ORIGINAL ARTICLE

Pathologic Grading of Malignant Pleural Mesothelioma: An Evidence-Based Proposal

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ABSTRACT

Introduction: A pathologic grading system (PGS) for malignant pleural mesothelioma (MPM) is warranted to better identify different risk categories of patients, plan therapeutic options, and activate clinical trials.

Methods: A series of 940 patients with MPM (328 in a training set and 612 in a validation set) that was diagnosed between October 1980 and June 2015 at the participant institutions was retrospectively assembled. A PGS was constructed by attributing to each histologic parameter, independent at multivariate analysis with excellent reproducibility ($\kappa > 0.75$), different scores based on the increase in corresponding hazard ratios. The relevant PGS score thus ranged from 0 to 8 points for individual patients with MPM.

Conclusions: The PGS was constructed by taking into consideration the histological subtyping of MPM (epithelioid/biphasic = 0 points; sarcomatoid = 2 points), necrosis (absent = 0 points versus present = 1 point), mitotic count per 1 mm^2 (cutoffs as follows: 1–2 = 0 points, 3–5 = 1 point, 6–9 = 2 points, or ≥10 = 4 points), and Ki-67 labeling index based on 2000 cells (<30% = 0 points versus ≥30 = 1 point), all of which are independent factors in both patient sets after adjustment for stage and age at diagnosis. No heterogeneity was seen across the validation centers ($p = 0.19$). Epithelioid/biphasic MPM patterning and biopsy versus resection did not affect survival, whereas the PGS outperformed mitotic count and Ki-67 LI in both the training (area under the curve receiver operating characteristic = 0.76) and validation sets (area under the curve receiver operating characteristic = 0.73) ($p < 0.01$). Patient survival progressively deteriorated from a score of 0 (median times of 26.3 and 26.9 months) to a score 1 to 3 (median times of 12.8 and 14.4 months) and a score of 4 to 8 (median times of 3.7 and 7.7 months) in both sets of patients, with the hazard ratio for a 1-point increase in score being 1.46 (95% confidence interval: 1.36–1.56) in the training set and 1.28 (95% confidence interval: 1.22–1.34) in the validation set (after adjustment for age and [when available] tumor stage). The PGS was effective even in subgroup analysis (epithelioid, biphasic, and sarcomatoid tumors).

Discussion: A simple and reproducible multiparametric PGS effectively predicted survival in patients with MPM.

Keywords: Mesothelioma; Pleura; Grading; Survival; Score

Introduction

Malignant pleural mesothelioma (MPM) is a rare and aggressive neoplasm with ominous prognosis *quoad vitam* and *quoad valetudinem*, the incidence of which has been steadily increasing worldwide and leveling off in Western countries.[1–5] Epithelioid (EMPM), biphasic (BMPM), and sarcomatoid (SMPM) mesothelioma account for the main histologic variants,[6,7] with EPMPs likely to behave less aggressively in comparison with BMPMs and especially in comparison with SMPMs.[8–10] Few studies have supported the notion that EMPM patterns can actually affect survival,[11–13] although the pleomorphic variant is thought to be an independent predictor of a dismal prognosis.[11,14]

A constellation of factors has been adopted in the clinical management of patients with MPM,[2,8,9,15–18] especially in cases of young subjects with early-stage disease, good performance status, and the epithelioid histologic variant.[1,2,19] These patients account for most of the 15% to 20% of patients with MPM who survive more than 3 years[8,11,14,20–22] and in whom a more aggressive treatment is clinically warranted.[1,23,24] It is tempting, however, to speculate about how quickly the disease will evolve at the level of an individual patient's cancer after the initial morphologic diagnosis.[8,15,16,23,25] Several investigations have confirmed that MPMs, as well as their peritoneal counterpart, encompass a case mix of diversely behaving tumors, with more indolent lesions being recorded within the epithelioid variant (also because of an earlier stage of disease).[2,8,11,13,21,22,26–29] Although most patients with MPM relentlessly die of illness over time, treatments are generally decided on the basis of managerial clinical criteria in which pathologic grading does not yet play an established role.[1,2]

A concept of grading in patients with MPM is included in the current WHO histologic classification,[6,7] although recent proposals have credited a role to nuclear atypia,[22,30] mitotic count,[22,30] or Ki-67 antigen labeling index (LI),[21] at least in the epithelioid variant, with similar results being described in the peritoneum.[26] Other studies have focused on necrosis, nucleolus presence and size, atypical mitoses, or growth patterns as

grading criteria.[13,30] Ideally, a grading system should offer timely prognostic information for individual patients with MPM regardless of the therapy being administered, the type or size of the diagnostic material, and histologic appearance. Grading could also assist clinicians in assigning patients to different outcomes, planning of individualized treatments, follow-up strategies, and clinical trial design. Knowledge of tumor aggressiveness is clinically beneficial to providing the right drug to the right patient at the right time.[1,19]

This large multi-institutional study was designed to develop and validate an innovative and reproducible subtyping score that is readily evaluable by clinicians and based solely on the evaluation of common and reproducible histopathologic parameters.

Materials and Methods

Patients with Tumors

A large multicenter, retrospective, observational study including 940 consecutive patients with a diagnosis of MPM based on either biopsy or resection specimens and follow-up information spanning from October 1980 to June 2015 was undertaken at the participating institutions. The challenge was to construct an innovative pathologic grading system (PGS) by using common and easy-to-assess histologic criteria to reliably predict tumor behavior for different kinds of patient cancers. Five secondary or tertiary Italian independent institutions that are devoted to and leaders in the diagnosis and treatment of MPM took part in the study. One center (the Bari set) contributed 328 patients to the training cohort, and four centers (the Milan, Modena, Padua, and Turin sets) contributed 612 patients to the validation cohort. Part of the Padua case series (24 cases) came from the Golnik Center (Slovenia), which is another center devoted to the diagnosis and treatment of this kind of tumor. These cases were in part processed in Padua (for Ki-67 antigen immunostaining) and additionally used for the validation cohort (data not shown). The main demographic and clinicopathologic characteristics of the patients in the training and validation cohorts are presented in Table 1.

