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Wiegman, EM; Meertens, H; Konings, AWT; Kampinga, HH; Coppes, RP

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Loco-regional differences in pulmonary function and density after partial rat lung irradiation


a Department of Radiation and Stress Cell Biology, University of Groningen, Ant. Deusinglaan 1, 9713 AV Groningen, Germany
b Department of Radiation Oncology, University Hospital Groningen, P.O. Box 30.001, 9700 RB Groningen, Germany

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Abstract

Purpose: The purpose of this study was to explore regional differences in radiosensitivity of rat lung using lung function and computed tomography (CT) density as endpoints.

Methods: At first, CT scans were used to determine rat lung volumes. The data obtained enabled the design of accurate collimators to irradiate 50% of the total lung volume for the apex, base, left, right, mediastinal and lateral part of the lung. Male Wistar rats were irradiated with a single dose of 18 Gy of orthovoltage X-rays. Further rat thorax CT scans were made before and 4, 16, 26, and 52 weeks after irradiation to measure in vivo lung density changes indicative of lung damage. To evaluate overall lung function, breathing frequencies were measured biweekly starting 1 week before irradiation.

Results: Qualitative analysis of the CT scans showed clear density changes for all irradiated lung volumes, with the most prominent changes present in the mediastinal and left group at 26 weeks after radiation. Quantitative analysis using average density changes of whole lungs did not adequately describe the differences in radiation response between the treated groups. However, analysis of the density changes of the irradiated and non-irradiated regions of interest (ROI) more closely matched with the qualitative observations. Breathing frequencies (BF) were only increased after 50% left lung irradiation, indicating that the hypersensitivity of the mediastinal part as assessed by CT analysis, does not result in functional changes.

Conclusions: For both BF and CT (best described by ROI analysis), differences in regional lung radiosensitivity were observed. The presentation of lung damage either as function loss or density changes do not necessarily coincide, meaning that for each endpoint the regional sensitivity may be different.

Keywords: Lung damage; Ionising radiation; Rat; Volume

1. Introduction

The radiotherapeutic treatment of malignant diseases in the thorax is limited by the tolerance of lung tissue. This is due to the high radiosensitivity of lung parenchyma. The subsequent clinical sequelae of radiation-induced lung damage can be separated into three or four phases. During the first sub-clinical phase, no symptoms are present. Thereafter, acute radiation pneumonitis, for which the first clinical symptoms appear 2–3 months after treatment characterized by cough, dyspnea and respiratory failure, characterizes the second phase. Thirdly, a state of lung fibrosis starts to appear from 6 months to years after treatment [2,6,13]. The transformation from pneumonitis to fibrosis may be either continuous or separated by a second sub-clinical phase, dependent on the dose used. These sequelae can be life threatening and therefore limit the dose that can be applied to the tumour. Modern techniques in radiotherapy like intensity modulated radiation therapy (IMRT) aim to reduce normal tissue damage while escalating the dose to the tumour region. This may lead to completely new dose distribution in the tissue with a high dose delivered to a small volume and a low dose to a large volume. It is not known whether these changes in dose distribution will have an effect on the radiation-response of the lung. The detailed study of radiation-induced lung complications in humans is difficult due to pre-existing diseases like emphysema, chronic bronchitis and differences in life-style, which may result in substantial differences in functional reserve [3]. Consequently, the interpretation of
follow-up lung function measurements after radiotherapy in the chest may be uncertain. Furthermore, the application of specific dose-volume irradiations necessary to study mechanisms behind radiation damage cannot be performed in humans. For this reason a variety of animal studies have been set up in the past to evaluate lung function after irradiation.

Studies in mice [10,16,17] not only showed that the response of the lung to radiation is dependent on the irradiated volume, but also suggested the existence of loco-regional differences in lung sensitivity to radiation. As such, signs of radiation pneumonia were significantly more pronounced in case of irradiation of the base of the lung compared to irradiation of the apex of the lung. It was concluded that a spatial heterogeneity of the volume effect exists for radiation pneumonitis in the mouse lung. This was suggested to be due to a difference in the distribution of target cells throughout the lung. In our laboratory, rat lungs have been shown to respond to irradiation in a pattern similar as to that in humans [19,20]. In agreement with this, an increase in breathing frequency was observed 16 weeks after hemithoracic right-lung irradiation with a dose of 18 Gy. Both function and histological data suggested the presence of lung fibrosis [20] similar to that observed in humans, indicating the usefulness of the rat as a model to study radiation-induced damage to the lung. In rats, only one study on regional lung sensitivity differences has been reported [8] using micronuclei formation as an end-point. These data were similar to the mice data in that a higher proportion of micronuclei containing cells were observed in cultures of fibroblasts obtained from the base, when compared to the apex of the lungs.

