Biologic effects of radiation from cardiac imaging: New insights from proteomic and genomic analyses

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An ongoing debate exists in the literature about the risk of carcinogenesis from radiation associated with medical imaging tests. Some position papers and societal guidelines advocate that the choice of a diagnostic imaging modality for any clinical indication be influenced by whether the test is associated with ionizing radiation.¹ However, there is sparse scientific evidence underlying the current estimates of cancer risk from low-level (doses <100 mSv) radiation, which are based on the linear no-threshold (LNT) model. Although the Biological Effects of Ionizing Radiation (BEIR) VII report² from the US National Academies recommends the use of the LNT model as the best simple model for purposes of radiation protection, as do reports from the United Nations³ as well as the leading international⁴ and US⁵ radiological protection organizations, several other widely divergent models have also been posited, including the theory of hormesis (protective effect of low dose radiation) on one extreme to a theory of hypersensitivity (to low dose compared to high dose) to the other.⁶ A major confounding factor in analyzing the observational data on this subject is the high population incidence of cancer, which makes it difficult to identify any small additional risk attributable to radiation from medical tests. Figure 1 from the BEIR VII report illustrates this point.

A complementary approach to exploring the association between radiation and carcinogenesis is to determine whether exposure results in the activation of DNA repair pathways (activation of protein and genes

involved in DNA repair, apoptosis, cell cycle regulation, and chromatic remodeling), indicating that DNA damage has occurred. Lobrich and colleagues have demonstrated proof of principle and validation of enumerating γ -H2AX foci on peripheral blood lymphocytes as a marker of double-stranded DNA damage after a chest or abdominal CT scan, and the loss of these foci as a marker of DNA damage repair.⁷ The near-simultaneous publication of three recent articles using these surrogate markers of DNA damage to determine the genotoxic effects of radiation from cardiac imaging provides new and valuable insights on the subject.

Won He Lee and colleagues⁸ from the laboratory of Joseph Wu at Stanford University, prospectively determined the activation of DNA response pathways in 63 patients having standard dose (average rest and stress activity 6.9 ± 0.5 and 23.8 ± 1.8 mCi, respectively; average rest, stress, and total effective dose 2.7 ± 0.6 , 8.1 ± 0.4 , and 10.7 ± 0.4 mSv, respectively) Tc-99m SPECT myocardial perfusion imaging. Notably, radiation doses to the peripheral lymphocytes were not determined. Twelve patients undergoing cardiac x-ray fluoroscopy (mean effective doses 18.2 ± 10.6 mSv) for coronary angiography and 15 undergoing echocardiography were used as positive and negative controls, respectively. Markers of DNA damage evaluated in peripheral blood T-lymphocytes collected before and after imaging were phosphorylation of DNA damage-marker proteins (H2AX, P53 and ATM) by flow cytometry and immunohistochemistry, and serial changes (up to 48 hours) in mRNA expression of DNA damage response genes (*Bax*, *Mdm2*, *Ddb2*, and *Tp53*). As expected, patients undergoing echocardiography had no changes in protein phosphorylation or gene expression after the procedure, compared to baseline. This control group served to mitigate against the possibility of chance or circadian variation being the explanation

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for the findings observed in the SPECT and fluoroscopy groups. In the SPECT group overall, the investigators found no significant differences in the phosphorylation of the three proteins or in gene expression, before and after imaging. Analysis of individual data revealed that most (two-thirds) patients did not have significant changes in phosphorylation of DNA damage marker proteins, and the majority (70-90%) had either down-regulated or unchanged mRNA expression of DNA damage genes at 48 hours. However, one-third of patients had evidence of increased phosphorylation in at least one protein maker, and these patients also had evidence of gene upregulation. In contrast, all 12 patients undergoing fluoroscopy had increased levels of phosphorylation in at least one protein marker, and consistent upregulation of genes at 24 hours.

