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# Advancement in Biodosimetry Techniques and Their Growing Role in Nuclear Medicine

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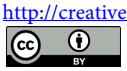



## Abstract

Biodosimetry, a technique for estimating radiation doses by assessing chromosomal aberrations in both accidental and occupational exposures, has played a crucial role in radiological protection, clinical contexts, and monitoring exposed populations. Recent advancements in biodosimetry aim to enhance accuracy, sensitivity, and clinical applicability. These innovations contain novel biomarkers (e.g., metabolomic, exosomal, and proteomic markers) for detecting radiation-induced DNA damage. Additionally, progress in high-throughput technologies and automated scoring systems facilitates quicker and more efficient chromosomal aberration analysis, rendering biodosimetry suitable for extensive studies. This review provides an overview of recent biodosimetry developments and their application in radiation emergencies and clinical practices, especially in nuclear medicine.

## Keywords

Radiation, Chromosomal Aberrations, Biomarkers, Biodosimetry, Nuclear Medicine

## 1. Introduction

Ionizing radiation, from natural and human-made sources, averages around 6.2 mSv (0.0062 Sv) annually for adults. Instances of increased radiation exposure occur due to equipment malfunctions, nuclear accidents, misplaced radioactive materials, improvised nuclear devices, and nuclear terrorism. Severe cases, like the Fukushima Dai-Ichi nuclear plant incident, exemplify the consequences [1]. Additionally, the widespread use of radiological instruments in medical field poses a growing public health threat, with mortality risks from acute radiation syndrome

varying from 3.5 Gy to 7.5 Gy, depending on treatment availability. The international Atomic Energy Agency (IAEA) and the International Commission on Radiological Protection (ICRP) issue guidelines regularly for radiation exposure prevention and practices aiming to mitigate health impacts. Thermoluminescent dosimeters (TLDs) are standard for occupational exposures, but non-compliance and detection limits can lead to inaccuracies, especially in unplanned incidents. Physicians involved in radiology and in fluoroscopic procedures (FG) may also underestimate their exposure due to insufficient training and negligence. Some operators also ignore protective measures, including dosimeters and lead shielding. Hence, accurately assessing absorbed radiation doses is vital for initiating appropriate medical responses in case of overexposure. Biodosimetry becomes essential in such situations, helping assess absorbed doses when physical reconstruction is uncertain [2]. Bender and Gooch [3] pioneered the use of dicentric chromosomes (DCs) detection in peripheral blood lymphocytes (PBLs) to estimate absorbed radiation doses in humans, leading to the routine use of the Dicentric Chromosome Assay (DCA) for assessing radiation doses in occupational and accidental exposures.

Biological dosimetry measures DNA damage at a cellular level, detecting chromosomal aberrations like DCs, centric rings (CRs), and micronuclei (MN). The frequency of these aberrations serves as a biological indicator of absorbed radiation dose, predicting short, medium, and long-term health consequences [4] [5]. Unlike physical measurements with TLDs, biodosimetry reflects actual biological harm and accounts for individual radiosensitivity, making them relevant for deterministic syndromes and stochastic cancer risks [6]. Currently, post-dose estimation of biological sample is the most reliable method for assessing absorbed dose [7] [8]. Biodosimetry markers have long been effective in radiation protection, measuring exposure in incidents, accidents, workplaces, and high-radioactivity environments. They are valuable in medical fields like nuclear medicine, radiology, radiation oncology, and radiation pathology, complementing other radiation health risk and toxicity predictors [9] [10].

In recent decades, several validated biodosimetry methods have emerged, mainly based on radiation-induced DNA damage and misrepair, detected through cytogenetic assays like DCA, cytokinesis-block micronucleus (CBMN) assay, premature chromosome condensation (PCC) assay, $\gamma$-H2AX assay, "Omics" technologies, and fluorescence in situ hybridization (FISH) assay for translocation analysis. DCA is widely used and considered as "gold standard" due to its radiation specificity and low background frequency. The PBLs derived from pluripotent stem cells in bone marrow, stimulated by phytohemagglutinin (PHA) *in vitro*, can be arrested in metaphase using colchicine for biodosimetry. Since PBLs circulate throughout the body, they serve as circulating dosimeters, averaging the dose from all body parts. Biodosimetry is valuable when TLDs are unavailable or inconclusive due to radiosensitivity variations. For clinical applications, biodosimetry helps measure absorbed radiation dose by diverse organs or systems. Gamma-H2AX and FISH are

sensitive and high-throughput tools, though standardization for clinical applications remains incomplete. Therefore, advancements in high-throughput cytogenetic biodosimetry are crucial for radiological accidents and for effective radiation diagnosis and therapy. This article reviews the development of key biodosimetry methods, discussing their potential applications and limitations in radiation emergencies, nuclear medicine, diagnostic, and interventional radiology.

## 2. Biodosimetry Techniques

Biodosimetry relies on cytogenetic assays in PBLs to estimate radiation exposure. These assays measure chromosomal aberrations, including DCs, MN, PCC, and translocations (FISH), to quantify radiation dose. DCA, CBMN assay, and FISH assay are standardized methods for this purpose, while PCC analysis is under consideration for standardization [11]. Comparison of these four major biodosimetry techniques is outlined in Table 1.

