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Presence of pleomorphic features but not growth patterns improves prognostic stratification of epithelioid malignant pleural mesothelioma by 2-tier nuclear grade

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Presence of pleomorphic features but not growth patterns improves prognostic stratification of epithelioid malignant pleural mesothelioma by 2-tier nuclear grade

Abstract: *Aims*: Nuclear grade has been recently validated as a powerful prognostic tool in epithelioid malignant pleural mesothelioma (E-MPM). In other studies histological parameters including pleomorphic features and growth patterns were also shown to exert prognostic impact. The primary aims of our study are (i) externally validate the prognostic role of pleomorphic features in E-MPM and (ii) investigate if evaluating growth pattern in addition to 2-tier nuclear grade improves prognostication. *Methods and results*: 614 consecutive cases of E-MPM from our institution over a period of 15 years were retrospectively reviewed, of which 51 showed pleomorphic features. E-MPM with pleomorphic features showed significantly worse overall survival compared to those without (5.4 versus 14.7 months). Tumours with predominantly micropapillary pattern showed the worst survival (6.2 months) followed by solid (10.5 months), microcystic (15.3 months), discohesive (16.1 months), trabecular (17.6 months) and tubulo-papillary (18.6 months). Sub-classification of growth patterns into high grade (solid, micropapillary) and low grade (all others) led to good separation of overall survival (10.5 versus 18.0 months) but did not predict survival independent of 2-tier nuclear grade. A composite score comprised of growth pattern and 2-tier nuclear grade did not improve prognostication compared with nuclear grade alone. Intra-tumoural heterogeneity in growth patterns is ubiquitous.

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Conclusions: Our findings support the incorporation of E-MPM with pleomorphic features in the epithelioid subtype as a highly aggressive variant distinct from 2-tier nuclear grade. E-MPM demonstrates extensive heterogeneity in growth pattern but its evaluation does not offer additional prognostic utility to 2-tier nuclear grade.

Keywords: mesothelioma, growth patterns, pleomorphic features, nuclear grade, heterogeneity

Introduction

Epithelioid malignant pleural mesothelioma (E-MPM), the most common subtype of malignant pleural mesothelioma, morphologically resembles primary thoracic epithelial malignancies with the propensity to form diverse growth patterns.[1–3] The prognostic impact of these patterns has been investigated in a limited number of studies[4–6] although none fully explored the relationship with nuclear grade, which has only been recently proposed and validated as a robust prognostic tool.[1,7–9] Also yet to be elucidated is the extent of intra-tumoural heterogeneity in terms of growth patterns, and their relation with nuclear grade and prognosis.

Distinct from pattern- and nuclear grade-based classification, E-MPM with pleomorphic features (also termed pleomorphic E-MPM) has been reported in recently years as an uncommon (incidence 5.1–14.7% of all E-MPM) and aggressive (median OS 3.0–8.1 months) variant of pleural mesothelioma.[4–6,10,11] However, the relatively small number of published cases (103 over the past 10 years) poses challenges to the ongoing discussion within major guideline committees in relation to its classification as a variant of epithelioid or non-epithelioid subtype.[1]

The primary aims of this study were (i) external validation of the prognostic impact of pleomorphic features in E-MPM and (ii) evaluation of the prognostic impact of growth patterns both alone and in combination with 2-tier nuclear grade. The secondary aims of the study were (i) characterisation of intra-tumoural heterogeneity of growth patterns by assessing predominant and co-existing secondary (non-predominant) patterns, (ii) investigation of the association of growth patterns with 2-tier nuclear grade[7,8] and (iii) evaluation of the prognostic impact of three growth patterns additional to those currently recommended for routine diagnostic usage[1] and identified during review of the cohort: discohesive, cribriform and well differentiated papillary mesothelioma (WDPM)-like features.[12]

Methods

STUDY POPULATION

A previously characterised cohort[7] of 614 consecutive cases of histologically confirmed epithelioid MPM between 2003 and 2017 from our institution was used for this study. Clinical and histopathological information were collected from an institutional mesothelioma database curated and maintained in conjunction with the National Centre of Mesothelioma Research (NCMR; National Heart & Lung Institute, Imperial College London) (M.F.M., W.O.C.M.C.). Institutional review board (IRB) approval was obtained for this study. Additional outcome data were retrieved from the National Health Service Spine repository, surgical records and regional thoracic oncology service (P.L.M, S.J., E.K.L., L.L.L., S.B., M.D., V.A., E.B., J.F., N.A., S.P.). Median OS was defined as time (measured in months) between the date of initial procedure from which a definitive diagnosis of MPM was made, and the date of death or last follow-up. TNM staging, metabolic uptake and chemotherapy or radiotherapy treatment information were not included in the study as these were incompletely recorded in the electronic patient record. Censor fraction in our study was 23% (141/614).

MICROSCOPY AND IMAGING

Microscopic assessment was performed using a Nikon Eclipse Ci-L microscope (Nikon Corporation, Japan) with a field area measuring 0.24 mm^2 per high power field (HPF, ×400 magnification). Microscopic images in 300 dpi TIF format were taken from representative cases using Nikon Digital Sight DS-L3 camera (Nikon Corporation, Japan), annotated using GNU Image Manipulation Program 2.10.10 (http://www.gimp.org, retrieved on 20.04.2019).

HISTOPATHOLOGICAL ASSESSMENT

All cases were diagnosed by at least one specialist pulmonary pathologist (C.B., A.R., J.L.R., A.G.N.) using current histological and immunohistochemical

criteria.[1–3] Histopathological parameters were assessed and recorded independently, blinded to outcome, by one pathologist. All available haematoxylin & eosin (H&E) sections were reviewed, with an average of 3.9 sections per case (range: 1–47). Common growth patterns (solid, tubulo-papillary, trabecular, micropapillary, microcystic) and the presence of pleomorphic features were evaluated using the current diagnostic criteria.[1,3] A minimum of 10% of tumour cells exhibiting severe nuclear atypia was required for the diagnosis of E-MPM with pleomorphic features. For the same reason in this study it was not regarded as a growth pattern.

