Radiology

Intravenous Iodinated Contrast Agents Amplify DNA Radiation Damage at CT[1]

Eike I. Piechowiak, MD
Jan-Friedrich W. Peter, MD
Beate Kleb, RT
Klaus J. Klose, MD, PhD
Johannes T. Heverhagen, MD, PhD

Purpose: To determine the effect of the use of iodinated contrast agents on the formation of DNA double-strand breaks during chest computed tomography (CT).

Materials and Methods: This study was approved by the institutional review board, and written informed consent was obtained from all patients. This single-center study was performed at a university hospital. A total of 179 patients underwent contrast material–enhanced CT, and 66 patients underwent unenhanced CT. Blood samples were taken from these patients prior to and immediately after CT. In these blood samples, the average number of phosphorylated histone H2AX (γH2AX) foci per lymphocyte was determined with fluorescence microscopy. Significant differences between the number of foci that developed in both the presence and the absence of the contrast agent were tested by using an independent sample *t* test.

Results: γH2AX foci levels were increased in both groups after CT. Patients who underwent contrast-enhanced CT had an increased amount of DNA radiation damage (mean increase ± standard error of the mean, 0.056 foci per cell ± 0.009). This increase was 107% ± 19 higher than that in patients who underwent unenhanced CT (mean increase, 0.027 foci per cell ± 0.014).

Conclusion: The application of iodinated contrast agents during diagnostic x-ray procedures, such as chest CT, leads to a clear increase in the level of radiation-induced DNA damage as assessed with γH2AX foci formation.

[1]From the Department of Diagnostic Radiology, Philipps University, Marburg, Germany (E.I.P., J.F.W.P., B.K., K.J.K., J.T.H.); and Department for Diagnostic, Interventional and Pediatric Radiology, Inselspital, Freiburgstr 10, 3010 Bern, Switzerland (E.I.P., J.T.H.). Received December 30, 2013; revision requested February 7, 2014; revision received August 14; accepted September 10; final version accepted November 20. **Address correspondence to** J.T.H. (e-mail: *Johannes.Heverhagen@insel.ch*).

X-rays, discovered in 1895 by W.C. Roentgen, have enabled many important diagnostic and therapeutic inventions, including computed tomography (CT) and angiography (1). However, currently, approximately 17% of the total annual exposure of humans to ionizing radiation is contributed by diagnostic x-ray procedures. Thus, these procedures are the largest man-made source of ionizing radiation exposure to humans (2–4). In addition, CT provides the largest contribution to medical radiation doses (2,4,5). Because of x-ray exposure, the risk of developing cancer is increased as a result of additional DNA damage, primarily in the form of DNA double-strand breaks (5).

In addition, investigations regularly require the application of iodinated contrast agents. While their nephrotoxic and cytotoxic effects are widely known, no effect on the DNA is considered in daily routine (6–11). However, it has been shown in small subject groups of five to 37 patients that these contrast agents also increase the radiation-induced DNA damage after CT or angiographic treatment (12–14). To analyze this damage, measurement of the phosphorylated histone H2AX (γH2AX) foci is an easily applicable approach, enabling one to enumerate the induction of double-strand breaks due to irradiation (12,15,16).

The purpose of this study was to evaluate the effect of iodinated contrast agents on the development of DNA double-strand breaks in patients undergoing diagnostic chest CT examinations in a larger population then in prior studies.

Advance in Knowledge

- The use of iodinated contrast agents during chest CT significantly increases the amount of DNA radiation damage by 107% ± 19 (standard error of the mean) when compared with the unenhanced scans (mean increase, 0.056 foci per cell ± 0.009 vs 0.027 foci per cell ± 0.014).

Materials and Methods

This prospective study was approved by the institutional review board of Marburg University, and written informed consent was obtained from all patients prior to the study procedures.

