Article

MIB1 expression is increased in meningiomas with histopathological evidence of CNS invasion

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Simple Summary: Meningiomas are the most common primary intracranial tumors. Although, most meningiomas are benign and slow growing, approximately 20% show tumor recurrence after surgical resection. One factor that facilitates the identification of patients at increased risk of tumor recurrence is the evidence of invasive tumor growth into CNS tissue. This characteristic has been integrated into the WHO classification of 2016 as a stand-alone criterion for higher grading. However, its prognostic value has since been debated due to conflicting results of retrospective analyses. Overall, more evidence is necessary to clarify its future role in meningioma grading. We have recently published data demonstrating a shorter progression-free survival of meningiomas with CNS invasion, detected by combined intraoperative and histopathological assessment. We now present additional results of the computed-assisted expression analysis of the proliferation marker MIB1 and the independent association with CNS invasion. Our results further strengthen the importance of CNS invasion in meningioma.

Abstract:

Background: Meningiomas are the most common benign intracranial neoplasms. CNS invasion in meningiomas has been integrated into the 2016 WHO classification of CNS tumors as a stand-alone criterion for atypia. Since then, its prognostic impact has been debated based on contradictory results from retrospective analyses. The aim of the study was to elucidate whether recurrence of meningiomas with CNS invasion is associated with increased proliferative potential.

Methods: We have conducted a quantified measurement of the proliferation marker MIB1 and analyzed its association with CNS invasion determined by histology together with other established prognostic markers of progression. Routine, immunohistochemical staining for MIB1 were digitalized and automatic quantification was done using Image J software.

Results: Overall, 1718 meningiomas were assessed. Histopathological CNS invasion was seen in 108 cases (6.7%). Uni- and multivariate analysis revealed a significantly higher MIB1 proliferation rate in meningiomas with CNS invasion ($p<0.0001$ and $p=0.0098$, respectively).

Conclusions: Meningiomas with histopathological CNS invasion show a higher proliferative activity.

Keywords: meningioma; CNS invasion; brain invasion; MIB1; Ki67; proliferation

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1. Introduction

Meningioma is the most common benign tumor of the central nervous system and makes up one third of primary intracranial tumors[1]. These tumors are usually slow growing and arise from the arachnoid cap cells of the meninges[2]. Treatment by microsurgical excision is sufficient for curing most patients, while radiation therapy is reserved for selected and recurrent cases[3]. About 20% of meningiomas recur[4] and some sources claim an even higher recurrence rate of up to 47% with a long follow-up of 25 years[5]. Therefore, it is of great importance to identify patients with an increased risk of meningioma recurrence, in order to guide postoperative management. Besides the long-established histopathological assessment according to the WHO classification of central nervous system tumors[4], the detection of infiltrative meningioma growth into brain parenchyma has been added as a stand-alone criterion for atypia[4]. However, its prognostic significance has since been questioned based on contradictory results of retrospective analyses[6-9] and its role for tumor grading in the WHO classification is frequently discussed[10,11].

We have recently compared the prognostic role of the histopathological and intraoperative detection of CNS invasion in a multivariate model in a large meningioma cohort. While each detection by itself did not reach prognostic significance in the multivariate analysis, the combination of both methods did[7]. The reasons for the conflicting evidence on CNS invasion meningioma are most likely the unstandardized sampling and non-uniform histopathological criteria applied[7,11]. Before abandoning CNS invasion for meningioma risk stratification prematurely, we believe it is important to keep up interdisciplinary efforts to generate more evidence in this field.

We have therefore applied a quantification analysis of the immunohistochemical expression of the proliferation marker MIB (Ki-67) in our meningioma cohort to investigate a possible association of proliferation and infiltrative growth in meningioma.

2. Materials and Methods

In this single center retrospective analysis, we investigated CNS invasion and other clinical factors regarding its association with the immunohistochemical expression of the proliferation marker MIB1 in a large cohort of meningiomas. Overall, 2156 meningiomas were surgically treated in the authors' institution between October 2003 and March 2017. 330 cases with missing consent for scientific utilization, incomplete clinical data and missing or poor-quality tissue were excluded (Figure 1).