The use of rats allows more accurate irradiation of the thorax, since rats have a 10-fold larger lung volume than mice [9]. Moreover, irradiation fields can be precise, using thorax, since rats have a 10-fold larger lung volume than compared to the apex of the lungs. These data were similar to the mice data in that a higher proportion of micronuclei containing cells were observed in cultures of fibroblasts obtained from the base, when compared to the apex of the lungs.

2. Methods and materials

2.1. Animals

The experiments were performed with adult male albino Wistar rats of the strain Hsd/Cpb:WU (Harlan-CPB, Rijswijk, The Netherlands). The rats were housed six each in a polycarbonate cage under a 14:10-h light cycle. Bedding consisted of wood shaves. Food (RMH-B, Hope Farms, Woerden, The Netherlands) and water were given ad libitum. At the time of arrival, rats were 8 weeks old and weighed approximately 240 g. Before starting the experiments, they were habituated for 2 weeks. All experiments were performed in agreement with the Netherlands Experiments on Animals Act (1977) and the European Convention for the Protection of Vertebrate Animals Used for Experimental Purposes (Strasbourg, 18.III.1986).

2.2. Design of the collimators

In order to design accurate collimators, five non-irradiated rats (300–340 g) were anaesthetised using an intraperitoneal (i.p.) injection of ketamine (67.5 mg/kg) and xylazine (4.5 mg/kg). Rats were positioned in a Perspex irradiation holder, using the upper incisors as a fixed point, resulting in a vertical positioning of the animals. This holder was used for the CT scans for lung topography, volume determination and also for the irradiation procedure, ensuring an identical position in all situations. Using this method, the precision of the positioning of the rats was within 0.3 mm (1 SD). A CT unit (Philips Tomoscan SR 7000 High Resolution) was used to scan the rat thorax with a slice thickness of 1.5 mm, an inter-slice distance of 1.0 mm and a pixel size of 0.31 × 0.31 mm². Using a general purpose computer program OSIRIS (OSIRIS Medical Imaging, Geneva University Hospital [11], for the display, manipulation, and analysis of digital medical images, the lung contours were outlined, and the volumes were calculated. The data obtained enabled us to accurately calculate 50% of the total lung volume for the apex, base, left, right, mediastinal and lateral parts of the lung and delineate the corresponding borders of these partial volumes. Six different radiation ports were then constructed by projecting all lung contours plus a margin of 5 mm in the collimator plane and constructing a final contour that enclosed all other contours. At the site of the 50% lung boundaries, no margin was taken to determine the radiation field edge. Six collimators were constructed, sized 140 × 110 mm consisting of 3 mm Pb for the irradiation of the apex, base, left, right, mediastinal and lateral parts of the lung. Outside the irradiation window (50 × 70 mm), an extra layer of 3 mm brass was used for reinforcing the collimator. In order to achieve the same dose in every region, the conversion from air to tissue was done using backscatter factors [7].
region was irradiated, we chose a single dose of 18 Gy, which is known to exert a pronounced radiation response. This dose was delivered by a single horizontal anterior–posterior field at source to skin distance of 22 cm, at a dose rate of 2.05 Gy min$^{-1}$. Irradiation was performed using an X-ray machine (Mueller MG 300, Philips, Eindhoven, the Netherlands) operated at a high voltage of 200 kV (filters: 0.5 mm copper and 0.5 mm aluminium; HVL = 1.05 mm copper) and a beam current of 15 mA. Dose was measured in air with a calibrated electrometer and Baldwin Farmer type ionisation chamber combination (Keithley electrometer type 35040 and Nuclear Enterprises ionisation chamber, type NE 2571). The dose inhomogeneity across the lung was estimated to be about 40% at maximum, i.e. the exit dose is about 60% of the entrance dose at the site where the depth of the lung is at largest.