CT Examination

A total of 245 patients (96 women, 149 men; mean age, 64 years ± 14 [standard deviation]; age range, 19–89 years) who were scheduled to undergo diagnostic chest CT were prospectively included in the study. The recruitment of patients ran from December 2009 to October 2011. Scheduled patients were not considered for the study if they currently had leukemia or lymphoma or if they had had leukemia or lymphoma in the past, if they had undergone radiation therapy within the past 6 months, if they had undergone diagnostic x-ray or nuclear medicine studies within 3 days, or if they were younger than 18 years. The patient group that received additional contrast media did not undergo unenhanced scanning prior to contrast material application. Each patient (Table 1) underwent clinically indicated CT with a Sensation 64 dual-source 64-detector CT unit (Siemens Medical Solutions, Forchheim, Germany). Sixty-six patients (21 women, 45 men; mean age, 64 years ± 17) underwent unenhanced CT, while 179 patients (75 women, 104 men; mean age, 64 years ± 13) underwent contrast material–enhanced CT after intravenous administration of an average of 18.651 mg iodine (62 mL, Ultravist 300; Bayer Vital, Leverkusen, Germany). The following imaging parameters were used: tube voltage, 120 kVp; rotation time, 0.5 second, and matrix, 512 × 512. The dose-length product, which was calculated as the product of the volumetric CT dose index and the scan length, was recorded for every patient. In addition, to correct for differences in radiation doses, the number of foci was standardized to a reference dose of 312 mGy·cm for each patient, reflecting the reported linear relationship between foci per cell and irradiation dose (15,17).

Sample Processing and Lymphocyte Separation

Blood samples were taken from patients from the cubital or dorsum mani vein immediately before and immediately after CT examinations. At the previously mentioned time points, 5 mL of blood was collected in heparin-containing vials at 37°C, diluted at a 1:1 ratio with phosphate-buffered saline (PBS), and processed immediately.

Lymphocyte separation was performed by using Ficoll density-gradient centrifugation with lymphocyte separation medium 1077 (PAA Laboratories, Pasching, Austria) (18). In addition, 3 mL of lymphocyte separation medium in a 50-mL centrifuge tube (Sarstedt, Nümbrecht, Germany) was covered with 3–8 mL of diluted blood and centrifuged at 1000 *g* for 10 minutes at room temperature. For deceleration, the centrifuge brake was not used. Lymphocytes from

Implications for Patient Care

- The potential biologic effect of radiation exposure is altered through additional contrast media in CT examinations.
- Despite the unknown effect of γH2AX foci on cancer risk, the application of contrast agents should be carefully considered.

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Abbreviations:
γH2AX = phosphorylated histone H2AX
PBS = phosphate-buffered saline

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Conflicts of interest are listed at the end of this article.

See also Berrington de Gonzalez and Kleinerman in this issue.

Table 1

Patient Characteristics and Dose Parameters

Characteristic	Unenhanced CT ($n = 66$)	Contrast-enhanced CT ($n = 179$)	*P* Value
No. of patients			NA
Male	21 (32)	75 (42)	
Female	45 (68)	104 (58)	
Age (y)			NA
Male	68.9 ± 15.5	63.7 ± 14.2	
Female	61.2 ± 17.2	64.8 ± 12.2	
Age range (y)			NA
Male	31–88	31–89	
Female	21–83	19–89	
Age (y)	64 ± 17	64 ± 13	.78
Height (cm)	170 ± 9	172 ± 9	.10
Weight (kg)	84 ± 16	79 ± 18	.08
Volumetric CT dose index (mGy)	9.4 ± 3.5	8.1 ± 3.1	.007
Dose-length product (mGy · cm)	342 ± 116	301 ± 120	.02
Scan length (cm)	36.7 ± 3.8	37.0 ± 3.4	.26

Note.—Unless otherwise indicated, data are mean ± standard error of the mean. Data in parentheses are percentages. NA = not applicable.

the interphase were washed three times in 10 mL of PBS at 250 *g* for 10 minutes, spotted onto slides, and left to dry for 10 minutes.

Immunofluorescence and Fluorescence Microscopy

The lymphocytes were fixed for 20 minutes in 100% methanol at −20°C and for 1 minute in acetone. The cells were washed in PBS three times for 10 minutes each. The samples were made permeable with 1% octoxinol (Triton X-100; Merck Chemicals, Darmstadt, Germany) in PBS for 30 minutes at room temperature and blocked with 10% fetal bovine serum in PBS for 60 minutes at room temperature. The cells were washed in PBS three times for 10 minutes each, incubated with anti-phospho-histone H2A.X (Ser139) antibody (clone JBW301; Millipore, Schwalbach am Taunus, Germany) at a 1:400 dilution in PBS with 1% fetal bovine serum overnight at 4°C, washed in PBS three times for 10 minutes each, and incubated with antimouse immunoglobulin G (H + L) antibody ($F[ab']_2$ fragment, Alexa Fluor 488 conjugate; Cell Signaling Technology, Danvers, Mass) at a 1:400 dilution for 1 hour at room temperature. The cells were then washed in PBS three times for 10 minutes each and mounted by using Roti-Mount FuorCare with 4',6-Diamidin-2-phenylindol mounting medium (Carl Roth, Karlsruhe, Germany) (16,19).

