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Biological Dosimetry of Ionizing Radiation

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1. Introduction

A worsening of the accidental hazards linked to the use of ionizing radiation is currently being observed for four reasons. First, the increasing need for radiation sources in numerous industrial applications (food sterilization, construction, engineering…) leads to an increasing probability of loss of the sources or abnormal/unsuitable use and storage. Second, advances in medicine generate new protocols and tools that are more efficient but also much more complex to carry out, increasing the risk of accidental overexposure. Third, the possibility of a terrorist attack using radiological or nuclear devices has to be taken into account. Finally, recent events in Fukushima (Japan) highlight the risks of exposure in the case of nuclear power plant accidents. All these issues could lead to the accidental exposure of one to several thousand individuals not wearing dosimeters. Thus, it is essential to be able to estimate the exposure level of victims. Nowadays, this evaluation is based on clinical diagnosis (mainly irradiation symptoms and hematological variations) supplemented with biological dosimetry and physical dose reconstruction. Biological dosimetry is especially important when the personal dosimeter is lacking or when the accidental context is unclear. All this information should help the medical staff to deliver appropriate medical care and to manage the long-term medical follow-up, if required.

It has been known since the last century that ionizing radiation causes DNA damage and that DNA misrepair can induce chromosome aberrations: stable (translocations, deletions, insertions) or unstable (dicentrics, centric rings, acentric fragments). These aberrations are observed in metaphase cells. A misrepair can be also observed after anaphase in the form of micronuclei. The applicability of the available assays of biodosimetry is based on the analysis of the chromosome damage present in peripheral blood lymphocytes, which is a convenient because its collection is non-invasive and it is easy to obtain.

Dicentric assay is currently the gold standard method for classic biodosimetry in cases of recent accidental exposure. The scoring of dicentrics allows assessment of the whole-body dose received by the individual. Moreover, in numerous accident contexts the exposure of victims is heterogeneous. In these cases, the fraction of the body irradiated and the dose received by this fraction can be estimated by dicentric scoring. In the case of large-scale accidents the dicentric aberration is always used as well as the micronuclei because its analysis is faster and easier. However, dicentrics and micronuclei are unstable aberrations and their rate decreases with time. Consequently, for past accidental exposure, the analysis of stable chromosome aberrations like translocations is required (Figure 1).

Nevertheless, these bioindicators have many limitations. Currently, a lot of research is performed to find new indicators of exposure (such as γH2AX and gene expression) (Figure 1). It is important to note that there are several essential requirements for biological parameters as meaningful dosimeters: low background level, clear dose-effect relationship for different radiation qualities and dose rates, specificity to ionizing radiation, noninvasive, fast availability of dose estimate, good reproducibility and comparability of in vitro and in vivo results (Romm, 2009).

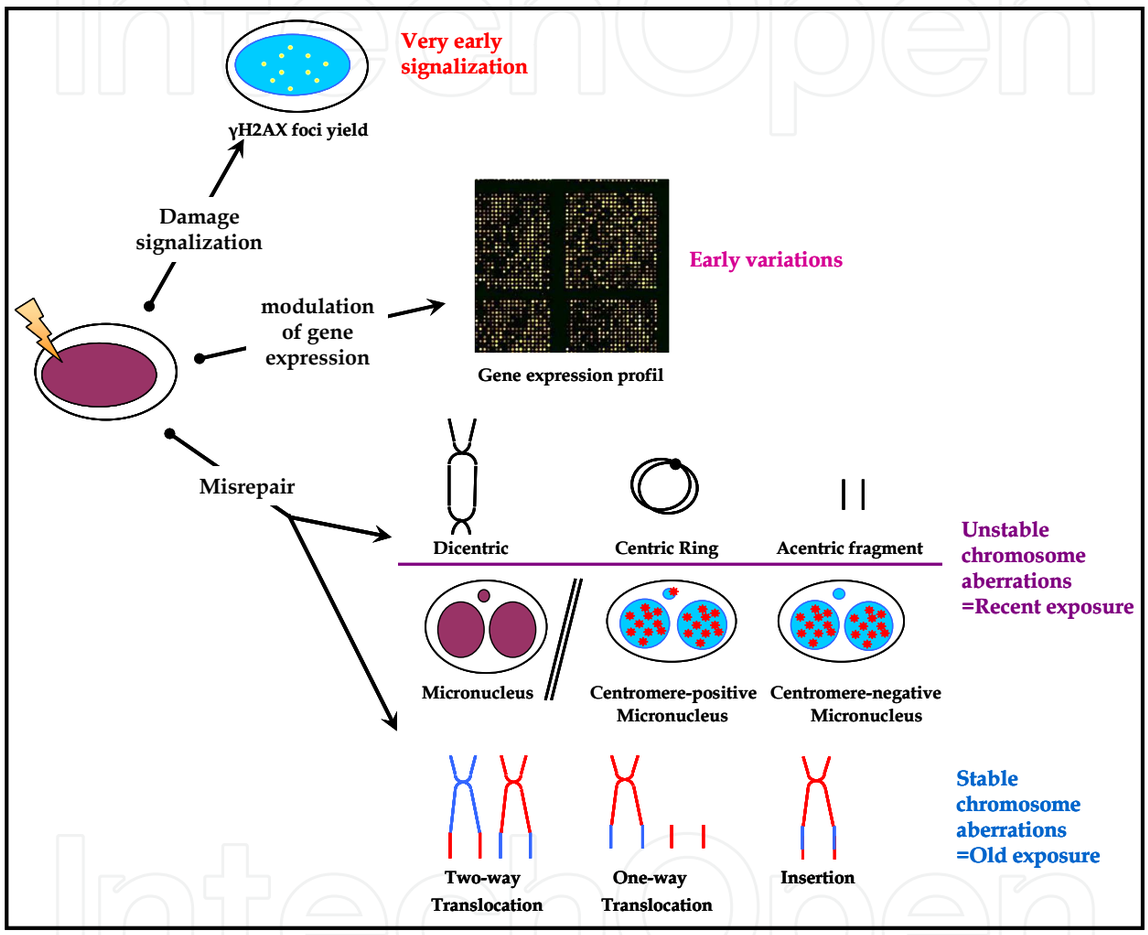


Fig. 1. After irradiation of cells, the induced DNA damage was observed i) very early with γH2AX foci yield, ii) early with variations of gene expression profile, and iii) later with chromosome aberrations.