Table 1. Comparison of four major cytogenetic biodosimetry techniques.

| | Dicentric (and ring) chromosome aberration (**DCA**) assay | Cytokinesis block micronucleus (**CBMN**) assay | Fluorescent *in situ* hybridization (**FISH**) (**Translocation**) assay | Premature chromosome condensation (**PCC**) assay |
|---|---|---|---|---|
| Typical aberrations scored for biodosimetry applications | Dicentrics (and rings) | Micronuclei | Dicentrics* (and rings)<br>Translocations* | Excess chromosome Fragments<br>Dicentrics* (and rings)<br>Translocations* |
| Typical radiation scenario applications | Low-level acute<br>Protracted prior exposure | acute | Protracted<br>Prior exposure | Acute (including high doses) |
| Photon equivalent, acute dose range (Gy) for whole-body dose assessment | 0.1 to 5 | 0.3 to 5 | 0.25 to 4 | 0.2 to 20 |
| Useful for partial-body exposure applications | yes | NA | NA | yes |
| Useful for triage dose assessment | yes | yes | NA | yes |
| Standardization of assay | ISO standard reference assay (1000 metaphase spreads or 40 dicentrics)<br>ISO standard for triage assay (20 - 50 metaphase spreads)-pending | ISO standard for reference assay pending | NA | NA |
| Advantages | Gold standard; Low baseline frequency | Less time required; easy to score | Retrospective assay | Suitable for high exposure |
| Disadvantages | Time consuming | Non-specific to radiation | More expensive; expertise dependent | Technical expertise required |

Table modified from TMT Handbook (ref: https://www.who.int/publications/m/item/tmt-handbook, 2009). *Specific chromosome aberrations are typically detected by the use of centromeric and whole-chromosome-specific DNA hybridization probes. NA = not applicable.

### 2.1. Dicentric Chromosome Assay (DCA)

The DCA is a widely used and highly effective method for assessing radiation exposure by DCs in human PBLs. DCs, characterized by having two centromeres, result from the fusion of different chromosome segments, each with its centromeric locus (**Figure 1**). The frequency of DCs directly correlates with the radiation dosage [12]. Normally, regardless of age, the baseline occurrence of DCs is about 1 in 1000 cells. DCA's sensitivity, capped at 0.1 Gy, has established it as the gold standard in biological dosimetry for emergency medicine, endorsed by organizations like the IAEA. It has been successfully used in various historical nuclear incidents, including Chernobyl in 1986 [13], the Goiania accident in 1987 [14], Japan's JCO Tokaimura incident in 1999 [15], and the Fukushima Dai-Ichi nuclear power plant disaster in 2012 [1], among others.

However, the DCA has some methodological limitations. It involves lengthy cell culture processes (often spanning 48 hours), complex preparation steps including collection, fixation, staining, and the labor-intensive task of microscopically analyzing a large number of metaphase cells. This becomes even more challenging in the case of a large-scale nuclear incident with numerous samples to process. Additionally, the inherent instability of DCs and their tendency to induce cell death during mitotic division limit the assay to post-exposure evaluations spanning several months to years. The DCA's reliability is compromised when doses exceed 50 Gy due to increased chromosomal aberration frequencies within cells. Over the past two decades, efforts have been made to enhance the efficiency of the DCA, particularly in the context of nuclear or radiological incidents [16]-[18]. Studies suggest that assessing as few as 50 metaphases in triage mode or up to 1000 metaphases or 100 DCs in full-scoring mode can expedite the sorting of exposed individuals. An innovative scoring technique called "DCA QuickScan" has been introduced but is awaiting adoption by the IAEA [19].

Recent research indicates that the DCA takes approximately 52 hours to produce results for medical triage [20], making it challenging to use in scenarios involving large-scale radiation incidents and significant numbers of populations. Collaborative efforts among multiple laboratories have become crucial in such situations, leading to inter-laboratory comparison initiatives that facilitate the exchange of knowledge, protocols, workloads, scoring methodologies, and microscopic analyses [21]. Technological advancements, including automated systems like Metasystems and IMSTAR with software-driven image acquisition for dose estimation, have further improved the practical application of DCA in assessing biodosimetry for extensive population cohorts.

While DCA is the gold standard for external acute exposures, its application to internal emitters like I-131 presents unique challenges. Developing precise *in vivo* calibration curves for internal emitters is complex due to non-uniform dose distribution and continuous low-dose-rate exposure. Most studies for I-131 biodosimetry rely on *in vitro* dose-response curves established using external gamma or X-ray sources. This introduces a discrepancy as the biological effects of internal

beta and gamma emissions from I-131 might differ from external acute exposures. The dose rate and protracted nature of internal exposure can influence the yield of dicentrics, potentially leading to an underestimation compared to acute exposures of the same total dose. The metabolism and biological half-life of I-131 vary significantly among individuals, influencing the actual absorbed dose to circulating lymphocytes. Factors such as patient age, thyroid function, presence of metastatic disease, and other co-morbidities can act as confounders, impacting the dicentric yield and complicating dose estimation. Additionally, the heterogeneity of I-131 distribution within the body means that lymphocytes may not receive a uniform dose, making a whole-body dose assessment based solely on peripheral blood challenging. Despite its frequent use in I-131 exposure scenarios, the DCA lacks specific ISO standardization for internal emitters. Guidelines for sampling time, particularly for protracted low-dose-rate exposures from internal contamination, need further refinement to accurately capture the peak biological effect while accounting for the instability of dicentrics. Robust inter-laboratory validation studies using biologically relevant internal emitter models are crucial for establishing reliable protocols.