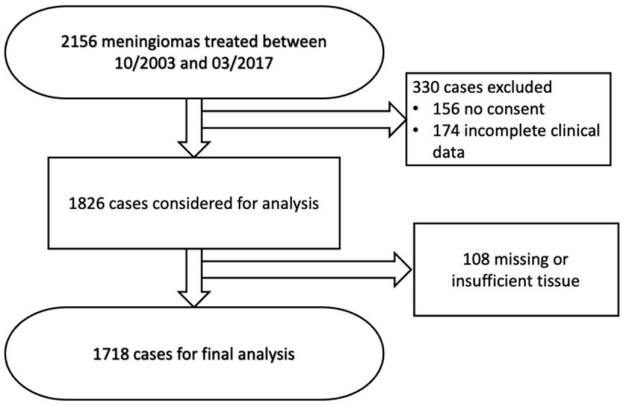

Figure 1. Flow chart of the composition of the study cohort.

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The following clinical factors were collected for all included cases via a systematic review of available clinical documents and radiographic imaging: age at diagnosis, gender, tumor status (primary/recurrent), radiotherapy prior to surgery, diagnosis of neurofibromatosis type 2, tumor location, extent of resection (according to Simpson[12]). In the authors' institution, CNS invasion was determined based on the histologic criteria defined by Perry[13]. Histopathological reports were reviewed and cases with clearly stated CNS invasion identified. If no statement regarding CNS invasion was documented, cases were graded as non-invasive. To analyze CNS invasion as an independent co-factor, brain-invasive but otherwise benign meningiomas were graded as outlined by the WHO classification of 2007[14], since it does not incorporate CNS invasion as sole grading criterion for atypia in comparison to the current classification of 2016[4].

Immunohistochemical staining for MIB1, that were routinely prepared during the histopathological diagnostic process, were retrieved and quantitatively reassessed. Digital images were taken of representative areas of each MIB1 staining and quantitative measurements of areas of immunopositivity were done with the Image J software (Version 1.51j8, NIH, Bethesda, MD, USA) and the plugins Bio-Formats (Release 5.4.1; Open Microscopy Environment, Madison, NJ, USA) and ImmunoRatio (Version 1.0c, Institute of Biomedical Technology, University of Tampere, Finland) (Figure 2).

For statistical analysis the JMP® Statistical Discovery Software was used (Version 15.1.0, Cary, NC: SAS Institute Inc.; 1989). Univariate analysis of clinical and histopathological factors regarding differences in MIB1 expression were done with ANOVA and a linear regression was done for multivariate analysis. The level of significance was set at $\alpha < .05$.

