Estimation of the effects of medical diagnostic radiation exposure based on DNA damage

Lin Shi and Satoshi Tashiro*

Department of Cellular Biology, Research Institute for Radiation Biology Medicine, Hiroshima University, Kasumi 1-2-3,
Minamiku, Hiroshima 734-8553, Japan

*Corresponding author. Department of Cellular Biology, Research Institute for Radiation Biology and Medicine, Hiroshima University, Kasumi 1-2-3,
Minamiku, Hiroshima 734-8553, Japan. Tel: +81-82-257-5827; Fax: +81-82-256-7104; Email: ktashiro@hiroshima-u.ac.jp

ABSTRACT

X-rays are widely applied in the medical field for the diagnosis and treatment of diseases. Among the uses of X-rays in diagnosis, computed tomography (CT) has been established as one of the most informative diagnostic radiology examinations. Moreover, recent advances in CT scan technology have made this examination much easier and more informative and increased its application, especially in Japan. However, the radiation dose of CT scans is higher than that of simple X-ray examinations. Therefore, the health risk of a CT scan has been discussed in various studies, but is still controversial. Consequently, the biological and cytogenetic effects of CT scans are being analyzed. Here, we summarize the recent findings concerning the biological and cytogenetic effects of ionizing radiation from a CT scan, by focusing on DNA damage and chromosome aberrations.

Keywords: CT scan; medical radiation; DNA damage; chromosome aberration; biological dosimetry

INTRODUCTION

Applications of radiation in medicine, such as for the diagnosis of diseases or injuries, started just after the discovery of X-rays in 1895. One of the most common usages of radiation in current medical diagnosis is computed tomography (CT) using X-rays, since CT scan images provide more detailed information than that provided by simple X-ray examinations. In addition to facilitating the diagnosis of various diseases, including cancer and vascular diseases, CT scans are useful in the follow-up care of cancer patients (for estimating the effectiveness of therapy, by examining the tumor size and detecting metastasis). Recent advances in CT scanning technology now allow the acquisition of appropriate images within a short scanning time and enable the examination of children without anesthesia [1]. Due to these benefits and technological advancements, CT scans are gaining popularity around the world.

However, the use of X-rays is a double-edged sword. On the one hand, doctors can make an accurate diagnosis of diseases with the information provided by diagnostic imaging, and improve the prognosis of cancer patients through radiation therapy. On the other hand, medical radiation has become a major source of radiation exposure for the general population. Since the increased risk of leukemia and solid cancer after high-dose irradiation has been reported in various studies, including the epidemiological studies of A-bomb survivors in Hiroshima and Nagasaki, the effects of medical radiation have become a public health issue [2–4]. Several reports concerning the cancer risk after medical radiation imaging, such as CT scans, have been published. Brenner *et al.* first reported the quantitative estimates of radiation risks associated with pediatric CT [5]. In 2012 and 2013, large cohort studies of the cancer risks in people exposed to CT scans in childhood provided evidence of the association between cancer risk and CT scans [6–8]. In contrast, other studies failed to observe the association of childhood cancer risk with medical diagnostic radiation [9, 10]. Therefore, it is difficult to draw a conclusion about the health effects of medical diagnostic imaging radiation from these cohort studies [11].

Ionizing radiation induces various types of DNA damage, including DNA double-strand breaks (DSBs). The DNA damage is normally repaired by the corresponding DNA repair system. However, if mistakes are made in the repair of the damaged DNA, then the genetic information may be changed, leading to health effects such as cancer and vascular events. Therefore, accurately quantifying the amounts of DNA damage, especially DSBs, induced by ionizing radiation is important in studying the effects of medical radiation exposure. Various proteins related to the DNA damage response or DNA repair, including the phosphorylated form of histone H2AX (γ-H2AX), form 'radiation-induced foci' at sites

with DNA damage. Therefore, the analysis of γ-H2AX foci has been applied to examining the radiation effects of CT scans.

Several methods have been developed for estimating the radiation dose in the field of radiation emergency medicine. Among the biological dosimetry procedures applied in radiation emergency medicine, conventional chromosome analysis using Giemsa-stained samples to detect dicentric and ring chromosomes has been established as the gold standard [12]. However, the analysis of Giemsa-stained chromosomes is time-consuming and requires well-trained technicians. Therefore, other methods, such as the fluorescence *in situ* hybridization (FISH) technique and the micronuclei (MN) assay, have been developed to overcome the weak points of Giemsa analysis and to facilitate dose estimation.

The biological effects of low-dose medical radiation exposure, including that from CT scans, have been investigated by using these molecular biological and cytogenetic methods, normally applied in radiation emergency medicine. Here, we summarize the reports related to the effects of CT scans, one of the major radiation sources in medicine, to discuss the biological effects of medical radiation exposure on human chromosomal DNA (Fig. 1).