In a similar study,⁹ the same group evaluated evidence of DNA damage in 67 patients undergoing cardiac computed tomography (CT). CT procedures included CT coronary angiography (CTA) and chest CT prior to transcatheter aortic valve implantation, using protocols with prospective and retrospective gating, and equipment of varying vintage. The median (interquartile range) radiation effective dose to patients was 36.9 (26.1-61.3) mSv, the wide range reflecting the heterogeneity in protocol and equipment. The median dose to blood was 29.8 (18.8-48.8) mSv. Patients undergoing CTA using contemporary equipment (dual-source scanner, prospective gating) had effective doses ≤ 7.5 mSv. Blood samples were collected after CT at varying time intervals for flow cytometric and immunohistochemical analysis of protein biomarkers of DNA damage and apoptosis, and genomic profiling, similar to the study on SPECT. Important study findings were that (1) there was no evidence of DNA damage in patients with dose (it is unclear whether this is effective dose or blood dose) levels ≤ 7.5 mSv, (2) Doses >7.5 mSv showed evidence of significant DNA damage on both the proteomic and genomic analyses, and apoptosis, (3) most patients did not have detectable residual DNA damage 2 hours after exposure to radiation, while in a minority, changes were discernible up to 1 month after the exposure.

The third recent study of relevance was from Leige, Belgium. Lancellotti and colleagues¹⁰ evaluated the effects of a cardiac magnetic resonance (CMR) study on peripheral blood counts, activation of leukocyte subsets and platelets, evidence of DNA damage in the form of H2AX phosphorylation, and evidence of cell death (apoptosis evaluated by the Annexin V apoptosis detection kit, and necrosis measured by caspase 3/7 activation). Twenty healthy men who underwent an unenhanced (without gadolinium, for which there are limited data suggesting possible genotoxic effects¹¹)

Figure 1. Diagrammatic representation of the calculated risk of cancer (1%, *star*) from a single exposure to 100 mSv of low linear energy transfer radiation relative to the lifetime risk of any cancer (42%, *solid circles*) among 100 individuals, each represented by a *circle*. This diagram illustrates the challenge, due to the high background incidence of cancer, in observational studies of quantifying risk attributable to low-level radiation. Reproduced with permission from Ref. 2.

CMR study using a 1.5 T magnet had blood drawn before and at several time points (1-2 hours, 2 days, 1 month, and 1 year) after the study. Five age-matched subjects who did not have a CMR study were recruited as controls. The salient finding was an increase in H2AX phosphorylation which was evident at 2 days (but not prior) and persisted at 1 month (but not 1 year). There was a strong correlation between γ H2AX levels and the specific absorption rate, a measure of the amount of radiofrequency energy delivered during the CMR study, perhaps suggesting a causal relationship. There was no evidence of increased apoptosis or cell necrosis, and no change in peripheral blood cell counts.

How do these studies influence our current sparse understanding of the potential mechanisms of radiation-induced genotoxic effects? These results suggest that the occurrence and severity of genotoxic effects on lymphocytes may vary depending on the type of radiation.⁸ Radiation associated with SPECT MPI is from a gamma-emitting isotope given intravenously, usually in two doses separated by several minutes to hours. The majority of patients did not manifest genotoxic effects after SPECT MPI, while a minority (approximately one-third) demonstrated both protein phosphorylation and increased expression of DNA damage genes, indicating

a heightened sensitivity to the effects of radiation. On the other hand, patients undergoing x-ray imaging of the chest involving continuous bursts of radiation during fluoroscopy or CT (>7.5 mSv) manifest much higher prevalences of DNA damage, apoptosis, and mRNA expression of DNA damage proteins in lymphocytes. DNA damage in lymphocytes was also evident in patients undergoing a CMR study.

