Histopathological features of epithelioid malignant pleural mesotheliomas in patients with extended survival☆☆

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Summary Diffuse malignant mesothelioma (DMM) of the pleura is a rare and aggressive disease, wherein the long-term survival (LTS) rate is low. The epithelioid subtype is the most prevalent form of DMM with the best prognosis. To study prognostic histopathologic factors associated with extended survival in epithelioid DMM, we examined 43 tumors from patients with survival more than five years (LTSs) and compared the findings with 84 tumors from a reference group (RG) with average survival. We analyzed the tumors considering previously published histopathological prognostic features and attempted to identify additional morphological features predictive of extended survival. Most of the LTS
1. Introduction

Diffuse malignant mesothelioma (DMM) of the pleura is a rare tumor, occurring mainly after asbestos exposure [1]. DMM is the most frequent type of mesothelioma. Other mesothelioma types, with a better prognosis, include localized malignant mesotheliomas and well-differentiated papillary mesotheliomas (WDPMs). DMM is histologically divided into three main subtypes, namely, epithelioid, biphasic, and sarcomatoid [2]. Many studies have shown that histology is the most important prognostic marker and that the epithelioid subtype is the most common type and it has the best prognosis [1,3]. The largest prospectively collected report revealed a median survival of 13 months in unselected patients with epithelioid DMM, whereas a study on surgically treated patients with epithelioid DMM reported a median survival of 19 months [1,4].

Long-term survivals (LTSs) in pleural DMM have been previously studied primarily in patients undergoing surgical or multimodality therapy [5–7]. Young age, female gender, epithelioid subtype, and the type of surgery were observed to predict long-term and cancer-specific survival in these surgically fit patients. To the best of our knowledge, no study has systematically evaluated the histopathological findings of LTSs. Several different prognostic features have been reported for the epithelioid subtype. Arguably, the best-characterized prognostic marker is the nuclear grading scale, which uses a combination of nuclear atypia and mitotic count. Several studies have recognized this scale as an independent predictor of survival in both pleural and peritoneal mesothelioma [8,9]. Histological subtyping of epithelioid DMM is another previously reported prognostic factor; pleomorphic and solid morphological subtypes are associated with aggressive behavior, whereas trabecular or tubulopapillary growth pattern has a better prognosis [10]. In addition, the absence of necrosis and pronounced myxoid stroma is associated with better survival [11,12]. Currently, these factors are not included in the classification of tumors of the pleura [2]. However, it has recently been proposed that nuclear grading, architectural features of mesotheliomas, and other prognostic indicators should be routinely included in the classification of mesotheliomas [13].

In this study, we sought to determine histopathological features predictive of survival more than five years in a group of patients with DMM of the pleura. To accomplish this, we used published morphological features and sought to identify novel features associated with prolonged survival.

2. Materials and methods

2.1. Patients

A description of the mesothelioma patient cohort in this study has been previously published [14]. In brief, a total of 1010 patients with DMM of the pleura from the years 2000–2012 were identified from the Finnish Cancer Registry. From this group of patients, we identified those with survival who exceeded five years. We evaluated the original pathology reports of the mesotheliomas that fulfilled the criteria for DMM, including a full set of immunohistochemical (IHC) analyses. We excluded one case of clerical error in the cancer registry, two WDPMs, and two localized malignant mesotheliomas (better prognosis is thought to be related to the lack of diffuse spread, while the histology is considered to be undistinguishable from DMM), and two nonpleural samples (the location might affect the histological picture of metastatic tumors). The remaining tumors were assigned to the LTS group. For comparison, we included a group of pleural DMMs with average survival from the same cohort as the patients in the LTS group; these patients formed the reference group (RG). Only the epithelioid subtype was included in the RG as all the LTSs were epithelioid tumors. We also had access to the clinical and radiological information of these patients [15]. Survival is calculated from the first biopsy that was diagnostic for DMM, and the study follow-up ended on September 9, 2018.
As per Finnish law, a forensic evaluation of the cause of death should be performed if death is suspected to have been caused by an occupational disease. In some of the cases, from these forensic autopsies, analysis of asbestos fiber burden in lung tissues was available. The analyses of asbestos burden were performed by the Finnish Institute of Occupational Health by transmission electron microscopy. The fiber counts are quantified as million fibers per gram of dry lung (mf/g).