Discohesive pattern was considered morphologically distinct from micropapillary pattern in that tumour cell clusters consisted of no more than 3 cells, frequently manifest as a single-cell infiltration in variable stroma. Cribriform pattern was defined as tumours with either sieve-like or atypical glandular architecture resembling cribriform pattern in lung adenocarcinoma,[13] but without nuclear attenuation to distinguish it from microcystic pattern (Table S1, Figure S1). WDPM-like was defined using published criteria for WDPM (Table S2),[12] after a *de novo* pleural WDPM had been ruled out and unequivocal invasion confirmed (Figure S2). Representative images illustrating the growth patterns are shown (Figure 1).

Predominant pattern was defined as the most abundant pattern, usually representing >50% of the entire tumour. Secondary pattern was defined as any non-predominant pattern representing at least 5% of the tumour. High grade pattern was defined as predominant solid or micropapillary patterns, and low grade pattern was defined as any other predominant pattern. A pattern-nuclear grade composite score was generated by giving one point to predominant growth pattern (high grade = 1, low grade = 0) and nuclear grade (high grade = 1, low grade = 0) then adding both components (range 0–2).

Data on patient age, surgical resection, 2-tier nuclear grade, necrosis and atypical mitosis, which were established as independent predictors of OS in E-MPM, were extracted from a previously published study from our group.[7]

STATISTICAL ANALYSIS

Descriptive statistics were employed to analyse the baseline demographic and clinicopathological parameters. Fisher's exact test was used to evaluate associations between categorical variables. Kruskal-Wallis test was used to evaluate differences on continuous variables by categorical variables. OS was estimated using the Kaplan-Meier method. Exact P values were recorded and $P < 0.05$ denotes statistical significance. Multivariate Mantel-Cox regression model was used to evaluate the effect size and statistical significance of each variable which demonstrated $P < 0.05$ in univariate analysis and with ≥10 events. All statistical analyses were performed using SPSS 26 (IBM Corp., Armonk, NY, USA). Kaplan-Meier curves were generated using GraphPad Prism Version 8 (GraphPad Software, La Jolla, California, USA).

Results

DEMOGRAPHIC AND CLINICOPATHOLOGICAL CHARACTERISTICS

The median age was 70 years (range 32–91), with 75.6% (464/614) of patients being male and 57.8% (355/614) right-sided disease. 87.0% (534/614) of patients underwent biopsy only with no documented history of neoadjuvant treatment, via video-assisted thoracoscopic surgery (VATS). This is defined as cases not having undergone maximal cytoreductive surgery (MCS) and generally comprised a lesser amount of tissue.[1,14] E-MPM with pleomorphic features represented 8.3% (51/614) of the study population.

After excluding those with pleomorphic features, tubulo-papillary was the most common predominant growth pattern (47.8%, 269/563), followed by solid (37.7%, 212/563), trabecular (8.2%, 46/563), discohesive (4.5%, 25/563), micropapillary (1.1%, 6/563) and microcystic (0.9%, 5/563). High grade predominant pattern was present in 38.7% (218/563) and low grade in 61.3% (345/563). The median OS of the study population was 13.5 months (95% confidence interval (CI) 12.2–14.8 months). Necrosis and atypical mitoses were present in 37.5% (230/614) and 73.0% (448/614) of cases respectively. Lymphatic and vascular invasion were present in 9.1% (56/614) and 8.1% (50/614) of cases, and were considered underestimates of the real incidence due to the biopsy-heavy setting of our service.

PLEOMORPHIC FEATURES IN E-MPM PREDICTS UNFAVOURABLE PROGNOSIS IN UNIVARIATE AND MULTIVARIATE SETTINGS

E-MPM with pleomorphic features was associated with significantly worse median OS (5.4 months) than those with predominant tubulo-papillary (18.6 months), trabecular (17.6 months), discohesive (16.1 months) and solid (10.5 months) patterns (all $P < 0.001$) (Table 1) (Figure 2A). A non-significant