2. Recent accidental exposure

2.1 Individual biodosimetry

Due to their instability and low background level, the dicentric rate in lymphocytes is an indicator of recent exposure. For dose assessment using dicentric assay, it is necessary to perform a dose-effect curve range to 0.1-5 Gy representing the relationship between the yield of dicentrics and the dose. The yield of dicentrics is obtained by manual scoring of dicentrics, rings and acentric fragments among 500 or more metaphase cells obtained from

peripheral blood lymphocytes after Giemsa staining (Figure 2). This technique requires a lymphocyte culture time from 48 to 50 hours. Additionally, the manual scoring of unstable aberrations on 500 metaphase images requires about 10 hours for a trained operator. This technique has a minimum detection threshold ranging from 0.1 to 0.2 Gy depending on the number of metaphase cells scored, background level and the radiation used (quality and dose-rate) (Agency, 2001).

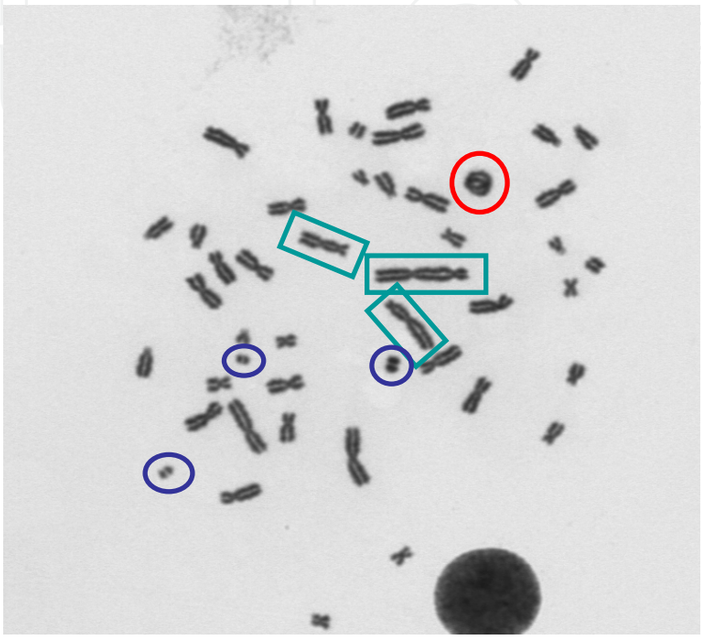


Fig. 2. Metaphase of peripheral blood lymphocytes. Unstable chromosomal aberrations are analyzed with Giemsa staining. Dicentrics are framed in green, rings in red and acentric fragments in blue.

2.1.1 Dose-effect relationship

Ionizations induce damages in the DNA (base damage, double-strand break, single-strand break…), their number in cells increases with the ionizing capacity of the radiation. Besides, the damage distribution between cells will differ according to the type of radiation because the quantity of ionization induced per track varies. Indeed, a track is formed by one more or less ionizing particle. This can be defined by the linear energy transfer (LET) value which describes the energy deposition per micrometer of matter.

Dicentric damage requires at least two double-strand breaks to be formed. The frequency of production of dicentrics induced by one track is proportional to the dose. By contrast, the frequency of production of dicentrics induced by two tracks is proportional to the square of the dose.

For low LET radiation (X or gamma rays) produces many tracks containing few primary events. The distribution of tracks is more randomized. Therefore, the distribution of damage between cells will be more uniform. There is a greater probability that two tracks induce one dicentric in the same cell. The dose-effect relationship is then linear in the low dose range (dicentrics induced exclusively by two tracks) and becomes quadratic at high doses (Figure 3). The dose-effect curve then fits the following equation:

$$Y = \alpha D + \beta D^2 + c$$

where β is the dose squared coefficient and the constant is the background frequency (Edwards, 1979). In this case, the distribution of dicentrics per cell follows a Poisson law. The coefficients α, β and the constant, with their standard errors, are calculated using a maximum likelihood method (Papworth, 1975).