Approval to use tissues and patient information was received from the national authority, Valvira (752/06.01.03.01/2016), and the Finnish Institute for Health and Welfare. The study protocol was approved by the local Institutional Review Board and the Ethics Committee of the Hospital District of Helsinki and Uusimaa (418/13/03/02/2015).

2.2. Histopathological evaluation

In many cases, multiple biopsies had been performed, and tumor material was also available from surgical specimens. In general, the first histological sample that resulted in the unequivocal diagnosis of malignant mesothelioma was studied. If the sample included several tissue blocks, all were studied, but one slide was selected to represent the sample. A more representative sample was evaluated instead of the diagnostic biopsy if it was obtained within three months of the first sample and if the initial biopsy sample was marginal. A senior pathologist (H.W.) with experience in mesothelioma diagnostics scored the histopathological samples in a blinded fashion using a structured scoring sheet. Another experienced pulmonary pathologist (M.M.) provided a second opinion in cases wherein significant differential diagnostic possibilities existed. The tumor samples were classified by size (large, small, and scant) as part of the scoring. The large biopsies included surgical material and material from surgical biopsies. The small biopsies included thick-needle biopsies and other small biopsies. The tumor sample was classified as scant if it was marginally sufficient for the diagnostic evaluation. If sufficient tumor tissue was available, we constructed formalin-fixed paraffin-embedded tissue microarray (TMA) blocks. We performed IHC staining for BRCA-associated protein 1 (BAP1) from these TMA samples [16].

The nuclear grade was assessed by a previously published method, in which the nuclear atypia and the mitotic count were separately scored and then summed up to yield the nuclear grade [8]. The nuclear size and irregularity were first evaluated at 400× magnification and graded from one to three (1 = mild, 2 = moderate, and 3 = severe atypia). After identifying the spots with the highest mitotic activity, the mitoses were then counted in 50 high-power fields (HPF) and counted as an average per 10 HPF. Six (4%) patients had only small areas of viable tumor, but reliable mitotic count could be assessed in the equivalent of 10 full HPFs. In eight (6%) cases, this could not be assessed owing to inadequate sample size. The tumors were then divided into a 3-point mitotic score as follows: 1 = low mitotic count (0–1 per 10 HPF), 2 = intermediate mitotic count (2–4 per 10 HPF), and 3 = high mitotic count (>5 per 10 HPF). Finally, the nuclear grade was assessed as follows: grade I = score 2–3, grade II = score 4–5, and grade III = score 6.

The epithelioid morphological subtypes were recorded as five percentage increments, and the tumors were classified by the predominant growth pattern (trabecular, tubulopapillary, solid, micropapillary, pleomorphic). We classified these subtypes into low-grade (trabecular, tubulopapillary) or high-grade (solid, micropapillary, pleomorphic) based on their dominant (>50%) pattern in survival analyses as per previous publications [10,17].

In addition, for each tumor sample, we recorded the number of tumor sample blocks, sample type, tumor density (percentage of tumor in the sample), superficial growth of the tumor (not applicable to small biopsy), presence of necrosis, invasiveness and invasion to adjacent structures, myxoid stroma (positive if it contains >50% of the tumor volume), size of nucleoli (inconspicuous, conspicuous <3 μm, large >3 μm), cytological type (microcystic, clear cell, deciduoid, small cell) and tumor (scale 0–3), or pleural inflammatory infiltrate (positive, negative).