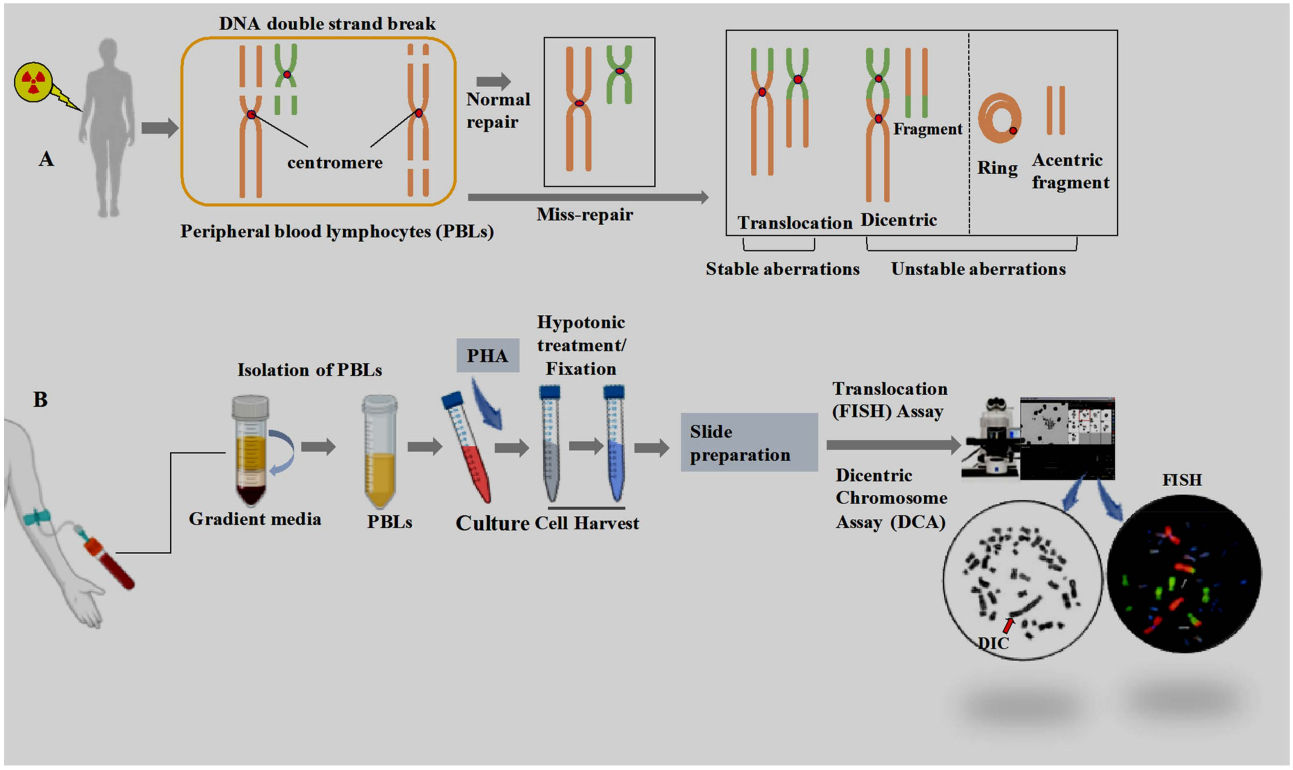


**Figure 1.** A schematic outline (A-B) of the formation of aberrant chromosome and their assay methods. (A) Formation of stable (Translocation and unstable (Dicentric) aberrant chromosome. (B) Assay of dicentric and translocation of chromosome.

## 2.2. Cytokinesis Block Micronucleus (CBMN) Assay

The CBMN assay quantifies MN in human PBLs, which form after DNA damage caused by ionizing radiation during cell division (**Figure 2**). These MN are distinct

spherical structures in the cytoplasm of daughter cells mirroring the nucleus in morphology and staining properties. Fenech and Morel [22] introduced the reliable CBMN assay for the first time using a cytokinesis inhibitor, Cytochalasin-B, to identify binucleated cells streamlining MN scoring and excluding non-dividing cells. Consequently, it emerged as the standard method for evaluating MN in cultured lymphocytes. Subsequent advancements allowed MN scoring in mononucleated cells and introduced the CBMN-centromere assay using FISH and centromeric probes to enhance sensitivity, particularly in lower radiation doses [23]-[26]. Fenech [27] introduced the CBMN-Cytome assay (CBMN-Cyt), an advanced version of the CBMN assay. It assesses not only micronuclei (MN) in binucleated and mononucleated cells but also quantifies nucleoplasmic bridges and nuclear buds in binucleated cells. Additionally, the CBMN-Cyt assay evaluates various cellular parameters, including the proportions of mono-nucleated, binucleated, and multinucleated cells, as well as identifying necrotic and apoptotic cells. This comprehensive analysis provides insights into cellular proliferation and cell death rate, which are significant in biological dosimetry. This refined assay not only assesses genomic damage through MN but also offers a broader perspective on cellular dynamics, enriching our understanding of the biological effects of genotoxic agents. The baseline occurrence of MN varies significantly, ranging from 0 to 40 per 1000 binucleated cells [28]-[31], with age and gender being important determinants [32]. Besides radiation-induced effects, other genotoxic stressors also lead to DNA fragmentation, resulting in MN within the cell cytoplasm [33] [34].