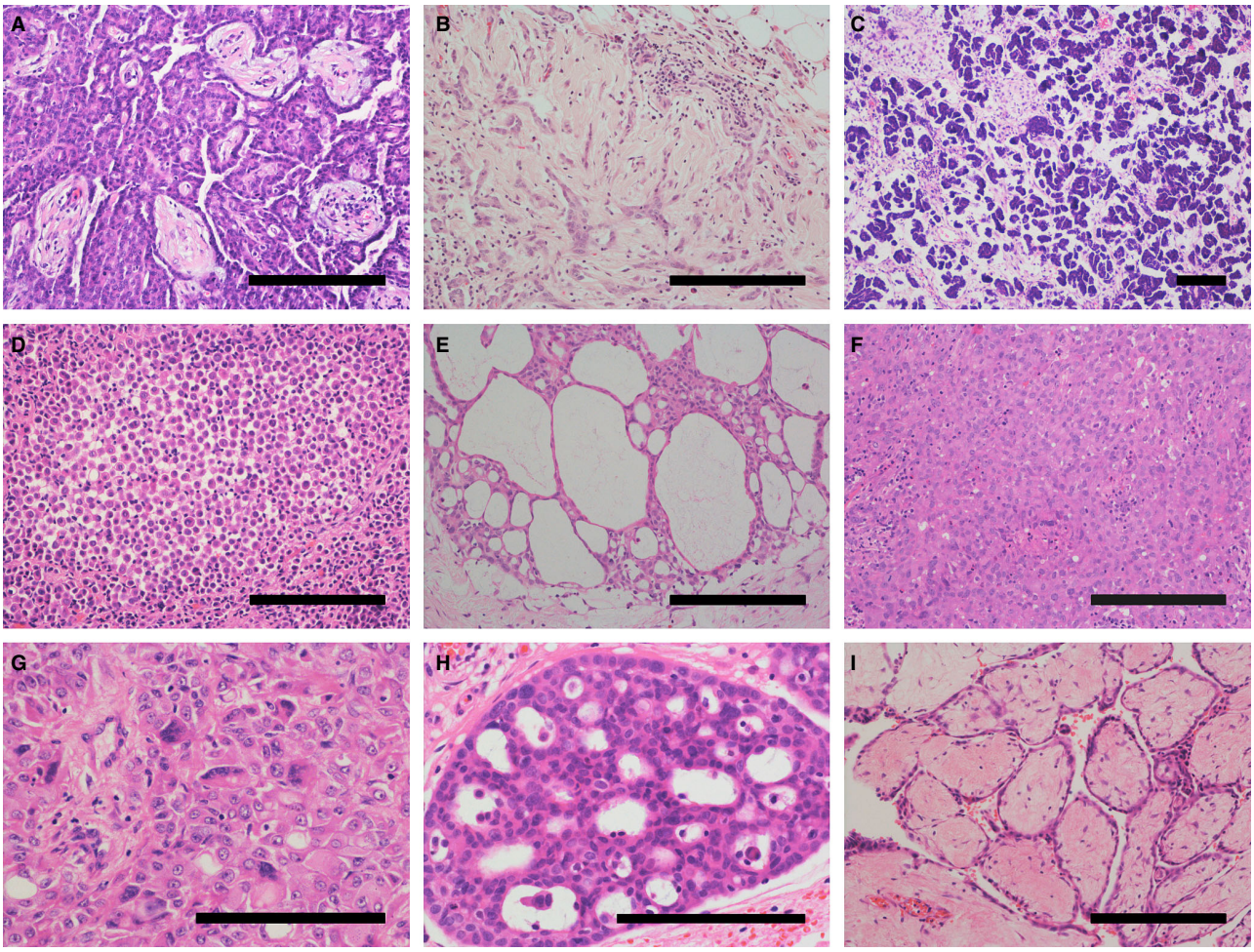

Figure 1. Growth patterns of epithelioid malignant pleural mesothelioma. (**A**) Tubulo-papillary pattern. This is a combination of frequently co-existent tubular (single luminal spaces, allowing branching) and papillary patterns. (**B**) Trabecular pattern. Tumour cells are arranged in linear fashion with or without formation of arcades. (**C**) Micropapillary pattern. This pattern resembles "miniaturised" papillary pattern but without its fibrovascular core. In the context of our study an epithelioid group of at least 4 cells is required to fulfil the diagnostic criteria. (**D**) Discohesive pattern. Tumour cells frequently show single-cell infiltration, occasionally forming clusters of no more than 3 cells. They can be dispersed in variable stroma or pseudoglandular spaces. (**E**) Microcystic pattern. Tumour cells with attenuated nuclei form enlarged glandular spaces. These can be nested so give rise to sieve-like appearance. (**F**) Solid pattern. Tumour cells form cohesive nests and nodules without discernible architecture. (**G**) Pleomorphic features. Tumour cells exhibit severer atypia including but not limited to multinucleation and formation of tumour giant cells (**H**) Cribriform pattern. The lack of intervening stroma and nuclear attenuation distinguish it from tubulo-papillary and microcystic patterns respectively. (**I**) WDPM-like features. This image shows an area indistinguishable from WDPM but there was unequivocal invasion elsewhere in the tumour (Figure). Scale bar = 100 μm.

trend towards worse OS was also observed when compared with microcystic (15.3 months, $P = 0.069$) and micropapillary (9.4 months, $P = 0.075$) patterns although the numbers of cases were small. One- and 2-year survival rates were 23.4% and 6.2% respectively (Figure 2B). The majority (82.4%, 42/51) of tumours consisted of between 10 and 20% of tumour cells exhibiting severe nuclear atypia (median 15%, range 10–50%) (Figure S3A). Using a 20% cut-off we observed no significant difference in OS ($P = 0.423$) (Figure S3B).

In multivariate analysis, pleomorphic features predicted OS independent of age, type of procedure, necrosis and atypical mitosis ($P < 0.001$ versus tubulo-papillary pattern, $P = 0.007$ versus solid pattern) (Table 2). This confirmed the findings from Kadota *et al.*[6] Furthermore, if we consider it as a function of nuclear features and include 2-tier nuclear grade instead of growth pattern as a covariate, pleomorphic features remained independently prognostic (HR 3.01 versus low grade, $P < 0.001$; HR 1.47 versus high grade, $P = 0.029$) (Table 3).

Table 1. Univariate analysis in predicting overall survival

Variable	Patients (%)	Median OS (months)	*P*
All patients	614 (100.0)	13.5	–
Predominant growth pattern			
Tubulo-papillary	269 (43.8)	18.6	6.8×10^{-13}
Trabecular	46 (7.5)	17.6	3.2×10^{-4}
Discohesive	25 (4.1)	16.1	0.001
Microcystic	5 (0.8)	15.3	0.069
Solid	212 (34.5)	10.5	4.7×10^{-5}
Micropapillary	6 (1.0)	9.4	0.075
Pleomorphic	51 (8.3)	5.4	Ref.
Grade (growth pattern)*			
Low grade	345 (61.3)	18.0	Ref.
High grade	218 (38.7)	10.5	1.0×10^{-6}
Composite score*			
Score 0	271 (48.1)	19.8	Ref.
Score 1	169 (30.0)	13.4	0.001
Score 2	123 (21.8)	8.1	1.0×10^{-17}
Predominant-secondary growth patterns*			
Low grade (predominant) + low grade (secondary)	123 (21.9)	20.8	Ref.
Low grade (predominant) + high grade (secondary)	222 (39.4)	15.3	0.026
High grade (predominant) + low grade (secondary)	197 (35.0)	12.0	6.0×10^{-6}
High grade (predominant) + high grade (secondary)	21 (3.7)	5.7	3.5×10^{-10}
Cribriform growth pattern**			
Absent	214 (79.6)	18.9	Ref.
Predominant	9 (3.3)	28.4	0.672
Secondary	46 (17.1)	12.8	0.952
WDPM-like features**			
Present	11 (4.1)	78.7	0.001
Absent	258 (95.9)	18.0	Ref.