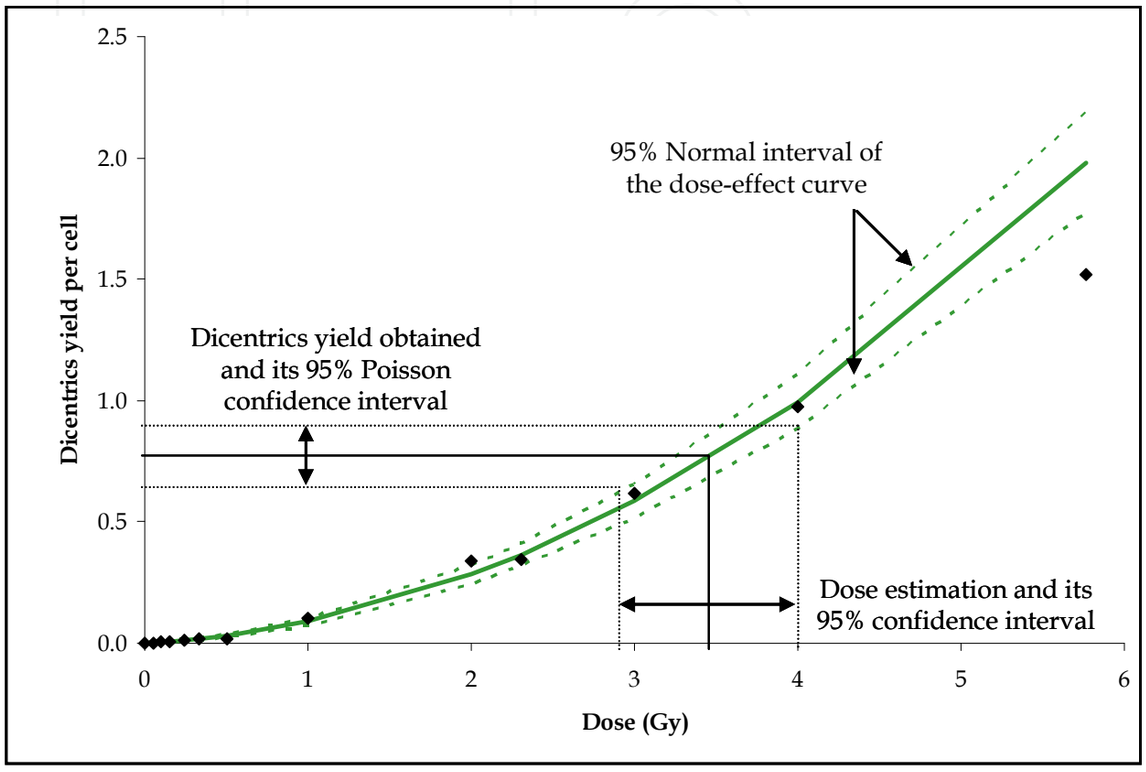


Fig. 3. Dose-effect relationship fitted by scoring of dicentrics induced in peripheral blood lymphocytes after in vitro irradiation by 137Cs gamma rays (dose rate of 0.5 Gy/min). The equation of the curve is Y=0.0338D+0.0536D²+0.0010.

From the dicentric yield obtained for an individual a 95% Poisson confidence interval is calculated using the Poisson table. The dose is estimated by correspondence of the dicentric yield to the dose-effect curves (Figure 3). The confidence interval of the dose is estimated by correspondence of the lower and upper confidence interval of the dicentric yield on the upper and lower curves, respectively (Papworth, 1975).

High LET radiation (neutrons or alpha particles) produces few tracks with many primary events (ionizations, excitations) very close together. There is a lot of damage at the same point and the misrepair of these damages induces multi-aberrant cells. With high LET radiation exposure, there is a high probability of one track inducing one dicentric. In this case, the dose-effect relationship is linear (Figure 4) and the dose-effect curve fits the following equation:

$$Y = \alpha D + c$$

where Y is the dicentric yield, α is the linear coefficient and D is the dose (Lloyd, 1976).

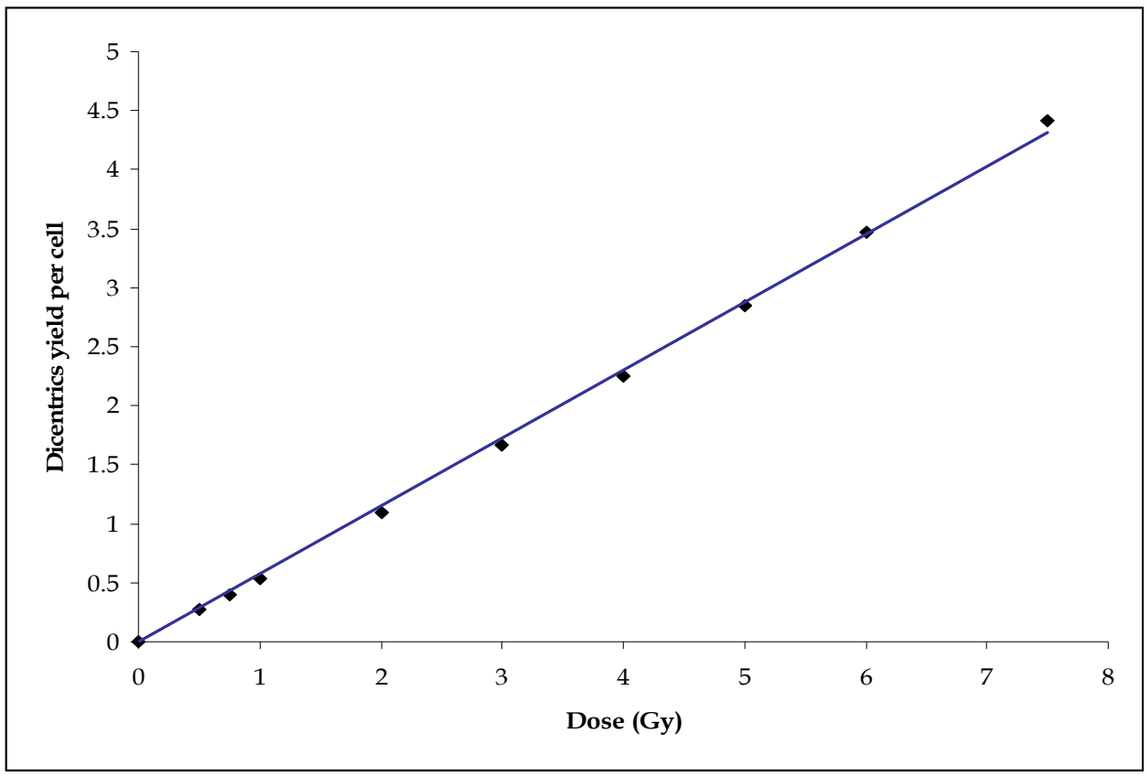


Fig. 4. Dose-effect relationship fitted by scoring of dicentrics induced in peripheral blood lymphocytes after in vitro irradiation with fission neutrons (1 MeV). The equation of the curve is $Y=0.5356D+3x10^{-16}$

2.1.2 Case of partial-body exposure

During exposure to low LET radiation, a dicentric is considered a rare event. It is mostly accepted that its distribution among the analyzed metaphases follows Poisson's law. In biodosimetry, we check that the dicentric distribution obtained is in agreement with Poisson distribution by u-test. In the case of partial-body exposure, mathematical models have been developed to estimate the fraction of the body irradiated and the dose received by this fraction, the contaminated Poisson's method (Dolphin, 1969) and the Qdr method (Sasaki&Miyata, 1968).