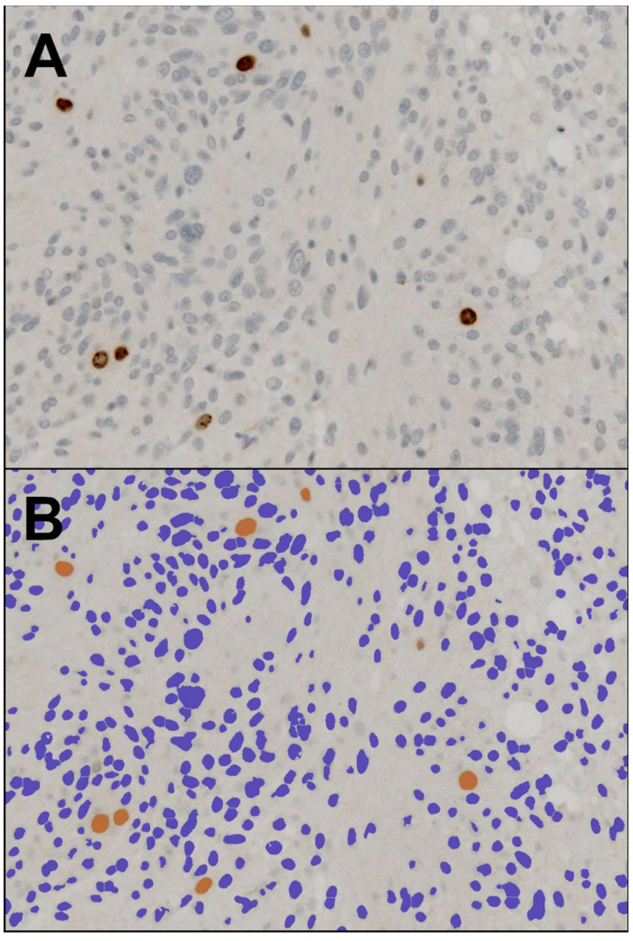

Figure 2. Example of the digital quantification of the immunohistochemical expression of MIB1 in tumor nuclei. Panel A shows the MIB1 diaminobenzidine staining (brown chromogen) and negative tumor cells counterstained with hematoxylin (blue) and panel B the corresponding quantitative computer-assisted measurement.

3. Results

3.1. Cohort characteristics

Overall, 1718 meningiomas were included for final analysis, consisting of 1229 female and 489 male patients (female to male ratio 2.51). The mean age of the cohort was 57.23 years, ranging from 3.83 to 90.96 years. The majority of meningiomas were primary tumors (n=1504, 87.5%) while 214 cases were recurrent tumors (12.5%). Eighty meningiomas received radiotherapy prior to surgical resection (4.7%). One-hundred and three tumors were from patients suffering from neurofibromatosis type 2 (6.0%). The tumor location was categorized into convexity/falx (n=649, 37.8%), skull base (n=893, 52%) and spinal (n=176, 10.2%). According to the WHO classification of central nervous system tumors, 1412 meningiomas were graded as WHO grade I (82.2%), 285 as grade II (16.6%) and 21 as grade III (1.2%). CNS invasion was histopathologically detected in 108 cases (6.7%) (for details see Table 1).

Table 1. Cohort characteristics and MIB1 expression.

Variable	n(%)	MIB1 expression (% immunopositive)	p-value (ANOVA)	p-value (linear regression)
Gender				
F	1229 (71.5)	2.64	<.0001*	0.0014*
M	489 (28.5)	3.77		
Age				
>=70.5	353 (20.5)	3.31	0.0125*	0.3385
<70.5	1365 (79.5)	2.87		
Tumor status				
Primary	1504 (87.5)	2.62	<.0001*	<.0001*
Recurrent	214 (12.5)	5.36		
Prior RT				
Yes	80 (4.7)	7.68	<.0001*	<.0001*
No	1638 (95.3)	2.73		
Neurofibromatosis type 2				
Yes	103 (6.0)	2.67	0.3007	
No	1615 (94.0)	2.98		
Tumor location				
Convexity/Falx	649 (37.8)	3.60	<.0001*	0.0002*
Skull base	893 (52.0)	2.54		
Spinal	176 (10.2)	2.77		
WHO classification of 2007				
I	1412 (82.2)	2.42	<.0001*	<.0001*
II	285 (16.6)	4.99		
III	21 (1.2)	12.14		
CNS invasion				
Yes	108 (6.7)	5.33	<.0001*	0.0098*

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No	1610 (93.7)	2.81

3.2. Univariate analysis of MIB1 expression

Meningiomas in male patients showed a significant higher MIB1 expression than in females (3.77% and 2.64%, p<0.0001). Regarding the influence of age, the largest difference of MIB1 expression was seen with a cutoff at 70.5 years of age according to a CART analysis. Patients a with an age equal or above 70.5 years had a mean MIB1 expression of 3.31% compared to 2.87% for younger patients (p=0.0125). Recurrent tumors showed a higher rate of immunopositivity for MIB1 as compared to primary meningiomas (5.36% compared to 2.62%, p<0.0001). Similarly, increased MIB1 immunostaining was found in patients with prior radiotherapy (7.68% with radiotherapy compared to 2.73% without radiotherapy, p<0.0001). There was no significant difference in MIB1 expression for NF2 patients. Meningiomas located at the convexity of falx had the highest immunopositivity (3.60%), followed by spinal tumors (2.77%) and the lowest rate was seen for skull base meningiomas (2.54%, p<0.0001). With increasing WHO grade, higher mean MIB1 expression scores were seen (WHO grade I: 2.42%, WHO grade II: 4.99% and WHO grade III: 12.14%, p<0.0001). Meningiomas with CNS invasion showed almost a double mean immunopositivity for the proliferation marker (5.33% compared to 2.81%, p<0.0001). Details of the univariate analysis are displayed in Figure 3 and Table 1.

