An Exploratory Study of DNA and Chromosomal Damage in UK Nuclear Medicine Technologists– Supplementary Information

SUPPLEMENTARY METHODS

DNA damage: γH2AX

Blood sample preparation

Venous blood samples were collected from nuclear medicine technologists at the beginning of a work day before and after radiopharmaceutical administration to a patient (350 MBq [^{18}F]FDG for PET, 170–220 MBq [^{99m}Tc]Tc-Myoview for general NM, 19.6-22 GBq [^{18}F]FDG for PET radiopharmacy and 11 GBq [^{99m}Tc]Tc-Myoview for NM radiopharmacy) and from control participants who do not work with radiation. A second blood sample was taken from technologists within 30 minutes of drawing up and administering a radiopharmaceutical to a patient. Samples from controls participants were collected in the morning before they started their workday. All blood samples were immediately placed on ice following collection and maintained under these conditions until sample preparation. Samples were diluted 1:1 with phosphate-buffered saline (PBS; Sigma-Aldrich), layered over Histopaque-1077 (Fisher Scientific) and centrifuged at 2000g (MSE Falcon 6/300) for 15 minutes to isolate the lymphocytes. The lymphocyte-containing buffy coat was collected, washed in 10 mL PBS, and centrifuged at 1000g for 5 minutes. The cell pellet was then resuspended in minimum essential medium (MEM; Thermo-Fisher).

Fixing and permeabilization for γH2AX

Samples were centrifuged at 1000g for 5 minutes, the supernatant was discarded and cells pelleted onto a Teflon/silane-coated 14-well slide (Tekdon) and allowed to adhere for 15 min at room temperature. 2% Formaldehyde (Sigma-Aldrich) in PBS was then added to each well for 5 minutes at room temperature to fix cells. Excess liquid was removed and 0.5% Triton-X(Sigma-Aldrich)/PBS mix was added to each well for 5 minutes to permeabilise cells. This was then replaced with 1% bovine serum albumin (Roche Diagnostics) in PBS to block non-specific epitopes.

γH2AX immunofluorescence labelling

Following fixation, permeabilization and blocking, cells were incubated with 50 µL primary γH2AX antibody (BioLegend; 1:500 in 1% BSA/PBS) for 45 min and washed with 1% BSA/PBS followed by a secondary staining with antibody/DAPI mix of goat anti-mouse antibody (1:500 in 1% BSA/PBS, AF488-conjugated; Invitrogen) and DAPI (Roche Diagnostics) for 30 min. Slides were then washed with 1% BSA/PBS, mounted with Vectashield Vibrance (Vector laboratories), and analysed under a fluorescent microscope at x40 magnification for γH2AX foci.

Chromosomal damage: Dicentric chromosomes

Blood sample preparation

Venous blood samples from technologists and controls were cultured in minimum essential medium (MEM; Thermo-Fisher). 40 mL of MEM was supplemented with heparin (2,000 U/mL in 0.85% saline; Thermo-Fisher), L-glutamine (200 mM; Thermo-Fisher;), penicillin (10,000 U/mL; Thermo-Fisher), streptomycin (10,000 µg/mL;Thermo-Fisher), bromodeoxyuridine (BrdU; 20 µM Merck), phytohaemagglutinin (PHA; at 2 µg/mL;Thermo-Fisher), and foetal bovine serum (FBS, 5 mL; Thermo-Fisher).

Dicentric chromosome preparation

4.6 mL of the prepared medium was added to 0.4 mL of blood in a cell culture tube and incubated at 37°C in a humidified atmosphere with 5% CO_2. for 45 h. Colcemid (0.1 mL; 0.5 µg/mL final concentration) was added for 3 h at 37°C to arrest cells in metaphase. Cultures were resuspended at 48h and centrifuged at 300 g for 5 min for lymphocyte separation. The supernatant was removed, and 6 mL of pre-warmed potassium chloride (KCL 75 mM; VWR) in distilled water containing 16 U/mL heparin was added. Tubes were incubated in a 37°C water bath for 7 minutes and centrifuged as before. The supernatant was discarded, and cells were fixed with methanol: acetic acid (3:1), centrifuged, and washed three times with fresh fixative. The cell pellet was resuspended in fixative.

