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ORIGINAL RESEARCH

Assessment of the Radiation Effects of Cardiac CT Angiography Using Protein and Genetic Biomarkers

Patricia K. Nguyen, MD,*†‡ Won Hee Lee, PhD,*‡ Yong Fuga Li, PhD,§ Wan Xing Hong, BS,*‡ Shijun Hu, PhD,*‡ Charles Chan, PhD,‖ Grace Liang, BS,* Ivy Nguyen, BS,* Sang-Ging Ong, PhD,*‡ Jared Churko, PhD,*‡ Jia Wang, PhD,¶ Russ B. Altman, PhD,§ Dominik Fleischmann, MD,*# Joseph C. Wu, MD, PhD*‡#

ABSTRACT

OBJECTIVES The purpose of this study was to evaluate whether radiation exposure from cardiac computed tomographic angiography (CTA) is associated with deoxyribonucleic acid (DNA) damage and whether damage leads to programmed cell death and activation of genes involved in apoptosis and DNA repair.

BACKGROUND Exposure to radiation from medical imaging has become a public health concern, but whether it causes significant cell damage remains unclear.

METHODS We conducted a prospective cohort study in 67 patients undergoing cardiac CTA between January 2012 and December 2013 in 2 U.S. medical centers. Median blood radiation exposure was estimated using phantom dosimetry. Biomarkers of DNA damage and apoptosis were measured by flow cytometry, whole genome sequencing, and single cell polymerase chain reaction.

RESULTS The median dose length product was 1,535.3 mGy·cm (969.7 to 2,674.0 mGy·cm). The median radiation dose to the blood was 29.8 mSv (18.8 to 48.8 mSv). Median DNA damage increased 3.39% (1.29% to 8.04%, $p < 0.0001$) and median apoptosis increased 3.1-fold (interquartile range [IQR]: 1.4- to 5.1-fold, $p < 0.0001$) post-radiation. Whole genome sequencing revealed changes in the expression of 39 transcription factors involved in the regulation of apoptosis, cell cycle, and DNA repair. Genes involved in mediating apoptosis and DNA repair were significantly changed post-radiation, including *DDB2* (1.9-fold [IQR: 1.5- to 3.0-fold], $p < 0.001$), *XRCC4* (3.0-fold [IQR: 1.1- to 5.4-fold], $p = 0.005$), and *BAX* (1.6-fold [IQR: 0.9- to 2.6-fold], $p < 0.001$). Exposure to radiation was associated with DNA damage (odds ratio [OR]: 1.8 [1.2 to 2.6], $p = 0.003$). DNA damage was associated with apoptosis (OR: 1.9 [1.2 to 5.1], $p < 0.0001$) and gene activation (OR: 2.8 [1.2 to 6.2], $p = 0.002$).

CONCLUSIONS Patients exposed to >7.5 mSv of radiation from cardiac CTA had evidence of DNA damage, which was associated with programmed cell death and activation of genes involved in apoptosis and DNA repair.
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From the *Stanford Cardiovascular Institute, Stanford University School of Medicine, Stanford, California; †Veterans Administration Palo Alto, Palo Alto, California; ‡Department of Medicine, Division of Cardiology, Stanford University School of Medicine, Stanford, California; §Department of Genetics, Stanford University School of Medicine, Stanford, California; ‖Department of Surgery, Stanford University School of Medicine, Stanford, California; ¶Environmental Health and Safety, Stanford University School of Medicine, Stanford, California; and the #Department of Radiology, Stanford University School of Medicine, Stanford, California. This work was supported by the American Heart Association (10SDG4280129), National Institutes of Health (HL093172 and HL09551), and Stanford Cardiovascular Institute. The authors have reported that they have no relationships relevant to the contents of this paper to disclose. Drs. Nguyen, Lee, and Li contributed equally to this work.

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ABBREVIATIONS AND ACRONYMS

BAX = BCL2-associated X protein

CTA = computed tomographic angiography

DDB2 = damage-specific deoxyribonucleic acid binding protein 2

H2AX = H2A histone family, member X

XRCC4 = x-ray repair complementing defective repair in Chinese hamster cells 4

The application of cardiac computed tomographic angiography (CTA) has risen dramatically over the last decade (1–3). Cardiac CTA is now commonly used to manage patients with suspected coronary artery disease (4), aortic stenosis in preparation for transcatheter aortic valve replacement (5), atrial fibrillation prior to ablation (6), and aortic dissection post-surgical repair (7). Radiation exposure from this procedure can be significant because of the need for gating to compensate for cardiac motion. A single cardiac CTA can expose patients to a radiation dose equivalent to having at ≥150 chest x-rays (8). Not surprisingly, the widespread use of this procedure has raised concern among physicians and patients about the potential deleterious effects of radiation exposure from cardiac CTA (9).

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It is well known that exposure of cells to therapeutic doses of radiation triggers a complex network of signal transduction pathways that induce changes in gene expression and protein structure (10). This results in apoptosis (e.g., programmed cell death), cell cycle arrest or progression, and deoxyribonucleic acid (DNA) repair to minimize the risk of mutagenesis (11). Whether radiation doses from medical imaging tests (<100 mSv) causes similar damage and activates the same biological pathways is less certain. Although previous studies have demonstrated that proteins involved in the DNA damage response pathway are phosphorylated after exposure to radiation from medical imaging (12–14), these clinical studies have been limited by the use of semiqualitative measures, specifically counting the formation of gamma H2A histone family, member X (H2AX) foci in a small subset of cells (12–14). Furthermore, prior studies have not measured the effects of radiation exposure from medical imaging on other key signaling proteins in the DNA damage response pathways, which are also altered after exposure to therapeutic doses of radiation (15), nor have they determined whether radiation from medical imaging is associated with programmed cell death. Finally, no human studies to date have measured the effects of radiation exposure from medical imaging on changes in gene expression *in vivo* (16), which have been shown to be significantly up-regulated after radiation therapy (17–19).

The purpose of our prospective study is to determine whether radiation exposure from cardiac CTA is associated with DNA damage and whether the extent of damage is associated with the activation of pathways responsible for repairing or eliminating cells to minimize mutation risk. The results of this study will help clinicians better understand the risks associated with radiation exposure from medical imaging so that clinicians and patients can make informed decisions about this procedure. This study will also help determine whether additional strategies are needed to protect patients against radiation exposure from CTA.

METHODS

Please refer to the Online Appendix for a more detailed description of the methods.