2.4. CT density measurements

The whole thorax was scanned with the same CT unit, using a slice thickness of 1.5 mm, an interslice distance of 1.0 mm and a pixel size of $0.59 \times 0.59 \text{ mm}^2$. Prior to scanning, the rats were anaesthetised as above. The rats used for the total lung volume determination were used for control lung CT density measurements. Further CT scans were performed 4, 16, 26 and 52 weeks after irradiation. At each time point, the same three randomly chosen rats were scanned in a specially designed CT-holder, which could hold three rats at a time. This holder also contained two different plastic rods for the verification of the CT density scale. The dose received due to the CT scanning was less than 1 cGy per session and was neglected.

Two different methods for analysis of the lung CT density were used. At first, the whole lung densities were analysed by calculating mean pixel value and standard deviation (SD) of the density distribution. This method involves outlining the lung contours on all slices, and calculating the mean density therein. This method included not only irradiated lung parts, but also the shielded parts. The SD of the density distribution provided information about the homogeneity of the density within lung tissue. As fibrosis is a focal process, with either fibrosis or regions of

![Fig. 1. Portal films of the radiation fields. All radiation fields consist of 50% of the total lung volume, as calculated with computerized tomography. For the lateral field, each portal consists of 25% of the total lung volume adding up to 50%. Differences in intensity have occurred as a result of varying exposure times. White contours involve shielded lung tissue. The pinpoints (black arrow) were used as reference points. Left 50% (a), right 50% (b), apex 50% (c), base 50% (d), mediastinal 50% (e), lateral 50% (f).](image-url)
emphysema [9] a rise in SD value was expected to indicate a radiation response.

In the second method, small regions of interest (ROIs) were analysed. Here, a polygon of 50 mm² was drawn in the first three CT slices located ventrally of the trachea, both in shielded and irradiated parts. As such, both hyperinflation and shrinkage of the lung was taken into account while drawing the ROIs. ROIs of 50 mm² were large enough to prevent the manipulating of the ROI location and thus, reduced the observer’s bias. Since the interslice thickness was 1.0 mm, the density was analysed in a volume of 150 mm³. Using this method, density changes in both irradiated and shielded lung parts could be quantified separately. Since the dose gradient across the lung volume in the direction of the radiation beam was about 1.6% mm⁻¹, the dose fall-off in the three CT slices in which the ROIs were drawn in was (3* 1.6%) 4.8%.

All CT value measurements were performed using OSIRIS. Density data were given as CT numbers and expressed as Hounsefield units (HU). CT numbers were related to attenuation coefficients. CT numbers ranged from $-1000$ (black in the images) for air to about $+1000$ for bone (white in the images), water was set at $0$ (grey in the images). Other typical values were muscle CT $= 45$ H, and fat CT $= -65$ H. The data obtained were used to construct frequency distributions, showing the distribution of the CT values of the pixels in a region of interest. After irradiation, changes in the lung parenchyma caused the curve to shift to the right in case of density increase (fibrosis, edema) or to the left in case of a density decrease (emphysema, hyperinflation).

2.5. Overall lung function

Lung function was monitored as previously described [20], once before and biweekly after irradiation. Rats were placed in an airtight 1500 ml plethysmograph, connected to a pressure transducer, which enabled us to measure the breathing frequency. Each rat was measured when at rest showing stable breathing patterns. Five periods were used to calculate mean breathing rate.

2.6. Data analysis

CT density measurements were noted as the mean CT pixel value of the whole lung, with the standard deviation (SD) of the distribution. Density measurements for the whole lung were performed at 0, 4, 16, 26 and 52 weeks after irradiation. Using a Kolmogorov–Smirnov test, we tested whether the distributions of the five control rats were normally distributed. After that, a Student’s t-test was performed to verify if the distributions were equal.

Data of the small ROIs are noted as mean densities and standard error of the mean (SEM). The results were compared with the mean density of the five control rats, and analysed using a one-sample Student’s t-test. Breathing frequencies were noted as the mean breathing frequency per group, measured in breaths per minute. Data were subjected to SPSS ANOVA for repeated measurements, using the ε-procedure recommended by Quintana and Maxwell [15] to analyse the whole curve. When the main analysis indicated a significant interaction ($\alpha = 0.05$) between factors, follow-up analyses were performed, adjusting error rates according to Bonferroni. The data were subjected to multiple group analysis (ANOVA) followed by Bonferroni correction, in order to analyse differences at each time point.

3. Results

3.1. Animals

At the time of irradiation, the rats weighed 298–340 g and growth of the rats was not affected by the radiation. Two animals died during the course of the experiments (one left and one lateral) due to problems with anaesthesia required for the CT analysis 16 weeks after irradiation. No detrimental side effects caused by the radiation other than the ones analysed were observed.

