Detecting Radiation-Induced Injury Using Rapid 3D Variogram Analysis of CT Images of Rat Lungs

Richard E. Jacob, PhD, Mark K. Murphy, MS, Jeffrey A. Creim, BS, James P. Carson, PhD

Rationale and Objectives: To investigate the ability of variogram analysis of octree-decomposed computed tomography (CT) images and volume change maps to detect radiation-induced damage in rat lungs.

Materials and Methods: The lungs of female Sprague-Dawley rats were exposed to one of five absorbed doses (0, 6, 9, 12, or 15 Gy) of gamma radiation from a Co-60 source. At 6 months postexposure, pulmonary function tests were performed and four-dimensional (4D) CT images were acquired using a respiratory-gated microCT scanner. Volume change maps were then calculated from the 4DCT images. Octree decomposition was performed on CT images and volume change maps, and variogram analysis was applied to the decomposed images. Correlations of measured parameters with dose were evaluated.

Results: The effects of irradiation were not detectable from measured parameters, indicating only mild lung damage. Additionally, there were no significant correlations of pulmonary function results or CT densitometry with radiation dose. However, the variogram analysis did detect a significant correlation with dose in both the CT images ($r = -0.57$, $P = .003$) and the volume change maps ($r = -0.53$, $P = .008$).

Conclusion: This is the first study to use variogram analysis of lung images to assess pulmonary damage in a model of radiation injury. Results show that this approach is more sensitive to detecting radiation damage than conventional measures such as pulmonary function tests or CT densitometry.

Key Words: Octree; variogram; lung; irradiation; CT imaging.

With the onset of certain diseases, lung tissue becomes more heterogeneous (ie, tissue density may present increased spatial variability), as is frequently evident in computed tomography (CT) images (1,2). Recent work by Subramaniam et al. (3) used quadtree decomposition in two-dimensional (2D) slices of lung CT images to analyze heterogeneity. Selected slices were iteratively subdivided into quadrants based on an intensity range threshold and heterogeneity measured as the number of squares per area. However, this 2D approach neglects large portions of the lung and ignores three-dimensional (3D) spatial relationships. We extended the quadtree concept to 3D by using octrees to iteratively and nonsubjectively divide an entire 3D image into homogeneous cubes. This approach focuses on the parenchyma by eliminating tissue boundaries and reducing the influence of the vasculature (4). Instead of simply calculating the cube density, we analyzed spatial relationships for indications of heterogeneity using variograms, a well-established geostatistics tool for measuring spatial variability that compares sample variances to the distance of separation without a priori assumptions about the spatial relationships (5,6). Recent biologic applications of the variogram include the characterization of brain white matter in magnetic resonance images (7).

In this article, we demonstrate the use of noninvasive imaging and a novel 3D image analysis approach using variograms of octree-decomposed images to detect subtle injury in radiation-exposed rat lungs. It has been shown that lung injury in radiation-exposed rat lungs includes acute inflammation in the weeks after exposure and chronic fibrosis in the months after exposure while providing a relatively uniform injury over the dosed area (8–10). We show that the results of variogram analysis on octree-decomposed CT images and 4DCT-based volume maps of irradiated lungs correlates with radiation dose better than physiologic measurements, conventional pulmonary function tests, or CT density measurements.

MATERIALS AND METHODS

All animal use followed a protocol approved by the Institutional Animal Care and Use Committee of our institution. Twenty-five female Sprague-Dawley rats weighing 216 ± 9 g were used.

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From Systems Toxicology (R.E.J., J.A.C., J.P.C.) and Instrumentation Services and Technology (M.K.M.), 902 Battelle Blvd., Pacific Northwest National Laboratory, Richland, WA 99352; present address for M.K.M.: Hanford Radiological Instrumentation Program, Mission Support Alliance, Richland, Washington. Received May 14, 2013; accepted July 2, 2013. **Address correspondence to:** R.E.J. e-mail: richard.jacob@pnnl.gov

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Lung irradiations employed an ~6400 Ci Co-60 gamma source with an ~30-cm-thick lead collimator. The collimator was trapezoidal to match the basic outline of the lungs, with dimensions based on a 3D image of a weight-matched control rat: 15 mm wide at the top, 30 mm wide at the bottom, and 26 mm high. The bottom was convexly tapered by 5 mm in the center to minimize exposure to the liver. The resulting beam was then calibrated in terms of absorbed dose to tissue (Gy/min) using a tissue-equivalent ionization chamber (Exradin model A12, Standard Imaging, Middleton, WI) connected to an electrometer (model 617, Keithley Instruments, Cleveland, OH) to collect the resulting current. Appropriate corrections were applied to convert from exposure in air to absorbed dose in tissue (11). The measured absorbed dose rate for the estimated location of the lung center (~1.5 cm from collimator face) was 3.74 Gy/min. It was determined that 1.5 cm of tissue results in approximately 5% reduction in absorbed dose rate, resulting in an estimated 3.55 Gy/min at lung center. One of five calculated doses of 0.0, 5.9, 8.8, 11.8, and 14.7 Gy (hereafter referred to as 0, 6, 9, 12, and 15 Gy) was delivered to the thorax; this dose range has been shown to cause significant injury in other rat strains (10,12,13). The dose rate to the body was measured to be ≤0.3% of that at the lung center. The dosed region was confirmed in a weight-matched rat using an x-ray source and Polaroid radiographic film.

