Molecular characteristics and prognostic biomarkers of central neurocytoma

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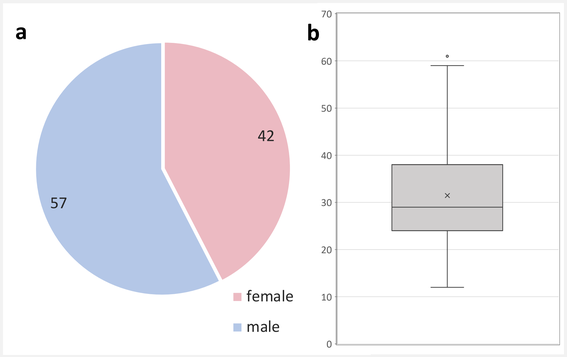
Background

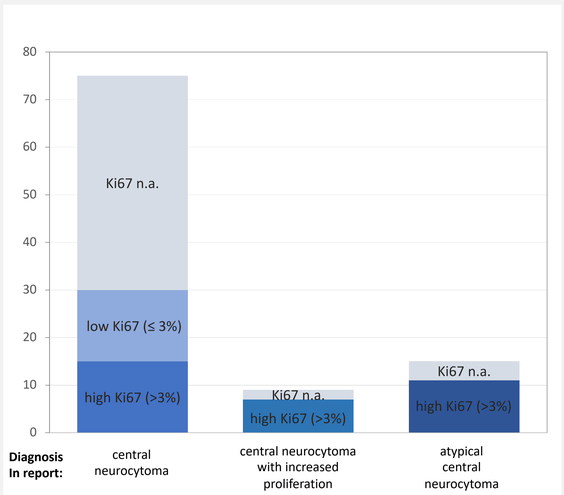
Central neurocytomas (CNs) are brain tumours that predominantely occur in young adults. Although associated with a favourable prognosis in many cases, they potentially recur, particularly when resection is incomplete. Occasionally, aggressive behaviour and rapid progression with multiple recurrences 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 tumour progression are not known. Hence, the project will elucidate the molecular background of CN based on epigenetic profiling and next-generation sequencing in a retrospective cohort of n = 99 patients. Ki67 proliferation index and genome-wide methylation will be integrated in a random survival forest risk model to identify clinically relevant subgroups and future patient stratifications.

A new retrospective multi-institutional cohort



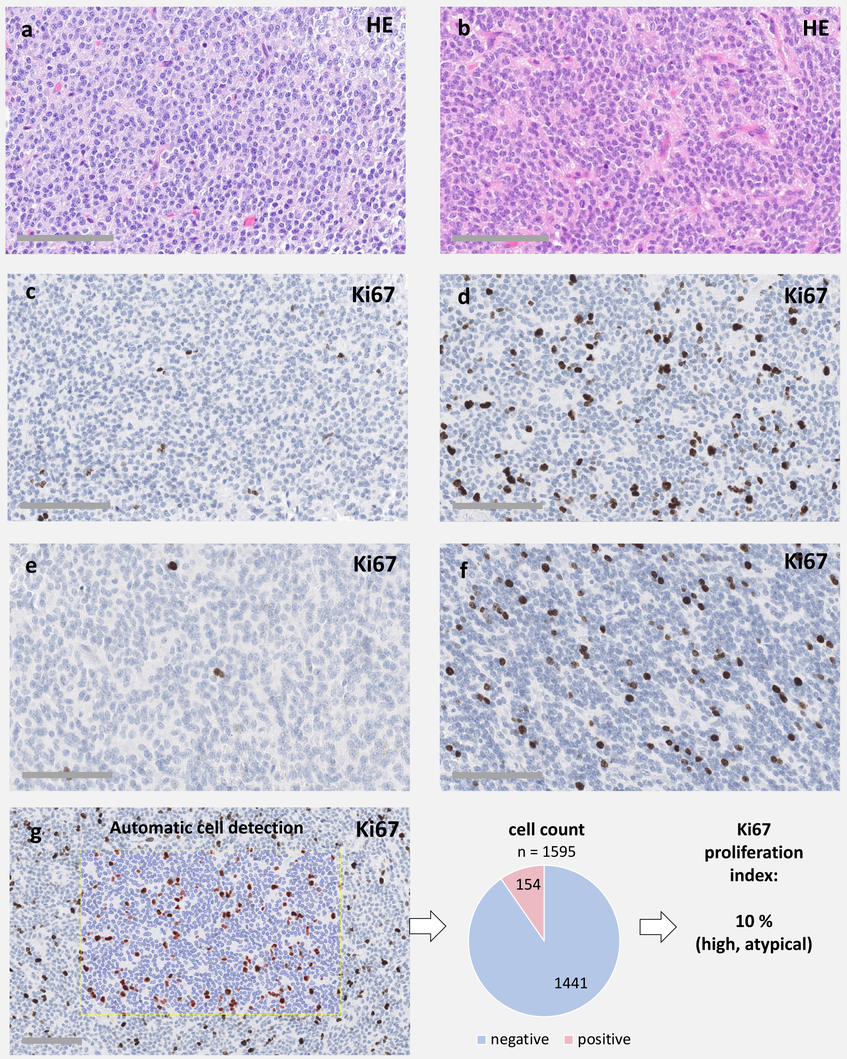
a The patients included in the current cohort (n = 99) are uniformly distributed among sexes ($\chi 2$ goodness-of-fit test, *p*-value = 0.1317). **b** Median patient age is 29 (range 12-61) years.

