Grading of oligodendroglialomas based on Ki67 labeling index assessment performed manually and automatically in hot-spot fields

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Introduction

Oligodendrogliomas are diffusely infiltrating neuroepithelial brain neoplasms composed of cells resembling oligodendrocytes and harboring IDH1 or IDH2 mutation with 1p/19q chromosomal codeletion.

- 5-10% of all giomas
- Most often occur in frontal lobe of cerebral hemispheres
Current WHO Classification of Tumours of the Central Nervous System (2016) distinguishes two malignancy grades for oligodendrogliomas:

- GII for well differentiated tumours
- GIII for anaplastic oligodendrogliomas.

The most important morphological criteria for diagnosing GIII oligodendrogliomas are:

- Microvascular proliferation
- Brisk mitotic activity
Introduction

- Assessment of mitotic activity is often supported by immunohistochemical studies of Ki67/MIB-1 expression by neoplastic cells.
- Time-consuming quantitative evaluation of immunostained slide is required to establish the proliferation index of tumor cells.
Introduction

- Estimation of Ki67 labeling index (LI) of tumor cells can be supported by additional digital modalities such as whole slide image acquisition and introduction of computer algorithms for counting immunostained cells.
The aim of study

- To assess concordance of Ki67 LI counting results in oligodendrogliomas performed manually in light microscope and by developed automatic computerised algorithm in WSIs.

- To evaluate utility of developed automatic computerised algorithm for Ki67 LI assessment in oligodendrogliomas grading.

- To estimate Ki67 LI cut-off value useful in grading of oligodendrogliomas.
Eighteen cases of WHO GII and twelve WHO GIII FFPE oligodendroglialomas were stained with monoclonal FLEX Ki-67 (clone MIB-1) antibody (Dako, Demark).

- VS: EnVision FLEX+/Autostainer Link (Dako, Denmark).

WSIs of stained specimens were acquired on 3DHistech Pannoramic II Flash scanner under the 20x magnification of lens with a resolution 0.38 μm per pixel.
Methods

- The manual quantitation of Ki67 LI (Ki67 score) was performed in light microscope by selection of ten high-power fields (HPFs) with highest density of Ki67+ve cells (hot-spots).
- In each selected HPF pathologist performed counting of immunostained and immunonegative tumor cell nuclei and estimated Ki67 LI.
- Mean value of Ki67 LI from all ten HPFs was the result of manual assessment of Ki67 score for analysed oligodendroglioma case.
Methods

- Selection of twenty hot-spots on WSIs was done automatically by the developed software with the gradual extinction scheme.
- Area of each selected ROI was equal to HPF area in light microscope with eyepiece lens 10x/22.
- Next, the same computed-based system performed quantitation of Ki67 LI in selected tumor areas.
- Mean value of Ki67 LI from all selected areas was the result of automatic assessment of Ki67 score for analysed oligodendroglioma case.
Methods – automatic algorithm

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Methods – automatic algorithm

For detection of hot-spots in WSIs the developed computer system used:

- mathematical morphology methods,
- textural descriptions,
- classifiers,
- penalty function.

Methods

The statistical analysis of results was performed with the Spearman, Wilcoxon and Chi-square tests.
Manual vs automatic Ki67 LI assessment

**G II:**
- range: 1.52 – 28.56% vs 0.62 – 27.87%
- mean: 4.84% vs 5.16%
- median: 3.35% vs 3.36%

**G III:**
- range: 11.8 – 55.2% vs 9.47 – 41.23%
- mean: 23.8% vs 24.88%
- median: 20.95% vs 25.1%
Correlation analysis of the manually and automatically estimated Ki67 LI showed good accordance of both methods with $R=0.9293$ ($p<0.000005$; Spearman rank).
Results

- Ki67 LI evaluated manually and automatically correlated well with tumour grade with $p=0.000041$ and $p=0.00002$, respectively (Wilcoxon test).

- For 8% Ki67 LI cut-off in tumour grading, Chi-square test gave $p<0.0001$ for both evaluation methods.
Conclusions

- The results of the study showed good accordance of manual and automatic Ki-67 LI examination in oligodendrogliomas.
- Computed-based Ki-67 LI assessment can help in grading of oligodendrogial neoplasms.
- Application of automatic algorythms can help in standardisation of quantitaive evaluation of immunostains.
- Further investigation and validation of presented algorythm on larger grups of tumours is needed.
Take-home message 😊

https://miap.wim.mil.pl
Thank you for your attention 😊