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REVIEW OF RETROSPECTIVE DOSIMETRY TECHNIQUES FOR EXTERNAL IONISING RADIATION EXPOSURES

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The current focus on networking and mutual assistance in the management of radiation accidents or incidents has demonstrated the importance of a joined-up approach in physical and biological dosimetry. To this end, the European Radiation Dosimetry Working Group 10 on 'Retrospective Dosimetry' has been set up by individuals from a wide range of disciplines across Europe. Here, established and emerging dosimetry methods are reviewed, which can be used immediately and retrospectively following external ionising radiation exposure. Endpoints and assays include dicentrics, translocations, premature chromosome condensation, micronuclei, somatic mutations, gene expression, electron paramagnetic resonance, thermoluminescence, optically stimulated luminescence, neutron activation, haematology, protein biomarkers and analytical dose reconstruction. Individual characteristics of these techniques, their limitations and potential for further development are reviewed, and their usefulness in specific exposure scenarios is discussed. Whilst no single technique fulfils the criteria of an ideal dosemeter, an integrated approach using multiple techniques tailored to the exposure scenario can cover most requirements.

INTRODUCTION

The word retrospective comes from the Latin, retrospectare, 'to look back', and generally refers to events that already have taken place. Thus, many dosimetry techniques can be defined as retrospective because they involve measuring a dose received in the past, even if it was only a few hours ago. In contrast, in the cytogenetic context, retrospective dosimetry often refers to stable endpoints, i.e. those which persist long enough to measure doses received months or years before blood sampling takes place. In the context of this work, for which the main aim is to compare physical and biological retrospective dosimetry techniques for individual external exposures, retrospective dosimetry can be defined simply as:

> 'The estimation of a radiation dose received by an individual recently (within the last few weeks), historically (in the past) or chronically (over many years).'

Such dosimetry methods are usually implemented when conventional 'prospective' dose estimation systems such as film badge dosimetry are not available or require independent verification[1].

Traditionally, the physical and biological dosimetry communities have been somewhat separate; however, the current focus on worldwide networking and mutual assistance has demonstrated the importance of a joined-up approach. To this end, the European Radiation Dosimetry Working Group 10 on 'Retrospective Dosimetry' has been set up by individuals from a wide range of disciplines across Europe.

Here, a review is presented of the current status of retrospective dosimetry techniques, which can be used to provide estimates of external radiation doses for individuals. A brief description of each dosimetry method, including cytogenetic, physical, genetic, immunochemical and computational techniques is followed by a review of the similarities and differences between the techniques. A few examples are given to discuss how these methods can be used to complement each other in different exposure scenarios. Also considered is the relevance/extension of these methods for emergency situations in which large numbers of casualties may have been exposed to varying doses of radiation.

Few of the discussed techniques will be the method of choice when assessing doses received from internal emitters. This is especially true for those radionuclides that are not deposited homogenously in the human body. For this reason, the present paper focuses on external radiation exposures. It should be mentioned, however, that a few radionuclides (^{137}Cs and ^{3}H), do distribute homogeneously and biological dosimetry has been successful. An example is the Goiania accident in 1987. It must also be noted, that there had been attempts in the past to use cytogenetic biodosimetry in accidents involving incorporation of radionuclides that are deposited non-uniformly, in order to supplement dosimetric information based on radioactivity measurements and modelling. Indeed, individual retrospective dosimetry may be important in cases of malevolent acts and mass casualty situations when information needed for modelling of doses may be incomplete due to delayed data collection or a lack of information about important exposure parameters.

TECHNIQUES FOR RETROSPECTIVE DOSIMETRY

Cytogenetic techniques

Analysis of cytogenetic damage in peripheral blood lymphocytes (PBL) induced by ionising radiation is commonly used for biodosimetry. The applicability of the available assays is based on the stability of the chromosomal damage. Dicentric, premature chromosome condensation fragment and micronucleus frequencies fall with the turnover of lymphocytes, and so these assays are best applied to assessing dose from more recent exposures. For exposures that have taken place years or decades ago or are chronic, the assay of choice is fluorescence *in situ* hybridisation (FISH) to detect stable translocations.

The dicentric chromosome assay

Dicentric chromosomes are almost exclusively induced by ionising radiation. Dicentric frequencies in PBL show a clear linear quadratic dose–effect relationship up to ~5 Gy for acute photon exposures. Numerous studies on both low- and high-linear energy transfer (LET) radiations have demonstrated that exposures *in vitro* and *in vivo* produce similar yields of dicentrics per unit dose. The spontaneous frequency of dicentrics is very low in the healthy general population (about one dicentric per 1000 cells). Due to this low background, the sensitivity of the dicentric assay is relatively good; being able to detect whole-body doses down to about 0.1 Gy from the analysis of 500–1000 metaphase spreads[2, 3]. Ideally, the dicentric assay is performed on blood samples within a few days of the exposure. Blood sampling after weeks or months requires the intrinsic exponential removal rate of dicentrics (half-time between 6 months and 3 y) to be taken into account. Mathematical procedures exist to modify the dose-squared coefficient in case of dose protraction or to provide dose estimation after partial-body exposure[3]. Furthermore, free data analysis software is available, which includes

curve-fitting and dose-calculating modules for full, partial-body and protracted exposure[4].

In the case of a mass casualty event requiring many individuals to be evaluated, the dicentric assay can be used in a 'triage' mode by initially analysing a smaller number of cells per subject. Dose estimations based on 20–50 cells will have larger confidence limits ($\pm$ 0.5 Gy) but are sufficient for supplementing early clinical triage, at least for whole-body exposures[5, 6]. Due to the requirement for a culture time of 48 h for stimulated lymphocytes, the assay takes at least 51 h for sample preparation. The subsequent analysis effort for each case is 1–2.5 person hours for the 50 cell triage mode and 5–25 h per scorer per 500 cell analysis, depending on the level of automation.

Premature chromosome condensation

Visualisation of chromosome aberrations during interphase in both cycling and non-cycling cells is possible with the premature chromosome condensation (PCC) technique. Chromosome condensation can be achieved without the completion of DNA replication by employing various agents. These include either polyethylene glycol-mediated cell fusion with mitotic cells or chemically induced PCC using calyculin A or okadaic acid[3]. In the fusion, PCC assay on unstimulated interphase cells, the excess number of PCC fragments (above the normal of 46 chromosomes) is counted. In general, 4–5 excess fragments per cell per gray are observed for low LET radiation. The frequency of spontaneously occurring PCC fragments is in the range of the dicentric frequency, 1–3 in 1000 cells. For the PCC assay, unstimulated lymphocytes should be immediately isolated following exposure in order to perform fusion with mitotic Chinese hamster ovary cells. If sampling is delayed, the repair kinetics for PCC fragments must be taken into account. PCC fragments were found to be 2-fold elevated at 4-h post-irradiation in comparison with 1 and 7 d, whereas no significant difference was observed between 1 and 7 d[7]. The whole process from collecting blood to slide preparation takes 3 h at most. Conventional microscope scoring of Giemsa-stained preparations is time consuming, since a large number of objects need to be counted. However, utilisation of an automated metaphase finder can speed up the analysis, and automated systems for scoring PCC fragments are currently being developed. FISH chromosome painting assays can be combined with PCC for identification of exchange-type aberrations[7].

