Automated quantification of Ki-67-positive cells on whole-slide images in pediatric high-grade glioma may have more prognostic value than WHO grade

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Background and objectives

- WHO adult glioma grading system may not be suitable for pediatric high-grade gliomas
- Considerable inter- and intraobserver variability of its criteria
- A clinical, radiological or biological signature of these tumours having a prognostic value is most wanted
- It should be robust, i.e. less prone to interobserver variability

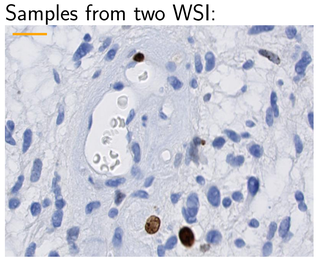
Cohort

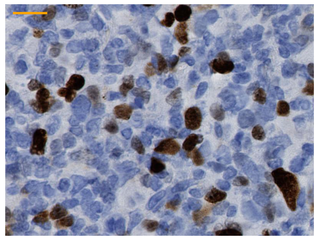
- Population of 128 patients (3 to 18 years old) suffering pediatric high-grade gliomas of WHO grades III (30 patients) and IV (98 patients)
- Recruited in 51 participating sites in 14 countries during the HERBY trial, with informed consent

Slide material for automated Ki-67 quantification

- Cohort used for automated Ki-67 quantification comprised 114 patients. Each one had a whole-slide image (WSI) of a biopsy or surgery sample marked with Ki-67 (MIB) brown immunostaining over hemalun counter-staining and digitized with a Hamamatsu Nanozoomer.
- 7 WSI had to be excluded from the study because of technical problems (insufficient sample or image quality, or artefact cytoplasmic staining by the Ki-67 immunomarker).

Samples from two WSI:





scale bar = 20 microns

Methods: Ki-67 quantification

Using a combination of software (NDPITools [2], in-house C and Perl programs and ImageJ macros), each WSI was segmented the following way [3]:

1. Area occupied by tissue was selected (excluding lumina of blood vessels).
2. Blurred regions were automatically detected then excluded from the tissue area because they could bias the result (segmentation quality of cell nuclei is not guaranteed in such regions) [4].
3. Clusters of red blood cells, detected as tissue in the first step of the treatment, were automatically detected then excluded from the tissue area because they could bias the result (artificially augmenting the area of the tissue region). In addition, on some WSI, they are artefactually stained like Ki-67-positive cell nuclei, leading to a wrong result.
4. Reference zones for the blue (hemalum) and brown (Ki-67) stains were selected automatically to calibrate staining on each slide.

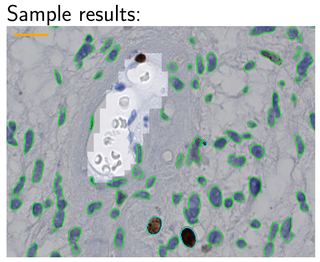
Methods: Ki-67 quantification (cont'd)

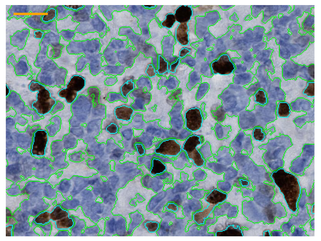
5. A color deconvolution was performed and objects (Ki-67-positive and Ki-67-negative nuclei) were segmented on the deconvoluted channels using automatically defined thresholds on channel intensities.
6. Objects smaller than $6.13 \mu m^2$ were discarded (false positives due to dust and impurities).
7. Final result: Ki-67 index = percentage of Ki-67-positive nuclei among the total number of detected nuclei on the WSI. (Typically 100,000 nuclei on one WSI).

Quality check procedure of the automated Ki-67 quantification

Each WSI, alogn with the automated segmentation results (mask of non-blurred tissue areas, masks of Ki-67-positive and -negative nuclei inside this area), was uploaded to a webserver for interactive review by a human expert.
Automated segmentation was found of very good quality, except for the 7 WSI which had been discarded from this study (see above).

