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THE CYTOKINESIS-BLOCK MICRONUCLEUS ASSAY ON HUMAN CRYOPRESERVED WHOLE BLOOD AND ISOLATED PERIPHERAL BLOOD MONONUCLEAR CELLS.

Introduction & aim of the study

The cytokinesis-block micronucleus (MN) assay is a widely used technique in:

- Human biodosimetry studies
- Occupational genotoxicity studies
- *In vitro* radiosensitivity testing

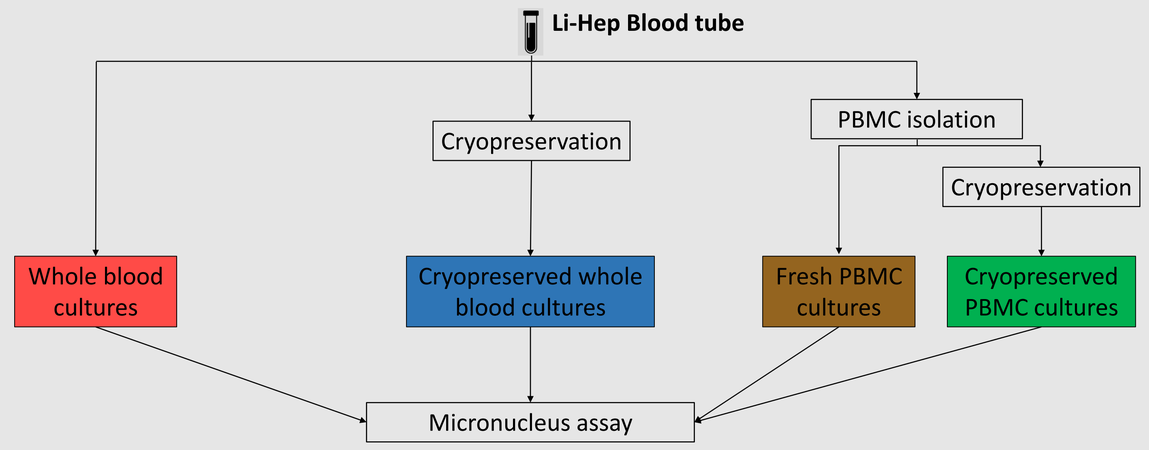
Micronuclei (MN) are small, extra nuclear bodies that are formed as a result of whole chromosomes that lag during mitosis, or as a result of chromosome fragments that are not incorporated into the main daughter nuclei.

Fresh whole blood cultures are commonly used, however:

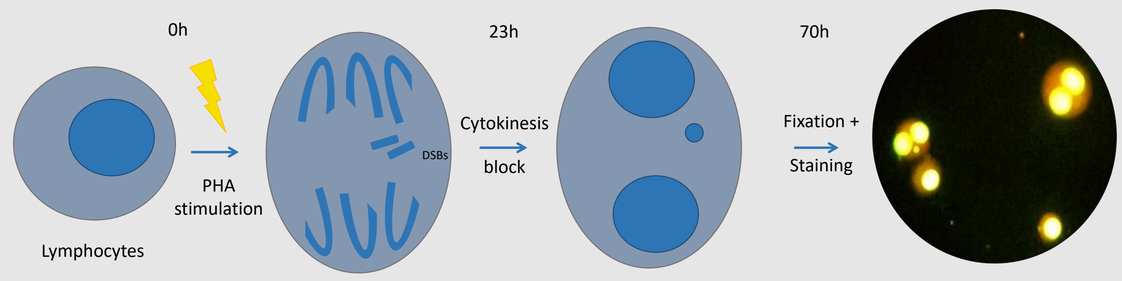
! Immediate processing of the fresh samples can be logistically challenging!

Aim: To establish and evaluate two novel protocols for the MN assay on cryopreserved whole blood and fresh or cryopreserved isolated peripheral blood mononuclear cells (PBMC).