Slide preparation

Cell suspensions were dropped onto cold microscope slides, dried and treated with 100 µL of RNAase (0.5 mg/mL; Merck) in a moist chamber at 37°C for 10 minutes. Slides were briefly rinsed in distilled water, washed in fixative and dried on a 37°C hot plate. To assess for second divisions, slides were submerged for 25 minutes in 0.5 µg/mL Hoechst (Sigma). The slides were rinsed in distilled water and Hoechst solution was added to each slide before being mounted on a coverslip, placed under UV light for 25 minutes and

rinsed in distilled water. The slides were then stained with 5% Giemsa stain (GWR) for 2-3 minutes and rinsed in distilled water before drying on a hotplate for at least 30 minutes. Once dry, slides were dipped in xylene (Fischer Scientific) for 5 minutes and mounted under a coverslip with Shandon mounting medium (Fisher Scientific) then dried on a hot plate for 2 h.

Scoring

Metaphases were scored at 63x magnification using the Metafer 4 Slide Scanning System (Metasystems Altlussheim, Germany), which scans and locates metaphases and captures high-power images for relocation and subsequent analysis. Metaphases were manually verified and dicentric chromosomes scored, consistent with best practice.

SUPPLEMENTARY TABLE

Table S1. Overview of nuclear medicine technologists' body and finger doses after administering a radiopharmaceutical to a patient or after handling a radiopharmaceutical in pharmacy.

TECHNOLOGIST	BODY (µSv)	FINGER (µSv)	RADIOPHARMACEUTICAL	ACTIVITY ADMINISTERED OR HANDLED (MBq)
T1	3	326	[^{18}F]FDG	350
T2	2	253.8	[^{18}F]FDG	350
T3	1	163.1	[^{18}F]FDG	350
T4	1	88.3	[^{18}F]FDG	350
T5	2	378	[^{18}F]FDG	350
T6	1	120	[^{18}F]FDG	350
T9	4.5	Not recorded	[^{18}F]FDG	22,000
T10	3	203	[^{18}F]FDG	350
T11	5.1	Not recorded	[^{18}F]FDG	21,000
T12	8	Not recorded	[^{99m}Tc]Tc-Myoview	11,000
T13	1	183	[^{18}F]FDG	350
T16	2.6	Not recorded	[^{18}F]FDG	19,600
T17	1	Not recorded	[^{99m}Tc]Tc-Myoview	220
T18	1	43.1	[^{99m}Tc]Tc-Myoview	220
T19	1	119	[^{99m}Tc]Tc-Myoview	250
T20	1	24	[^{99m}Tc]Tc-Myoview	170

Table S2. Unadjusted and adjusted linear regression analyses of the association between historical annual whole-body dose and dicentric chromosome frequency. The adjusted model included sex, smoking history and family history of cancer. Current and former smokers were combined into an ever-smoker category and compared with never-smokers. β denotes the regression coefficient and CI denotes confidence interval.

Model	Predictor	β	95% CI	p-value
Unadjusted	Historical annual whole-body dose	−0.0238	−0.0699 to 0.0224	0.292
Adjusted	Historical annual whole-body dose	0.000848	−0.0579 to 0.0596	0.976
	Family history of cancer: Yes vs No	0.0706	−0.00968 to 0.151	0.08
	Sex: Male vs Female	0.0085	−0.0543 to 0.0713	0.775
	Smoking: Ever vs Never	−0.0212	−0.102 to 0.0596	0.581

SUPPLEMENTARY FIGURES

Please **initial** box if you agree with the following

1. I confirm that I have read and understand the information sheet dated 21/03/2023 (Version 3), for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my legal rights being affected. ☐
3. I understand that relevant sections of any of my medical notes and data collected during The study, may be looked at by responsible individuals from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐
4. I agree to have a blood sample taken as described in the information sheet. ☐
5. I agree that my samples may be stored anonymously and used in future related approved research projects. ☐
6. I agree that my samples maybe transferred anonymously to UK laboratories external to the university for further analysis. ☐

Participant	**Person responsible for collecting the informed consent.**
Date:	Date:

Figure S1. Consent form for staff.

