Molecular characteristics and prognostic biomarkers of central neurocytoma

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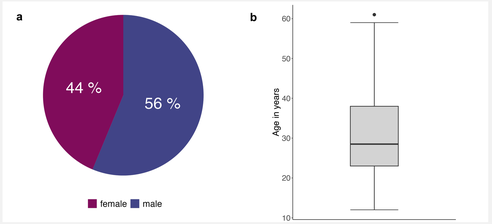
Background

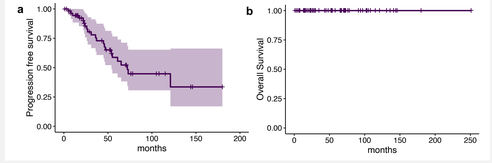
Central neurocytomas (CNs) are rare neuroepithelial tumors mainly affecting young adults. Although associated with a favorable prognosis in many cases, they potentially recur, particularly when resection is incomplete. Occasionally, aggressive behavior and rapid progression with multiple recurrences and dissemination is observed. An increased risk for progression is currently assessed morphologically based on the presence of atypical features and an elevated Ki67 proliferation index. The histomorphological and immunhistochemical criteria are inconsistently defined and applied. Molecular biomarkers for risk stratification or tumor progression are not known. Hence, we want to elucidate the molecular background and clinical characteristics of the entity in a large retrospective and epigenetically pure cohort of n = 126 central neurocytoma.

A novel multi-institutional cohort of CN



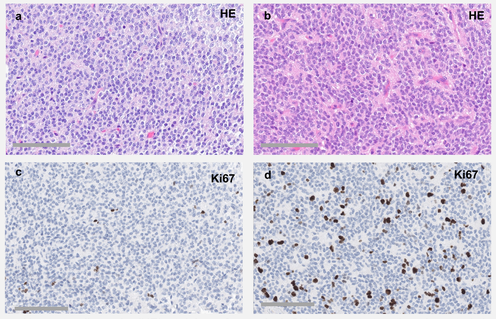
a The patients included in the current cohort (n = 96) are uniformly distributed among sexes ($\chi 2$ goodness-of-fit test, *p*-value = 0.254). **b** Median patient age is 29 (range 12-61) years.

Progression Free Survival of CN patients



a Recurrence was observed in 22 out of 60 patients with available clinical data. The median progression free survival was 72 months. **b** None of the patients died within the observation time (median 56, range 1 - 256 months, n = 60).

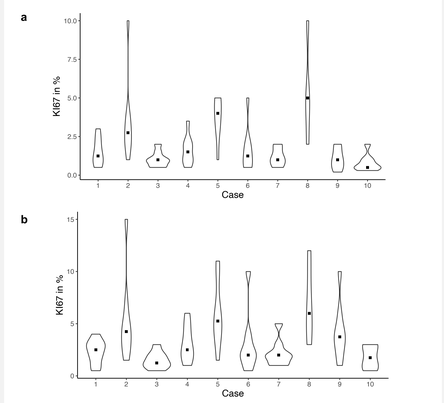
Histopathology in classical and atypical CNs



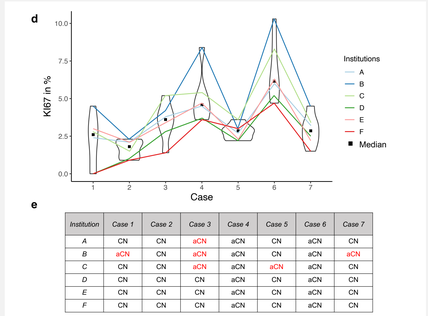
a Haematoxylin-eosin (HE) staining of a typical CN demonstrates the characteristic morphology comprising monomorphic round cells with round nuclei. **b** A similar morphology is exhibited by the atypical CN with mild endothelial proliferation. While the typical CN is characterized by a low Ki67 index of about 1% (**c**), the atypical CN shows a much higher proliferation rate of about 10% (**d**). Several studies have shown increased aggressiveness in cases with atypical features and/or Ki67 proliferation index > 2-3% [1]. Since signs of malignancy like necrosis, glomeruloid vascular proliferation or a high mitotic count are seen rarely, Ki67 index is used for patient stratification in most cases, even though the optimal threshold for predicting prognosis is still under debate.

Interobserver and interlaboratory variability of the Ki67 proliferation index

The Ki67 proliferation index is currently considered one of the most important prognostic markers in CN [1], though its inconsistency of assessment and practical use raises the question about the meaningfulness of the Ki67 as a reliable criterium for the classification and future stratification of CNs.



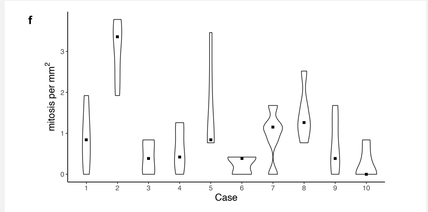
For demonstration of the interobserver variability, Ki67 index was estimated by 8 different neuropathologists in 10 CN cases. Differences in the estimations of the global Ki67 index (whole slide) are shown in **a**, whereas **b** displays the discrepancy in the ratings of the focally highest Ki67 index of the "hotspot" region, chosen by each rater (**c**). Surprisingly, even if there was a high agreement on the hotspot region between the neuropathologists (e.g. Case 9) the estimation of Ki67 index differed widely (1-6%).