EPP, Extrapleural pneumonectomy; EPD, Extended pleurectomy and decortication; PD, Pleurectomy and decortication; OS, Overall survival.
*Epithelioid MPM with pleomorphic features were excluded.
**Only tumours with tubulo-papillary predominant growth pattern were included.

E-MPM DEMONSTRATES EXTENSIVE HETEROGENEITY IN GROWTH PATTERNS

We sought to explore the heterogeneity in growth patterns in E-MPM by assessing the diversity and abundance of its secondary patterns. Growth patterns co-exist, frequently as a mixture of high and low grade patterns. For example, 77.8% (165/212) of solid-predominant tumours had a tubulo-papillary secondary pattern, whereas 56.1% (151/269) of

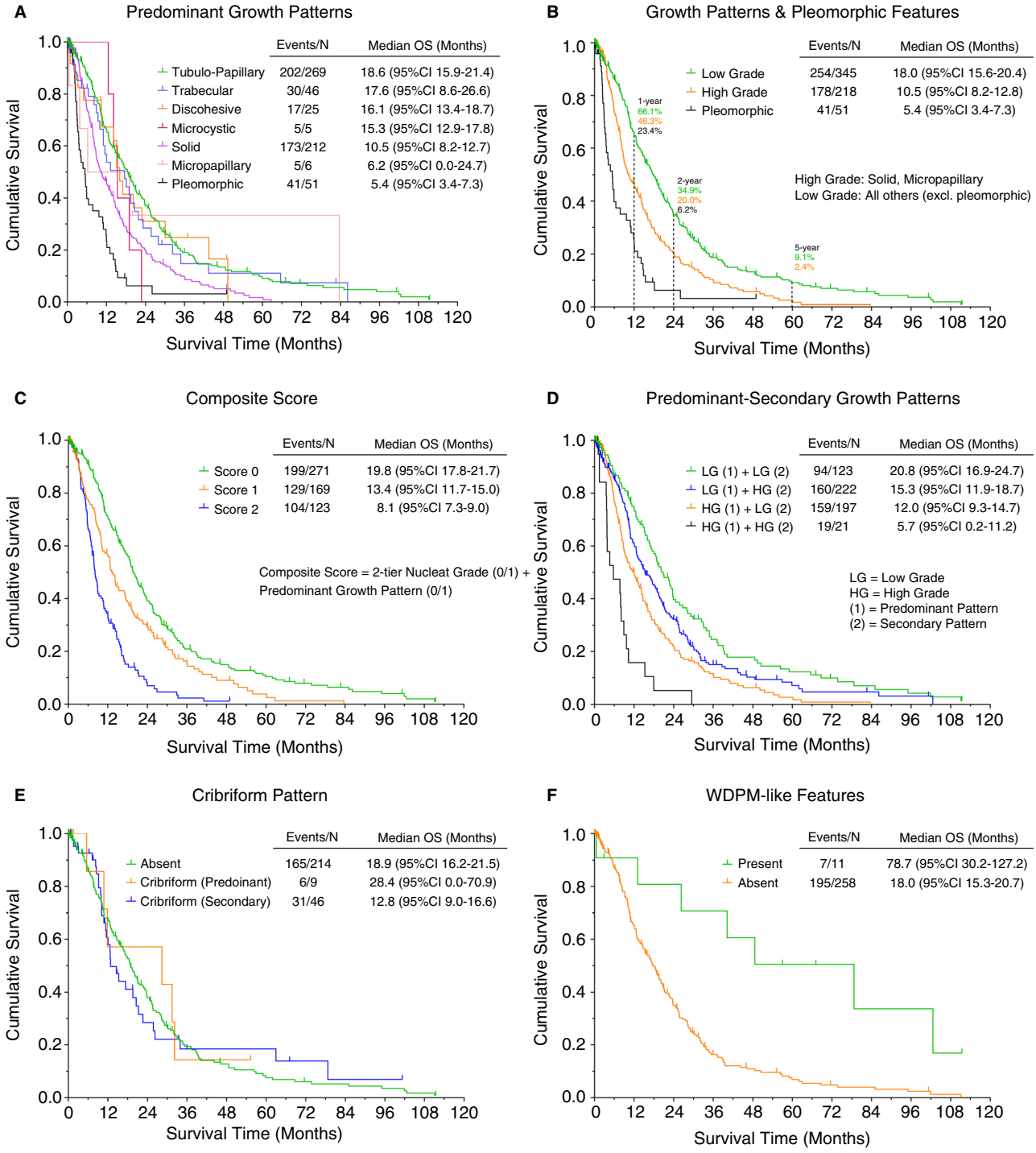

Figure 2. Overall survival by growth patterns. (**A**) Predominant growth patterns showed different survival characteristics. (**B**) Prognostic stratification was achieved by classifying predominant growth patterns into high grade and low grade. Pleomorphic features were associated with significant worse survival. (**C**) Composite score offered superior separation of median OS in univariate setting. (**D**) Evaluation of secondary patterns further modified prognostic stratification by high grade and low grade growth patterns. (**E**) Presence of cribriform pattern did not influence median OS. (**F**) E-MPM with WDPM-like features was associated with superior median OS compared with tubulo-papillary-predominant tumours without.

