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A nuclear grading system is a strong predictor of survival in epitheloid diffuse malignant pleural mesothelioma

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Abstract

Epithelioid mesothelioma is the most prevalent subtype of diffuse malignant pleural mesothelioma in which only staging is prognostic for survival. In this study of epithelioid diffuse malignant pleural mesothelioma, we investigate the prognostic utility of nuclear features. The slides of 232 epithelioid diffuse malignant pleural mesothelioma patients (14 stage I, 54 stage II, 130 stage III, and 34 stage IV) from a single institution were reviewed for the following seven nuclear features: nuclear atypia, nuclear/cytoplasmic ratio, chromatin pattern, intranuclear inclusions, prominence of nucleoli, mitotic count, and atypical mitoses. MIB-1 immunohistochemistry was performed using tissue microarray, and MIB-1 labeling index was recorded as the percentage of positive tumor cells. Median overall survival of all patients was 16 months and correlated with nuclear atypia (P<0.001), chromatin pattern (P=0.031), prominence of nucleoli (P<0.001), mitotic count (P<0.001), and atypical mitoses (P<0.001) by univariate analysis. Multivariate analysis revealed nuclear atypia (P=0.012) and mitotic count (P<0.001) as independent prognostic factors, and these two factors were utilized to create a three-tier nuclear grade score. The resulting nuclear grade stratified patients into three distinct prognostic groups: grade I (n=107, median overall survival = 28 months), grade II (n=91, 14 months), and grade III (n=34, 5 months). Not only was nuclear grade an independent predictor of overall survival (P<0.001), but it was also a stronger discriminator of survival than all currently available factors. Furthermore, nuclear grade was associated with time to recurrence (P=0.004) in patients who underwent complete surgical resection (n=159). MIB-1 labeling index correlated with mitotic count (P<0.001) and nuclear atypia (P=0.037) and stratified overall survival (P<0.001) and time to recurrence (P=0.048),

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confirming the prognostic value of the nuclear grade. Nuclear grading in epithelioid mesothelioma provides a simple, practical, and cost-effective prognostic tool that better stratifies clinical outcome and time to recurrence than currently available clinicopathologic factors.

Keywords

epithelioid mesothelioma; MIB-1; mitosis; nuclear atypia; nuclear grade; survival

Diffuse malignant pleural mesothelioma is an aggressive malignancy of the pleural cavity known to develop after asbestos exposure.[1] Due to prolonged latency period following asbestos exposure, the incidence is projected to increase in many industrialized countries until 2020.[2] Despite aggressive trimodality therapy, the prognosis of diffuse malignant pleural mesothelioma remains poor with a median survival of 9–12 months.[3-5] Challenges in managing diffuse malignant pleural mesothelioma stem from a paucity of prognostic factors in stratifying patients for therapy and clinical outcomes.[3,4] At this time, therapeutic decisions are based on histology and the TNM staging system. The prognostic utility of the TNM staging is limited to differentiating between early stage (I–II) from late stage patients (III–IV) and lacks precision.[5] Improved prognostic stratification is necessary to optimize treatment options as well as to better stratify patients in clinical trials.

Epithelioid histology is a strong prognostic factor in diffuse malignant pleural mesothelioma and confers a better prognosis compared with biphasic and sarcomatoid histology.[4-9] Beyond histology, nuclear grading system has been shown to have prognostic utility in breast,[10,11] renal cell,[12,13] and bladder carcinomas.[14,15] Moreover, studies in breast and bladder carcinomas have shown associations between nuclear grading and molecular features,[16,17] underscoring the importance of nuclear grading beyond prognostic stratification. Yet, no attempts have been made to stratify prognosis by nuclear features within epithelioid diffuse malignant pleural mesothelioma. We herein report a comprehensive pathologic review of 232 epithelioid diffuse malignant pleural mesothelioma patients from a single institution with particular attention to nuclear features. Based on the multivariate analysis of a large series of epithelioid diffuse malignant pleural mesothelioma, we propose a simple prognostic nuclear grading system based on nuclear atypia and mitotic count.

Materials and methods

Patients

Clinical and pathological information on 232 patients diagnosed with epithelioid diffuse malignant pleural mesothelioma between 1989 and 2009 at Memorial Sloan-Kettering Cancer Center was collected through the Thoracic Surgery mesothelioma database and the Department of Pathology data file. Institutional Review Board approval was obtained for this study. Clinical variables recorded in the prospectively maintained database included age, gender, laterality, TNM stage, and surgical procedure. TNM staging was based on the reported imaging findings, the surgeon’s intraoperative findings, and the pathologic evaluation of the resected specimens using the sixth edition of the American Joint

Commission on Cancer Staging Manual.[18] All patients were followed until date of death or last follow-up.

Pathologic diagnosis was based on standard histologic, histochemical, and immunohistochemical criteria.[1,19,20] As a positive marker of immunohistochemistry for malignant pleural mesothelioma, standard immunohistochemical markers included calretinin, WT-1, cytokeratin 5/6, and D2-40. As negative markers for malignant pleural mesothelioma, we used carcinoembryonic antigen, CD15, B72.3, BerEP4, and thyroid transcription factor-1. In cases before positive mesothelial markers were available, negative markers were used for making the diagnosis of malignant pleural mesothelioma. In order to confirm original diagnosis, for specimens where tumor blocks were available, we performed immunohistochemistry for calretinin and WT-1. Only one case was negative for both markers; however, this particular patient's clinical and radiological findings were characteristic of diffuse malignant pleural mesothelioma. The pathological diagnosis was correlated with the gross distribution of tumor and the absence of an intrapulmonary mass lesion on radiologic imaging.