PATIENTS AND DIAGNOSTIC IMAGING STUDIES. Adult patients age ≥18 years who underwent a clinically indicated cardiac CTA were recruited from Stanford Hospital (Stanford, California) and the Veterans Affairs Palo Alto Health Care System (Palo Alto, California). This study complies with the institutional review boards of Stanford University and the Veterans Affairs Health Care System Palo Alto. All subjects gave informed consent.

ESTIMATION OF RADIATION DOSE. Radiation dose to the body and blood was estimated using phantom dosimetery, the ImPACT Computed Tomography Patient Dosimetry Calculator spreadsheet (ImPACT, London, England) (13,20). Only doses calculated from the ImPACT Computed Tomography Patient Dosimetry Calculator were used in the analysis.

SAMPLE COLLECTION FOR IN VIVO STUDIES. Whole blood was collected at baseline and at multiple time points after cardiac CTA, as detailed in the Online Appendix.

PROTEOMIC BIOMARKER ASSAYS. Analyses of protein biomarkers of DNA damage and apoptosis by flow cytometry and immunohistochemistry were performed using standard protocols. DNA damage biomarkers included phosphorylated H2AX, ataxia telangiectasia mutated (ATM), and tumor protein p53 (p53). Ten thousand cells were evaluated. Biomarkers of apoptosis, including annexin V and BCL2-associated X protein (BAX), were measured by flow cytometry and immunohistochemistry, respectively. A total of 100,000 cells and 100 cells were evaluated for the expression of apoptotic markers by flow cytometry and immunohistochemistry, respectively.

GENOMIC BIOMARKER ASSAYS. Whole genome profiling using ribonucleic acid sequencing ($n = 3$) and single cell polymerase chain reaction ($n = 51$) of selected genes (Online Table 1) were performed using standard protocols.

STATISTICAL ANALYSIS. Continuous variables with normal distribution were expressed as mean ± SD

and those that were not normally distributed as median (first quartile to third quartile), respectively. Observations from dichotomous variables were summarized as proportions. Differences in continuous variables that were normally distributed, continuous variables that were not normally distributed, and proportions were compared using the Student *t*, Wilcoxon sign ranked, and Fisher exact tests respectively. Spearman correlations (rho, 95% confidence intervals, p value) were used to assess associations between continuous variables. Analysis of whole genome sequencing data is detailed in the Online Methods. Multivariate logistic regression models were used to evaluate the association between blood radiation dose and the following 3 outcomes: 1) DNA damage; 2) programmed cell death; and 3) gene activation. In addition to blood radiation dose, the following 7 covariates were evaluated for the presence of a significant association or correlation to the 3 outcomes identified above: 1) age; 2) sex; 3) body mass index; 4) race; 5) history of smoking; 6) history of cancer; and 7) iodine content. Only those covariates with significant associations with the 3 outcomes were included in the model. Significant DNA damage was defined as having ≥2% increase in phosphorylation of at least one DNA marker. A 2% cutoff was chosen based on findings in patients undergoing echocardiography (n = 9), an imaging study that does not produce radiation (data not shown). Patients who underwent echocardiography had <1% change in phosphorylation; thus, this cutoff is well above the level detected in our negative control group. Statistical analysis was performed using Intercooled Stata, version 12.1 (Stata Corp, College Station, Texas). Tests had an alpha level for significance set at $p < 0.05$ for single comparisons. The Bonferroni method was used to adjust the p values for multiple testing. Unadjusted p values are shown, and an asterisk is noted when comparisons were significant.