Table 2. Multivariate analysis in predicting overall survival (Pleomorphic features as a function of growth pattern)

Variable	Hazard ratio	95% CI	*P*
Age			
>65 versus ≤65 years	1.39	1.14–1.70	0.001
Procedure			
Resection versus biopsy only	0.33	0.24–0.46	5.6×10^{-11}
Necrosis			
Present versus absent	2.10	1.67–2.63	1.6×10^{-10}
Atypical mitosis			
Present versus absent	1.71	1.36–2.16	4.0×10^{-6}
Predominant growth pattern			
Solid versus pleomorphic	0.62	0.44–0.88	0.007
Tubulo-papillary versus pleomorphic	0.51	0.35–0.74	4.0×10^{-4}
Trabecular versus pleomorphic	0.65	0.39–1.08	0.098
Micropapillary versus pleomorphic	0.79	0.30–2.08	0.637
Microcystic versus pleomorphic	0.92	0.36–2.39	0.871
Discohesive versus pleomorphic	0.57	0.32–1.03	0.061

CI, Confidence interval.

tubulo-papillary-predominant tumours had a solid secondary pattern (Table 4). Pure growth pattern (without a secondary pattern) was seen in only 9.6% (54/563) of cases, and in just under a quarter of cases showed at least three co-existing secondary patterns (Table 5). Solid-predominant growth was associated with reduced heterogeneity ($P = 0.002$), but the number of secondary growth patterns had no significant impact on survival (Table S3). There was no association with two-tier nuclear grade ($P = 0.122$) (Table S4).

The number of resections in our study population was limited ($n = 80$, $n = 73$ after excluding pleomorphic E-MPM). Comparing biopsy-only and resection cohorts, the former was associated with smaller number of detected secondary patterns ($P < 0.001$) in keeping with sampling bias (Table S5). However there was no significant difference with regard to the detection of high grade/low grade predominant pattern ($P = 0.400$) (Table S6). Our findings were in keeping with a multi-institutional study showing moderate agreement in the assignment of subtype, presence of necrosis and nuclear grade (in epithelioid subtype) between biopsy and subsequent resection.[15]

EVALUATION OF GROWTH PATTERN DOES NOT OFFER ADDITIONAL PROGNOSTIC UTILITY TO TWO-TIER NUCLEAR GRADE

Univariate analysis showed high grade predominant pattern was associated with worse median OS compared with its low grade counterpart (10.5 versus 18.0 months, $P < 0.001$) (Table 1) (Figure 2B). Further improvement was achieved via the composite score where score 2 was associated with the worst median OS (8.1 months) ($P < 0.001$ versus score 0) followed by score 1 (13.4 months) ($P = 0.001$ versus score 0) and 0 (19.8 months) (Table 1) (Figure 2C). However in multivariate analysis, despite predicting OS independent of the established prognostic variables ($P = 0.010$) (Table 6), the composite score exhibited inferior prognostic separation (HR 1.65, score 2 versus 0) compared with 2-tier nuclear grade (HR 2.02, high grade versus low grade).[7]

We found by incorporating secondary growth patterns in univariate analysis the presence of secondary growth patterns significantly modified OS (Table 1) (Figure 2D). In the multivariate setting, however, only a pure high grade pattern (3.7%, 19/563) predicted OS independent of 2-tier nuclear grade

Table 3. Multivariate analysis in predicting overall survival (Pleomorphic features as a function of nuclear features)

Variable	Hazard ratio	95% CI	*P*
Age			
>65 versus ≤65 years	1.38	1.13–1.69	0.002
Procedure			
Resection versus biopsy only	0.31	0.23–0.43	3.0×10^{-12}
Necrosis			
Present versus absent	1.28	0.89–1.83	0.180
Atypical mitosis			
Present versus absent	1.54	1.22–1.95	3.3×10^{-4}
2-tier nuclear grade			
High grade versus low grade	2.03	1.39–2.98	2.8×10^{-4}
Pleomorphic features			
Present versus low grade	3.01	1.92–4.73	2.0×10^{-6}
Present versus high grade	1.47	1.04–2.09	0.029

CI, Confidence interval.

Table 4. Co-existence of growth patterns in epithelioid MPM

	Secondary growth pattern					
Predominant growth pattern	SOL (%)	TP (%)	TRAB (%)	MPP (%)	MC (%)	DIS (%)
SOL (*n* = 212)	–	165 (77.8)	88 (41.5)	30 (14.2)	7 (3.3)	58 (27.4)
TP (*n* = 269)	151 (56.1)	–	107 (39.8)	87 (32.3)	34 (12.6)	106 (39.4)
TRAB (*n* = 46)	28 (60.9)	40 (87.0)	–	5 (10.9)	1 (2.2)	11 (23.9)
MPP (*n* = 6)	5 (83.3)	5 (83.3)	2 (33.3)	–	1 (16.7)	3 (50.0)
MC (*n* = 5)	1 (20.0)	5 (100.0)	4 (80.0)	0 (0.0)	–	3 (60.0)
DIS (*n* = 25)	15 (60.0)	19 (76.0)	10 (40.0)	13 (52.0)	6 (24.0)	–

DIS, Discohesive pattern; MC, Microcystic pattern; MPP, Micropapillary pattern; MPM, Malignant pleural mesothelioma; TP, Tubulo-papillary pattern; TRAB, Trabecular pattern; SOL, Solid pattern.

alongside the other established prognostic variables ($P < 0.001$) (Table 7). The presence of any high grade pattern irrespective of whether it was predominant or secondary only showed a non-significant trend towards worse OS ($P = 0.061$) (Table S7).

