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An Evaluation Into the Robustness of Grading of Pleural Mesothelioma Outside of Specialist Centres

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ABSTRACT

The 2021 WHO classification of thoracic tumours recommends grading pleural mesothelioma to aid prognostication. Robustness of grading and morphological characterisation is key to its clinical utility, though validation of this grading system has largely been conducted by expert thoracic pathologists. We conducted a survey inviting pathologists across a range of practices and expertise to grade digitised images of 50 epithelioid pleural mesotheliomas that had been graded by an expert in thoracic pathology. We included slides that were considered potentially problematic such as small biopsies, focal necrosis, and rare subtypes that may affect grading (small cell and deciduoid features). Using the Sectra Uniview web viewer, participants were asked to score atypia, mitotic count, and necrosis and choose from a list of cytological and architectural features. Seventy-four pathologists anonymously participated. There was 90% agreement of consensus scores with expert opinion using the WHO 2-tier grade and 72% for the 3-tier nuclear grade but only 70% for nuclear atypia, 56% for mitoses, and 84% for necrosis. Both 3-tier nuclear grade and WHO 2-tier grading systems were significantly associated with survival. Our study affirms the overall robustness and utility of grading for pleural mesothelioma, reveals variances, and suggests the need for dedicated training.

1 | Introduction

Mesothelioma is a malignant tumour of the serosal surface of the pleura, peritoneum, pericardium, and tunica vaginalis, typically caused by asbestos exposure. Although it is considered a rare disease, there were 30,870 new cases of mesothelioma recorded globally in 2020 [1]. Asbestos has been banned in many countries [2], but due to the decades-long latency of

disease development from exposure, new cases are predicted to continue to be diagnosed for years to come [3]. Prognosis is poor, with patients' survival ranging from 8 to 14 months [4, 5]. A recent multicentre randomised trial revealed that extended pleurectomy decortication and chemotherapy together worsened outcomes compared to chemotherapy alone [6]. Immunotherapy in mesothelioma shows some promise, but outcomes are difficult to predict [5] and better predictive markers for efficacious treatment outcomes are being sought.

Initial studies by Kadota et al. established the prognostic significance of histological features such as nuclear atypia and mitotic counts in resections and biopsies from pleural mesothelioma. They proposed a 3-tiered classification of nuclear grade in pleural mesothelioma by combining scores on nuclear atypia and mitotic counts [7]. This nuclear grading system was found to be predictive of survival in subsequent studies [8–11]. Rosen et al. added scores from necrosis to nuclear grade. This further separated tumour groups based on overall survival, proving the prognostic and possible predictive value of necrosis [9]. The prognostic role of nuclear atypia, mitotic counts, necrosis and atypical mitoses was also validated on pleural mesothelioma biopsies by Habougit et al. [12]. The work from Kadota et al. and Rosen et al. paved the way for a 2-tiered grading system [7, 9]. Following the EURACAN/IASLC proposal, the WHO incorporated tumour grading for diffuse pleural epithelioid mesothelioma into the classification guidelines, and grading is now a core element of pathological reporting in the USCAP cancer protocols and International Collaboration on Cancer Reporting (ICCR) guidelines [13–16]. (Table 1) The 2021 WHO classification of tumours of the pleura and pericardium also recommends inclusion of cytological, architectural, and stromal features [13] (Table 2).

Some of the known prognostic factors related to survival in pleural mesothelioma include age, gender, TNM staging, and histological subtype [17–19]. Epithelioid mesothelioma has a better prognosis than the other subtypes, and grading is only relevant to epithelioid subtypes [15, 20]. Other studies have shown that a neutrophil-lymphocyte ratio, haemoglobin levels, platelet counts, BAP1 IHC status, and microvessel density can have prognostic significance [21–24]. We here focus on markers relevant to pathology reporting that are endorsed by the WHO and included in the ICCR structured report.

TABLE 1 | Grading of Pleural Mesothelioma [13].

Nuclear Atypia Score: mild (1), moderate (2), severe (3)
Mitotic Count: 0–1/2 sq. mm (1), 2–4 mitosis/2 sq. mm (2), > 5/2 sq. mm (3)
Necrosis: present (1) or absent (0)
3-tier Nuclear Grade
• Sum of nuclear atypia and mitotic count
• Grade I (2, 3), grade II (4, 5) and grade III (6)
2-tier Grade
• Low grade: grade I and II tumours without necrosis
• High grade: grade II with necrosis and grade III with or without necrosis

The WHO Classification of tumours of the peritoneum, however, does not currently specify the grading system for use in the histological diagnosis of peritoneal mesothelioma, although studies suggest the WHO grading can be used [25, 26]. Tumour grades are not provided on cytology samples as mitotic counts based on square mm and architectural features are not applicable to fluids. However, as an isolated feature, concordance of nuclear atypia has been reported in paired histology and cytology samples of epithelioid mesothelioma [27, 28]. Grading is applicable only to diffuse mesothelioma and cannot be assessed for in situ mesothelioma. There are currently no recommendations integrating tumour grade into patient selection for specific treatments [28].

This 2-tier WHO grading method was utilized to assess its role in predicting prognosis in some studies [8, 29, 30] but has not been as widely validated as the 3-tiered grading system for grading mesothelioma [9, 28, 31]. Grading in these studies was predominantly performed by pathologists who were experts in thoracic pathology. One study compared three non-expert pathologists' grading scores with two expert thoracic pathologists scores in mesothelioma. Agreement on the 3-tiered nuclear grade was moderate, with improved agreement with the 2-tiered grading system [32].

Molecular profiling showed an association between the WHO low nuclear grade and alterations in BRCA-associated protein 1 (*BAP1*) [33]. Low grade was shown to correlate with improved survival with BAP1 loss on immunohistochemistry in all histological subtypes. In their article, Fuchs et al. graded all histological subtypes using the WHO grading method and found prognostic significance only in the epithelioid and biphasic subtypes [34]. Currently, however, the WHO grade is only recommended for epithelioid mesothelioma, and no grading guidelines exist for non-epithelioid subtypes. Hence, the possibility of associations of tumour grade in mesothelioma with clinical or molecular features warrants consideration. Interestingly, high asbestos fibre counts in the lung have been associated with mesothelioma of high grade [31]. Initial studies on the association of tumour grade with treatment response have suggested a definitive link [35, 36].

Architectural patterns and cytological features in mesothelioma are known to have prognostic implications [37, 38], and these features are suggested to be included in reporting, but their reproducibility for prognostication has been suboptimal. This is probably due to tumour heterogeneity, the small number of samples in the subgroups, and variations in host defence and microenvironment [30, 39]. Studies have shown that mesothelioma with rhabdoid morphology predicts aggressive behaviour [40]. Pleomorphic features in mesothelioma arising from the pleural and peritoneum are also considered to run an aggressive course with poor prognosis [37, 41, 42]. Interestingly, myxoid stroma is associated with a more favourable course [43, 44]. Though mesothelioma with deciduoid features was considered to denote tumour aggressiveness [45, 46], others do not suggest poor prognosis for this variant [47, 48]. According to the WHO classification of thoracic tumours (2021), cytological features such as deciduoid, small

TABLE 2 | Architectural patterns, cytological features and stromal characteristics of epithelioid mesothelioma [13].