PARTICIPANT CONTACT

Name:

Address:

Email address:

Telephone number:

PARTICIPANT INFORMATION

Today's Date:

Male or Female:

Age: 20-39 ☐ 40-59 ☐ 60+ ☐

Height:

Weight:

PART 1 WORK HISTORY

1. Which year did you first start working in nuclear medicine?
2.

For each job you have worked please answer the following questions.

You do not need to name the facility you worked at, just the year. Some dates may be difficult to recall but your best estimates will be valuable for the study

2. Number of years at first job: Year Started

Year left

3. How many hours per week did you work?

4. How many scanners did the department have?

1 ☐ 1-2 ☐ 2-4 ☐ More than 4 ☐

5. Was the hot waiting room within the department?

Yes ☐ No ☐

6. How many times per week did you perform the following diagnostic procedures?

Scans	**Never (0)**	**Frequency per week (1 or more)**
Bone		
Cardiac		
Parathyroid		
Indium White Blood Cell		
Indium		
Thyroid		
DAT		
Renograms		
V/Q		
HIDA		
Gastric emptying		
PET (brain)		
PET (oncology)		
PET (other)		
Other diagnostic procedures not listed		**Frequency per week (1 or more)**

7. How many times per week did you perform the following therapeutic procedures?

Therapy	**Never (0)**	**Frequency per week (1 or more)**
Thyroid Ablation		
Ytrium		
Radium		
Other therapies not listed		**Frequency per week (1 or more)**

<u>The following questions are in relation to scanning procedures only.</u>

8. Was the acquisition station where you sat during the scan in the same room as the scanner?

Yes ☐ No ☐

If Yes, how close were you to the patient?

1 meter or less ☐ 2-3 meters ☐ 4 -5 meters ☐ More than 5 meters ☐

9. Was there a shield (movable or fixed) between you and the patient?

Yes ☐ No ☐

10. How many times per week did you perform the following non imaging procedures?

Procedure	Never (0)	Frequency per week (1 or more)
Elute the generator		
Draw up radiopharmaceuticals		
Inject radiopharmaceuticals		
Perform cardiac stress testing		
Administer iodine for thyroid therapy		

11. When performing non-imaging procedures, did you use the following?

Lead shielded	Always	>50% of the time	<50% of the time	Never
Vial				
Syringe				

PART 2 MEDICAL HISTORY

1. How many hours per week do you exercise strenuously? (Examples: running, vigorous swimming)

None, ½ hr, 1hr, 1½ hr, 2-3 hrs, 4-6 hrs, 7-9 hrs, 10 hrs or more

2. How many hours per week do you exercise moderately? (Examples: walking for exercise, light swimming, golf).

None, ½ hr, 1hr, 1½ hr, 2-3 hrs, 4-6 hrs, 7-9 hrs, 10 hrs or more

3. How many hours per week do you do weight training or resistance exercise?

None, ½ hr, 1hr, 1½ hr, 2-3 hrs, 4-6 hrs, 7-9 hrs, 10 hrs or more

4. Do you currently smoke cigarettes?

Yes ☐ No ☐

If yes, how many cigarettes per day do you usually smoke?

1-10 11-20 21-30 31-40 41 or more

5. Have you smoked cigarettes in the past?

Yes ☐ No ☐

6. How old were you when you started smoking?

7. How old were you when you stopped smoking?

8. How many cigarettes per day did you usually smoke? (SELECT THE ONE BEST ANSWER)

1-10 11-20 21-30 31-40 41 or more

9. Have you ever been diagnosed with cancer?

Type:

Date of diagnosis:

10. Have any of your blood-related parents, siblings or children ever been diagnosed with cancer?

Type:

Age at diagnosis:

11. Have you had the following diagnostic ionizing radiation procedures performed on you in the last year?

Procedure	**Part of the body**	**How many times** (Count twice if done with and without contrast)	**When** (year)
CT Scan			
X-Ray			
Fluoroscopy			
Nuclear Medicine scan			
PET/CT scan			

Thank you for taking the time to complete the questionnaire. Following are extra pages for each job. Should you require more pages, please email and we shall send them to you. Your participation in the study is valuable and greatly appreciated.

Figure S2. Questionnaire for technologists (4 pages).