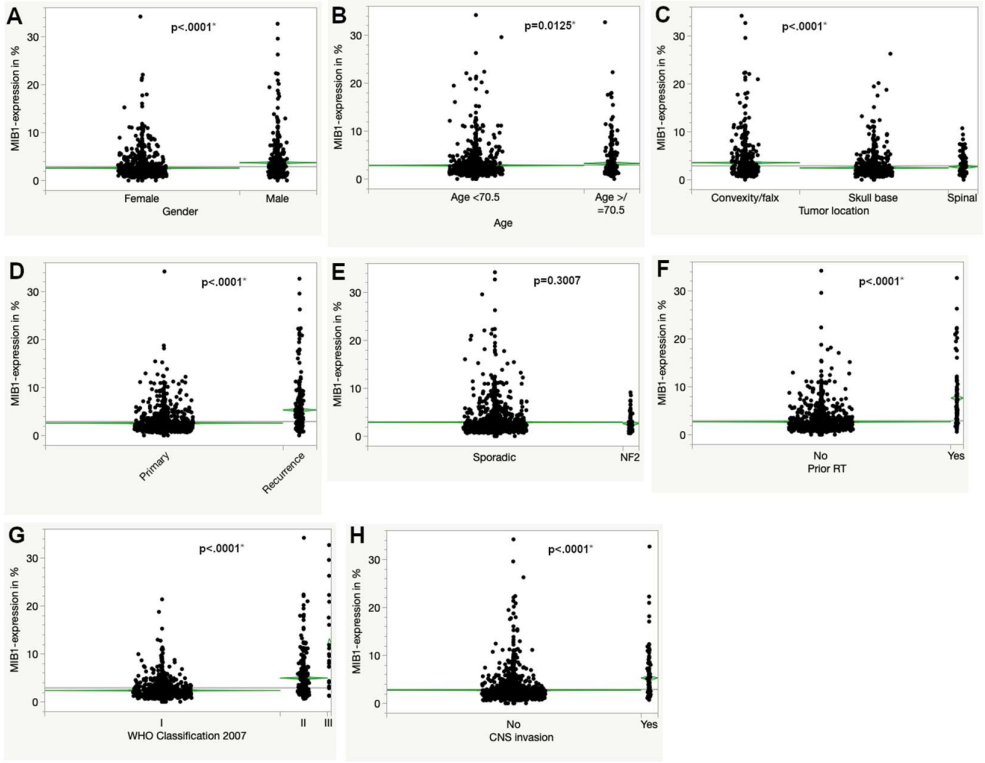

Figure 3. Univariate analysis of the immunohistochemical expression of MIB1 according to gender (A), age (B), tumor location (C), tumor status (D), neurofibromatosis type 2 (E), prior radiotherapy (F), WHO classification 2007 (G) and CNS invasion (H).

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3.3. Linear regression analysis

All factors that showed significant association with MIB1 expression in the univariate analysis were integrated into the multivariate linear regression. Presence of histopathological CNS invasion was an independent factor for increased MIB1 expression rates (p=0.0098). Furthermore, male gender, recurrent tumor status, prior radiotherapy and convexity/falx tumor location were also independently associated with higher proliferation marker values (Details are shown in Table 1).

