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

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PII: S0046-8177(20)30048-4

DOI: https://doi.org/10.1016/j.humpath.2020.02.007

Reference: YHUPA 4972

To appear in: *Human Pathology*

Received Date: 6 February 2020

Accepted Date: 27 February 2020

Please cite this article as: Paajanen J, Laaksonen S, Kettunen E, Ilonen I, Vehmas T, Salo J, Räsänen J, Sutinen BHc E, Ollila BHc H, Mäyränpää MI, Myllärniemi M, Wolff H, Histopathological features of epithelioid malignant pleural mesotheliomas in patients with extended survival, *Human Pathology*, https://doi.org/10.1016/j.humpath.2020.02.007.

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

Running title: Histological features in mesothelioma long-term survivals

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Declarations of interest: none

Funding for this study was provided by the Finnish Work Environment Fund, the Finnish Cancer Foundation (JP), the Finnish Medical Foundation (MIM), and the Nummela Foundation (HW). The

funders played no role in study design; in the collection, analysis, and interpretation of data; in the writing of the report or implementation of this manuscript.

Abstract

Diffuse malignant mesothelioma (DMM) of the pleura is a rare and aggressive disease, where the long-term survival (LTS) rate is low. The epithelioid subtype is the most prevalent form of DMM with the best prognosis. In order to study prognostic histopathologic factors associated with extended survival in epithelioid DMM, we examined 43 tumors from patients with survival over five years (long-term survivals [LTS]) and compared the findings with 84 tumors from a reference group with average survival (RG). 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 tumors presented with nuclear grade I (n = 34,90%) and a tubulopapillary growth pattern (n = 30,70%). One LTS tumor had necrosis. In contrast, nuclear grade II (n = 49,61%) and solid growth pattern (n = 59,70%) were more frequent in RG, and necrosis was present in 16 (19%) tumors. We also evaluated the association of asbestos lung tissue fiber burden quantified from autopsy samples with histopathological features and found that elevated asbestos fiber was associated with higher nuclear grade ($p < 0.001$) and the presence of necrosis ($p = 0.021$). In univariate survival analysis, we identified the following three novel morphological features associated with survival: exophytic polypoid growth pattern, tumor density, and single mesothelium layered tubular structures. After adjustments, low nuclear grade ($p < 0.001$) and presence of exophytic polypoid growth ($p = 0.024$) were associated with prolonged survival. These results may aid in estimating DMM prognosis.

Keywords: epithelioid mesothelioma, histopathology, survival, grade, asbestos

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, includes 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 epithelioid DMM patients, whereas a study on surgically treated epithelioid DMM patients reported a median survival of 19 months [1,4].

Long-term survivals (LTS) in pleural DMM has 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 LTS.

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 have 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 over 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]. Briefly, a total of 1010 patients with DMM of the pleura from the years 2000 to 2012 were identified from the Finnish Cancer Registry. From this group of patients, we identified those with survival that exceeded five years. We evaluated the original pathology reports of the mesotheliomas that fulfilled the criteria for DMM, including a full set of immunohistochemistry (IHC). 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 non-pleural 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 LTS patients; these patients formed the reference group (RG). Only the epithelioid subtype was included in the RG, as all the LTS 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 9 September 2018.

According to 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, asbestos fiber analysis of lung tissues from these forensic autopsies was available. The asbestos analyses

were made by the Finnish Institute of Occupational Health using transmission electron microscopy (TEM). 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 biopsy had been taken 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 where 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, where the nuclear atypia and the mitotic count were separately scored and then summed to yield the nuclear grade [8]. The nuclear size and irregularity were first evaluated at 400x magnification and graded from one to

three (1 = mild, 2 = moderate, 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 due to inadequate sample size. The tumors were then divided into a 3-point mitotic score as follows: 1 = low mitotic count (0-1/10 HPF), 2 = intermediate mitotic count (2-4/10 HPF) and 3 = high mitotic count (>5/10 HPF). Finally, the nuclear grade was assessed follows: grade I = score 2-3, grade II = score 4-5, grade III = score 6.

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

In addition, for each tumor sample, we recorded the number of tumor sample blocks, sample type, tumor density (% 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), nucleoli size (inconspicuous, conspicuous <3µm, large >3µm), 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) (Figure 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 (Figure 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, Figure 1D, 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 taken with a Leica 4000B microscope equipped with a Leica DFC 480 camera.