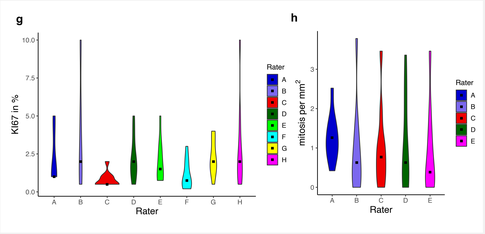
e

Institution	*Case 1*	*Case 2*	*Case 3*	*Case 4*	*Case 5*	*Case 6*	*Case 7*
A	CN	CN	aCN	aCN	CN	aCN	CN
B	aCN	CN	aCN	aCN	CN	aCN	aCN
C	CN	CN	aCN	aCN	aCN	aCN	CN
D	CN	CN	CN	aCN	CN	aCN	CN
E	CN	CN	CN	aCN	CN	aCN	CN
F	CN	CN	CN	aCN	CN	aCN	CN

Slides of 7 CNs were stained in 6 different neuropathological institutions against Ki67. Ki67 was determined manually by counting cells in a 10um^2 sized area. **d** Institution's stains varied greatly across different cases. Accordingly, CN diagnosis given depending on a Ki67 index ≤ 3% or > 3% differed between the institutions (**e**).

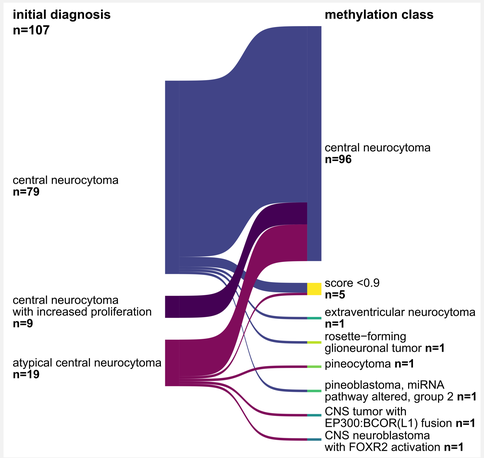


f To explore if the mitotic count could be an alternative and eventually better stratification marker for CN patients compared to Ki67 index, 5 neuropathologist were advised to count mitosis in 10 randomly chosen High Power Fields (HPF) in the same 10 CN cases used for Ki67 evaluation. Counts were afterwards calculated into mitosis per mm^2.



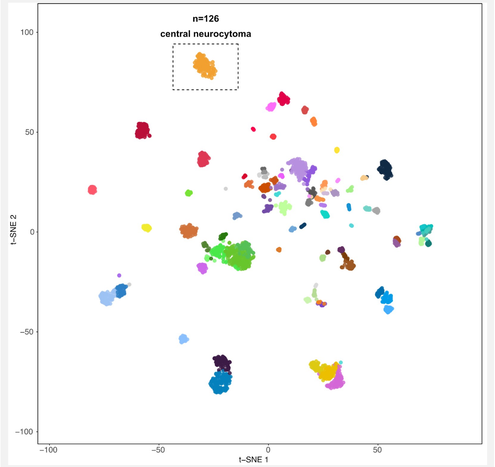
Further we evaluated if raters rather tended to rate low or high concerning the Ki67 proliferation index (**g**) respectively count many or less mitosis per 10 HPF (**h**).

Epigenetic profiling reveals histological misdiagnosis



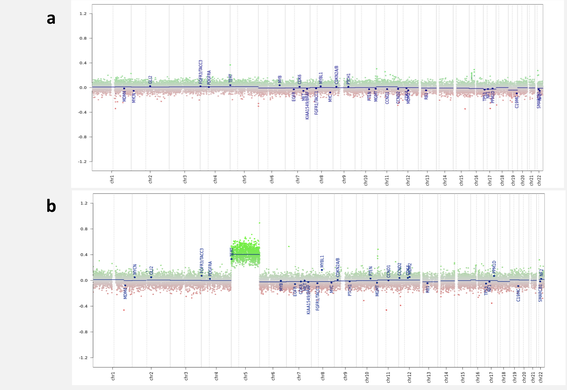
Methylation data of 107 cases diagnosed histologically as CN was classified using the Heidelberg Brain Classifier v12.5. 6 cases fell into other methylation classes and 5 cases did not yield a sufficient prediction. The concordance of 89% between histology-based diagnoses and methylation-based classification is in line with previous reports from Capper *et al.* 2018 on all CNS entities [2].

CNs show distinct and homogeneous epigenetic profiles



In *t*-distributed stochastic neighbour embedding (*t*-SNE) representation, methylation data of n = 96 cases and 8 recurrences clustered together with the methylation class of central neurocytoma (n = 22) of the Heidelberg classifier v11b4 from Capper *et al.* 2018 [2]. Extensive clustering analysis within the CN class revealed no subgroups.

Most CNs are characterized by flat CNVs profiles



a Methylation analysis reveals that the majority of cases show no chromosomal alterations.
b Some cases (9/96) show individual alterations, for instance a chr5 gain (n = 3).

Summary and Conclusions

Our data suggests that interobserver reliability of Ki67 estimation was poor. To further investigate the reliability of this index, additional 20 CN cases will be assessed by our neuropathologists. Subsequently the interclass correlation coefficient (ICC), a widely used index to stratify interrater reliability [3], will be determined concerning the estimation of the Ki67 index and the mitotic count. ICC estimates and their 95% confidence intervals, will be calculated based on single-rating, absolute-agreement and 2-way mixed effects model.

Epigenetic profiling led to a change in diagnosis in some of the cases former classified as CN. Based on DNA methylation analysis no subgroups within CN class were identified. Survival analysis will compare the WHO criteria to an elastic net regularized Cox regression integrating molecular (CNV profile, CNV load, variable CpG sites) and clinical data, in hope to find more reliable markers for predicting prognosis in CN patients.

References

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The authors declare that they have no conflicts of interest.