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ORIGINAL ARTICLE

Prognostic Characterization of Higher-Grade Meningiomas: A Histopathological Score to Predict Progression and Outcome

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Abstract

Higher-grade meningiomas (WHO grade II and III) represent a diagnostic and prognostic challenge. We assessed the pathological and molecular characteristics of 94 higher-grade meningiomas (85 grade II, 9 grade III) to identify novel prognostic parameters. Higher mitotic count ($p = 0.018$), diffuse (≥50%) prominent nucleoli ($p < 0.001$), and sheeting ($p < 0.001$) were associated with recurrence. Lower SSTR2a-positive cells median rate ($p = 0.048$) and *TERT* promoter mutations ($p = 0.014$) were associated with recurrence and patient death, respectively; further analyses did not identify other outcome associations. Presence of Ki67 hot spots was associated with a shorter progression-free survival (PFS), independently of WHO grade at multivariate analysis (HR = 3.35, $p = 0.008$). Necrosis was related to a poorer overall survival (OS) at univariate (focal: HR = 4.55, $p = 0.041$ and diffuse: HR = 7.38, $p = 0.020$) and Kaplan-Meier analyses. A prognostic score was designed based on previous results: Presence of diffuse (≥50%) prominent nucleoli (0/1 point), diffuse (≥50%) sheeting (0/1 point), focal (<50%) or diffuse (≥50%) necrosis (0/1/2 points), and Ki67 hot spots (0/1 point). A total score ≥4 predicted poorer PFS and OS by Kaplan-Meier (PFS: 1.7 vs 6.4 years, $p < 0.001$ and OS: 5.2 vs 10.8 years, $p = 0.001$) and multivariate (PFS: HR = 5.98, $p < 0.001$ and OS: HR = 2.99, $p = 0.048$) analyses. These results were confirmed in an independent series of 58 grade II meningiomas (PFS: HR = 7.22, $p = 0.002$ and OS: HR = 9.69, $p = 0.003$). These associations and the integrated score could complement WHO grading.

Key Words: Ki67, Meningioma, Prognosis, Prognostic factors, Score, SSTR2a, TERT.

INTRODUCTION

Meningiomas are the most common primary tumors of the CNS with an incidence of 8.14/100 000 population according to the latest Central Brain Tumor Registry of the United States (CBTRUS) (1). According to the World Health Organization (WHO) classification criteria, a tumor grade between I and III is assigned based upon the assessment of specific morphological features (2). Diagnosis of atypical grade II meningioma is defined by an increased mitotic count (≥4/10 high-power fields [HPF]) or histological evidence of brain invasion or the presence of 3 out of 5 characteristics: Prominent nucleoli, sheeting, hypercellularity, small cells with a high nucleus/cytoplasm ratio, and foci of spontaneous necrosis. Grade III anaplastic meningiomas are defined by elevated mitotic activity (≥20/HPF) or overtly malignant cytology. Also, some histotypes harbor an independent grading implication. Overall, higher-grade meningiomas (II and III) represent a significant subgroup of patients, amounting to 25% and 5% of all meningiomas, respectively (3–5).

Surgical resection is considered the first-line option for patients needing treatment and gross total resection should be achieved whenever possible, limiting patient observation to selected cases (i.e., small asymptomatic meningiomas). Although most grade I meningiomas are cured by resection alone, grade II and grade III tumors show high recurrence rates and may thus require adjuvant treatments (6). Adjuvant radiotherapy is usually advised for incompletely resected grade II

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and for all grade III meningiomas (7); however, this approach is not universally agreed upon and grey zones do exist, thus making meaningful and reproducible prognostic factors especially warranted when tailoring patient treatment. For recurrent meningiomas, if additional surgery or re-irradiation is not feasible or indicated, medical therapies can be considered although efficacy is usually very limited (7–11); however, this could change in the near future thanks to targeted approaches (12).

WHO grading criteria suffer from subjective interpretation, which could explain the different frequencies of grade II and III tumors observed among institutions and, more importantly, the wide differences in patients' outcome reported within the same grade group. Although phosphohistone H3 (PHH3)-based mitotic count can improve interobserver reproducibility and prognostic stratification by increasing mitotic count reliability (5, 13), the evaluation of other criteria heavily depends on pathologists' subjective evaluation. Correlation between tumor grade and Ki67 labeling index is known, but at present it is not considered a grading variable (2). Moreover, in our experience, a significant number of meningiomas show Ki67 hot spots (i.e., focal areas of high Ki67 labeling index) despite a low overall median index, but no data have been reported about the possible prognostic significance of this feature. Regarding other immunohistochemical prognostic factors, expression of the progesterone receptor was found to be associated with a better outcome, but its prognostic role could overlap with tumor grade (14). Somatostatin receptors are commonly expressed by meningiomas and their prognostic role has been shown in other tumors (15). In meningiomas, no relationship with outcome was found, but most of the analyzed cases were grade I meningiomas, thus it could be of interest to specifically evaluate a larger series of higher-grade meningiomas (16). More recently, the absence of H3K27 trimethylation, detected by immunohistochemistry, was found to be prognostic of a poorer outcome (17).