2.3 Statistical analysis

Categorical variables are presented as number of patients with percentage and the differences between the study groups were analyzed by chi-square test. Continuous variables are expressed as median with interquartile range (IQR) and statistical differences were tested by non-parametric 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 analysis was 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 (CI). 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 in patients with pleural DMM were analyzed. From these patients, 43 (34%) were LTS and 84 (66%) formed the RG. A median of three (IQR 1-5) hematoxylin and eosin-stained (H&E) slides were reviewed from each tumor. The analyzed sample was from pneumectomy in 5 (4%), surgical biopsy in 84 (66%), and thick-needle or other types of non-surgical biopsies in 38 (30%) cases. We classified 42 (33%) samples as being small, out of which seven (6%) were evaluated as scant. There were no significant differences in the distribution of small samples between the study groups (LTS: n = 10, 23% and RG: n = 32, 38%; p = 0.093). The presence of tumor invasion in the evaluated samples (95% in LTS and 92% 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 was available in 57 (45%) patients and was negative in 12/14 (86%) LTS patients and 25/43 (58%) in RG patients (p = 0.150).

The main histopathological differences between the groups are summarized in Table 1. LTS tumors presented mostly with 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 1/12 (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), superficial growth of the tumor (p = 0.130), tumor invasion into adjacent structures (p = 0.627), nucleoli size (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 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 LTS was 79.3 months (IQR 69.3 – 99.3 months) and for the RG 11.3 months (IQR 5.6 – 19.2 months). None of the RG and eight (19%) LTS patients were alive at the end of the study follow-up. Out of these patients, two had only minimal signs of DMM in their last follow-up visits; one had no signs of recurrence after surgery, while 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 LTS patients is summarized in Table 2.

The nuclear grade, histologic subtype, tumor density and the presentation of necrosis, exophytic polypoid growth, and single layer predicted survival in univariate survival analysis (Table 3). We

found that both mitosis (HR 1.16, 95% CI 1.11 – 1.22) and nuclear atypia (moderate versus mild atypia: HR 1.97, 95% CI 1.16 – 3.33 and severe versus mild atypia: HR 3.13, 95% CI 1.64 – 5.99) had prognostic value when nuclear grade was separated to its initial factors. The multivariate analysis was adjusted on these significant markers along with age, gender, and treatment information. The findings are presented in Table 4. In summary, we found that low nuclear grade and the presence of exophytic polypoid growth pattern were the only independent prognostic factors for longer survival.

As the differential diagnosis between DMM with papillary features and WDPM, including WDPM with invasive foci is demanding [2,18], we re-evaluated the tumors with polypoid changes. In 2/14 (14%) cases, the first biopsy that resulted in the original diagnosis of DMM was on re-evaluation compatible to a WDPM, and these patients were subsequently excluded from the analyses. In addition, in 3/14 (21%) cases, the diagnosis of WDPM with invasive foci could be considered. All three cases showed invasion within the papillary myxoid stroma and in one of the cases a minor invasive focus in the adipose tissue. As these WDPM-like tumors originally had been diagnosed as DMM, and their clinical behavior was similar to the others in the group of tumors with polypoid features, they were included as DMM:s for the statistical evaluation. The survival analyses were also performed after removing these three patients and is shown in Supplementary Table 3. This additional model reduced the statistical significance of polypoid features ($p = 0.057$), without affecting other results. In the other cases, the histological picture indicated the diagnosis of DMM of the pleura. The individual clinical information and outcomes of patients with polypoid features is shown in supplementary Table 2.

