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REVIEW

The micronucleus assay as a biological dosimeter of *in vivo* ionising radiation exposure

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Biological dosimetry, based on the analysis of micronuclei (MN) in the cytokinesis-block micronucleus (CBMN) assay can be used as an alternative method for scoring dicentric chromosomes in the field of radiation protection. Biological dosimetry or Biodosimetry, is mainly performed, in addition to physical dosimetry, with the aim of individual dose assessment. Many studies have shown that the number of radiation-induced MN is strongly correlated with dose and quality of radiation. The CBMN assay has become, in the last years, a thoroughly validated and standardised technique to evaluate *in vivo* radiation exposure of occupational, medical and accidentally exposed individuals. Compared to the gold standard, the dicentric assay, the CBMN assay has the important advantage of allowing economical, easy and quick analysis. The main disadvantage of the CBMN assay is related to the variable micronucleus (MN) background frequency, by which only *in vivo* exposures in excess of 0.2–0.3 Gy X-rays can be detected.

In the last years, several improvements have been achieved, with the ultimate goals (i) of further increasing the sensitivity of the CBMN assay for low-dose detection by combining the assay with a fluorescence *in situ* hybridisation centromere staining technique, (ii) of increasing the specificity of the test for radiation by scoring nucleoplasmic bridges in binucleated cells and (iii) of making the assay optimally suitable for rapid automated analysis of a large number of samples, viz. in case of a large-scale radiation accident. The development of a combined automated MN-centromere scoring procedure remains a challenge for the future, as it will allow systematic biomonitoring of radiation workers exposed to low-dose radiation.

Introduction

Biological dosimetry for radiation exposure

Biological dosimetry, based on the analysis of dicentric chromosomes, has been used since >40 years (mid 1960s) and has become the standard test for dose assessment in the framework of radiological protection programmes (1). In case of a radiation accident, the first information comes especially from physical dose reconstruction, blood count data and from the clinical symptoms that exposed persons might display. All these sources of information may be combined with the results of biological dose assessments to obtain a clearer evaluation of the case. Biological dosimetry using cytogenetic methods is of particular importance because it takes into account inter-individual variation in susceptibility to radiation damage. Numerous studies, performed both on animals and humans, have demonstrated a close relationship between dicentric chromosomes induced in peripheral blood lymphocytes (PBLs) under *in vitro* and *in vivo* conditions. This allows dose estimation of an accidentally exposed person by comparing the observed aberration yield of dicentrics to an *in vitro* calibration curve. The power of dicentrics for dose estimation is related to the low and constant spontaneous dicentric rate in the healthy population (about one dicentric per 1000 metaphases), and by the fact that dicentrics are specifically induced by ionising radiation (2). After whole-body exposure with low linear energy transfer (LET) radiation, doses down to ~0.1 Gy can be detected. However, in cases of exposure to low doses of radiation, a disadvantage of the dicentric assay is the time needed for microscopic scoring analysis of a sufficient number of metaphases.

For many years, the dicentric assay performed in PBL was the only method available; and still today, it is the gold standard for cytogenetic radiation dosimetry. However, in the past years, a number of additional assays (3) have been worked out and validated, including the now well-established micronucleus (MN), translocation and premature chromosome condensation assays. More recently, molecular biomarker methods such as the gamma-H2AX assay have been proposed (4).

The range of biodosimetry options now available have led to proposals for a multi-parametric approach to investigate overexposed subjects (5).

The MN assay as biological dosimeter

The cytokinesis-block micronucleus (CBMN) assay has been developed by Fenech and Morley in 1985 (6). MN are small extranuclear bodies resulting from chromosome breaks or whole chromosomes lagging behind during anaphase. They are scored in PBL in the first interphase after cell division. These cells can be identified as binucleated (BN) cells by addition of the cytoplasmic division inhibitor cytochalasin B during cell culture.

As MN can arise from exposure to various clastogenic agents in the form of acentric chromosome fragments, as well as to aneugenic agents as whole chromosomes, they are not radiation specific. As a consequence, the CBMN assay is often used as a general toxicology test (7). However, because ionising radiation is a strong clastogenic agent, and thus a potent inducer of MN, the CBMN assay has proven to be a very reliable, thoroughly validated and standardised

technique in the field of radiation biology (i) to evaluate *in vivo* radiation exposure of occupational, medical and accidentally exposed individuals and (ii) to assess individual *in vitro* radiosensitivity or cancer susceptibility (8–23). Radiation-induced chromosome aberrations such as MN observed in PBL are mainly the result of unrepaired or misrepaired double-strand breaks by the non-homologous end joining (NHEJ) repair pathway (22,24).

MN dose response

Many studies showed that the number of radiation-induced MN is strongly correlated with radiation dose and depends on the radiation quality (25–29). For low LET radiation, linear-quadratic dose–response functions of the form $y = c + \alpha D + \beta D^2$ are reported, while a linear dependence ($y = c + \alpha D$) is observed for high LET radiation, with the latter being more effective in generating MN at the same dose levels. In the equations, y represents the yield of MN/BN cells, c the background frequency, D the absorbed dose and the α and β coefficients refer to MN per BN cell per Gy and per Gy^2, respectively. These curve fittings follow the same shape as described for the standard dicentric assay. However, due to inter-laboratory differences in MN dose response caused by the use of different protocols, scoring criteria, etc., any laboratory intending to perform biological dosimetry, should determine and periodically re-establish its own *in vitro* dose–response calibration curves (10). Ideally, about eight dose points should be used in the range up to 5 Gy. For doses higher than 5 Gy, the response levels off. This phenomenon is well known also for other cytogenetic end points such as dicentrics and is interpreted as selection against heavily damaged cells that cannot enter mitosis. In Figure 1, a typical example of a MN dose–response curve for low LET radiation (^{60}Co γ-rays, dose rate 0.5 Gy/min), based on the average MN yields per 1000 BN cells of 10 donors (13), is shown.

