multi-institutional study. Mod Pathol 2018;31 (04):598–606

10 Nicholson AG, Sauter JL, Nowak AK, et al. EURACAN/IASLC proposals for updating the histologic classification of pleural mesothelioma: towards a more multidisciplinary approach. J Thorac Oncol 2020;15(01):29–49
11 Kadota K, Suzuki K, Sima CS, Rusch VW, Adusumilli PS, Travis WD. Pleomorphic epithelioid diffuse malignant pleural mesothelioma: a clinicopathological review and conceptual proposal to reclassify as biphasic or sarcomatoid mesothelioma. J Thorac Oncol 2011;6(05):896–904
12 Mlika M, Limam M, Benzarti A, Mezni F. Impact on survival of nuclear atypia in epithelioid malignant mesothelioma. Adv Respir Med 2019;87(02):90–95
13 Demirag F, Ünsal E, Yilmaz A, Caglar A. Prognostic significance of vascular endothelial growth factor, tumor necrosis, and mitotic activity index in malignant pleural mesothelioma. Chest 2005; 128(05):3382–3387
14 Habougit C, Trombert-Paviot B, Karpathiou G, et al. Histopathologic features predict survival in diffuse pleural malignant mesothelioma on pleural biopsies. Virchows Arch 2017;470(06): 639–646
15 Bueno R, Reblando J, Glickman J, Jaklitsch MT, Lukanich JM, Sugarbaker DJ. Pleural biopsy: a reliable method for determining the diagnosis but not subtype in mesothelioma. Ann Thorac Surg 2004;78(05):1774–1776
16 Hashimoto M, Sato A, Kuroda A, et al. Clinical feature of diagnostic challenging cases for pleural biopsy in patient with malignant pleural mesothelioma. Gen Thorac Cardiovasc Surg 2020;68(08): 820–827
17 Rodríguez Panadero F. Diagnosis and treatment of malignant pleural mesothelioma. Arch Bronconeumol 2015;51(04): 177–184