Despite its limitations, the CBMN assay shows a significant trend: an increase in micronuclei (MN) frequency with higher radiation dose and quality, validating its reliability for measuring radiation exposure. The assay's utility shines through its adoption as a pivotal triage biodosimetry tool in diverse scenarios including post-Chernobyl [35], Istanbul radiation incidents [36], and brachytherapy cases [37]. One notable advantage is its rapid turnaround time, requiring just 200 binucleated cells to achieve a sensitivity of 1.0 Gy [38]. This makes it particularly valuable in resource-limited settings where access to specialized equipment might be restricted. Other challenges of this method include complex quantification of different cell types and extended cell culture times (68 - 72 hours). To address these issues, imaging flow cytometry has been proposed for automated MN identification across cell types [39]. Additionally, Repin *et al.* [40] introduced the RABiT-II automated MN assay system to reduce result turnaround time, especially in large-scale radiological incidents. Ongoing advancements include a recent triage application using manual MN scoring in shorter 48-hour cultures, enhancing the CBMN assay's practicality for swift ionizing radiation exposure assessment [41].

While CBMN assay is quicker than DCA, its validation for internal emitters like I-131 is still evolving. Studies often use *in vitro* curves derived from external sources, which may not fully represent the chronic, low-dose-rate exposure characteristic of I-131 therapy. The continuous exposure could lead to repair mechanisms being activated differently, affecting MN formation. The CBMN assay is

less specific to radiation-induced damage compared to DCA, as MN can be induced by various genotoxic agents, chemical exposures, and even lifestyle factors [33] [34]. This lack of specificity is a significant confounder when assessing I-131 exposure, making it challenging to attribute observed MN increases solely to the radioisotope. Individual variations in metabolic rates, immune responses, and baseline MN frequencies further complicate accurate dose estimation. Although ISO standardization for reference assay is pending, specific guidelines for applying CBMN to internal emitters, particularly regarding optimal sampling times to capture the peak MN frequency after protracted exposure, are needed.

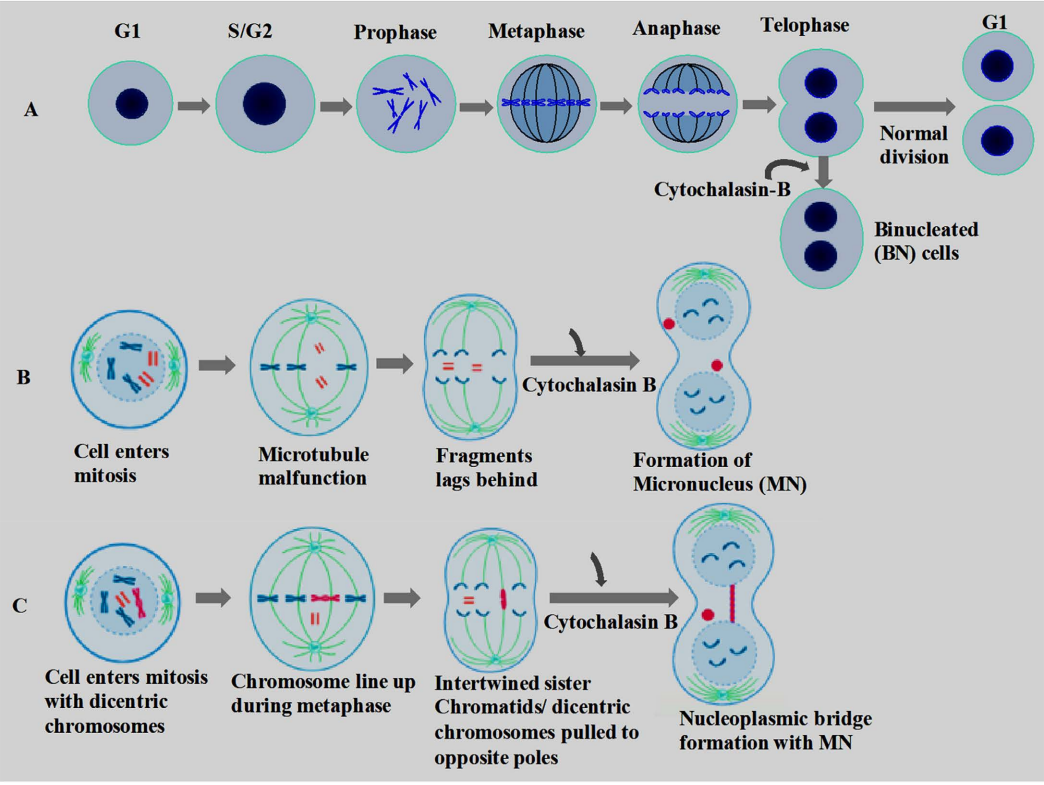


**Figure 2.** (A)-(C) A schematic outline of binucleated (BN) and micronucleus (MN) formation. (A) Formation of BN cells *in vitro* using Cytochalasin-B. (B) MN originating from acentric fragments. (C) MN with nucleoplasmic bridges (NPB) originating from dicentric chromosomes (DICs).

## 2.3. Fluorescence *in Situ* Hybridization (FISH) Assay

Fluorescence in situ hybridization (FISH) is a crucial tool for assessing radiation-induced chromosomal aberrations, particularly when dealing with the long-term effects of radiation exposure. While traditional methods like counting DCs and MN have limitations due to their instability over time, FISH allows for the reliable detection of stable chromosomal aberrations, such as translocations (**Figure 1**), which persist over decades [42]-[44].