Pattern/features	Favourable	Unfavourable
Architectural patterns • Tubulopapillary • Trabecular • Adenomatoid • Solid • Micropapillary	Architectural patterns • Tubulopapillary • Trabecular • Adenomatoid	Architectural patterns • Solid (> 50%) • Micropapillary
Cytological features • Rhabdoid • Deciduoid • Small-cell • Clear-cell • Signet ring • Lymphohistiocytoid • Pleomorphic	Cytological features • Lymphohistiocytoid • Low nuclear grade	Cytological features • Rhabdoid • Pleomorphic • High nuclear grade
Stromal features • Myxoid	Stromal features • Myxoid (if predominant, i.e., when > 50% solid pattern contains myxoid stroma)	Necrosis (included in grading)

cell, clear cell, and signet ring cell do not carry any prognostic significance. Architectural patterns such as the tubulopapillary, trabecular, and adenomatoid are associated with favourable prognosis, though solid and micropapillary patterns are associated with unfavourable prognosis [13].

This study aimed to assess the robustness of grading in a real live setting by assessing the level of agreement on grading pleural mesothelioma by pathologists across a range of practices and levels of experience. We used virtual microscopy on digitised images, which are increasingly being utilised for routine diagnosis and specialist examinations. This study compared concordance and accuracy in pathologists' grading of diffuse pleural mesothelioma outside of specialist centres using whole slide digital imaging, scoring by an expert thoracic pathologist (SK) as reference, and explored associations with survival and BAP1 status by IHC.

2 | Methods

2.1 | Patient Population

This study consisted of a cohort sourced from archival tissue for 50 patients with epithelioid pleural mesothelioma diagnosed at the Department of Anatomical Pathology at Flinders Medical Centre between 1991 and 2021. Case information was recorded as part of a clinician-initiated quality assurance study. Ethics approval for the study was received from the Southern Adelaide Clinical Human Research Ethics Committee (HREC/19/SAC/28) and Central Adelaide Local Health Network Human Research Ethics Committee (R20190415). Two were resection samples, and 48 were pleural biopsies. All cases had been diagnosed by experts (S.K. and the late D.W.H), graded by S.K. at SA Pathology, Flinders Medical Centre, Adelaide, Australia, and where applicable were re-classified according to 2021 WHO guidelines. Patient inclusion criteria consisted of a histological diagnosis of pleural mesothelioma and access to clinical follow-up information.

2.2 | Survey Details

The study was conducted using an online survey on the Microsoft Forms platform between November 2021 and May 2022. Slides were selected to reflect various levels of difficulty and a wide range of morphological subtypes. Invitation to participate in the survey was disseminated via the newsletter of the Royal College of Pathologists of Australasia (RCPA) and emails on the RCPA network to individuals with interests in lung pathology. Invitations were also sent to members of the International Mesothelioma Interest Group (iMiG) and members of the Pulmonary Pathology Society (PPS). The invitation included a link to a video demonstrating the use of the WHO classification recommended grading system. All data from pathologists were anonymized, and no personal identifying information was collected with the survey. Selected cases can be accessed from Table S1.

The survey consisted of 50 H&E-stained whole slide images scanned using an Olympus VS200 slide scanner (Olympus, Japan) and visualised with OlyVIA version 3.2 software (Olympus). Whole slide images were stored in the Sectra digital pathology solution (Sectra AB, Linköping, Sweden) and were viewed by participants using Sectra Uniview. Each slide image was accompanied by multiple choice options for participants to select architectural and cytological features, nuclear atypia, mitotic count, necrosis, and nuclear grade, as well as overall grade. There were no time constraints, and respondents were free to return and complete the survey at any time. Participants could skip cases or multiple-choice questions.

2.3 | Histological Procedures and Scoring

Formalin-fixed paraffin-embedded tissue was used to prepare haematoxylin and eosin-stained slides. For BAP1 immunohistochemical analysis, sections were cut at 0.4 μm thickness. Staining was conducted as per the manufacturer's instructions

on a Ventana BenchMark Ultra platform (Ventana, USA), using Mouse monoclonal BAP1 C-4 antibody (sc-28383, 1:50 dilution; Santa Cruz Biotechnology, USA). Antigen retrieval was carried out using Ventana reagents Cell Conditioning Solution 1 (CC1) and Ventana Amplification Kit (760-080). Internal positive control cells labeling with BAP1 were required for assessment. Diagnostic clinical procedures were performed in an Australian (NATA)-approved laboratory that uses tests validated by a Quality Assurance Program (QAP).

All slides were graded in accordance with the 2021 WHO classification recommendations (Table 1) and the 3-tier nuclear grade and the 2-tier WHO tumour grade were derived from participants' responses after viewing whole-slide images as proposed by Kadota and Nicholson et al. respectively [7, 15] (Tables 1 and 2).

2.4 | Statistical Analysis

Scores from Microsoft Forms were downloaded to Microsoft Excel. For each slide, a consensus score was determined based on the maximum scores from participants. Overall agreement between the experts scores (SK) and participants consensus scores was expressed as percentages.

For pairwise comparisons, kappa and accuracy were computed for every given pair having sufficiently many observations for a 2×2 table. Kappa and weighted kappa values were calculated using the R package psycho. Accuracy values were produced in R with a custom function according to the common formula:

$$\text{Accuracy} = \frac{TP + TN}{TP + TN + FP + FN}$$

TP, TN, FP, FN represent the number of true positives, true negatives, false positives, and false negatives, respectively. One reviewer was arbitrarily assigned as "truth" and the other as "test". Since accuracy is a strictly binary input, the 3-tier scores were divided into higher vs. lower scores by median (i.e. either 1 and 2 or 2 and 3 were merged). In comparisons against the expert, scores of the latter were considered as "truth" The numbers TP, TN, FP, FN represented how many patients received the higher score from both reviewers, from none, from "test" alone, and from "truth" alone, respectively.

Survival analysis using Kaplan–Meier log-rank analysis was conducted with SPSS version 28 (IBM, USA). Bar charts were created using GraphPad Prism 9.0 for Windows (GraphPad Software, USA).

3 | Results

3.1 | Histological Grading

Seventy-four pathologists participated in the survey and assessed whole slide images. Specialist pathologist (SK) scores using glass slides were used as a comparator for 3-tiered nuclear grade and 2-tiered WHO tumour grade as shown in Table 3 and correlated with survival.

TABLE 3 | Histological grading by expert pathologist (SK).

3-tier nuclear grade	2-tier overall tumour grade
Grade I = 8	Low grade = 32
Grade II = 32	High grade = 18
Grade III = 10	

TABLE 4 | Level of agreement with expert pathologist's scores and grading 50 epithelioid mesothelioma.

	Agreement	%
Atypia	35/50	70
Mitoses	28/50	56
Nuclear grade (3-tier)	36/50	72
Necrosis	42/50	84
Overall grade (2-tier)	45/50	90

BAP1 loss on IHC was seen in 31 of 50 slides, retained in 17, and data unavailable for 2 cases. Twenty-two of the 31 tumours with BAP1 loss were low grade, and 9 were high grade. Architectural features were predominantly tubulopapillary, trabecular, or a combination; 2 cases were described as deciduoid, 3 with myxoid stroma (example case 2 in Table S1), 3 with solid features (example case 28 in Table S1), 2 with small cell (example case 51 in Table S1) and 1 pleomorphic (example case 8 in Table S1). The solid and pleomorphic types were considered high grade by all raters. Of the two tumours with deciduoid and small cell features each, one was scored as low grade and another as high grade (case 29 in Table S1) by the expert pathologist.