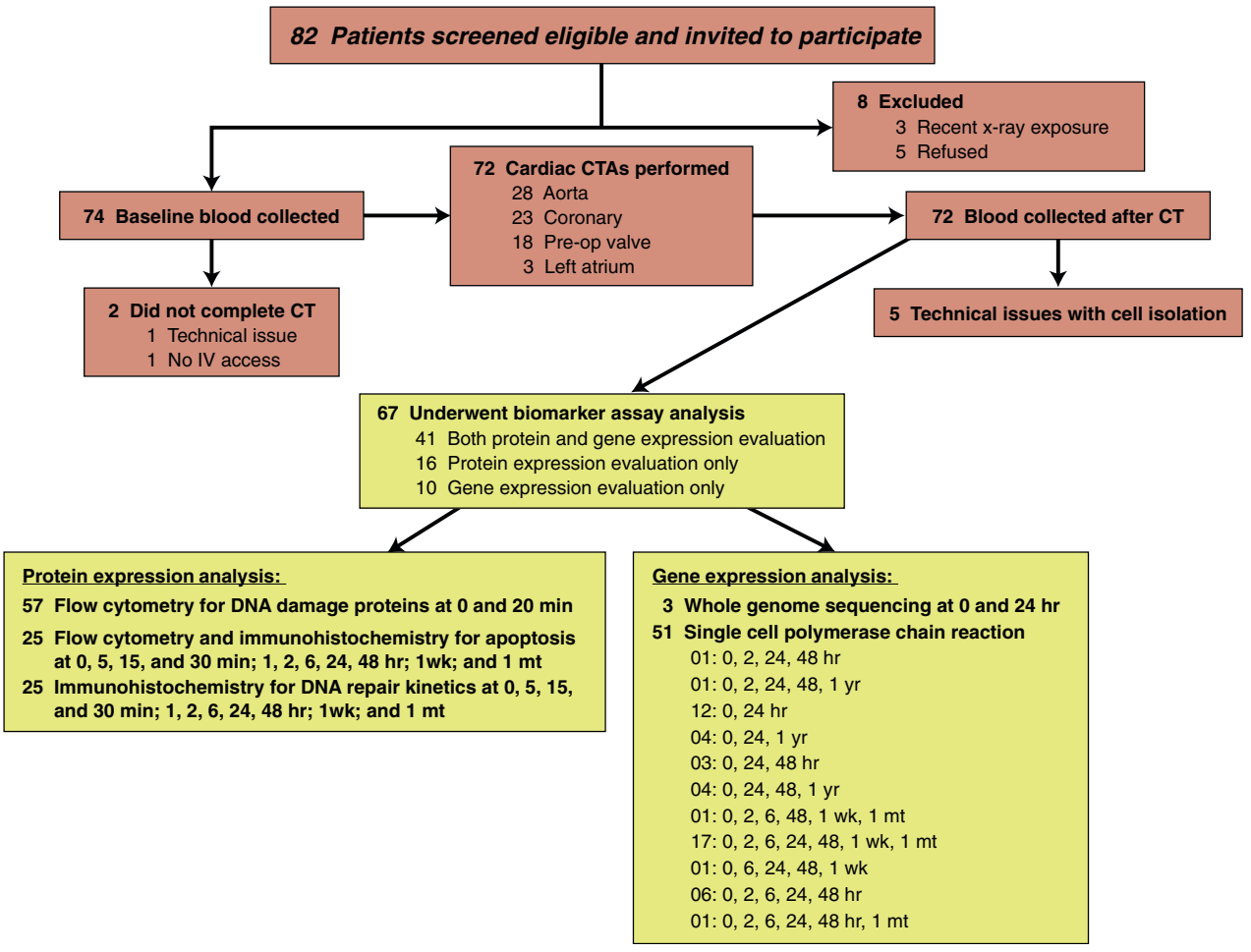
RESULTS

CLINICAL AND SCAN PARAMETERS. Of the 82 patients who were eligible to participate in the study, 67 patients underwent biomarker analysis before and after exposure to cardiac CTA (**Figure 1**), using standard scanning protocols detailed in Online Table 2 and in the Online Appendix. Clinical and scan parameters are detailed in **Table 1**.

EXTENT OF DNA DAMAGE AND ITS ASSOCIATION WITH CLINICAL AND SCAN PARAMETERS. A total of 70% of patients (36 of 57) had ≥2% increase in phosphorylation of at least 1 DNA damage marker post-radiation exposure (**Figures 2A and 2B**), indicating that at least 200 of 10,000 cells/patient had evidence of DNA damage after cardiac CTA. The median change in phosphorylation of any DNA damage marker was 3.39% (1.29% to 8.04%; *$p < 0.0001$). Although H2AX is more commonly used to estimate the extent of DNA damage (12–14,21), the median change in phosphorylation was higher for ATM (1.7% [0.7% to 8.0%]; *$p < 0.0001$) than both H2AX (0.2% [0% to 0.7%]; *$p < 0.0001$) and p53 (0.5% [−0.2% to 2.2%]; *$p < 0.0001$). This suggests that phosphorylated ATM may be a more sensitive biomarker for DNA damage. Median change in phosphorylation of any DNA damage marker was higher in patients exposed to ≥20 mSv of radiation compared with those exposed to <20 mSv (3.6% [1.6% to 12.6%] vs. 1.3% [0.1% to 3.7%]; *$p = 0.03$). Importantly, patients receiving radiation doses ≤7.5 mSv had no significant changes in phosphorylation, suggesting an absence of detectable DNA damage at very low doses ($p > 0.05$) (**Figure 2C**). **Table 1** presents descriptive and bivariate analysis of patients with or without DNA damage with clinical and scan parameters. Of the clinical and demographic parameters evaluated, only radiation dose was significantly associated with the presence or absence of DNA damage (39.6 mSv [23.6 to 53.8 mSv] vs. 23.2 mSv [8.8 to 31.5 mSv]; *$p < 0.0001$). The extent of DNA damage was also correlated with the amount of radiation exposure ($r = 0.48$, *$p = 0.0001$) (**Figure 2D**, Online Figure 1). Although iodine dose was higher in patients who had evidence of DNA damage, this parameter was not significant after adjustment for multiple comparisons. To assess whether DNA damage was primarily due to radiation and not contrast effects, we performed in vitro whole blood irradiation experiments in the absence of contrast. Similar to our in vivo findings, biomarkers of DNA damage were consistently increased at radiation doses above 25 mSv (Online Figure 2).

EXTENT OF PROGRAMMED CELL DEATH AND ITS ASSOCIATION WITH DNA DAMAGE AND RADIATION DOSE. We next measured levels of apoptotic cell death in a subset of patients before and after undergoing cardiac CTA (n = 25). A total of 60% (15 of 25) of patients had at least 2-fold increase in apoptosis. The median increase in apoptosis was 3.1-fold (1.4- to 5.1-fold; $p < 0.0001$) post-radiation. In absolute terms, however, the median number of cells undergoing programmed cell death was estimated at 0.7% (0.5% to 1.28%), which is equivalent to the death of 700 of 100,000 lymphocytes evaluated (**Figure 3A**). Median fold apoptosis was highest in patients exposed to ≥20 mSv compared to those exposed to <20 mSv (2.3-fold [1.8- to 3.0-fold] vs. 0.7-fold [0.7- to 1.0-fold]; *$p = 0.03$) (Online Figures 3A to 3C). The degree of apoptosis was more strongly correlated

FIGURE 1 Consort Diagram



A consort flow diagram summarizing the participant flow through the study. CT = computed tomography; CTA = computed tomographic angiography; DNA = deoxyribonucleic acid; hr = hour; IV = intravenous; mt = month; wk = week.

with the extent of DNA damage ($r = 0.78$; $^*p < 0.0001$) (**Figure 3B**) than the amount of radiation exposure ($r = 0.42$; $p = 0.03$) (Online Figure 3D). The majority of damaged cells, however, were repaired (**Figure 3C**). Although the rate of response of repair and apoptotic pathways to DNA damage (i.e., disappearance of excess foci counts per nucleus) varied across individuals, most patients did not have detectable DNA damage 2 h after exposure to radiation from cardiac CTA (**Figure 3D**, Online Table 3), which is consistent with a previous study (14).

WHOLE GENOME PROFILING TO EVALUATE CHANGES IN BIOLOGICAL PATHWAYS AFTER RADIATION EXPOSURE FROM CARDIAC CTA. Using the transcriptome data of T lymphocytes from 3 patients (Online Table 4), we performed gene functional enrichment analyses to identify biological processes, signaling/metabolic pathways, and transcription factors that were significantly associated with radiation exposure. In total, 33 signaling/metabolic pathways, 39 transcription factors, and 17 biological processes were significantly changed after multiple test correction (q value cutoff 0.1, p values obtained by Fisher exact test) (**Figure 4**, Online Figure 4, Online Tables 5 to 8). The active transcription factors formed a densely connected regulatory network (Online Figure 4B), with many transcriptional regulations among them. This suggests that the activity change of some of the significant transcription factors may be driven by other upstream active transcription factors.