To provide more stratification in the evaluation, we noted the relative presence of tubular structures covered with a single layer of mesothelial cells (single layer) (Fig. 1F). We also decided to record presence of exophytic growth, stout fibrovascular papillae referred to as polypoid in the text, sometimes forming confluent areas or polyp-like structures (Fig. 1A, B, C). Sometimes, exophytic growth presented as delicate papillary structures, and this was noted separately. Furthermore, we noted the presence of large tubular structures (large tubule, Fig. 1D and E). These features were recorded as being present if any of these features were noted or absent if none was seen. The complete list of all evaluated features with definitions and explanations is found in Supplementary Table 1. The tumor slides were examined, and pictures were taken using a Leica 4000B microscope (Leica Microsystems, Wetzlar, Germany) equipped with a Leica DFC 480 camera (Leica Microsystems, Wetzlar, Germany).

2.3. Statistical analysis

Categorical variables are presented as number of patients with percentage, and the differences between the study groups were analyzed using the chi-square test. Continuous variables are expressed as median with interquartile range (IQR), and statistical differences were tested using the nonparametric Mann-Whitney U test or Kruskal-Wallis test, as indicated in the tables. The Bonferroni correction for multiple tests was used if multiple comparisons were made. Bivariate correlations were assessed...
using the Spearman correlation coefficient. Cox proportional hazards univariate and multivariate survival analyses were used to assess how histopathological factors predict survival. Age and gender were used as covariates in multivariate models. Based on our findings on the impact of treatment on prognosis, the survival analyses were adjusted with treatment status (Paajanen et al., submitted). The results of survival analysis are presented as hazard ratios (HR) and associated 95% confidence intervals (CIs). Statistical analyses were performed using IBM Statistics 25.0 (IBM SPSS Statistics, Chicago, IL). $P$-values $<0.05$ were considered significant.

3. Results

3.1. Histopathological factors related to the patient groups

A total of 127 tumor specimens from patients with pleural DMM were analyzed. From these patients, 43
(34%) and 84 (66%) formed the LTS group and the RG, respectively. A median of three (IQR = 1−5) hematoxylin and eosin (H&E)–stained slides was reviewed from each tumor. The analyzed sample was from pneumonectomy in 5 (4%), surgical biopsy in 84 (66%), and thick-needle or other types of nonsurgical biopsies in 38 (30%) cases. We classified 42 (33%) samples as being small; of which, seven (6%) were evaluated as scant. There were no significant differences in the distribution of small samples between the study groups (LTS group: n = 10, 23% and RG: n = 32, 38%; P = 0.093). The presence of tumor invasion in the evaluated samples (95% in the LTS group and 92% in the RG) was similar in the study groups (P = 0.444). The tumor sample size affected the frequency of invasion; all nine tumors with no invasion were associated with small samples (P < 0.001). BAP1 IHC staining was available for 57 (45%) patients and was negative for 12 of 14 (86%) patients in the LTS group and 25 of 43 (58%) in RG patients (P = 0.150).

The main histopathological differences between the groups are summarized in Table 1. LTS tumors presented mostly with a tubulopapillary growth pattern with nuclear grade I. In contrast, a solid growth pattern and grade II were more common in the RG (P < 0.001). Only 1 of 12 (1%) tumors with exophytic polypoid patterns was seen in the RG tumors (P < 0.001). We did not find differences in the presence of diffuse exophytic growth (P = 0.053), supraventricular growth of the tumor (P = 0.130), tumor invasion into the adjacent structures (P = 0.627), size of nucleoli (P = 0.415), tumor (P = 0.380) or pleural inflammation (P = 0.386), or cytological features (P = 0.412). A sensitivity analysis was performed after removing small biopsy samples, but this did not affect the distributions between the study groups (data not shown). Not surprisingly, the sample size affected the presence of large tubules (none in small samples; P = 0.015) and exophytic polypoid growth (one in small samples; P = 0.052). Tubules with a single layer of the mesothelium were also more common in larger samples (n = 38, 45% versus n = 12, 29%), but the difference was less distinct (P = 0.080).