Previously, translocation analysis using G-banding techniques required expert evaluation of karyotypes, but FISH revolutionized this process by using distinct chromosome-specific colors to "paint" each chromosome pair. This technique has

been in use since 1996 and has evolved with various methodologies and probes that can depict chromosomes in different colors, making inter-chromosomal translocation detection possible [45]. For instance, multiplex-FISH (mFISH) and spectral karyotyping allow the simultaneous use of all 24-human whole-chromosome painting probes. The main challenge with FISH is subtle shade variations between colors, which may make differentiation difficult to the human eye. To address this, the current practice involves focusing on three pairs of chromosomes, with each pair having a distinct color or sharing a color with another pair [46]. This simplifies translocation detection while maintaining accuracy. FISH offers precise and sensitive dose calculations, maintaining reliability even after extended periods, such as 20 years post-exposure [47]. In studies involving mice, translocations were found to be three times more prominent than dicentrics when exposed to doses between 1 to 20 mGy/day [48].

FISH has been applied in various scenarios, including estimating radiation doses for atomic bomb survivors [49] [50], radiation technologists with long-term occupational exposure [51], and accidents like Goianna [14], and the Georgian accident [52]. To simplify the process and reduce costs, it is now common to focus on three larger chromosomes (Chromosome No. 1 to 12), which constitute approximately 20% of the genome with a 33% translocation detection efficiency [11]. Different combinations of FISH probes for these chromosomes have been developed, and their combined genomic translocation frequencies can be calculated.

For retrospective biological dosimetry, a single-color FISH for a triple paint of target chromosomes is often sufficient [53]. In comparison to traditional G-banding, the FISH assay-based translocation measurement proves swift and uncomplicated, surpassing conventional DCs and MN analysis for retrospective biological dosimetry. While FISH may have lower sensitivity compared to other methods with a detection limit of around 0.30 Gy, it is highly regarded for its precision in detecting radiation-induced translocations [47]. It has proven effective in capturing subtle genetic alterations that other biodosimetry methods may miss. Additionally, FISH can be used in conjunction with other techniques like CBMN assays to provide a comprehensive assessment of chromosomal damage and cellular responses to genotoxic stress.

FISH is particularly valuable for retrospective biodosimetry of internal emitters due to the stability of translocations. While FISH effectively detects translocations from I-131 exposure, the establishment of specific *in vivo* calibration curves for internal emitters remains a challenge. The prolonged, low-dose-rate nature of I-131 exposure can lead to different dose-response relationships for stable aberrations compared to acute, high-dose external exposures. *In vitro* curves derived from external sources may not fully account for the repair kinetics and cellular environment under chronic internal irradiation. Although translocations are highly specific to radiation, other confounding factors could potentially influence their frequency, such as genetic predisposition, age, and previous exposures to genotoxic agents. While less susceptible to short-term fluctuations than unstable aberrations, long-

term cellular turnover and individual radiosensitivity can still affect the measured translocation yield. There is currently no specific ISO standard for FISH application in internal dosimetry. Developing standardized protocols for sampling, cell culture, and scoring for long-term follow-up of internal exposure is essential.

### 2.4. Premature Chromosome Condensation (PCC) Assay

Biodosimetry typically involves assessing chromosomal abnormalities like DCs and translocations in cells during initial mitosis. This process uses mitogen phytohemagglutinin (PHA) to stimulate cell division, followed by cell cycle arrest using colcemid. However, these methods have limitations, including delays and cell death, leading to underestimated radiation exposure. In radiation emergencies, waiting 48 - 72 hours for results is often impractical. The Premature Chromosome Condensation (PCC) assay offers a solution. It induces early chromosome condensation, reducing the culture time and mitigating potential delays or cell death. It can be applied to inactive lymphocytes, providing rapid exposure insights just hours after collection. This technique also avoids issues related to post-irradiation stimulation and cell cycle progression.

The PCC method was initially developed by Johnson and Rao [54], fusing interphase cells with mitotic cells from other cell lines using agents like Sendai virus or polyethylene glycol. Despite its advantages, PCC adoption for biodosimetry has been limited. In 1995, Gotoh *et al.* introduced a simpler PCC induction method using Calyculin A, which is highly efficient across various cell types [55]. This method allows visualization of interphase chromatin and enables rapid chromosome analysis within 2-hour timeframe, which reduces the risk of losing information due to interphase cell death.

The Calyculin A-induced PCC assay has gained widespread acceptance, demonstrating effectiveness in constructing dose-effect curves for high levels of exposure [56]-[58]. It has a remarkable minimum dose detection threshold of 0.05 Gy and has been applied in various scenarios, including the Tokaimura incident [59] and non-human primate studies [60]. The RENEB network has also utilized G0-lymphocyte PCC for dose assessments [61]. Efforts are ongoing to automate the PCC assay and explore novel approaches, such as using the longest-to-shortest chromosome length ratio [62], and mitotic Akodon cells [63] to enhance dose estimation accuracy. Combining PCC with other techniques, like mFISH and mBAND, has shown promise in detecting chromosome aberrations, making the G0-PCC technique a valuable biodosimetry tool for assessing radiation dose and identifying chromosome abnormalities in unstimulated lymphocytes [64].