TABLE 1 Clinical and Scan Parameters for Patients With and Without DNA Damage

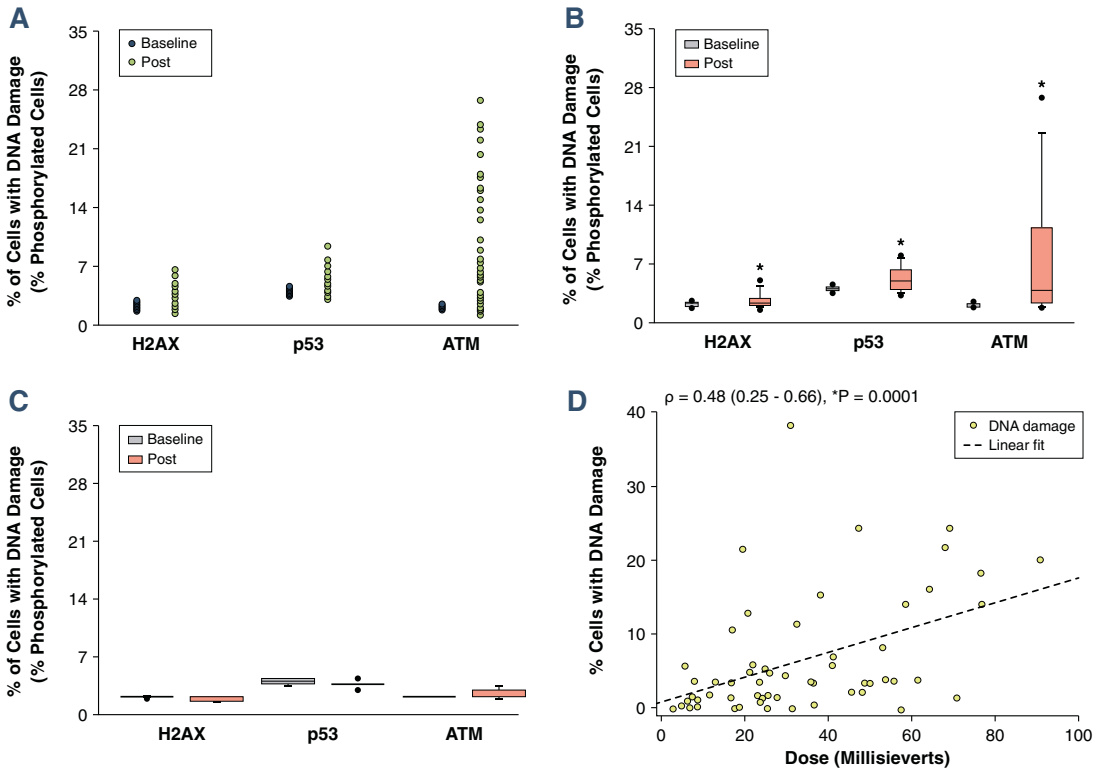
	Total (n = 57)	No DNA Damage (n = 21)	DNA Damage (n = 36)	p Value
Clinical parameters				
Age at enrollment, yrs	67 (56–79)	73 (63–77)	66.5 (54–88)	0.67
Sex				0.33
Male	78.9 (45/57)	71.4 (15/21)	83.3 (30/36)	
Female	21.1 (12/57)	28.6 (6/21)	16.7 (6/36)	
BMI, kg/m^2	26.5 (24.4–29.4)	25.7 (24.2–30.1)	27.1 (24.4–30.2)	0.66
Race				
White	80.7 (46/57)	71.4 (15/21)	86.1 (31/36)	0.18
Nonwhite	19.3 (11/57)	28.6 (6/21)	13.9 (5/36)	
Current smoking	21.1 (12/57)	14.2 (3/21)	25.0 (9/36)	0.27
History of cancer	22.8 (13/57)	23.8 (5/21)	22.2 (8/36)	0.57
Scan parameters*				
Median DLP, mGy·cm	1,511.0 (969.7–2,589.1)	1,105.9 (568.3–1,431.0)	2,137.0 (1,293.0–2,740.9)	<0.0001*
Median total effective dose, mSv	36.9 (26.1–61.3)	30.2 (17.0–45.1)	49.2 (32.6–71.3)	0.004*
Median blood radiation dose, mSv	29.8 (18.8–48.8)	23.2 (8.8–31.5)	39.6 (23.6–53.8)	<0.0001*
Median iodine content, g	38.6 (33.3–46.9)	35.0 (25.9–37.8)	41.4 (33.3–48.0)	0.01

Values are median (1st to 3rd quartiles) or % (n/N). *Scan parameters reflect only the cohort of patients that underwent biomarker testing for DNA damage (n = 57) and do not reflect the entire cohort (n = 67).

BMI = body mass index; DLP = dose length product; DNA = deoxyribonucleic acid.

CHANGES IN EXPRESSION OF INDIVIDUAL GENES ASSOCIATED WITH DNA REPAIR AND APOPTOSIS. To validate the overall differences in gene expression after radiation found in the ribonucleic acid sequencing analysis, we next measured the expression levels of a select number of genes involved in the regulation of apoptosis, cell cycle, and cell repair that were found to be up-regulated in the whole genome transcription analysis (Online Tables 1 and 9 to 11). Radiation exposure from cardiac CTA elicited a statistically significant change in the expression levels of damage-specific DNA binding protein 2 (*DDB2*), x-ray repair complementing defective repair in Chinese hamster cells 4 (*XRCC4*), and *BAX* over time (*$p \leq 0.01$) as shown in Online Figure 5 and Online Table 10. These genes are known to play important roles in response to DNA damage. *DDB2*, for example, facilitates DNA binding for nuclear excision repair and regulates cell fate by promoting cell cycle progression and programmed cell death (22), whereas *XRCC4* has been found to complex with DNA ligase IV to complete the final steps of nonhomologous repair of DNA double stranded breaks (23). Finally, *BAX* mediates p53-dependent apoptosis by inserting into the mitochondrial membrane and releasing pro-apoptotic factors (24). The maximum relative fold change in gene activation of these genes were significantly increased after cardiac CTA: *DDB2*: 1.9-fold (1.5- to 3.0-fold); *$p < 0.001$; *XRCC4*: 3.0-fold (1.1- to 5.4-fold); *$p = 0.005$; *BAX*: 1.6-fold (0.9- to 2.6-fold); *$p < 0.001$ (**Figures 5A and 5B**). Although DNA repair was complete within 2 h post-radiation exposure, peak changes in gene expression occurred 24 h post-radiation exposure. This finding is consistent with previous studies that have reported that transcription is arrested by DNA damage, but subsequently recovers after repair is complete to prevent the production of aberrant transcripts and interference between the transcription and repair machinery (25–27). Although gene changes did not vary significantly by dose (Online Figure 5), patients with evidence of DNA damage had significantly higher activation of these genes compared with those with no DNA damage (*DDB2*: 2.55-fold [1.74- to 4.42-fold] vs. 1.26-fold [1.16- to 1.77-fold]; *$p = 0.003$; *XRCC4*: 4.9-fold [2.8- to 6.6-fold] vs. 1.0-fold [0.9- to 1.9-fold]; *$p = 0.005$; *BAX*: 2.1-fold [1.1- to 2.9-fold] vs. 1.0-fold [0.9- to 1.3-fold], *$p = 0.001$) (**Figure 5B**). In support of these findings, gene activation could not be detected in patients receiving a radiation dose of ≤7.5 mSv who also had no evidence of DNA damage as detailed in the previous text ($p > 0.05$) (**Figure 5C**). These findings suggest that more extensive DNA damage is associated with greater transcriptional changes in repair and apoptotic genes. To assess whether changes were primarily due to radiation and not from contrast effects, experiments were repeated in vitro in the absence of any contrast. Similar to our findings in vivo, increased expression of several genetic biomarkers associated with cell repair and death were found after radiation exposure (Online Figure 6). Importantly, patients who underwent echocardiography (n = 11) showed no significant