3.2. Histopathological features and their association with overall survival

The median survival for the LTS group was 79.3 months (IQR = 69.3–99.3 months) and was 11.3 months for the RG (IQR = 5.6–19.2 months). Eight (19%) patients in the LTS group and none of the RG patients were alive at the end of the study follow-up. Of these patients, two had only minimal signs of DMM in their last follow-up visits; one had no signs of recurrence after surgery, whereas the other one was lost to follow-up before study closure. Other six patients had either progressive multifocal clinical or radiological findings matching DMM. The follow-up of the patients in the LTS group is summarized in Table 2.
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Table 3 Univariate analysis associated with overall survival.

| Variable                        | HR (95% CI) | P-value |
|---------------------------------|-------------|---------|
| Nuclear grade                   |             |         |
| Grade I                         | 1.00        |         |
| Grade II                        | 3.05 (2.05–4.55) |         |
| Grade III                       | 15.82 (5.19–48.11) |         |
| Histologic subtypes, low-grade | 0.43 (0.30–0.62) | <0.001 |
| Necrosis, yes                   | 2.48 (1.45–4.23) | 0.001  |
| Exophytic polypoid growth, yes  | 0.43 (0.23–0.80) | 0.008  |
| Single layer, yes               | 0.52 (0.35–0.75) | 0.001  |
| Large tubule, yes               | 0.72 (0.37–1.37) | 0.312  |
| Myxoid stroma, yes              | 0.96 (0.44–2.06) | 0.908  |
| Invasion, yes                   | 1.33 (0.65–2.74) | 0.434  |
| Tumor density (continuous)      | 1.01 (1.01–1.02) | 0.001  |

Univariate determinants of survival using the Cox proportional hazard model.
Abbreviations: HR, hazard ratio; CI, confidence interval.

Table 4 Multivariate analysis associated with overall survival.

| Variable                        | HR (95% CI) | P-value |
|---------------------------------|-------------|---------|
| Age (continuous)                | 1.01 (0.98–1.03) | 0.674  |
| Gender, male                    | 1.25 (0.71–2.18) | 0.440  |
| Nuclear grade                   | <0.001      |         |
| Grade I                         | 1.00        |         |
| Grade II                        | 4.43 (2.51–7.82) |         |
| Grade III                       | 16.39 (4.57–58.76) |         |
| Histologic subtypes, low-grade  | 0.71 (0.39–1.26) | 0.241  |
| Necrosis, yes                   | 1.26 (0.66–2.42) | 0.484  |
| Exophytic polypoid growth, yes  | 0.43 (0.21–0.89) | 0.024  |
| Single layer, yes               | 0.76 (0.46–1.26) | 0.288  |
| Tumor density (continuous)      | 1.00 (0.99–1.01) | 0.883  |

The Cox proportional hazard model adjusted for age, gender, nuclear grade, histologic subtypes, necrosis, exophytic polypoid growth, single layer, tumor density, and treatment.
Abbreviations: HR, hazard ratio; CI, confidence interval.

3.3. Asbestos fiber count and its association with histopathological parameters

In a separate study, we evaluated the association of asbestos fiber count and LTS (Paajanen et al. submitted). We observed that the patients in the LTS group had less asbestos fiber in lung tissues than those in the RG. However, asbestos fiber analysis was available from just 14 (33%) patients of the LTS group and 60 patients (71%) from the RG. The overall median concentration was 3.95 mf/g (IQR = 0.40–77.50 mf/g). We found an association with the total concentration of lung fibers and nuclear grade ($P < 0.001$, Fig. 2). When nuclear grade was separated into its initial factors, we found an association with asbestos concentration and mitosis ($r = 0.41; P = 0.001$), but not with nuclear atypia ($P = 0.071$). In addition, higher tissue fiber content was more likely to be found in tumors with necrosis (median = 64.50 mf/g, IQR = 3.40–182.50 mf/g versus median = 1.90 mf/g, IQR = 0.40–24.75 mf/g; $P = 0.021$). No other relevant associations were found between the fiber concentration and histopathological findings.