QUANTIFICATION OF MEDICAL IRRADIATION-INDUCED DNA DAMAGE BY THE γ-H2AX FOCI ASSAY

γ-H2AX focus formation after induction of DNA damage

The histone variant H2AX becomes phosphorylated at Ser139 by PIKK family kinases, such as ataxia telangiectasia mutated (ATM), ATR or DNA-PK, after the induction of DNA damage, and is referred to as γ-H2AX. γ-H2AX accumulates at a site containing a

DSBs to form a focus within several minutes after ionizing irradiation exposure (Fig. 1A) [13–15]. The number of γ-H2AX foci in cultured cells reaches a peak within 60 min after irradiation *in vitro* and shows a linear correlation with the exposed dose, even within the low-dose range of <100 mGy [16, 17]. Given their easy sampling and circulation throughout the body, human peripheral blood lymphocytes (PBLs) are most commonly used to assess the DNA damage induced by CT scans. Several studies have reported the increase in γ-H2AX foci in PBLs after CT scans *in vitro* and *in vivo* (Table 1). The CT dose index (CTDI), the dose length product (DLP) and the size-specific dose estimates (SSDEs) are the parameters used for estimating the dose absorbed during a CT scan. The γ-H2AX foci assay revealed that the DNA damage induced by a CT scan correlated with the CTDI, DLP and SSDE [18–20]. These findings suggest that CT scans induce DNA damage in PBLs in a dose-dependent manner.

Following the formation of γ-H2AX foci, the DNA repair pathway is activated and DNA repair proteins accumulate at sites with damaged DNA. It is commonly accepted that the disappearance of γ-H2AX foci suggests that the DNA damage has been repaired. After the peak of the γ-H2AX focus formation, ~1 h after the induction of DNA damage, the number of foci decreases and returns to almost the basal level at 24 h after radiation exposure [18, 21]. Therefore, the rapid kinetics of γ-H2AX foci formation may lead to underestimation of the amount of DNA damage induced by radiation. To avoid this underestimation, the phosphatase inhibitor calyculin A has been employed to prevent the disappearance of γ-H2AX foci. Calyculin A itself does not induce DNA damage, and a treatment with 1 nM calyculin A reportedly inhibited the decrease in γ-H2AX foci in irradiated cells [22, 23]. When compared with
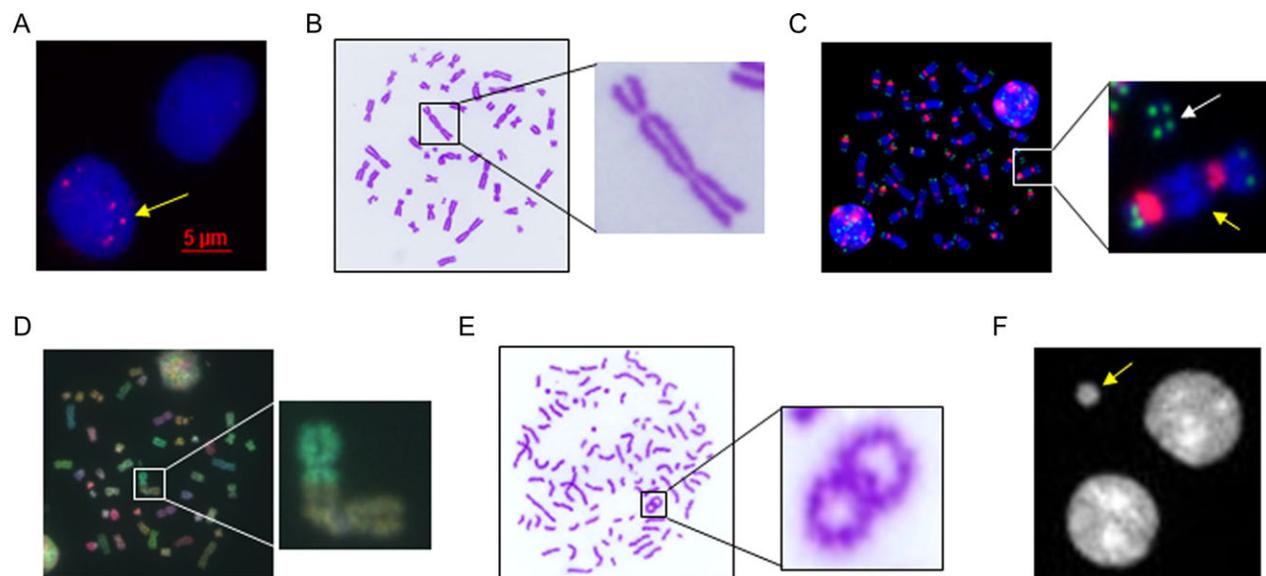

Fig. 1. Various methods used for the estimation of the biological effects of medical radiation. (A) γ-H2AX foci (yellow arrow) in PBLs after irradiation; (B) dicentric chromosome in a metaphase lymphocyte after Giemsa staining; (C) dicentric chromosome (yellow arrow) and acentric fragment (white arrow) in metaphase chromosomes after PNA-FISH using telomere/centromere probes; (D) chromosome aberration in metaphase chromosomes after multi-color FISH using chromosome painting probes; (E) PCC-ring chromosome in PBLs after irradiation; (F) MN (yellow arrow) in binucleated cells after DAPI staining.