Based on consensus scores, agreement with expert pathologist's scores revealed the variability and percentage of agreement or non-agreement as shown in Table 4 and Figure 1. Agreement on the overall WHO 2-tier tumour grade was higher (90%) compared to the 3-tier nuclear grade (72%). Agreement on the presence of necrosis was higher than that of atypia or mitotic counts. Disagreement with expert pathologist's scores was highest (44%) for mitotic count scores and lowest (10%) for the 2-tier tumour grade proposed by the WHO.

We then evaluated interrater agreement pairwise, in all possible pairs of the 74 reviewers (left plots in Figure 2). Cohen's kappa has been a popular scoring approach in similar contexts. For 3-tier scores we also calculated weighted kappa, which accounted for the relative disagreement (i.e. mis-assignments of score 1 instead of 3 received higher weights than e.g. 2 instead of 3). Although, Cohen's kappa has been criticized for suffering from missing values (when a patient was not evaluated by both raters) and from high imbalance of assigned scores (when higher or lower scores dominate and are highly likely to be assigned by both raters). Also, it neither allows for significance estimation nor leans on zero as a baseline. We therefore complemented kappa with accuracy estimates. Next, we compared each reviewer's scores to the expert's assignments (right plots in Figure 2).

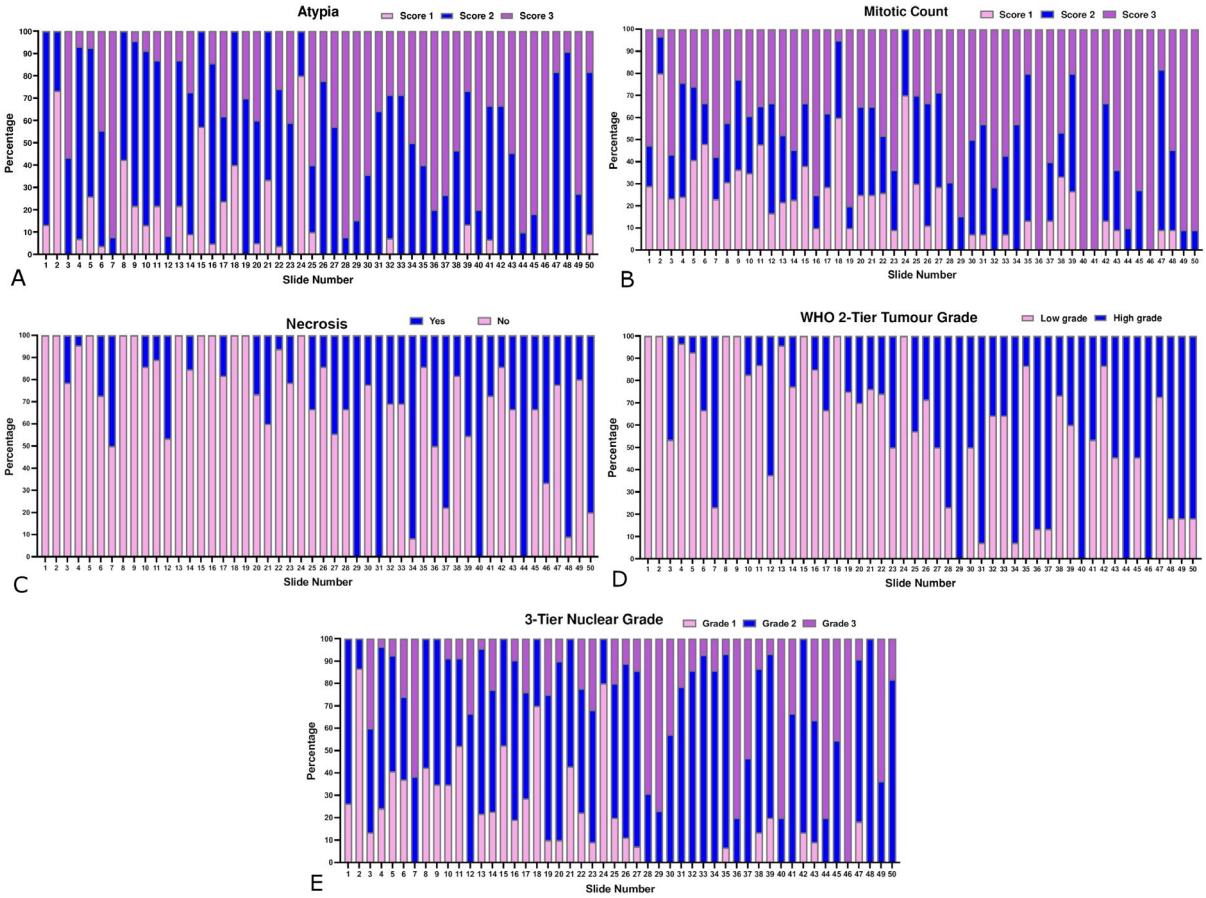

FIGURE 1 | (A–E) Variability in scores for atypia, mitotic counts, necrosis, 3-tier nuclear grade and 2-tier WHO grade provided by multiple graders. Bar graphs showing variability in scoring atypia in 50 pleural epithelioid mesotheliomas.

All in all, the pairwise analysis indicated that the overall agreement between the reviewers was modest, but consistently better than expected by chance. Median kappa and accuracy values exceeded the reference value expected by chance for all gradings except for "mitotic" on which the reviewers performed worse than on the other four gradings. Agreement with the expert was even better; the fraction of reviewers showing agreement better than random was 70.8%–93.4% (Figure 2). Examples of slides with variable scores are seen in Figure 3.

3.2 | Survival Analysis

Both expert's scores and consensus scores on 3-tier nuclear grade and 2-tier WHO tumour grade correlated with survival. For the 2-tier WHO tumour grade, median survival for expert's scores was 15 months for low grade (CI 7.7–22) (CI = confidence interval), and 7 months for high grade (CI 2.1–11.9), log rank $p = 0.002$. (Figure 4A) Median survival with consensus scores was 15 months for low grade (CI 7.6–22.3) and 6.5 months for high grade (CI 4.2–8.7) log rank $p < 0.001$. Consensus scores for nuclear atypia, mitotic counts, and presence of necrosis in the cohort significantly associated with survival ($p = 0.002$, 0.027 and 0.002, respectively, figure not shown).

For the 3-tier nuclear grade scores, median survival for experts' scores was 22 months for Grade I (CI 0–56), 13 months for Grade II (CI 9.7–16.2) and 6.5 months for Grade III (CI 5.03–7.96), log rank $p = 0.004$ (Figure 4C). Median survival with consensus scores was 22 months for Grade I (CI 11.9–32), 13.5 months for Grade II (CI 10.6–16.3), and 6 months for Grade III (CI 3.9–8.0), log rank $p < 0.001$ (Figure 4D).

In the group with BAP1 loss on IHC (31 of 50), median survival for 2-tier WHO tumour grade with specialist and consensus scores was 17.5 months for low grade (CI 4.8–30) and 6.5 months for high grade (CI 5.0–7.9), log rank $p = 0.003$ (Figure 4E). In the group with BAP1 retained on IHC (17 of 50), median survival with specialist scores was 13 months for low grade (CI 9.2–16.7) and 9 months (CI 1.79–16.2) for high grade, log rank $p = 0.164$. For consensus scores, median survival was 13 months for low grade (CI 8.8–17.1) and 6 months for high grade (CI 0–16.2), log rank $p = 0.164$ (Figure 4F).