Recently, a number of specialised curve-fitting computer programs for cytogenetic end points such as dicentrics, translocations and MN have been developed (30), like the 'chromosomal aberration calculation software' (CABAS) and the 'Dose Estimate' tools. CABAS was developed by Deperas *et al.* (31) and uses maximum likelihood methods to fit calibration data to the linear quadratic equation. Dose Estimate is a similar tool, which was developed at the UK Health Protection Agency (32), allowing both linear quadratic and linear fitting. Both CABAS and Dose Estimate include additional tools that assist with processing data from radiation accident cases in order to derive dose estimates when the circumstances of the overexposure are deviating from acute or whole-body exposure.

MN background frequencies

A drawback of the CBMN assay compared to the dicentric assay, for applications in the low-dose range, is the inter-individual variability of the spontaneous MN frequency (33). Values ranging from 2 to 36 per 1000 BN cells have been recorded (34). Due to this variable background, the CBMN assay in its basic form could only detect *in vivo* exposures in excess of 0.2–0.3 Gy X- or γ-rays (10,29,35).

The two most important factors influencing the MN background levels, besides diet (36) and exposure to environmental mutagens, are age and gender (37,38). Large-scale biomonitoring studies have shown that the spontaneous MN yield increases systematically with age. Values between 0.24 and 0.44 MN/1000 BN cells/year were obtained on healthy male populations in the frame of studies performed by the Ghent group on nuclear power plant and hospital workers (9,11,12). Also in the large-scale Fenech study (39), investigating variables influencing baseline MN frequencies, an increase of 0.31 MN/1000 BN cells/year was measured in a male population. In females, the spontaneous MN yields are

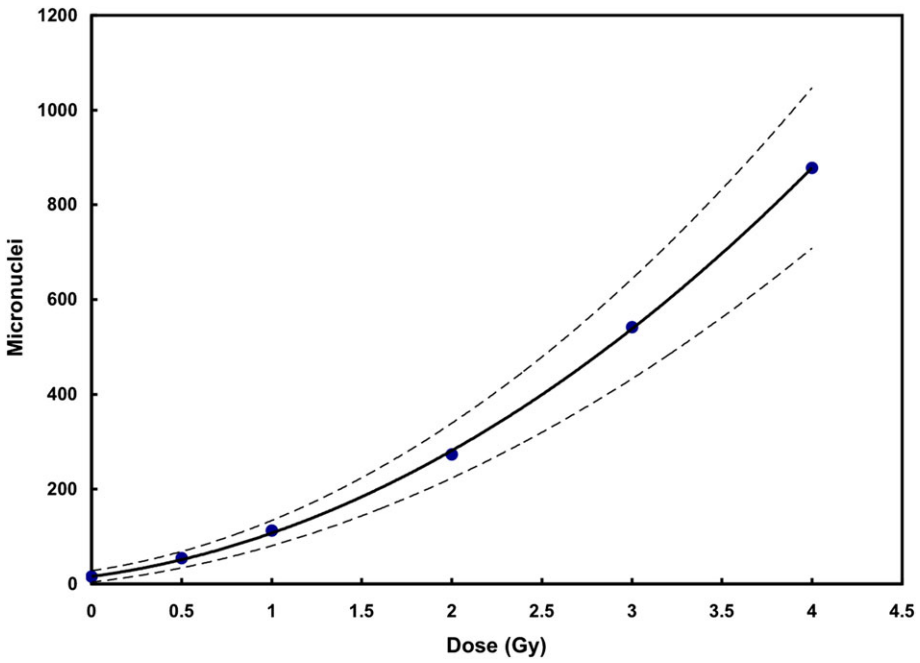

Fig. 1. Linear-quadratic MN dose response for low LET ^{60}Co γ-rays. The average MN yields per 1000 BN cells of 10 donors, together with the curve fittings through these points, are shown (full line). The fitted curves for the 95% upper confidence limit and the 95% lower confidence limit are shown as dashed lines.

higher compared to males and the increase of MN with age is more prominent: an increase of 0.58 MN/1000 BN cells/year was observed by the Ghent group (12), this in good agreement with the increase of 0.52 MN/1000 BN cells/year reported by Fenech (39).

Investigation of the MN content using a pan-centromeric fluorescence *in situ* hybridisation (FISH) probe showed that the age increase of baseline MN frequencies can be attributed almost completely to centromere-positive MN, reflecting an increased chromosome loss with age (10–12). By using an X-chromosome-specific centromeric probe, it was shown that the X-chromosome is almost always involved in this spontaneously occurring chromosome loss (40,41). This finding may explain also the gender difference observed in spontaneous MN frequencies. In the MN study mentioned above performed on hospital workers using a pan-centromeric probe, a mean spontaneous total MN yield of 16.4 was obtained for males (mean age = 41.4 years) while for females (mean age = 41.8 years), an MN yield of 23.5 was found. The number of centromere-negative MN detected in this study was not significantly different between males (6.7) and females (7.7) (12).

Applications of the CBMN assay for biological dosimetry

To assess the suitability of the CBMN assay for biological dosimetry, MN yields have been analysed in PBLs of different groups of patients treated with fractionated partial body radiotherapy, for e.g. cervical cancer, prostate cancer or Hodgkin's disease. The doses estimated by MN analysis agree quite well with averaged whole body doses calculated from the radiation treatment plans (8,42–45). Studies performed in thyroid cancer patients undergoing radioiodine treatment further demonstrated that the CBMN assay is sensitive enough to detect low average whole-body doses from internal exposure scenarios (16,23,46).

As the above-mentioned patient studies have shown that the CBMN assay is a reliable biomarker for radiation exposure, the CBMN assay has been used frequently to measure radiation exposure in radiation accidents and has been applied for large-scale biomonitoring.