M&M: Different cell culture set-ups



M&M: Cytokinesis-block micronucleus assay



Results

1. Comparison of the different CBMN assays

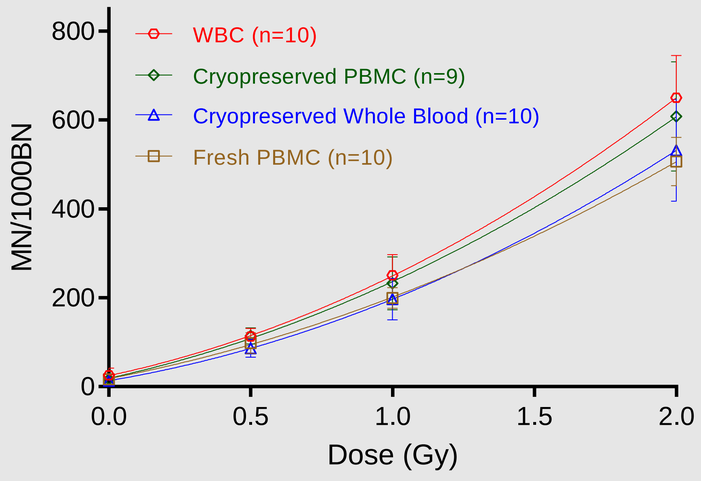


Figure 1. Linear quadratic fits of the MN results of the different sample types are represented. The error bars represent SD of the mean.

2. Effect of cryopreservation time on MN results.

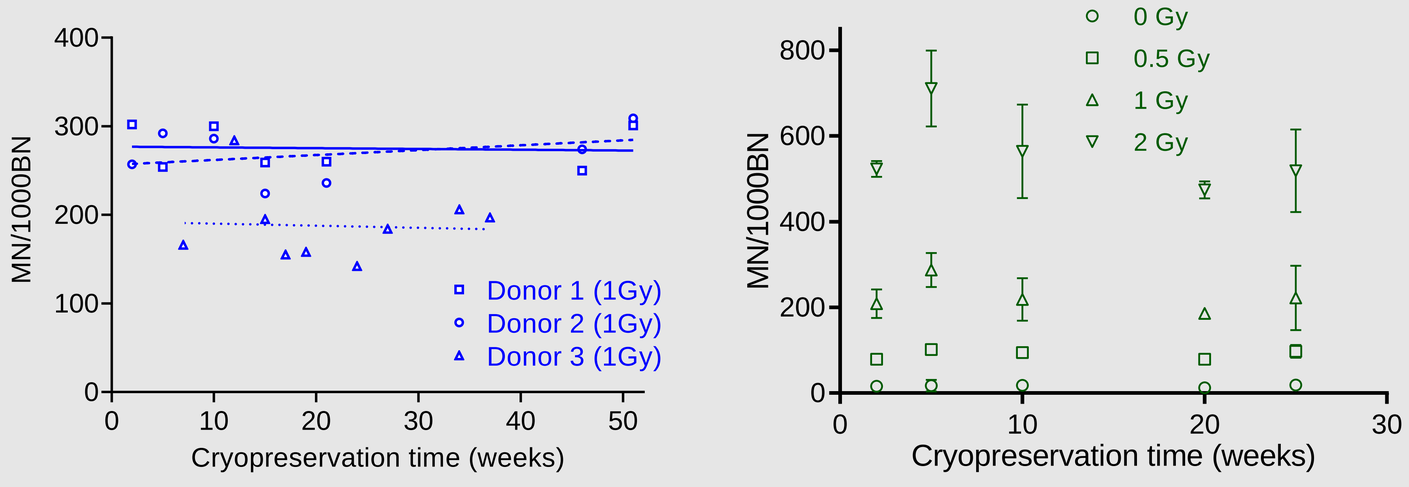


Figure 2. The left graph shows the MN results of 3 different cryopreserved whole blood samples, exposed to 1 Gy (Blue). On the right, the MN results of 3 cryopreserved PBMC samples, that were exposed to 0, 0,5, 1 and 2 Gy (Green). The error bars indicate SD of the mean.