Histologic Evaluation

All available hematoxylin and eosin (H&E)-stained slides of each epithelioid diffuse malignant pleural mesothelioma patient (median: 9, range: 1–43 slides/case) were reviewed by a pathologist, and problem cases were reviewed by two pathologists. For each case, we evaluated the following nuclear features: (1) nuclear atypia, (2) nuclear/cytoplasmic (N/C) ratio, (3) chromatin pattern, (4) intranuclear inclusion, (5) prominence of nucleoli, (6) mitotic count, and (7) atypical mitoses. The presence of lymphatic and vascular invasion was also noted if at least one tumor cell cluster was visible within an endothelial lined lymphatic vessel or vein, respectively.

Nuclear features were evaluated using high-power-field (HPF) at ×400 magnification (0.237 mm^2 field of view) using an Olympus BX51 microscope (Olympus, Tokyo, Japan) with a standard eyepiece of 22 mm diameter. Mitoses were evaluated in 50 HPF areas (11.85 mm^2), with the highest mitotic activity identified after scanning through all tumor slides[21-24] and counted as an average of mitotic figures per 10 HPF.[22]

For nuclear atypia, area with the highest degree of atypia (nuclear size and irregularity) was assessed and graded as follows: mild atypia—uniform nuclei in size and shape (Figure 1a), moderate atypia—nuclei in intermediate size between mild and severe, with slight irregularity in shape (Figure 1b), and severe atypia—bizarre, enlarged nuclei of varied sizes, with some nuclei at least twice as large as others (Figure 1c).[11,14,23] Nuclear atypia was recorded only if it consisted of >5% of the entire tumor area. For each specimen, the most predominant N/C ratio and chromatin patterns were evaluated. N/C ratio was graded as by the following three categories: low—<1/3 nucleus-to-cytoplasm area (Figure 1d), intermediate—1/3–2/3 (Figure 1b), or high—>2/3 (Figure 1e). Chromatin pattern was graded as homogeneous (Figure 1e), fine granular (Figure 1a), or coarse granular (Figure 1c).[25,26] Prominence of nucleoli was evaluated using as reference nearby red blood cells, which measured approximately 7 μm, and graded as the following three categories by the measurement of predominant size: indistinct—inconspicuous or very small (Figure 1a),

distinct—<3 μm (Figure 1b), and large—≥3 μm (Figure 1d).[27] Intranuclear inclusions were determined as present or absent by examining 10–50 HPF (Figure 1f), depending on the number of available tumor slides for each case.[27]

We used the following criteria to distinguish mitotic figures from pyknotic cells: absence of a nuclear membrane or a central clear zone, presence of hairy rather than triangular or spiky projections that reflected a mitotic spindle and cytoplasmic basophilia rather than eosinophilia.[24] Areas of necrosis and prominent stromal fibrosis or inflammation were avoided whenever possible. In the cases in which only small areas of viable tumor were available for review, the best attempt was made to assess the equivalent of 10 full HPF of viable tumor for mitosis counting.[22] Tumors were graded into the following three groups by mitotic count number using optimal cutoff values associated with the difference in overall survival: low—0–1/10 HPF, intermediate—2–4/10 HPF, and high—≥5/10 HPF. Atypical mitoses were defined as the presence of abnormal chromosome spread, tripolar or quadripolar forms, circular, or indescribably bizarre forms.[28] Atypical mitoses were determined as present or absent by examining 10–50 HPF (Figure 1c), depending on the number of available tumor slides for each case.

Additionally, since we recently proposed that pleomorphic epithelioid malignant pleural mesothelioma should be reclassified as biphasic or sarcomatoid mesothelioma due to the poor prognosis,[29] we further analyzed this data set after excluding the pleomorphic cases.

Tissue Microarray

Formalin-fixed, paraffin-embedded tumor specimens were used for tissue microarray construction. Briefly, six representative tumor areas were marked on H&E-stained slides, and cylindrical 0.6-mm tissue cores were arrayed from the corresponding paraffin blocks into a recipient block using an automated tissue arrayer ATA-27 (Beecher Instruments, Sun Prairie, WI, USA), resulting in four tissue microarray blocks. From each tissue microarray, 4-μm-thick paraffin sections were prepared for immunohistochemistry. In all, 158 cases with adequate cores were available for immunohistochemical analysis.

Immunohistochemistry and Scoring of MIB-1

Briefly, 4-μm sections from the tissue microarray blocks were deparaffinized in xylene and dehydrated in graded alcohols. Standard avidin–biotin complex peroxidase technique was used for immunohistochemical stain of anti-Ki-67 antibody (clone MIB-1, Immunotech, Westbrook, ME, USA; diluted at 1 : 100). Sections were stained using a Ventana Discovery XT automated immunohistochemical stainer (Ventana, Tucson, AZ, USA) according to the manufacturer's guidelines. Diaminobenzidine was used as the chromogen and hematoxylin as the nuclear counterstain. Positive control tissues were stained in parallel with the study cases.

MIB-1 labeling index was recorded as the percentage of tumor cells with positive nuclear immunostaining in each tissue microarray core. The average values of the cores were considered as MIB-1 labeling index for each patient. For the purpose of survival analysis,

tumors were classified into the following two groups by median MIB-1 labeling index (10.0%): low <10.0%, and high ≥10.0%.