PARTICIPANT CONTACT

Name:

Address:

Email address:

Telephone number:

PARTICIPANT INFORMATION

Today's Date:

Male or Female:

Age: 20-39 ☐ 40-59 ☐ 60+ ☐

Height:

Weight:

PART 1 WORK HISTORY

1. What is your current occupation?

2. How many years have you worked at this job?

3. Do you work with ionizing radiation?

Yes ☐ No ☐

4. Do you work with any chemicals?

Yes ☐ No ☐

5. If yes to (4) which chemicals do you use?

PART 2 MEDICAL HISTORY

1. How many hours per week do you exercise strenuously? (Examples: running, vigorous swimming)

None, ½ hr, 1hr, 1½ hr, 2-3 hrs, 4-6 hrs, 7-9 hrs, 10 hrs or more

2. How many hours per week do you exercise moderately? (Examples: walking for exercise, light swimming, golf).

None, ½ hr, 1hr, 1½ hr, 2-3 hrs, 4-6 hrs, 7-9 hrs, 10 hrs or more

3. How many hours per week do you do weight training or resistance exercise?

None, ½ hr, 1hr, 1½ hr, 2-3 hrs, 4-6 hrs, 7-9 hrs, 10 hrs or more

4. Do you currently smoke cigarettes?

Yes ☐ No ☐

If yes, how many cigarettes per day do you usually smoke?

1-10 11-20 21-30 31-40 41 or more

5. Have you smoked cigarettes in the past?

Yes ☐ No ☐

6. How old were you when you started smoking?

7. How old were you when you stopped smoking?

8. How many cigarettes per day did you usually smoke? (SELECT THE ONE BEST ANSWER)

1-10 11-20 21-30 31-40 41 or more

9. Have you ever been diagnosed with cancer?

Type:

Date of diagnosis:

10. Have any of your blood-related parents, siblings or children ever been diagnosed with cancer?

Type:

Age at diagnosis:

11. Have you had the following diagnostic ionizing radiation procedures performed on you in the last year?

Procedure	**Part of the body**	**How many times** (Count twice if done with and without contrast)	**When** (year)
CT Scan			
X-Ray			
Fluoroscopy			
Nuclear Medicine scan			
PET/CT scan			

Thank you for taking the time to complete the questionnaire. Your participation in the study is valuable and greatly appreciated.

Figure S3. Questionnaire for non-technologist participants (3 pages).

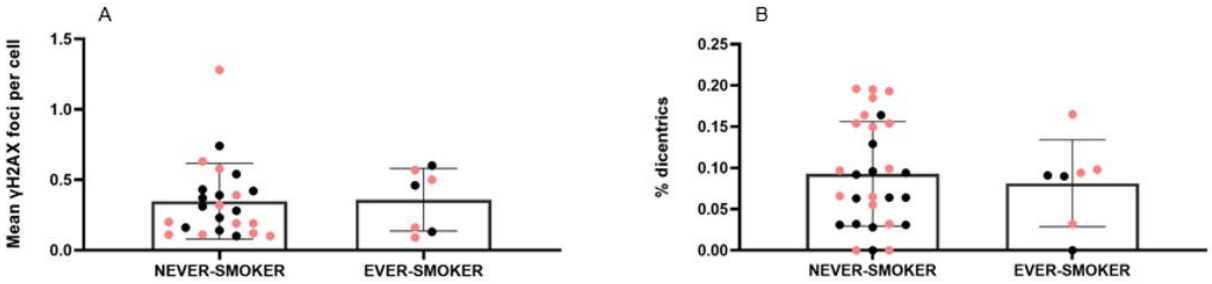

Figure S4. γH2AX and dicentric chromosomes in blood lymphocytes from control participants and technologists plotted against smoking history. (A) γH2AX foci/cell and (B) percentage of dicentric chromosomes in never-smokers and ever-smokers. Samples from technologists were taken before handling of radiopharmaceuticals. Coloured dots indicate technologists. Samples with unsuccessful γH2AX staining were excluded from the analysis. In contrast, dicentric chromosome analysis was successfully performed for all 20 technologists and 16 controls.