Besides morphological and immunohistochemical features, molecular factors could play a prognostic role as well. In particular, both DNA methylation-based classification (18, 19) and telomerase reverse transcriptase (*TERT*) promoter mutations (a common alteration of many human cancers, although rare in meningiomas) (20–23) showed promising results. However, the cost of comprehensive molecular analyses like methylation-based classification is not negligible, thus its widespread implementation for routine diagnostic workup will require some time, especially for a pathology with a relatively high incidence like meningioma.

Lastly, most studies focused on discerning between benign (grade I) and aggressive (grade II–III) meningiomas, while only few data are available regarding possible prognosticators within the latter group, despite the potential clinical usefulness.

Thus, we analyzed a retrospective series of grade II and III meningiomas with the aim of improving their prognostic characterization. Specifically, we (1) assessed the prognostic significance of the morphological features currently used for meningioma grading adopting specific predetermined definitions and cut-off values; (2) evaluated the role of Ki67 labeling index (including the specific assessment of Ki67 hot spots), somatostatin receptor subtype 2a (SSTR2a) expression and *TERT* promoter status; and (3) built and evaluated an integrated score based upon the association of the previous variables and aimed at predicting patients' outcome in addition to WHO grade.

MATERIALS AND METHODS

Case Series and Tissue Samples

Grade II and grade III meningiomas, diagnosed at the Pathology Unit of AOU Città della Salute e della Scienza University Hospital of Turin between January 1994 and December 2015, were retrospectively collected. All patients underwent surgical resection at the Neurosurgery Unit of the same institution. Histological diagnosis was reviewed and confirmed by a senior neuropathologist (P.C.) according to the 2016 WHO classification. Follow up data were retrieved from patients' charts and the Piedmont Tumor Registry. The study was conducted in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans and within the guidelines and regulations defined by the Research Ethics Committee of the University of Turin. This study was approved by the Research Ethics Committee of the University of Turin; considered the retrospective nature of the research protocol and that it had no impact on patient care, no specific written informed consent was required.

Integrated Prognostic Score External Validation

A series of grade II meningiomas (according to WHO 2016 criteria) with follow up data was collected at the Neuropathology Unit of Fondazione IRCCS Istituto Neurlogico "C. Besta" (Milan, Italy) and morphological features required for score assessment were evaluated.

Morphological Features and Immunohistochemistry: Qualitative and Quantitative Evaluation

Tumor histotype, mitotic count, presence of hypercellularity (evaluated as absent vs present or as involving $<50\%$ vs $\geq 50\%$ of tumor), small cells (absent/present), prominent nucleoli (absent/present or $<50\%/\geq 50\%$), sheeting (patternless growth) (absent/present or absent/$<50\%/\geq 50\%$), and spontaneous necrosis (absent/present or absent/$<50\%/\geq 50\%$) were assessed. Small cell presence was defined as presence of meningioma cells with a size up to 3 times that of a lymphocyte, involving $\geq 5\%$ tumor sample. Immunohistochemistry for Ki67 (clone 30–9, Ventana Medical Systems, Tucson, AZ) and SSTR2a (clone UMB1, Abcam, Cambridge, UK) were performed on the BenchMark ULTRA platform (Ventana Medical Systems). Ki67 labeling index was assessed by manually counting 1000 cells, while Ki67 hot-spots were arbitrarily defined as intermediate power fields ($\times 200$) displaying at least a triple labeling index compared with the mean tumor labeling index. SSTR2a expression was evaluated both as the overall rate of positive

cells and scored as previously described (24): Score 0: absence of immunoreactivity; score 1: pure cytoplasmic immunoreactivity, either focal or diffuse; score 2: membranous reactivity in <50% of tumor cells, irrespective of the presence of cytoplasmic staining; and score 3: circumferential membranous reactivity in >50% of tumor cells, irrespective of the presence of cytoplasmic staining.

TERT Promoter Sequencing

DNA extraction from formalin-fixed and paraffin-embedded (FFPE) tumor samples was performed as previously described (25), and concentrations/purity were measured by a Nanodrop 1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA).

Mutational status of the *TERT* promoter region from position –27 to –286 from ATG start site, including the polymorphic site represented by rs2853669, were determined by PCR and Sanger sequencing using the following primer pair: Promoter forward 5′-CAGCGCTGCCTGAAACTC-3′ and reverse 5′-GTCCTGCCCCTTCACCTT-3′, as described by Horn et al (26). PCR products were purified and used as template for the sequencing reactions that were performed with a BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA). After purification, the sequences were analyzed by Sanger direct sequencing using the ABIPRISM 3130 Genetic Analyzer (Applied Biosystems).

Statistical Analyses

Statistical analyses were performed using Stata/MP 15.0 Statistical Software (StataCorp, College Station, TX). Categorical variables were compared using Pearson's χ^2 test. Kruskal-Wallis has been used to compare not normally distributed variables. The variables tested were as follows: Age at diagnosis, gender, tumor histotype/grade, tumor site, brain invasion and other morphological features, Ki67 labeling index, Ki67 hot-spots, SSTR2a expression, *TERT* promoter status, tumor recurrence, progression-free survival (PFS) and overall survival (OS).