All the original histologic slides were reviewed by two pathologists experienced in MPM from each center to ensure diagnostic consistency; they were blinded to patient identity and initial tumor categorization. Information about demography, asbestos exposure, cancer-related overall survival (OS), tumor staging, adjuvant and/or neoadjuvant therapy, and types of material (a small biopsy specimen if the tumor measured $\leq$2 cm on gross examination; a large biopsy if the tumor measured $>$2 cm or pleurectomy was performed; and a surgical resection specimen if major surgical procedures were carried out) was obtained from the original pathology reports, clinical charts, referring physicians, the patients, and their families. These data were subsequently collected in a database.

Tumor tissues were fixed in a 4% buffered formaldehyde solution for 12 to 24 hours and embedded in paraffin according to the standard histopathologic methods. All diagnoses were also supported by appropriate immunohistochemistry according to current guidelines (two mesothelial markers and two carcinoma cell markers).[31] A list of commonly assessed histologic parameters was tested in the training set for devising the PGS. They included percentage of necrosis on the whole tumor tissue specimens present on slides; tumor subtyping according to the 2015 WHO classification[7]; growth patterning of the EMPM or the epithelioid component of the BMPM assessed; cell atypia of the EMPM or the epithelioid component of the BMPM, which was classified as mild, moderate, or severe according to the resemblance of tumor cells in terms of nuclear shape and size and discernible amount of cytoplasm with nonneoplastic mesothelial cells within each tumor case; nucleoli (inconspicuous, distinct, macronucleoli); mitotic count per 1 mm^2 evaluated in representative tumor blocks by scanning preserved tumor areas with the highest activity after scrutiny of all available tumor slides; and Ki-67 LI based on hot spot areas, counting 2000 cells or 1 mm^2 in the same tumor block and areas as for the mitotic count. During evaluation of the Ki-67 LI, particular attention was paid to exclude Ki-67–decorated inflammatory cells from the final counting by allowing only pathologists in experienced MPM to participate in the study. Cutoff points for continuous variables (i.e., mitotic count and Ki-67 LI) were identified according to the distribution of individual data across the training set and then maintained in the validation set.

Furthermore, 128 tumors stemming from the Milan (79 cases) and Turin (49 cases) sets were reviewed in blinded fashion by two different pairs of pathologists for assessing interobserver variability of the histologic parameters under evaluation.

Ethics

The study was approved by the independent ethics committee of the University Hospital Polyclinic of Bari, Bari, Italy (accession number 5062; date June 22, 2016). All patients gave their written informed consent for diagnosis and research activities when they were admitted to the hospital.

Statistical Analysis

The main study outcome was OS, which was calculated from the date of diagnosis of mesothelioma to the

date of death or last contact with the patient. Associations between clinicopathologic characteristics and patient survival were assessed by using Cox proportional hazards regression models. The assumption of proportional hazards was verified by visual inspection of the log of the negative log transformation of the survival functions for the single covariates included in the multivariable model. Models were fitted on the whole set of patients, with missing values represented by dummy variables (missing indicator method). Pathologic characteristics significantly associated with OS at univariate analysis and showing good reproducibility (or agreement) between pathologists were selected for inclusion in the PGS. Agreement between pathologists for the determination of tumor necrosis, histologic variant, nucleolus presence, atypia, Ki-67 LI, and mitotic count was assessed by using the Cohen κ: a κ value greater than 0.75 was considered excellent agreement beyond chance, a κ value between 0.40 and 0.75 was considered good agreement beyond chance, and a κ value less than 0.40 was considered poor agreement beyond chance.[32] For the sake of simplicity and to allow clinicians to readily calculate the PGS score, factors with a statistically significant hazard ratio (HR) less than 2.0 were given 1 point, those with an HR between 2.0 and 4.0 were given 2 points, and those with an HR greater than 4.0 were given 4 points. The PGS score was obtained by adding up the points for the four identified factors (i.e., tumor necrosis, histologic variant, Ki-67 LI, and mitotic count). Survival curves according to the PGS classes (0 point, 1–3 points, and 4–8 points) were constructed by using the Kaplan-Meier method. Difference in survival between groups was assessed by the log-rank test. The risk of death associated with a 1-point increase in the PGS score was also calculated with adjustment for patients' age at diagnosis and tumor stage. We checked for linearity, plotting β estimates obtained from a model substituting the continuous PGS for eight dummy variables corresponding to each value of the PGS score and using a β value of 0 for the reference category. In the training set, the respective β estimates for PGS scores 0 to 8 were 0, 0.63, 1.22, 1.37, 1.95, 1.98, 2.09, 3.92, and 4.03, indicating a linear relationship. We also assessed the performance of the score with calibration curves, plotting the observed 12-month mortality with 95% confidence intervals (CIs) obtained from actuarial survival against the predicted 12-month mortality derived from the Cox proportional hazards regression model with PGS score set as a continuous variable, for groups of patients with different PGS scores. The predicted 12-month mortality in the validation cohort was calculated by using the baseline survival estimate S_0 and the covariate estimate β obtained from the testing set, and it was compared with the observed mortality in the validation set.