Those who underwent surgical resection within our study population were a statistically underpowered cohort and subject to bias due to heterogeneity in surgical techniques and adjuvant treatment. 2-tier nuclear grade and pleomorphic features remained predictive of OS in the univariate setting (Figure S4). The former also predicted OS independent of high grade/low grade predominant growth patterns ($P = 0.009$) (Table S8). These findings are supportive of the above conclusions but we advise cautious interpretation. The prognostic impact of pleomorphic features relative to high nuclear grade in the setting of surgical resection in particular warrants independent validation using a large surgical cohort. We observed a non-significant trend towards worse

Table 5. Heterogeneity in growth patterns in epithelioid MPM

No. of secondary growth patterns	All patients (%)	SOL (%)	TP (%)	TRAB (%)	MPP (%)	MC (%)	DIS (%)
0	54 (9.6)	21 (9.9)	31 (11.5)	1 (2.2)	0 (0.0)	0 (0.0)	1 (4.0)
1	189 (33.6)	84 (39.6)	86 (32.0)	14 (30.2)	0 (0.0)	1 (20.0)	4 (16.0)
2	187 (33.2)	69 (32.5)	86 (32.0)	22 (47.8)	2 (33.3)	1 (20.0)	7 (28.0)
≥3	133 (23.6)	38 (17.9)	66 (24.5)	9 (19.6)	4 (66.7)	3 (60.0)	13 (52.0)

DIS, Discohesive pattern; MC, Microcystic pattern; MPP, Micropapillary pattern; MPM, Malignant pleural mesothelioma; TP, Tubulo-papillary pattern; TRAB, Trabecular pattern; SOL, Solid pattern.

survival (5.9 versus 20.6 months, $P = 0.085$) but the number of cases of pleomorphic E-MPM was small ($n = 7$).

WDPM-LIKE BUT NOT CRIBRIFORM GROWTH PATTERN MODIFIED SURVIVAL IN E-MPM WITH PREDOMINANT TUBULO-PAPILLARY PATTERN

Cribriform pattern was seen in 20.4% of tumours with a predominant tubulo-papillary pattern (55/269). There was a positive association with high nuclear grade (38.2%, 21/55) compared with those without (17.8%, 38/214). No statistical significance was reached by predominant ($P = 0.672$) or secondary patterns ($P = 0.952$) in the univariate setting compared with tubulo-papillary-predominant epithelioid MPM without such growth pattern (Table 1, Figure 2E).

WDPM-like features pattern was seen in 4.1% of tumours (11/269). All but one case showed low nuclear grade. We observed significantly prolonged median OS compared with those without (78.7 versus 18.0 months, $P = 0.001$) (Table 1, Figure 2F). The small number of cases in our population precluded multivariate analysis.

INTERACTION BETWEEN PLEOMORPHIC FEATURES, GROWTH PATTERNS AND CLINICOPATHOLOGICAL VARIABLES

Finally, we investigated the interaction between pleomorphic features, growth patterns and other clinicopathological variables. E-MPM with pleomorphic features were primarily solid-predominant (84.3%, 43/51). They retained expression of mesothelium-associated immunohistochemical markers (Calretinin, Wilms tumour protein, cytokeratin 5/6) expected of E-MPM (Table S9). Along with solid growth pattern they were associated with a higher frequency of necrosis and atypical mitosis (all $P < 0.001$) (Table 8).

Table 6. Multivariate analysis in predicting overall survival (Composite score)

Variable	Hazard ratio	95% CI	*P*
Age			
>65 versus ≤65 years	1.39	1.16–1.77	0.001
Procedure			
Resection versus biopsy only	0.33	0.23–0.47	6.1×10^{-10}
Necrosis			
Present versus absent	1.73	1.27–2.37	0.001
Atypical mitosis			
Present versus absent	1.59	1.25–2.01	1.2×10^{-4}
Composite score			
Score 1 versus 0	1.27	0.99–1.63	0.058
Score 2 versus 0	1.65	1.13–2.41	0.010

CI, Confidence interval.

A higher incidence of lymphovascular invasion (LVI) was seen in micropapillary and discohesive patterns, as well as those with pleomorphic features (all $P < 0.001$). High grade growth patterns were associated with high nuclear grade ($P < 0.001$) although such was not exclusive. We observed progressive transformation towards high nuclear grade with an increasing proportion of high grade growth pattern ($P < 0.001$) (Table S10).

With regard to sampling criteria, pleomorphic features were detected at higher frequency with increasing maximum tissue dimension ($P = 0.016$) but not the number of biopsy sites ($P = 0.842$) (Figure S5). This served as further supportive evidence to propose

Table 7. Multivariate analysis in predicting overall survival (Predominant-secondary growth patterns)

Variable	Hazard Ratio	95% CI	*P*
Age			
>65 versus ≤65 years	1.45	1.18–1.79	4.8×10^{-4}
Procedure			
Resection versus biopsy only	0.31	0.22–0.44	9.9×10^{-11}
Necrosis			
Present versus absent	1.29	0.87–1.94	0.209
Atypical mitosis			
Present versus absent	1.44	1.13–1.84	0.003
2-tier nuclear grade			
High grade versus low grade	1.95	1.28–2.96	0.002
Growth pattern			
Low grade (predominant) + high grade (secondary) versus low grade (predominant) + low grade (secondary)	1.28	0.98–1.67	0.074
High grade (predominant) + low grade (secondary) versus low grade (predominant) + low grade (secondary)	1.26	0.95–1.68	0.110
High grade (predominant) + high grade (secondary) versus low grade (predominant) + low grade (secondary)	2.67	1.59–4.49	2.1×10^{-4}

CI, Confidence interval.

extensive sampling recommendations.[1,7,16] Neither sampling criteria had significant impact on the detection of high grade growth patterns.