Since there is no influence of cell death and mitotic delay in the PCC assay, it is possible to detect partial-body exposure as low as 3 and 6 % in *in vitro* and *in vivo* studies, respectively[8].

The chemically induced PCC assay uses the phosphatase inhibitors calyculin A and okadaic acid, which induce chromosome condensation in S and G_2 phase cells but not in unstimulated lymphocytes. This assay therefore takes at least 40 h. It has been found to be suitable for the analysis of ring chromosomes, especially at higher doses. This variant has been successfully used in assessing dose in the Tokai-mura radiation accident in Japan[9].

More recently the chemically induced PCC technique has been validated for triage following exposure to high, partial-body doses by analysing ring chromosomes. Both this technique and the dicentric assay used in triage mode were found to have limitations for this exposure scenario[10].

The micronucleus assay

The *in vitro* cytokinesis-block micronucleus (CBMN) assay is another established method for biodosimetry. Micronuclei (MN) arise from acentric fragments or whole chromosomes that are not incorporated into the daughter nuclei during cell division. They are seen as distinctly separate small spherical objects that have the same morphology and staining properties of nuclei, within the cytoplasm of the *binucleated* daughter cell[3].

MN are not radiation specific: they can be caused by exposure to many clastogenic and aneugenic agents. The CBMN assay for PBL is a thoroughly validated and standardised technique to evaluate the exposure of occupationally, medically and accidentally exposed individuals[1, 11]. Like dicentrics, MN represent unstable chromosome aberrations, which disappear with time after exposure, and thus their use is rather limited for exposures that occurred many years ago.

Compared to the dicentric assay, scoring of MN is simple and quick and does not require extensive experience in cytogenetics. Together with the fact that MN scoring can be automated, the characteristics make the CBMN assay very attractive for high throughput analysis. The efficacy of automated MN scoring has been confirmed for fast mass casualty triage in a multi-centre setting[12]. One disadvantage is that lymphocytes require 3 d to enter cytokinesis following stimulation, so that the time to a first dose estimate is at least 75 h.

The lower limit for dose detection of the MN assay as employed in many laboratories is restricted to 0.2–0.3 Gy[3]. This is due to the relatively high and variable spontaneous MN yield that tends to increase with age and is more pronounced in females[13]. Almost all the age-dependent increase of baseline MN frequencies is due to centromere-positive MN reflecting an increased aneuploidy with age. By restricting scoring to centromere-negative

MN, the detection limit is lowered to 0.05–0.1 Gy for individual and population dose estimates[14].

The CBMN assay has been validated as a good dosimetric tool in a limited number of small radiation accidents. In the Istanbul accident where 10 workers were irradiated by an unshielded radiotherapy ^{60}Co source[3] and the accident of a hospital worker exposed to radiotherapy X-ray device[14], dose estimates were in excellent agreement with values obtained from dicentrics. The CBMN assay does not allow assessment of partial-body irradiation, as MN are inherently overdispersed.

Fluorescence in situ *hybridisation*

FISH techniques for assessment of past exposures have been in use for many years. The technique most commonly used is single colour FISH (sFISH), which enables the detection of inter-exchanges, such as dicentrics and translocations. In order to assess induced translocations among different labelled chromosomes, multi-colour FISH and for whole genome analysis M-FISH have been developed. Furthermore, pancentromeric and telomeric probes are combined with chromosome paint probes in order to discriminate accurately between translocations and dicentrics, and between two-way and one-way translocations. Translocations are the aberration of choice in cases of either protracted exposure, e.g. occupational doses, or for historic exposure assessment. Translocation frequencies have been shown to persist for many years in circulating lymphocytes[15–18], particularly when the analysis is restricted to stable cells. However, background frequencies increase significantly with age[19, 20] and can vary greatly between individuals of similar age and dose history. No significant effects of gender or race have been observed but smoking habit has been suggested to be of significance[20]. Due to these confounding factors the lower detection limit is around 0.5 Gy cumulative lifetime dose[18] for individual dose assessment, although in younger non-smoking individuals it may be possible to detect doses down to 0.2 Gy. In partial-body exposures, cells containing translocations are often unstable and therefore the frequency is reduced with time[18]. The need for mitotic lymphocytes and lengthy hybridisation protocols mean that first results are available only ~5 d after receipt of a blood sample.

Most retrospective dosimetry has been undertaken on individuals exposed to low LET radiation (reviewed in [18, 21–23, 24]). FISH techniques have also been used to retrospectively assess chromosome damage in individuals with exposure to high LET radiation. Increased translocation frequencies have been observed in plutonium workers many years post-exposure[25, 26]. However, their situation is confounded by significant external gamma irradiation, making the interpretation of results difficult. Other aberrations have been suggested as biomarkers of high LET exposure, such as insertions, intra-chromosomal and complex aberrations. Increased frequencies of intra-aberrations have been reported in plutonium workers using the multi-coloured banding (mBAND) technique[27, 28] but this hypothesis has not been confirmed elsewhere[25, 29]. Two EU concerted actions aimed at standardising sFISH concluded that only 'complete' cells, i.e. those with all 'painted' material present and ~46 chromosomes, should be used and frequencies calculated using stable cells only. For population-based studies analysis of ~300 genome equivalent cells per individual is recommended. Accurate assessment of individual dose requires a minimum of ~1000 genome equivalent cells.

Genetic techniques

Somatic mutations glycophorin A/hypoxanthine-guanine-phosphoribosyl transferase

Two somatic mutation assays have been suggested for use as alternative biodosemeters to chromosome aberration analysis: the Glycophorin A (GPA) and hypoxanthine-guanine-phosphoribosyl transferase (HPRT) mutation assays. Several studies have compared one or both of these assays with chromosome aberration analysis but all have concluded the latter to be the technique of choice for retrospective biodosimetry[23, 30, 31].

GPA is a glycoprotein expressed on the surface of red blood cells in two allelic forms: M and N. The assay involves labelling the different allelic forms with different monoclonal antibodies measured by flow cytometry. A major disadvantage of the assay is that only individuals with the MN genotype are suitable for analysis, and thus it is only applicable to 50 % of any population. Also, there is no *in vitro* model system available and the assay cannot be used during the first months after the exposure because GPA mutations can only arise in red blood cell precursors. After blood sampling, it takes only a few hours to process and analyse samples to obtain a dose estimate. Background frequencies have been observed to increase significantly with age[32] but do not appear to be associated with other confounding factors, such as smoking[30]. Studies of exposure to external radiation (reviewed in refs (1, 22)) have demonstrated mixed results with large inter-individual variability being reported, particularly at the higher doses. Studies on the Japanese A bomb survivors and radiation accident cases with high acute doses have shown a positive correlation with GPA mutation frequency. However, studies where the doses were lower and/or chronic, including Chernobyl clean-up workers and local residents,

residents of the Semipalatinsk nuclear test site and occupational radiation workers, showed a much shallower dose response. The large inter-individual variation in GPA mutation frequencies restricts the use of this assay to average dose estimations for populations rather than for individuals[1].