We performed stratified analyses based on the whole set of patients (training plus validation sets) to assess the validity of the PGS in different patient subgroups. A forest plot was generated to visually assess the variation of the HR across all subgroups. Receiver operating characteristic curves with the respective areas under the curves (AUCs) were drawn to illustrate the prognostic ability of the PGS to determine 12-month mortality, set as a binary end point in comparison with those of Ki-67 LI and mitotic count when considered alone. Comparison of AUCs was done by using the nonparametric approach suggested by DeLong et al.[33] We also plotted the time-dependent AUCs, with 95% CIs in the training cohort and in the validation cohort for follow-up times ranging from 0 to 60 months, and we calculated the integrated AUC over time.

All analyses were performed with SAS software (version 9.4, SAS Institute Inc., Cary, NC). All p values were two sided, and p values less than 0.05 were considered statistically significant.

Results

Clinicopathologic Traits of Patients

As detailed in Table 1, most patients were male and older than 60 years in either tumor set, with a slight prevalence of asbestos exposure and tumors diagnosed predominantly on the basis of small biopsy specimens measuring 2 cm or less. Tumor stage information was available for 39.3% of patients (369 of 940), with an expected distribution across II to IV stage. Palliative chemotherapy had been administered in 68% of patients in the training set, but only in 39.5% of the those in the validation set (the set containing the most patients for whom information on palliative chemotherapy was missing).

Tumor necrosis was detected in about one-third of cases in both tumor sets regardless of the amount. However, EMPMs were prevalent over BMPMs or SMPMs. EMPMs and the epithelioid component of biphasic tumors predominantly showed a solid, tubular, or papillary architecture, whereas SMPMs were mostly composed of spindled tumor cells, with fewer cases featuring a desmoplastic or pleomorphic appearance.

Microscopic examination showed inconspicuous to distinct nucleoli in the nuclear area, whereas eosinophilic macronucleoli were observed in about one-fourth of tumors. Tumors featured moderate to severe cell atypia in 50% or more of cases, with most of them having a Ki-67 LI greater than 30% and an increased mitotic count (>3 mitoses per mm^2).

Selection, Scoring, and Confirmation of the Predictor

The first concern was to identify the most objective histologic parameters so as to minimize interobserver

Table 1. Clinicopathologic Characteristics of the 940 Patients of the Testing and Validation Cohorts

Variable	Training Cohort, n (%)	Validation Cohort, n (%)
Total population	328 (100)	612 (100)
Center		
Bari	328 (100)	–
Milan	–	79 (12.9)
Modena	–	255 (41.7)
Padua	–	69 (11.3)
Turin	–	209 (34.2)
Period of diagnosis		
<2000	140 (42.7)	69 (11.3)
2000-2004	64 (19.5)	106 (17.3)
2005-2009	59 (18.0)	161 (26.3)
≥2010	65 (19.8)	276 (45.1)
Sex		
Male	247 (75.3)	450 (73.5)
Female	81 (24.7)	162 (26.5)
Age at diagnosis, y		
<60	81 (24.7)	125 (20.4)
60-69	104 (31.7)	202 (33.0)
70-79	108 (32.9)	223 (36.4)
≥80	35 (10.7)	62 (10.1)
Asbestos exposure		
No	102 (31.1)	144 (23.5)
Yes	226 (68.9)	331 (54.1)
Missing cases	–	137 (22.4)
Histologic variant		
Epithelioid	221 (67.4)	478 (78.1)
Pattern mostly solid	148 (45.1)	214 (35.0)
Pattern mostly tubular	40 (12.2)	60 (9.8)
Pattern mostly papillary	6 (1.8)	135 (22.1)
Pattern mostly microcystic	8 (2.4)	23 (3.8)
Pattern mostly Indian files	19 (5.8)	46 (7.5)
Biphasic	73 (22.3)	84 (13.7)
Pattern mostly solid	61 (18.6)	60 (9.8)
Pattern mostly tubular	12 (3.7)	17 (2.8)
Pattern mostly papillary	–	5 (0.8)
Pattern mostly microcystic	–	2 (0.3)
Sarcomatoid	34 (10.4)	50 (8.2)
Classic (high cellularity)	12 (3.7)	35 (70.0)
Classic (low cellularity)	8 (2.4)	2 (4.0)
Desmoplastic	8 (2.4)	11 (22.0)
Pleopmorphic	6 (1.8)	2 (4.0)
Nucleoli		
Inconspicuous	23 (7.0)	290 (47.4)
Distinct	201 (61.3)	206 (33.7)
Macronucleolus	96 (29.3)	114 (18.6)
n/e (desmoplastic)	8 (2.4)	2 (0.3)
Atypia		
Mild	16 (4.9)	173 (28.3)
Moderate	141 (43.0)	277 (45.3)
Severe	163 (49.7)	153 (25.0)
n/e	8 (2.4)	9 (1.5)
Ki-67 LI		
<10%	52 (15.9)	108 (17.6)
10%-19%	82 (25.0)	239 (39.1)
20%-29%	67 (20.4)	56 (9.2)
≥30%	127 (38.7)	187 (30.6)
Missing cases	–	22 (3.6)
Mitosis number		
1-2	50 (15.2)	187 (30.6)
3-5	140 (42.7)	211 (34.5)
6-9	99 (30.2)	120 (19.6)
≥10	39 (11.9)	94 (15.4)
Stage		
I	52 (15.9)	2 (0.3)
II	51 (15.5)	8 (1.3)
III	28 (8.5)	18 (2.9)
IV	29 (8.8)	181 (29.6)
Missing cases	168 (51.2)	403 (65.8)
Chemotherapy		
None	105 (32.0)	13 (2.1)
Palliative	223 (68.0)	242 (39.5)
Missing cases	–	357 (58.3)
Sampling material		
Small biopsy specimen	190 (57.9)	408 (66.7)
Large biopsy specimen (pleurectomy)	107 (32.6)	183 (29.9)
Surgical specimen	31 (9.5)	21 (3.4)
Necrosis		
Absent	247 (75.3)	415 (67.8)
Present	81 (24.7)	197 (32.2)