Differences were considered significant when $p < 0.05$ was reported for two-sided p values. PFS was calculated from the initial diagnosis until there was radiologically confirmed tumor progression, with data censored at the last available date of follow-up. OS was defined as the interval from the initial diagnosis until death, with data censored at the last available date of follow-up. All patient data were updated as of December 31, 2016. Survival distribution curves were plotted using the Kaplan-Meier method and the statistical comparisons were performed using the log-rank test. Cox regression analyses were carried out on PFS and OS to calculate crude and adjusted hazard ratios (HRs) and 95% confidence intervals for the different study groups. A model was created for evaluating the prognostic role of different variables. The proportional hazard assumption was assessed with the Schoenfeld residuals which gave no reason to suspect violation of this assumption.

RESULTS

Clinical Characteristics

Ninety-four grade II and III meningiomas were retrospectively collected: 50/94 (53.2%) were female, median age at diagnosis was 61 (range: 25–83). Median follow up time was 3.9 years. Tumor site was supratentorial in 77/94 (81.9%), posterior fossa in 8/94 (8.5%), and cranial base in 7/94 (7.5%). Data were unavailable for 2/94 (2.1%) patients. Forty-seven (50%) patients received radiotherapy after surgical resection. Gender, age and tumor site were not significantly correlated with tumor grade (Table 1). Twenty-nine patients (30.9%) developed tumor recurrence and 70/94 (74.5%) patients were alive at the end of follow up. No association was found between recurrence and gender ($p = 0.523$), age ($p = 0.294$), tumor site ($p = 0.272$), and previous radiotherapy ($p = 0.925$).

Tumor Grade and Histological Features

Eighty-five cases (90.4%) were grade II meningiomas and 9/94 (9.6%) were grade III. Among grade II tumors, 3/85 (3.5%) were clear cell and 3/85 (3.5%) were chordoid meningiomas. Histological features according to tumor grade are listed in Table 1. In particular, mitotic count ranged from 0 to 25/10 HPF (median: 4) when considering all cases, whereas it was 0–14 (median: 4) and 6–25 (median: 20) when evaluating grade II and III meningiomas, respectively. Focal hypercellularity was present in almost all cases (92/94, 97.9%).

In our series, WHO tumor grade was associated with recurrence ($p < 0.001$), while brain invasion was not ($p = 0.361$; Table 1). Among the histological features, higher mitotic count ($p = 0.018$), diffuse (≥50%) prominent nucleoli ($p < 0.001$), and presence of sheeting ($p < 0.001$) were associated with recurrence. Necrosis was neither associated with recurrence when considered as a dichotomic variable (absent vs present) ($p = 0.090$) nor as a stratified variable (absent vs focal vs diffuse) ($p = 0.055$). Example images of the assessed morphological features are presented in Figure 1A, B.

Ki67 Labelling Index

Median Ki67 labeling index was significantly different between grade II and grade III meningiomas (10% vs 15%, $p = 0.014$), but not between nonrecurrent and recurrent cases (10% vs 12%, $p = 0.155$; Table 1). Presence of Ki67 hot spots (Fig. 1C) was not significantly different based on tumor grade ($p = 0.396$) or recurrence ($p = 0.419$).

SSTR2a Expression

Considering the ratio of SSTR2a-positive cells only (irrespective of the staining pattern), a significant lower median rate was observed in recurrent meningiomas (40% vs 70%, $p = 0.048$; Table 1), whereas no difference was observed between grade II and III meningiomas ($p = 0.293$; Table 1; Fig. 1D). Conversely, no specific association was found between the SSTR2a score and tumor grade ($p = 0.451$) or recurrence ($p = 0.582$).

TABLE 1. Clinicopathological Characteristics of the Higher Grade Meningiomas and Tumor Grade/Tumor Recurrence