Fluorescence images were obtained by using an AxioObserver Z1 fluorescence microscope (Carl Zeiss, Jena, Germany) equipped with a charge-coupled device camera and AxioVision software. For the quantitative analysis, foci were counted by eye with original magnification (×630). Monocytes and granulocytes were identified by using morphologic criteria and were excluded from the analysis. For each in vivo data point, cell counting was performed independently in two different areas on duplicate slides until at least 200 cells and 200 foci were counted each time (16,19).

Statistical Analysis

Data are displayed as mean ± standard error of the mean. The baseline numbers of γH2AX foci were subtracted from the postirradiation values to correct for intraindividual variances in the baseline foci numbers between patients. The statistical difference between the number of foci in patients (with and without administration of contrast agents) was tested by using an independent sample *t* test. These differences were considered significant when the *P* value was less than .05. All calculations were performed by using SPSS software (version 14.0; SPSS, Chicago, Ill).

Results

The patient group exhibited no significant differences ($P > .05$) in patient characteristics (Table 1).

The radiation dose parameters (Table 1) were significantly different between patient groups (dose-length product for unenhanced CT, 342 mGy · cm ± 116; dose-length product for contrast-enhanced CT, 301 mGy · cm ± 120; $P = .02$). The mean dose-length product was 312 mGy · cm ± 120 for the entire patient cohort.

Prior to CT scanning, both groups showed similar levels of γH2AX foci per cell (Fig 1). The group undergoing unenhanced CT had 0.073 foci per cell ± 0.002 compared with 0.071 foci per cell ± 0.001 in the contrast-enhanced group (Table 2, Fig 2). No significant difference was detected between the two groups ($P = .94$).

Immediately after CT scanning, the number of γH2AX foci increased significantly for both groups ($P < .03$). The patients who underwent contrast-enhanced CT showed an additional 0.056 foci per cell ± 0.009 (Table 2; Fig 2). This increase was 107% higher than that in the patients who underwent unenhanced CT and showed an additional 0.027 foci per cell ± 0.014 (Table 2, Fig 2). This difference was not significant ($P = .44$).

The standardization to a mean radiation dose of 312 mGy · cm resulted in an even greater difference in foci per cell after CT scanning (Table 3). The group that underwent unenhanced CT showed 0.093 foci per cell ± 0.015, while the contrast-enhanced group exhibited 0.149 foci per cell ± 0.015. This difference resulted in an increase of 0.021 foci per cell ± 0.013 in the group that underwent unenhanced CT and an increase of 0.077 foci per cell ± 0.012