Statistical Analysis

Associations between clinicopathologic variables and histologic findings were analyzed using Fisher's exact test for categorical variables, Cochran Armitage test for ordinal variables, and Wilcoxon test for continuous variables. Overall survival following surgery was estimated using the Kaplan–Meier method, with patients censored if they were alive at the time of last follow-up. An analysis of time to recurrence was restricted to patients who underwent surgery that was deemed to be a complete resection. Non-parametric group comparisons were performed using log-rank test adjusted for stage.[30] Multivariate analyses were performed using the Cox proportional hazard regression model to study the effects of different variables on overall survival. All *P*-values were based on two-tailed statistical analysis, and a *P*-value <0.05 was considered to indicate statistical significance. All analyses were performed using SAS statistical software (version 9.2; SAS Institute, Cary, NC, USA) and the 'clinfun' package in R (http://www.r-project.org).

Results

Clinicopathologic Demographics and Their Association with Overall Survival

Clinicopathologic profile of all patients is outlined in Table 1. There were 232 patients with a median age 64 years (range: 29–85), 72% of which were males. The tumor involved the left pleura in 45% (*n* = 104) of the cases. Fourteen (6%) of the patients were stage I, 54 (23%) were stage II, 130 (56%) were stage III, and 34 (15%) were stage IV. By procedure, 115 (50%) underwent extrapleural pneumonectomy, 91 (39%) pleurectomy-decortication, and the remaining 26 (11%) had other procedures (11 biopsies, 10 exploratory thoracotomies, 3 palliative pleurectomies, and 2 video-assisted thoracoscopic surgeries). Lymphatic invasion was detected in 44% (*n* = 102), and vascular invasion in 23% (*n* = 54).

Among all patients, median overall survival was 16 months, with a 2-year overall survival of 34% and 5-year overall survival of 11%. On univariate analyses, older age (>65 years) (*P* = 0.046), right-sided disease (*P* = 0.040), higher T stage (T3–4) (*P* = 0.013), advanced stage (stages III–IV) (*P* = 0.007), lymphatic invasion (*P*<0.001), and vascular invasion (*P*<0.001) were associated with worse overall survival (Table 1).

Nuclear Features and Their Association with Overall Survival

Nuclear features and their association with overall survival are outlined in Table 2. Of the seven nuclear features examined, five were significant predictors of overall survival: nuclear atypia (*P*<0.001), chromatin pattern (*P* = 0.031), prominence of nucleoli (*P*<0.001), mitotic count (*P*<0.001), and atypical mitoses (*P*<0.001). Nuclear atypia (*P* = 0.005), mitotic count (*P* < 0.001), and the presence of atypical mitoses (*P* < 0.001) were prognostic of overall survival in the 68 early stage patients (stages I–II). As well, nuclear atypia (*P* < 0.001), prominence of nucleoli (*P* = 0.002), mitotic count (*P* < 0.001), and presence of atypical mitoses (*P* < 0.001) correlated with overall survival in the 130 stage III patients.

For nuclear atypia, patients with severe atypia had the worst median overall survival (n = 46, 8 months), followed by moderate (n = 74, 15 months) and mild (n = 112, 23 months) (Figure 2a). Overall survival was significantly different between severe and moderate atypia as well as moderate and mild atypia (P = 0.003 and P = 0.003, respectively). For chromatin pattern, patients with coarse granular chromatin had the worst median overall survival (n = 96, 11 months), followed by fine granular (n = 112, 19 months) and homogeneous (n = 24, 25 months) (Figure 2b). Patients with large nucleoli had the worst median overall survival (n = 55, 11 months), followed by distinct (n = 122, 16 months) and indistinct (n = 55, 25 months) (Figure 2c). Mitotic count ranged from 0 to 64 per 10 HPF (median, 3.0; mean ± s.d., 5.0 ± 6.9), and patients with high mitotic counts had the worst median overall survival (n = 81, 10 months), followed by intermediate (n = 76, 17 months) and low mitotic counts (n = 75, 31 months) (Figure 2d). Overall survival was significantly different between high and intermediate mitotic counts as well as intermediate and low (P < 0.001 and P = 0.003, respectively). The presence of atypical mitoses were associated with worse median overall survival (n = 55, 8 months) compared with absence (n = 177, 19 months) (P < 0.001). N/C ratio was not a significant prognostic factor. Intranuclear inclusion was seen only in seven cases, which was not insufficient to perform overall survival analysis.

On multivariate analysis of nuclear features, nuclear atypia (hazard ratio (HR) = 1.89, 95% confidence interval (CI) = 1.15–3.10, P = 0.012) and mitotic count (HR = 2.79, 95% CI = 1.69–4.59, P < 0.001) were found to be independent prognostic factors (Table 3).

Nuclear Grading System in Epithelioid Diffuse Malignant Pleural Mesothelioma

We developed a nuclear grading system based on the two independent prognostic factors on multivariate analysis—nuclear atypia and mitotic count. For nuclear atypia, tumors were scored as 1 for mild, 2 for moderate, and 3 for severe atypia. For mitotic count, tumors were scored as 1 for low, 2 for intermediate, and 3 for high. In each case, a total score was computed as the sum of the two-parameter scores, ranging from 2 to 6. Figure 3a shows the overall survival curve in all patients by this scoring scheme. Scores of 2 (n = 60, median overall survival = 31 months) and 3 (n = 47, 24 months) showed the best overall survival. Scores of 4 (n = 52, 15 months) and 5 (n = 39, 12 months) showed similar outcome with intermediate overall survival. A score of 6 showed the worst overall survival (n = 34, 5 months). On the basis of these results, we simplified our scoring scheme into a three-tier grade: grade I for total scores 2 or 3, grade II for total scores 4 or 5, and grade III for a total score 6. Patients with nuclear grade III (n = 34) had the worst median overall survival (5 months), followed by grade II (n = 91, 14 months) and grade I (n = 107, 28 months) (P < 0.001) (Figure 3b), with significant differences seen between each grade (P < 0.001 for grades I *vs* II, P = 0.001 for grades II *vs* III). These observations were replicated in a cohort of 68 early stage patients (stages I–II) (P < 0.001) and in 130 stage III patients (P < 0.001).