Variable		All Cases	WHO Grade II	WHO Grade III	p	No Recurrence	Recurrent Cases	p
Gender	Female	50	44	6	0.394	36	14	0.523
	Male	44	41	3		29	15	
Median age at diagnosis (range)		61 (25–83)	61 (25–83)	53 (25–80)	0.946	62(25–83)	58 (25–80)	0.294
Tumor site	Supratentorial	77	70	7	0.202	51	26	0.272
	Posterior fossa	8	7	1		6	2	
	Cranial base	7	7	0		7	0	
	Data unavailable	2	1	1		1	1	
Death	No	70	65	5	0.226	56	14	**<0.001**
	Yes	24	20	4		9	15	
Histotype	Atypical	79	79	0	Not applicable	59	20	**0.001**
	Clear cell	3	3	0		2	1	
	Chordoid	3	3	0		3	0	
	Anaplastic	9	0	9		1	8	
WHO grade	II	85	–	–	–	64	21	**<0.001**
	III	9	–	–	–	1	8	
Brain invasion	Absent	76	69	7	0.805	51	25	0.361
	Present	18	16	2		14	4	
Hypercellularity	Absent	2	2	0	0.642	2	0	0.340
	Present	92	83	9		63	29	
Hypercellularity	<50%	60	59	1	**0.001**	43	17	0.483
	≥50%	34	26	8		22	12	
Prominent nucleoli	Absent	20	19	1	0.433	15	5	0.523
	Present	74	66	8		50	24	
Prominent nucleoli	<50%	79	75	4	**0.001**	61	18	**<0.001**
	≥50%	15	10	5		4	11	
Small cells	Absent	72	65	7	0.930	49	23	0.678
	Present	22	20	2		16	6	
Sheeting	Absent	66	66	0	**<0.001**	53	13	**<0.001**
	Present	28	19	9		12	16	
Sheeting	Absent	66	66	0	**<0.001**	53	13	**<0.001**
	<50%	8	6	2		6	2	
	≥50%	20	13	7		6	14	
Necrosis	Absent	31	31	0	**0.027**	25	6	0.090
	Present	63	54	9		40	23	
Necrosis	Absent	31	31	0	**<0.001**	25	6	0.055
	<50%	46	43	3		32	14	
	≥50%	18	11	6		8	9	
Median mitotic count (range)		4 (0–25)	4 (0–14)	20 (6–25)	**0.001**	4 (0–23)	6 (2–25)	**0.018**
Median Ki67% (range)		10 (2–30)	10 (2–25)	15 (10–30)	**0.014**	10 (2–25)	12 (2–30)	0.155
Ki67 hot spots	Absent	44	41	3	0.394	32	12	0.481
	Present	50	44	6		33	17	
Median SSTR2a positive cells (range)		55 (0–95)	60 (0–95)	30 (0–95)	0.293	70 (0–95)	40 (0–95)	**0.048**
SSTR2a score	0	7	6	1	0.451	6	1	0.582
	1+	15	12	3		10	5	
	2+	14	13	1		8	6	
	3+	58	54	4		41	17	

Significant p values ($p < 0.05$) are listed in bold.

TERT Promoter Sequencing

TERT promoter was successfully analyzed in 64/94 cases (68%). In the remaining cases (30/94, 32%), Sanger sequencing results were not conclusive or the available material was insufficient for analysis. Canonical *TERT* promoter mutations were detected in 5/64 (8%) cases (4 grade II and 1 grade III): The 1 295 228 C > T (C228T) and the 1 295 250 C > T (C250T) mutations, positioned respectively at 124 and 146 base pairs upstream of the ATG translational start site of *TERT*, were observed in 4/5 and 1/5 cases, respectively. In 23/64 (36%) noncanonical mutations were found, while the remaining 36/64 (56%) cases were wild type (Table 2).