If the exposure is partial, this distribution does not follow Poisson's law. Indeed, unirradiated cells are now scored within the population of analyzed metaphases and increase the number of undamaged cells. This contamination produces an overdispersion of the distribution and this is tested by u-test on each distribution obtained. The deviation of the variance was calculated and used to calculate the u value which approximates to a unit normal deviate. Thus, the values of u were compared with the theoretical value of 1.96, and this was used to identify a significant under- or overdispersion of the experimental distributions ($u > 1.96$ then p-value < 0.05) (Edwards, Lloyd, 1979).

If the distribution of dicentrics per cell is overdispersed due to nonuniform exposure ($u > 1.96$), two mathematical models can be used to calculate the yield of dicentrics of the fraction irradiated: contaminated Poisson's (Dolphin, 1969) and Qdr (Sasaki&Miyata, 1968) allowing the calculation of the dose received by the fraction.

The calculation of the dicentric yield of the fraction irradiated, with the contaminated Poisson method, uses the following equation:

$$\frac{y}{\left(1-e^{-y}\right)}=\frac{x}{\left(N-n_0\right)}$$

where y is the mean yield of dicentrics of the irradiated fraction, e^{-y} represents the Poisson probability of cells without dicentric in the irradiated fraction, x is the number of dicentrics observed, N is the number of cells scored and n0 is the number of cells free of dicentrics.
The 95% confidence intervals of y are calculated with the following equation:

$$y_{min/max}=y*\pm 1.96\left[Var\left(y*\right)^{0.5}\right]$$

where y* is the yield of dicentrics per unstable cell (x/ (N - n_0)) and Var(y*) is its variance (x(1 + y - y*) / (N - n_0)²) (Barquinero, 1997).
The dose of the irradiated fraction and its 95% confidence interval are estimated by correspondence of the yield of dicentrics per unstable cell and its 95% confidence interval to dose-effect curve.
Furthermore, the fraction of cells exposed (F) and its 95% confidence interval are estimated using the following equation:

$$F=\frac{f}{p}\left(1-f+\frac{f}{p}\right)$$

where f is the fraction of cells scored that were irradiated (x/(yN)) and p is the fraction of surviving cells, taking into account the selective loss of irradiated cells due to interphase death and mitotic delay. For each condition, p is estimated using the following equation:

$$p=e^{-\frac{D}{D_0}}$$

where D is the estimated dose for the irradiated fraction (f) and D_0 is the dose for which 37% of irradiated cells survived. Various values have been reported for D_0 depending on the studies and the irradiation conditions: 2.7 Gy (Lloyd, 1973) and 3.8 Gy (Barquinero, Barrios, 1997) for X-rays and 3.5 Gy for 60Co γ-rays (Matsubara, 1974).
The yield of dicentrics of the irradiated fraction can also be calculated with a second method, Qdr (Sasaki&Miyata, 1968). This method uses the same parameters as the contaminated Poisson method and in addition the excess acentric fragments yield. Acentric fragments are eliminated rapidly during mitosis, so their presence indicates that aberrant cells counted are due to exposure. It is necessary to plot a dose-effect curve based on the excess acentric fragments yield observed per exposure dose. The Qdr method uses the following equation:

$$\frac{x}{\left(N-n_0\right)}=\frac{y*}{\left(1-e^{-\left(y*-y_{ace}\right)}\right)}$$

where y_{ace} is the yield of excess acentric fragments per unstable cell (Sasaki&Miyata, 1968).

The 95% confidence intervals of y are calculated with a similar equation to the contaminated Poisson method. The dose of the irradiated fraction and its 95% confidence intervals are estimated by correspondence of the yield obtained with the Qdr method and its 95% confidence intervals to the dose-effect curve.

2.1.3 Case of protracted exposure

Protracted exposures have an effect on the yield of dicentrics. Indeed the time to repair the induced damage is lower than the exposure time. Some double-strand break can be repaired before and to coexist with new double-strand break produced later. Despite that, studies have shown that there is also a good relationship between the dose received and the dicentric yield (Bauchinger, 1979). For low LET radiation, protracted exposures modify the number of dicentrics produce by two tracks. For this reason and to correct the β coefficient obtained at the acute irradiation the G-function is need. Several studies have shown that the mean time to repair a lesion is about 2 hours (Schmid, 1976), (Purrott&Reeder, 1976), (Virsik&Harder, 1980). The equation representing the dose-effect relationship can be written as:

$$Y = \alpha D + \beta G(t / t_0) D^2 + c$$

where the G-function follows this equation:

$$G(t / t_0) = \frac{2}{(t / t_0)}\left[(t / t_0) - 1 + e^{-(t/t_0)}\right]$$

where t is the time over which irradiation occurs and t_0 represents the mean lesion repair time estimated (2 hours). If the time of exposure is long, the G-function may be reduced to zero (Lloyd, 1984). Therefore, in case of fractioned irradiations if the time between exposures is greater than 6 hours, the different exposures will be considered as separate and the effect as additive. For high LET radiation, no effect on the yield of dicentric produced will be expected.

2.1.4 Case of high dose exposure

The dose range detectable with the dicentric assay is 0.1-5 Gy. In the case of exposure to a highest dose, there is a strong influence of cell death and mitotic delay. It is then necessary to observe chromosome aberrations induced by ionizing radiation in interphase cells. In this sense, two different techniques based on premature chromosome condensation (PCC) have been proposed. The first method used the fusion of interphase lymphocytes with mitotic cells, it does not need to stimulate the cell division. With this methodology, excess fragments can be detected (Pantelias&Maillie, 1984) and results can be obtained in a few hours after sample reception. The second method is able to condensate chromatide before metaphase, it is used chemical agents (calyculin A or okadaic acid). In this second method lymphocytes need to be stimulated and aberrations can be detected in G1 and G2 phases of the cell cycle (Kanda, 1999). One of chromosome aberrations observed are ring chromosomes (Figure 5). For both chromosome aberrations (excess fragments, rings), the dose-effect curves are fitted to a linear model. The methodology dose up to 25 Gy is estimated (Lamadrid, 2007). The PCC assay, using chemical agent, was successfully used to estimate dose in the Tokai-Mura radiation accident (1999, Japan) (Hayata, 2001).