4 | Discussion

The interest in developing guidelines for mesothelioma grading on pathological specimens arose from the need to develop a prognostic tool that is simple to use, yet robust. It is also hoped that grading will improve the selection of patients for specific treatments and/or clinical trials. The current grading system recommended by the WHO classification derives from earlier work by Kadota et al. who developed the 3-tiered nuclear grade [7], and included necrosis as a prognostic factor in addition to nuclear atypia and mitotic counts

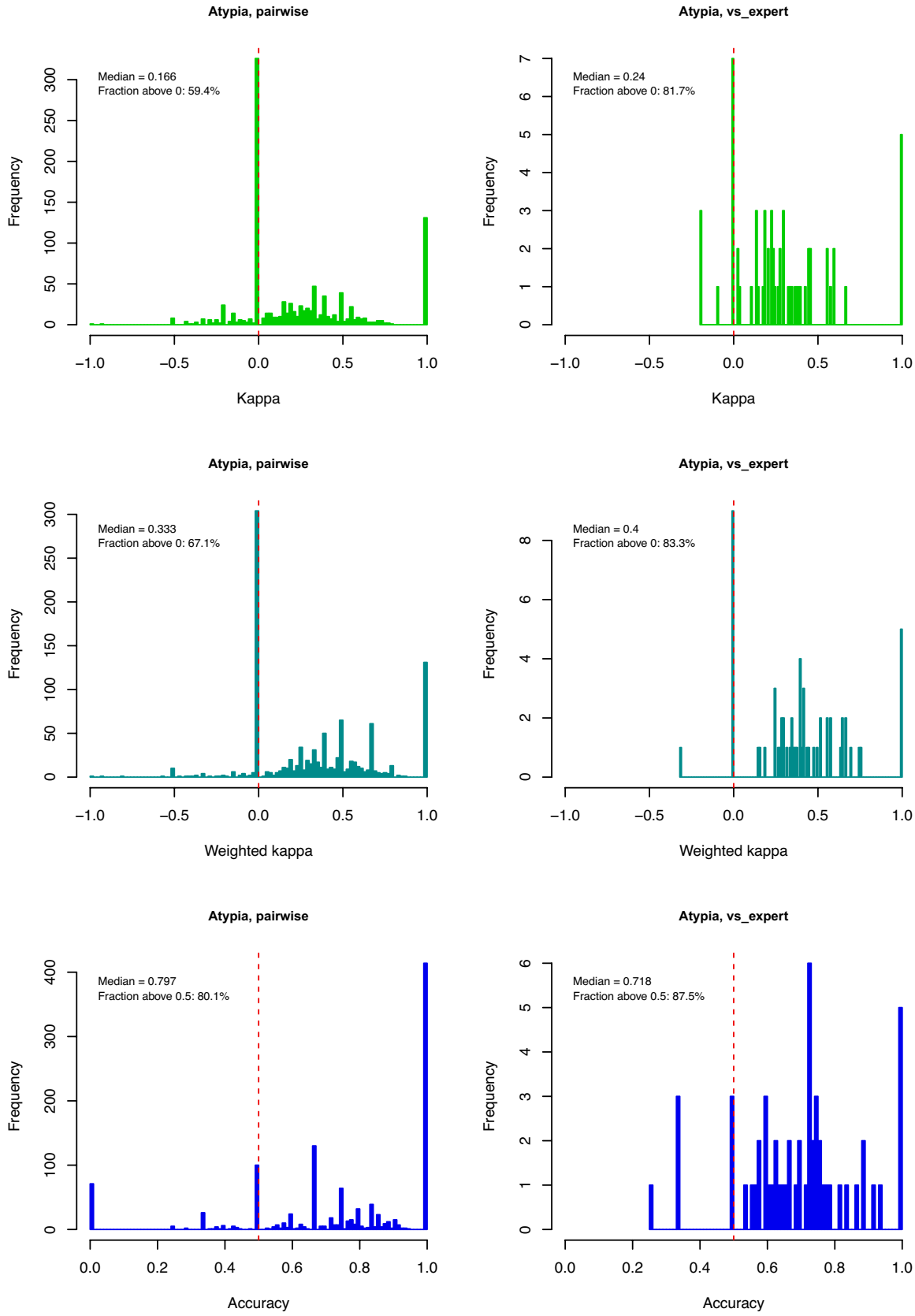

FIGURE 2 | Pairwise analysis of interrater agreement in assignment of five grading scores. Left plots: All-versus-all pairs of the 74 reviewers. Right plots: Comparisons of each of the 74 reviewers against the expert. Red dotted line: No agreement, reference so that agreement cases to the right of it are better than random.