In specific radiation accident studies, the CBMN assay was applied to assess protracted exposure, due to the incorporation of long-lived radionuclides by residents in the vicinity of the Chernobyl nuclear power plant (47) and of the Semipalatinsk nuclear test site (19). MN frequencies measured in a large number of residents were significantly associated with the estimated internal absorbed dose.

In case of small accidents involving only few patients, the dicentric assay has generally been used to assess radiation damage soon after the accident, and only a limited number of studies on MN-based dosimetry are available. In the study describing the Istanbul accident where 10 scrap metal workers were irradiated by an unshielded former radiotherapy ^{60}Co source (48,49) and in the study reporting the accident of a hospital worker exposed to a 50-kV contact radiotherapy X-ray device during maintenance (50), several cytogenetic end points were scored. In both radiation accidents, blood was sampled at different time points, varying between 1 month (48,49) and 6 months (50), after the accident took place. In both studies, MN-derived dose estimates were in striking agreement with dose values obtained from dicentric studies.

Large-scale biomonitoring studies on radiation workers, like nuclear power plant workers and hospital staff, showed that the CBMN assay, and especially the CBMN assay combined with FISH staining for centromeres can detect radiation-induced chromosomal damage at the population level for accumulated doses received occupationally exceeding 50 mSv (9–13,15,17). These biomonitoring studies, examining fairly large populations (ranging between 70 and 270 subjects) of radiation workers occupationally exposed to accumulated doses ranging from 10 to 248 mSv, showed a clear dependence of MN formation on the accumulated dose. In the study performed by the Ghent group on nuclear power plant workers (9), a linear regression of age-corrected individual MN frequencies showed an increase of 0.0175 MN/1000 BN cells/mSv. Application of the CBMN centromere assay in a second study performed by our group on nuclear power plant workers (11) resulted in a comparable dose-dependent increase of MN, i.e. 0.025 MN/1000 BN cells/mSv. This second study further demonstrated that dose-dependence is completely due to centromere-negative MN, thus pointing to the clastogenic effect of ionising radiation. In the biomonitoring study of Vaglenov *et al*. (51), an increase of 0.03 MN/1000 BN cells/mSv was also found in nuclear power plant workers.

Significant achievements

The many applications of the CBMN assay in biodosimetry, of which some have been described above, highlight the important role of this cytogenetic test in assessing radiation exposure but also its shortcomings. By tackling as well its strong and weak points, important improvements have been achieved during the last years. The ultimate goals of these improvements are (i) to further increase the sensitivity of the CBMN assay in the low-dose range, (ii) to make the assay more specific for radiation by scoring nucleoplasmic bridges (NPBs) in BN cells and (iii) to adapt the assay for rapid analysis of a large number of samples.

Development of a CBMN centromere assay

As it has been shown that most of the radiation-induced MN originate primarily from acentric fragments while spontaneous MN contain especially whole chromosomes (10,52–55), the application of the CBMN centromere assay, which uses a pan-centromeric probe to discriminate between centromere-positive and -negative MN (Figure 2a and b), substantially increases the sensitivity of the CBMN assay in the low-dose range (10,52). In the two studies performed by the Ghent group (10,52), it was demonstrated that the majority of spontaneous MN were centromere positive (73 and 71%, respectively) while most radiation-induced MN were centromere negative. The number of centromere-positive MN only showed a very small increase with dose (5.3 and 3.7 MN/Gy/1000 BN cells, respectively). By manual scoring of centromere-negative MN in 2000 BN cells, a detection limit, at the 95% confidence limit, of 0.1 Gy was achieved. According to the results of Pala *et al*. (55), this detection limit could be even lowered to 0.05 Gy.

Scoring of NPBs in cytokinesis-blocked BN cells as a biomarker of dicentric chromosome formation

In the cytokinesis-block micronucleus cytome (CBMN cyt) assay (56), it is possible to score NPBs joining the two nuclei in a BN cell. NPBs originate from dicentric chromosomes (Figure 3), which are induced by misrepair of chromosome