On multivariate analysis including all factors found to be prognostic in our series, in addition to right-sided disease (HR = 1.36, 95% CI = 1.01–1.83, P = 0.046) and lymphatic invasion (HR = 1.50, 95% CI = 1.09–2.08, P = 0.014), nuclear grade (II *vs* I: HR = 2.11, 95% CI = 1.52–2.94, P < 0.001; III *vs* II: HR = 4.16, 95% CI = 2.47–7.03, P < 0.001) was a strong independent predictor of worse overall survival, as shown in Table 4.

As we have recently proposed to reclassify the pleomorphic subtype epithelioid diffuse malignant pleural mesothelioma as biphasic or sarcomatoid due to poor prognosis,[29] we repeated our analysis of nuclear features in a cohort of epithelioid diffuse malignant pleural mesothelioma excluding the 34 pleomorphic cases. In these 198 patients, our findings of nuclear features remained significant. Patients with severe atypia had the worst median overall survival (n = 12, 5 months) followed by moderate (n = 74, 15 months) and mild (n = 112, 23 months) ($P < 0.001$). Patients with high mitotic count had the worst median overall survival (n = 56, 11 months), followed by intermediate (n = 68, 18 months) and low (n = 74, 31 months) ($P < 0.001$). Nuclear grading remained prognostic with grade III patients exhibiting the worst median overall survival (n = 9, 5 months), followed by grade II (n = 82, 13 months) and grade I (n = 107, 28 months) ($P < 0.001$).

Association Between Nuclear Grade and Time to Recurrence

Having shown the value of nuclear features in predicting overall survival, we next sought to determine their prognostic value in predicting recurrence. Among 159 patients who underwent complete resection, patients with severe nuclear atypia (n = 31) had the shortest median time to recurrence (14 months), followed by moderate atypia (n = 53, 18 months) and mild atypia (n = 75, 25 months) ($P = 0.037$; Figure 4a). Patients with high (n = 57) and intermediate mitotic counts (n = 60) had shorter median time to recurrence (14 and 16 months, respectively) than low (n = 42, 67 months) ($P < 0.001$; Figure 4b). Patients with nuclear grade II (n = 69, 16 months) had shorter median time to recurrence than grade I (n = 68, 37 months), while the median was not met in patients with grade III (n = 22) ($P = 0.004$; Figure 4c). Similar observations by nuclear atypia, mitotic count, and nuclear grade were made in a cohort of 53 early stage patients (stages I–II) ($P = 0.019$, 0.003, and 0.012, respectively) and 99 stage III patients ($P = 0.023$, 0.035, and 0.017, respectively).

Association Between Nuclear Features and Clinicopathologic Factors

Severe nuclear atypia was associated with lymphatic invasion ($P < 0.001$), vascular invasion ($P < 0.001$), and increased T stage ($P = 0.037$). High mitotic count was associated with lymphatic invasion ($P < 0.001$), vascular invasion ($P < 0.001$), lymph node metastasis ($P < 0.001$), and advanced stage (stages III and IV) ($P = 0.008$). Higher nuclear grade was associated with male gender ($P = 0.043$), lymphatic invasion ($P < 0.001$), vascular invasion ($P < 0.001$), advanced overall stage ($P = 0.003$), and showed an increased tendency for higher T stage ($P = 0.065$) and lymph node metastasis ($P = 0.054$) (Table 5). Nuclear grade showed no association with age or laterality.

MIB-1 Labeling Index and Their Association with Nuclear Grade and Prognosis

To confirm our finding of nuclear grade on H&E-stained slides, we then compared our results to MIB-1 labeling index, a known marker of proliferation. When comparing MIB-1 labeling index and mitotic count as continuous variables, a moderately significant positive correlation was found (rank correlation coefficient = 0.44, $P < 0.001$). Tumors with high mitotic count (n = 59) had the highest MIB-1 labeling index (mean ± s.d., 22.0 ± 15.8), followed by intermediate (n = 53, 12.5 ± 11.4) and low (n = 46, 9.0 ± 7.8) ($P < 0.001$). Tumors with severe nuclear atypia (n = 34) had the highest MIB-1 labeling index (mean ±

s.d., 21.6 ± 17.8), followed by moderate (n = 50, 14.5 ± 12.8) and mild (n = 74, 12.3 ± 10.7) (P = 0.037).

Furthermore, patients with high MIB-1 labeling index (n = 88) had significantly worse median overall survival (12 months) than low MIB-1 labeling index (n = 70, 23 months) (P < 0.001; Figure 5a). Among patients who underwent complete resection, patients with high MIB-1 labeling index (n = 68) had shorter median time to recurrence (16 months) than low MIB-1 labeling index (n = 55, 25 months) (P = 0.048; Figure 5b).

Discussion

Among the three main histologic types of diffuse malignant pleural mesothelioma in the 2004 WHO Classification—epithelioid, biphasic, and sarcomatoid,[20] epithelioid subtype is the most prevalent and has the best prognosis.[4-9] Currently, there is a lack of prognostic factors to further stratify clinical outcomes in epithelioid diffuse malignant pleural mesothelioma patients. The available clinical parameters in our series (T1–2 *vs* T3–4, N0 *vs* N1–3, and stages I–II *vs* III–IV) at the best can differentiate patients by overall survival between 15 and 20 months, with a distribution of ~60 *vs* 40%. The available pathological features (lymphatic and vascular invasion) at the best can differentiate outcome by overall survival of 10 *vs* 20 months, with a distribution of ~70 *vs* 30%. In an effort to further stratify clinical outcome and investigate the biology of epithelioid diffuse malignant pleural mesothelioma, we have recently proposed a morphological classification.[29] In this study, we propose and demonstrate the prognostic utility of a nuclear grading system in epithelioid diffuse malignant pleural mesothelioma.