Table 1. Overview of published reports about γ-H2AX foci formation after CT scans

Publication	Type of study	Number of patients	Radiation effect of CT	Dose-enhancing effect of contrast material (CM)	Foci count	Toxicity of CM itself
Löbrich et al., 2005; [18]	In vitro & in vivo	Adult patients: n = 23	Yes		Manual	
Rothkamm et al., 2007; [55]	in vitro & in vivo	Adult patients: n = 13	Yes		Manual	
Jost et al., 2009; [28]	In vitro		Yes	No (within diagnostic dose range)	Automatic	
Grudzenski et al., 2009; [25]	In vitro & in vivo	Adult patients; contrast-enhanced CT: n = 18; unenhanced CT: n = 19	Yes	Yes (in vitro: 33 mg/ml blood; in vivo: 27–49 g/patients)	Manual	No
Kuefner et al., 2010; [56]	In vivo	Adult patients: n = 36	Yes		Manual	
Kuefner et al., 2010; [33]	In vivo	Adult patients: n = 34	Yes		Manual	
Pathe et al., 2011; [21]	In vitro & in vivo	Contrast-enhanced CT: n = 15; unenhanced CT: n = 15	Yes	Yes (37–44.4 g/patient)	Manual	No
Geisel et al., 2012; [57]	In vivo	Adult patients: n = 56	Yes		Automatic	
Beels et al., 2012; [19]	In vitro & in vivo	Adult patients: n = 69 (contrast-enhanced CT)	Yes	No (in vitro)	Manual	No
May et al., 2012; [58]	In vivo	Adult patients: n = 33	Yes (^{18}F-Fdg PET/CT)		Manual	
Brand et al., 2012; [59]	In vivo	Adult patients: n = 66	Yes		Manual	
Kuefner et al., 2013; [23]	In vitro & in vivo	Adult patients: n = 7	Yes		Manual	
Halm et al., 2014; [34]	In vivo	Pediatric patients: n = 3	Yes		Manual	
Piechowiak et al., 2015; [26]	In vivo	Contrast-enhanced CT: n = 179; unenhanced CT: n = 66	Yes	Yes (18.651 g/patient)	Manual	
Nguyen et al., 2015; [60]	In vivo	Adult patients: n = 67 (cardiac CT angiography)	Yes		Flow cytometry & manual	
Vandevoorde et al., 2015; [17]	In vitro & in vivo	Pediatric patients: n = 51	Yes		Manual	
Fukumoto et al., 2017; [20]	In vitro & in vivo	Adult patients: n = 45	Yes		Automatic	
Wang et al., 2017; [27]	In vivo	Adult patients; contrast-enhanced CT: n = 48; unenhanced CT: n = 22	Yes	Yes (33.3 g/patient)	Manual	Yes

patients' blood treated with calyculin A, the number of CT-induced γ-H2AX foci in blood without treatment was lower by ~35% [23]. Hence, calyculin A would be useful for enhancing the accurate detection of CT-induced DNA damage by disturbing the time-dependent disappearance of γ-H2AX foci.

Enhancement of DNA damage induction by contrast media

Contrast media are used to enhance the contrast of human internal organs, structures or fluids within the body in CT imaging. Contrast media reportedly lack intrinsic genotoxicity detectable by the γ-H2AX assay [24, 25]. However, increases in CT-induced DNA damage by contrast media have been reported by several groups [16, 21, 24, 26]. In those studies, the number of radiation-induced γ-H2AX foci was associated with the concentration of the contrast media. A significant increase in radiation-induced DSBs caused by contrast media was observed when the blood concentration of iodine was >17.5 mg/ml, in an in vitro study [24]. Wang et al. reported a slight but significant increase in γ-H2AX foci in PBLs from patients after the injection of contrast media but before the CT scan [27]. However, several studies have also shown that the administration of contrast media within the pharmacologically appropriate concentration range and a radiation dose corresponding to that of a CT scan failed to enhance the induction of DNA damage [17, 19, 28].

Although further studies are required in order to clarify the enhancement of DNA damage by and the organ toxicity of contrast medium, the enhanced radiation effect associated with contrast medium may be due to the secondary electrons generated during a contrast-enhanced CT scan [29, 30]. Through dose estimation from the change in attenuation measured in Hounsfield units, some studies have revealed an increase in the radiation dose in several organs after contrast-enhanced CT as compared with un-enhanced CT [31, 32]. Therefore, the enhancement of radiation-induced DNA damage by contrast media is still controversial.

The advantages and disadvantages of the γ-H2AX foci assay

Among the various methods for detecting radiation-induced DNA damage, the γ-H2AX foci assay is one of the fastest methods, and can be completed within several hours of the collection of blood samples. Furthermore, the γ-H2AX foci assay is a sensitive method that can detect DNA damage induced by X-ray irradiation at ~1 mGy [33, 34]. Therefore, the γ-H2AX foci assay is an ideal method for analyzing the DNA damage induced by medical diagnostic radiation exposure, such as from a CT scan.

Manual quantification of the γ-H2AX foci, however, is laborious and time-consuming work. Therefore, image analysis software, such as Image J and FociCounter, is used for the quantification of γ-H2AX foci [13, 35]. Several microscope systems allow automatic image capture and quantification of foci, including the MetaCyte (MetaSystems GmbH, Germany) and In Cell Analyzer (GE Healthcare) systems [20, 36]. Although it is possible to count the numbers of γ-H2AX foci automatically using these software and microscope systems, the parameter values used to detect γ-H2AX foci, such as focus size or intensity, must be determined and implemented subjectively by

operators. This could lead to inter- or intralaboratory variations, even for the analysis of the same samples [37]. Besides this problem, differences in the quality or sensitivity of the antibodies used for the detection of γ-H2AX foci, could also affect the quantification of γ-H2AX foci. Therefore, a standardized method for detecting γ-H2AX foci, including how to prepare the internal control, should be established for the practical use of the γ-H2AX foci assay in the evaluation of DNA damage induced by medical radiation exposure.