The five rats scanned for lung volume determination, in order to design the collimators, had a mean lung volume of 6.15 cm³ (range 5.50–6.46 cm³). Usage of the six different designed collimators enabled us to irradiate the partial lung volumes accurately with respect to the field edges. This means that the volumes were within the range 48–51% of volume for the left and right radiation field (range 43–60%) for the apex and base, and the range 18–31% and the range 20–30% for, respectively, the left and right lateral/mediastinal fields. To achieve 50% irradiation volume in the lateral group, the left and right volumes were added.

3.2. Lung density

3.2.1. Qualitative analysis of CT

The five control rats showed similar distributions in lung density ($p < 0.001$), which means that the lung tissue in all rats was radiologically identical. The mean lung density was $-593 \pm 13$ H (1 SEM). Already 4 weeks after irradiation, a clear density increase was seen in the rats irradiated to the lateral 50% of the lungs (Fig. 2b), as compared to control rats (Fig. 2a). Evidently, this density increase is only present in the irradiated parts of the lung. Also the 50% mediastinal-irradiated rats show a density increase in the irradiated lung parts, although to a lesser extent (Fig. 2c). The other groups did not show any signs of density changes at this time-point. These density changes are probably alveolar infiltrates. This is supported by the fact that the infiltrates had resolved after 5 weeks (data not shown). Since the density changes, which occurred 4 weeks after irradiation were of edematous origin and dissolved after 5 weeks, no further analysis was performed.
At 16 weeks after irradiation, another set of CT scans was made. In the present study no clear changes in lung density were visible at this time point. At week 26 after irradiation, clear fibrotic lesions had occurred in all irradiated lung parts. In contrast to the diffuse lesions observed after 4 weeks, the changes observed at this time point were more focal. Patches of fibrosis were visible in the radiation fields, most prominent in the 50% mediastinal (Fig. 3a) and 50% left (Fig. 3c and d) irradiated groups. In addition, anatomical changes had occurred, which appeared as pronounced shrinkage of the irradiated parts of the lung with a compensatory hyperinflation of the shielded parts (Fig. 3d) and an elevation of the diaphragm (Fig. 3b and c). One year after irradiation, the shrinkage of the irradiated parts was more pronounced, complicating the distinguishability between fibrosis and normal anatomical structures as the heart and chest wall. At this time-point, the apex group showed fibrotic signs too, as shown in Fig. 3e. These density changes, however, were not accompanied by shrinkage of the irradiated lung parts. After the last CT scans, the rats were sacrificed. The histological data confirmed the CT data. The lungs showed major fibrotic reactions and also an extensive inflammatory response was present. Finally, shielded lung parts showed hyperinflation. These observations could not be dedicated to regions and were therefore not analysed.

3.2.2. Quantitative analysis of CT: whole lung density

To quantify density changes, the mean pixel value of the whole lung was calculated, using outlined lung contours and averaging therein. If anything, a slight mean lung density decrease in time was observed in all groups using this type of analysis (data not shown), indicative of mild hyperinflation of the shielded lung tissue. Analysis of the standard deviation (SD) of the density distribution, indicative of changes in lung tissue homogeneity revealed that the SD increased over time in all groups, demonstrating a diminished homogeneity of lung tissue. This analysis did not yield any significant differences between the different irradiated groups (data not shown) despite the clear qualitative changes observed (Fig. 3). Thus, quantitative analysis of average lung densities and standard deviation do not seem to be adequate to describe the qualitative changes in lung density following irradiation.

3.2.3. Quantitative analysis of CT: ROIs

As the analysis of whole lung density was non-conclusive even though clear qualitative changes were visible on the CT scans, we changed to the analysis of ROIs, as described in Section 2. This enabled us to study density changes separately in the irradiated and shielded parts of the lung. Analysis of the ROIs at 16 weeks after irradiation revealed a significant density decrease of the shielded mediastinal lung parts. After 26 weeks, this density decrease was accompanied by a significant ($p < 0.05$) density increase of the irradiated parts of the mediastinal fields (Fig. 4). Although the left 50% irradiated group qualitatively seemed to show marked density changes on the CT slides, statistically only a tendency could be shown.