The HPRT somatic mutation assay involves the determination of mutations of the HPRT gene in T-lymphocytes by measuring the ability of cultured cells to grow in the presence of 6-thioguanine. The method commonly used is a clonogenic assay whereby T-lymphocytes are grown under selective and non-selective conditions. Mutant frequencies are calculated from the ratio of the two cloning efficiencies. However, the technique is quite complex and time consuming to perform. Also, it takes several weeks of cell culture to obtain a result for one blood sample. The assay has been used to ascertain if there is a relationship between dose and mutation frequency in radiation workers[33, 34], Chernobyl clean-up workers and residents[30, 31, 35], victims of the Goiania accident in Brazil[36] and radiotherapy technicians[37]. However, results have been inconclusive in most cases. Mutant frequencies have been reported to be associated with age[33, 36] and smoking status[33, 34] but there is also evidence that the mutation frequency is not stable over time[35, 36] making the HPRT somatic mutation assay unsuitable as a retrospective biodosemeter[1].

Gene expression assays

Expression levels of many genes are modulated in response to ionising radiation exposure. Gene expression profiles have been assessed in radiation workers and radiotherapy patients[38–41]. An overview of the current literature concerning ionising radiation exposure and microarray approaches (used to quantify modulation of gene expression) shows that the exposure conditions found in the different studies are heterogeneous in terms of the doses used but also in terms of the time between exposure and analysis. Thus, it is difficult to reach consensus with regard to these factors. Nevertheless, several conclusions can be drawn.

The key steps in application of the assay in array format are RNA extraction, labelling and hybridisation and could take 2 d before a dose estimate can be obtained for less than 10 samples. Well-established and standardised protocols exist for each of these. While gene expression arrays are excellent tools for identification of radiation-responsive genes in a small number of samples, quantitative real-time reverse transcriptase polymerase chain reaction methods can be used instead of gene expression arrays for determining expression levels of a small set of radiation-responsive genes within a few hours for dozens to hundreds of blood samples. The modulation of gene expression in response to ionising radiation is a dynamic mechanism—both the level of gene expression and the types of modulated genes may change over time[42, 43]. However, it is likely that the specific 'time pattern' could be identified, and the instability of response could be accounted for when this assay is applied. Additionally, modulation occurs even at very low doses (around some millisievert)[39, 41, 44–46] which is indicative of a very low baseline level for this assay.

Studies of radiation specificity are still rare and the confounding influence of many exogenous factors remains to be analyzed. The variability of response to different qualities of radiation is currently unknown. Also the uncertainty and suitability of the assay for detection of more complex exposure scenarios, such as partial-body exposure, must be evaluated before the assay can be reliably used for dosimetry.

Haematological techniques

A differential blood cell count is the first quantitative bio-indicator that can be applied after irradiation. The assay is readily available, automated and inexpensive because it is a standard diagnostic tool for investigating many clinical conditions. Measurements take only a fraction of an hour for multiple samples. For radiation exposures, the assay is quantified with respect to detecting acute and whole -, or nearly whole-, body exposures that might lead to the haematological component of the acute radiation syndrome. Although chronic radiation syndrome undoubtedly exists, and is characterised by continuously lowered cell counts, there are far fewer data for fractionated or low dose-rate exposures compared with the single brief irradiation response[47].

Fluctuations in cell counts commence at a threshold whole-body dose of ~0.5 Gy. However, normal inter- and intra- individual variations in counts impose a background 'noise' such that it requires a dose of 1.0 Gy or higher before values depart from the normal ranges. Reference background ranges are 1.5–4.0 and $4–9\times10^9$/l for lymphocytes and granulocytes, respectively[48]. Having reached a point where values fall outside these ranges, the most informative early responses are the counts of lymphocytes and granulocytes. The platelet count is slower to respond because their lifespan in the circulating blood is longer.

Because the pre-irradiation background values of any particular patient are unknown, it is essential to take a blood sample as soon as possible after exposure. The differential count in this is then used as the baseline from which to plot any subsequent changes. Therefore, frequent repeated sampling,

initially every few hours over the first few days and then daily, is needed throughout the time course of clinical management. The rates and extents of the measured changes in the counts are dependent on the magnitude of the dose. They therefore provide an early indication of the likely severity of later sequelae and, in particular, the degree of bone marrow damage and the need to intervene to restore its function.

Various authors have suggested further subdivision of depressed cell counts into ranges whereby patients may be placed into severity bands ranging from mild to lethal. Some of these categorisations include an estimate of the likely dose range[49] whilst the METREPOL scheme[50] dispenses with estimating doses and considers just the constellation of clinical signs and symptoms.

Following an exposure severe enough to carry a risk of lethality the first change to be noted is a marked elevation above normal in the granulocyte count. However, this is not an exclusive diagnosis of irradiation; other possible causes such as severe septicaemia need to be considered. With acute external radiation exposures, this elevation persists over the first 2–3 d and is then followed by a dramatic fall. Alongside this the lymphocytes count also falls steeply.

One practical problem arises when there is delayed discovery of exposure so that the patients come to medical attention after these first most informative days. Patients then present with lowered counts, which may continue to fall, although over the period 10–20 d post-exposure there may be abortive rises in the counts. Without the earlier data, it is much more difficult to characterise the extent of the exposure from haematology alone and other even less quantitative signs such as a history of nausea and vomiting may be informative. Of course a cytogenetic examination is then particularly important. For delayed discovery events a 'rule of thumb' has been proposed for interpreting lymphocyte counts made on Day 6 into six severity bands[49]. Thus, for example, $0.7–1.5\times10^9$/l correlates with a mild degree of acute radiation syndrome and a dose of 1–2 Gy, whereas $0.1–0.3\times10^9$/l indicates a very severe exposure in the range of 6–8 Gy of acute, external X- or gamma rays.

Protein biomarkers

Numerous changes in protein abundance and localisation as well as enzymatic modifications occur as a consequence of biological responses to irradiation at the cellular, tissue or systemic level. Such changes can be identified in urine or blood samples using a range of proteomic approaches. Various antibody-based techniques, including western blotting, enzyme-linked immunosorbent assays, flow cytometry, immunohistochemistry or immunofluorescence microscopy have been used to produce dose–response curves and time-course data for specific proteins following radiation exposure. The time between sample receipt and result is typically on the order of a few hours for these assays. A number of promising protein markers for human radiation exposure have been suggested in recent literature reviews [51, 52]. Here, three relatively mature markers will be discussed in more detail.

γ-*H2AX*

The radiation-induced activation, stabilisation or expression of DNA damage signalling factors like ATM, γ-H2A histone family member X (γ-H2AX), TP53 and CDKN1A/p21/Waf1 contribute to the key cellular responses to ionising radiation and facilitate DNA damage-induced cell cycle checkpoint activation and DNA repair. As such, these changes are thought to be specific to ionising radiation, when analysed in non-cycling white blood cells. Especially the immunofluorescence microscopic detection of foci of the phosphorylated histone γ-H2AX—which form at the sites of DNA double-strand breaks—has been tested for its usefulness in biological dosimetry in multiple clinical settings, including diagnostic CT scans[53] and interventional cardiology[54], making this a sensitive biomarker for radiation exposure. γ-H2AX foci form within minutes after irradiation in a dose-dependent manner. Foci levels peak at <1 h but then rapidly decay until they return to baseline levels within one to several days, depending on the dose received. Considerable inter-individual variation of baseline levels and rapid loss of foci over time severely reduce the sensitivity of this assay for post-exposure times of 1 d or more. Automated foci scoring techniques have been developed (reviewed in ref. (55)), which ensure more reproducible scoring criteria. Instead of scoring the number of microscopic foci, attempts are also being made to determine γ-H2AX *intensity* as a measure of radiation dose, using either flow cytometry or ELISA-type assays. Results so far suggest a potentially higher throughput than foci scoring but also relatively low sensitivity and large inter-individual variation[56]. γ-H2AX analysis can detect partial-body exposure, at least when samples are obtained shortly after exposure[53]. As with most other biological dosimetry assays, internal exposures would be detectable but it would be difficult to provide a reliable dose estimate, due to a lack of available reference data for different intake routes, nuclides and chemical compositions. Some attempts are underway to optimise the γ-H2AX assay for rapid triage in a large-scale emergency. In summary, this assay appears to work well as a sensitive biodosemeter for planned (medical) exposures where

pre-exposure levels and the timing of the exposure can be determined for each individual. It cannot be used to assess past exposures that occurred a week or more ago but may be useful as a rapid triage tool to identify individuals with high levels of DNA damage for priority follow-up monitoring and treatment in situations where the exposure occurred more recently.