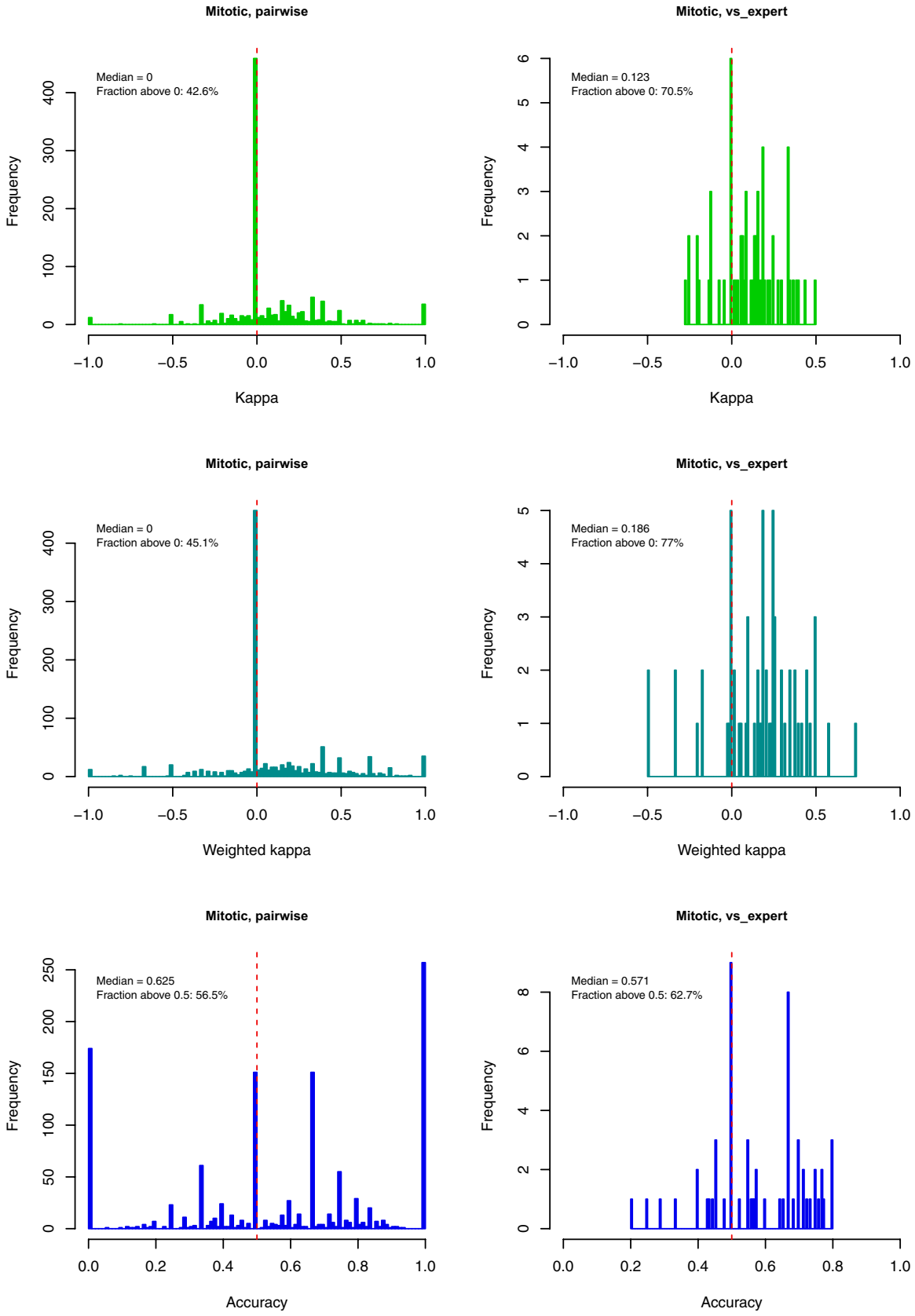

FIGURE 2 | (Continued)

[13]. However, reproducibility of the grading system in a variety of clinical settings is key to endorsing its use in regular pathology diagnosis and assessing the reliability of results. Most studies on grading mesothelioma were conducted by expert pathologists in research settings and may not be representative of everyday reporting by a range of pathologists.

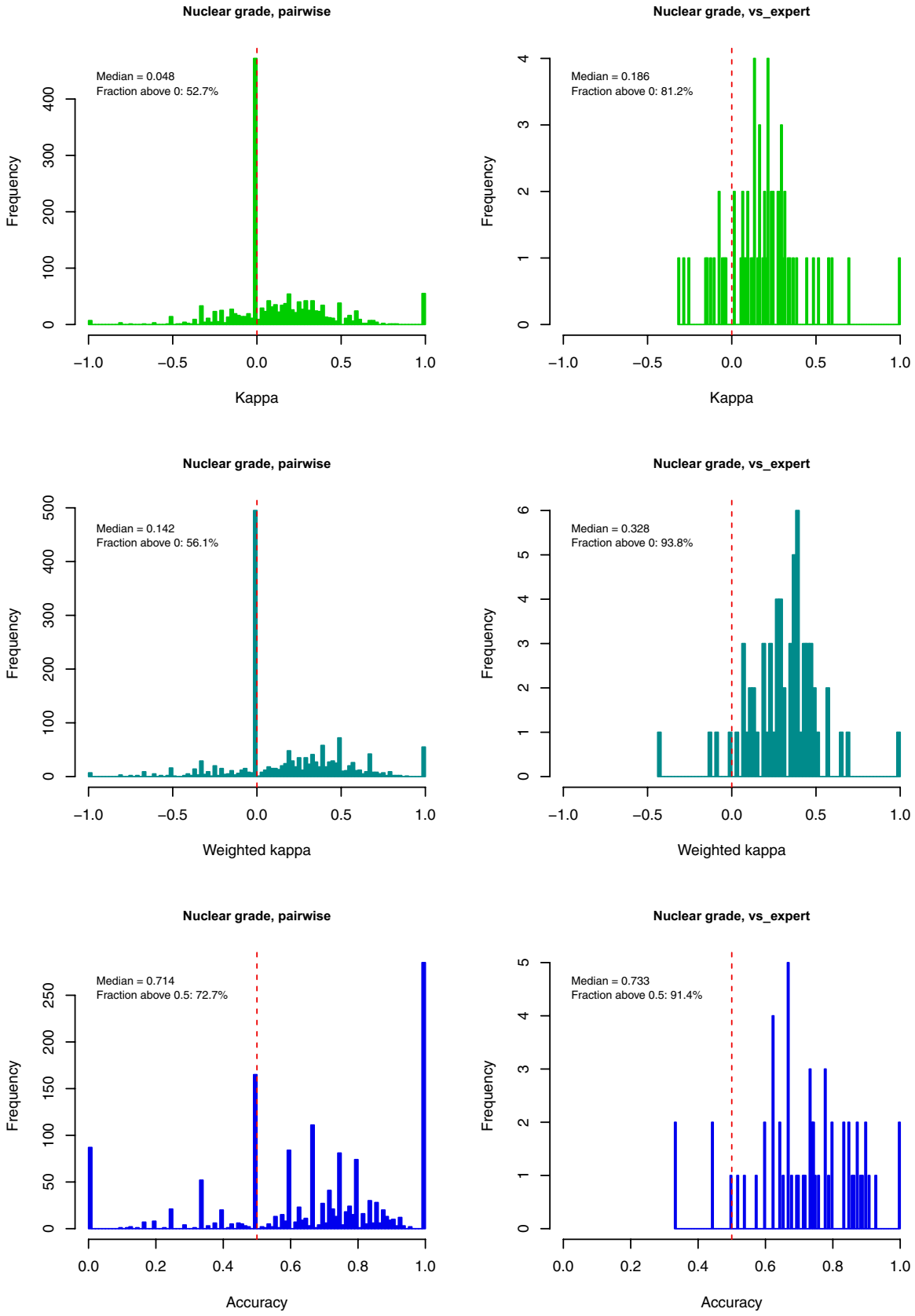

FIGURE 2 | (Continued)

One limitation is that this grading is only applicable to epithelioid mesothelioma, but grading methods suitable for all mesothelioma subtypes have been proposed. The method proposed by Pelosi et al. added points for necrosis, histological type, Ki67, and mitotic count, as these variables showed good interobserver agreement. All subtypes, including the desmoplastic variant of sarcomatoid mesothelioma, were included [49]. Fuchs et al. proposed a grading method that included clinical variables and