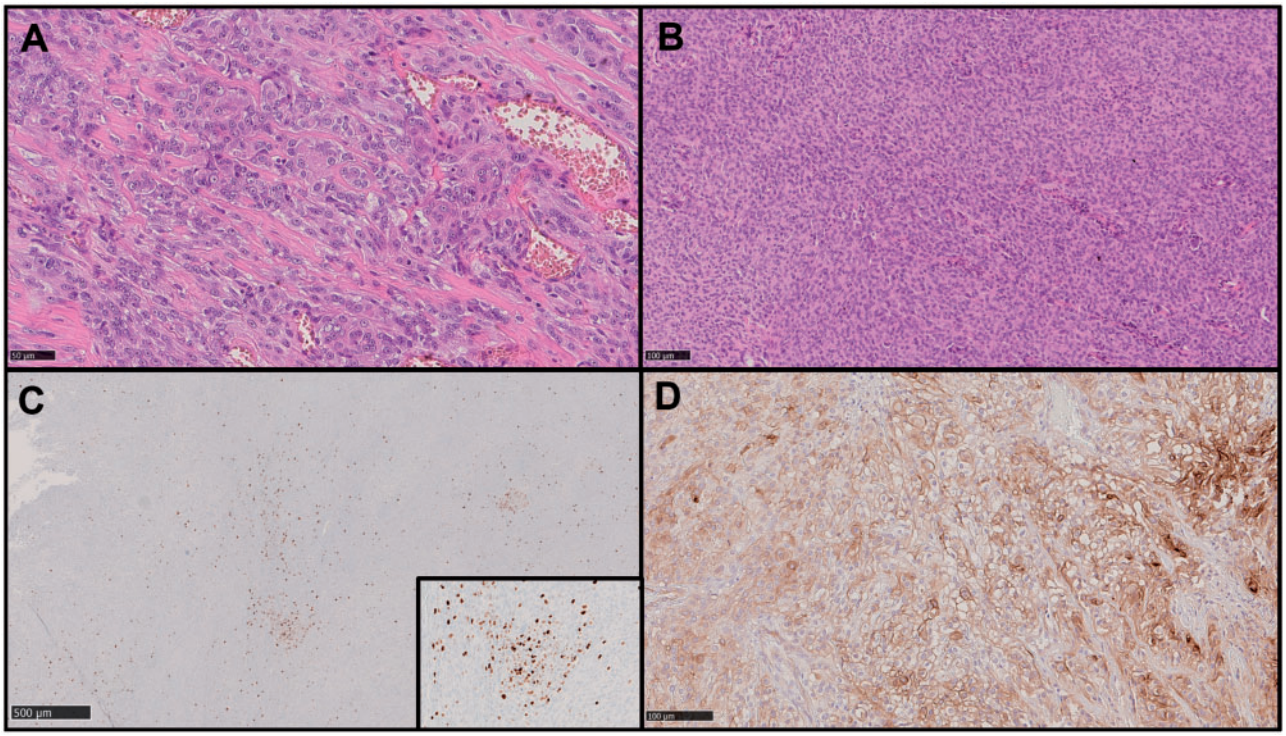

FIGURE 1. Example images showing some of the morphological and immunohistochemical features assessed. Hematoxylin and eosin (H&E) images showing diffuse prominent nucleoli **(A)** and sheeting **(B)**. Original magnification: ×200. Immunohistochemical images showing Ki67 hot spots **(C)** and only partial staining of tumor cells for SSTR2a **(D)** in a meningioma which recurred during follow up.

TABLE 2. *TERT* Promoter Mutations and SNPs According to WHO Tumor Grade

***TERT* Promoter Status**			**n**	**Grade II**	**Grade III**
Mutations	Canonical mutations	–124 C>T	4/64	3	1
		–146 C>T	1/64	1	None
	Other mutations	–67 C>T	1/64	1	None
		–111 C>T	1/64	1	None
		–125 C>T	2/64	2	None
		–125_126 CC>TT	1/64	1	None
		–126 C>T	1/64	1	None
		–128 C>T	2/64	2	None
		–144 C>T	1/64	1	None
		–149 C>T	1/64	1	None
		–150 C>T	2/64	2	None
		–150 C>T –111 C>T	1/64	1	None
		–150 C>T –128 C>T	1/64	1	None
		–156 C>T	2/64	2	None
		–159 C>T	4/64	3	1
		–159 C>T –101 C>T	1/64	1	None
		–166 C>T	2/64	1	1
SNP	SNP rs2853669		31/64	26	5
	Other SNPs		9/64	8	1

Approximately half of the cases (31/64 [48%]) cases harbored the rs2853669 single nucleotide polymorphism (SNP), while other SNPs were present in 9/64 (14%) cases (Table 2). Different SNPs were mutually exclusives. In our series, no association was identified between *TERT* promoter mutations and SNP or between them and clinical variables, histopathological features or outcome, except for *TERT* promoter mutations and patients' death ($p = 0.014$ when considering canonical mutations [C228T and C250T]; $p = 0.045$ when considering all the observed mutations). Regarding this association, 4/5 patients with canonical *TERT* promoter mutations were no longer alive at the end of follow up: 2 died because of meningioma progression, while the cause of death of the remaining 2 patients was not available.

PFS Analysis

Overall, median PFS was 6.1 years (1-year PFS: 90.2%, 2-year PFS: 79.3%, and 5-year PFS: 52.6%). By univariate analysis, grade III (HR = 2.52, $p = 0.044$) and presence of Ki67 hot spots (HR = 2.93, $p = 0.015$) were associated with a poorer PFS, whereas gender, age at diagnosis and previous radiotherapy were not (Table 3). Kaplan-Meier analysis also confirmed these results: Tumor grade (1.6 vs 6.1 years, log-rank test $p = 0.038$; Fig. 2A) and presence of Ki67 hot spots

TABLE 3. Univariate Analysis of the Effect of the Clinicopathological Variables on PFS and OS

Variable		PFS			OS		
		HR	95% CI	p	HR	95% CI	p
Gender (M vs F)		1.02	0.48–2.19	0.952	2.78	1.14–6.72	**0.024**
Age at diagnosis (linear)		0.99	0.98–1.03	0.984	1.08	1.03–1.13	**0.001**
Brain invasion (present vs absent)		0.29	0.09–0.99	**0.049**	0.68	0.23–2.00	0.487
WHO grade (grade III vs II)		2.52	1.02–6.22	**0.044**	1.25	0.42–3.74	0.679
Hypercellularity (≥50% vs <50%)		1.32	0.60–2.90	0.493	1.08	0.47–2.48	0.851
Prominent nucleoli (present vs absent)		0.79	0.29–2.13	0.644	2.38	0.55–10.21	0.243
Prominent nucleoli (≥50% vs <50%)		1.44	0.63–3.31	0.387	0.76	0.28–2.04	0.587
Small cells (present vs absent)		0.66	0.26–1.67	0.386	1.23	0.50–3.01	0.645
Sheeting (present vs absent)		1.95	0.89–4.25	0.092	0.83	0.35–1.95	0.671
Sheeting	Absent	1			1		
	<50%	1.61	0.36–7.26	0.543	0.67	0.09–5.21	0.709
	≥50%	2.03	0.89–4.61	0.088	0.86	0.35–2.12	0.745
Necrosis (present vs absent)		1.74	0.69–4.35	0.233	4.55	1.06–19.40	**0.041**
Necrosis	Absent	1			1		
	<50%	1.40	0.52–3.76	0.499	3.63	0.82–16.17	0.090
	≥50%	2.59	0.91–7.41	0.074	7.38	1.55–34.98	**0.020**
Mitotic count (linear)		1.06	0.99–1.12	0.061	1.02	0.95–1.10	0.612
Ki67 (linear)		1.05	0.98–1.13	0.122	1.05	0.98–1.12	0.142
Ki67 hot spots (present vs absent)		2.93	1.23–6.93	**0.015**	1.46	0.62–3.42	0.376
SSTR2a positive cells (linear)		0.99	0.65–1.52	0.971	1.00	0.67–1.51	0.968
SSTR2a score	0	1			1		
	1+	1.02	0.11–9.48	0.984	0.34	0.05–2.51	0.389
	2+	1.31	0.15–11.07	0.807	1.92	0.39–9.42	0.733
	3+	1.05	0.14–8.05	0.960	0.71	0.16–3.21	0.657
TERT promoter status (mutated vs wildtype)		1.10	0.45–2.67	0.831	1.87	0.74–4.73	0.184
TERT promoter status	Wildtype	1			1		
	Canonical mutations	1.29	0.35–4.76	0.704	1.69	0.50–5.67	0.394
	Other mutations	1.03	0.38–2.77	0.951	2.02	0.69–5.95	0.201
TERT promoter SNP rs2853669 (present vs absent)		1.71	0.68–4.26	0.251	0.81	0.32–1.99	0.647