CHROMOSOME ABERRATIONS INDUCED BY MEDICAL IRRADIATION

Misjoining of the wrong DNA ends of DSBs leads to structural abnormalities of chromosomes. Among the chromosome aberrations, dicentric and ring chromosomes are unstable abnormalities, since cells carrying these abnormalities are defective in chromosome segregation, leading to cell death. Therefore, the scoring of dicentric chromosomes in lymphocytes arrested at the first mitosis after stimulation with phytohemagglutinin (PHA) has been established as the gold standard for the estimation of radiation dose after a recent exposure. In contrast, reciprocal translocations, which can be transferred to daughter cells, are stable abnormalities persisting for many years after radiation exposure. Scoring of translocations is useful for chronic radiation exposure estimations or retrospective studies of past radiation exposure. Although the dose response of the increase in abnormal chromosomes after low-dose irradiation (<100 mGy) is still unclear, there are several reports concerning chromosome analysis for patients receiving a CT scan, in which the sensitivity of the analysis was increased by analyzing >1000 cells per sample.

Conventional Giemsa staining analysis

Conventional Giemsa staining analysis is the most common method applied for the biological dosimetry of ionizing radiation, because of the short, easy staining procedures and low cost (Fig. 1B). Using conventional Giemsa staining analysis, the increase in dicentric chromosomes after in vitro exposure of PBLs to radiation from a CT scan was observed, and the relationship between the chromosome abnormalities and the tube voltage/phantom size of the CT scanner was suggested [38]. For accurate evaluation of the radiation dose, only chromosome abnormalities in the first mitosis after the PHA stimulation of PBLs should be counted. For this purpose, the fluorescence plus Giemsa (FPG) method is used to identify the lymphocytes arrested at the first mitosis. Using the FPG staining method, a significant increase in dicentric chromosomes was observed in the lymphocytes from 10 pediatric patients <10 years old after a CT scan, while the increase in the older group of children from 10 to 15 years old was not significant [39]. This finding suggests that children <10 years old may be more sensitive to the low-dose radiation from a CT scan, as compared with older children. In contrast, a significant increase in chromosome aberrations in PBLs from adult patients after a CT scan has also been reported [40, 41]. These different results regarding the increase in chromosome abnormalities after a CT scan could be due to the radiation dose/conditions of the CT scan (Table 2). Another possibility is the interlaboratory variability in the scoring of dicentric

chromosomes using Giemsa staining [42, 43]. The identification of abnormal chromosomes by Giemsa analyses is difficult and requires skilled technicians. Therefore, the IAEA recommends that laboratories involved in radiation dosimetry establish their own dose–response curves [12]. About 10 dicentric chromosomes in 100 cells are reportedly formed after 1 Gy γ-ray irradiation of human blood *in vitro* at a dose rate of ~110 mGy/min [44]. Therefore, the scoring of >1000 cells is recommended for the estimation of radiation doses of <100 mGy, because of the low numbers of abnormal chromosomes. It would not be practical to apply the Giemsa-staining method to analyzing many samples manually for the evaluation of the effects of low-dose irradiation, including that from CT scans.

Chromosome analyses using FISH

The FISH technique can score abnormal chromosomes sensitively, easily and accurately, by marking specific DNA regions or chromosomes with different fluorescent dyes (Fig. 1C and D). Therefore, to overcome the difficulty in identifying the chromosomal aberrations in Giemsa-stained lymphocytes, the FISH technique has been applied for karyotyping. The probes used in the FISH analysis, however, are very expensive, especially those for differentially painting 24 chromosomes. This has led to widespread application of the analysis using three chromosome painting probes to estimate the numbers of chromosome translocations in biological dosimetry. FISH analysis using three chromosome painting probes failed to show an increase in translocations or chromosome aberrations in metaphase lymphocytes after a CT scan [41, 45]. This could be due to confounding factors, such as smoking and aging, which are also associated with chromosome translocations [46]. Chromosome translocations are stable abnormalities that can persist for many years, and a lifelong accumulation of chromosome translocations in hematopoietic stem cells may also make it difficult to detect the slight effects of low-dose irradiation in lymphocytes. The other possibility is the low sensitivity of the analysis, due to the detection of chromosome translocations involving only three chromosomes. To increase the accuracy of the translocation assay, FISH analyses using probes differentially painting 24 chromosomes $(22 + X + Y)$ should be performed.

PNA-FISH, using telomere and centromere PNA probes, can identify dicentric and ring chromosomes in PBLs after high-dose irradiation more easily and accurately than a Giemsa analysis [47]. Moreover, the PNA probes for centromeres and telomeres are not expensive. A telomere/centromere PNA-FISH analysis revealed an increase in dicentric chromosomes in lymphocytes from 10 patients after a CT scan [41]. Therefore, at present, the PNA-FISH analysis of dicentric and ring chromosomes could detect a slight increase in abnormal chromosomes after low-dose irradiation, such as from a CT scan. Further study is required to establish the usefulness of the PNA-FISH analysis in this point.