3. Use of the PBMC MN assay for biological dosimetry

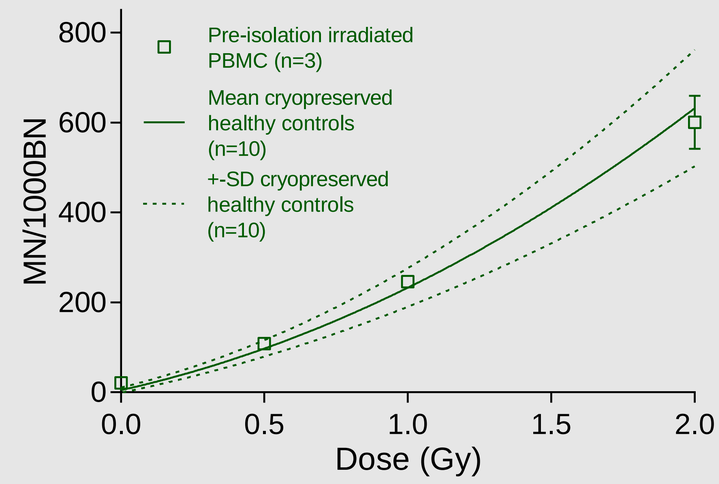


Figure 3. Whole blood was exposed to 0.5, 1 and 2 Gy, before PBMC isolation. The mean MN +-SD of three donors is plotted against the linear quadratic fit of the mean +-SD of ten cryopreserved PBMC controls.

4. Radiosensitivity assessment of patients

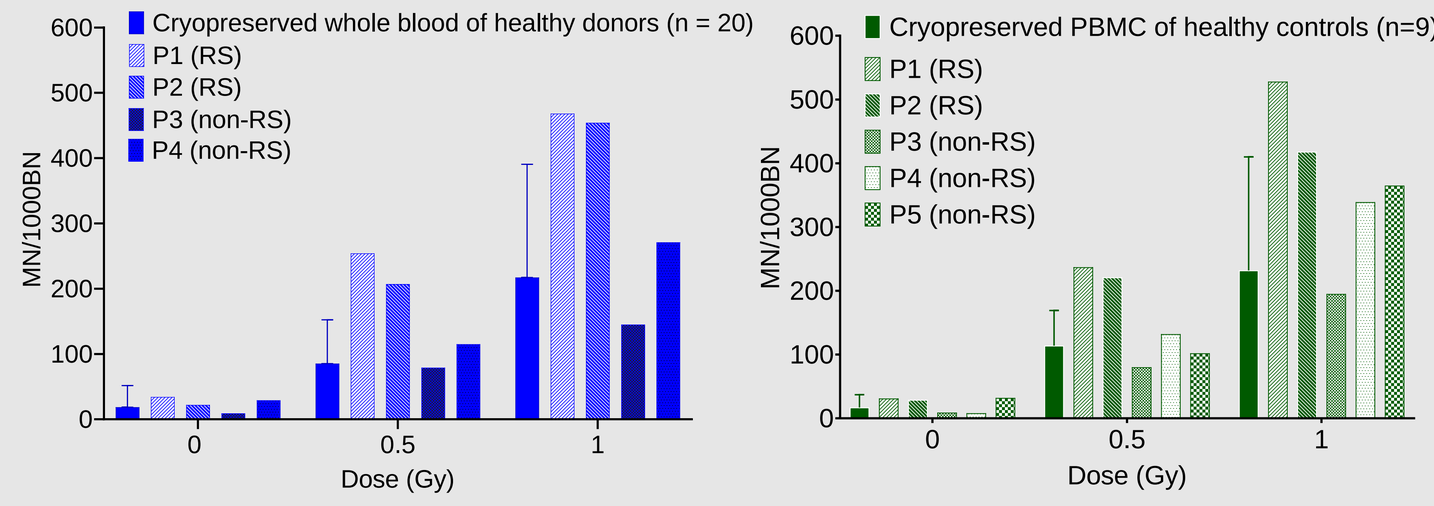


Figure 4. Patients are considered radiosensitive when their MN values exceed the mean +3 SD of a healthy control group, for each dose (Indicated by error bars). According to clinical data, patients 1 and 2 were considered radiosensitive while patients 3, 4 and 5 were not radiosensitive. Both assays indicated these outcomes correctly.

Conclusions

Our new MN assay protocols on fresh PBMC, cryopreserved PBMC and cryopreserved whole blood demonstrate to be reliable tools for radiosensitivity and biodosimetry studies.

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