Based on an investigation of a large series of epithelioid diffuse malignant pleural mesothelioma, the nuclear grading system we propose is (1) cost-effective in that the assessment can be performed on routine H&E slides, (2) practical to implement as most pathologists are very familiar with nuclear atypia and mitotic counts that are commonly utilized in other cancers, (3) stratifies epithelioid diffuse malignant pleural mesothelioma patients into three distinct groups with relatively larger median overall survival differences (28 *vs* 14 *vs* 5 months) compared with the available clinicopathological factors discussed above, and (4) stratifies patients into groups with distinct distribution (46 *vs* 39 *vs* 15%). The strength of our proposed nuclear grading is demonstrated by the fact that it remained significant in subcohorts of stages I–II and stage III patients and also in its ability to predict time to recurrence. Furthermore, these findings remained significant after excluding patients with a pleomorphic subtype, which we recently reported as the subtype showing the poorest prognosis among epithelioid diffuse malignant pleural mesothelioma.[29]

In addition to prognostic stratification, nuclear grade also showed associations with tumor biology such as lymphatic invasion, vascular invasion, higher TNM stage, and an increased tendency for higher T stage and lymph node metastasis. These findings are consistent with what has been observed in other malignancies[15,17,26,31,32] and confirms that our grading system correlates with the malignant potential of epithelioid diffuse malignant pleural mesothelioma.

We evaluated seven nuclear features in the current study, out of which two—nuclear atypia and mitotic count—were independent prognostic factors. Nuclear atypia has been reported as a prognostic factor in various tumors such as lung,[23,32] breast,[11] renal cell,[12,13] and bladder carcinomas.[14,15] Similar to what is observed in other cancers,[33] nuclei of individual diffuse malignant pleural mesothelioma tumor cells vary in size, shape, and chromatin pattern, both in comparison with normal nuclei and also among the tumor cells. In our study, nuclear atypia, chromatin pattern, and prominence of nucleoli were closely related to each other, and each factor was associated with worse overall survival. Multivariate analysis of nuclear features revealed nuclear atypia as an independent prognostic factor.

In our series, mitotic count was one of the most important prognostic variables. This result is consistent with other studies in non-small cell lung carcinoma,[34,35] breast carcinoma,[10,11,21] bladder carcinoma,[15] and sarcoma.[36-38] In mesothelioma, mitotic count in peritoneal mesothelioma[27] and MIB-1 labeling index in pleural mesothelioma[39,40] have been previously reported as prognostic factors. To the best of our knowledge, however, this is the first report to show the prognostic significance of mitotic count in a large series of epithelioid diffuse malignant pleural mesothelioma. Our findings on H&E-stained slides were confirmed by comparing with MIB-1 labeling index. MIB-1 labeling index correlated with mitotic count and nuclear atypia, and stratified overall survival and time to recurrence in our cohort. An important advantage of mitotic count over MIB-1 is that it can be determined using H&E slides within routine clinical work.

Atypical mitoses have been well recognized as one of the histologic factors to determine malignant potential in adrenocortical tumors and pheochromocytomas of the adrenal gland.[28,41] In peritoneal mesothelioma, Cerruto *et al*[27] reported that the presence of atypical mitoses was a significant indicator of poor survival. In this study, we observed similar results for diffuse malignant pleural mesothelioma patients with atypical mitoses but observed mitotic count as being a stronger prognostic factor.

One important issue in reporting histopathological results is the issue of interobserver variability, which has been addressed in nuclear grading,[10,42] mitoses,[43,44] and atypical mitoses.[45] Concerns of interobserver variability and reproducibility of nuclear grading have been answered in earlier publications by utilizing well-defined criteria,[24,28] which were used in our study.

In recent years, several attempts have been made to develop a better classification for epithelioid diffuse malignant pleural mesothelioma.[5,9,46-49] Our comprehensive evaluation of a histologic nuclear grading system suggests that nuclear features are important prognostic markers in epithelioid diffuse malignant pleural mesothelioma and can be incorporated into future studies investigating clinical, molecular, and radiographic findings. This nuclear grading system provides simple and useful prognostic information for individual patients and will help physicians in deciding clinical management. Most pathologists are familiar with grading systems based on nuclear features as similar methods are applied to tumors in other organs.[10,11] As such, our findings in nuclear grading of epithelioid diffuse malignant pleural mesothelioma by nuclear atypia and mitosis should be readily applicable in clinical diagnosis and research.