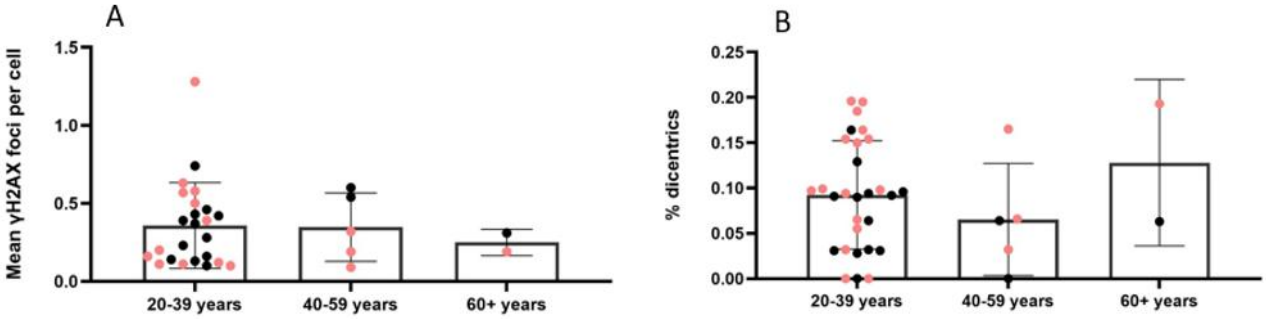

Figure S5. γH2AX and dicentric chromosomes in blood lymphocytes from control participants and technologists plotted against age. (A) γH2AX foci/cell and (B) percentage of dicentric chromosomes in different age groups. Samples from technologists were taken before handling of radiopharmaceuticals. Coloured dots indicate technologists. Samples with unsuccessful γH2AX staining were excluded from the analysis. In contrast, dicentric chromosome analysis was successfully performed for all 20 technologists and 16 controls.

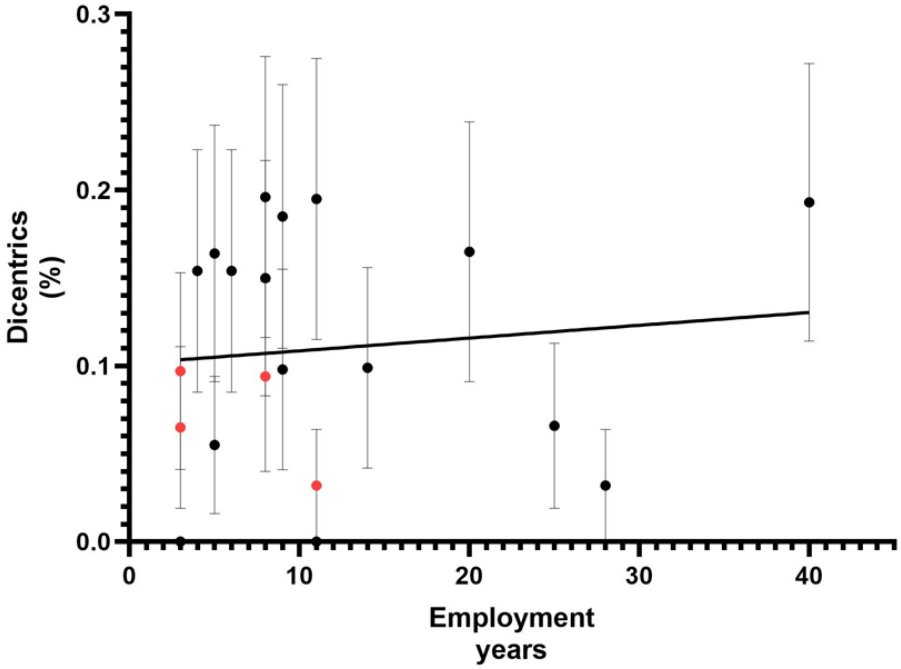

Figure S6. Dicentric chromosomes in blood lymphocytes of technologists plotted against years in employment. Points represent individual technologists and error bars indicate ± SEM. Red points indicate technologists with beta-radiation exposure. Pearson's correlation showed a weak, non-significant positive correlation, r(18) = 0.11, p = 0.65.(n=20).