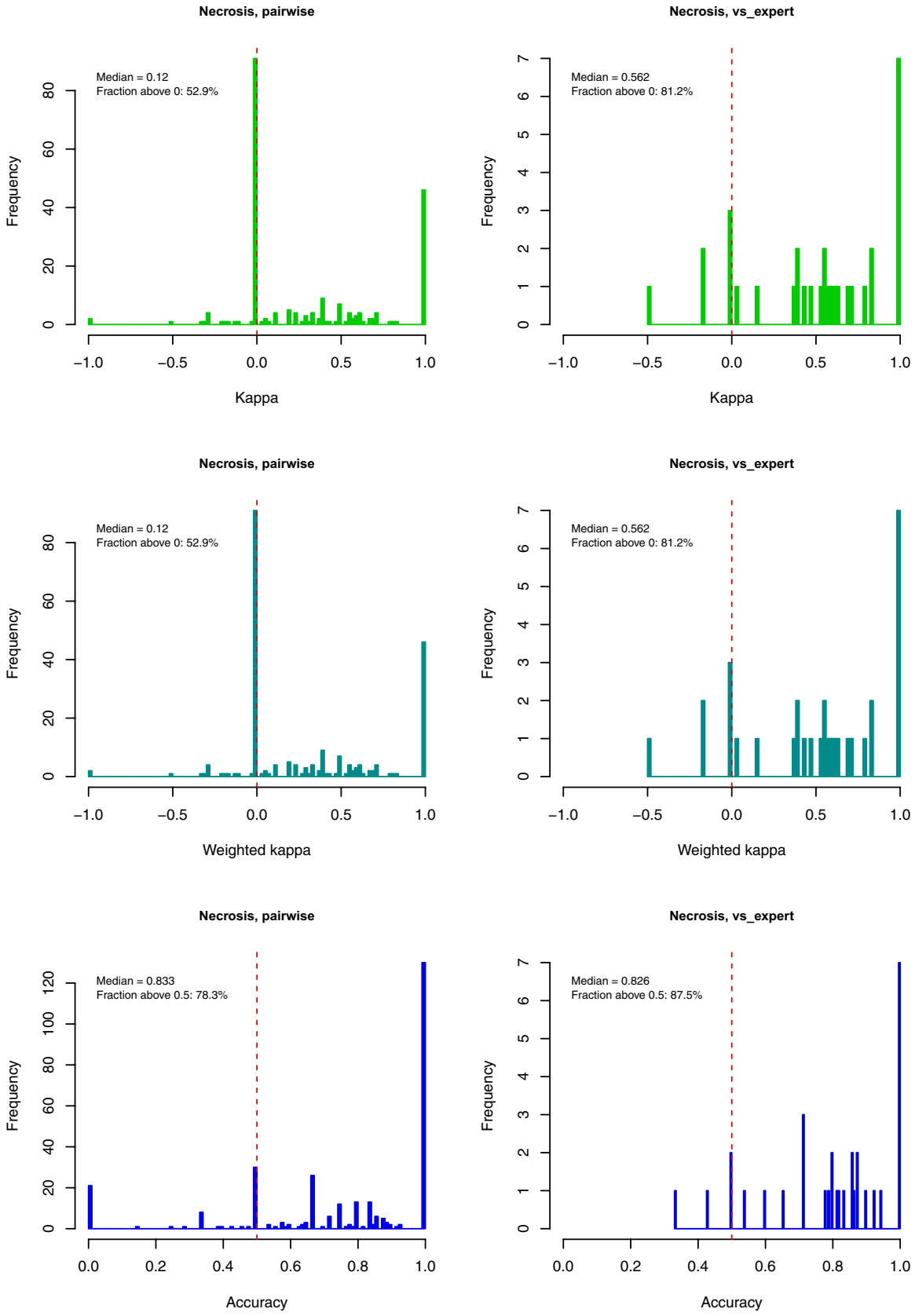

FIGURE 2 | (Continued)

used scores on age, necrosis, histological type, mitotic counts, nuclear atypia, and BAP1 expression on IHC. They also performed grading on all subtypes with the WHO grading method and found that prognostic significance was only found in the epithelioid and biphasic subtypes, but not in sarcomatoid mesothelioma [34]. Currently, though, grading is recommended only

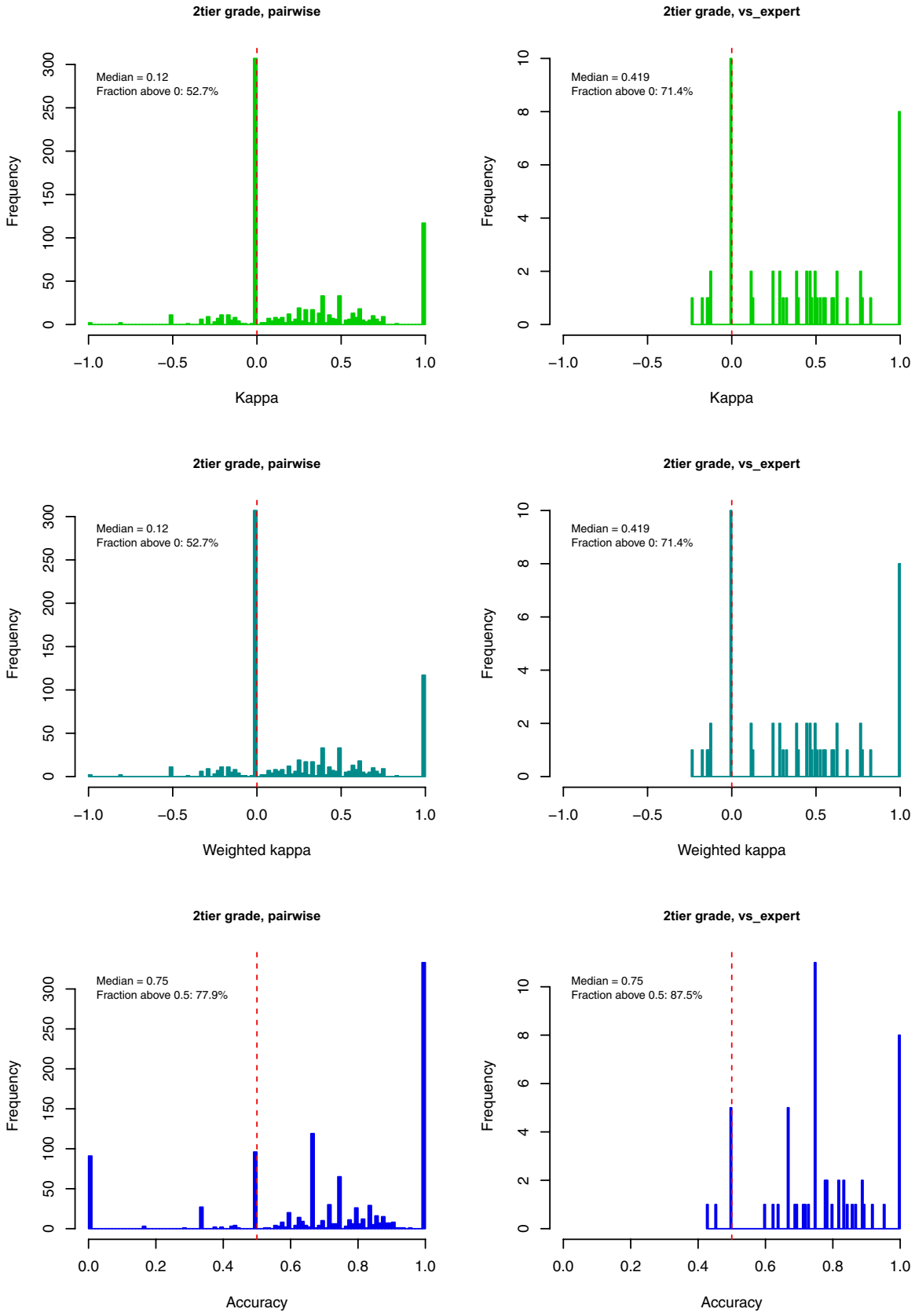

FIGURE 2 | (Continued)

for the epithelioid subtype. A slight modification to the WHO grading by Benzerdjeb et al. provided similar scoring criteria for nuclear atypia and mitotic counts; however, the presence of necrosis received a score of 2 rather than 1 (as proposed in the WHO grading) [50]. All grading methods in these studies could independently predict survival.