4. Discussion

As the epithelioid subtype is the most common in DMM and is associated with the best prognosis, several attempts have been made to further characterize these tumors. The heterogeneity in histology and inconsistent clinical behavior of these tumors complicate the prognostic evaluation in clinical practice. In this study, we identified several histopathological factors that are associated with a more indolent tumor type. Nuclear grade I, low-grade histological subtype, lower tumor density, absence of necrosis among with the presence of polypoid exophytic growth,
large tubules, and tumor growth on a single layer were more prevalent in LTS tumors. In contrast, we did not observe a survival association with tumor myxoid stroma (not including myxoid in exophytic polypoid structures), tumor superficial growth, size of nucleoli, the level of invasion in the initial biopsy, invasion to the adjacent structures, cytological type, or inflammatory cells.

The separation of benign from malignant mesothelial proliferation can sometimes be challenging, especially with limited low-quality diagnostic samples. Tumor invasion of the stroma is considered to be central for diagnosing DMM; if such invasion is absent, atypical mesothelial hyperplasia or mesothelioma in situ should be considered as an initial diagnosis [2,19]. However, an epithelioid DMM diagnosis can be made from cytological samples (in which invasion cannot be evaluated) if IHC, clinical, and radiological characteristics are consistent with invasive DMM [20]. In our evaluation, invasion could not be seen in nine tumor samples; all of which were in small biopsies. However, there were no differences in the presence of invasion between the study groups, and the presence of invasion did not predict survival in crude analyses. We assessed the clinical behavior of the patients in the LTS group who had no evident invasion: they had either radiological or thoracoscopical signs of DMM, and one had rebiopsy, which was consistent with invasive DMM. A small sample size can be seen to increase the possibility of mesothelial proliferations as DMM. However, in this study, the small sample size was more prevalent in the LTS group than in the RG group. BAP1 loss detected in IHC staining can distinguish between benign and malignant mesothelial proliferation: malignant mesotheliomas are often negative for BAP1, whereas reactive and normal mesothelium, adenomatoids, and at least most WDPMs are positive [2,16,21]. We were able to stain 45% of the tumors for BAP1. Negativity for BAP1 was more frequent in the LTS group than in the RG, although the difference was not significant. During follow-up, all but two patients (one of which was lost to follow-up) presented with additional findings consistent with DMM. Taken together, we are confident that the results of this study cannot be explained by misdiagnosis of reactive mesothelial changes as DMM [22].

The three-tier nuclear grading score is a previously published independent predictor for survival, which combines nuclear atypia and mitotic count [8]. The initial studies on this grading system were conducted primarily with larger surgical samples, but a similar prognostic impact has also been observed with smaller pleural biopsy samples [11]. Similarly, it has been linked to better prognosis in epithelioid peritoneal mesothelioma [9]. In this study, we confirmed that nuclear grade is an independent prognostic marker in epithelioid DMM. The presence of nuclear grade I in LTS tumors was more than twice that of the control group. The advantage of nuclear grading is that it is easy to perform on basic H&E-stained slides. However, assessing nuclear atypia is subjective, and mitosis count can be time-consuming and prone to errors [23]. These problems could be solved with computer-assisted automation, which have shown superior reproducibility and good correlation with other pathological reviews [24].

Stratifying epithelioid DMMs into secondary morphological subtypes is another previously reported prognostic marker. This approach has strong reproducibility with good
interobserver agreement [25]. Previous reports have shown differences in the prevalence of these subtypes, which may be due to differences in the sample sizes used in these studies. For example, Brčić et al. [25] found that the solid subtype was the most prominent and only some tumors were characterized as micropapillary, trabecular, or pleomorphic. Kadota et al. [10] used mainly larger surgical specimens and observed that other subtypes were more commonly distributed. When we combined all tumors, we observed that the solid subtype was the most frequent (54%), followed by the tubulopapillary subtype (43%); only single tumors were micropapillary, pleomorphic, or trabecular. For survival analyses, we combined these into low-grade and high-grade in accordance with previous publications [10,17]. These low-grade compound subtypes were more common in LTS tumors than in the RG tumors. The survival analysis showed significant findings only in univariate analysis but not after adjustments. As stratifying tumors into secondary morphological subtypes has been shown to be reproducible and simple to perform, we believe this approach should be further investigated and used, either alone or in combination with other prognostic markers.