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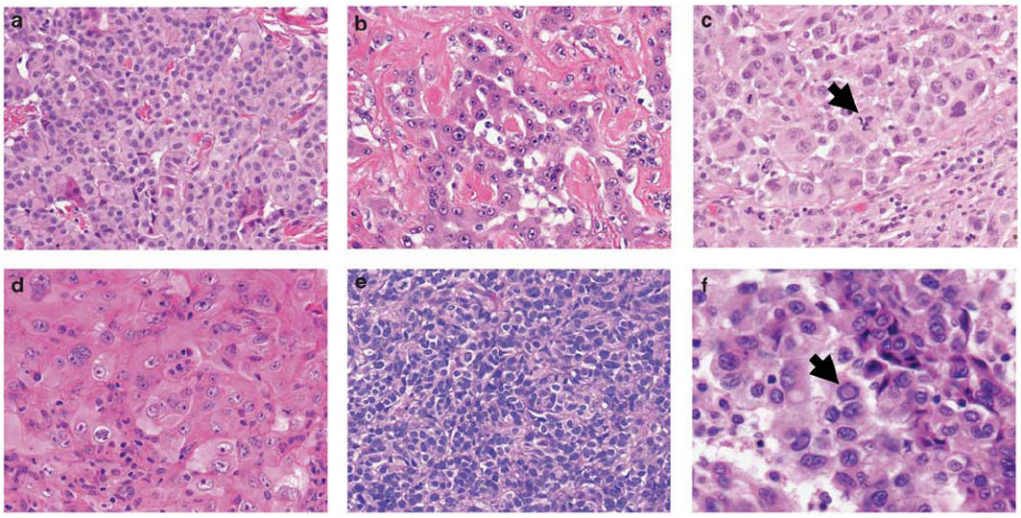

Figure 1.

Nuclear features of epithelioid diffuse malignant pleural mesothelioma (H&E stain; original magnification, ×400: **a–e**, ×600: **f**). (**a**) Tumor cells showing mild nuclear atypia, fine granular chromatin, and indistinct nucleoli. (**b**) Tumor cells showing moderate nuclear atypia, intermediate N/C ratio, and distinct nucleoli. (**c**) Tumor cells showing severe atypia, coarse granular chromatin, and atypical mitosis (arrow). (**d**) Tumor cells showing low N/C ratio and large nucleoli. (**e**) Tumor cells showing high N/C ratio and homogeneous chromatin. (**f**) Tumor cells with intranuclear inclusion (arrow).

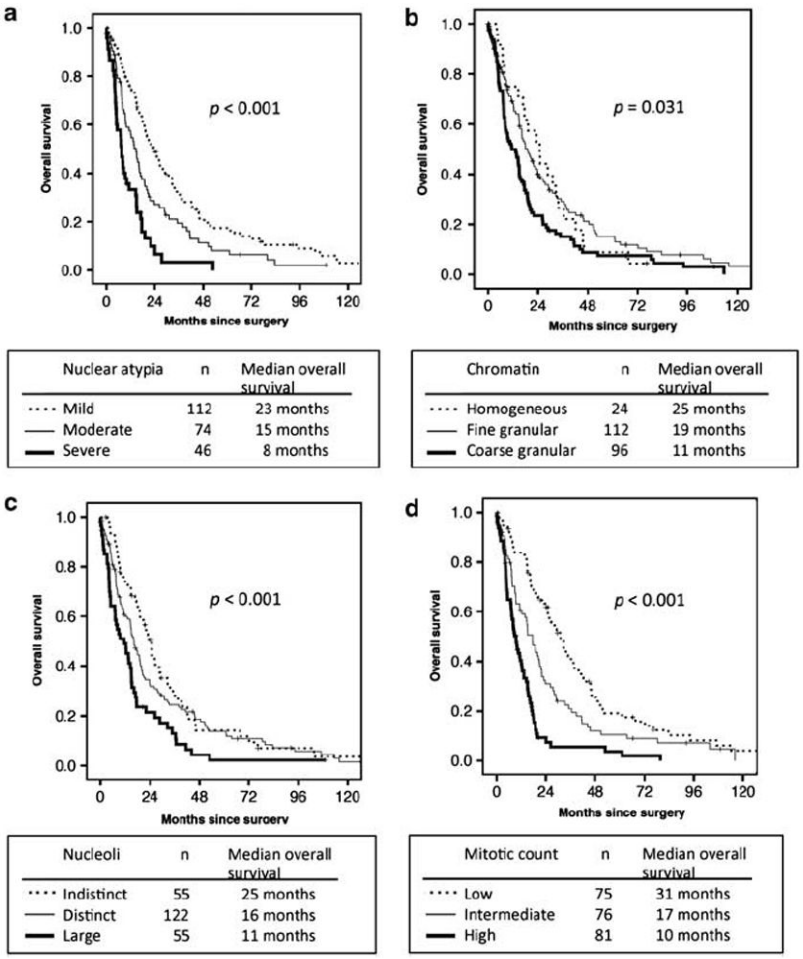

Figure 2.

Overall survival by nuclear features in all patients. (**a**) Patients with severe nuclear atypia had the worst median overall survival, followed by moderate and mild. (**b**) Patients with coarse granular chromatin had the worst median overall survival, followed by fine granular and homogeneous. (**c**) Patients with large nucleoli had the worst median overall survival, followed by distinct and indistinct. (**d**) Patients with high mitotic count had the worst median overall survival, followed by intermediate and low.