Fig. 5. PCC of peripheral blood lymphocytes in G2. Unstable chromosomal aberrations are analyzed with Giemsa staining. The ring is framed in blue.

2.2 Biodosimetry for population triage purposes

The standard biological dosimetry technique, based on dicentric, is labor-intensive and time-consuming. In the case of a large-scale radiological event, the standard dicentric assay cannot be used to perform rapid triage of the numerous potential victims and triage of badly injured victims would have to be done rapidly. Besides, following this triage step it will be necessary to estimate the dose received as accurately as possible in order to manage the long-term medical follow-up of the victims.

2.2.1 Application of the dicentric assay

Currently, the strategy for triage is to use the dicentric assay by reducing the number of metaphases scored. The IAEA advises to score unstable chromosomal aberrations among only 50 metaphases. This increases the minimum detection threshold from 0.1-0.2 Gy to 0.5-0.6 Gy depending on the calibration curve (Agency, 2001). In vitro studies have shown good dose assessment for whole-body exposure (Lloyd, 2000), (Romm, 2011). The triage mode was applied after accident in Georgia (1998) where 85 people were potentially exposed. The results showed an under-estimation of the dose for 82% of individuals when the scoring of 50 metaphases was compared with the scoring of 250 metaphases (Voisin, 2001). This trend seemed to be correlated with another accident in Senegal (2006) where 63 people were potentially exposed.. Indeed, under-estimation was observed for 50% of individuals with the scoring of 50 metaphases (Vaurijoux, 2009). These results indicate that the good agreement observed in in vitro experiments is not left with real cases of accident.

Additionally, it should be expected that triage mode estimation of partial-body exposure will become much less accurate, because there is a low probability to observe an

overdispersion with few metaphases scored. The results of in vitro simulation of partial-body exposure suggested that the estimation of the irradiated fraction was good in 25% of cases for doses below 3 Gy and in 75% of cases for doses above 3 Gy (Lloyd, Edwards, 2000). There are no published results on the detection of partial-body exposure with scoring of 50 metaphases in comparison with scoring of 500 metaphases in the case of real accidents.

2.2.2 Application of the micronucleus assay

The other alternative is the in vitro cytokinesis-block micronucleus (CBMN) assay performed on peripheral blood lymphocytes (Fenech&Morley, 1985). The micronucleus is a small spherical object of same appearance as that nuclei after Giemsa staining. It is composed of an acentric fragment or a whole chromosome that is not included in the daughter nuclei during cell division. It is observed in binucleated cells, though if the micronucleus contains an acentric fragment (Figure 6) it will be eliminated after anaphase.



Fig. 6. Binucleated cell of peripheral blood lymphocytes containing a micronucleus (Giemsa straining).

Scoring micronuclei is easier than scoring dicentrics and therefore allows a faster analysis. Moreover, the precision of the micronucleus assay is better than that of the dicentric assay on 50 metaphases (Roy, 2007). The dose-effect response fits the linear quadratic model for low LET radiation and the linear model for high LET radiation (Vral, 2011). Dose is estimated using the correspondence between micronucleus yield and the dose-effect curve. However, the CBMN assay does not allow the detection of partial-body exposure. Furthermore, the micronucleus is not radiation-specific and can be induced by many genotoxic agents. There is also marked inter-individual variability due to age and gender (Fenech, 1993).

It is well known that most micronuclei induced by ionizing radiation are formed of acentric fragments because they are the result of chromosome breakage. However, a minority of

micronuclei contain a whole chromosome because of time lag during anaphase caused by some defect in the spindle or the kinetochore protein (Vral, 1997). The use of fluorescence in situ hybridization (FISH) to highlight chromosome centromere indicates whether the micronucleus contains an acentrics fragment ($MNCM^-$) or if it contains one or more chromosome ($MNCM^+$). The scoring of centromere-negative micronuclei ($MNCM^-$) reduces the detection threshold to 0.1 Gy for 2000 binucleated cells scored (Vral, Thierens, 1997). However to apply FISH technique increase the time need to obtain the result and more expensive and for these reasons in large-scale radiation seems to be less suitable.

3. Past exposure to ionizing radiation

Past exposures to ionizing radiation are evaluated differently from recent exposures. Dicentric is an unstable aberration and its yield decreases with time, so an accurate dose estimate can only be obtained up to 1 year after exposure (Lloyd, 1998). For past dose assessment the most appropriate assay is to score of stable chromosome aberrations in stable cells, since cells containing unstable aberrations or complex aberrations decrease in number over time (Edwards, 2005). Non-reciprocal translocations are not stable because there is loss of genetic information (Pala, 2001), (Gregoire, 2006), so to obtain an accurate estimation of the dose it is important to score only two-way translocations.