For further analysis of the density changes in the 50% left and the 50% mediastinal-irradiated groups, frequency distributions of the CT values of the pixels were constructed for illustrative purposes (Fig. 5). For the 50% mediastinal-irradiated rats, the irradiated parts (grey line) showed a shift to the right, with the pixels H-values moving towards 0, indicative of fibrosis. The shielded part of the lung showed more pixels with values towards $-1000$ H indicating hyperinflation. The frequency distribution supports that the 50% left irradiated group showed a tendency towards fibrosis as the discrimination between the shape of the irradiated and shielded distribution was less pronounced when compared to the 50% mediastinal-irradiated rats.
Fig. 4. Local density changes 16 and 26 weeks after irradiation, depicted as mean local density changes ($\Delta H$) ± SEM compared to control, for irradiated parts (grey), and shielded lung parts (black). In case of 50% mediastinal irradiation, a significant density decrease of the shielded lung parts has occurred ($p < 0.05$) 16 weeks after irradiation, as compared to the control rats. A marked density increase ($p < 0.05$) of the irradiated lung parts was observed only at 26 weeks after mediastinal irradiation, furthermore, the density decrease of the shielded lung parts has progressed ($p = 0.006$). The left group only showed a tendency. The remaining groups only showed a density decrease for both irradiated and shielded lung parts.

Fig. 5. Frequency distributions of the local density showing pixels within 50 mm$^2$ ROIs with a certain density, 26 weeks after 50% mediastinal and 50% left irradiation. Increasing lung densities have values towards 0 H (fibrosis), while decreasing lung densities have values towards -1000 H (hyperinflation). After 50% mediastinal irradiation, the irradiated parts (grey line) showed a shift to the right, indicating that pixels have H-values towards 0 (fibrosis). Shielded lung showed more pixels with values towards -1000 H, indicating hyperinflation. The 50% left irradiated group only showed a tendency, as supported by the fact that the difference between the shape of the irradiated and shielded distributions was less pronounced.
According to the above described, density changes the mediastinal part of the rat lung seems to be the most radiosensitive.

3.3. Breathing frequency

Breathing frequencies were measured to evaluate overall lung function. Control animals had an average breathing frequency of 150 breaths/min and remained constant with age. This is depicted in Fig. 6 where the area of the average ± 1 SEM is indicated by the dotted lines. The values are in agreement with previously published ones [19,20]. All groups showed a slight increase in breathing frequency at 22 weeks after irradiation. Surprisingly, only the 50% left irradiated group showed a marked increase in breathing frequency thereafter (Fig. 6). Although the SPSS ANOVA for repeated measurements, using the ε-procedure did not reveal a statistical difference for the whole curve, multiple group analysis followed by Bonferoni correction showed significant differences at several time-points as indicated by asterisks (p < 0.05). Meanwhile, the breathing frequency of the rats irradiated to the 50% mediastinal part of the lung were unaffected, despite the fact that they revealed the largest density changes as analysed from CT scans. The data suggest that the 50% left part of the lung is more sensitive to radiation-induced changes in function than the other parts.

Surprisingly, we found no effect on BF when irradiating the 50% right part of the lung unlike in earlier studies from our laboratory [19,20] where right hemithoracic irradiation with 18 Gy caused substantial BF increases. Recalculation of the volume of previously used hemithoracic irradiation, however, revealed that under these conditions approximately 60% of the total lung volume must have been irradiated. Although an increase of 10% volume seems small, it should be realized that this volume contains the remaining part of the right lung. Damage to this small volume might lead to a decrease in compensatory capabilities of the right lung, and thus resulting in profound lung function loss. Hence, sparing 10% of the total lung volume may already reduce the toxicity on overall lung function, indicative of steep dose–volume dependence.