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 LTS patients had less asbestos fiber in lung tissues than the RG. However, asbestos fiber analysis was available from just 14 (33%) of the LTS group and 60 (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 ($p < 0.001$, Figure 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 complicates 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 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, nucleoli size, the level of invasion in the initial biopsy, invasion to 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 (where invasion cannot be evaluated) if IHC and 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 LTS patients that had no evident invasion: they had either radiological or thoracoscopical

signs of DMM and one had re-biopsy that was consistent with invasive DMM. Small sample size can be seen to increase the possibility of mesothelial proliferations as DMM. However, in this study small sample size was more prevalent in the LTS group than the RG group. BAP1 loss detected in IHC can distinguish between benign and malignant mesothelial proliferation: malignant mesotheliomas are often negative for BAP1, while 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 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 over 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, the 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, Brcic et al found that

the solid subtype was the most prominent and only some tumors were characterized as a micropapillary, trabecular, or pleomorphic [25]. Kadota et al, used mainly larger surgical specimens and observed that other subtypes were more constantly distributed [10]. 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 compounded subtypes were more common in LTS-tumors when compared to the RG. 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 employed, 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 the study of Habougits but was too low to reliably test the combination with nuclear grade [11]. 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 polypoid exophytic growth in the scoring was an independent prognostic factor in multivariate analysis (Figure 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 3/14 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 pleura is less common and seems to have a degree of association with asbestos. When comparing to peritoneal WDPM, pleural cases seems 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 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]. Germline BAP1 loss is associated with an inherited predisposition to mesothelioma as well as other tumors. Mesotheliomas with germline BAP1 loss have a better overall survival compared with mesothelioma patients without these mutations [30]. It is possible that germline BAP1 mutations could explain in part the prolonged survival in the LTS group. Although the presence of these exophytic polypoid structures seem 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 seem to be more closely associated with the survival than the degree of tumor invasiveness, although the low number of cases preclude 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 smaller 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 (Figure 1 D, 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 mesothelium in a single layer in tubular structures (Figure 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 since normal mesothelium occurs in a single layer. This is also analogous to findings in well-differentiated adenocarcinomas where 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 LTS patients had a lower amount of asbestos fibers in the lung tissue compared to 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, the amount asbestos fiber count associated with tumor necrosis.

4.1 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 polypoid growth pattern along with nuclear grade were the only independent predictive factors 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.

Acknowledgements: We wish to thank Sauli Savukoski for expert technical assistance and the personnel at the Helsinki University Biobank for preparing the TMA.

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Table 3. Univariate analysis associated with overall survival

Variable	HR[1] (95% CI[2])	p-value
Nuclear grade		<0.001
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 Cox proportional hazard model. 1. HR, hazard ratio; 2. CI, confidence interval

Table 1. Main histopathological findings

	LTS[1]	RG[2]	p-value
Conventional parameters			
Nuclear grade, n (%)			<0.001
Grade I	34 (90%)	28 (34%)	
Grade II	4 (10%)	49 (61%)	
Grade III	0	4 (5%)	
Histologic subtypes, n (%)			<0.001
Trabecular	2 (5%)	0	
Tubulopapillary	30 (70%)	24 (29%)	
Solid	9 (20%)	59 (70%)	
Micropapillary	2 (5%)	1 (1%)	
Compound histologic subtype[3], n (%)			<0.001
High-grade	11 (25%)	60 (71%)	
Low-grade	32 (75%)	24 (29%)	
Myxoid stroma, n (%)			0.605
Absent	40 (93%)	80 (95%)	
Present	3 (7%)	4 (5%)	
Necrosis, n (%)			0.007
Absent	42 (98%)	68 (81%)	
Present	1 (2%)	16 (19%)	
Novel parameters			
Exophytic polypoid growth pattern, n (%)			<0.001
Absent	32 (74%)	83 (99%)	
Present	11 (26%)	1 (1%)	
Large tubule, n (%)			0.029
Absent	36 (84%)	80 (95%)	
Present	7 (16%)	4 (5%)	
Single layer, n (%)			<0.001
Absent	16 (37%)	61 (73%)	
Present	27 (63%)	23 (27%)	
Tumor density, %, median (IQR)	50.0 (30 – 60)	70.0 (45 – 81)	<0.001*

P-values are for the comparison of LTS and RG and were calculated by chi-square tests except for *, where Mann-Whitney U-test was used. 1. LTS, long-term survival over five years; 2. RG, reference group; 3. Histologic subtypes combined for survival analyses: high-grade (solid, micropapillary), low-grade (trabecular, tubulopapillary)

Table 2. Survival data of long-term survival (n = 43)