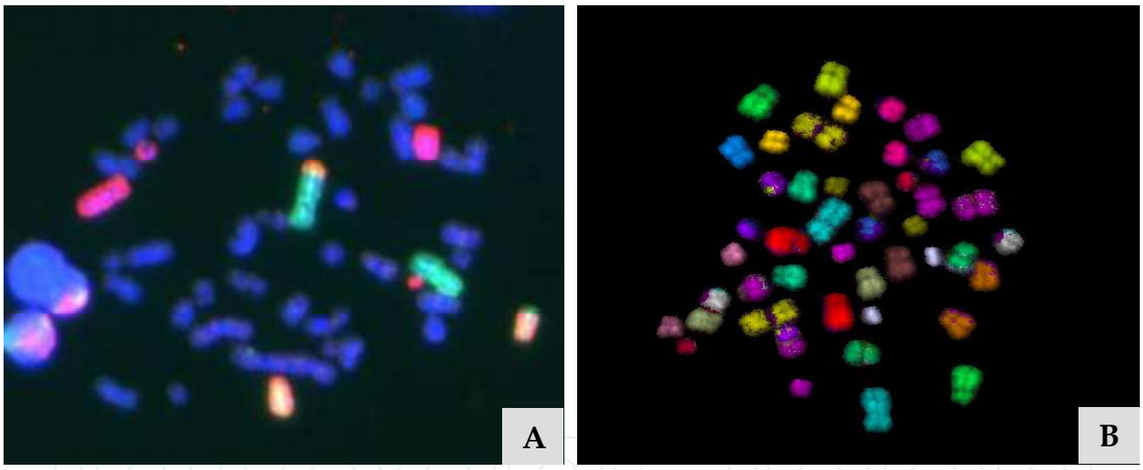


Fig. 7. FISH staining of peripheral blood lymphocytes in metaphase. A) Three chromosomes are painted: 2 (green), 4 (red) and 13 (orange) and B) All chromosomes are painted with a combination of fluorochromes (multicolor-FISH).

Translocations are analyzed by FISH, which paints one or more chromosome with the aid of DNA probes associate with a fluorochrome (Figure 7). In case of a part of genome is visualized, the yield of translocations obtained represents is not directly applicable to full genome. Lucas et al have developed mathematical models to reconstitute the yield of the full genome (Lucas, 1997):

$$F_G = \frac{F_P}{2.05 f_P \left(1 - f_P\right)}$$

where F_G is the yield of the full genome, F_P is the yield of translocations observed by FISH, and f_P is the fraction of the genome hybridized. This equation is general. In the case where more than two colors are used, the following equation is used (Lucas, 1997):

$$F_G = \frac{F_P}{2.05\left[\sum_i f_i(1-fi) - \sum_{i<j} f_i f_j\right]}$$

However, stable chromosome aberrations are less specific to ionizing radiation exposure than dicentrics. Furthermore, translocations persist for many years in circulating lymphocytes and their frequency increases significantly with age and the lifetime conditions (alcohol, tobacco, pesticide exposure…) (Whitehouse, 2005), (Sigurdson, 2008), (Grégoire, 2010). This increase has to be taken into consideration in evaluation of exposure and corrected for as a function of the age of the individual. The relationship between translocation yield and age is based on a linear-exponential model (Sigurdson, Ha, 2008):

$$F_T = \alpha + \beta_{age} e^{(\gamma_{age})}$$

where F_T is translocation yield, α represents the translocation yield at age 0, β_{age} represents a linear slope indicating the increase in translocation yield with age and γ_{age} represents a loglinear curvature parameter.

In a study of blood from nuclear test veterans, 50 years after exposure to ionizing radiation (Wahab, 2008), the frequency of total chromosome translocations was three times higher than the one observed in the control group. Analysis of potential confounding factors suggested that this high frequency was most likely attributable to radiation exposure. It is hard to estimate a dose accurately 50 years after the fact, but exposure may be assumed if the frequency of translocation is above the background level in the population (Lindholm&Edwards, 2004).

4. Improving biological dosimetry

Current research in biological dosimetry is seeking shorter analysis time, lower threshold detection and accurate localization and dose estimation in the case of partial-body exposure. New methods of scoring dicentrics and micronuclei, as well as new biomarkers such as foci γH2AX, and gene expression are investigated. Advantages and limits have been observed in all cases, and further research is needed remains to be done in the development of ionizing radiation biodosimetry.

4.1 Reducing analysis time

4.1.1 Automation of dicentric scoring

Dicentrics assay is the gold standard method in biodosimetry. One improvement has been automation of scoring using image analysis software (DCScore software; MetaSystems). Briefly, the software identifies as chromosomes all objects corresponding to the shape and size of benchmarks and detects dicentrics among them (Schunck, 2004) (Figure 8). Putative dicentrics are validated by an operator.

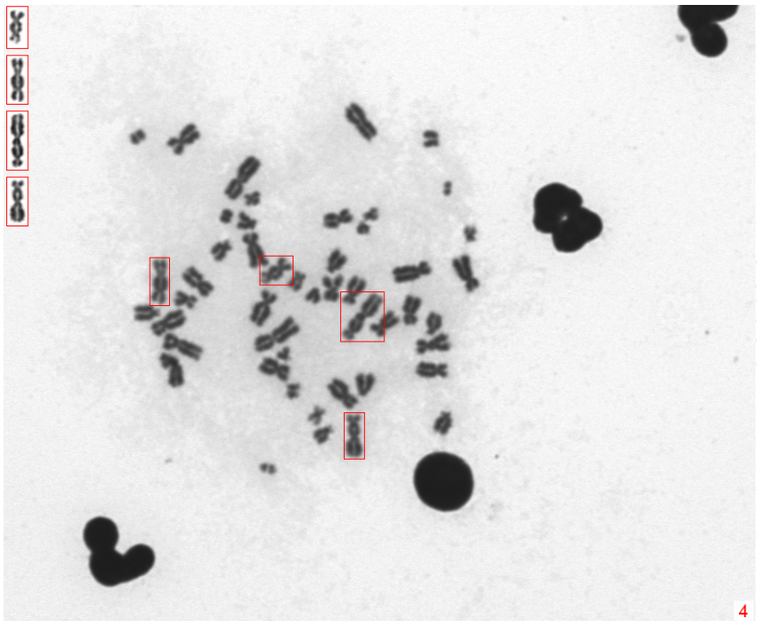


Fig. 8. Image of metaphase obtained after analysis by DCScore software (MetaSystems). Chromosomes detected as dicentrics are framed in red.