4. Discussion

The clinical impact of CNS invasion in meningioma is increasingly critically discussed since its integration into the WHO classification for CNS tumors in 2016[4,11]. The current knowledge is primarily based on multiple retrospective analyses that found no prognostic impact of CNS invasion[6,8,15]. However, it is important to keep in mind that two issues with the detection of CNS invasion exist, that have likely impacted retrospective studies. First of all, the histopathological characteristics used to determine infiltrative growth are not clearly defined[10,11] and possibly vary between departments and neurooncological centers. Additionally, intraoperative tumor sampling is non-standardized and especially areas of interest may not always be amenable to appropriate sampling[16]. We recently compared the prognostic potential of histopathological and intraoperative detection of infiltrative growth in 1517 meningiomas. We found that both methods do not show an independent prognostic impact by itself, as they are currently applied, but if they are combined. Our findings underlined the need to further assess the prognostic impact by other methods and to investigate the histopathological and intraoperative detection of CNS invasion in a prospective and controlled fashion[7].

The tumor cell proliferation rate is an integral part of the WHO classification for CNS tumors. The assessment of the mitotic index by detection of mitoses per 10 high-power fields is an established measure when considering the diagnosis of atypical or anaplastic meningioma[2]. Immunohistochemical expression of MIB1 as direct visualization of proliferating cells[17], has long been suggested as a prognostic marker in meningioma[18]. However, variations in interobserver interpretation and different staining protocols make it difficult to establish clear cut off values. The consideration of the mitotic index for prognostic assessment is still essential for tumor grading in the upcoming WHO classification while inclusion of proliferation has been recommended by some authors [2,19]. We have therefore used a computerized quantification method to control for an interobserver bias and to obtain continual numerical values. We have recently demonstrated the independent significant prognostic impact of quantified MIB1 expression in our meningioma cohort[20].

In this study we showed that MIB1 expression is independently associated with histopathological detection of CNS invasion suggesting that meningiomas with infiltrative growth have a higher proliferation rate compared to tumors of the same grade where CNS invasion is absent. To our knowledge, this is the first study to show this relationship. It underlines the prognostic potential of CNS invasion in meningioma. However, if the nature of infiltrative growth is biologically associated with the proliferative activity of meningioma cells remains unclear. The mechanism of CNS invasion may occur independently from tumor cell proliferation. But our data clearly show, that meningiomas that have developed invasive features, have a significant higher proliferative marker expression, and thus can be considered as a more aggressive entity. This supports the decision expressed in the new WHO classification for CNS tumors, which still incorporates CNS invasion as a stand-alone criterion for atypia[2]. Understandably, the role of infiltrative growth in brain parenchyma is still controversial, especially due to non-standardized sampling and histopathological grading as recently expressed[16,11]. Our data provide a contribution to this topic, but more robust studies are needed to further our understanding of the mechanism of CNS invasion. This may also reveal targets for specific therapies that could

possibly extend the few treatment options currently available, especially for patients with advanced meningiomas, when surgical and radiotherapeutic options have been exhausted.

5. Conclusions

Histopathological detection of CNS invasion in meningioma is an independent factor for increased expression of the proliferation marker MIB1, underlining the association of infiltrative growth and proliferative activity.

Supplementary Materials: Not applicable.

Author Contributions: Conceptualization, F.B., J.S. and M.S.; methodology, F.B. and J.S.; software, J.S.; validation, F.B., G.T., J.G., M.T., J.S. and M.S..; formal analysis, F.B., C.F., J.S. and M.S.; investigation, F.B., C.F., J.S. and M.S.; resources, G.T., J.H., M.T., J.S. and M.S.; data curation, F.B., C.F., S.W., J.M.H., E.H., J.S. and M.S.; writing—original draft preparation, F.B., J.S. and M.S..; writing—review and editing, F.B. and M.S.; visualization, F.B.; supervision, F.B.; project administration, F.B.; All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was approved by the Clinical Ethics Committee of the University of Tübingen (Project number: 618/2014BO2).

Informed Consent Statement: Tumor tissue and clinical data were only used if patient consent was available as defined by the Clinical Ethics Committee of the University of Tübingen.

Data Availability Statement: The dataset is available from the corresponding author upon reasonable request.

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Conflicts of Interest: The authors declare no conflict of interest.

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