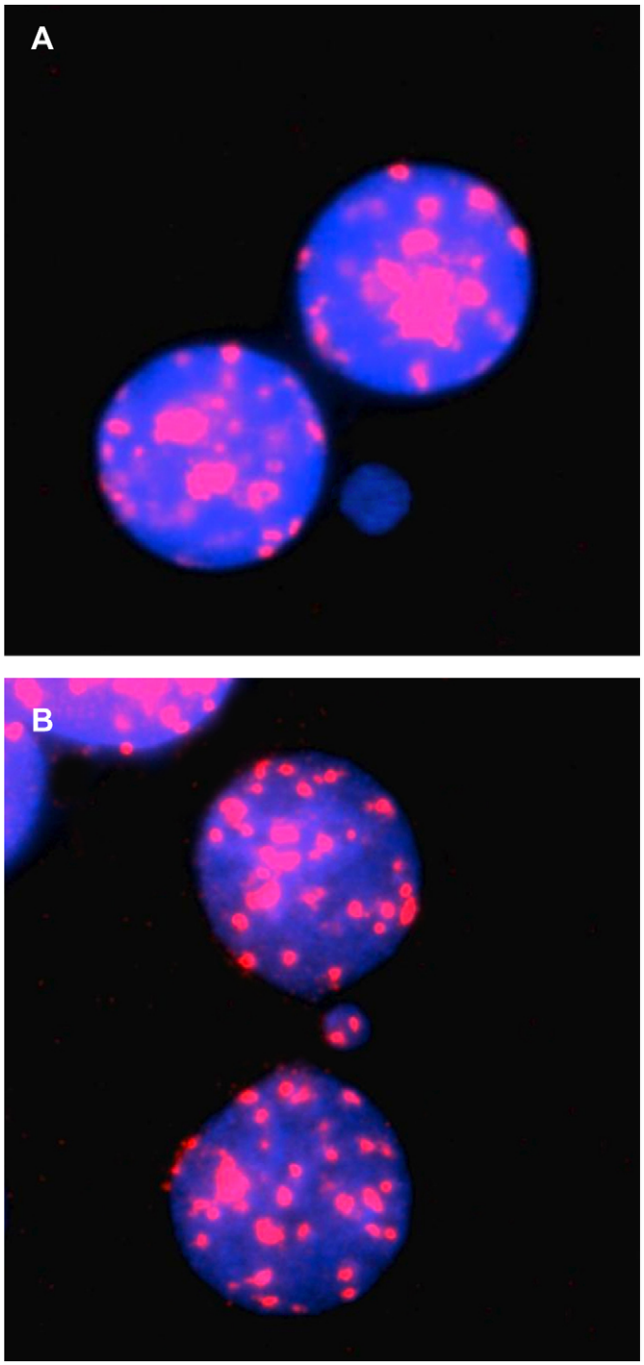

Fig. 2. (**a** and **b**) BN lymphocytes with a centromere-negative MN (a) and a centromere-positive MN (b). The centromeres are stained with a FISH pan-centromeric probe (spectrum orange) and the nuclei and micronuclei are counterstained with DAPI.

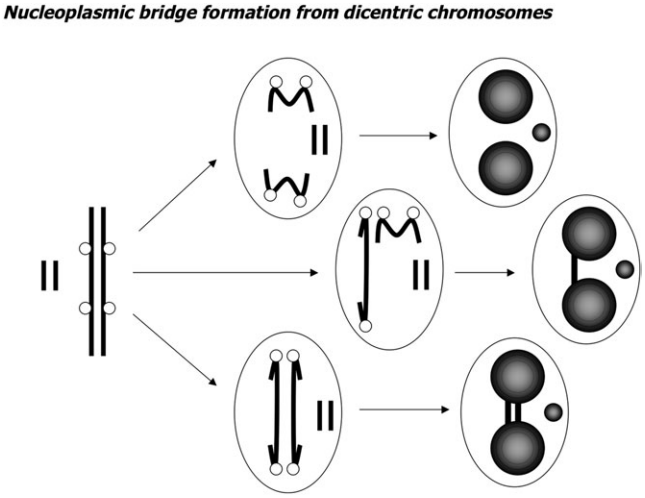

Fig. 3. Formation of NPBs from dicentric chromosomes. Top panel, centromeres of dicentric chromatids travel to the same pole of the cell and no NPB are formed. Middle panel, centromeres of one of the dicentric chromatids are pulled to opposite poles of the cell leading to the formation of one NPB. Bottom panel, centromeres of both dicentric chromatids are pulled to opposite poles of the cell leading to the formation of two NPBs. In each of the above cases, one MN is produced from the lagging acentric chromosome fragment accompanying the dicentric chromosome.

breaks. Scoring NPBs in the CBMN cyt assay for radiation biodosimetry is important because (i) the NPB index has a lower background frequency than MN frequency; (ii) unlike MN background yields, the NPB frequency is not affected by gender; (iii) NPBs in BN cells provide a direct method of measuring asymmetrical chromosome rearrangement in cells after a single-cell division and (iv) NPBs can be scored efficiently because the CBMN cyt assay allows a large proportion of BN cells to be accumulated and (v) furthermore, MN/NPB ratio may provide a fingerprint of specific genotoxic exposure. NPBs in lymphocytes are increased in a dose-related manner following exposure to ionising radiation and correlate well with dicentric and ring chromosome frequencies in metaphases of the same lymphocyte cultures (Figure 4a and b; 57).

Automation of the CBMN assay

Compared to the labour-intensive dicentric assay, the easy and rapid scoring of MN makes this method very attractive for population triage in case of large-scale radiation accidents, as well as for large-scale assessment of genetic damage in radiation workers receiving a high radiation dose. Algorithms for automated MN image analysis were already developed in the 1990s (58,59). These systems, however, showed a high inaccuracy in classification of BN cells. More recently, improved image analysis systems for automated MN scoring have been developed. The MN software module integrated in the metaphase finder system MSearch of Metasystems (MetaSystems Hard & Software GmbH, Altlussheim, Germany) automatically identifies BN cells by the occurrence of two adjacent similarly 4′-6-diamidino-2-phenylindole-stained nuclei. In a second step, MN are scored automatically in a circular region defined around the two nuclei of the BN cell (60,61). A further evaluation of the detected yield of MN by a manual scorer is not needed. As the recognition of a BN cell is based on the occurrence of two adjacent but unattached nuclei, this MN software module does not allow to detect NPBs. The system described by Decordier *et al.* (62) uses a PathFinder™ Cellscan™ capture station and two MN analysis workstations. This system, which has been developed for use in biomonitoring of *in vivo* exposure to mutagenic agents, identifies firstly the cytoplasm of Giemsa-stained cells, and subsequently detects the number of nuclei in the cell, allowing the identification of BN cells. MN are then scored in a third final step.

A collaborative study performed by the Ghent group using the Metasystems software (29) demonstrates the suitability and advantage of automated MN scoring for population triage in

case of large-scale radiation accidents, where it is important to distinguish and isolate severely exposed individuals (≥1 Gy), who require early medical follow-up and treatment.

The fully automated MN scores obtained in our study were highly correlated with manual MN scores and demonstrated that a visual validation step was not needed for this application (29). The reference dose–response curve obtained for automated MN scoring, based on MN data of 10 individuals, showed that a dose of 1 Gy can be detected with an accuracy of 0.2 Gy (Figure 5). The 95% confidence intervals of the 0 Gy and 1 Gy doses do not overlap. Accurate dose estimations were also achieved at the higher doses of 2 and 3 Gy. Therefore, the automated MN scoring system validated in this study is able to distinguish exposures with doses of 1, 2 or 3 Gy, at very high speed: on one slide, 2000 BN cells can be scored for MN in <8 min.