PCC's rapid turnaround time makes it appealing for internal emitter exposure, especially in high-dose scenarios. While PCC can rapidly assess chromosomal damage, establishing specific *in vivo* dose-response curves for internal emitters like I-131 is still an area of active research. The dose rate and continuous nature of I-131 exposure might influence the kinetics of PCC induction and aberration formation differently than acute external exposures. Most existing calibration

curves are based on acute external irradiation, and their direct applicability to protracted internal exposures needs further validation. PCC, particularly in its basic form, can detect chromosome fragments which are highly sensitive to radiation. However, the presence of non-radiation induced DNA damage or pre-existing chromosomal instability from other factors could act as confounders. The interpretation of PCC results for internal emitters requires careful consideration of the background levels of aberrations and potential non-radiation-induced sources of damage. PCC is currently not ISO standardized. For its widespread adoption in internal dosimetry, clear guidelines on sample handling, induction protocols, scoring criteria, and interpretation of results, especially for protracted internal exposures, are essential.

### 2.5. Other Prospective Methods for Biodosimetry

Apart from conventional biodosimetry techniques, emerging methods are under development to assess radiation-induced DNA damage. One notable approach is the measurement of phosphorylated histone H2AX ($\gamma$-H2AX foci) levels, which promptly increase following DNA double-strand breaks caused by ionizing radiation, peaking around 30 minutes post-exposure and gradually decreasing [65]. Studies have shown a positive correlation between $\gamma$-H2AX foci and radiation doses from conventional CT scans [66] [67]. However, $\gamma$-H2AX is more relevant for short-term estimation of radiation exposure, as it is detectable shortly after exposure and valuable for immediate assessment after accidental incidents [68] [69]. The GPA mutation assay, another promising biodosimetry method, estimates accidental exposure by examining allele loss in the GPA, a surface glycoprotein on red blood cells. This method is applicable to about half of the population (only on MN heterozygotes), making it less versatile. Ha *et al.* applied the GPA assay on 32 hospital workers, revealing correlation between NO and NN variants and exposure doses [70]. Additionally, research has explored [71] correlations between ionizing radiation and DNA methylation and oxidative damage in occupational exposure consisting of 117 interventional physicians and 117 controls. While these approaches have shown promise, their complexity and time requirements limit their regular use. The Comet assay is a well-known technique used to measure DNA damage from low-dose ionizing radiation in PBLs [72] [73]. It detects DNA damage at the single-cell level, primarily through comet-like structures formed during electrophoresis. While widely used, it has lower sensitivity, although enhancing sensitivity could be valuable for detecting low-dose exposures.

These emerging methods hold promise for I-131 biodosimetry, but require further validation.

While rapid, the transient nature of $\gamma$-H2AX foci limits its use for protracted internal exposures like I-131, especially for retrospective assessment. The continuous low-dose-rate exposure from I-131 may lead to a sustained but lower level of foci, making it difficult to establish acute dose equivalents. The kinetics of foci

formation and repair in response to internal beta emitters require more specific investigation and calibration curves. The main limitation of GPA mutation assay for I-131 application is its applicability to only a subset of the population (MN heterozygotes), significantly limiting its widespread utility for internal dosimetry. While promising for understanding biological effects, the correlation between DNA methylation, oxidative damage, and specific I-131 doses is not yet robustly established.

A key gap for all these techniques, when applied to internal emitters, is the development of robust *in vivo* dose-response relationships and the understanding of how continuous, low-dose-rate exposure from radioisotopes like I-131 affects these biomarkers compared to acute external exposures. Confounders specific to patient metabolism and disease state also need thorough investigation. Significant standardization efforts are required before these methods can be routinely adopted for I-131 biodosimetry.

## 3. Application of Biodosimetry in Nuclear Medicine, Especially in Radioiodine Therapy

Biodosimetry plays a crucial role in nuclear medicine by measuring radiation doses received by individuals exposed to ionizing radiation through the assessment of chromosomal damage in their blood cells. Nuclear medicine utilizes radiopharmaceuticals containing radioactive isotopes emitting ionizing radiation for both diagnostic and therapeutic purposes. This radiation can harm cells, and the level of damage depends on the absorbed radiation, which can be estimated by analyzing chromosomal aberrations. In diagnostic nuclear medicine, low radiation doses are used, whereas therapeutic doses are higher and require careful monitoring to minimize harm to healthy tissues. Biodosimetry is instrumental in monitoring radiation doses, reducing damage, and adjusting treatment plans.

In the context of internal exposure from radiopharmaceuticals, particularly I-131, various biodosimetry techniques have been employed to assess genotoxic effects.

### 3.1. Dicentric Chromosome Assay (DCA) in I-131 Exposure

Early studies utilized DCA to estimate biological dosage in patients undergoing therapeutic radioiodine (I-131) administration. Lloyd *et al*. developed an algorithm for this purpose [74]. Subsequent research consistently reported increased frequencies of dicentric chromosomes following I-131 treatment for thyroid tumors, non-cancerous thyroid conditions, and other ailments [30] [75]-[94]. For instance, Harnandez-Jerdines *et al*. observed a higher post-treatment frequency of radiation-induced aberrations in patients with metastatic conditions compared to those without metastases, attributed to greater exposure of circulating PBLs to cancerous tissue retaining I-131 [86]. While specific fold-changes in dicentrics varied across studies due to differences in administered activity and patient characteristics, the general trend was a dose-dependent increase. Some studies also

generated *in vitro* dose-response calibration curves using external sources (60Co, 137Cs, or X-rays) to benchmark *in vivo* biomarker measurements [75]-[77] [79] [81] [82] [85] [88].