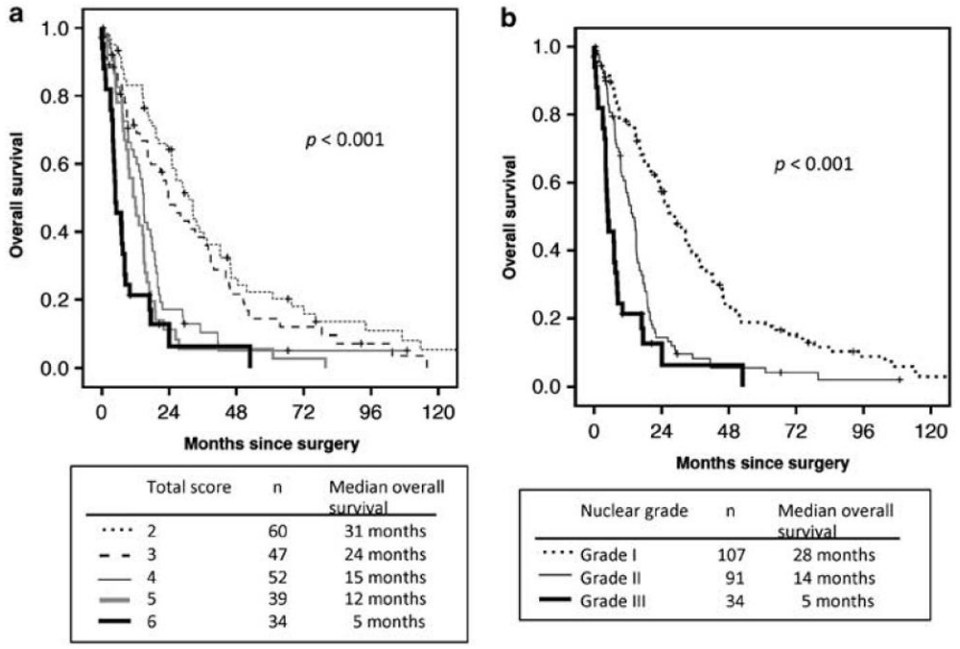

Figure 3.

Overall survival by nuclear grade in all patients. (**a**) Nuclear grading scores of 2 and 3 had the best overall survival. Score of 4 and 5 showed similar overall survival curves, with an intermediate overall survival. Total score of 6 showed the worst overall survival. (**b**) Patients with grade III had the worst median overall survival, followed by grade II and grade I.

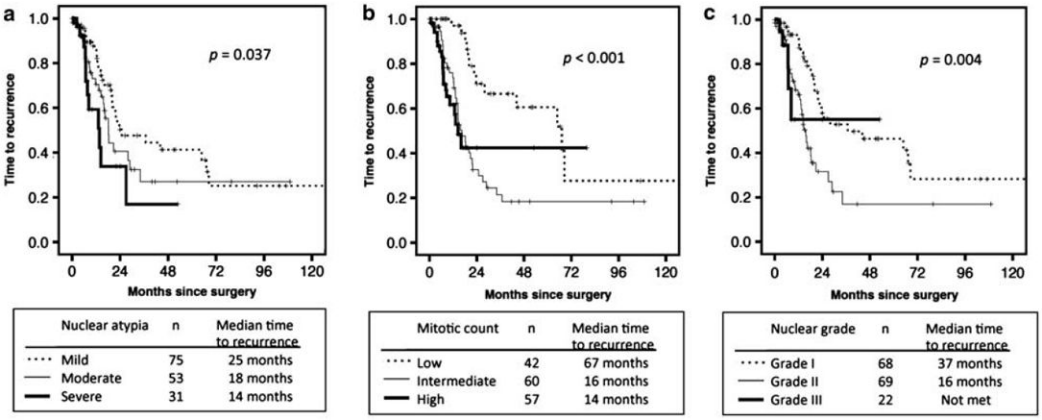

Figure 4.

Association of time to recurrence with nuclear grade in all patients. (**a**) Patients with severe nuclear atypia had the shortest median time to recurrence, followed by moderate and mild atypia. (**b**) Patients with high and intermediate mitotic count had shorter median time to recurrence than low. (**c**) Patients with nuclear grade II had shorter median time to recurrence than grade I.

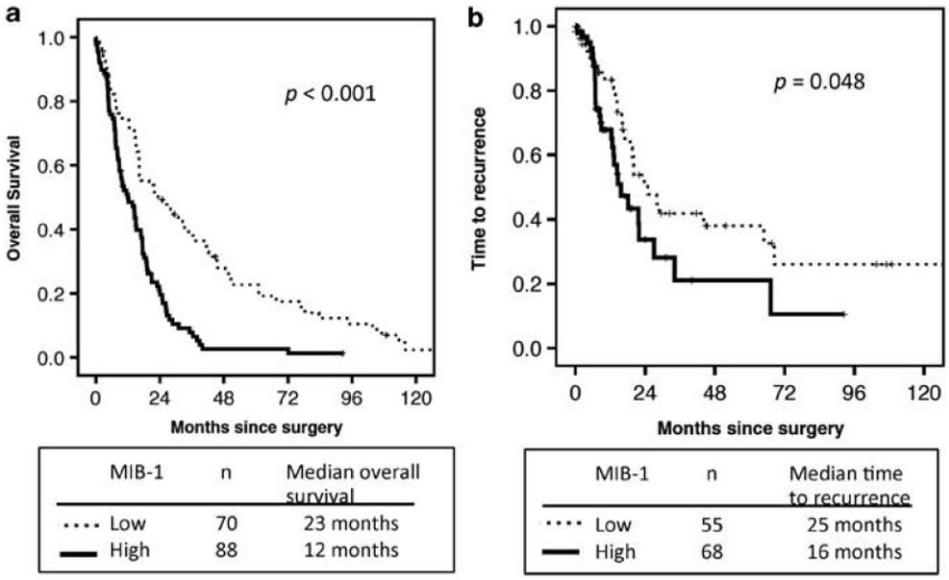

Figure 5.

Overall survival and time to recurrence by MIB-1 labeling index. (**a**) Patients with high MIB-1 labeling index had significantly worse median overall survival than low MIB-1 labeling index. (**b**) Among patients who underwent complete resection, patients with high MIB-1 labeling index had shorter median time to recurrence than low.