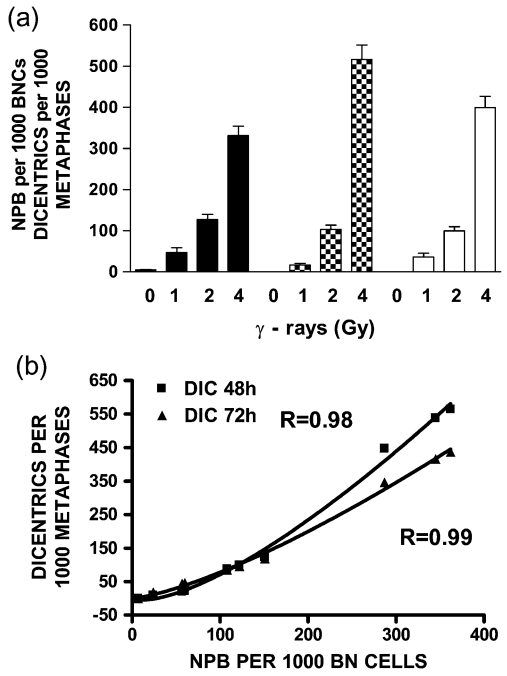

Fig. 4. (**a** and **b**): Comparison of γ-ray dose response in lymphocytes for nucleoplasmic bridges in BN cells and dicentric chromosomes in metaphases. In Figure 4a, the full bars represent NPB at 72 h, the squared bars represent dicentrics at 48 h and the white bars give the yield of dicentrics at 72 h [from Thomas *et al*. (57)].

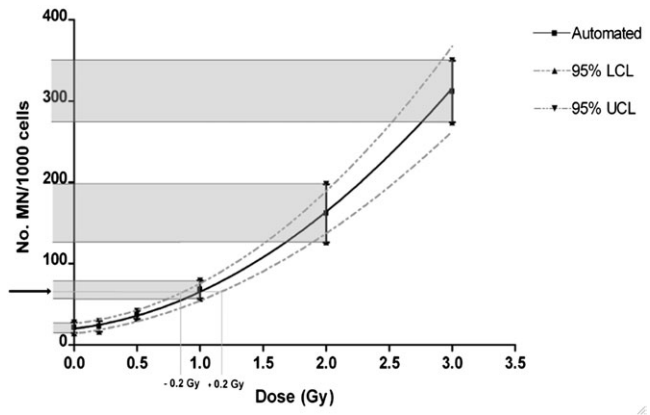

Fig. 5. The fitted dose–response curve of the average MN yields per 1000 BN cells obtained by automated MN scoring (in black), with the 95% confidence interval (CI). The 95% CIs are based on the raw data of 10 donors. Fitted curves for the 95% lower confidence limit (95% LCL) and the 95% upper confidence limit (95% UCL) were added to guide the eye (grey, dashed lines). The black arrow demonstrates a dose estimation of 1 ± 0.2 Gy, based on the fitted curves for average MN yields and the 95% CI [from Willems *et al*. (29)].

Limitations of the CBMN assay

The major limitations of the CBMN assay are related to retrospective dosimetry and accidents involving partial body irradiation.

In the studies already described in Applications of the CBMN Assay for Biological Dosimetry (48–50), where blood samples were taken 1–6 months after the accident took place, MN and dicentric data were also compared with data obtained from FISH translocation tests, which are the end point of choice for retrospective dosimetry. In the Istanbul accident, the dose estimates obtained by MN and dicentric scoring were in good agreement with each other, while the dose estimates obtained by scoring translocations were ∼20–30% higher. In the study reporting the accident of a hospital worker exposed to a 50-kV contact radiotherapy X-ray device (50), a blood sample was taken 1 year after the first sample was taken and on this sample MN, translocations and dicentrics were scored. The results obtained here showed that MN dissappear with a half-life of 342 days, very close to the value of 377 days, obtained for dicentrics. This is in agreement with the decline in MN frequency with post-irradiation time down to ∼60% after 1-year post-treatment, observed in radiotherapy patients (45). Correction of the MN values for the delayed blood sampling in the hospital worker resulted in a dose estimate that was in good agreement with the estimated dose resulting from retrospective dosimetry performed using FISH translocations in the same study (50).

The tendency to underestimate radiation doses in situations of delayed blood sampling is due to the fact that MN and dicentrics represent unstable chromosome aberrations, which have a limited *in vivo* persistence, especially after high doses. Therefore, these diagnostic systems are less suitable for old or long-term exposures (retrospective biodosimetry), whereas FISH analysis for stable translocations remains at present the method of choice.

A second limitation of the CBMN assay, which applies also for the dicentric assay, is related to the fact that in practice most accidents involve partial body exposures, whereby undamaged PBL present outside the irradiation field will dilute the aberration yield, leading to an underestimation of the dose of the exposed tissue. In these cases, the dicentric assay, however, allows an estimation of the unexposed part of the body by comparing the distribution of aberrations among the cells with respect to the Poisson distribution. This calculation is only useful when a significant part of the body has received little or no dose, when the exposed body parts received a relatively high dose (>3 Gy) and when the exposure has taken place over a very short period of time (63). As the distribution of MN is slightly overdispersed (25,64), the power of an MN frequency distribution analysis with respect to a partial body irradiation is questionable and still needs further investigation.

Recommendations for future research

Important issues on which future research on the MN cytogenetic assay should focus include the following.

● The development of a combined automated MN-centromere scoring procedure remains a challenge for the future, as it will allow systematic biomonitoring of radiation workers exposed to low doses. Furthermore, this implementation will allow, in the case of mass radiation casualities, a more accurate assessment of the exposure in a second phase—after early triage—when the time constraint will be less strict. It will combine high-speed MN analysis with a more accurate assessment in the low-dose range.