C-reactive protein

C-reactive protein (CRP) is a well-established biomarker for inflammation for which robust and sensitive assay systems are routinely used in a number of clinical settings. High-level radiation induces an inflammatory response which, through cytokines, triggers CRP induction for a few days after the exposure. Given that CRP is increased in a large number of acute or chronic medical conditions, it is not specific for radiation and therefore unsuitable as a stand-alone biodosimetry tool. However, it has been proposed as one component of a multi-parametric biodosimetry approach[57] or could perhaps be used as a first of several diagnostic layers in a mass casualty incident to support clinical triage and management of large numbers of casualties, especially for cases of combined injuries. In such a scenario, however, it should not be regarded as a specific marker for ionising radiation exposure. As CRP is not induced in *ex vivo*-irradiated blood samples, *in vivo* studies are required to obtain dose–response and time-course data. Studies with non-human primates have reported significantly increased CRP levels between 8 and 24 h post-exposure to 6–6.5 Gy whole-body X- or gamma rays[57, 58]. CRP levels were reported to correlate with clinical outcome in patients who had been irradiated during the Chernobyl accident[59]. Increasing CRP levels were also observed during different radiotherapy treatments (see ref. (60)). The CRP assay is already fully automated and can be performed rapidly (within a few hours) at any modern hospital with a clinical biochemistry department. Also, hand-held deployable CRP assay systems are in routine use. The CRP assay cannot distinguish between partial- or whole-body radiation exposures. For protracted and internal exposures, only doses and dose rates sufficiently high to induce significant inflammatory responses may increase CRP levels but reliable dose quantification would be difficult.

Serum amylase

Irradiation of the salivary tissue induces acute inflammatory and degenerative changes that result in increased serum amylase activity (hyperamylasaemia). Serum amylase levels increase in a dose-dependent manner, peak at 18–30 h post-exposure and return to baseline within a few days[61]. Such responses have been reported for patients undergoing internal and external radiotherapy and for the three individuals exposed during the Tokai-mura criticality accident[62]. The speed and sensitivity of this routine diagnostic assay appears similar to that of CRP. One obvious limitation is its restriction to the dose received by the salivary gland. Irradiation of other tissues would not change amylase levels significantly. Also, the extent of partial-body exposure cannot be assessed with this system. Finally, large inter-individual variation and responsiveness to a number of different factors (including emotional stress) limit the usefulness of amylase as stand-alone radiation biodosemeter but, as with CRP above, it may be one useful marker in a multi-parametric system[57].

Physical techniques

'Physical' methods used for retrospective dosimetry conventionally include the techniques of electron paramagnetic resonance (EPR), thermoluminescence, optically stimulated luminescence and nuclear activation. The terminology stems simply from the fact that these methods are typically used in the physical science studies. In contrast with biological endpoints, the physical ones do not reflect a biological response, even when performed in biological tissues such as hair, fingernails and tooth enamel/bone. In general, the time from sample receipt to dose estimate is between 1 and 48 h, depending on the required accuracy.

EPR dosimetry

The EPR technique gives an estimate of absorbed dose by detection of the paramagnetic centres, such as radicals or point defects that are specifically generated by ionising radiation.

The most advanced physical method for retrospective dose assessment for individuals is EPR spectroscopy with tooth enamel[63, 64]. Several international intercomparisons have been organised on tooth enamel dosimetry[65, 66–70] and P. Fattibene *et al.*, submitted for publication. It has been extensively employed for historical and chronic exposures[71, 72], such as the A bombs[73], Chernobyl[74] and Southern Urals radiation incidents[75, 76]. In cases of acute exposure and severe accidents, when bone biopsies are available, bone tissues can be used especially for localised or heterogeneous irradiation cases[77].

Tooth enamel and bones require invasive collection. Other materials are more suitable for fortuitous EPR dosimetry because they can be collected with non-invasive procedures (e.g. sugar, plastics, glass, wool, cotton, hair and nails). Preparation of samples for EPR dosimetry is usually relatively simple.

Depending on the material, a single measurement can take between some minutes up to a few hours. The readout is non-destructive, allowing for repeated measurements of the same sample. A drawback is that EPR spectrometers are expensive and highly qualified personnel are required for their operation. EPR detection limits vary widely between ~100 mGy for tooth enamel and 10 Gy for cotton. A procedure for uncertainty analysis has been proposed for tooth enamel[78]. Data interpretation can suffer from the presence of background EPR non-radiation-induced signals. Few studies have been carried out regarding the effects of different qualities of radiation on some of the above-mentioned materials. The time stability of the EPR signal varies widely between materials ranging from 5 to 7 d for plastics[79] to $\sim 10^6$ years for tooth enamel[129]. Storage of samples at low temperatures ($<4^{\circ}$C) slows down the recombination of paramagnetic centres. EPR dosimetry is particularly suitable for application after partial-body or non-uniform exposure because dose heterogeneity can be assessed by using several objects in contact with different parts of the body. For further information, the reader is referred to a recent review[80] and references therein.

Techniques for *in vivo* EPR measurements of teeth are under development. These techniques use microwave frequencies of 1 GHz, i.e. lower than those used for conventional *in vitro* measurements (about 10 GHz). With low-frequency microwaves a loss in sensitivity of a factor of 5–10 compared with X-band spectrometry is expected from calculations. Hence, the limit of detection is expected to be in the range of 0.5–1 Gy. At present a prototype system is operating with a whole-body magnet. Measurements of extracted whole teeth were found to be possible with an approximate associated standard error of ± 0.5 Gy. This leads to a limit of detection close to 2 Gy with measurement time of about 10 min[81]. In contrast to low microwave frequency, frequencies higher than X-band offer a better sensitivity that compensates for smaller sample volumes. This allows measurement of tooth enamel biopsies, whose collection is less invasive than extraction of a whole tooth. A detection limit of 190 mGy has been evaluated for a 4-mg sample[82].

Luminescence dosimetry

Ionising radiation absorbed by an insulator or a semiconductor produces free charge carriers that can be trapped at lattice defects of the material. Luminescence dosimetry is based on the stimulated emission of light from these materials by release of the trapped charge carriers and subsequent recombination. Stimulation is performed either thermally (thermoluminescence, TL) or optically (optically stimulated luminescence, OSL).