Table 1

Univariate analysis in predicting overall survival by clinicopathologic factors

Variables	Patients (%)	Median overall survival (months)	*P*-value[a]
All patients	232 (100)	16	
Age (years)			0.046
≤65	132 (57)	18	
>65	100 (43)	15	
Gender			0.065
Female	64 (28)	21	
Male	168 (72)	15	
Laterality			0.040
Left	104 (45)	17	
Right	128 (55)	15	
T stage			0.013
T1	20 (9)	24	(T1–2 *vs* T3–4)
T2	88 (38)	18	
T3	97 (42)	15	
T4	27 (12)	14	
N stage			0.092
N0	139 (60)	19	(N0 *vs* N1–3)
N1	17 (7)	8	
N2	74 (32)	11	
N3	2 (1)	Not applicable	
Stage			0.007
I	14 (6)	22	(I–II *vs* III–IV)
II	54 (23)	19	
III	130 (56)	14	
IV	34 (15)	15	
Surgical procedure			0.472
EPP	115 (50)	13	
PD	91 (39)	19	
Other procedures	26 (11)	15	
Lymphatic invasion			<0.001
Absent	130 (56)	22	
Present	102 (44)	11	
Vascular invasion			<0.001
Absent	178 (77)	18	
Present	54 (23)	9	

EPP, extrapleural pneumonectomy; PD, pleurectomy/decortication.

[a] All *P*-values are adjusted for stage, except for those corresponding to T stage, N stage, and stage.

Table 2

Univariate analysis in predicting overall survival by nuclear features

Variables	Patients (%)	Median overall survival (months)	*P*-value[a]
All patients	232 (100)	16	
Nuclear atypia			<0.001
Mild	112 (48)	23	
Moderate	74 (32)	15	
Severe	46 (20)	8	
Nuclear/cytoplasmic ratio			0.572
Low	86 (37)	15	
Intermediate	104 (45)	17	
High	42 (18)	19	
Chromatin pattern			0.031
Homogeneous	24 (10)	25	
Fine granular	112 (48)	19	
Coarse granular	96 (41)	11	
Intranuclear inclusion			0.686
Absence	225 (97)	16	
Presence	7 (3)	18	
Prominence of nucleoli			<0.001
Indistinct	55 (24)	25	
Distinct	122 (53)	16	
Large	55 (24)	11	
Mitotic count			<0.001
Low	75 (32)	31	
Intermediate	76 (33)	17	
High	81 (35)	10	
Atypical mitosis			<0.001
Absence	177 (76)	19	
Presence	55 (24)	8	

[a] All *P*-values are adjusted for stage.

Table 3

Multivariate analysis in predicting overall survival by nuclear features

Variables	Hazard ratio	95% CI	*P*-value
Nuclear atypia			
Moderate *vs* mild	1.30	0.92–1.83	0.138
Severe *vs* mild	1.89	1.15–3.10	0.012
Chromatin pattern			
Fine granular *vs* homogeneous	1.52	0.82–2.81	0.181
Coarse granular *vs* homogeneous	1.06	0.74–1.50	0.754
Prominence of nucleoli			
Distinct *vs* indistinct	0.79	0.48–1.31	0.365
Large *vs* indistinct	0.81	0.55–1.19	0.280
Mitotic count			
Intermediate *vs* low	1.55	1.05–2.28	0.028
High *vs* low	2.79	1.69–4.59	<0.001
Atypical mitosis			
Presence *vs* absence	1.02	0.60–1.72	0.948

CI, confidence interval.

Table 4

Multivariate analysis in predicting overall survival

Variables	Hazard ratio	95% CI	*P*-value
Age (>65 *vs* ≤65) (years)	1.26	0.95–1.67	0.105
Laterality (right *vs* left)	1.36	1.01–1.83	0.046
Stages (III–IV *vs* I–II)	1.39	0.99–1.94	0.152
Lymphatic invasion	1.50	1.09–2.08	0.014
Vascular invasion	0.88	0.57–1.37	0.578
Nuclear grade (II *vs* I)	2.11	1.52–2.94	<0.001
Nuclear grade (III *vs* II)	4.16	2.47–7.03	<0.001

CI, confidence interval.

Table 5

Distribution of clinicopathologic factors by nuclear grade

Variables	All patients	Grade I	Grade II	Grade III	*P*-value
All patients (%)	232 (100)	107 (46)	91 (39)	34 (15)	
Age (years)					0.867
Median	64	62	61	61	
Range	29–85	34–85	29–79	35–81	
Gender (%)					0.043
Female	64 (28)	34 (32)	26 (29)	4 (12)	
Male	168 (72)	73 (68)	65 (71)	30 (88)	
Laterality (%)					0.810
Left	104 (45)	49 (46)	40 (44)	15 (44)	
Right	128 (55)	58 (54)	51 (56)	19 (56)	
Lymphatic invasion (%)					<0.001
Absence	130 (56)	77 (72)	43 (47)	10 (29)	
Presence	102 (44)	30 (28)	48 (53)	24 (71)	
Vascular invasion (%)					<0.001
Absence	178 (77)	100 (93)	67 (74)	11 (32)	
Presence	54 (23)	7 (7)	24 (26)	23 (68)	
T stage (%)					0.065
T1–2	108 (47)	52 (49)	48 (53)	8 (24)	
T3–4	124 (53)	55 (51)	43 (47)	26 (76)	
N stage (%)					0.054
N0	139 (60)	73 (68)	47 (52)	19 (56)	
N1–3	93 (40)	34 (32)	44 (48)	15 (44)	
Stage (%)					0.003
I–II	68 (29)	39 (36)	26 (29)	3 (9)	
III–IV	164 (71)	68 (64)	65 (71)	31 (91)	