FIGURE 2 DNA Damage Detected After Radiation Exposure

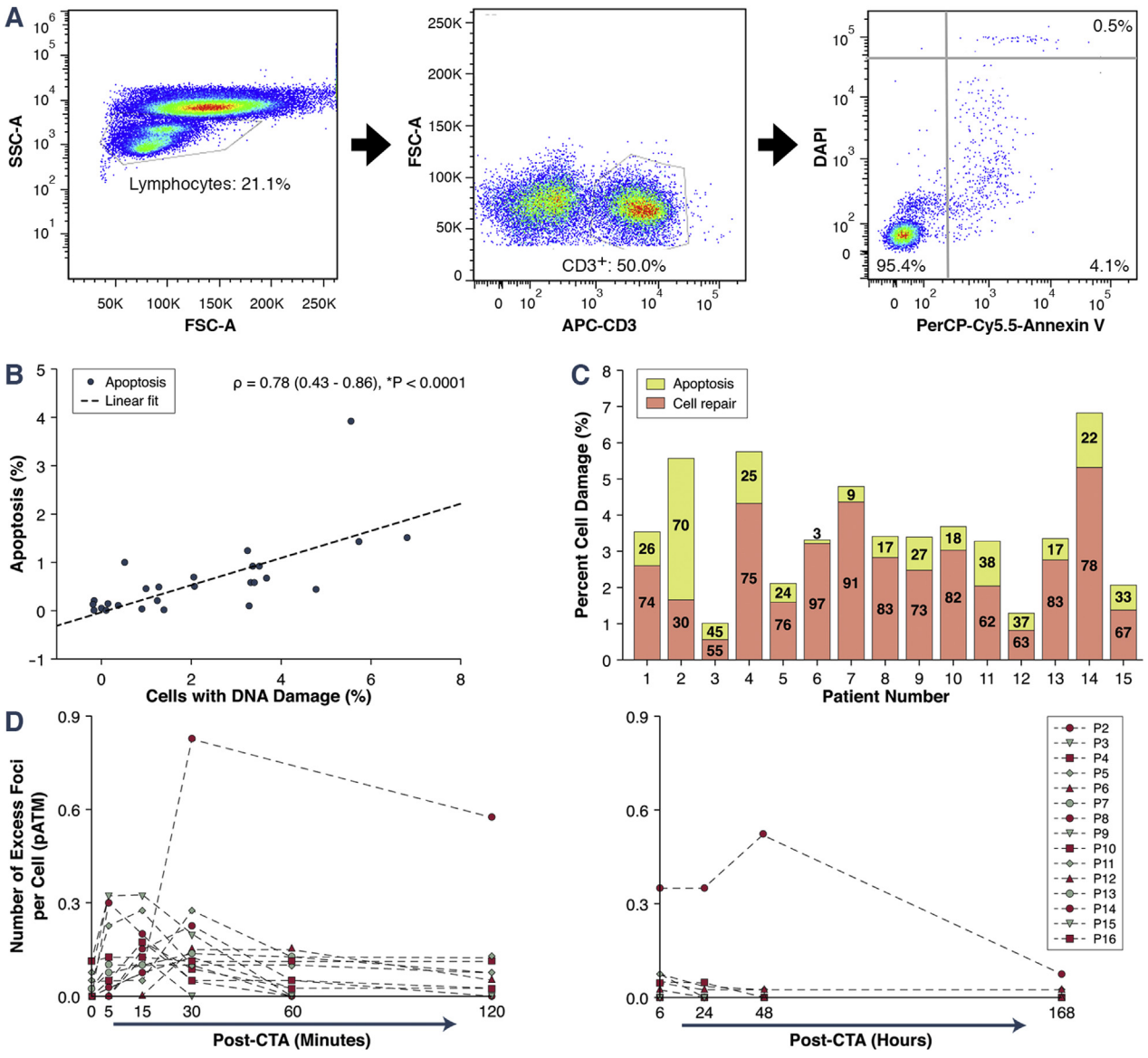


(A) Scatter plot graph of levels of phosphorylated H2A histone family, member X (H2AX), ataxia telangiectasia mutated (ATM), and tumor protein p53 (p53) at baseline and after exposure to radiation from cardiac CTA (**left**, n = 57). **(B)** Quantitative assessment of protein biomarkers of DNA damage (i.e., levels of phosphorylated H2AX, ATM, and p53) in patients undergoing cardiac CTA (**right**, n = 57). The **horizontal line within the box** marks the median, and the **length of the box** represents the interquartile range, which is the difference between the 75th and 25th percentiles. **Whiskers (error bars) above and below the box** indicate the 90th and 10th percentiles. In addition, outliers are indicated by a **dot**. *Statistically significant at $p < 0.01$. **(C)** Quantitative assessment of protein biomarkers of DNA damage in patients receiving very low doses of radiation (e.g., ≤7.5 mSv). **(D)** Scatterplot graph of the correlation analysis between DNA damage estimate and radiation dose (n = 57). Abbreviations as in Figure 1.

change in gene expression post-imaging (data not shown).