Quartz extracted from bricks and other fired-building materials is currently the main mineral used for retrospective luminescence dosimetry purposes. Sample preparation techniques and measurement protocols are well established, although, in general, they take more than 1 d. Various studies were performed with quartz to evaluate the external exposure in the area of Chernobyl, in areas affected by fallout from the Semipalatinsk and Nevada nuclear test site and in the Southern Urals[83]. Minimum detectable doses in the order of 20–25 mGy can be obtained using bricks of a few tens of years old. The possibility of using quartz extracted from unfired building materials (mortar, concrete ...) was also tested[83]. However, in such cases, a detection limit higher than 100 mGy has been found.

In addition to quartz, other phosphors have recently been studied, which can be found either in the urban environment or in materials carried on or close to the body by the general population[84]. Examples of such materials include memory chip modules from telephone, ID, health insurance, cash and credit cards[83–87], ceramic resistors of portable electronic devices such as mobile phones[87, 88], materials used for dental restoration[83, 89], tooth enamel[90, 91], household and workplace chemicals[92, 93] and glass[94]. Inorganic dust extracted from natural materials or personal items has also been investigated[95, 96].

Most of these items show a linear dose–response over a wide dose range. The radiation sensitivity and time stability of the response strongly depend on the type of material but detection limits of the order of 10 mGy can be achieved for most materials. For tooth enamel however they are presently more in the range of 1–5 Gy. In general, personal objects have the common feature of showing partial signal fading with storage time. Procedures for sample preparation and measurement protocols vary but for most materials are comparatively quick and easy: processing of a sample from a personal object can be achieved within less than an hour. Similarly, the type of measurement to be preferred for dose assessment depends on the specific properties of each material. Since in general OSL does not require heating of the sample to high temperatures, it may be chosen for those materials that cannot tolerate heating, provided that optically active defects are present.

Activation techniques

Neutron activation techniques are based on the measurement of radioactivity induced by neutron interaction with biological tissues, such as blood, hairs or nails, or metallic elements worn by the victims, such as coins, jewellery or belt buckles. Activation techniques can be used in emergency management of criticality accident and in dose

reconstruction many years following exposure to neutrons, such as for A bomb survivors.

Criticality accidents usually involve a small number of victims; however, irradiation can be severe and heterogeneous[97]. Activation techniques permit very rapid dose estimation and is also used to complement individual dosimetry by giving pertinent information on dose heterogeneity. Thus, in the early phase of the management of a criticality accident, rapid and efficient triage can be performed using the measurement of sodium activation in humans [^{23}Na(n,γ)^{24}Na, $T_{1/2}$=14,96 h, E_γ=1.36 MeV (100 %) and 2.75 MeV (99.85 %)]. At the site of an accident, very rapid measurements of gamma radiation emitted by ^{24}Na with a simple direct gamma survey instrument positioned against the abdominal area of victims are considered a good indicator of the severity of the neutron exposure[98–100].

As the activity is directly proportional to the thermal neutron fluence, the total neutron kerma and the total dose can be also deduced if the neutron spectrum and the gamma to neutron dose ratio are known (1 Bq of ^{24}Na is related to 0.5–3 mGy of total (n+γ) dose). Later, a more precise estimate of the sodium activity in victims can be performed with a whole-body counter or by gamma spectrometry of blood samples[100, 101]. At this stage, the estimate can also be corrected to allow for the victim's orientation and weight. With the whole-body counter, the detection limit for thermal neutron doses is approximately a few tens of microgray but is somewhat higher for fast neutrons.

In addition to activated sodium, measurement of activated sulphur in hair and nails [^{32}S(n,p)^{32}P, $T_{1/2}$=14,28 d, $E_\beta$max=1.710 MeV (100 %)] has also been used for dose reconstruction following accidents[101, 102]. The activity can be measured directly using a Geiger-Müller counter or by liquid scintillation techniques, following simple chemical procedures. Hair can be collected from different parts of the victim's body and thus useful information on dose distribution and the victim's orientation can be derived. Using sulphur, the detection limit is about 0.05 Gy for 1 mg of hair (0.05 g of sulphur per g of hair).

In the case of the Tokai-mura accident, for example, post-mortem analysis of activation in bones was also performed to estimate the neutron dose distribution by measuring ^{32}P and ^{45}Ca activities[103].

For A bomb survivors, neutron doses were revaluated by measuring long-lived activated nuclei in environmental samples (^{63}Ni in copper samples; ^{152}Eu, ^{60}Co, ^{59}Ni, ^{41}Ca, ^{39}Ar, ^{36}Cl, ^{14}C, ^{10}Be in granite gravestones) or biological materials (^{41}Ca in tooth enamel)[104, 105].

For the above techniques, procedures and protocols have been established for several decades and some countries offer the possibility of regular training of interventional teams and medical analysis laboratories[106].

Computational techniques

Analytical dose reconstruction ('time and motion' calculations)

The techniques applied for analytical reconstruction of individual doses following radiation accidents have been established for decades. A state-of-the-art analytical method, known as realistic analytical dose reconstruction with uncertainty estimation (RADRUE), was developed by an international group of experts[107] for estimation of external exposure of Chernobyl clean-up workers. The method is based on a time-and-motion approach so the subject's exposure can be estimated as time spent in certain locations multiplied by exposure rate at this location and taking account of applicable shielding factors. Stochastic modelling is applied to dose calculations in order to estimate uncertainty. It could be easily expanded to any other accidental situation where exposure rates are mapped and individual exposure itineraries are available.

Methods have been implemented for gamma exposures, but related software for exposure estimation taking into account both neutron exposures and volumetric activity, 'Rockville,' has been recently developed (Kryuchkov, personal communication).

Dose reconstruction consists of several steps. First, a personal interview is carried out. A trained interviewer, familiar with the location and chronology of the exposure event (i.e. clean-up), adds details of the subject's occupancy within the zone of interest to a specially designed questionnaire, which is then processed by an expert dosimetrist who checks and interprets the information and subsequently inputs the data into the calculation program. Finally, the expert runs the stochastic modelling unit to obtain the stochastic distribution of individual dose estimates for each exposed subject.

The RADRUE program does not include a dose threshold and is applicable to a large range of exposures. It is suitable for air kerma and organ dose reconstruction using embedded exposure-to-dose conversion coefficients (e.g. red bone marrow, thyroid). However, neither partial-body exposures nor internal exposures are covered by RADRUE. The method has been applied for case–control studies of haematological malignancies and of thyroid cancer[108, 109].

Dose reconstruction by numerical approaches

There is a large variety of numerical tools used to estimate dose retrospectively to individuals. Most of

these are nowadays based on Monte Carlo (MC) radiation transport codes. With such codes the transport of particles can be simulated in a defined geometry and thus a dose map calculated. It has been used for a wide range of applications.

In contaminated territories, for example, MC codes were used to determine radiation fields generated by contaminated soils and calculate chronic exposure levels to inhabitants. Calculations are based on activity measurements of soil samples, dose-rate measurements in air and estimation of accumulated dose in building materials by luminescence techniques[110].

With the help of numerical phantoms of the human body, it is possible to estimate dose distributions in the organism, effective doses or doses to specific organs[111]. This has been widely used for planned or accident situations, for radiation protection purposes or dose reconstruction for overexposed individuals. These approaches have recently been used for accidents during interventional radiology procedures, in processing facilities and with lost or orphan sources [68, 112–114]. In cases of localised and severe irradiations, calculated dose distributions enable the surgical removal of lethally exposed tissue before necrosis occurs. In such a case, calculations are performed with voxel phantoms derived from MRI or CT scans to take into account the individual anatomy of the patient.