● The establishment of an international network including several cytogenetic reference laboratories establishing and optimising International Standardisation organisation (ISO) standards for the conventional and automated CBMN assay. By the creation of such a network of trained laboratories using similar equipment for MN automation and the same classifiers, standardised fixation protocols, etc., comparable results can be obtained and the throughput of automated MN scoring can be increased to allow a rapid response to large-scale radiation accidents. A European programme has been started whereby multi-disciplinary biodosimetry tools, including the CBMN assay, will be developed in 15 European groups, to manage high-scale radiological casualties and to increase European capabilities in radiological incident response.

● Further refinement of the CBMN assay is needed to optimise its use in retrospective biodosimetry and for the analysis of cases of protracted exposure and partial body exposure. To date, only limited and diverse data are available concerning the disappearance of MN, and further research and validation is needed. Appropriate calibration curves need also to be established for more complex exposure scenarios.

Conclusions

The conventional CBMN assay is a thoroughly validated and standardised technique for biological dosimetry. New approaches such as centromere staining, NPB scoring and automation of MN scoring, which have been recently optimised, and are still under development will render the CBMN assay more sensitive and specific for radiation dose estimations and make it of special interest for large-scale screening applications.

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References

1. International Atomic Energy Agency (1986) *Biological dosimetry: chromosomal aberration analysis for dose assessment. Technical Reports Series No. 260*. IAEA, Vienna, Austria.
2. International Atomic Energy Agency (2001) *Cytogenetic analysis for radiation dose assessment. Technical Reports Series No. 405*. IAEA, Vienna, Austria.
3. Ainsbury, E. A., Livingston, G. K., Abbott, M. G., Moquet, J. E., Hone, P. A., Jenkins, M. S., Christensen, D. M. and Lloyd, D. C. Rothkamm, K. (2009) Interlaboratory variation in scoring dicentric chromosomes in a case of partial-body X-Ray exposure: Implications for biodosimetry networking and cytogenetic ''triage mode'' scoring. *Radiat. Res.*, **172**, 746–752.
4. Rothkamm, K. and Horn, S. (2009) γ-H2AX as protein biomarker for radiation exposure. *Ann. Ist. Super. Sanità*, **45**, 265–271.
5. Bertho, J. M., Roy, L., Souidi, M. *et al.* (2008) New biological Indicators to evaluate and Monitor radiation-induced damage: an accident case Report. *Radiat. Res.*, **169**, 543–550.
6. Fenech, J. and Morley, A. A. (1985) Measurement of micronuclei in lymphocytes. *Mutat. Res.*, **147**, 29–36.
7. Fenech, M. (2006) Cytokinesis-block micronucleus assay evolves into a ''cytome'' assay of chromosomal instability, mitotic dysfunction and cell death. *Mutat. Res.*, **600**, 58–66.
8. Thierens, H., Vral, A., van Eijkeren, M., Speleman, F. and De Ridder, L. (1995) Micronucleus induction in peripheral blood lymphocytes of patients under radiotherapy treatment for cervical cancer or Hodgkin's disease. *Int. J. Radiat. Biol.*, **67**, 529–539.
9. Thierens, H., Vral, A. and De Ridder, L. (1996) A cytogenetic study of radiological workers: effect of age, smoking and radiation burden on the micronucleus frequency. *Mutat. Res.*, **360**, 75–82.
10. Thierens, H., Vral, A., De Ridder, L., Touil, N., Kirsch-Volders, M., Lambert, V. and Laurent, C. (1999) Scoring of different cytogenetic endpoints after in vitro low dose γ-exposure: interlaboratory comparison for biomonitoring of radiological workers. *Int. J. Radiat. Biol.*, **75**, 23–34.
11. Thierens, H., Vral, A., Barbé, M. *et al.* (1999) A cytogenetic study of nuclear power plant workers using the micronucleus-centromere assay. *Mutat. Res.*, **445**, 105–111.
12. Thierens, H., Vral, A., Morthier, R. *et al.* (2000) Cytogenetic monitoring of hospital workers occupationally exposed to ionizing radiation using the micronucleus-centromere assay. *Mutagenesis*, **15**, 245–249.
13. Thierens, H., Vral, A., Barbé, M. *et al.* (2002) Micronucleus assay reveals no radiation effects among nuclear power plant workers. *Health Phys.*, **83**, 178–182.
14. Thierens, H., De Ruyck, K., Vral, A. *et al.* (2005) Cytogenetic biodosimetry of an accidental exposure of a radiological worker using multiple assays. *Radiat. Prot. Dosimetry*, **113**, 408–414.
15. Sari-Minodier, I., Orsière, T., Auquier, P. *et al.* (2007) Cytogenetic monitoring by use of the micronucleus assay among hospital workers exposed to low doses of ionizing radiation. *Mutat. Res.*, **629**, 111–121.
16. Monsieurs, M., Bacher, K., Brans, B. *et al.* (2003) Patient dosimetry after ^{131}I lipiodol therapy. *Eur. J. Nucl. Med. Mol. Imaging*, **30**, 554–561.
17. Dias, F. L., Antunes, L. M. G., Rezende, P. A. *et al.* (2007) Cytogenetic analysis in lymphocytes from workers occupationally exposed to low levels of ionizing radiation. *Environ. Toxicol. Pharmacol.*, **23**, 228–233.
18. Song, E. Y., Rizvi, S. M., Qu, C. F. *et al.* (2008) The cytokinesis-block micronucleus assay as a biological dosimeter for targeted alpha therapy. *Phys. Med. Biol.*, **3**, 319–328.
19. Tanaka, K., Iida, S., Takeichi, N., Nailya, J., Chaizhunusova, N. J., Gusev, B. I., Apsalikov, K. N., Inaba, T. and Hoshi, M. (2006) Unstable-type chromosome aberrations in lymphocytes from individuals living near Semipalatinsk nuclear test site. *J. Radiat. Res.*, **47** (Suppl. A), A159–A164.
20. Fucic, A., Brunborg, G., Lasan, R. *et al.* (2008) Genomic damage in children accidentally exposed to ionizing radiation: a review of the literature. *Mutat. Res.*, **658**, 111–123.
21. Baeyens, A., Thierens, H., Claes, K. *et al.* (2002) Chromosomal radiosensitivity in breast cancer patients with a known or putative genetic predisposition. *Br. J. Cancer*, **87**, 1379–1385.
22. Willems, P., Claes, K., Baeyens, A. *et al.* (2009) Polymorphisms in non homologous end-joining genes associated with breast cancer risk and chromosomal radiosensitivity. *Genes Chromosomes Cancer*, **47**, 137–148. Erratum Genes Chromosomes Cancer, 48, 381.
23. Iarmarcovai, G., Ceppi, M., Botta, A. *et al.* (2008) Micronuclei frequency in peripheral blood lymphocytes of cancer patients: a meta-analysis. *Mutat. Res.*, **659**, 274–283.
24. Iliakis, G., Wang, H., Perrault, A. R. *et al.* (2004) Mechanisms of DNA double strand break repair and chromosome aberration formation. *Cytogenet. Genome Res.*, **104**, 14–20.
25. Vral, A., Verhaegen, F., Thierens, H. *et al.* (1994) Micronuclei induced by fast neutrons versus 60Co-rays in human peripheral blood lymphocytes. *Int. J. Radiat. Biol.*, **65**, 321–328.
26. Verhaegen, F. and Vral, A. (1994) Sensitivity of micronucleus induction in human lymphocytes to low LET radiation qualities: RBE and correlation of RBE and LET. *Radiat. Res.*, **139**, 208–213.
27. Wuttke, K., Muller, W. U. and Streffer, C. (1998) The sensitivity of the in vitro cytokinesis-blocked micronucleus assay in lymphocytes for different and combined radiation qualities. *Strahlenther. Onkol.*, **174**, 262–268.
28. Gersey, B., Sodolak, J., Hada, M. *et al.* (2007) Micronuclei induction in human fibroblasts exposed *in vitro* to Los Alamos high-energy neutrons. *Adv. Space Res.*, **40**, 1754–1757.