Ki-67 LI, Ki-67 labeling index; n/e, not evaluable.

variability of the defining criteria. Reproducibility testing showed excellent agreement beyond chance for necrosis ($\kappa = 0.81$), histologic subtyping ($\kappa = 0.93$), Ki-67 LI ($\kappa = 0.81$), and mitotic count ($\kappa = 0.76$) but not nucleoli ($\kappa = 0.69$) or cell atypia ($\kappa = 0.66$). Therefore, histologic subtyping, necrosis, Ki-67 LI, and mitotic count entered the prediction system for tumor grading (Supplementary Table 1). Supplementary Table 2 presents the results of univariate analysis for all variables and the results of the multivariate analysis with consideration for the four aforementioned histologic parameters. For the sake of simplicity, we decided to group patients with BMPM (regardless of the amount of sarcomatoid component or other subtype), together with patients with EMPM and patients with a Ki-67 LI less than 30%, as none of these subgroups demonstrated significant differences in survival.

Scoring attribution was performed in the training set on the basis of HR, with 1 point awarded for the presence of necrosis (HR = 1.60, 95% CI: =1.22–2.09), 2 points awarded for the SMPM histologic variant

Table 2. Histopathologic Traits Associated with Overall Survival at Multivariate Analysis in the Training Cohort (n = 328 patients) and Assignation of Relative Points for Construction of Scoring of the Pathologic Grading System

Variable	Multivariable HR (95% CI)	Assignment of Points
Necrosis		
Absent	1	0
Present	1.60 (1.22-2.09)	1
Histologic variant		
Epithelioid/biphasic	1	0
Sarcomatoid	2.04 (1.39-2.99)	2
Ki-67 LI		
<30%	1	0
≥30%	1.54 (1.19-2.00)	1
Mitosis number		
1-2	1	0
3-5	1.90 (1.32-2.74)	1
6-9	2.79 (1.89-4.12)	2
≥10	4.26 (2.61-6.95)	4

Note: For details on the assessment of the defining criteria (necrosis, histologic variant, Ki-67 LI, and mitosis number), see the Materials and Methods section. Statistically significant HR values less than 2 are scored as 1 point, HR values of 2 to 4 are scored as 2 points, and HR values greater than 4 are scored as 4 points.
HR, hazard ratio; CI, confidence interval; KI-67 LI, Ki-67 labeling index.

(HR = 2.04, 95% CI: 1.39–2.99), 1 point awarded for a Ki-67 LI of at least 30% (HR = 1.54, 95% CI: 1.19–2.00), 1 point awarded for a mitotic count of 3 to 5 (HR = 1.90, 95% CI: 1.32–2.74), 2 points awarded for a mitotic count of 6 to 9 (HR = 2.79, 95% CI: 1.89–4.12), and 4 points awarded for a mitotic count of 10 (HR = 4.26, 95% CI: 2.61–6.95) (Table 2). The distribution of the resulting score in the training cohort and in the validation cohort, by center, is presented in Supplementary Table 3. The prediction power of this multiparametric PGS compared with that of the single-event evaluation was subsequently confirmed by the AUC receiver operating characteristic analysis of the 12-month mortality rate, which showed an AUC far wider for the PGS than that for mitoses or the Ki-67 LI, as assessed separately in either tumor set (Fig. 1). The integrated AUC for the PGS over time was 0.761 in the training cohort and 0.731 in the validation cohort (Fig. 2). There was a highly significant correlation between Ki-67 LI and mitotic count in both the training and validation sets ($p < 0.0001$), albeit with a wide dispersion of values (Pearson correlation coefficient values of 0.41 and 0.35, respectively). A schematic flowchart illustrating the development and validation of this PGS is depicted in Supplementary Figure 1. Representative histologic pictures of MPM cases according to the four defining criteria being used in this study are depicted in Supplementary Figure 2.

PGS score was demonstrated to be an excellent prognostic factor in both the training and the validation sets (log-rank $p < 0.0001$) (Fig. 3). The risk of death increased by 45% (training set) or 31% (validation set) for a 1-point increase in the PGS score. After adjustment for age at diagnosis and (when available) for tumor stage, the respective HRs for a 1-point increase in PGS score were 1.46 (95% CI 1.36–1.56) in the training set and 1.28 (95% CI: 1.22–1.34) in the validation set. Patient survival progressively deteriorated from a score of 0 (median 26.3 and 26.9 months in the training and validation sets, respectively) to a score of 1 to 3 (median times of 12.8 and 14.4 months, respectively) and a score of 4 to 8 (median times of 3.7 and 7.7 months, respectively), with no heterogeneity across validation centers in the death risk attribution ($p = 0.19$) (see Fig. 3). The predicted 12-month mortality derived from the Cox model with the PGS score set as a continuous variable showed a good concordance with the observed 12-month mortality obtained from actuarial survival analysis (Fig. 4). When the prediction model derived from the testing cohort was applied to the validation cohort, the predicted 12-month mortality was again in agreement with that observed (see Fig. 4).

With regard to the whole tumor series of 940 patients, there was a 34% increase in risk of death per 1-point increase in PGS score, with narrow 95% CIs (Fig. 5). Such excess risk was present in all variables under evaluation (center, sex, age, type of material, stage, chemotherapy, and histologic subtyping); it was lower only among patients unable to receive chemotherapy and in those with sarcomatoid tumor. The excess risk was also lower in patients with missing information about stage (HR = 1.29, 95% CI: 1.23–1.35) than in those with available information (HR = 1.42, 95% CI: 1.34–1.52), with a significant interaction ($p = 0.002$) between PGS score and stage missingness (see Fig. 5). Similarly, the ability of the PGS score to predict 12-month mortality was high in all subgroups, with AUCs around 0.70. Interestingly, PGS score was associated with deterioration of median survival of patients, while increasing its value regardless of histologic variant (Table 3).