PERFORMANCE OF TECHNIQUES IN DIFFERENT RADIATION EXPOSURE SCENARIOS

Table 1 summarises some of the characteristics of the different dosimetry techniques described above. Stability of the dosimetric signal has a direct impact on the time window after exposure during which an assay can be used. Whilst most assays can be used for very recent exposures where samples can be obtained within days of the event, only very few methods pick up radiation-induced signals suitable for dosimetry years or decades after the exposure. Signal stability is also directly linked to the assays' ability to quantify protracted exposures. The biological assays can be grouped into three categories corresponding to the biological nature of the signal used for dosimetry.

(i) Unrepaired DNA damage and early damage responses: PCC fragments and γ-H2AX foci represent unrepaired DNA breaks, which are induced in large numbers by ionising radiation but are typically completely repaired within a few days after the exposure. Accordingly, these assays are potentially very sensitive when used within a few hours after the exposure but their usefulness for unplanned or chronic exposures is severely hampered by rapid signal loss. Similarly, changes in blood cell counts, gene expression and serum proteins reflect early cellular effects and tissue responses to the radiation exposure and typically last only for a number of days.

(ii) Unstable rearrangements: dicentrics, PCC rings and micronuclei in lymphocytes result from misrepaired DNA damage. These rearrangements persist in non-dividing cells but cannot be passed on to daughter cells. Consequently, they are depleted with the rate of lymphocyte renewal and have a half-life of 0.5–3 y.

(iii) Stable rearrangements and mutations: translocations and mutations are generally mitotically stable and can be passed through from stem cells to mature blood cells. Therefore, any replacement over time of originally irradiated blood cells with newly matured ones would not be expected to significantly affect the frequencies of translocations or mutations in stable cells. This notion has been confirmed experimentally at least for translocations, though only for low to moderate doses.

Materials used or envisaged for application in physical dosimetry can be grouped into three categories, according to the lifetime of the radiation-induced signal:

(1) Radiation-induced free radicals in calcified tissue have a very low yield of recombination, making dose estimation by EPR possible over decades in living tooth enamel while for living bones it may be affected by bone remodelling in the years following irradiation. In case of neutron exposure, it was demonstrated that measurement of activated calcium allows dose estimations for up to a few decades post-exposure. Certain luminescence signals in quartz (extracted e.g. from brick or concrete) are thermally stable over decades or even hundreds of thousand years and are also extensively used in archaeological and geological dating.

(2) Sugars, salts and manufactured materials such as glass, electronic components and chip cards show signal fading, but with a sufficiently low yield of recombination to allow the measurement of a radiation-induced signal for up to several weeks after an exposure. However, rapidly changing material specifications used in personal items make it necessary to maintain an up-to-date database of dose–response and kinetic data for commonly used materials.

Table 1. Comparison of retrospective dosimetry assays.

	Time since exposure			Exposure			Time (h) from sample receipt to dose estimate	Agent specificity	Dose range (Gy) for photon equivalent acute whole-body exposure 24 h ago	Triage use	Automated analysis	Dose uncertainty analysis available	Standardisation
	Days	Months	Years	Acute	Protracted	Partial body							
Dicentrics, full	✓	✓	—	✓	✓	✓	55	IR	0.1–5	—	✓	✓	ISO 19238[128]
Dicentrics, triage	✓	✓	—	✓	✓	—	52	IR	0.5–5	✓	✓	✓	ISO 21243[6]
PCC fragments	✓	—	—	✓	—	✓	2[a]	IR	0.2–20	✓	Underway	—	—
PCC rings	✓	✓	—	✓	✓	—	40[b]	IR	1 to >20	✓	Underway	—	—
Micronuclei	✓	✓	—	✓	✓	—	75	Genotoxins	0.2–4	✓	✓	✓	ISO pending; scoring criteria[11]
FISH	✓	✓	✓	✓	✓	—	120	IR	0.25–4	—	Underway	✓	—
GPA	—	✓	✓	✓	—	—	3	Mutagens	>1	—	✓	—	—
HPRT	✓	✓	—	✓	—	—	400	Mutagens	>1	—	—	—	—
Gene expression	✓	—	—	✓	—	—	4/36[c]	Genotoxins	>0.1	✓	✓	—	—
EPR (teeth/bone)	✓	✓	✓	✓	✓	—	1–48	IR	>0.1	—	—	✓	ISO in preparation
EPR (p.b.)	✓	—	—	✓	—	✓	1–48	IR	>2	✓	—	—	—
TL/OSL (bricks)	✓	✓	✓	✓	✓	—	<24	IR	>0.03	—	✓	✓	—
TL/OSL (p.b.)	✓	✓	—	✓		—	<1	IR	>0.01	✓	✓	—	—
Activation	✓	✓	✓	✓	✓	—	<24	Neutrons	>0.0001	✓	✓	—	—
Haematology	✓	—	—	✓	—	—	<1	Wide range	>1	✓	✓	—	Routine diagnostics
γ-H2AX	✓	—	—	✓	—	✓	3	Genotoxins	0.5 to >8	✓	✓	—	—
CRP	✓	—	—	✓	—	—	1	Wide range	>1	✓	✓	—	Routine diagnostics
SA	✓	—	—	✓	—	—	1	Wide range	>1	✓	✓	—	Routine diagnostics
Computational	✓	✓	✓	✓	✓	✓	<1	IR	0 to ∞	✓	✓	✓	—

p.b., personal belongings.
[a]PCC fusion method.
[b]PCC chemically induced.
[c]PCR/array analysis.

(3) Synthetic and biological materials such as polymers, hair and nails exhibit significant signal fading, restricting dose estimations to hours or a few days after the incident.

Another important aspect is radiation specificity. Whilst all the physical dosimetry methods are intrinsically specific for ionising radiation, this is not the case for all biological endpoints. Some are either sensitive to a wider range of genotoxic agents or, in the case of haematology, CRP and serum amylase, they may in addition reflect responses related to stress, inflammation and infection. Lack of radiation specificity severely compromises the usability of some of these endpoints as stand-alone dosemeters. They may, however, provide rapid initial triage tools for mass casualty scenarios where more specific endpoints cannot offer the throughput required for screening everybody. Alternatively, they could be combined into a multi-parametric biodosimetry system[58].

To further illustrate the specific characteristics of the different methods, their usefulness in a few specific scenarios is discussed:

Acute exposure

Acute exposure covers a large set of possible scenarios. Most acute exposures are related to partial-body irradiation or localised irradiation. Localised irradiations are mainly associated with overexposure either during radiotherapy treatment and interventional surgery, or due to manipulation of orphan or lost sources. It should be underlined that even in the case of whole-body exposure; the dose distribution in the victim's body is usually heterogeneous, as with neutrons in the case of a criticality accident. Moreover, some reported cases comprised both localised and whole-body exposure, e.g. the accident in the Nesvizh radiation processing unit. Personal dosemeters worn by victims are often not sufficient, especially in cases of partial-body exposure, for an accurate dose estimate. Moreover, many of the accidents involve members of the public, and for most of the recently reported cases, no dosemeters were worn by the workers involved. Therefore, retrospective dosimetry is an essential tool in victim management. Acute exposures require medical care and advice on associated health risks. Clinical assessment and treatment of radiation casualties benefit from support and guidance from retrospective dosimetry efforts aiming to assess the dose distribution in the victim's body or in specific organs, such as for example the haematopoietic system.