The prognostic value of histological tumor necrosis has been shown in various solid malignancies [26]. Similar results have been published in pleural DMM [11]. In addition, a multi-institutional study revealed that overall survival was further stratified when the nuclear grade was added to the presence of necrosis [27]. We found necrosis only in one LTS tumor in contrast to sixteen in the control group. The proportion of necrosis in the control group was similar to that in the study of Habbougit et al [11] but was too low to reliably test the combination with nuclear grade. The low prevalence of necrosis could also explain the significance in univariate analysis but not in multivariate analysis.

We also attempted to identify novel histological parameters to further define tumor differentiation. The feature designated as polypoid exophytic growth in the scoring was an independent prognostic factor in multivariate analysis (Fig. 1A, B, C), although its significance diminished in sensitivity analyses (Supplementary Table 3). In contrast, the exophytic delicate papillary diffuse growth had no prognostic value. These polypoid features are similar to those seen in WDPM [22]. The differential diagnosis between pleural DMM with papillary formations with broad fibrovascular cores and pleural WDPM is challenging, especially for a recently introduced group of WDPM with limited invasion. Thus, we evaluated the polypoid tumors in this regard and observed that 3 of 14 could be considered to exhibit features of WDPM including three cases with limited invasion (Supplementary Table 2) [2,18]. As noted earlier, we have excluded two cases of classical WDPM from the main analyses. The peritoneal WDPM occurs typically in women and is not associated with asbestos exposure, and it has a relatively good prognosis [28]. WDPM in the pleura is less common and seems to have a degree of association with asbestos. When comparing with peritoneal WDPM, pleural cases seem to be more aggressive, including cases that subsequently behave as DMM [22]. Our findings are consistent with this observation: the deaths of four patients with WDPM-like features were attributed to DMM, and the only one alive had clear signs of diffuse invasive disease. IHC staining for BAP1 was available and negative in three of the WDPM cases. Interestingly, it has been reported that while BAP1 negativity is rare in WDPM, it is associated with synchronous or metachronous DMM [29]. Germ line BAP1 loss is associated with an inherited predisposition to mesothelioma and other tumors. Mesotheliomas with germ line BAP1 loss have a better overall survival than patients with mesothelioma without these mutations [30]. It is possible that germ line BAP1 mutations could explain in part the prolonged survival in the LTS group. Although the presence of these exophytic polypoid structures seems to predict prolonged survival, patients with this feature developed progressive DMM, with the median survival of 70 months. Somewhat surprisingly, the outcome was similar in cases compatible with WDPM, WDPM with focal invasion, and DMM with polypoid features (Supplementary Table 2). This suggests that the presence of these polypoid features in mesothelial lesions seems to be more closely associated with survival than the degree of tumor invasiveness, although the small number of cases precludes definite conclusion. On the other hand, the finding of pleural WDPM seems to have predicted a fully fledged DMM in these cases, as also indicated by an earlier study on pleural DMM [22].

We attempted to estimate the amount of tumor within the diagnostic sample, identified as tumor density. The tumor density was lower in LTS and prognostic in univariate survival analysis but not after adjustments. Another feature associated with LTS was the presence of large tubular structures covered with mesothelial cells (Fig. 1D, F). The nature of these structures is unclear. In addition to being large tubular structures, a possibility could be distended lymphatic vessels covered with mesothelial cells. Although these structures were more frequent in LTS tumors, the proportions were low in both groups. Another morphological finding was growth of the mesothelium in a single layer in tubular structures (Fig. 1E). This was also more frequently observed in the LTS group. This finding only had prognostic value in univariate analysis (HR = 0.52) if present and is probably related to the degree of differentiation in the tumor because the normal mesothelium occurs in a single layer. This is also analogous to findings in well-differentiated adenocarcinomas in which the glandular structures often are described to be in a single layer.