Discussion

In the recent past, several histologic traits have been proposed as prognosticators in patients with pleural or peritoneal malignant mesothelioma, including Ki-67 LI,[21,22,27] cell/nuclear atypia,[22,26,30] nuclear-to-cytoplasmic ratio,[22] chromatin pattern,[22] intranuclear inclusions,[22] nuclear grade/size,[13,30] nucleolar prominence,[22,30] necrosis,[29,30] (atypical) mitoses,[13,22,26,28–30] lymphocyte infiltration,[28] and growth pattern,[28] mostly being effective in EMPM. They have been considered only as single or independent factors, with no interaction

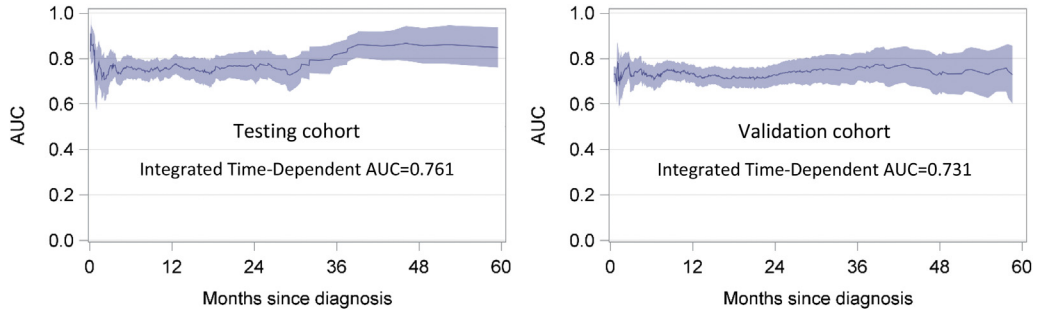

Figure 1. Receiver operating characteristic curves with the relevant area under curve (AUC) for the pathologic grading system, mitotic count, and Ki-67 LI as predictors of mortality at 12 months in the training and the validation cohorts of patients. The AUC values for the pathologic grading system, mitotic count, and Ki-67 LI as predictors of 2-year and 5-year OS were, respectively, 0.76, 0.71, and 0.69 and 0.85, 0.81, and 0.80 in the training cohort and 0.73, 0.72, and 0.64 and 0.73, 0.77, and 0.56 in the validation cohort.

analysis of variables or predictive evaluation for the type of patient cancer. It is thus difficult to attribute to these parameters a decision-making role in the clinical work-up of patients with MPM once tumors have been diagnosed on the basis of biopsy samples or surgical specimens so as to plan the subsequent clinical handling. A recent interaction analysis of prognostic variables in risk groups of patients with MPM has taken into account histologic subtyping solely among pathologic factors,[8] with the result that the clinical request for an effective PGS continues to remain an unanswered question.[1,8,9,15–17,19]

The goal of this study was to set up a PGS for use in patients with MPM that is able to identify tumor aggressiveness in the individual patient as exemplified by median survival thresholds according to different scores obtained by using a combination of mitotic count, Ki-67 LI, necrosis, and histologic subtyping.[34] We did not include any molecular information because we wanted to create a simple, reproducible, and inexpensive tool to be applied anywhere to help clinicians in patient management. Accordingly, we assembled the largest series of patients with MPM thus far investigated for grading purposes, which was labeled as a training cohort to fit the model (the Bari set), and a validation cohort to confirm the validity of the proposed model (the Milan, Modena, Padua, and Turin sets). An interobserver agreement test served to select the most reproducible variables to insert in the prediction model. For the construction of a widespread prediction model, we took into consideration only those histopathologic parameters that show good reproducibility between centers/pathologists and are well-known parameters by most pathologists.

Of note, the four parameters fitting the model are widely known by pathologists faced with malignant mesothelioma, either pleural or peritoneal,[13,21,22,26–30]

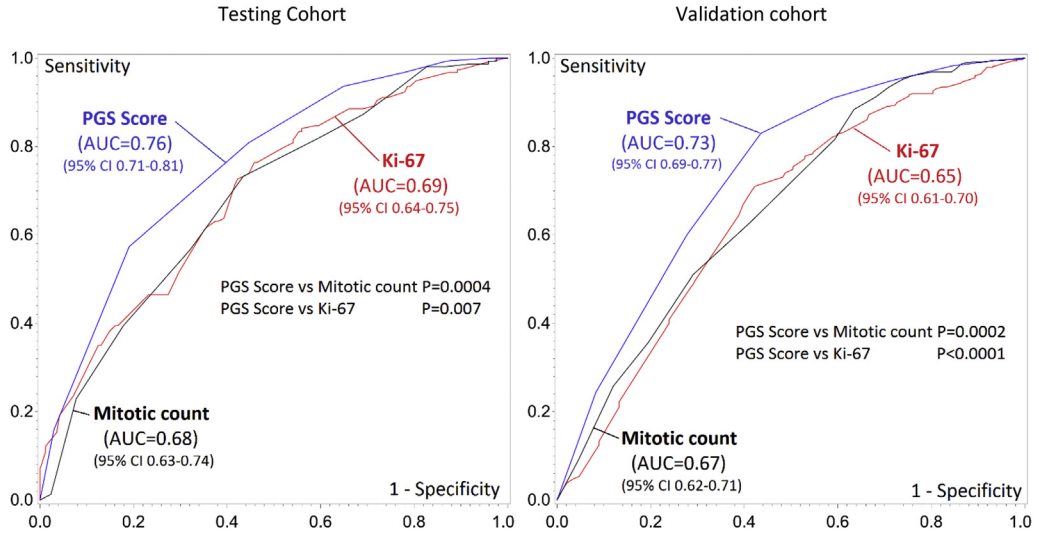

Figure 2. Time-dependent area under the curve (AUC) in the training cohort and in the validation cohort. The shaded area represents the 95% confidence intervals (CIs). Abbreviation: PGS, pathologic grading system.