Due to the complex and often uncertain circumstances of typical radiation accidents (exposure time, distance, position, ...), a multi-technique approach is usually chosen. As a matter of fact, there is no gold standard method that can be universally applied.

The 'gold standard' biological assay for acute exposure scenarios is the dicentric assay. It estimates the dose to the circulating and tissue-associated blood lymphocytes and allows some indication on the dose heterogeneity. To determine doses to critical organs or the dose distribution, calculation codes (analytical or MC) associated with a mathematical or voxel phantom are currently used[108]. These codes are powerful tools, but need accurate input data (distance, position, exposure duration, ...). Nevertheless, if some parameters are not accurately known, they can be adjusted based on dose estimates from cytogenetic, EPR, OSL or activation measurements[112, 114]. For localised irradiation, when no detailed information on accident circumstances is available, only physical dosimetry is currently able to determine a dose in one or several points in or close to the irradiated region. EPR dosimetry is for example currently used in such cases on bone biopsies, where available[68].

The main conclusions are that for most of case of acute exposure, a multi-technique approach is needed and the different dosimetric tools are complementary[115].

Criticality accident

To date there have been approximately 60 criticality accidents[97], most of which occurred in the 1950s and 1960s at military nuclear industry enterprises, processing facilities or nuclear research institutions. In the last 20 y, only two events have been registered. Usually, the number of casualties is limited but injuries are severe and lead to death in many cases. The individuals affected are mainly radiation workers who are under dose monitoring programs. Retrospective biological dosimetry is usually used to validate the doses measured and calculated by physical dosimetry and ^{24}Na and ^{32}P activation assays. There is a limited need to follow-up radiation exposure of larger groups of individuals or of the general public.

One of the few criticality accidents that involved many individuals, potentially to follow with retrospective dosimetry, was the K-431 submarine reactor accident in 1985 near Vladivostock, where about 2000 people involved in the cleanup needed to be followed up with dose assessments. Generally, doses to members of the public are moderate or relatively low. After the Tokai-mura accident in Japan in 1999, in addition to the radiation workers, a group of non-radiation workers and neighbouring residents were followed up for dose assessments. None of the 436 assessed individuals, including 56 radiation workers, received doses >50 mSv. In almost all cases, the doses were due to gamma exposure. This implies

that for retrospective dose assessment, for the workers and the public, a relatively sensitive assay like dicentrics will be the method of choice. This category of scenarios also includes one of the catastrophic incidents—nuclear detonations. In an urban environment, such an incident would be a mass casualty event which is addressed later in this paper.

Dosimetry many years after exposure

The most suitable assays to estimate doses many years following exposure are EPR on tooth enamel, translocation analysis by FISH and luminescence on building materials in combination with computational modelling. This is largely because the radiation-induced signals and translocations detected with these assays have been shown to persist for a very long time.

Uniform body exposures can for example be expected for inhabitants of contaminated territories (Chernobyl area, Techa River region in the Southern Urals), for which EPR and FISH analysis are in principle applicable. For the Techa River region, an additional challenge arises due to the uptake of ^{90}Sr with contaminated water, milk and food and subsequent incorporation into tooth enamel. Therefore, independent evaluation of the internal dose due to ^{90}Sr is necessary to correct the measured EPR dose[116, 117]. An alternative approach involves TL/OSL measurements of absorbed doses in building materials like bricks and tiles. By mapping the exposure dose rates in a larger area in front of the sampled building and by performing photon transport calculations, doses in bricks can be converted into integral air kerma values at given reference points. These in turn can be used to estimate integral external exposure of the inhabitants by making assumptions about the average time spent in specific locations. They can also be used to independently evaluate dosimetry systems used in epidemiological studies such as the Techa river dosimetry system.[116, 118]

For cases of non-uniform body exposure, information or assumptions on exposure geometry will be required for reconstruction of air kerma or organ doses from measurements of absorbed dose in tooth enamel by EPR and red bone marrow dose by FISH. Comparison of reconstructed air kerma from measurements by EPR and FISH can be a tool for validating conditions of exposures in the past. It is applied in epidemiological studies with Mayak PA workers to validate assumptions on historical exposure conditions of Mayak PA workers. These are needed to reconstruct air kerma and organ doses from the workers' film badge doses[66].

Regarding the usefulness of the FISH assay in non-uniform body exposure scenarios, there are several issues in obtaining dose estimates: (1) FISH is routinely performed on only a few of the chromosomes, which means that typically only ~30 % of all translocations in the genome are detected. This means that fewer cells with multiple exchanges would be scored, compared with the dicentric assay, even if in total three times as many cells are analysed for translocations. Given that calculations of the irradiated fraction, using the Dolphin or Qdr methods[3], are based on the frequency of cells with multiple chromosome exchanges, the sensitivity of FISH for this purpose is not as good as that of the dicentric assay. M-FISH would overcome this problem but is very costly; (2) the Dolphin and Qdr methods assume that there are no exchanges in the unirradiated fraction. This is more or less true for dicentrics, but translocations accumulate with increasing age, so that different mathematical algorithms would have to be used that can 'unmix' two distributions from each other and (3) bone marrow stem cells with multiple translocations may be less likely to divide and mature into lymphocytes than cells with only one exchange. Moreover, even stable cells with only one translocation have been shown to disappear, albeit more slowly than cells carrying unstable aberrations such as dicentrics. Therefore, the frequency of peripheral lymphocytes with multiple translocations may decrease over time. This would change the perceived irradiated fraction over the years after a partial-body exposure. For all these reasons, FISH may be able to *detect* non-uniform body exposure (especially if M-FISH is used) when applied several years after the exposure, but any estimates of the irradiated fraction or non-uniform body doses would carry large uncertainties[119].

A mass casualty event

Mass casualty events require the coordinated response of a wide range of emergency services. Depending on the scenario, response teams may include radiation assessment support teams, emergency medical personnel, search and rescue teams, medical triage units, police and fire fighters. The main objective of the early response is the preservation of life. While the life-saving objective is aimed at the general public, the safety and health of response workers is also critical. Triage in a large casualty scenario is, therefore, of major importance to define which patients will derive most benefit from prompt medical attention, considering the expected limited availability of resources. The key points for early triage and management are the casualties' spatio-temporal coordinates relative to the radiation source, physical examination, dosimetry predictions from initial models and from real-time physical dosimetry (dose measurements) and from available clinical laboratory studies. Treatment of trauma injuries takes priority over all actions relating to the radiation exposure. Importantly,

prognosis of patients with combined injuries will be worse than a patient injured with either of each alone. The TMT Handbook (www.tmthandbook.org) provides comprehensive guidance on triage, monitoring and treatment of people exposed to ionising radiation in such an event.

When a large number of individuals may have been exposed, blood cell counts can help identify critically exposed individuals. Also, the 'gold standard' dicentric assay could be used in triage mode, reducing the number of cells scored per sample from 500 to 50 or even 20, to increase the throughput, at the cost of sensitivity which would drop from ~0.1 to ~0.5 or ~0.8 Gy, respectively. However, even when international assistance networks like WHO BioDoseNet[120], IAEA RANET or the emerging European biodosimetry network[121] are activated to share the burden of sample processing and scoring, the throughput may not be sufficient to rely solely on the dicentric assay for triage. Automated dicentric scoring and the use of the automated micronucleus assay may provide further improvements in throughput. However, the intrinsic delay of >50 h associated with these cytogenetic assays and the complexity of fusion-based PCC (which could in principle provide results within a day) mean that there is currently a capability gap for assays that enable triage of hundreds or thousands of people within hours after the event. Deployable protein biomarker and gene expression assays as well as fast luminescence and EPR dosimetry approaches are being developed specifically to address this need. Until such methods become available for use in emergencies, initial triage has to rely on clinical symptoms, blood counts and modelling of individual doses based on the location of casualties during the event.