MULTIVARIATE REGRESSION ANALYSIS. Only dose and iodine content were associated with DNA damage, programmed cell death, and gene activation. Findings from the bivariate analysis suggest that iodine content may be a potential confounder, although the difference between the iodine content in patients who did and did not have DNA damage was not significant after adjustment for multiple comparisons (Table 1). Iodine content was found to correlate with dose ($r = 0.67$; *$p < 0.0001$). To determine the effects of iodine content on the relationship between dose and DNA damage, we performed the following set of regressions: 1) DNA damage on dose alone; 2) DNA damage on iodine content alone; and 3) DNA damage on dose, iodine, and their interaction. Although both DNA damage and iodine content were significant ($p < 0.01$) when analyzed separately, only DNA damage remained significant when both were analyzed together. Given the confounding effects of iodine content, subsequent analyses excluded this covariate. Results of separate multivariate regressions are summarized in Table 2. Higher radiation dose was associated with a greater odds of having DNA damage (odds ratio [OR]: 1.8 [95% confidence interval (CI): 1.2 to 2.6]; *$p = 0.003$). Patients with a greater extent of DNA damage had greater odds of gene activation (OR: 2.8 [95% CI: 1.2 to 6.2]; *$p = 0.002$) and apoptosis (OR: 1.9 [95% CI: 1.2 to 5.1]; *$p < 0.0001$). Patients with a greater extent of DNA damage and at least a 2-fold activation of at least 1 gene had greater odds of apoptosis (OR: 1.6 [95% CI: 1.2 to 2.7]; *$p <$ 0.0001). Radiation dose was not a statistically

FIGURE 3 Apoptosis Detected After Radiation Exposure



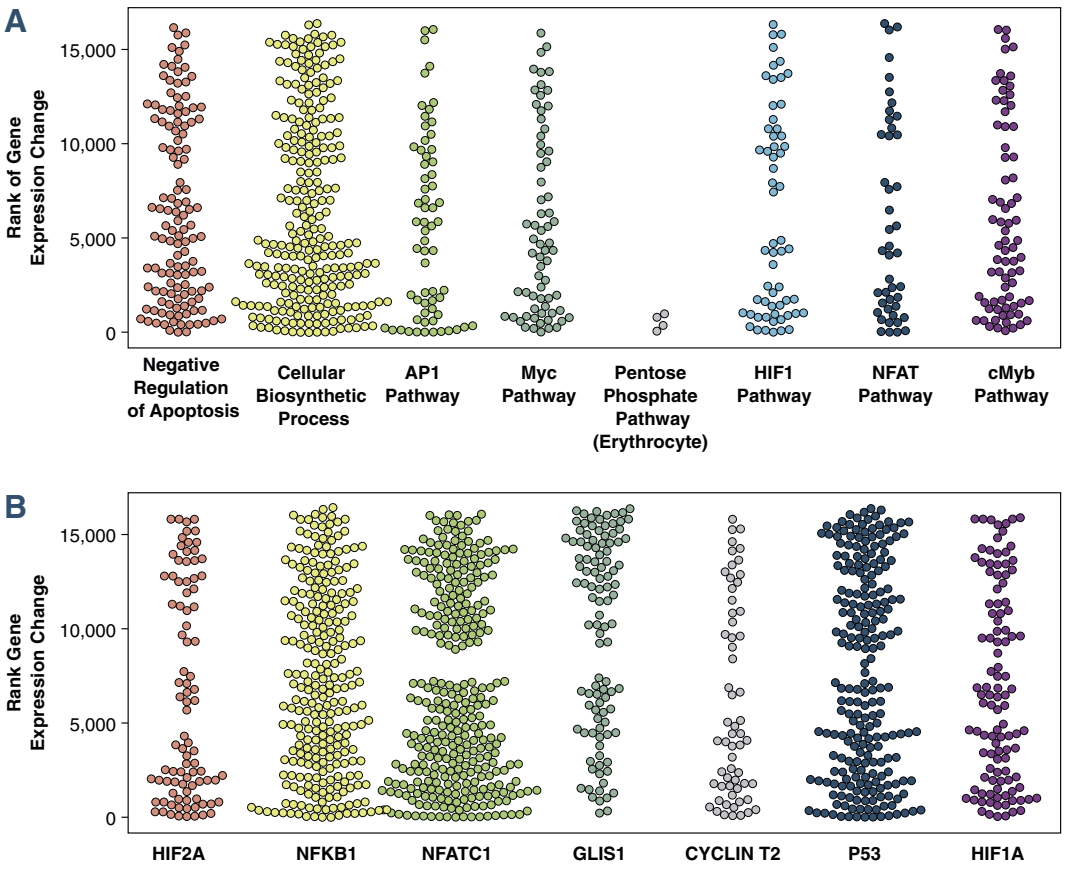
(A) Sample flow cytometry dot plot from a patient after cardiac CTA. To identify apoptotic cells, lymphocytes were first isolated using forward and side scatter profiles **(left)**. $CD3^+$ T cells were then identified from within this subset **(middle)**. Last, apoptotic T cells were detected using Annexin V, an early marker of apoptosis, and DAPI, an intracellular marker for dead cells, which are either necrotic or at the later stages of apoptosis **(right)**. The estimate of apoptotic cell death includes the sum of cells that express Annexin only or Annexin-DAPI. This patient had 4.1% of apoptotic cells that express Annexin only and 0.5% of dead cells that express both Annexin and DAPI. **(B)** Scatterplot graph of the correlation analysis between the estimates of apoptosis and DNA damage (n = 25). **(C)** Bar graph displaying the relative percentages of cells that were repaired or had undergone programmed cell death in individual patients with evidence of cell death by flow cytometry (n = 15). The remainder of the patients had no cell death and complete repair (n = 10) (not shown in graph). **(D)** Scatter plot graph of the disappearance of the phosphorylated ataxia telangiectasia mutated (pATM) over time as damaged cells are repaired or eliminated (n = 25). APC-CD3 = allophycocyanin-cluster differentiation 3; DAPI = 4′,6-diamidino-2-phenylindole; FSC-A = forward-scatter area; PerCP-Cy5.5 = peridinin chlorophyll protein cyanine 5.5; SSC-A = side-scatter area; other abbreviations as in Figure 1.

significant predictor of gene activation or apoptosis. Taken together, it appears that an estimate of radiation dose does not capture the entire spectrum of biological changes associated with radiation exposure from cardiac CTA.