While these findings throw some light on the mechanism and prevalence of radiation-induced DNA damage, the association with carcinogenesis still remains speculative. Importantly, these data are all limited to radiation's effects in lymphocytes, and not in the critical organs receiving the highest doses from cardiac imaging procedures. There is very little direct evidence to indicate that the low levels of DNA damage produced by exposure to radiation from diagnostic imaging, and usually repaired effectively shortly thereafter, actually result in an increased risk of cancer in *adult* patients. Limited data¹²⁻¹⁴ from patients who underwent many fluoroscopic studies do show an increased risk of breast cancer and appear consistent with risks in atomic bomb survivors; however, the cumulative doses in these cohorts were far higher than those of most cardiac imaging patients.¹⁵ Retrospective data suggest that pediatric populations exposed to radiation from medical imaging, specifically from CT scanning, have an increased risk of cancer in adulthood that can be attributed to the radiation (2-6 cancers per 10,000 patients exposed).¹⁶ While ongoing epidemiological studies of CT scans in adults will address radiation risk to adults,¹⁷ at present, similarly compelling data simply do not exist in the adult population. Existing studies that have attempted to explore an association in adults are confounded by differences in the population studied (atomic bomb survivors, radiation workers, and recipients of radiation therapy), resulting in significant uncertainty in extrapolating the result to diagnostic medical imaging.¹⁸ One study, the Quebec post-MI study,¹⁹ specifically explored the relationship between radiation exposure from cardiac procedures (radionuclide ventriculography, myocardial perfusion imaging, diagnostic coronary angiography, and percutaneous coronary intervention) and incident cancer in patients treated for an acute myocardial infarction, and found an astonishing 3% increase in the risk of incident age and gender-adjusted cancer over a mean follow-up period of just 5 years. While the context and patient characteristics of this study were perhaps the most suitable to answer the question of whether radiation from diagnostic medical imaging is indeed associated with an attributable cancer risk, the vastly higher risk observed so early after exposure appears inconsistent with current knowledge. The observed rate of incident

cancer was at least double the expected rate in this population. Furthermore, several methodological limitations confound interpretation of the results, including the use of typical average radiation dose estimates for each procedure (rather than actual doses received by patients), and lack of adjustment for known carcinogens such as smoking.

Another intriguing question is that of the cumulative effects of radiation from serial imaging. Studies of patients undergoing CT of the chest and abdomen suggest that most patients repair double-stranded DNA damage to baseline levels within 24 hours of testing, as evidenced by the loss of γ -H2AX foci on lymphocytes.⁷ However, persistent repair defects have been identified in individual patients.⁷ The concern is that, if DNA defects are not effectively repaired or cells with residual defects not eliminated, cumulative effects from repeated radiation could result in a significant prevalence of mutated cells. The study by Nguyen et al.⁹ showed that there was almost complete resolution of radiation-induced DNA damage in the majority of patients within 2 hours of the CT scan. In a few patients, persistent damage was evident up to 1 month after exposure, but the number of cells with residual damage was very small ($<1\%$). Clearly, more study is required to explore the effects of cumulative radiation from serial testing.

Thus, there are now evolving data obtained by direct measurement of the effects of DNA damage induced by radiation associated with cardiac imaging. This approach avoids a limitation of observational studies of carcinogenesis, which is the difficulty of identifying a very small, if any, cancer risk attributable to radiation from diagnostic testing in the presence of a very high background incidence of cancer. At the same time, however, it only provides a surrogate biological endpoint which may or may not translate to the development of cancer, and the experiments are limited to radiation's effect on lymphocytes. Nevertheless, these new data give us several important insights. First, as has been previously demonstrated as well, the prevalence and magnitude of DNA damage appear to be dependent on the specific type and dose of radiation associated with the imaging test. Second, repair and elimination mechanism are rapidly effective in the majority of patients. Finally, non-ionizing radiation associated with CMR also appears to induce DNA damage.^{10,20,21}

These data do not obviate the need for continued vigilance, and the application of the ALARA (As Low As Reasonably Achievable) principle to medical tests and interventions involving radiation. When choosing a diagnostic imaging modality for any clinical indication, the physician should select the test that is most likely to provide the optimal clinical information required to

manage the patient, and, in case of tests involving ionizing or non-ionizing radiation, apply the ALARA principle to perform the test at the lowest possible radiation dose while maintaining quality. There is little evidence to suggest that the choice of test be based on whether it involves radiation or not. In the case of properly selected patients with known or suspected CAD, the benefit from the diagnostic and prognostic information provided by cardiac imaging is likely to far exceed the potential risk from radiation.

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