One thousand metaphases can be analyzed for triage in 1 hour and 3000 for individual dosimetry in 3 hours, with a 3-fold reduction in analysis time. For triage the threshold obtained with automatic scoring is better than that with manual scoring of 50 metaphases. For triage the automatic detection of dicentrics has been validated of the accident in Senegal (2006). We have show that use of this methodology for a large population dose estimation as it can replace the usual manual scoring of both the 50MS and 500MS methods (Vaurijoux, 2008).

4.1.2 Automation of micronucleus scoring

The scoring of micronuclei poses a problem of inter- and intra-laboratory variability (Fenech, 2003), and in response to this and to speed up scoring use is made of image analysis software (Metafer MicroNuclei; MetaSystems). Briefly, the software first detects binucleated cells according to morphometric criteria: size, ratio of the longest to the shortest diameter, relative concavity depth, and distance between objects. Then, it detects the presence of a micronucleus with the same criteria (Varga, 2004). It is interesting to note that the correlation is good between manual and automatic scoring. When 1000 binucleated cells are scored, the detection threshold is 0.4 Gy (Willems, 2010). When 5000 binucleated cells are scored, the detection threshold is 0.2 Gy. This scoring requires 40 min (Baeyens, 2011). Inter-laboratory variability appears to be limited by the use of this software (Willems, August, 2010).

It is describe that the scoring of centromere-negative micronuclei (MNCM-) improves the accuracy of the dose estimation. Semi-automation of MNCM- scoring (automation of micronucleus detection and manual analysis of MNCM-) enables analysis of 5000 binucleated cells in approximately 2 hours. This is longer than automation of all micronuclei seems more accurate in the low dose range (Baeyens, Swanson, 2011).

4.2 Application of early biomarkers

Dicentric assay and micronucleus assay have two main limitations. First, the lymphocyte culture step requires 48 or 64 hours, respectively. Second, both methods are not able to estimate a dose below 0.1 Gy. Current research is investigating the relevance of early biomarkers after radiation exposure for biodosimetry purposes.

4.2.1 H2AX protein

H2AX protein is a histone whose serine 139 is phosphorylated in response to a DNA double-strand break. This phosphorylated form is called γH2AX and is visualized in the nucleus after immunofluorescence staining with specific fluorescent antibodies (Rogakou, 1998) (Figure 9).

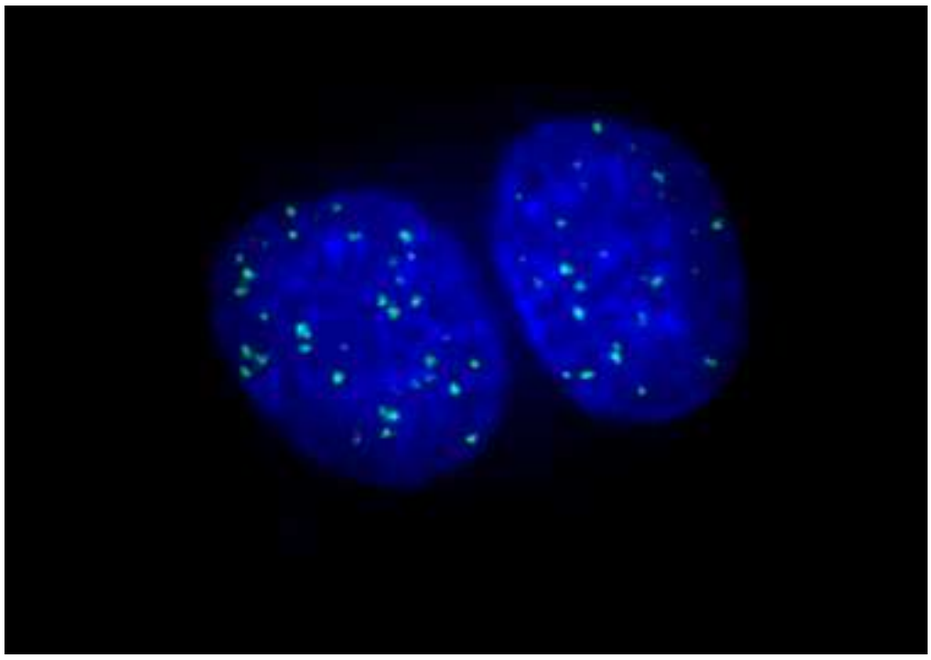


Fig. 9. Nuclei of peripheral blood lymphocytes with γH2AX foci visualized by fluorescence.

The number of γH2AX foci and their relative fluorescence show a linear relationship with the dose received (Leatherbarrow, 2006). Doses as low as 0.05 Gy can be detected with this marker after in vivo exposure (Rothkamm, 2007). The linear relationship is maintained for 30 min to 16 hours in human peripheral lymphocytes after irradiation with γ rays for doses in the range 0.05 to 2 Gy (Roch-Lefevre, 2010).
The main limitations of γH2AX foci quantification are the marked inter-individual variation in baseline values and the decrease of γH2AX foci yield with time (Roch-Lefevre, Mandina, 2010).

4.2.2 Gene expression

Study of gene expression profiles in the response to radiation exposure is an alternative approach to biodosimetry (Amundson, 2000), (Amundson, 2001). Cellular damages usually induce cellular stress which leads to a response through activation of several cellular pathways that result in modulations of gene expression. Microarray technology is used to

study these modulations with the aim of identifying the corresponding gene or group of genes whose profile shows a dose-effect relationship (Paul, 2011). Amundson et al in 2000 were among the first to use gene expression as biological dosimetry in peripheral blood lymphocytes. They showed that modulation of several target genes of p53 protein seems to be correlated with the dose in the range 0.2 to 2 Gy. The maximal response for doses of 0.5 Gy or less could be observed early (about 4 hours after exposure), but this ex-vivo model seems to be limited for times longer than 48 h post-exposure (Amundson, Do, 2000).
Studies of doses below 0.1 Gy, especially on human blood cells, are still rare (Gruel, 2008), (Morandi, 2009). Globally, the level of induction of known target genes of p53 protein appears to decrease with dose and even becomes undetectable at very low doses (>0.025 Gy). This suggests loss of the characteristics of a typical stress response at very low doses and that the response is more diverse and less specialized. It is interesting to note that many genes modulated at this level of dose are known to play a role in mechanisms such as cytoskeleton metabolism, cell-cell signaling, chromatin modeling, RNA and protein processing, proliferation, etc (Gruel, Voisin, 2008), (Morandi, Severini, 2009).
There are two main limitations on immediate implementation of these results in operational dosimetry. First, given the time limit of current studies (48-64 hours), it is hard to keep peripheral blood lymphocytes in culture without creating bias in the analysis. Second, RNA is not stable in blood collection tubes with anticoagulant which poses a problem for the storage and handling of samples during shipment from the sampling site to the specialized laboratory.