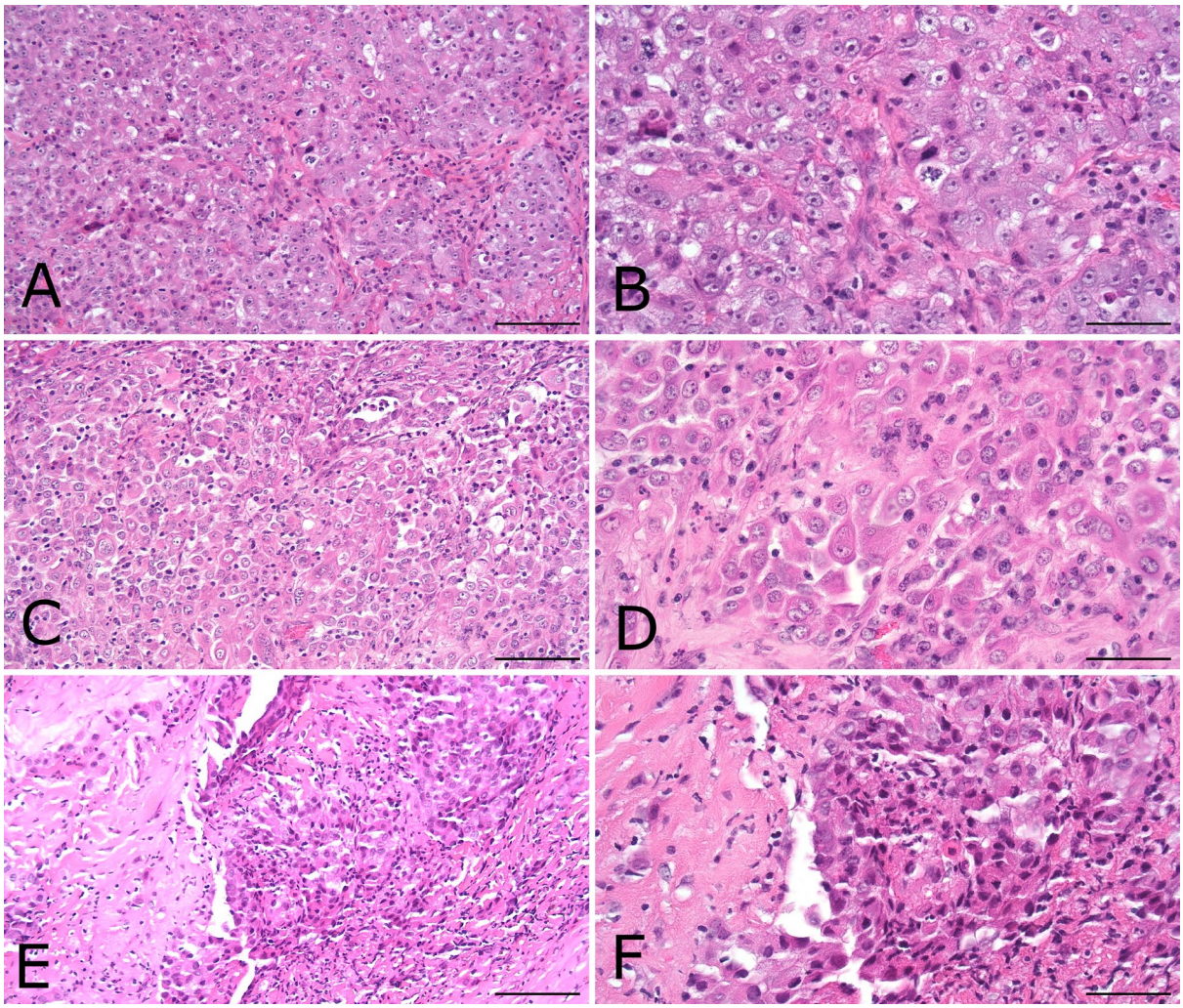

FIGURE 3 | Histopathological examples of slides with discordance in scores on atypia and necrosis. (A, B) Slide scored as 2 for atypia by the expert and 3 by majority of graders. (20× and 40× respectively) (C, D) Slide scored as atypia score 2 by the expert but scores 2 or 3 by equal number of graders. (20× and 40×, respectively) (E, F) Slide scored as positive for presence of necrosis by the expert but negative by majority of graders. (20× and 40×, respectively). Scale bars represent 100 μm at 20× and 50 μm at 40×, respectively.

Architectural patterns such as trabecular, tubulopapillary, acinar, adenomatoid, and tumours with myxoid stroma are associated with better prognosis [13, 39]. Patterns that are associated with unfavourable prognosis include the micropapillary pattern, solid, transitional, rhabdoid and pleomorphic features [13, 14]. Although deciduoid features were considered a poor prognostic factor [45, 46], other studies do not associate deciduoid features with unfavourable prognosis [47, 48]. Incidentally, there were two tumours with deciduoid features in our study, and one received a high grade while the other received a low grade on consensus scores. Pleomorphism in deciduoid mesothelioma may underlie their classification as high grade, which means the histological feature does not by itself denote an aggressive tumour [15, 51]. All patterns are thus recommended to be mentioned in diagnostic reports [15, 52]. Variations in assessing architectural patterns and cytological features, as well as small numbers within these subtypes, render their role suboptimal for prognostication in pleural epithelioid mesothelioma [28, 39]. Pleomorphic features in mesothelioma were suggested to be classified as a separate group, as prognosis was worse than high-grade pleural mesothelioma [41]. However, they have not revealed any unique mutations on whole exome sequencing [53].

Discrepancies in pathologists' scores on prognostic factors such as nuclear atypia, mitotic counts, and tumour grade have been identified individually in previous studies [34]. Fuchs et al. noted that interrater agreement on mitotic count and necrosis was moderate but poor for agreement on atypia. Mlika et al. found that the current grading system resulted in poor agreement on grading between two experienced pathologists [54]. Li et al. suggest that training provided to pathologists, especially on scoring nuclear atypia in mesothelioma, will improve the reproducibility of grading mesothelioma and reduce discrepancies [32]. In their study, there was excellent agreement between pathologists on the 2-tiered WHO tumour grade; agreement on the 3-tier nuclear grade was moderate, though substantial for necrosis and mitotic counts, and fair for nuclear atypia. Similar to our findings, remarkable improvement in agreement was found when the 2-tiered WHO tumour grade was determined, underlining its advantage as a robust prognostic tool [32]. Although we found good agreement with necrosis, we believe