Assays for chromosomal aberrations require a lot of time, usually several days from the blood sample collection to the completion of the analysis, as compared with the γ-H2AX foci assay. Lymphocytes must be cultured in medium containing PHA for 48 h, for cell-cycle progression to obtain metaphase cells. The time and cost required for the staining and analyses differ quite a lot between the Giemsa and FISH staining methods. Giemsa staining is the fastest and least

expensive, but the identification and scoring of chromosome aberrations is time-consuming work. FISH using chromosome painting probes is the most expensive and requires long-term hybridization of the probe with the target DNA, usually more than 12 h. As compared with Giemsa staining, FISH using chromosome painting probes is faster, easier and more accurate for chromosome analysis. PNA-FISH overcomes the disadvantages of Giemsa staining and FISH using chromosome painting probes, due to the short hybridization time, low cost and easily identified chromosome aberrations.

Due to the low numbers of chromosome aberrations induced by low-dose radiation, including that from CT scans, the analysis of more than 1000 cells is recommended to increase the accuracy. More than 3 h are required for the analysis of 1000 metaphase chromosomes, even in the PNA-FISH analysis of dicentric and ring chromosomes. Computer-assisted microscopy, such as the Metafer microscope platform (MetaSystems, Germany), can be used to search for all of the metaphase cells on slides and obtain high-resolution images automatically. Similar to the γ-H2AX foci assay, the scoring of abnormal chromosomes can also be processed by software, such as DCScore and Ikaros. Since the detection efficiency of dicentric chromosomes by these software programs is low compared with that of human observers, dose–response curves for automatic or semi-automatic analyses are required for dose estimation [48, 49]. Automatic or semi-automatic chromosome aberration scoring has been suggested as a useful method for the rapid triage of potentially exposed individuals in a large-scale radiation accident [48, 49]. Therefore, the development of software to automatically and accurately identify abnormal chromosomes is awaited for the detection of the effects of low-dose medical irradiation.

Premature chromosome condensation

The fusion of interphase cells with mitotic cells or drug treatment can induce the condensation of chromosomes, in a phenomenon referred to as premature chromosome condensation (PCC) (Fig. 1E). The combination of PCC with the chromosome staining technique is applied to the detecting of chromosome fragments or ring chromosomes in interphase lymphocytes after ionizing radiation exposure. Since few ring chromosomes are induced by low-dose irradiation, the PCC ring assay is suited for the estimation of the radiation doses of samples after high-dose radiation, usually >10 Gy [12]. For chromosome analyses, PCC fusion using mitotic cells is the fastest method of evaluating the effects of ionizing radiation, since the chromosome aberrations can be scored within several hours after blood sampling. PCC associated with the FISH technique using painting probes seems to be more sensitive, compared with the FISH analysis of metaphase lymphocytes using three chromosome painting probes. The numbers of translocations in metaphase lymphocytes showed no significant changes after a CT scan, using the samples from the same patients, but this could be due to the increase in chromosomal fragments in interphase lymphocytes immediately after a CT scan [45].

The PCC assay requires skilled technicians, as is the case for chromosome analysis of Giemsa-stained samples. Some reports have noted the usefulness of software in the PCC analysis of samples after high-dose irradiation [50, 51]. The application of such software

Table 2. Overview of published reports about chromosome aberrations induced by CT scans

Publication	Type of study	Number of patients	Type of chromosome aberrations	Staining method	Exposed dose	Number of lymphocytes analyzed	Radiation effect of CT
M'kacher et al., 2003; [45]	In vitro & in vivo	Adult patients (n = 10)	Translocations, dicentrics, rings, insertions and acentric fragments of painted chromosomes in metaphase and chromosome fragments in interphase (PCC)	FISH (chromosome DNA probes 1, 3, 4)	In vivo: 0.057 Gy; in vitro: 0.05, 0.1, 0.2 Gy		Significant increase in chromosomal fragments (PCC) but not chromosome aberrations in metaphase
Stephan et al., 2007; [39]	In vivo	Pediatric patients (n = 10)	Dicentric (dic) & excess acentrics (ace)	Fluorescence plus Giemsa (FPG) staining	Mean dose to blood: 12.9 mGy	>20 000 cells (average: 1000 cells/subject)	Yes (only in patients <10 years old)
Jost et al., 2009; [28]	In vitro		Dicentric	Fluorescence plus Giemsa (FPG) staining	0.025, 0.05, 0.1, 1 Gy	>1000 metaphase/subject (0, 0.025, 0.05, 0.1 Gy); >200 metaphase for subjects after 1 Gy IR	Yes
Golfier et al., 2009; [61]	In vitro		Dicentric	Fluorescence plus Giemsa (FPG) staining	0.025, 0.05, 0.1, 1 Gy	>1000 metaphase/subject (0, 0.025, 0.05, 0.1 Gy); >200 metaphase for subjects after 1 Gy IR	Yes
Abe et al., 2015; [41]	In vivo	Adult patients (n = 10)	Dicentric	Giemsa staining & PNA-FISH	5.78–60.27 mSv: mean 24.24 mSv	2000 metaphase/subject	Yes
Kanagaraj et al., 2015 [40]	In vivo	Adult patients (contrast scan: n = 14; brain plain scan: n = 13)	Chromosome aberrations (dicentric, chromosome/chromatid break) & MN	Giemsa staining	Eye: 2~520 mGy; forehead: 0.84–210 mGy; thyroid: 1.79~ 185	250–300 metaphase/subject (chromosome aberrations); 1000 binucleated cells (MN)	Yes
Abe et al., 2016; [62]	In vivo	Adult patients (n = 12)	Translocation	FISH (chromosome painting DNA probes 1, 2, 4)	5.78–60.27 mSv: mean 24.24 mSv	>5000 metaphase/subject	No significant increase in translocation after CT scan

for studies of the effects of low-dose irradiation, such as that from a CT scan, is awaited.