Anesthetized rats were placed in a custom-made contoured holder to facilitate reproducible positioning of the rat thorax directly in front of the collimator. Rats were randomly assigned a radiation dose, with five rats per group. Irradiations were blind to the staff performing other measurements in order to reduce bias.

Following irradiation, rats were returned to the animal facility where they were individually housed, provided food and water ad libitum, and observed daily for general well-being. One rat from the 6-Gy group developed a ~3 cm growth on its back and was eliminated from the study. Otherwise, no mortality or outward signs of poor health were observed. Two additional rats were kept in the same room as health sentinels, and at the end of the study they were confirmed to be seronegative for common rat pathogens.

At 6 months postirradiation, rats were subjected to pulmonary function tests (PFT) and microCT imaging. First, a whole-body plethysmograph (Buxco Research Systems, Wilmington, NC) was used to measure breathing rate, tidal volume, and minute volume of unanesthetized, unrestrained rats for ~5 minutes. Rats were acclimated to the chamber for ~10 minutes per day for several days before the test.

Next, rats were imaged using four-dimensional (4D) CT (multi-time-point 3D imaging). Details of animal preparation, ventilation, and 4DCT imaging closely follow those described in (14). Rats were anesthetized with 4% isoflurane in oxygen, orally intubated with a 14-gauge catheter tube, and connected to a computer-controlled mechanical ventilator (model 830/AP, CWE Inc., Ardmore, PA). Rats were maintained on isoflurane and ventilated with 30% O_2 (balance N_2) at 54 breaths per minute, with a 500-ms inhale duration and no breath-hold. Periodic sighs were delivered every 100 breaths to maintain lung recruitment. The ventilator recorded tracheal pressure, inspiratory volume, and expiratory volume. Peak inspiratory volume (PIV) was ~2.1 mL. No positive end-expiratory pressure was used so that images could be acquired at full passive exhalation when the lung volume was at functional residual capacity (FRC). A microCT scanner (eXplore 120, GE Healthcare, Waukesha, WI) with ventilatory gating was used to acquire 11 images throughout the breathing cycle in 26 minutes with 100-ms temporal resolution (we note that only the images at the breathing cycle extremes—FRC and PIV—were analyzed for this study). Gating was tested by comparing the ventilator gate signal to a signal sent by the scanner on firing x-rays; the delay between the ventilator trigger and x-ray firing was found to be ≤250 microseconds. CT imaging parameters were: 80 kVp, 32 mA, 16-millisecond exposure time, and 360 projections with 1° angular separation. The estimated radiation dose from all images was 940 mGy. Images were reconstructed to 200 μm isotropic resolution using supplied software. We empirically found that this resolution provided sufficient detail for later analysis.

Following imaging, PFT were performed. Rats were anesthetized with an intraperitoneal injection of ketamine/xylazine and surgically intubated for measurement of inspiratory volumes, forced expiratory volumes, and quasistatic chord compliance using a Forced Maneuvers system (Buxco Research Systems). PFT measurements took ~5 minutes. Immediately after the pulmonary function tests, animals were euthanized via CO_2 asphyxiation and lungs were excised to obtain wet and dry weights.

Acquired images were processed with a 5-pixel-diameter 3D median filter to remove noise while maintaining feature boundaries. These filtered images were then masked to assign intensity value 0 to nonlung regions; filtered images were multiplied with a binary image created using the 3D connected threshold tool in the 3D Plugins Toolkit of ImageJ (15,16) to delineate lung from nonlung-based on intensity. From these masked images, the mean and standard deviation σ of the distribution of Hounsfield units (HU) in the lung were determined. The coefficient of variation (CoV) was then calculated by taking the ratio of σ to the mean.

Octree subdivision reduces the original volume into eight octants of equal dimension (Fig 1). Images were first zero-filled to 256 × 256 × 256 so that octree subdivision would result in isotropic cubes. Iteratively, each octant was further subdivided if it contained any mask voxels or if its σ exceeded a threshold value t, unless the octant reached a minimum size of 2 × 2 × 2. All octants containing the mask value of 0 were discarded from further analysis. Remaining octants larger than 2 × 2 × 2 represented relatively homogeneous sections of the lung, whereas the 2 × 2 × 2 cubes defined boundaries and regions with high spatial variability. In developing this approach, we empirically determined the optimal t to be approximately two-thirds the mean of the control group's