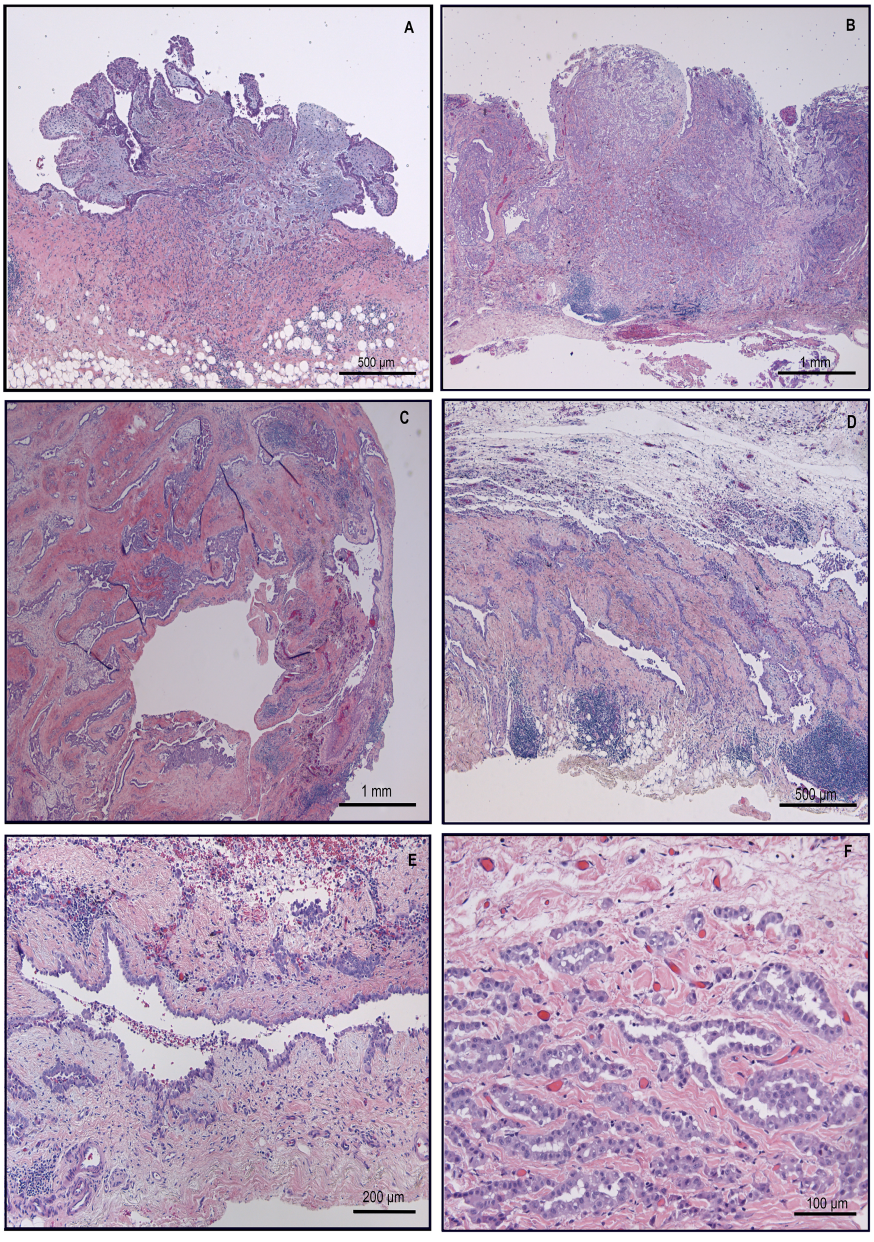
	Median survival, months (IQR)	Deceased due to DMM	Deceased for reason other than DMM	Alive with disease	Alive with minimal stable signs of DMM
Values	79.3 (69.3-99.3)	32 (74%)	3 (7%)	6 (14%)	2 (5%)