Figure 1

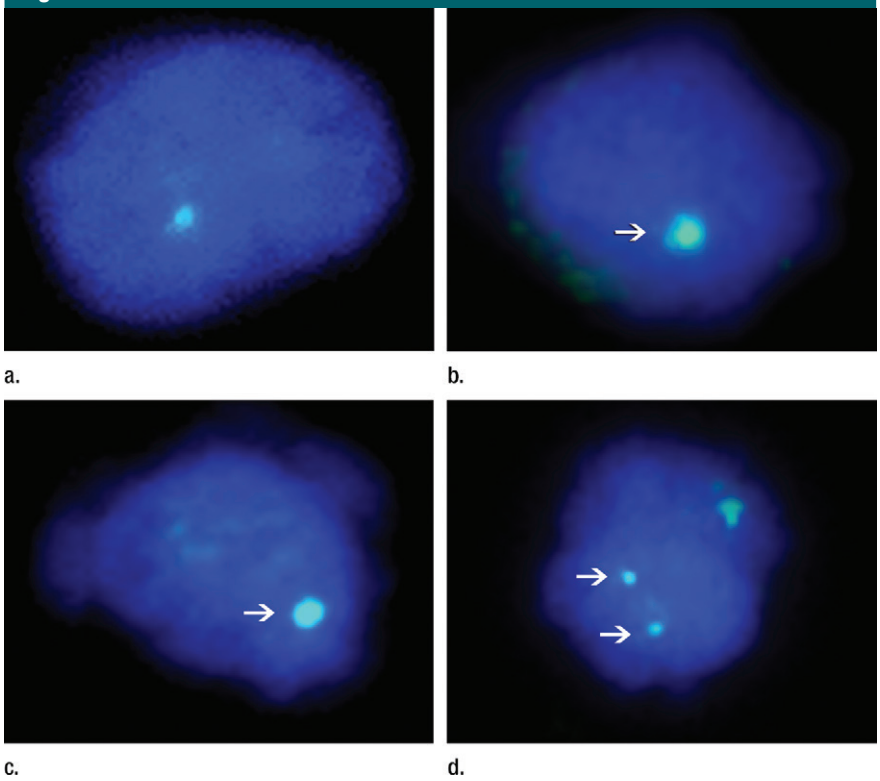

Figure 1: Peripheral blood lymphocytes exhibit increased γH2AX foci formation after CT in the presence of contrast agent. The significantly higher increase in γH2AX foci (arrows) when an iodinated contrast agent is present is shown. **(a, b)** Before CT scanning, the baseline foci levels are low. **(c, d)** After CT scanning, the number of foci increases in the absence (**c**) and presence (**d**) of iodinated contrast agents. Foci levels in the same individuals before and after CT scanning are shown without (**a**, **c**) and with (**b**, **d**) the application of an iodinated contrast agent. The increase is significantly higher ($P < .05$) when the contrast agent is present.

Table 2

Mean Foci per Cell in Patients before and after CT Scanning

Patient Group	Unenhanced CT	Contrast-enhanced CT	*P* Value
Before CT	0.073 ± 0.015	0.071 ± 0.009	.94
After CT	0.100 ± 0.016*	0.128 ± 0.012*	.09
Difference	0.027 ± 0.014	0.056 ± 0.009	.44

Note.—Data are mean ± standard error of the mean.

* Significant increase compared to values before CT (unenhanced CT, $P = .03$; contrast-enhanced CT, $P = .00$).

in the group that underwent contrast-enhanced CT. This increase was 267% higher for the contrast-enhanced group. The difference was significant ($P = .001$).

Discussion

Recent reports about the influence of iodinated contrast agents on DNA damage during CT scanning (12,15,20) led us to study this influence in vivo in a large cohort of patients undergoing chest CT.

It is estimated that in the United States, approximately 6000 new cancer cases per year can be ascribed to the radiation applied during diagnostic procedures, such as CT, angiography, and conventional x-ray radiography (2–5,10,21). In addition, the direct cytotoxic effects of the iodinated contrast agents that are used in these procedures are well known (6,8,9,11). Some reports have shown that the presence of iodinated contrast agents can enhance the number of x-ray–induced DNA double-strand breaks in blood lymphocytes (12–14). However, the only investigations performed have been in vitro or in small patient cohorts (25–37 subjects) (12–14). Our goal was to investigate the effects of the application of iodinated contrast agents on the DNA damage caused by diagnostic x-ray procedures in a large patient cohort by using a standardized irradiation procedure (chest CT).

Our findings show that the presence of iodinated contrast agents during irradiation increases the number of induced γH2AX foci by approximately 107%. This increase is most likely caused by the generation of additional secondary electrons when x-rays are absorbed by the contrast agent. Because of their high density, iodinated contrast agents absorb more x-rays than do human soft tissues (10,22,23). In addition, the generation of secondary electrons is strongly dependent on the density of the absorbing material. Thus, these effects are synergistic, and the generation of secondary electrons is even more pronounced. These secondary electrons could potentially be the major cause of x-ray–induced DNA damage (24,25).

In medical practice, thousands of diagnostic x-ray procedures are performed each day by using iodinated contrast agents. Until now, precautions for the use of contrast agents have mostly been related to preventing allergic reactions and kidney damage caused by nephrotoxicity (26–28). Our study showed that applying iodinated contrast agents also increased DNA damage in peripheral blood lymphocytes because secondary electrons were generated when x-ray radiation interacted with the contrast agent. This result has two primary future implications: First, the potential risks of x-ray radiation cannot simply be assessed with dosimetry or by measuring the dose-length