MN assay

MN are considered to result from a lagging acentric chromosome, or chromosome aberrations not included in the daughter nuclei, due to failed attachment to the spindle during the segregation of chromosomes in anaphase. The cell cycle progression of PHA-stimulated lymphocytes can be blocked at the stage of cytokinesis after the first mitosis by using cytochalasin B, for the efficient detection of MN. Therefore, the cytokinesis-blocked MN (CBMN) assay has been established as a useful and sensitive method for the quantification of radiation effects on chromosomal DNA (Fig. 1F). A significant increase in MN has been reported in lymphocytes from patients undergoing plain or contrast CT scans by the CBMN assay [40]. Computer-assisted microscopy and software are also used in the MN assay [52, 53]. Lyulko *et al.* reported the automatic generation of dose–response curves of MN by using the RABiT image analysis software, and the numbers of MN scored correlated well with the manual scoring results [54]. The automatic MN assay, however, has not been applied for evaluation of the radiation effects of CT scans.

SUMMARY

Adverse health effects from low-dose irradiation by a CT scan, especially possible associations with cancer risk, have been suggested, but such effects remain controversial. However, the induction of DNA damage by a CT scan examination has been clearly demonstrated by the γ-H2AX foci assay. Several reports have also suggested the induction of chromosome aberrations after a CT scan, using conventional chromosome analysis methods, but the technical difficulty of chromosome analysis has prevented the large-scale study required for evaluation of the effects of low-dose irradiation by diagnostic exposure. Recent advances in microscopy and image analysis will overcome this technical difficulty of chromosome analysis, by enabling high-throughput analysis of the minor increase in chromosome aberrations after low-dose radiation exposure. Moreover, recent advancements in biomedical science, such as the development of next-generation sequencers, will also make it possible to perform high-throughput analyses of genome information. Therefore, the combined applications of these new technologies will enable a breakthrough in understanding the biological and cytogenetic effects of low-dose irradiation, including CT scan examinations, in the near future. The development of these new technologies will also facilitate the establishment of precision medicine, by providing information about an individual's genome instability.

ACKNOWLEDGEMENTS

CONFLICT OF INTEREST

The authors declare that there are no competing interests.

FUNDING

REFERENCES

1. Brenner DJ, Hall EJ. Computed tomography—an increasing source of radiation exposure. *N Engl J Med* 2007;357:2277–84.
2. Preston DL, Shimizu Y, Pierce DA et al. Studies of mortality of atomic bomb survivors. Report 13: solid cancer and noncancer disease mortality: 1950–1997. *Radiat Res* 2003;160:381–407.
3. Preston DL, Ron E, Tokuoka S et al. Solid cancer incidence in atomic bomb survivors: 1958–1998. *Radiat Res* 2007;168:1–64.
4. Preston DL, Pierce DA, Shimizu Y et al. Effect of recent changes in atomic bomb survivor dosimetry on cancer mortality risk estimates. *Radiat Res* 2004;162:377–89.
5. Brenner D, Elliston C, Hall E et al. Estimated risks of radiation-induced fatal cancer from pediatric CT. *Am J Roentgenol* 2001;176:289–96.
6. Pearce MS, Salotti JA, Little MP et al. Radiation exposure from CT scans in childhood and subsequent risk of leukaemia and brain tumours: a retrospective cohort study. *Lancet* 2012;380:499–505.
7. Mathews JD, Forsythe AV, Brady Z et al. Cancer risk in 680,000 people exposed to computed tomography scans in childhood or adolescence: data linkage study of 11 million Australians. *BMJ* 2013;346:f2360.
8. de Gonzalez AB, Salotti JA, McHugh K et al. Relationship between paediatric CT scans and subsequent risk of leukaemia and brain tumours: assessment of the impact of underlying conditions. *Br J Cancer* 2016;114:388–94.
9. Hammer GP, Seidenbusch MC, Schneider K et al. A cohort study of childhood cancer incidence after postnatal diagnostic X-ray exposure. *Radiat Res* 2009;171:504–12.
10. Hammer GP, Seidenbusch MC, Regulla DF et al. Childhood cancer risk from conventional radiographic examinations for selected referral criteria: results from a large cohort study. *Am J Roentgenol* 2011;197:217–23.
11. Costello JE, Cecava ND, Tucker JE et al. CT radiation dose: current controversies and dose reduction strategies. *Am J Roentgenol* 2013;201:1283–90.

12. IAEA. *Ctogenetic Dosimetry: Applications in Preparedness for and Response to Radiation Emergencies.* Vienna: International Atomic Energy Agency, 2011.

13. Jucha A, Wegierek-Ciuk A, Koza Z et al. FociCounter: a freely available PC programme for quantitative and qualitative analysis of gamma-H2AX foci. *Mutat Res* 2010;696:16–20.