DISCUSSION

In this study, we demonstrated that patients undergoing cardiac CTA at doses >7.5 mSv had evidence of DNA damage. This amount of radiation was

FIGURE 4 Strip Charts Showing the Associations of Selected Gene Sets With the Transcriptional Response of Blood Cells to Cardiac CTA Exposure

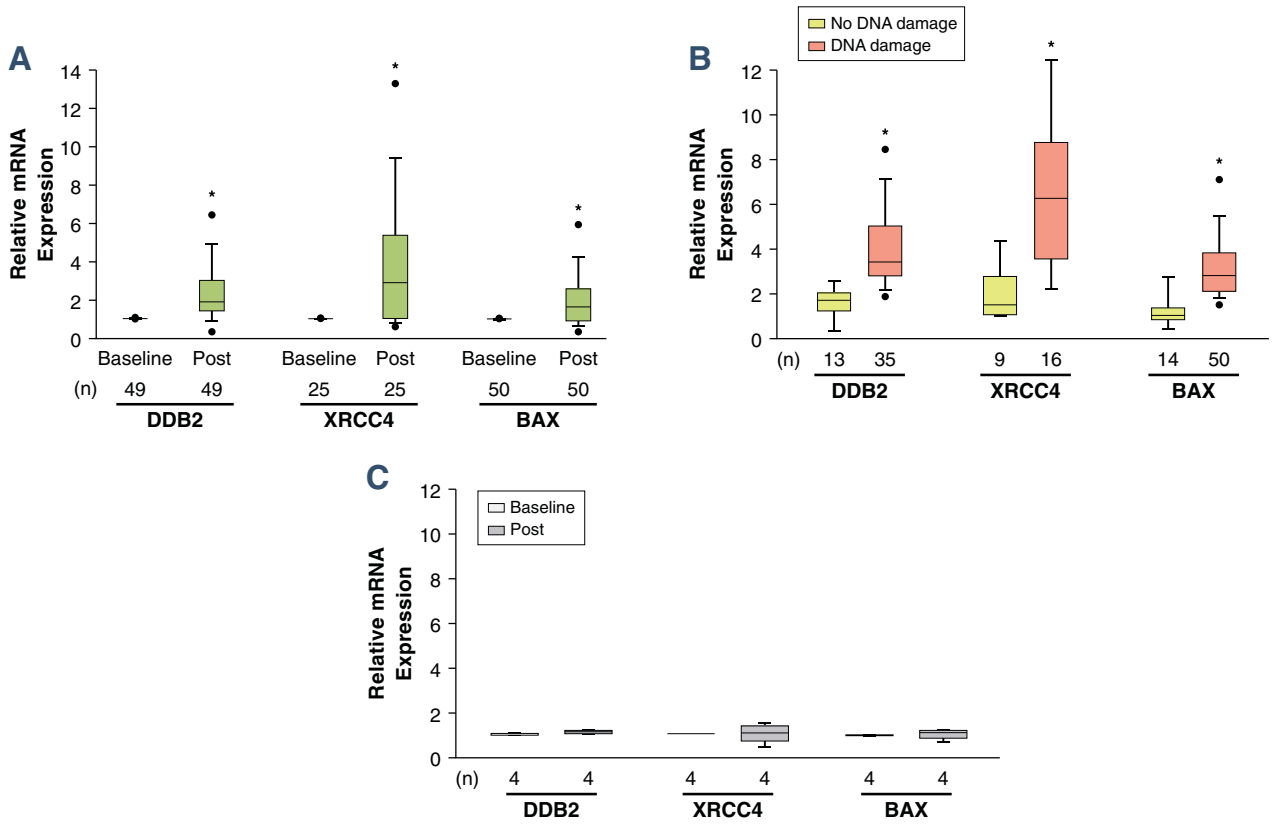


(A) Functional gene sets for biological processes, signaling, and metabolic pathways. **(B)** Regulation gene sets for transcription factor target genes. The transcriptome data is obtained through ribonucleic acid sequencing of 3 selected individuals before and 24 h after cardiac CTA. The color groups the genes (shown as **dots**) in different biological processes/pathways **(A)** or regulated by different transcription factors **(B)**. The y-axis corresponds to the rank of the gene's expression changes among 16,486 protein-coding genes. Low ranks correspond to decreased expression of genes after cardiac CTA exposure, and high ranks correspond to increased expression. When a set of genes are not associated with cardiac CTA exposure, we expect the ranks of the genes' expression changes to be evenly distributed; hence, an uneven distribution of the dots along the y-axis indicates association of a gene set with cardiac CTA exposure. GLS1 = kidney type glutaminase; HIF1A = hypoxia-inducible factor 1α; HIF2A = hypoxia-inducible factor 2α; NFATC1 = nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1; NFKB1 = nuclear factor kappa-light-chain-enhancer of activated B cells; p53 = tumor suppressor p53; other abbreviations as in Figure 1.

associated with activation of genes involved in regulating cell repair and programmed cell death. Although most damaged cells are repaired, a small percentage of cells die. These findings raise the possibility that radiation exposure at >7.5 mSv from cardiac CTA may cause DNA damage that can lead to mutations if damaged cells are not repaired or eliminated properly. Cumulative cell death after repeated exposures may also be problematic.

Although previous studies have reported cross-sectional associations between radiation exposure from medical imaging and DNA damage, these studies were conducted in either the pediatric population (12) or in small groups of adult patients undergoing CTA using a single biomarker of damage (13,14,28,29). The relationship between dosage and DNA damage has also not been fully explored in adult patients undergoing cardiac CTA (30). In our study, we performed a comprehensive evaluation of cellular effects of radiation exposure from diagnostic imaging, including measurements of multiple parameters of DNA damage and apoptosis as well as transcriptional changes in over 50 patients at serial time points. In a multivariate analysis, we found that radiation dose was associated with DNA damage, although the relationship was only roughly linear (31,32). Dose was not

FIGURE 5 Individual Genes Involved in Apoptosis and Repair Altered After Radiation Exposure



(A) Maximum median change in gene expression of *DDB2*, *XRCC4*, and *BAX* post-radiation compared to baseline in all patients. **(B)** Bar graph comparing the relative change in gene expression in patients with or without DNA damage. DNA damage is measured as the maximum change of any of the 3 DNA damage biomarkers 30 min post-radiation. Apoptosis is the maximum apoptotic cell death detected at any time point within 1-week post-radiation. **(C)** Maximum median change in gene expression of *DDB2*, *XRCC4*, and *BAX* post-radiation compared with baseline in the subset of patients who received a radiation dose of ≤7.5 mSv. *Statistically significant at $p < 0.01$. *BAX* = BCL2-associated X protein; *DDB2* = damage-specific deoxyribonucleic acid binding protein 2; mRNA = messenger ribonucleic acid; *XRCC4* = x-ray repair complementing defective repair in Chinese hamster cells 4; other abbreviations as in Figure 1.