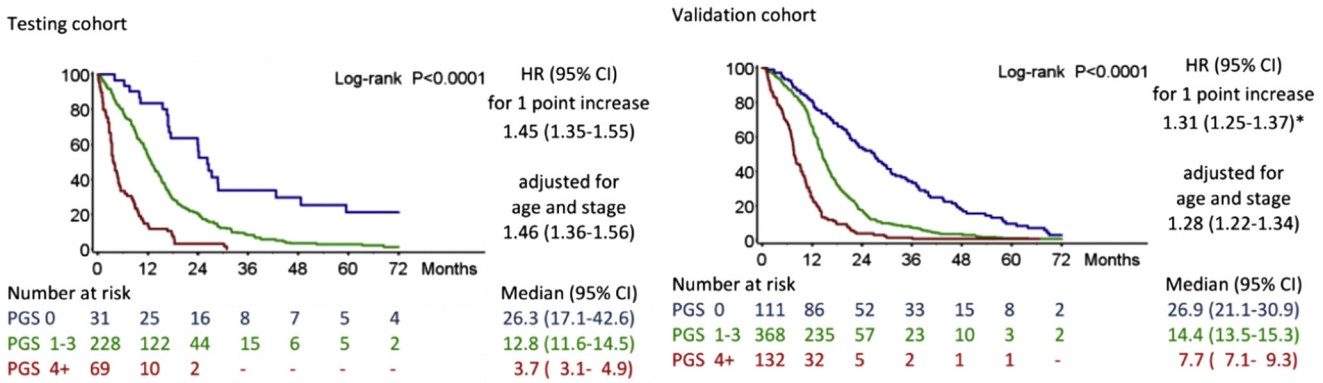

Figure 3. Overall survival after diagnosis of malignant pleural mesothelioma according to the pathologic grading system (PGS) in the training and the validation cohorts of patients. There was 31% to 45% death risk increase for every 1-point increment of the hazard ratio (HR) values, and median survival deteriorated over time according to the increase in PGS scoring. The Milan HR value was 1.19 (95% confidence interval [CI]: 1.06-1.34); the Modena HR value was 1.31 (95% CI: 1.23-1.41); the Padua HR value was 1.39 (95% CI: 1.17-1.65); and the Turin HR value was 1.39 (95% CI: 1.28-1.52); no heterogeneity was observed across the four validation centers ($p = 0.19$).

but only those showing excellent agreement beyond chance (κ score >0.75) eventually entered the predictor. Cell atypia[30] and prominence of nucleoli, which have been used in other studies,[13,22,30] were discarded inasmuch as the relevant κ value indicated only a good agreement beyond chance. We chose to assess interobserver rather than intraobserver agreement[35] for all potentially eligible variables because they should have been of clinical help in the practical management of patients with MPM.

The mitotic count was quantified per 1 mm^2 for a quick and easy assessment and hence was more practical than the system based on the evaluation of larger tumor areas,[22,26] also considering the well-known plateau effect when cell counting is expanded to larger tumor areas[34] and there is some variability in the definition of high-power microscopic fields.[22] Furthermore, guidelines in assessing mitoses in malignant mesothelioma are still lacking.[7,31,36] We evaluated Ki-67 LI on hot spot areas by counting 2000 cells or 1 mm^2 in the same tumor block and areas as mitotic count for the sake of comparison because this numeric value averaged 4.5 quantified fields each measuring 0.237 mm^2 (microscopic diameter 0.55 mm).[34] This procedure has been shown to provide reproducible results in biopsy samples and paired surgical specimens of lung neuroendocrine neoplasms, which are comparable in terms of tumor cellularity, also confirming the Ki-67 LI plateau effect during evaluation of larger tumor surfaces.[34,37]

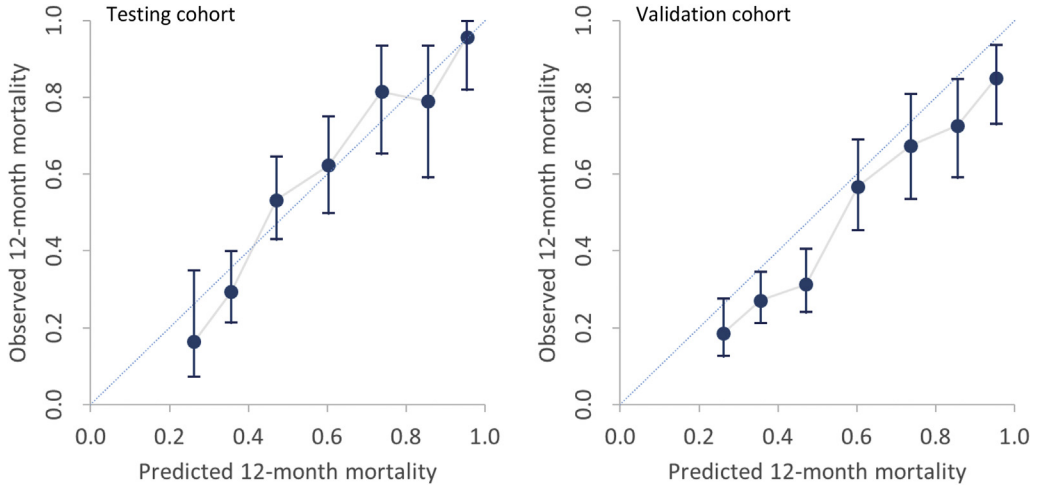

Figure 4. Calibration of the predicted 12-month mortality in the training cohort and in the validation cohort. The predicted 12-month mortality is derived from a univariate Cox proportional hazard regression model fitted in the testing cohort, with the pathologic grading system score set as a continuous variable ($1\text{-}S_0^{\exp(0.37086*PGS)}$, where the baseline survival estimate is $S_0 = 0.73840$ [for a subject with a pathologic grading system score of 0]).