29. Willems, P., August, L., Slabbert, P. *et al*. (2010) Automated micronucleus (MN) scoring for population triage in case of large radiation events. *Int. J. Radiat. Biol*., **86**, 2–11.
30. Ainsbury, E. A. and Barquinero, J. F. (2009) Biodosimetric tools for a fast triage of people accidentally exposed to ionising radiation: Statistical and computational aspects. *Ann. Ist. Super Sanita*, **45**, 307–312.
31. Deperas, J., Szluinska, M., Deperas-Kaminska, M. *et al*. (2007) CABAS: a freely available PC program for fitting calibration curves in chromosome aberration dosiometry. *Radiat. Prot. Dosimetry*, **124**, 115–123.
32. Ainsbury, E. A. and Lloyd, D. C. (2010) Dose estimation software for radiation biodosimetry. *Health Phys*., **98**, 290–295.
33. Bonassi, S., Fenech, M., Lando, C. *et al*. (2001) Human MicroNucleus Project: international database comparison for results with the cytokinesis-block micronucleus assay in human lymphocytes: I. Effect of laboratory protocol, scoring criteria, and host factors on the frequency of micronuclei. *Environ. Mol. Mutagen*., **37**, 31–45.
34. Bauchinger, M. and Braselmann, H. (1989) *''Use of micronuclei in biological dosimetry of absorbed radiation dose'' Chromosome Aberrations—Basic and Applied Aspects,* Springer-Verlag, Berlin, Germany, pp.202–211.
35. Thierens, H., Vral, A. and Deridder, L. (1991) Biological dosimetry using the micronucleus assay for lymphocytes: interindividual differences in dose-response. *Health Phys*., **61**, 623–630.
36. Fenech, M., Aitken, C. and Rinaldi, J. (1998) Folate, vitamin B12, homocysteine status and DNA damage in young Australian adults. *Carcinogenesis*, **19**, 1163–1171.
37. Fenech, M., Holland, N., Chang, W. P., Zeiger, E. and Bonassi, S. (1999) The HUMN Project—an international collaborative study on the use of the micronucleus technique for measuring DNA damage in humans. *Mutat.n Res*., **428**, 271–283.
38. Fenech, J. and Morley, A. A. (1986) Cytokinesis-block micronucleus method in human lymphocytes: effect of *in vivo* ageing and low-dose X-irradiation. *Mutat. Res*., **161**, 193–198.
39. Fenech, M. (1993) The cytokinesis-block micronucleus technique: a detailed description of the method and its application to genotoxicity studies in human populations. *Mutat. Res*., **285**, 35–44.
40. Catalan, J., Autio, K., Kuosma, E. and Norppa, H. (1998) Age-dependent inclusion of sex chromosomes in lymphocyte micronuclei of man. *Am. J. Hum. Genet*., **63**, 1464–1472.
41. Carere, A., Antoccia, A., Cimini, D. *et al*. (1999) Analysis of chromosome loss and non-disjunction in cytokinesis-blocked lymphocytes of 24 male subjects. *Mutagenesis*, **14**, 491–496.
42. Lee, T. K., O'Brien, K. F., Naves, J. L., Christie, K. I., Arastu, H. H., Eaves, G. S., Wiley, A. L., Karlsson, U. L. and Salehpour, M. R. (2000) Micronuclei in lymphocytes of prostate cancer patients undergoing radiation therapy. *Mutation Research*, **469**, 63–70.
43. Silva-Barbosa, I., Pereira-Magnata, S., Amaral, A., Sotero, G. and Melo, H. C. (2005) Dose assessment by quantification of chromosome aberrations and micronuclei in peripheral blood lymphocytes from patients exposed to gamma radiation. *Genet. Mol. Biol*., **28**, 452–457.
44. Le Roux, J., Slabbert, J., Smit, B. and Blekkenhorst, G. (1998) Assessment of the micronucleus assay as a biological dosimeter using cytokinesis-blocked lymphocytes from cancer patients receiving fractionated partial-body radiotherapy. *Strahlenther. Onkol*., **174**, 75–81.
45. Fenech, M., Denham, J., Francis, W. *et al*. (1990) Micronuclei in cytokinesis-blocked lymphocytes of cancer patients following fractionated partial-body radiotherapy. *Int. J. Radiat. Biol*., **57**, 373–383.
46. Livingston, G. K., Foster, A. E. and Elson, H. R. (1993) Effect of in vivo exposure to iodine-131 on the frequency and persistence of micronuclei in human lymphocytes. *J. Toxicol. Environ. Health*, **40**, 367–375.