### 3.2. Micronuclei (MN) in I-131 Exposure

The CBMN assay has been widely applied to assess genotoxic effects in patients undergoing I-131 therapy. Studies consistently reported significant increases in MN yields after I-131 treatment for thyroid cancer, thyrotoxicosis, neuroendocrine tumors, and hepatocellular carcinoma [30] [75] [76] [78] [80] [84] [90] [92] [93] [95] [96]. For example, patients with hyperthyroidism displayed persistent micronuclei even after total thyroidectomy, indicating sustained genomic instability [80]. The increase in MN frequency generally correlated with the administered I-131 dose, with fold-changes often ranging from 1.5 to 3 times the baseline levels, depending on the individual patient and dose. While some studies reported exceptions where MN levels did not increase or even decreased [84] [90], the overwhelming evidence supports MN as a sensitive indicator of I-131 induced DNA damage. The "human transferring receptor positive reticulocytes (Tf-Ret)" method, using MN in reticulocytes, also showed effectiveness in monitoring I-131 treated patients [30].

### 3.3. Translocations (FISH) in I-131 Exposure

FISH analysis for stable chromosomal aberrations, particularly translocations, has proven valuable for assessing long-term effects of I-131 exposure. Elevated stable translocation levels were documented through FISH analysis after approximately 3.5 years of I-131 treatment in thyroid cancer and thyrotoxicosis patients [81], highlighting the clinical utility of cytogenetic biodosimetry for retrospective assessments. Longitudinal studies conducted over 20 to 25 years confirmed sustained chromosomal aberrations (translocations) [92] [93], emphasizing their value for retrospective radiation exposure assessment and ongoing monitoring of chromosomal instability. While specific fold-changes for translocations are less frequently reported compared to dicentrics or MN, the persistence of these stable aberrations over long periods indicates their importance for long-term health surveillance.

### 3.4. Other Biomarkers in I-131 Exposure

Khvostunov *et al.* recently assessed chromosomal damage induced by I-131 therapy in differentiated thyroid cancer (DTC) cases, including a pediatric patient, showing increased aberrant cells after each therapy session and mostly unchanged patterns during follow-up [94]. This aligns with findings from mFISH analysis using buccal cells, which also showed long-term heightened chromosomal aberrations from I-131 exposure [81] [88]. These studies collectively demonstrate the utility of various cytogenetic biomarkers in assessing the genotoxic impact of I-131 therapy and monitoring long-term effects in nuclear medicine patients.

## 4. Future Prospect of Biodosimetry

Advancements in genomics, proteomics, metabolomics, and transcriptomics have enhanced the biodosimetry field's capabilities. These developments have also improved biodosimetry techniques, showcasing their potential for assessing radiation effects on large populations, aiding in individualized treatment planning, and evaluating long-term radiation risks based on individual sensitivity. Yet, traditional cytogenetic tests like DCs, MN, translocations, and PCC suffer from drawbacks such as time-consuming procedures, specialized expertise requirement, and limited accuracy. In the USA, an inovative "rapid on-site screening test" utilizing a finger-prick blood sample is under development, aiming to quickly identify radiation exposure levels and prioritize clinical care for affected individuals. This test employs enzyme-linked immunosorbent assay (ELISA) to detect radiation-induced changes in blood plasma proteins, providing results within 24 hours to 14 days post-exposure [97]. Notably, it has been successful in distinguishing radiation doses below and above 2 Gy during cancer radiation therapy [98]. The field of proteomics also promises to be a valuable asset in evaluating levels of radiation exposure and characterizing radiation injury biomarkers [99].

Another innovative approach, employing peptide nucleic acid (PNA) probes for scoring radiation-induced DNA damage, offers rapid, accurate, and cost-effective biodosimetry [100]. These probes have improved the scoring of dicentric and ring chromosomes in mitosis-induced lymphocytes and PCC in non-stimulated lymphocytes. Additionally, these probes can enhance the identification of MN in cells, making this common test more useful [101]. Experts suggest that this method could revolutionize DNA damage assessment, making radiation effects measurement more efficient through automation [102].

Furthermore, gene expression analysis is emerging as a promising on-site method for swiftly assessing radiation effects. A portable device employing nanopore sequencing has been introduced to detect radiation-induced changes in 46 human genes in PBLs [103]. Additionally, machine learning and deep learning techniques are being employed for high-throughput gene analysis and the evaluation of DCs and CBMN assays [104]-[106]. In the area of nuclear medicine, advancements in radiopharmaceuticals are paving the way for theranostics, where a single drug serves both diagnostic and therapeutic purposes, offering exciting prospects [107]. Moreover, artificial intelligence (AI) is being integrated into clinical practice, aiding in disease prediction and treatment planning. AI has shown success in predicting conditions like amyotrophic lateral sclerosis and diagnosing sarcoma based on DNA data [108]-[110].

Recently, machine learning and deep learning have made notable progress across various scientific fields. For instance, deep learning systems have been developed for automated dose estimation using the dicentric assay [111]. Machine learning has also been used to predict individual radiosensitivity based on telomere length data [112]. These developments underscore the versatile applications of machine learning in different scientific fields, including biodosimetry. The in-

tegration of machine learning and artificial intelligence-based algorithms with biodosimetry holds great promise. These algorithms can efficiently process vast datasets, accurately predict radiation doses, and play a crucial role in diagnosing and treating radiation-induced illnesses. It is imperative for the biodosimetry community to closely monitor the advancements in this emerging research area.