The criteria for atpyical neurocytoma are not consistently used in day-to-day diagnostic practice



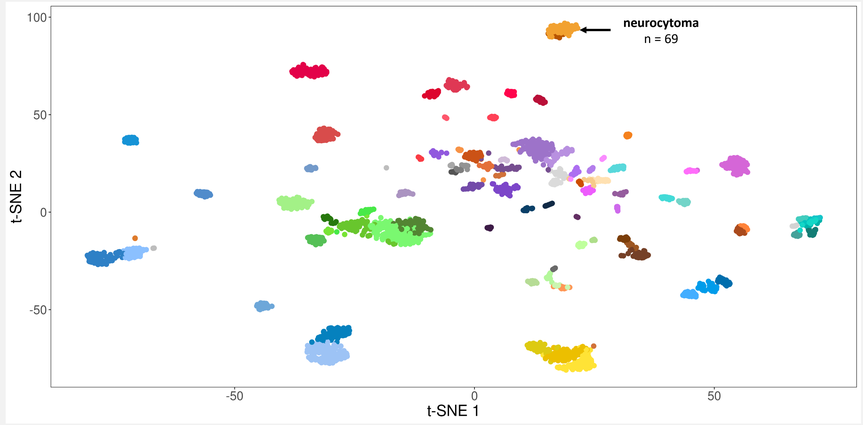
Several studies have shown increased aggressiveness in cases with atypical features and/or Ki67 proliferation index > 2-3% [1]. The optimal threshold for predicting prognosis is still under debate. The classification in and terminology of classical and atypical neurocytomas are not consistently applied by different neuropathologists. For example, in 15/30 cases of our series the diagnosis „central neurocytoma“ was given despite a reported Ki67 ≥ 3% (NA: n = 45). In 7/9 cases, neuropathologists diagnosed a „central neurocytoma with increased proliferation“ if the Ki67 was estimated ≥ 3%, but histological features of atypia were not seen (NA: n = 2). Furthermore, there are no established and validated standard protocols for the estimation of the Ki67 proliferation index. The inconsistency of assessment and practical use raises questions about the meaningfulness of the Ki67 as a reliable criterium for the classification and future stratification of CNs.

Differences in histopathology and Ki67 proliferation index in classical and atypical CNs



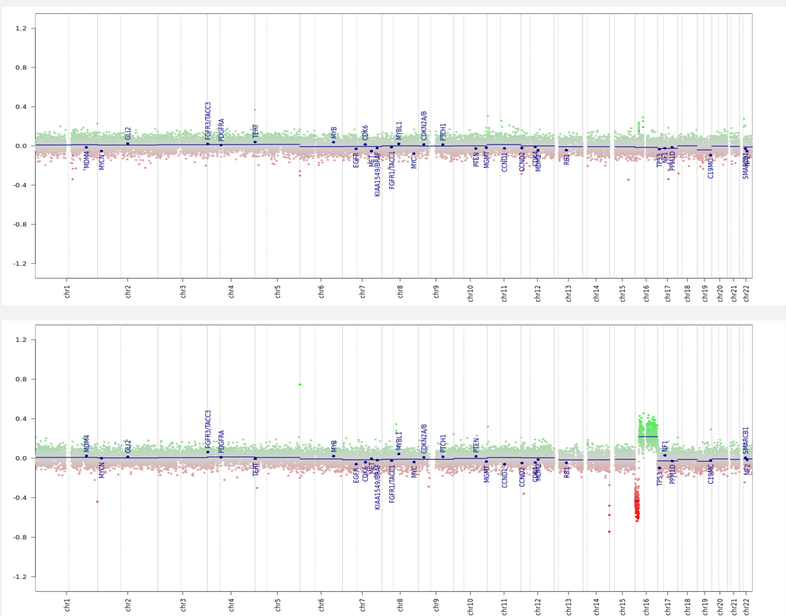
a Haematoxylin-eosin (HE) staining of a typical CN demonstrates the characteristic morphology of CNs comprising monomorphic round cells with round nuclei and "salt and pepper" chromatin. A similar morphology is exhibited by the atypical CN with mild endothelial proliferation shown in **b**. While the typical CN is characterised by a low Ki67 index of about 1% (**c**), the atypical CN shows a much higher proliferation rate of about 10% (**d,g**). Signs of malignancy like necrosis, glomeruloid vascular proliferation or a high mitotic count are absent in both examples, regardless of Ki67 indices. The Ki67 index of the same tumour (**e,f**) in two distinct areas demonstrates a high intratumoral heterogenity: While one region exhibts a low Ki67 (<1%, **e**), the other region shows a increased Ki67 (7%, **f**). The estimation of Ki67 by microscopy is difficult, and subjective. Determination of Ki67 may be facilitated by using digital pathology and automated counting by the QuPath software (**g**). Scale bar denotes 100 µm.

All neurocytoma of the study were selected based on their characteristic epigenetic profile



In t-distributed stochastic neighbour embedding (t-SNE) representation, all cases (n = 69) clustered together with the methylation class neurcytoma (dark red) established in the Heidelberg classifier v11b4 reference cohort from Capper *et al.* 2018 [2] and received high classifier scores (> 0.9).

Most CNs are characterized by flat CNVs profiles, but some show alterations



Methylation analysis reveals that the majority of cases show no chromosomal alterations (**a**). Some cases (6/70) show individual alterations, for instance a chr15q loss and chr16 gain (**b**).

References

1. Park S., Honavar M., Sievers P., Central neurocytoma. WHO Classification of Tumours Editorial Board. Central nervous system tumours, *International Agency for Research on Cancer* 5 edn. Vol. 6 (2021)
2. Capper, D., Jones, D., Sill, M. *et al.* DNA methylation-based classification of central nervous system tumours. *Nature* 555, 469–474 (2018).