5. Standardisation of biodosimetry

Biological dosimetry, based on the study of the radio-induced chromosomal aberrations, mainly the dicentric assay, has become a routine component of the accidental dose assessment. Experience of its application in hundreds of cases of suspected or proven overexposures has proved the value of the method and also defined its limitations.
Biological dosimetry is incorporated into radiation protection programmes of several countries, to confirm or discount a suspected radiation exposure. By contrast, the absence of real concurrence (only one or two lab per country) underlines the needs of homogeneously and largely established bases to assure its credibility. Therefore, an ISO standard was developed to address the critical aspects of the use of the dicentric assay as a biodosimeter. The first publication of 19238 ISO standard in 2004 provides for expertise, minimum requirement for experimental processes, quality assurance and quality control programmes, and evaluation of performance. Another 21243 ISO standard published in 2008 was intended to define performance criteria for cytogenetic triage. The primary purpose of this standard is to provide a guideline in order to perform the dicentric assay for dose categorization in triage mode using documented and validated procedures. The described approaches included pre-planning, reagent stockpiling, simplified sample processing, automation, networking, and modification of some of the ISO 19238 scoring criteria.
The standards are written in the form of procedures to be adopted for dicentric expertise/cytogenetic triage depending upon the application of the results: medical management when appropriate, radiation protection management, record keeping and medical/legal requirements. Whatever the laboratory in any country, the application of such standards ensures quality of practice which is very important for credibility. Second, it helps to compare the results obtained in one laboratory to another one, particularly in case of an

international collaboration or intercomparison. Finally, each new laboratory should get from this standard the most useful information to perform dicentrics assay in the best experimental and reproducible conditions.

To be qualified, the biological dosimetry laboratory must establish technical validation of the bioassay used, besides the precise description of the dose assessment process (relationship with customer, confidentiality of information, capability of laboratory staff, QA&QC program...) For instance, in order to test the influence of protocol variations the mitotic index and dicentrics rates were measured under different experimental conditions (L. Roy et al, 2011, Radiation Protection Dosimetry, submitted). The effect of seven parameters was tested: BrdU, PHA and colcemid concentrations, blood and medium volumes, culture duration and incubation temperature. The results show that mitotic index was influenced by the concentration of BrdU, medium and blood, the culture duration and the temperature. However none of the factors has a significant impact on the yield of dicentrics. We can conclude that the dicentric assay is robust against reagents variations within the range tested. These results could be used by relevant laboratories as element of the quality of their dose assessment and their procedures robustness in any event requiring such demonstration.

There is also some limitation to systematically introduce specific QA&QM programs in the normal activity of the biological dosimetry laboratory. While the quality system is a natural way for any R&D activity, the application of such standard is time consuming because all the process must be checked for deviation and this checking is required regularly. It is especially true when a specific ISO standard is required for supplementing classical accreditation process following more general ISO 17025:2000. For instance the 19238 ISO standard was heavily updated by more detailed description of the experimental and statistical steps for satisfying the accreditation requirements and this implementation come into force probably next year.

A new ISO standard on the use of micronuclei assay in individual biodosimetry and population triage is in preparation and it is expected a future ISO standard on automation in cytogenetic dosimetry.

6. Conclusion

Currently, the dicentric assay seems to be the best bioindicator of recent radiation exposure and their assay is the only one to offer all of the following advantages: low background level, clear dose-effect relationship for different radiation qualities and dose rates, specific to ionizing radiation, non-invasive, good reproducibility and comparability of in vitro and in vivo results. The only drawback is the time required to obtain a dose estimation, especially in the case of large-scale accidental exposure. However, new advances in the automation of dicentric scoring enormously reduce the time needed to estimate the dose and this method remains the most promising.

Nevertheless, the detection of accidental exposure below 0.1 Gy remains difficult. Markers as γH2AX foci and gene expression lead to reduce this detection threshold but some limitations shill exists to be used them at the real accidental biodosimetry.

Translocation is the only biomarker used in biodosimetry for past exposure. However, it is difficult to estimate an accurate dose because the background level depends on age and life conditions of the individual.

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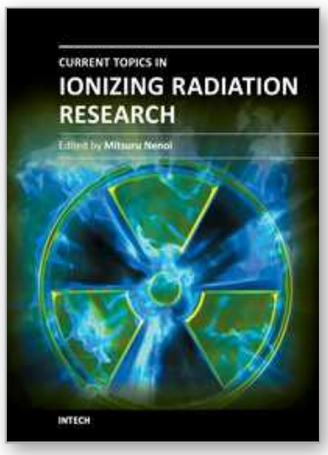
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Since the discovery of X rays by Roentgen in 1895, the ionizing radiation has been extensively utilized in a variety of medical and industrial applications. However people have shortly recognized its harmful aspects through inadvertent uses. Subsequently people experienced nuclear power plant accidents in Chernobyl and Fukushima, which taught us that the risk of ionizing radiation is closely and seriously involved in the modern society. In this circumstance, it becomes increasingly important that more scientists, engineers and students get familiar with ionizing radiation research regardless of the research field they are working. Based on this idea, the book "Current Topics in Ionizing Radiation Research" was designed to overview the recent achievements in ionizing radiation research including biological effects, medical uses and principles of radiation measurement.

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