Sample results:





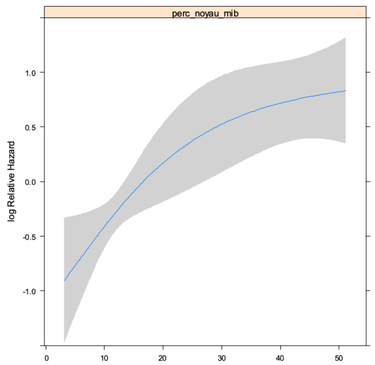
Scale bar = 20 microns. Shadowed area = unblurred tissue.
Cyan / green contours = Ki-67-positive / -negative nuclei.

Results: Ki-67 index has prognostic value

Statistical analysis of overall survival during follow-up (between 0.03 and 46.8 months, with median 24.1 months).

- Our Ki-67 index was significantly correlated with the overall survival (a 10% increase of Ki-67 index was associated with a hazard ratio of 1.53).

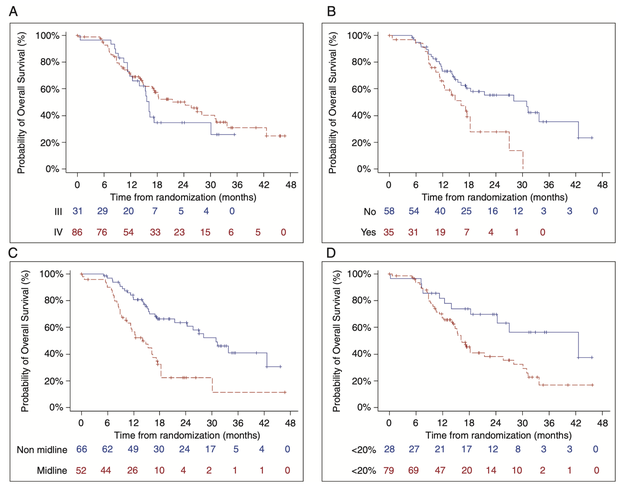
- Interestingly, it is a continuous prognostic factor (not on/off like presence/absence of a mutation).



Percentage of Ki-67-positive nuclei

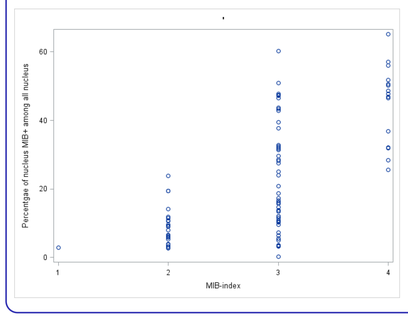
Results: WHO grade shows no significant prognostic value

Survival acc. to:
(A) 2007 WHO grade (B) H3F3AK27M mutation (C) tumour site (D) Ki-67 index.



- (B) and (C) are correlated (not shown). (D) is the only continuous factor.

Automated quantification is more robust than manual quantification



Plot of result of automated quantification vs. manually attributed index (1, 2, 3, or 4) shows good correlation and strong variability of manual estimate.

Conclusion and perspectives

- For pediatric high-grade gliomas, WHO grade is not reliable for prognosis.
- Our automatic WSI Ki-67 quantification provides an alternative with proven prognostic value and less prone to interobserver variability.
- It consumes less human specialist's time than standard procedure.
- It does not require any machine training phase.
- It can be applied to other diseases.

References

[1] P. Varlet et al., Neuro-oncology, **22**(1):116–127 (2020)
[2] C. Deroulers et al., Diagn. Pathol. 8:92 (2013)
[3] C. Deroulers et al., Diagn. Pathol. 2:209 (2016)
[4] D. Ameisen et al., Diagn. Pathol. 8(Suppl 1):S23 (2013)

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