Figure 2

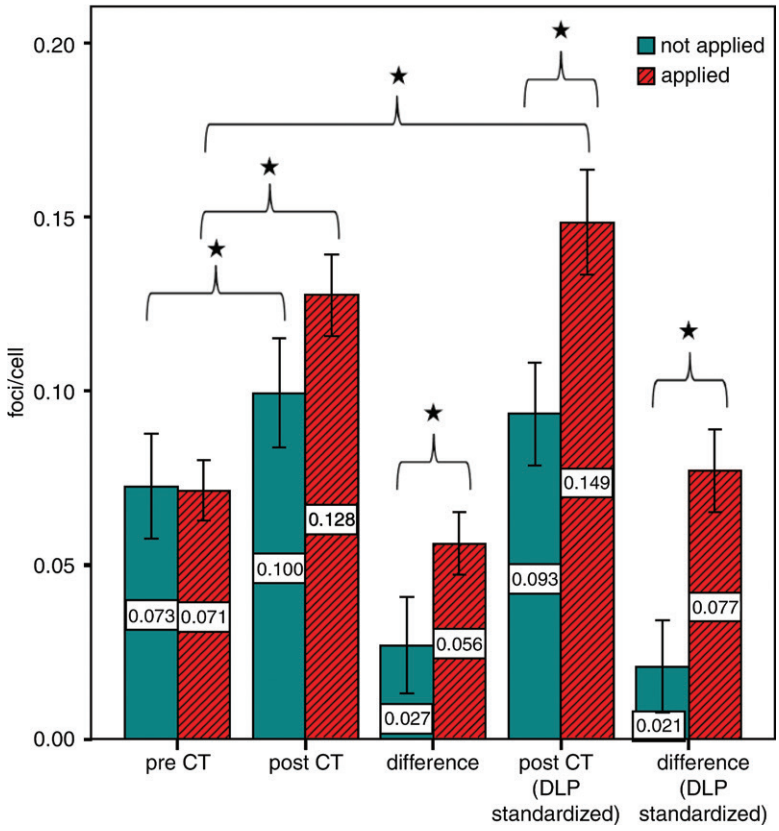

Figure 2: Differences in γH2AX foci numbers between patient groups. The differences in the mean numbers of γH2AX foci in patients undergoing unenhanced and contrast-enhanced CT are displayed. Error bars represent the standard error of the mean. The mean number of foci before and after CT and the differences between the groups are outlined. In addition, the standardized values were corrected to a dose-length product of 312 mGy · cm, and the baseline difference is shown. In the presence of the iodinated contrast agent, more γH2AX foci are generated during CT scanning. The standardization enhances this effect. ∗ = a significant difference between groups.

Table 3

Mean Foci per Cell in Patients after Standardization to a Reference Dose of 312 mGy/cm

Patient Group	Unenhanced CT	Contrast-enhanced CT	*P* Value
Before CT	0.073 ± 0.015	0.071 ± 0.009	.94
After CT	0.093 ± 0.015*	0.149 ± 0.015*	.005
Difference	0.021 ± 0.013	0.077 ± 0.012	.001

Note.—Data are mean ± standard error of the mean.

* Significant increase compared to values before CT (unenhanced CT, $P = .03$; contrast-enhanced CT, $P = .00$).

product with CT. In addition, the effect of administered iodinated contrast material should be considered. This may be assessed through evaluation of postexamination γH2AX foci. Second, the application of iodinated contrast agents in x-ray examinations should be considered even more carefully, for example, in repeated investigations, including follow-up examinations.

The strengths of the present study lie in the large patient cohort, which allowed us to show significant differences exist between groups. In addition, we investigated two clinically relevant patient groups undergoing chest CT with or without the application of contrast agents. Both groups are relevant in routine clinical practice and are representative of patients undergoing CT scanning. The only difference between the groups was the application of contrast agents. The radiation dose, age, weight, body mass index, and sex were not significantly different between the groups.

Our study did, however, have some limitations. First, there was potential bias from the underlying disease of the patients on the number of measured double-strand breaks. This topic is the focus of ongoing investigations in our department. Second, we investigated the DNA damage that occurred in peripheral blood lymphocytes. Lymphocytes were chosen because they are easily accessible through a blood draw and are present throughout the body. Similar damage should be expected in other white blood cells. The presence of this type of damage does not mean that similar damage also occurs in solid organ tissues because the concentration of contrast agent is lower in solid organs. DNA damage in solid organs should be addressed in appropriate animal models (29). Furthermore, the relationship between the measured DNA breaks and cancer risk is unknown and would require large-scale studies to reach a definitive conclusion on the issue. Finally, it all comes down to counterbalance of relative risks. The theoretical risk that a double-strand break–induced cancer would result from the application of additional contrast medium for diagnosis can be considered much smaller than the potential, and often very real, threat caused by the underlying disease. Thus, in most cases a greater adverse effect may originate from the failure to provide a proper diagnostic screening than from the diagnostic procedure itself. The involved risks

have to be estimated on a case-by-case basis (30,31).

In conclusion, our study showed that the application of iodinated contrast agents during chest CT scanning clearly increases the amount of peripheral lymphocyte DNA radiation damage. We know that DNA radiation damage causes cancer; therefore, if this damage is enhanced, the likelihood of cancer generation is theoretically increased. Consequently, the potential cellular effect in contrast-enhanced examinations is not exclusively dependent on the radiation dose and therefore cannot be assessed solely with conventional dosimetry. Individual patient characteristics and biologic dosimetry applications, such as the analysis of γH2AX foci, must be considered.

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