47. Livingston, G. K., Jensen, R. H., Dilberstein, E. B., Hinnefeld, J. D., Pratt, G., Bigbee, W. L., Langlois, R. G., Grant, S. G. and Shuklar, R. (1997) Radiobiological evaluation of immigrants from the vicinity of Chernobyl. *Int. J. Radiat. Biol*., **72**, 703–713.
48. International Atomic Energy Agency. (2000) *The Radiological Accident in Istanbul*. IAEA, Vienna, Austria.
49. Turai, I., Günalp, B., Lloyd, D. and Cosset, J. M. (2000) *''Medical Aspects of the Radio-logical Accident in Istanbul''*. Proc. 8th WHO/REMPAN Conf, NRPB, Oxford, UK.
50. Thierens, H., De Ruyck, K., Vral, A., de Gelder, V., Whitehouse, C. A., Tawn, E. J. and Boesman, I. (2005) Cytogenetic biodosimetry of an accidental exposure of a radiological worker using multiple assays. *Radiat. Prot. Dosimetry*, **113**, 408–414.
51. Vaglenov, A., Bliznakov, V. V. and Karadjov, A. (1997) Cytogenetic monitoring of workers from a nuclear power plant. *Centr. Eur. J. Occup. Environ. Med*., **3**, 40–47.
52. Vral, A., Thierens, H. and De Ridder, L. (1997) *In vitro* micronucleus-centromere assay to detect radiation-damage induced by low doses in human lymphocytes. *Int. J. Radiat. Biol*., **71**, 61–68.
53. Norppa, H., Renzi, L. and Lindholm, C. (1993) Detection of whole chromosomes in micronuclei of cytokinesis-blocked human lymphocytes by antikinetochore staining and in situ hybridization. *Mutagenesis*, **8**, 519–525.
54. Huber, R., Salassidis, K., Kulka, U., Braselmann, H. and Bauchinger, M. (1996) Detection of centromeres in vinblastine- and radiation-induced micronuclei of human lymphocytes using FISH with an alpha satellite pancentromeric DNA probe. *Environ. Mol. Mutagen*., **27**, 105–109.
55. Pala, F. S., Alkaya, F., Tabakcioglu, K. *et al*. (2008) The effects of micronuclei with whole chromosomes on biological dose estimation. *Turk. J. Biol*., **32**, 283–290.
56. Fenech, M. (2007) Cytokinesis-block micronucleus cytome assay. *Nat. Protoc*., **2**, 1084–1104.
57. Thomas, P., Umegaki, K. and Fenech, M. (2003) Nucleoplasmic bridges are a sensitive measure of chromosome rearrangement in the cytokinesis-block micronucleus assay. *Mutagenesis*, **18**, 187–194.
58. Castelain, P., Van Hummelen, P., Deleener, A. and Kirsch-Volders, M. (1993) Automated detection of cytochalasin-B blocked binucleated lymphocytes for scoring micronuclei. *Mutagenesis*, **8**, 285–293.
59. Verhaegen, F., Vral, A., Seuntjens, J., Schipper, N. W., De Ridder, L. and Thierens, H. (1994) Scoring of radiation-induced micronuclei in cytokinesis-block human lymphocytes by automated image analysis. *Cytometry*, **17**, 119–127.
60. Schunk, C., Johannes, T., Varga, D., Lorch, T. and Plesch, A. (2004) New developments in automated cytogenetic imaging: unattended scoring of dicentric chromosomes, micronuclei, single cell gel electrophoresis, and fluorescence signals. *Cytogenet. Genome Res*., **104**, 383–389.
61. Varga, D., Johannes, T., Jainta, S., Schuster, S., Schwarz-Boeger, U., Kiechle, M., Garcia, B. P. and Vogel, W. (2004) An automated scoring procedure for the micronucleus test by image analysis. *Mutagenesis*, **19**, 391–397.
62. Decordier, I., Papine, A., Plas, G., Roesems, S., Vande Loock, K., Moreno-Palomo, J., Cemeli, E., Anderson, D., Fucic, A., Marcos, R., Soussaline, F. and Kirsch-Volders, M. (2009) Automated image analysis of cytokinesis-blocked micronuclei: an adapted protocol and a validated scoring procedure for biomonitoring. *Mutagenesis*, **24**, 85–93.
63. Leonard, A., Rueff, J., Gerber, G. B. and Leonard, E. D. (2005) Usefulness and limits of biological dosimetry based on cytogenetic methods. *Radiat. Prot. Dosimetry*, **115**, Special Issue: Part 1 Sp. Iss. SI, 448–454.
64. Wojcik, A., Gregoire, E., Hayata, I., Roy, L., Sommer, S. and Stephan, G. Voisin, P. (2004) Cytogenetic damage in lymphocytes for the purpose of dose reconstruction: a review of three recent radiation accidents. *Cytogenet. Genome Res*., **104**, 200–205.