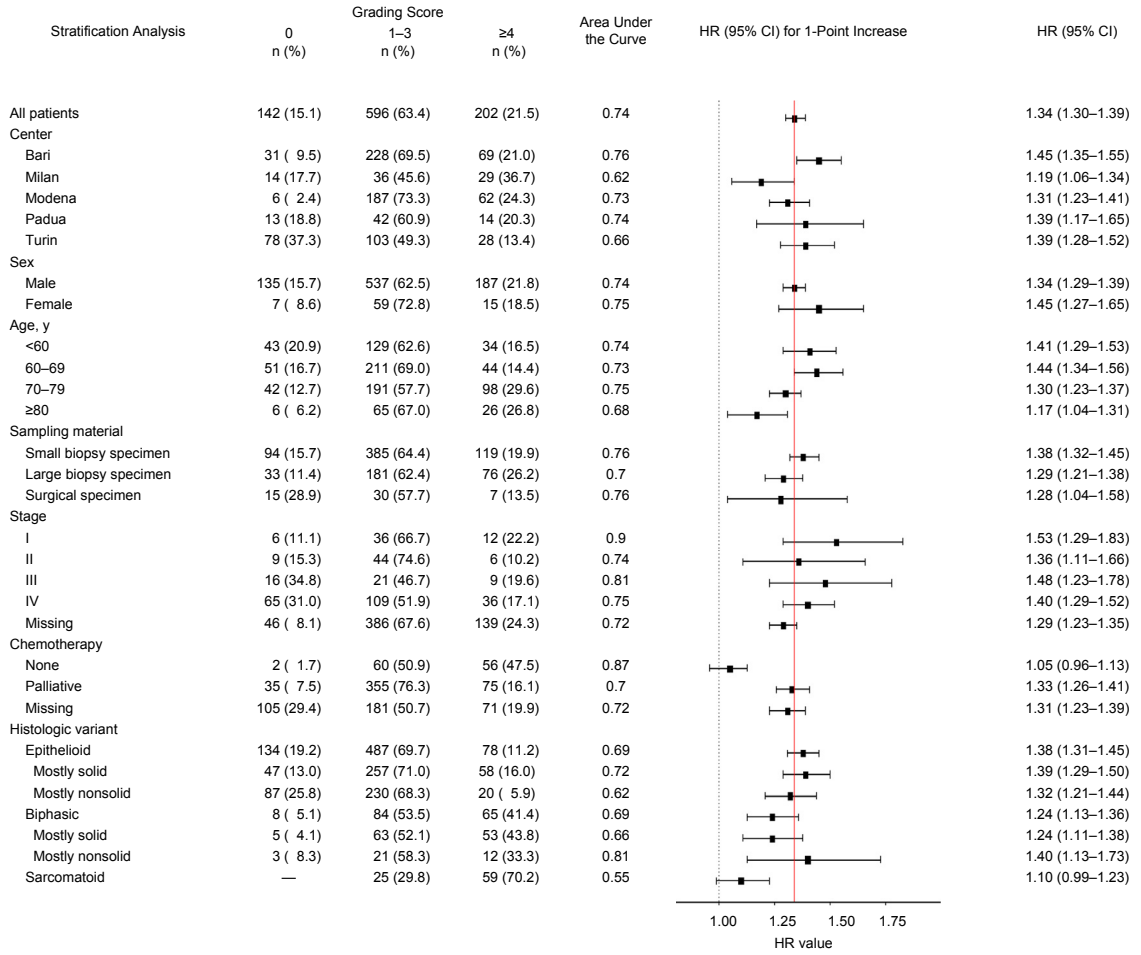

Figure 5. Stratified analysis by forest plot of scoring pathologic grading system for several variables along with area under the curve evaluation for each variable being investigated. HR, hazard ratio; 95% CI, 95% confidence interval.

It could be said that the more cells that were counted, the more interobserver differences were minimized until a plateau effect was reached, after which the results no longer changed. These considerations may explain why our predictor was somewhat independent of the material being analyzed, as was recently noted by others,[30] also suggesting a minor intratumor behavioral heterogeneity of MPM despite its claimed polyclonal origin[38] and genetic complexity.[39,40] Furthermore, we chose to count cells with a manual counter rather than by using automated analysis systems so as to make results more applicable in daily practice, where automated imaging systems can be inaccessible or cumbersome.

Table 3. Numbers of Patients and Their Median Overall Survival by Histologic Variant and Pathologic Grading System Scored

Histologic Variant	Median Overall Survival, mo (95% CI)		
	Score 0	Score 1-3	Score ≥4
Epithelioid	n = 134 27.1 (23.1-30.9)	n = 487 14.6 (13.7-15.3)	n = 78 9.4 (6.4-10.6)
Biphasic	n = 8 11.3 (4.6-28.9)	n = 84 11.1 (9.1-13.4)	n = 65 7.4 (5.3-8.9)
Sarcomatoid	—	n = 25 8.6 (4.4-10.2)	n = 59 4.2 (2.7-5.5)

Note: The testing and validation cohorts have been combined.
CI, confidence interval.

