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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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Date of submission 26 February 2020
Accepted for publication 22 April 2020
Published online Article Accepted 25 April 2020

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. The prognostic impact of these patterns has been investigated in a limited number of studies although none fully explored the relationship with nuclear grade, which has only been recently proposed and validated as a robust prognostic tool. 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 recent 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. 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.

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 and (iii) evaluation of the prognostic impact of three growth patterns additional to those currently recommended for routine diagnostic usage and identified during review of the cohort: discohesive, cribriform and well differentiated papillary mesothelioma (WDPM)-like features.

Methods

Study Population

A previously characterised cohort 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., 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 mm² 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. 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. A minimum of 10% of tumour cells exhibiting severe nuclear atypia was required for the diagnosis of E-PM 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, 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), 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-PM, were extracted from a previously published study from our group.

**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. E-PM 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-PM PREDICTS UNFAVOURABLE PROGNOSIS IN UNIVARIATE AND MULTIVARIATE SETTINGS**

E-PM 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
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).
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...

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               | $6.8 \times 10^{-13}$ |
| Trabecular                                    | 46 (7.5)     | 17.6               | $3.2 \times 10^{-4}$ |
| Discoid                                       | 25 (4.1)     | 16.1               | 0.001   |
| Microcystic                                   | 5 (0.8)      | 15.3               | 0.069   |
| Solid                                         | 212 (34.5)   | 10.5               | $4.7 \times 10^{-5}$ |
| 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               | $1.0 \times 10^{-6}$ |
| 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                | $1.0 \times 10^{-17}$ |
| 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               | $6.0 \times 10^{-6}$ |
| High grade (predominant) + high grade (secondary)| 21 (3.7)  | 5.7                | $3.5 \times 10^{-10}$ |
| 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.
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.
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-PM). 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 S3). However there was no significant difference with regard to the detection of high grade/low grade predominant pattern ($P = 0.122$) (Table S4).

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

| Variable                                | Hazard ratio | 95% CI     | $P$      |
|-----------------------------------------|--------------|------------|----------|
| Age                                     |              |            |          |
| $>65$ versus $\leq 65$ years            | 1.39         | 1.14–1.70  | 0.001    |
| Procedure                               |              |            |          |
| Resection versus biopsy only            | 0.33         | 0.24–0.46  | $5.6 \times 10^{-11}$ |
| Necrosis                                |              |            |          |
| Present versus absent                   | 2.10         | 1.67–2.63  | $1.6 \times 10^{-10}$ |
| Atypical mitosis                        |              |            |          |
| Present versus absent                   | 1.71         | 1.36–2.16  | $4.0 \times 10^{-6}$ |
| Predominant growth pattern              |              |            |          |
| Solid versus pleomorphic                | 0.62         | 0.44–0.88  | 0.007    |
| Tubulo-papillary versus pleomorphic     | 0.51         | 0.35–0.74  | $4.0 \times 10^{-4}$ |
| 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.
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 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| $3.0 \times 10^{-12}$ |
| Necrosis               |              |          |        |
| Present versus absent  | 1.28         | 0.89–1.83| 0.180  |
| Atypical mitosis       |              |          |        |
| Present versus absent  | 1.54         | 1.22–1.95| $3.3 \times 10^{-4}$ |
| 2-tier nuclear grade   |              |          |        |
| High grade versus low grade | 2.03         | 1.39–2.98| $2.8 \times 10^{-4}$ |
| Pleomorphic features   |              |          |        |
| Present versus low grade | 3.01         | 1.92–4.73| $2.0 \times 10^{-6}$ |
| 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

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

**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.

**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       | \( 6.1 \times 10^{-10} \) | |
| Necrosis                  |              |                 |        |
| Present versus absent     | 1.73         | 1.27–2.37       | 0.001  |
| Atypical mitosis          |              |                 |        |
| Present versus absent     | 1.59         | 1.25–2.01       | \( 1.2 \times 10^{-4} \) |
| 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...
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, 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 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. Based on the findings from this study and our previous study, E-MPM could be reliably subclassified 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 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.
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 x 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 x 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 x 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 x 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) |      |
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.\textsuperscript{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 \textit{CDC42} or \textit{TRAF7},\textsuperscript{17} in contrast to \textit{BAP1} and \textit{CDKN2A} alterations frequently registered in MPM.\textsuperscript{18,19} An evolutionary link between WDPM and subsequent malignant transformation to diffuse invasive mesothelioma was also proposed.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.
Acknowledgements

The authors thank the Clinical Data Warehouse (CDW) team at Royal Brompton and Harefield NHS Foundation Trust for their assistance in retrieving clinical outcome data for this study; the International Mesothelioma Panel (IMP) and members of the National Centre for Mesothelioma Research for meaningful discussions during the preparation of the manuscript. This study was funded by the National Centre for Mesothelioma Research, established via a Department of Health (United Kingdom) grant awarded to M.F.M. and W.O.C.M.C. Y.Z.Z. was supported by a clinical research fellowship from the National Centre for Mesothelioma Research.

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, Coviden, 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.