IQR, interquartile range; DMM, diffuse malignant mesothelioma

Table 4. Multivariate analysis associated with overall survival

Variable	HR[1] (95% CI[2])	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

Cox proportional hazard model adjusted for age, gender, nuclear grade, histologic subtypes, necrosis, exophytic polypoid growth, single layer, tumor density, and treatment; 1. HR, hazard ratio; 2. CI, confidence interval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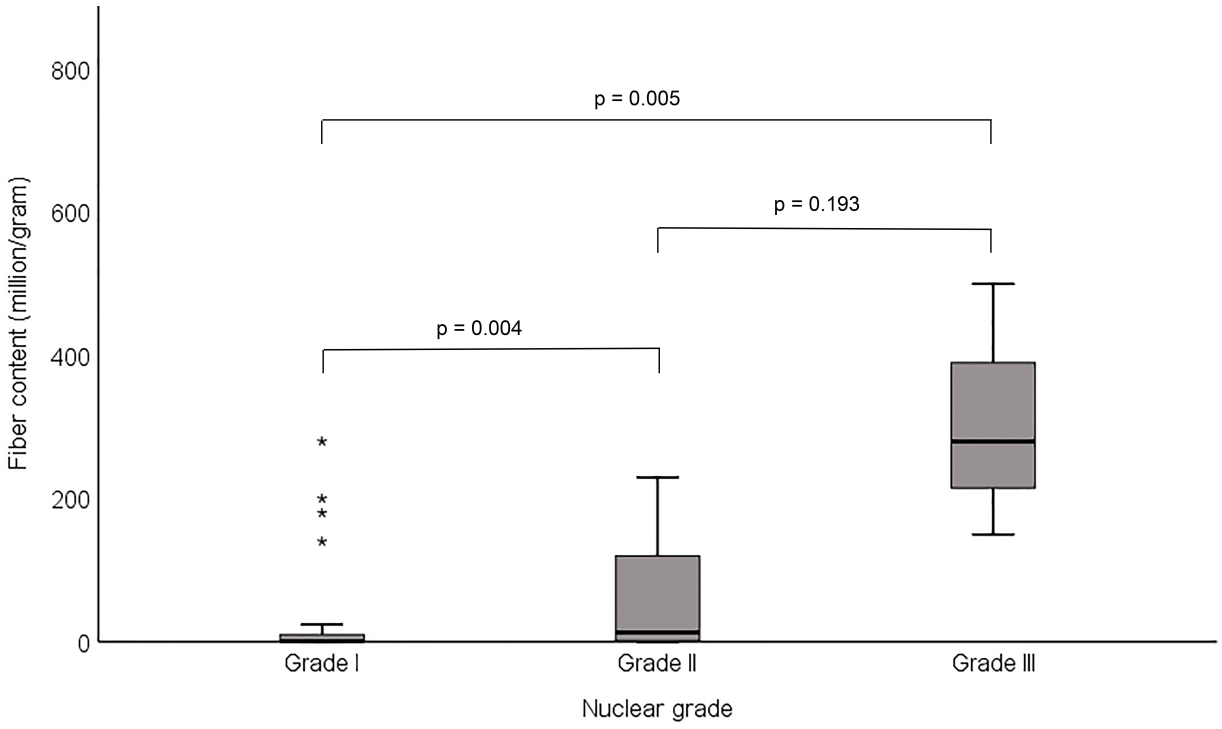

Figure legends

Figure 1. Histologic features of epithelioid diffuse malignant mesothelioma of the pleura (hematoxylin and eosin stain). A, papillary myxoid lesion with invasion. B, thick confluent area of partially myxoid exophytic mesothelial growth. C, tip of large exophytic polypoid-like lesion with internal mesothelial growth. D, large tubular structures in the pleura. E, higher enlargement of large tubular structure seen in previous picture. F, tubular structures covered mostly with a single layer of mesothelium.

Figure 2. The boxplot shows asbestos fiber content in patients divided by nuclear grade. The median concentration in grade I (n = 27) was 0.60 mf/g (IQR 0.20 – 8.60 mf/g), grade II (n = 39) 13.0 mf/g (1.0 – 140.0 mf/g), and grade III (n = 3) 280.0 mf/g (150 – NA mf/g). The Kruskall-Wallis test showed a p-value < 0.001. The p-values in pairwise comparisons showed in the figure were adjusted with Bonferroni correction for multiple tests.

Highlights

- A small portion of epithelioid DMM patients presents with prolonged survival
- Low nuclear grade and low-grade histological subtypes associates with better prognosis
- Polypoid features found in WDPM can also be found in cases of DMM
- Polypoid features were independent predictors of good prognosis

Author statement

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