The close association between mitotic count and Ki-67 with a relatively low coefficient indicative of wider dispersion of values was largely expected, as these biomarkers are related to different measures of cell cycle.[41–43] The combined use of both proliferation indices can compensate for assessment errors in either parameter, as suggested in other tumor grading systems based on mitoses and Ki-67 LI simultaneously.[44,45]

Histologic subtyping is currently considered the single most important parameter and basically represents the main contribution of pathology alongside tumor staging to the clinical work-up of malignant mesothelioma[7,13,21,22,26–30] and related clinical prediction models,[1,2,8,9,15–17,19] whether pleural or peritoneal. Accordingly, this criterion was considered a backbone

of the PGS. As the amount of the sarcomatoid component has been said to be relevant to prognosis of BMPM,[7,31] we investigated these tumors in a preliminary analysis of the testing cohort according to the amount of sarcomatoid component (data not shown). In 29 out of 73 patients with BMPM, the tumor was mostly epitheliod and in 44 it was mostly sarcomatoid. At univariate analysis, patients with mostly BMPM had a risk of death similar to that in patients with EMPM (HR = 1.22, 95% CI: 0.82–1.83), whereas patients with mostly sarcomatoid biphasic tumor had an increased risk of death somewhat similar to that in patients with pure sarcomatoid tumor (HR = 2.27, 95% CI: 1.63–3.17). However, in a fully adjusted model (for age, necrosis, tumor grade, nucleoli, cell atypia, Ki67, p16 alterations by fluorescence in situ hybridization, mitotic count, and tumor stage) this association for patients with mostly sarcomatoid BMPM lost any statistical significance (HR = 1.31, 95% CI: 0.91–1.87), whereas it remained strong and significant for pure SMPM (HR = 2.29, 95% CI: 1.43–3.65). Accordingly and for the sake of simplicity, we decided to regroup patients with epithelioid and biphasic tumors together, thereby assigning 2 points for PGS score only to patients with pure SMPM.

Necrosis in turn has been only occasionally addressed as a prognosticator in MPM, having a negative impact on prognosis alone or along with nuclear grade.[13,29,30] In our study, necrosis was the least powerful prognosticator in both the training and validation sets, likely because of some colinearity with mitoses and Ki-67 LI as a reflection of disturbed growth in cell proliferation-deregulated tumors.[46] Nonetheless, it was considered in the final model, as it contributed to forecasting prognosis deterioration independently of the other parameters.

The multiparametric approach of this PGS is the first innovative approach to prognosis assessment of MPM because it was based on the evaluation of reproducible histopathologic factors and provided information on specific cancers. As a matter of fact, we noticed steady deterioration of survival in both patient cohorts for each point increase in the PGS score. As an example, the median survival of patients in the validation set dropped from 26.9 months to 14.4 months and 7.7 months with PGS scores of 0, 1 to 3, and 4 to 8, respectively (see Fig. 2). The different institutions did not affect the validation results, thus further stressing the robustness and reproducibility of our four-tiered histologic model with which results were obtained by four independent pairs of pathologists. Stratification analysis based on the whole series of 940 tumors again confirmed a steady progression of risk of death as a function of scoring. Some apparent discrepancies emerging from the sensitivity analyses likely reflected minor representation of aggressive MPM in some validation centers (e.g., the Milan set had a minor presence of BMPM and SMPM, elderly patients suffering from comorbidities, no chemotherapy treatment in patients with poor performance status, and the well-known rarity of SMPM [84 of 940 (8.9%)] in our study) compared with the representation of other histologic subtypes.[7,31,36]

A limitation of our study could be the lack of information on tumor staging in a large proportion of patients (about 60%). This should not have played a decisive role in influencing the results, as the association between PGS score and OS was present and statistically significant both in patients with defined stage and in those with missing stage, although it was weaker among patients with missing stage (interaction $p = 0.002$). Even so, this consideration leaves open the interpretation that tumor stage actually represents the final clinical outcome according to duration of the preclinical phase, which in turn is likely to temporally depend on the intrinsic aggressiveness of individual tumors.

Thus far, the histologic subtypes of MPM have been considered monolithic entities for clinical strategies to manage, with a case mix of diversely surviving patients still obvious in the epidemiologically prevalent epithelioid subtype, to the extent that BMPM and SMPM are often merged as nonepithelioid tumors for cumulative risk of death to assess.[1,2,7,18] Of note, our PGS was also effective in subgroup analysis of MPM, in which similar survival was seen across different histologic subtypes according to the increase in PGS score, thus sustaining once again the validity of the model. Other potentially interfering factors for either biological or methodological issues, such as chemotherapy administration, tumor staging, and histologic subtyping within the categories EMPM and BMPM, did not significantly affect the attribution of risk of death and survival according to our prediction model.

The precise position of a PGS in the clinical scenario of patients with MPM is still unclear,[1,2,7] but our evidence-based PGS dissecting tumor aggressiveness in different forms of MPM could also be a useful model to enroll patients for chemoradiotherapy, target treatments or surgery and palliation, plan follow-up strategies, complement predictive and prognostic molecular information, and design innovative clinical trials based on a managerial vision of such a disease.

Acknowledgments

This work is dedicated to the memory of Carlotta, an extraordinarily lively girl who died an untimely death due to cancer in the prime of her life.

Supplementary Data

Note: To access the supplementary material accompanying this article, visit the online version of the *Journal of Thoracic Oncology* at www.jto.org and at https://doi.org/10.1016/j.jtho.2018.07.002.

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