The role of asbestos in DMM development is firmly established, although its prognostic significance has been
debated [31,32]. Previously, we have observed that patients in the LTS group had a lower amount of asbestos fibers in the lung tissue than those in the RG, and here we studied the association between asbestos fiber burden and histopathological features. We found that the concentration of asbestos fiber was associated with nuclear grade, which was driven by correlation to mitotic count. In addition, asbestos fiber count was associated with tumor necrosis.

5. Conclusions

We identified several histopathological parameters that are associated with LTS. We confirmed previous reports that low-grade histopathological subtypes (tubulopapillary or trabecular) along with low nuclear grade and the absence of necrosis have better prognosis. In addition, we present new morphological findings that are associated with LTS. The presence of the polypoid growth pattern along with nuclear grade was the only independent predictive factor for survival. Taken together, we believe that the features discussed in the article may be helpful in predicting prognosis in DMM. Whether these prognostic factors affect the efficacy of various treatment modalities remains to be studied.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.humpath.2020.02.007.

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References

[1] Beckett P, Edwards J, Fennell D, Hubbard R, Woolhouse I, Peake MD. Demographics, management and survival of patients with malignant pleural mesothelioma in the national lung cancer audit in England and Wales. Lung Canc 2015;88:344–8.
[2] Galateau-Salle F, Churg A, Roggli V, Travis WD. The 2015 world health organization classification of tumors of the pleura: advances since the 2004 Classification. J Thorac Oncol 2016;11:142–54.
[3] Husain AN, Colby TV, Ordóñez NG, et al. Guidelines for pathologic diagnosis of malignant mesothelioma: 2017 update of the consensus statement from the international mesothelioma interest group. Arch Pathol Lab Med 2018;142:89–108.
[4] Meyerhoff RR, Yang CFJ, Speicher PJ, et al. Impact of mesothelioma histologic subtype on outcomes in the Surveillance, Epidemiology, and End Results database. J Surg Res 2015;196:23–32.
[5] Yan TD, Boyer M, Tin MM, et al. Prognostic features of long-term survivors after surgical management of malignant pleureothelioma. Ann Thorac Surg 2009;87:1532–6.
[6] Sugarbaker DJ, Wolf AS, Chirieac LR, et al. Clinical and pathological features of three-year survivors of malignant pleural mesothelioma following extrapleural pneumonectomy. Eur J Cardio Thorac Surg 2011;40:298–303.
[7] Leuzzi G, Rea F, Spaggiari L, et al. Prognostic score of long-term survival after surgery for malignant pleural mesothelioma: a multicenter analysis. Ann Thorac Surg 2015;100:890–7.
[8] Kadota K, Suzuki K, Colovos C, et al. A nuclear grading system is a strong predictor of survival in epithelioid diffuse malignant pleural mesothelioma. Mod Pathol 2012;25:260–71.
[9] Valente K, Blackham AU, Levine E, et al. A histomorphologic grading system that predicts overall survival in diffuse malignant peritoneal mesothelioma with epithelioid subtype. Am J Surg Pathol 2016;40:1243–8.
[10] Kadota K, Suzuki K, Sima CS, Rusch VW, Adusumilli PS, Travis WD. Pleomorph epithelioid diffuse malignant pleural mesothelioma: a clinicopathological review and conceptual proposal to reclassify as biphasic or sarcomatoid mesothelioma. J Thorac Oncol 2011;6:986–904.
[11] Habouguit C, Trombert-Paviot B, Karpathiou G, et al. Histopathologic features predict survival in diffuse pleural malignant mesothelioma on pleural biopsies. Virchows Arch 2017;470:639–46.
[12] Shia J, Qin J, Erlandson RA, et al. Malignant mesothelioma with a pronounced myxoid stroma: a clinical and pathological evaluation of 19 cases. Virchows Arch 2005;447:828–34.
[13] 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;15:29–49.
[14] Laaksonen S, Ilonen I, Kuosma E, et al. Malignant pleural mesothelioma in Finland: regional and gender variation. Acta Oncol (Madrid) 2018;58:38–44.
[15] Paajanen J, Laaksonen S, Ilonen I, et al. Computed tomography in the evaluation of malignant pleural mesothelioma—association of tumor size to a sarcomatoid histology, a more advanced TNM stage and poor survival. Lung Canc 2018;116:73–9.
[16] Erber R, Warth A, Muley T, Hartmann A, Herpel E, Agaimy A. BAP1 loss is a useful adjunct to distinguish malignant mesothelioma including the adenomatoid-like variant from benign adenomatoid tumors. Appl Immunohistochem Mol Morphol 2020;28:67–73.
[17] Johansson L, Linden C. Aspects of histopathologic subtype as a prognostic factor in 85 pleural mesotheliomas”. Chest 1996;109: 109–14.
[18] Churg A, Allen T, Borczuk AC, et al. Well-differentiated papillary mesothelioma with invasive foci. Am J Surg Pathol 2014; 38:990–8.
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[19] Henderson DW, Shilkin KB, Whitaker D. Reactive mesothelial hyperplasia vs mesothelioma, including mesothelioma in situ: a brief review. Am J Clin Pathol 1998;110:397–404.