CI, confidence interval; HR, hazard ratio; OS, overall survival; PFS, progression free survival.
Significant p values ($p < 0.05$) are listed in bold.

(2.7 vs 8.3 years, log-rank test $p = 0.0103$; Fig. 2B) correlated with a shorter PFS. By multivariate analysis these parameters were confirmed to be independent (HR = 3.71, $p = 0.009$ for grade and HR = 3.35, $p = 0.008$ for Ki67 hot spots) when analyzed together with age at diagnosis (HR = 1.01, $p = 0.488$) and gender (HR = 0.94, $p = 0.888$).

OS Analysis

Overall median OS was 9.7 years (1-year OS: 95.7%; 2-year OS: 88.7%; and 5-year OS: 82.5%). By univariate analysis, gender (male) (HR = 2.78, $p = 0.024$) and age at diagnosis (HR = 1.08, $p = 0.001$) correlated with a shorter OS, as expected (Table 3). Kaplan-Meier analysis confirmed this finding: Male gender showed a poorer outcome (9.3 vs 19.6 years, log-rank test $p = 0.018$; Fig. 2C). Among the histopathological features, necrosis was a negative prognostic variable (HR = 4.55, $p = 0.041$), particularly if diffuse (HR = 7.38, $p = 0.020$). Kaplan-Meier analysis showed similar findings: Median OS was lower in presence of necrosis (9.3 vs 13.4 years vs median not reached for diffuse, focal and absent necrosis, respectively; log-rank test $p = 0.016$; Fig. 2D). At multivariate analysis, only the association between age and OS was confirmed (HR = 1.07, $p = 0.001$), whereas patients' gender (HR = 2.14, $p = 0.111$) and focal (HR = 3.09, $p = 0.149$) and diffuse necrosis (HR = 4.13, $p = 0.091$) were not significantly correlated with OS.

Integrated Prognostic Score

On the basis of the results previously observed, we designed a possible prognostic score aimed at identifying patients with shorter PFS and OS. This score is based on the following: (1) presence of diffuse (≥50%) prominent nucleoli (0 vs 1 point), (2) diffuse (≥50%) sheeting (0 vs 1 point), (3) focal (<50%) or diffuse (≥50%) necrosis (0 vs 1 vs 2 points), and (4) presence of Ki67 hot spots (0 vs 1 point); total score range: 0–5. Employing a cut-off value of ≥4 points, Kaplan-Meier analysis showed significant differences in median PFS (1.7 vs 6.4 years, log-rank test $p < 0.001$; Fig. 2E) and median OS (5.2 vs 10.8 years, log-rank test $p = 0.001$; Fig. 2F). By univariate analysis, this score was also significantly associated

