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First conclusions regarding the validation of an automated micronucleus counting microscope based on samples from prostate tumorous patients

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Introduction:
As the part of the validation of an automated micronucleus (MN) counting microscope, we tested its performance in the range of low doses. The MN assay makes it possible to determine the quantity of DNA damage as a biomarker of the received dose. For the testing process we examined blood samples from patients with prostate tumour undergoing low dose rate brachytherapy treatment. Our study aimed to identify the factors that can influence the accuracy of the automated system.

Methods: The cytokinesis-blocked micronucleus (CBMN) test was conducted with peripheral lymphocytes sampled just before the beginning of the therapy and at regular intervals during the treatment period (1 day, 3 months, 6 months after seed insertion). In the course of the slide preparation the cells are forced to start proliferation and arrested before the division of the cytoplasm and cell membrane. As a result the objects of interest, the so-called binucleated cells are created. They contain two main daughter nuclei which can be accompanied with small nucleus-like fragment residues, named micronuclei, in case of DNA damage. All of them have a well-defined contour and circular shape thus can be identified using automatic image processing. The average frequency of the MN found in binucleated cells correlates with the received dose. Although it is a high dose that the seeds are locally delivering to the tumour, the MN assay measures the whole body equivalent dose of the irradiation received by the healthy tissue surrounding the gross tumour volume. Thus the number of identified aberrations is in the range of the low doses (<500 mGy). The automatic slide scanning and image segmentation was done by Radosys Radometer-MN Series automated microscope dedicated to this assay.

Results: Factors that can influence the accuracy of the automatically determined MN frequency were identified. Afterwards sample subgroup-pairs were formed in a way that they differ only in one of the previously identified factors of the slide preparation or scanning. We defined the accuracy of the automatic counting system as the MN-frequency difference between the totally automatic and the supervised (semi-automatic) MN-scoring method. The factors can be classified into three groups according to the degree of their effect on the accuracy.

Conclusions: There are further factors that influence the scoring during automatic procedure compared to the standard manual inspection. Only those samples can be compared based on their MN-frequencies that behave in the same manner regarding the factors labelled "significant"in Table 1. The differences in staining and geometrical properties of the cells and (micro)nuclei found to be negligible. The automatic evaluation is found to be also robust for slightly out-of-focus images. Beside the inevitable difference of the individual cell stress tolerance of the different patients and the effect of the received dose the greatest contributor to the inaccuracy is the impurity of the sample. Thus the analysis of these artefacts has an utmost importance. Their well-designed classification should imply whether they can be eliminated by further cleaning cycles. Otherwise this task is delegated to a possible improvement of the image processing algorithm.

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