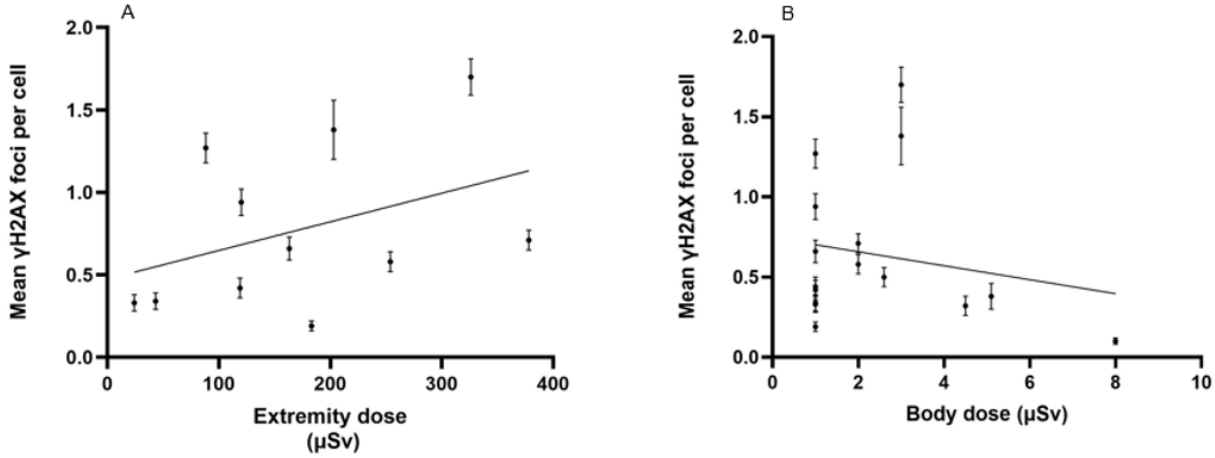

Figure S7. Correlation between γH2AX with measured doses. Mean foci per cell in technologists' blood lymphocytes versus live finger (A) and whole body (B) dosimeter readings after a radiopharmaceutical injection.

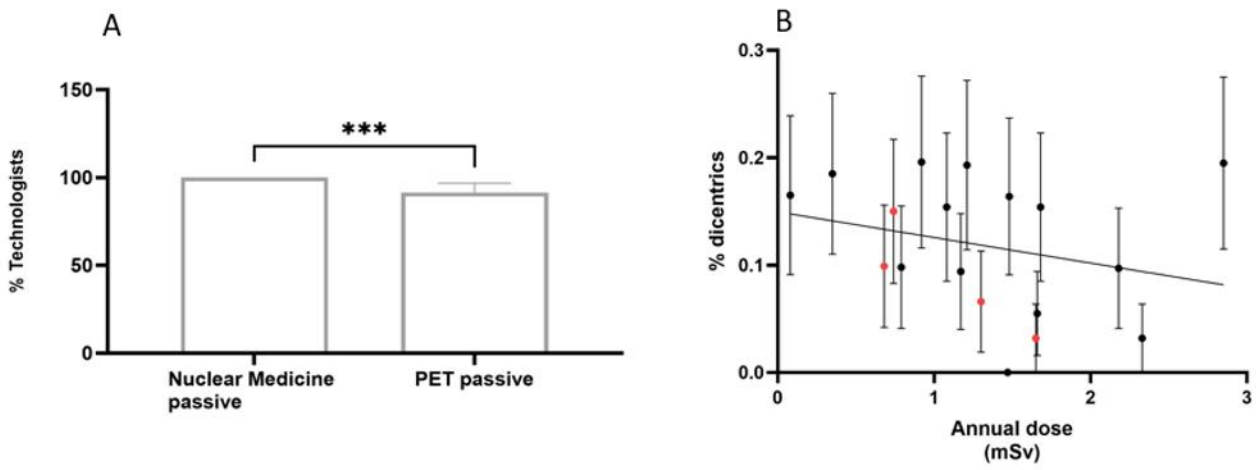

Figure S8. Observation of dosimeter practices and correlation of dicentrics with historical whole-body dose. (A) Compliance of passive whole-body dosimeter use over eight observation days in a nuclear medicine department (5–8 technologists per day) and a PET department (9–15 technologists per day). (B) Percentage of dicentric chromosomes in blood lymphocytes plotted against calculated annual radiation dose. Points represent individual technologists and error bars indicate ± SEM. Red points indicate technologists with beta-radiation exposure. Pearson's correlation showed a weak, non-significant negative correlation, $r(16) = -0.26$, $p = 0.29$; $n = 18$. Dose records were unavailable for two technologists and not included in the analysis. Asterisks indicate statistical significance ***$p < 0.001$