Discussion

With nuclear grade emerging as a robust tool in the prognostication of E-MPM, many questions remain,[1] not only for the next iteration of the WHO tumour classification but also day-to-day diagnostic practice. This study assessed the impact of other histological parameters on prognosis and how they interact with nuclear grade.

First, we validated findings from the previous reports on E-MPM with pleomorphic features, for prognosis showing statistical significance versus both low and high grade E-MPM (Table S11). Its prognostic impact and clinicopathological features warrant having its own diagnostic category. Based on available evidence from the literature[4–6,10,11] and our study, nearly all are thought to be an aggressive variant of E-MPM rather than non-epithelioid subtype, as the preservation of mesothelial lineage is still evident despite suggestions of early de-differentiation (Table S12). Although it is not possible to exclude sampling error with absolute certainty as 44 out of 51 cases did not undergo resection or autopsy based on available information, no cases with pleomorphic features were associated with sarcomatoid morphology elsewhere in our series. Therefore we propose pleomorphic features as a suffix equivalent to 2-tier nuclear grade as the diagnosis is based on nuclear features alone, as pleomorphic E-MPM has a significantly worse prognosis than conventional high grade E-MPM.[7] Based on the findings from this study and our previous study, E-MPM could be reliably sub-classified using biopsies into low grade (59.6%, median OS 19.3 months), high grade (32.1%, median OS 8.9 months) and pleomorphic (8.3%, median OS 5.4 months).

Second, whilst confirming the survival characteristics conferred by growth patterns in reported series (Table S13), we showed a combined approach utilising growth pattern and 2-tier nuclear grade did not improve prognostic stratification. This negative finding suggests that 2-tier nuclear grade alone remains the prognostic tool of choice for E-MPM, other than the addition of pleomorphic cases. The extensive intra-tumoural heterogeneity we observed offers a possible explanation: not only could high grade and

Table 8. Distribution of clinicopathological variables by predominant growth pattern

Variable	All patients	PLEO	SOL	TP	TRAB	MPP	MC	DIS	*P*
All patients (%)	614 (100.0)	51 (8.3)	212 (34.5)	269 (43.8)	46 (7.5)	6 (1.0)	5 (0.8)	25 (4.1)	–
Age (years)									
Median	70	69	70	70	68	58	71	71	0.084
Range	32–91	37–88	39–91	41–90	46–88	48–67	61–82	32–83	
Sex (%)									
Male	464 (75.6)	41 (80.4)	165 (77.8)	200 (74.3)	29 (63.0)	4 (66.7)	5 (100.0)	20 (80.0)	0.277
Female	150 (24.4)	10 (19.6)	47 (22.2)	69 (25.7)	17 (37.0)	2 (33.3)	0 (0.0)	5 (20.0)	
Laterality (%)									
Left	254 (41.4)	27 (52.9)	90 (42.5)	101 (37.5)	19 (41.3)	1 (16.7)	1 (20.0)	15 (60.0)	0.187
Right	355 (57.8)	24 (47.1)	121 (57.1)	166 (61.7)	26 (56.5)	5 (83.3)	4 (80.0)	9 (36.0)	
Not documented	5 (0.8)	0 (0.0)	1 (0.4)	2 (0.8)	1 (2.2)	0 (0.0)	0 (0.0)	1 (4.0)	
Procedure (%)									
Biopsy	534 (87.0)	44 (86.3)	190 (89.6)	229 (85.1)	46 (100.0)	3 (50.0)	4 (80.0)	18 (72.0)	4.3×10^{-4}
PD or EPD	72 (11.7)	7 (13.7)	21 (9.9)	35 (13.0)	0 (0.0)	2 (33.3)	1 (20.0)	6 (32.0)	
EPP	5 (0.8)	0 (0.0)	0 (0.0)	4 (1.5)	0 (0.0)	1 (16.7)	0 (0.0)	0 (0.0)	
Other procedures	3 (0.5)	0 (0.0)	1 (0.5)	1 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)	1 (4.0)	
Necrosis (%)									
Present	230 (37.5)	41 (80.4)	112 (52.8)	61 (22.7)	6 (13.0)	3 (50.0)	1 (20.0)	6 (24.0)	1.3×10^{-19}
Absent	384 (62.5)	10 (19.6)	100 (47.2)	208 (77.3)	40 (87.0)	3 (50.0)	4 (80.0)	19 (76.0)	
Lymphatic invasion (%)									
Present	56 (9.1)	9 (17.6)	18 (8.5)	20 (7.4)	1 (2.2)	3 (50.0)	0 (0.0)	5 (20.0)	4.5×10^{-4}
Absent	558 (90.9)	42 (82.4)	194 (91.5)	249 (92.6)	45 (97.8)	3 (50.0)	5 (100.0)	20 (80.0)	
Vascular invasion (%)									
Present	50 (8.1)	8 (15.7)	15 (7.1)	15 (5.6)	2 (4.3)	4 (66.7)	0 (0.0)	6 (24.0)	8.2×10^{-8}
Absent	564 (91.9)	43 (84.3)	197 (92.9)	254 (94.4)	44 (95.7)	2 (33.3)	5 (100.0)	19 (76.0)	

Table 8. *(Continued)*

Variable	All patients	PLEO	SOL	TP	TRAB	MPP	MC	DIS	*P*
Atypical mitosis (%)									
Present	448 (73.0)	50 (98.0)	178 (84.0)	176 (65.4)	23 (50.0)	4 (66.7)	2 (40.0)	15 (60.0)	6.4×10^{-10}
Absent	166 (27.0)	1 (2.0)	34 (16.0)	93 (34.6)	23 (50.0)	2 (33.3)	3 (60.0)	10 (40.0)	
Nuclear grade (%)*									
Low grade	366 (59.6)	–	92 (43.4)	210 (78.1)	39 (84.8)	3 (50.0)	4 (80.0)	18 (72.0)	2.2×10^{-14}
High grade	197 (32.1)	–	120 (56.6)	59 (21.9)	7 (15.2)	3 (50.0)	1 (20.0)	7 (28.0)	