14. Franken NA, ten Cate R, Krawczyk PM et al. Comparison of RBE values of high-LET alpha-particles for the induction of DNA-DSBs, chromosome aberrations and cell reproductive death. *Radiat Oncol* 2011;6:64.

15. Horn S, Barnard S, Rothkamm K Gamma-H2AX-based dose estimation for whole and partial body radiation exposure. *PLoS One* 2011;6:e25113.

16. Grudzenski S, Raths A, Conrad S et al. Inducible response required for repair of low-dose radiation damage in human fibroblasts. *Proc Natl Acad Sci U S A* 2010;107:14205–10.

17. Vandevoorde C, Gomolka M, Roessler U et al. EPI-CT: *in vitro* assessment of the applicability of the gamma-H2AX-foci assay as cellular biomarker for exposure in a multicentre study of children in diagnostic radiology. *Int J Radiat Biol* 2015;91:653–63.

18. Lobrich M, Rief N, Kuhne M et al. *In vivo* formation and repair of DNA double-strand breaks after computed tomography examinations. *Proc Natl Acad Sci U S A* 2005;102:8984–9.

19. Beels L, Bacher K, Smeets P et al. Dose-length product of scanners correlates with DNA damage in patients undergoing contrast CT. *Eur J Radiol* 2012;81:1495–9.

20. Fukumoto W, Ishida M, Sakai C et al. DNA damage in lymphocytes induced by cardiac CT and comparison with physical exposure parameters. *Eur Radiol* 2017;27:1660–6.

21. Pathe C, Eble K, Schmitz-Beuting D et al. The presence of iodinated contrast agents amplifies DNA radiation damage in computed tomography. *Contrast Media Mol Imaging* 2011;6:507–13.

22. Antonelli F, Belli M, Cuttone G et al. Induction and repair of DNA double-strand breaks in human cells: dephosphorylation of histone H2AX and its inhibition by calyculin A. *Radiat Res* 2005;164:514–7.

23. Kuefner MA, Brand M, Engert C The effect of calyculin A on the dephosphorylation of the histone γ-H2AX after formation of X-ray–induced DNA double-strand breaks in human blood lymphocytes. *Int J Radiat Biol* 2013;89:424–32.

24. Gould R, McFadden SL, Horn S et al. Assessment of DNA double-strand breaks induced by intravascular iodinated contrast media following *in vitro* irradiation and *in vivo*, during paediatric cardiac catheterization. *Contrast Media Mol Imaging* 2016;11:122–9.

25. Grudzenski S, Kuefner MA, Heckmann MB et al. Contrast medium-enhanced radiation damage caused by CT examinations. *Radiology* 2009;253:706–14.

26. Piechowiak EI, Peter JF, Kleb B et al. Intravenous iodinated contrast agents amplify DNA radiation damage at CT. *Radiology* 2015;275:692–7.

27. Wang L, Li Q, Wang XM et al. Enhanced radiation damage caused by iodinated contrast agents during CT examination. *Eur J Radiol* 2017;92:72–7.

28. Jost G, Golfier S, Pietsch H et al. The influence of x-ray contrast agents in computed tomography on the induction of dicentrics and gamma-H2AX foci in lymphocytes of human blood samples. *Phys Med Biol* 2009;54:6029–39.

29. Hill MA. The variation in biological effectiveness of X-rays and gamma rays with energy. *Radiat Prot Dosimetry* 2004;112:471–81.

30. Kraft G, Krämer M, Scholz M. LET, track structure and models. *Radiat Environ Biophys* 1992;31:161–80.

31. Amato E, Lizio D, Settineri N et al. A method to evaluate the dose increase in CT with iodinated contrast medium. *Med Phys* 2010;37:4249–56.

32. Amato E, Salamone I, Naso S et al. Can contrast media increase organ doses in CT examinations? A clinical study. *Am J Roentgenol* 2013;200:1288–93.

33. Kuefner MA, Hinkmann FM, Alibek S et al. Reduction of X-ray induced DNA double-strand breaks in blood lymphocytes during coronary CT angiography using high-pitch spiral data acquisition with prospective ECG-triggering. *Invest Radiol* 2010;45:182–7.

34. Halm BM, Franke AA, Lai JF et al. γ-H2AX foci are increased in lymphocytes *in vivo* in young children 1 h after very low-dose X-irradiation: a pilot study. *Pediatr Radiol* 2014;44:1310–17.

35. Cai Z, Vallis KA, Reilly RM. Computational analysis of the number, area and density of γ -H2AX foci in breast cancer cells exposed to ^{111}In-DTPA-hEGF or gamma-rays using Image-J software. *Int J Radiat Biol* 2009;85:262–71.

36. Moquet J, Barnard S, Staynova A et al. The second gamma-H2AX assay inter-comparison exercise carried out in the framework of the European biodosimetry network (RENEB). *Int J Radiat Biol* 2017;93:58–64.

37. Barnard S, Ainsbury EA, Al-hafidh J et al. The first gamma-H2AX biodosimetry intercomparison exercise of the developing European biodosimetry network RENEB. *Radiat Prot Dosimetry* 2015;164:265–70.

38. Jost G, Lengsfeld P, Voth M et al. The influence of tube voltage and phantom size in computed tomography on the dose–response relationship of dicentrics in human blood samples. *Phys Med Biol* 2010;55:3237–48.