OUTLOOK

For cytogenetic biodosimetry, current research is focused on automation of the techniques, validation through inter-laboratory comparisons[122] and the potential for sharing of workloads through national and international networks, such as the BIODOSENET project[120]. New strategies for the dicentric assay are being investigated to optimise the method and achieve faster throughput. These include automation of cell culturing[123], microscopes fitted with dicentric scoring software[124], rapid manual scoring approaches like QuickScan[125] and scoring of high-resolution images of metaphases via the internet (telescoring). When automated MN-centromere scoring is developed it will improve systematic biomonitoring of radiation workers exposed to low doses and in the case of mass radiation casualties, more accurate dose assessments in a second step after early triage. Automation of certain steps of the FISH assay is possible, enabling rapid metaphase finding and capture of images for analysis.

Research in genetic techniques is currently focused on further development of the use of microarray and quantitative polymerase chain reaction technologies, which should enable gene expression assays to produce and validate a reliable signature of human exposure to very low doses of ionising radiation in the near future. This signature will probably not be able to predict a given dose but will rather allow a distinction between exposed and non-exposed individuals, and as such could be helpful in identifying an exposure above a dose threshold, provided that the post-exposure time is within a defined period of time. Currently, the standard molecular biology protocols used in the assay are fully automated for applications other than biodosimetry and thus there is potential for automation for dosimetry approaches, although this has not yet been attempted.

For physical dosimetry, future research activities should be aimed at further investigating the EPR response and dosimetric properties of widely available materials such as glass, plastics and textile fibres, and of fingernails. In particular, efforts are required in order to standardise protocols for the measurement of EPR signals and to automate the procedures to deal with mass casualty situations. In addition, techniques of data analysis must be improved, for instance to better evaluate the radiation-induced signal and separate it from the background signal, which can be native or occur due to radical species produced by UV radiation and can lead to increased uncertainty in the dose estimate. Current research on *in vivo* EPR of tooth enamel is focused on development of portable intraoral and helmet magnets and there is a large potential for further developments to improve sensitivity and mobility of the system for application in the field. Further research on tooth enamel biopsies is also desirable.

In parallel to EPR, future research in luminescence dosimetry could mainly be focused on (i) the modification/development and harmonisation of measurement protocols in order to improve both precision and accuracy of the dose assessment[126, 127] and (ii) the study of the possibilities of new materials that could be valid in case of radiological emergency or accident employed as individual dosemeter (i.e. precious or semi-precious stones) or many other domestic or industrial materials to be applied for dose reconstruction in populated areas as an alternative to bricks or insulators (e.g. vitroceramics, electrode coatings, etc.). For tooth enamel, an improvement in the detection limit and in the understanding of the OSL characteristics needs to be achieved for a future development of a suitable *in vivo* method using a

portable OSL reader and fibre optics. In addition to TL and OSL, it could be of interest to determine the potential use of some other luminescence techniques: radioluminescence, cathodoluminescence, ionoluminescence, etc., for dosimetric purposes.

Both activation and haematological techniques are well established and procedures and protocols have been implemented. The quality standard should be maintained by continuous training and international exercises.

The immunocytochemical techniques discussed here are relatively new, and thus a large amount of work will be required before they can be used as reliable dosemeters. Nevertheless, protein biomarkers such as γ-H2AX, CRP or serum amylase have some clear advantages over cytogenetics assays, for example results can be obtained within hours rather than several days after sampling; sample processing and analysis can be optimised and automated for high throughput; non-invasive sampling may be possible (saliva, buccal cells, hair), depending on the marker, and deployable assay formats exist or are in development. However, a number of issues have to be considered before these techniques can really be used as robust biodosimetric tools. In particular, they are not as specific for ionising radiation as, say, the dicentrics assay, confounding factors need to be fully characterised and their levels change rapidly over time. Several calibration curves for different post-exposure times and exact timing between exposure and sampling are therefore required. In contrast to cytogenetic and DNA damage foci assays, dose–response curves for CRP and amylase cannot be performed *ex vivo*. *In vivo* experiments with suitable animal models and validation studies with radiotherapy patients are therefore required but the translation of animal or cancer patient data to the response of 'normal' humans needs to be considered carefully. Some of these markers may not be suitable as stand-alone biodosemeters but would perhaps work as part of a multi-parametric biodosimetry system, which produces a dose-dependent signature[57]. Further, they could be useful for rapid clearance of the 'worried well' in a multi-tiered triage setting, though their inter- and intra-individual variation in baseline levels and in response to radiation has not been fully determined yet. Available data suggest a larger variation than seen for the dicentric assay. Finally, there is very little known about their response to different radiation qualities.

Computation techniques are quite straightforward in their concept, but their implementation often requires sophisticated solutions. So, the automatic direct input of dose-rate measurement data into the databases, powerful inter- and extrapolation algorithms and tools for prediction of doses are the main routes of further development of time-and-motion techniques. In addition, unlike other retrospective dosimetry techniques, computational methods have potential for conversion into prognosis and optimisation tools for planning of post-accident response, finding the safest evacuation/transportation routes, optimisation of the activities of responders and public in different ways, i.e. by collective or individual doses, time before withdrawal from radiation hazard zone, etc. Once implemented, this approach would allow provision of retrospective assessment of individual and collective doses and estimate (predict) doses at the following time intervals.

Development of the complementarity of all the different techniques is now required, as worldwide networking efforts lead to a greater need for intercomparisons between techniques as well as laboratories. Effort is required to standardise the newer methods and develop rigorous statistical analysis methods to enable formal comparisons of techniques. This particular task is currently being addressed through the EU FP7 MULTIBIODOSE collaboration. Availability of techniques in Europe and around the world is also of interest, and current research efforts are additionally focused on training and dissemination of information about the different techniques.

For most radiation accident scenarios, none of the methods described above can in a satisfactory manner be used as a stand-alone tool. This situation will most probably never change despite ongoing research to improve each method. The reason for this is that each tool is inherently limited with respect to the requirements of an ideal (bio)dosemeter which are:

- specificity to ionising radiation,
- large discernable dose range from a few microgray to tens of gray,
- good signal stability to allow analysis of recent and distant exposures,
- ability to estimate the extent of partial-body exposure,
- ability to discriminate between internal and external exposure,
- well-defined dose response relationships for different radiation qualities and dose rates,
- possibility to generate an *in vitro* calibration curve,
- possibility to assess the uncertainty of the dose estimate,
- low inter-individual variation,
- absence of confounding factors,
- non- or minimally invasive sampling,
- standardised, rapid (automated) and cheap sample processing and analysis.

Given this, the way forward may be the development of an integrated dosimetry system consisting of many complementary tools which, between them,

fulfil most of the above requirements. The system will require a software-based analysis module that will combine the results and associated uncertainties from each tool in an attempt to generate a best estimate of the absorbed dose and the exposure scenario. For large-scale radiological casualties, this approach is currently being addressed through the EU FP7 MULTIBIODOSE collaboration.

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