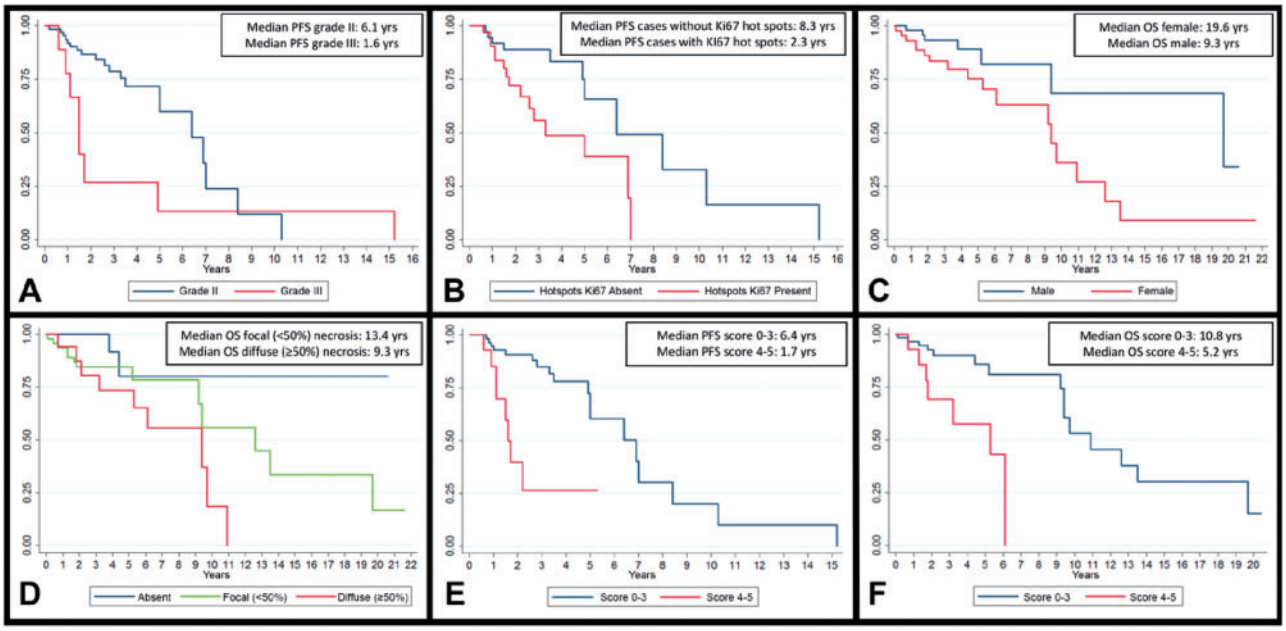

FIGURE 2. Kaplan-Meier analyses curves of outcome-associated variables. **(A)** WHO grade and PFS (log-rank test p = 0.038). **(B)** Ki67 hot spots and PFS (log-rank test p = 0.0103). **(C)** Gender and OS (log-rank test p = 0.018). **(D)** Necrosis and OS (log-rank test p = 0.016). **(E)** Integrated score and PFS (log-rank test p < 0.001). **(F)** Integrated score and OS (log-rank test p = 0.001).

TABLE 4. Multivariate Analysis of the Prognostic Role of the Proposed Integrated Score in Terms of PFS and OS

Variables	PFS			OS		
	HR	95% CI	p	HR	95% CI	p
Gender (M vs F)	0.83	0.37–1.87	0.662	2.83	1.05–7.62	**0.039**
Age at diagnosis	0.99	0.96–1.02	0.541	1.08	1.03–1.13	**0.002**
Integrated score (total score ≥4)	5.98	2.24–15.97	**<0.001**	2.99	1.01–8.86	**0.048**

CI, confidence interval; HR, hazard ratio; OS, overall survival; PFS, progression-free survival.
Significant p values (p < 0.05) are listed in bold.

TABLE 5. Multivariate Analyses of the Prognostic Role of the Proposed Integrated Prognostic Score in the External Validation Series

	PFS			OS		
	HR	95% CI	p	HR	95% CI	p
Gender (M vs F)	1.05	0.41–2.70	0.917	0.93	0.25–3.37	0.913
Age at diagnosis	0.99	0.95–1.03	0.775	1.05	0.98–1.12	0.167
Integrated score (total score ≥4)	7.22	2.12–24.6	**0.002**	9.69	2.21–42.5	**0.003**

CI, confidence interval; HR, hazard ratio; OS, overall survival.
Significant p values (p < 0.05) are listed in bold.

with both shorter PFS (HR = 5.44, p < 0.001) and OS (HR = 4.03, p = 0.009). Finally, multivariate analysis including age at diagnosis, gender and our score showed a significant independent association with PFS (HR = 5.98, p < 0.001) and OS (HR = 2.99, p = 0.048; Table 4).

To validate the integrated prognostic score, a series of 58 grade II meningiomas was collected at the Neuropathology Unit of the Fondazione IRCCS Istituto Neurologico "C. Besta" (Milan, Italy). It was decided to collect only grade II meningiomas since they represent the real grey zone in which a tool to stratify patients according to their outcome could be most useful for tailoring adjuvant treatments. Series characteristics are reported in Supplementary Data Table S1. Median follow up was 7.2 years. Multivariate analysis confirmed the prognostic capability of the integrated prognostic score both in terms of PFS (HR = 7.22, p = 0.002) and OS (HR = 9.69, p = 0.003; Table 5).

DISCUSSION

The present study identifies a set of variables significantly associated with tumor recurrence and patients' outcome in a series of higher-grade (II and III) meningiomas. Moreover, based on the results of this analysis, a possible prognostic score predictive of both PFS and OS is proposed and validated in an independent series. These results could prove useful for the management of these tumors in addition to WHO grade.

According to the latest WHO classification, grade II and III meningiomas represent 20%–25% and 1%–6% of all meningiomas (2), respectively, with a significant increment compared with the previous classifications (4.7%–7.2% for grade II and 1%–2.8% for grade III meningiomas) (27). It is difficult to ascertain the exact reasons behind this rise, but changes in the diagnostic criteria probably played a significant role (28). Whatever the reason, these data mean that the diag-

nosis of a higher-grade meningioma is a frequent occurrence in the clinical practice.