predictive of gene activation or programmed cell death. These results are not surprising, given that we currently rely on complicated computer simulations and mathematical models to extrapolate the "absorbed dose" and "biological equivalent dose" (33), and then use these values to estimate biological risk (i.e., cellular injury and cancer risk) (34). Our findings suggest that measurement of the degree of

TABLE 2 Multivariate Regression Model of the Association of DNA Damage, Gene Activation, and Apoptosis With Dose

	Multivariate Logistic Regression		Exact Logistic Regression
	DNA Damage (n = 57)	Gene Activation (n = 51)	Apoptosis (n = 25)
OR (95% CI)	1.8 (1.2–2.6)* for dose	1.0 (0.99–1.1) for dose 2.8 (1.2–6.2)* for DNA damage†	1.1 (1.0–1.2) for dose 1.9 (1.2–5.1)* for DNA damage† 1.6 (1.2–2.7)* for gene activation
R^2	0.2	0.16	–
Model score	13.1‡	8.2‡	16.4
p value	0.003	0.002	<0.0001

*$p < 0.01$. †Model that replaces dose with DNA damage given DNA damage captures the effects of dose. ‡Chi-square for likelihood ratio test.
CI = confidence interval; DNA = deoxyribonucleic acid; OR = odds ratio.

DNA damage is more predictive of both gene activation and apoptosis than estimated radiation dose and may be a better marker of biological risks.

Importantly, in this study, we purposely recruited patients who had a wide range of radiation dose to determine its effects on cellular damage. Patients with radiation exposure >20 mSv either underwent a CTA performed in a traditional scanner equipped with older technology (Discovery 750HD, GE, Milwaukee, Wisconsin) and retrospective gating or underwent an evaluation of their entire aorta (e.g. patients with a history of dissection or pre-transcatheter aortic valve replacement) using a state-of-the-art dual source scanner (Sensation Dual Source, Siemens Medical Solutions, Forchheim, Germany) and prospective gating. In patients undergoing coronary angiography using a dual source scanner where radiation doses were ≤7.5 mSv, no damage was observed, further supporting a dose-response relationship. Overall, these data are consistent with our hypothesis that higher radiation dose leads to more DNA damage.

In addition to evaluating the relationship between dose and DNA damage, we provide a comprehensive analysis of how cells respond to damage, which has not been evaluated in prior studies. Our study found that exposure to radiation from CTA, like exposure to therapeutic doses of radiation (17,18,35,36), activates biological pathways and genes and increases the activity of transcription factors involved in the regulation of cell repair, cell cycle progression, and apoptosis, which are critical in preventing the development of mutations, a finding that has not yet been previously reported. Although cells with DNA damage are mostly repaired, a small number of cells die after radiation exposure from CTA. These findings are consistent with known biological responses to DNA damage (11). Cells with insignificant damage can be repaired, whereas those cells with extensive damage undergo programmed cell death to minimize the risk of mutation. We found a complete resolution of DNA damage occurred within 2 h of exposure in the majority of patients, which is also consistent with prior studies (14). In a few patients, however, residual DNA damage persisted and continued activation of cellular response pathways was detectable up to 1 month post-exposure, which has been reported after exposure to radiation doses as low as 1 mGy in vitro. This supports a possible lack of efficient cellular activation of repair mechanisms at dosing levels typically used in medical imaging (21). If residual cells are not eventually repaired or eliminated, they can potentially retain mutations. The number of cells with residual DNA damage after radiation exposure from CTA, however, is small (<1%). The number of lymphocytes that died after imaging is also small (<1%). Nevertheless, cumulative cell death from repeated radiation exposure can be potentially harmful, especially in elderly patients who may have a limited pool of naïve T cells that respond to novel pathogens (37). Further study is needed to evaluate whether the subpopulation of naïve T cells is affected by radiation exposure from CTA.

STUDY LIMITATIONS. A potential limitation of the study is that we did not directly measure DNA damage. However, a direct measure of small changes in DNA damage is not possible with current techniques (38). This study also did not measure the risk of cancer from radiation exposure from cardiac CTA because only cells that evade cellular repair and programmed cell death survive and produce cancer long-term. Because identifying these cells is not yet feasible, interpretation of these findings should be limited to the cellular effects of radiation from CTA in the short-term. Although this study was not designed to assess cancer risk, it does provide valuable insight into the biological response to radiation from medical imaging.

CONCLUSIONS

Patients undergoing cardiac CTA have evidence of DNA damage in T lymphocytes, which is associated with death of a small fraction of cells and activation of biological pathways, transcription factors, and genes involved in cell repair and apoptosis. Although cardiac CTA is a valuable clinical tool in the management of patients with cardiovascular disease, awareness among physicians and patients that DNA damage and apoptosis can occur even after diagnostic imaging may encourage greater adherence to dose reduction strategies. Further research is needed to develop novel agents to protect patients from the potential adverse effects of radiation exposure from cardiac CTA.

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REPRINT REQUESTS AND CORRESPONDENCE: Dr. Joseph C. Wu OR Dr. Patricia Nguyen, 265 Campus Drive, Room 1120B, Stanford, California 94305. E-mail: joewu@stanford.edu OR pknguyen@stanford.edu

PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Cardiovascular imaging remains the cornerstone for the management of complex cardiovascular disease. Due to our rapidly growing reliance on imaging for diagnosis and monitoring, many patients now receive more radiation from medical imaging than ever before, a trend that will likely continue to accelerate. This raises growing concerns about the potential risk from exposure to low-dose radiation from medical imaging. For instance, lymphocyte DNA damage has been detected in patients who undergo computed tomographic imaging, with the amount of damage seemingly proportional to dosage. Notably in this study, DNA damage was not detected in patients who received doses lower than or equal to 7.5 mSv, which is equivalent to 50 chest x-rays or 1 cardiac CTA using state-of-the-art technology. These results support the need to adhere to dose reduction strategies and minimize radiation exposure in medical imaging, although more extensive studies need to be conducted to refine the thresholds of harmful exposure to other tissues.

TRANSLATIONAL OUTLOOK: Further studies are warranted to identify certain patients who may be at a greater risk of DNA damage from low-dose radiation as well as beneficial compounds that may protect patients from the potential adverse effects of imaging.

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APPENDIX For supplemental methods as well as figures and tables, please see the online version of this article.