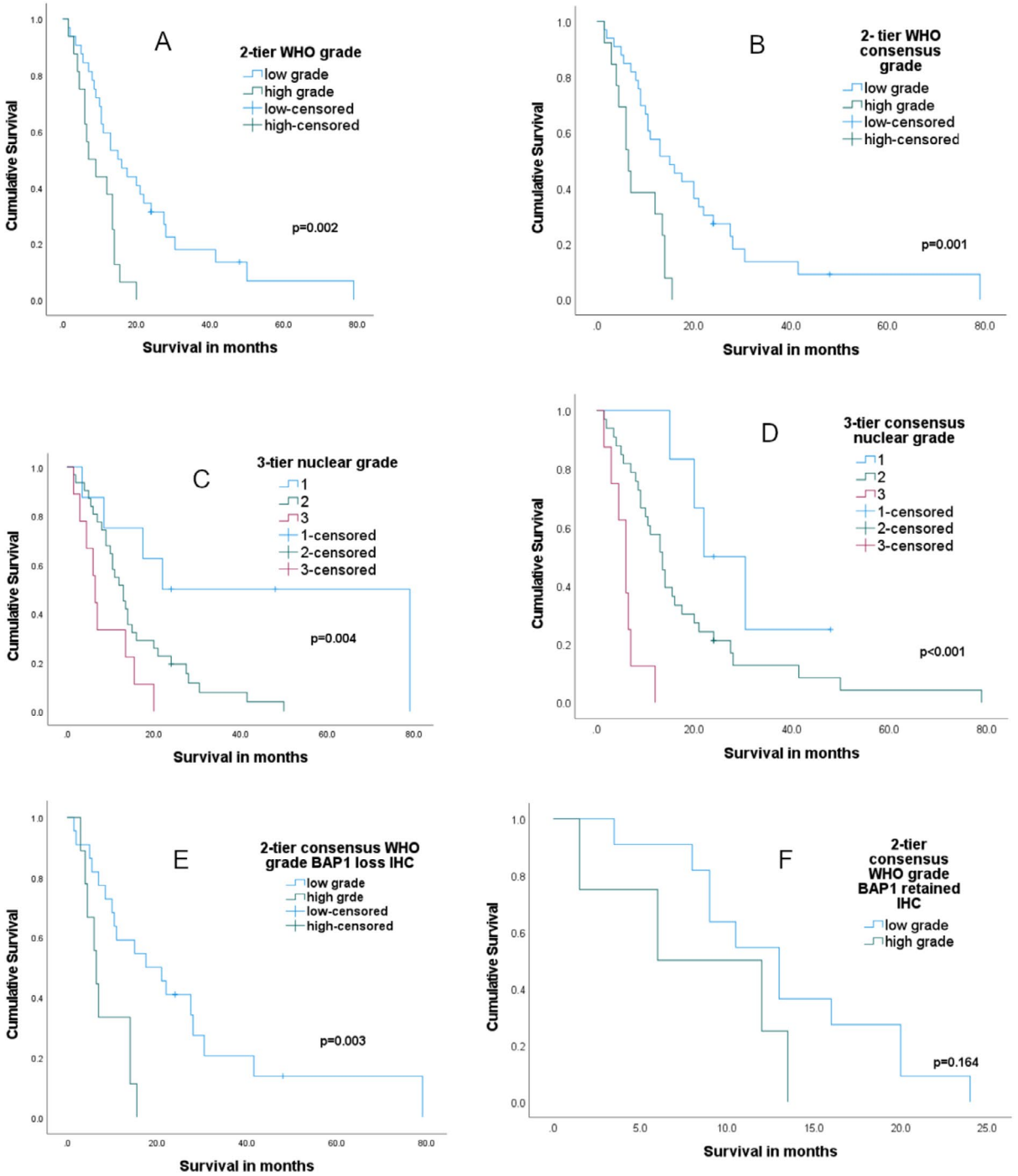

FIGURE 4 | Kaplan Meier survival curves showing the prognostic significance of the 2-tier grading. (A) low grade versus high grade (using expert's scores) $N = 48$ (B) low grade versus high grade (consensus scores) $N = 46$ (C) 3-tier nuclear grade (using expert's scores) $N = 48$ (D) 3-tier nuclear grade (consensus scores) $N = 47$ (E) low grade versus high grade in the group with BAP1 loss on IHC (consensus scores) $N = 31$ (F) low grade versus high grade in the group with BAP1 retained on IHC (consensus scores) $N = 17$.

that the use of digitalised slides led to lower agreement on mitotic counts.

Mesothelioma being a disease with inherent heterogeneity in its morphology, it is not surprising that interobserver agreement on mitotic counts, nuclear pleomorphism and architectural patterns occurs [55]. Subjectivity in assessing nuclear atypia and mitotic counts renders them susceptible to scoring bias [56]. Factors that affect mitotic counts include delayed fixation [57], while using digital pathology for mitotic counts has been suggested to improve mitotic counting [58]. Increasingly, pathologists are including whole slide imaging into their workflow. In the study by Benzerdjib et al. mitotic counts were assessed by expert pathologists on virtual slide microscopy to grade peritoneal mesothelioma [50]. Although grading in peritoneal mesothelioma has not been recommended, studies suggest grading can be applied to peritoneal mesothelioma as well [26]. The use of whole slide imaging (WSI) in mitotic count assessment for

breast cancer grading is now possible. When compared to mitotic counting on light microscopy, WSI assisted by an artificial intelligence (AI) algorithm has shown better results [59]. With the use of AI, slide raters in a study were noted to detect more numbers of mitotic figures, with fewer false positive detections [60]. Refinements in AI assisted algorithms may improve grading of mesothelioma.

Regarding the association of tumour grade with *BAP1* alterations, Chen-Yost et al. also found an association between *BAP1* alterations and low grade for pleural epithelioid mesothelioma, but not for peritoneal mesothelioma [33]. Another study found that WHO grade was only prognostic for tumours with BAP1 loss by IHC [34]. According to Paajanen et al. loss of BAP1 on IHC in pleural mesothelioma is more common in long-term survivors, who more often had low grade mesothelioma [31]. The proportion of germline *BAP1* mutations in this group is unknown, but BAP1 loss is more common in epithelioid mesothelioma and is considered an early event in tumour development. However, other studies found no association of tumour grade with *BAP1* and *NF2* status [61]. In our study, (with only epithelioid mesothelioma) we found significant differences in survival for the two grades in the group with BAP1 loss on IHC ($p = 0.003$). Forest et al. propose that tumour grade reflects expression patterns of genes such as *CXCR1*, *GPR176*, and *DPZD7*, though the molecular basis of this is not clear [29] This is an area under active investigation.

Interrater agreement on grading mesothelioma between two thoracic pathologists, where agreement on the 3-tier nuclear grade was 69.2% and 86.7% for the presence of necrosis, closely resembles our study result [62]. Our overall results were 72% and 84%, respectively, highlighting the reproducibility of assessing prognostic factors in mesothelioma.

Some of the limitations of our study relate to variable sample size, including small biopsies; though this reflects real-life situations. Also, familiarity with digital platforms may have influenced participants' use compared to glass slide examination. We were limited in expressing interrater agreement on assigning architectural patterns and cytological features due to variability in the results. We could not compare agreement on architectural and cytological features in a statistically meaningful manner due to incomplete data and a high level of variability. Furthermore, our survey was anonymous; it did not provide information on the experience or specialist practice of the participants. Some participants experienced technical difficulties (inability to correct grades once entered) and some participants only provided a final 2-tier WHO tumour grade but did not provide details on atypia, mitoses, or necrosis, which meant we could not determine a 3-tier nuclear grade. This may reflect the clinical practice of those pathologists who have a 'Gestalt' approach. These drawbacks were consequences of the survey design that aimed to encourage participation by ensuring anonymity, which may be addressed by a different design. Real-world studies on grading mesothelioma may be useful.

In conclusion, despite some shortfalls in the reproducibility of prognostic features, the WHO 2-tier grade proves itself as a robust prognostic tool for survival. A strength of our study is the large number of participants who graded the images, the inclusion of different levels of expertise, and the inclusion of cases with rare cytological features such as small cell and deciduoid. Whilst consensus scores were overall straightforward, there were some cases where opinions on low versus high grade were equally split between participants; hence, training on grading mesothelioma is required. To this end, a selection of cases is available to view on Sectra Uniview in Table S1.

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Conflicts of Interest

Sonja Klebe prepares medicolegal reports for the courts of Australia on the diagnosis and causation of occupational lung disease, outside of the submitted work. Victor L. Roggli consults with attorneys representing plaintiffs and defendants in asbestos litigation. All other authors do not have any conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author [S.K.] upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.