Higher-grade meningiomas, however, represent a peculiar grey zone both in terms of diagnosis and therapeutic management: The diagnostic criteria provided by the WHO classification are based, at least in part, upon a subjective evaluation and the clinical outcome can vary significantly among patients (2). In fact, reliability and reproducibility of many of the diagnostic criteria have been extensively questioned over time (29, 30) and cut-off values or clear definitions are still lacking, possibly favoring diagnostic inconsistencies between different institutions. In our study, trying to overcome this limitation, morphological traits were evaluated according to predetermined qualitative/quantitative criteria.

In terms of patient follow up and treatment, current guidelines suggest adjuvant radiotherapy for partially resected grade II and all grade III meningiomas (7), but the decision is not always easy, such as in completely resected grade II meningiomas with higher Ki67 labeling index. A better understanding of prognostic factors in this group of tumors is therefore warranted.

Our results show that only some of the routinely assessed morphological features are associated with tumor recurrence in higher-grade meningiomas: Higher mitotic count, diffuse prominent nucleoli and sheeting. In our series, no association was found between brain invasion and tumor recurrence. This parameter was added as a sufficient criterion for the diagnosis of atypical meningioma in the latest WHO classification based on its relationship with tumor recurrence (31), but conflicting evidence about its significance exists (32); differences in meningioma resection technique and sampling could explain, at least in part, our results as well as the discrepancies reported in literature (33–36). Accordingly, extensive sampling could be recommended in future prospective studies to reduce this possible source of variability.

Reduced expression of somatostatin receptors in higher-grade meningiomas has been reported in literature (16). A lower median rate of SSTR2a-positive cells was associated with tumor recurrence (40% vs 70%, $p = 0.048$) in our series, but we did not find significant associations between SSTR2a expression and other outcome measures.

TERT promoter status is reported to be mutated in a minority of meningiomas. As initially showed by Koelsche et al, this alteration is usually present in higher-grade meningiomas (20). Subsequent studies have demonstrated a relationship between this alteration and both malignant histological progression at recurrence (21) and a shorter time to progression (22). Our analysis found an association with patient death ($p = 0.014$ when considering canonical mutations only); recent data, specifically concerning nonbenign meningiomas, also support these negative prognostic correlations (23). Considering the overall limited number of mutated cases even in atypical and anaplastic meningiomas, evaluation of *TERT* promoter status does not seem to be warranted in routine diagnostics, but it can be taken into consideration in selected cases. Moreover, we also wanted to check for any possible effect of the *TERT* rs2853669 SNP considering its high frequency in the general population (~50%), but no relationship with outcome was observed.

In terms of survival analysis, presence of Ki67 hot spots and necrosis resulted associated with PFS and OS, respectively. In our experience, presence of brisk Ki67 hot spots is a relatively common finding, even in meningiomas with low average Ki67 indices. The prognostic role of Ki67 labeling index in hot spots has been taken into consideration for many other tumors (37), but it is difficult to decide whether to integrate the hot spot index in the overall quantification or not (38). To overcome this problem, we decided to assess the prognostic significance of Ki67 hot spots presence irrespective of the specific labeling index values. In our series, this feature resulted significantly associated with a shorter PFS at univariate ($HR = 2.93$) and Kaplan-Meier analyses (2.7 vs 8.3 years, log-rank test $p = 0.0103$); even more interestingly, this association was confirmed at multivariate analysis ($HR = 3.35$) and resulted independent of WHO grade. In the future, the role of Ki67 hot spots could also be explored in benign (grade I) meningiomas to verify if this variable could help distinguish a subgroup with worse outcome. Regarding necrosis, its extent is a well-known prognosticator in many tumors (39, 40): Thus, the observed association with a poorer OS is consistent with previous data.

In our case series, the variables associated with tumor recurrence differ from those associated with PFS/OS, likely due to sample size and to the overall limited strength of the associations. To overcome this limit, we reconsidered the factors associated with tumor recurrence (prominent nucleoli and sheeting), PFS (Ki67 hot spots) and OS (necrosis) in an integrated score. On the basis of previous results and to reduce subjectivity in evaluation, prominent nucleoli and sheeting were only considered if diffuse ($\geq$50% vs absent/$<$50%). A score value ≥ 4 was associated with both poorer PFS and OS at univariate (PFS: $HR = 5.44$ and OS: $HR = 4.03$) and Kaplan-Meier (PFS: 1.7 vs 6.4 years and OS: 5.2 vs 10.8 years) analyses. These associations were also confirmed by multivariate analysis (PFS: $HR = 5.98$, $p < 0.001$ and OS: $HR = 2.99$, $p = 0.048$) and by validation in an external series (PFS: $HR = 7.22$, $p = 0.002$ and OS: $HR = 9.69$, $p = 0.003$), thus this tool could provide valuable information, in addition to WHO grade. In particular, it could be useful for the management of grade II meningiomas or to "screen" cases in which additional testing, like DNA methylation-based classification, could be most useful. Further studies could specifically explore the reproducibility of this approach and possibly optimize the score parameters.

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