[20] Henderson DW, Reid G, Kao SC, Van Zandwijk N, Klebe S. Challenges and controversies in the diagnosis of mesothelioma: Part 1. Cytology-only diagnosis, biopsies, immunohistochemistry, discrimination between mesothelioma and reactive mesothelial hyperplasia, and biomarkers. J Clin Pathol 2013;66:847–53.

[21] Stevers M, Rabban JT, Garg K, et al. Well-differentiated papillary mesothelioma of the peritoneum is genetically defined by mutually exclusive mutations in TRAF7 and CDC42. Mod Pathol 2019;32:88–99.

[22] Galateau-Salle F, Vignaud J, Burke L, et al. Well-differentiated papillary mesothelioma of the pleura: a series of 24 cases. Am J Surg Pathol 2004;28:534–430.

[23] Yigit N, Gunal A, Kucukodaci Z, Karslioglu Y, Onguru O, Ozcan A. Are we counting mitoses correctly? Ann Diagn Pathol 2013;17:536–9.

[24] Puri M, Hoover SB, Hewitt SM, et al. Automated computational detection, quantitation, and mapping of mitosis in whole-slide images for clinically actionable surgical pathology decision support. J Pathol Inf 2019;10:1–12.

[25] Brčić L, Jakopović M, Brčić I, et al. Reproducibility of histological subtyping of malignant pleural mesothelioma. Virchows Arch 2014;465:679–85.

[26] Richards CH, Mohammed Z, Qayyum T, Horgan PG, McMillan DC. The prognostic value of histological tumor necrosis in solid organ malignant disease: a systematic review. Future Oncol 2011;7:1223–35.

[27] Rosen L, Karrison T, Ananthanarayanan V, et al. Nuclear grade and necrosis predict prognosis in malignant epithelioid pleural mesothelioma: a multi-institutional study. Mod Pathol 2018;31:598–606.

[28] Vogin G, Hettal L, Vignaud JM, et al. Well-differentiated papillary mesothelioma of the peritoneum: a retrospective study from the renape observational registry. Ann Surg Oncol 2019;26:852–60.

[29] Lee HE, Molina JR, Sukov WR, Roden AC, Yi ES. BAP1 loss is unusual in well-differentiated papillary mesothelioma and may predict development of malignant mesothelioma. Hum Pathol 2018;79:168–76.

[30] Ohar JA, Cheung M, Talarchek J, et al. Germline BAP1 mutational landscape of asbestos-exposed malignant mesothelioma patients with family history of cancer. Cancer Res 2016;76:206–15.

[31] Christensen BC, Godleski JJ, Roelofs CR, et al. Asbestos burden predicts survival in pleural mesothelioma. Environ Health Perspect 2008;116:723–6.

[32] Montanaro F, Rosato R, Gangemi M, et al. Survival of pleural malignant mesothelioma in Italy: a population-based study. Int J Canc 2009;124:201–7.