4. Discussion

Normal tissue damage is the main dose-limiting factor in radiotherapy. In case of malignancies in the thoracic cavity, the dose that can be delivered to the tumour is limited by the tolerance of normal lung tissue. Theoretically, the lung is thought to be a parallel-organised organ, composed of functional subunits (FSU) and radiation-induced lung function loss will be more severe with increasing number of damaged FSUs. However, studies by Travis et al. [10,16,17] have shown that the base of the mouse lung seems more sensitive to radiation than the apex of the lung, indicating that lung damage may not be purely proportional to the volume irradiated. In our present pilot study, we explored regional differences in rats, comparing breathing frequency and CT-based density measurements. The presence of radiation-induced lung damage is characterized by pleural thickening, interstitial and vascular fibrosis [2,6,13]. This damage causes an increase in lung density, as measured with CT in humans [12,18] and in rodents [4,5,9,14]. Patches of fibrosis are often adjacent to foci of low-density areas consisting of hyperinflation, possibly due to the release of surfactant [10]. Several techniques have been tested to objectify and quantify lung damage using CT. Outlining the lung contours and averaging the density therein seemed to be a reproducible but not very sensitive method, as fibrosis is a focal process with both density increase and density decrease [4,9]. Also the standard deviation of the distribution was used to quantify the homogeneity of the lung tissue. Analysis of small areas in the lung seemed a more sensitive method to track down focal fibrotic lesions [9]. The present study also showed that quantification of CTs by way of mean lung density changes cannot score the qualitative observed changes due to the coinciding appearance of increases in density at the irradiated parts with a hyperinflation of the shielded parts of the lung.

Although CT imaging has been used for the detection of radiation-induced damage to the lung previously [4,5,9,14], it has never been used in partial lung irradiation. In our present study, we compared density changes analysis of irradiated parts of the lung with shielded parts of the lung using small ROIs (polygons consisting of 50 mm²). This appeared to be a more sensitive method to quantify density

![Breathing Frequency Graph](image)
changes if compared with normal non-irradiated lung tissue. Using this technique, we were able to quantify fibrosis in irradiated lung tissue, but also reactions occurring in non-irradiated lung tissue. Moreover, the analysis was consistent with the qualitative assessments of the CT, showing a marked density change after mediastinal irradiation in the irradiated parts of the lung, which coincided with a density decrease of the shielded parts. The other fields did not show significant changes although qualitative changes could be observed.

Interestingly, the density changes observed in the mediastinal-irradiated rat lungs were not accompanied by lung function changes. Probably, the mediastinal lung part has a less important role in overall lung function. This would be consistent with the hypothesis that relatively radiosensitive gas exchange units might be more situated in the peripheral regions of the lung, whereas the mediastinal parts consisted more of relatively radioresistant vessels and bronchi [16]. We did observe a decrease in overall lung function first appearing after 22 weeks, for left 50% volume irradiation. Thus, irradiation of the left lung appears to have the most significant impact on lung function. Whether or not this is due to a higher intrinsic sensitivity of this part of the lung remains to be seen. When the left 50% lung was irradiated, part of the right lung had to be included, leading to irradiation of the mediastinum. This may have contributed to the function loss. It must be realized, however, that also the heart was partly irradiated in the 50% left irradiated group. Radiation-induced damage to the heart (heart failure) might have influenced the breathing frequency. This, however, seems to be contradicted by the observations after mediastinal irradiation, in which the heart also lies within the radiation field, but after which a rise in breathing frequency did not occur.

Furthermore, for the right 50% irradiation, part of the right lung was excluded. This excluded part of the lung may induce an extra compensatory reaction, which would explain the difference (lower) response when compared to our previously published whole right lung irradiation studies [19,20].

Travis et al. [10,16,17] found that the base of mouse lung appeared to be more radiosensitive than the apex of the lung. We must take into account, however, that the precision of mouse lung irradiations is likely low due to small overall volumes. Since rats have a larger total lung volume, the radiation will be delivered more precisely. Secondly, in mouse base radiation damage to the liver might be responsible for high circulating levels of TGF-β, increasing toxicity. Finally, the heart, which is positioned in the basal region of the thoracic cavity, might have contributed to the observed decrease in lung function, by either cardiac damage or pulmonary hypertension. In the present study, we found a functionally more sensitive left lung, while the mediastinal part of the lung showed major fibrotic changes. Possibly at higher doses a difference between apex and base may be observed.

In summary, comparison of CT density measurements of control with shielded and irradiated lung volumes using standard ROIs can quantitatively describe density changes. Secondly, differences in regional lung radiosensitivity seem to exist. The main contributing factors in regional sensitivity remain unknown, and we should be cautious by drawing early conclusions. The presentation of lung damage either as function loss or density changes do not necessarily coincide, meaning that for each end point, regional sensitivity may be different. Moreover, a pronounced volume effect may be present in the lung as an increase of about 10% of the irradiated lung volume results in major pulmonary disturbance. Based on the data of this study, more studies need to be developed using varying doses and varying volumes, to further explore regional differences and volume effects in rats and humans, in order to develop new treatment strategies in the future.

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