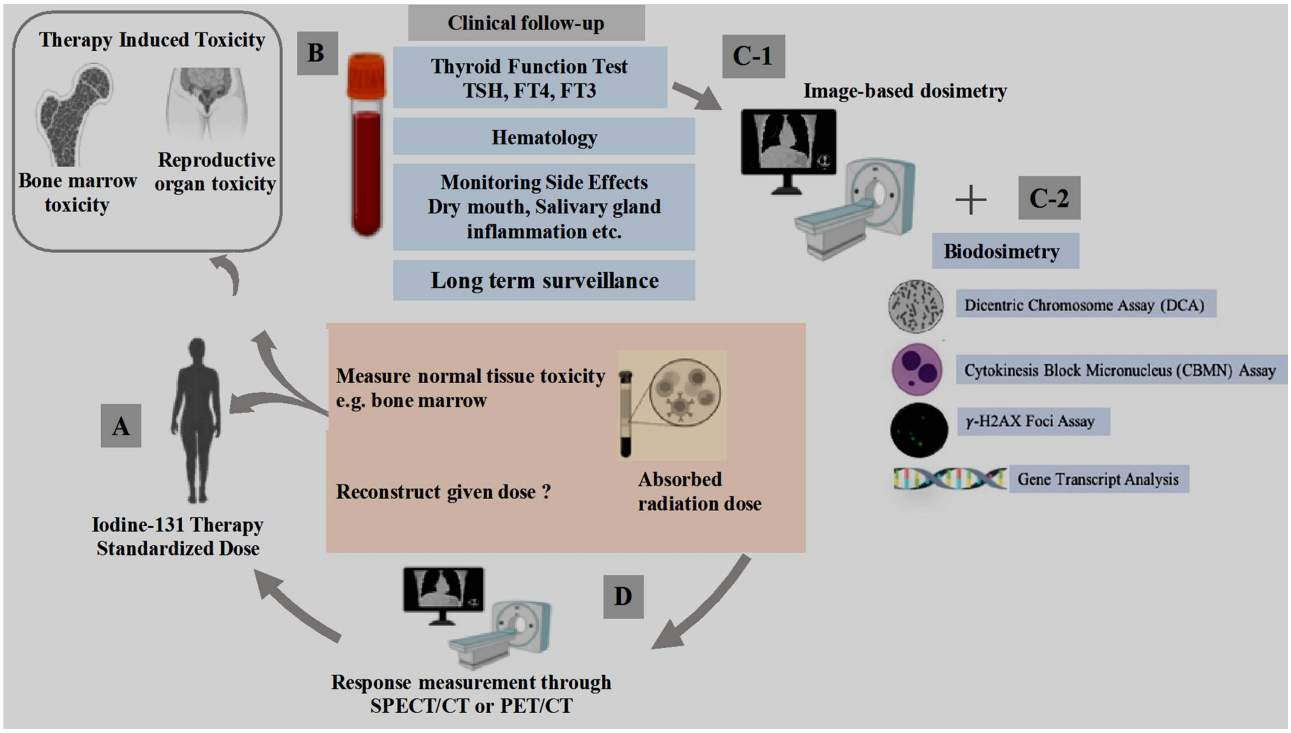


**Figure 3.** A proposed (A-D) updated scheme adding biodosimetry based investigation in Iodine-131 therapy.

## 5. Conclusions

Dosimetric biomarkers, utilizing advanced methods like genomics, proteomics, metabolomics, and EPR spectroscopy, have been effective in understanding the cellular impact of radiation, particularly in nuclear accidents and medical procedures, although there is limited radiobiology data available from the nuclear medicine clinics. This is due to the inherent characteristics of radioiodine-131 treatment procedures. Through incorporation of biodosimetry based investigation in clinical nuclear medicine trials, as shown in **Figure 3**, would increase radioiodine treatment data availability.

Multiparametric evaluations in biodosimetry are crucial for sensitivity and specificity across various exposure scenarios. Future research should focus on identifying markers for different types of radiation and internal exposure, areas where knowledge is currently limited. In radiation emergencies, rapid field-deployable technologies for blood and tissue sampling, along with comprehensive dose estimation techniques, need to integrate into emergency response strategies. Biodosimetry allows for individualized radiosensitivity assessment, which is challenging with traditional dose quantification. For radiation-based procedures, as-

sessing DNA damage through biodosimetry is crucial for long-term genotoxic effects. Gathering data on variations, confounding variables, inter-laboratory disparities, and reproducibility is essential for developing reliable dose estimation algorithms. As technology advances, full automation of cytogenetic analysis and AI-driven data analysis will likely enhance the accuracy of dose projections in biodosimetry.

## Disclosure Statement

The authors confirm that they do not have any associations or participation in any organization or entity that holds financial or non-financial interests related to the subject matter or materials discussed in this article.

## Author Contributions

Md. Ziaur Rahman conceived the idea and is responsible for drafting the initial manuscript. Safaiatul Islam and Abu Hena Mostofa Kamal also contributed to the drafting and preparation of the manuscript. A.Y.K. Md. Masud Rana and P. K. Roy provided revisions to the manuscript. All authors reviewed and approved the final version.

## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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