39. Stephan G, Schneider K, Panzer W et al. Enhanced yield of chromosome aberrations after CT examinations in paediatric patients. *Int J Radiat Biol* 2007;83:281–7.

40. Kanagaraj K, Abdul Syed Basheerudeen S, Tamizh Selvan G et al. Assessment of dose and DNA damages in individuals exposed to low dose and low dose rate ionizing radiations during computed tomography imaging. *Mutat Res Genet Toxicol Environ Mutagen* 2015;789–790:1–6.

41. Abe Y, Miura T, Yoshida MA et al. Increase in dicentric chromosome formation after a single CT scan in adults. *Sci Rep* 2015;5:13882.

42. Lloyd DC, Edwards AA, Prosser JS et al. A collaborative exercise on cytogenetic dosimetry for simulated whole and partial body accidental irradiation. *Mutat Res* 1987;179:197–208.

43. Lloyd DC, Edwards AA, Leonard A et al. Chromosomal aberrations in human lymphocytes induced *in vitro* by very low doses of X-rays. *Int J Radiat Biol* 1992;61:335–43.

44. Barquinero JF, Barrios L, Caballin MR et al. Establishment and validation of a dose–effect curve for gamma-rays by cytogenetic analysis. *Mutat Res* 1995;326:65–9.

45. M'Kacher R, Violot D, Aubert B et al. Premature chromosome condensation associated with fluorescence *in situ* hybridisation

detects cytogenetic abnormalities after a CT scan: evaluaton of the low-dose effect. *Radiat Prot Dosimetry* 2003;103:35–40.

46. Ramsey MJ, Moore DH II, Briner JF et al. The effects of age and lifestyle factors on the accumulation of cytogenetic damage as measured by chromosome painting. *Mutat Res* 1995;338:95–106.

47. Shi L, Fujioka K, Sun J et al. A modified system for analyzing ionizing radiation–induced chromosome abnormalities. *Radiat Res* 2012;177:533–8.

48. Vaurijoux A, Gruel G, Pouzoulet F et al. Strategy for population triage based on dicentric analysis. *Radiat Res* 2009;171:541–8.

49. Romm H, Ainsbury E, Barnard S et al. Automatic scoring of dicentric chromosomes as a tool in large scale radiation accidents. *Mutat Res* 2013;756:174–83.

50. Gonzalez JE, Romero I, Gregoire E et al. Biodosimetry estimation using the ratio of the longest:shortest length in the premature chromosome condensation (PCC) method applying autocapture and automatic image analysis. *J Radiat Res* 2014;55:862–5.

51. M'Kacher R, El Maalouf E, Terzoudi G et al. Detection and automated scoring of dicentric chromosomes in nonstimulated lymphocyte prematurely condensed chromosomes after telomere and centromere staining. *Int J Radiat Oncol Biol Phys* 2015;91:640–9.

52. Castelain P, Van Hummelen P, Deleener A et al. Automated detection of cytochalasin-B blocked binucleated lymphocytes for scoring micronuclei. *Mutagenesis* 1993;8:285–93.

53. Depuydt J, Baeyens A, Barnard S et al. RENEB intercomparison exercises analyzing micronuclei (Cytokinesis-block Micronucleus Assay). *Int J Radiat Biol* 2017;93:36–47.

54. Lyulko OV, Garty G, Randers-Pehrson G et al. Fast image analysis for the micronucleus assay in a fully automated high-throughput biodosimetry system. *Radiat Res* 2014;181:146–61.

55. Rothkamm K, Balroop S, Shekhdar J et al. Leukocyte DNA damage after multi-detector row CT: a quantitative biomarker of low-level radiation exposure. *Radiology* 2007;242:244–51.

56. Kuefner MA, Grudzenski S, Hamann J et al. Effect of CT scan protocols on x-ray–induced DNA double-strand breaks in blood lymphocytes of patients undergoing coronary CT angiography. *Eur Radiol* 2010;20:2917–24.

57. Geisel D, Zimmermann E, Rief M et al. DNA double-strand breaks as potential indicators for the biological effects of ionising radiation exposure from cardiac CT and conventional coronary angiography: a randomised, controlled study. *Eur Radiol* 2012;22:1641–50.

58. May MS, Brand M, Wuest W et al. Induction and repair of DNA double-strand breaks in blood lymphocytes of patients undergoing ^{18}F-FDG PET/CT examinations. *Eur J Nucl Med Mol Imaging* 2012;39:1712–9.

59. Brand M, Sommer M, Achenbach S et al. X-ray induced DNA double-strand breaks in coronary CT angiography: comparison of sequential, low-pitch helical and high-pitch helical data acquisition. *Eur J Radiol* 2012;81:e357–62.

60. Nguyen PK, Lee WH, Li YF et al. Assessment of the radiation effects of cardiac CT angiography using protein and genetic biomarkers. *JACC Cardiovasc Imaging* 2015;8:873–84.

61. Golfier S, Jost G, Pietsch H et al. Dicentric chromosomes and γ-H2AX foci formation in lymphocytes of human blood samples exposed to a CT scanner: a direct comparison of dose response relationships. *Radiat Prot Dosimetry* 2009;134:55–61.

62. Abe Y, Miura T, Yoshida MA et al. Analysis of chromosome translocation frequency after a single CT scan in adults. *J Radiat Res* 2016;57:220–6.