DIS, Discohesive pattern; EPP, Extrapleural pneumonectomy; EPD, Extended pleurectomy and decortication; MC, Microcystic pattern; MPP, Micropapillary pattern; PD, Pleurectomy and decortication; PLEO, Pleomorphic; SOL, Solid pattern; TP, Tubulo-papillary pattern; TRAB, Trabecular pattern.
*Epithelioid MPM with pleomorphic features does not receive nuclear grade.

low grade growth patterns co-exist, but tumour cells exhibiting high nuclear grade could commit to low grade growth patterns, and *vice versa*. This extended the previous observation where higher nuclear grade was associated with tumours with solid-predominant growth pattern.[7] Evaluation of growth pattern is still of value however, for example in aiding accurate diagnosis and identification of growth patterns associated with LVI.

Third, our findings on cribriform pattern and WDPM-like features warrant external validation as they might represent uncommon groups of tumours with unique survival and biological characteristics. The presence of the latter should also be considered as a potential diagnostic pitfall in mesothelioma especially in the small biopsy setting, albeit rarely encountered, highlighting the importance of correlation with clinicoradiological and intraoperative findings. Of note, although the authors studied only cases of peritoneal origin, true WDPM has been shown to harbour mutations in *CDC42* or *TRAF7*,[17] in contrast to *BAP1* and *CDKN2A* alterations frequently registered in MPM.[18,19] An evolutionary link between WDPM and subsequent malignant transformation to diffuse invasive mesothelioma was also proposed.[20] However as no genomic data are available it is uncertain if it represents a molecularly-distinct entity, or very early- stage diffuse mesothelioma characterised by minimal invasion.

Our study has two major limitations. First, it is retrospective, and we were not able to investigate the impact of additional parameters associated with pleomorphic features other than prognosis, such as metabolic uptake.[21] Secondly we were not able to evaluate response to treatment including maximal cytoreductive surgery as well as chemotherapy, in relation to pleomorphic features and growth patterns.

Our future work includes the investigation of the genetic basis and evolution of pleomorphic features in E-MPM compared with those showing low/high nuclear grades, evaluation of the prognostic impact of pleomorphic features in non-epithelioid MPM (biphasic, sarcomatoid), and assessment of inter-observer agreement which is known to be problematic.[22,23] We believe the questions around growth patterns in E-MPM should be revisited when we have acquired deeper understanding and novel tools to spatially deconvolute the cellular and molecular signatures of co-existing growth patterns. This is because for such morphologically distinct tumour architectures, a homogenous biological process is highly implausible.

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Author contributions

Y.Z.Z. and A.G.N. conceptualised and designed the study. Y.Z.Z., C.B., A.R., J.L.R. and A.G.N. performed the research. Y.Z.Z. analysed the data. J.L.Q., A.N.H., W.O.C.M.C. and M.F.M. contributed to data interpretation. P.L.M, S.J., E.K.L., L.L.L., S.B., M.D., V.A., E.B., J.F., N.A. and S.P. contributed to clinical data acquisition. Y.Z.Z. and A.G.N. wrote the manuscript. All authors reviewed and approved the final version of the manuscript.

Conflict of interest

E.K.L. received personal fees from the following: Abbott Molecular, Glaxo Smith Kline, Pfizer, Novartis, Covidien, Roche, Lily Oncology, Boehringer Ingelheim, Medela, ScreenCell and Ethicon; and grants from ScreenCell, Clearbridge Biomedics and Guardant Health. S.P. received honoraria from BMS, Roche, Takeda, AstraZeneca, Chugai, Novartis, Pfizer, MSD, EMD Serono, Guardant Health, AbbVie, Boehringer Ingelheim, Medscape, Tesaro and OncLive. None were relevant during the preparation of the manuscript. The other authors declared no conflict of interest exist.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Cribriform growth pattern in epithelioid MPM.

Figure S2. Example of invasive E-MPM with WDPM-like features.

Figure S3. Extent of pleomorphic features and association with survival.

Figure S4. Univariate analysis in predicting overall survival by 2-tier nuclear grade and pleomorphic features (Resections only).

Figure S5. Effect of sampling criteria on the detection of pleomorphic features and growth patterns.

Table S1. Assessment criteria for cribriform growth pattern.

Table S2. Assessment criteria for WDPM-like features.

Table S3. Univariate analysis in predicting overall survival by number of secondary growth patterns.

Table S4. Distribution of 2-tier nuclear grade by the number of secondary growth patterns.

Table S5. Heterogeneity in growth patterns in epithelioid MPM (Resections only).

Table S6. Detection of high grade/low grade predominant growth pattern by type of procedure.

Table S7. Multivariate analysis in predicting overall survival (Prognostic impact of the presence of any high grade growth pattern).

Table S8. 2-tier nuclear grade predicted OS independent of high/low grade predominant pattern (Resections only).

Table S9. Immunoprofile of pleomorphic epithelioid MPM (n = 51).

Table S10. Distribution of 2-tier nuclear grade by predominant-secondary growth patterns.

Table S11. Comparison of demographic and clinicopathological characteristics with previously published cohorts of pleomorphic epithelioid MPM.

Table S2. Arguments for and against pleomorphic epithelioid MPM being incorporated into non-epithelioid MPM subtype.

Table S13. Comparison with previously published studies on the prognostic impact of growth patterns by overall survival.

