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X-ray Dark-Field CT for Early Detection of Radiation-induced Lung Injury in a Murine Model[10,11]

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Conflicts of interest are listed at the end of this article.

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X-ray dark-field imaging has recently been introduced as a potential low-dose technique for the imaging of pulmonary diseases, both in animal studies and in humans (1). It measures the number of multiple refractions at air-tissue interfaces, such as in the alveoli, and the signal is reduced when the alveolar integrity is impaired (2,3). Irradiation of lung cancer or pulmonary metastases may lead to postirradiation changes. These include early transient radiation pneumonitis and subsequent radiation fibrosis, which has previously been investigated in multiple animal studies (4). Because lung fibrosis leads to the destruction of alveoli (a decrease in air-tissue interfaces), x-ray dark-field radiography has been shown to be particularly useful for its detection (5). A previous study has shown that a dark-field CT scanner was able to provide three-dimensional information on the alveolar structure and allow for the detection of pulmonary fibrosis at a single time point in an animal model (6). The aim of this study was to evaluate the benefit of dark-field CT imaging compared with conventional (attenuation-based) CT imaging in a longitudinal mouse model for the detection of radiation-induced lung injury.

Materials and Methods

Full methods are provided in Appendix E1 (online). In brief, we compared conventional CT imaging to dark-field CT imaging in a murine model with radiation-induced lung injury in the right lung (n = 6) and a nonirradiated control group (n = 6). Animals were scanned before irradiation and 12, 16, 20, and 24 weeks thereafter. Three radiologists assessed the images twice for the presence of lung injury and rated their confidence on a scale from 1 to 5. Sensitivity was defined as the percentage of readings in which irradiated mice were classified correctly. The interrater and intrarater reliability was determined and rated with the Fleiss κ and Cohen κ, respectively. For the quantitative analysis, the ratio of the mean pixel value of the right lung to that of the left lung was calculated[1]. Results from the irradiated group were compared with those of the control group by using the Student t test. $P <$ .05 was chosen as indicative of statistically significant difference[2].

1 Because R is typically used the as the symbol for multivariable correlation coefficient analysis in scientific studies, "R" has been replaced with a description of the ratio throughout. Please confirm or amend.

2 Edits OK in sentence beginning with "P < .05…"?

Results

Figure 1 shows a selection of sections of the CT scans in one mouse from the irradiated group before irradiation (0 weeks) as well as 16 and 24 weeks thereafter. Twelve weeks after irradiation, the sensitivity for lung fibrosis was 36.7% (95% CI: 18.4, 55.0; 11 of 30) for conventional CT and 53.3% (95% CI: 34.4, 72.3; 16 of 30) for dark-field imaging (P = .006) (Fig E1 [online[3]]). The greater sensitivity of dark-field imaging was maintained at 16 weeks (conventional CT, 50.0% [95% CI: 32.8, 67.2; 18 of 36] vs dark-field CT, 91.7% [95% CI: 82.2, 100.0; 33 of 36]; $P < .001$[4]). No differences were found in sensitivity at 20 or 24 weeks (Fig E1 [online[5]]). Intra- and interreader reliabilities can be found in Table E1 (online). The overall median confidence score was 3 for conventional imaging and 5 for dark-field imaging (Fig E2 [online]). In the control group, the ratio of the mean pixel value of the right lung to that of the left lung did not change over time for either method (Fig 2A, 2C). In the irradiated group, the ratio increased over time for conventional CT scans, while it decreased for dark-field CT scans (Fig 2B, 2D; Table E2 [online]). For conventional scans, the difference between the irradiated group and the control group was significant at 20 weeks and 24 weeks (Fig 2B), while the ratio for the dark-field scans was different starting at 16 weeks (Fig 2D, Table E3 [online[6]]).

3 Typically in Radiology, when the denominator is two digits, the derived percentage is expressed as two digits. Please consider rounding sensitivity percentages and the respective 95% CIs.

4 In the Results paragraph, please provide the units for sensitivity. (ie, is 11 of 30 the number of images? lesions?)

5 Edits OK in sentence beginning with "No differences…"?

Discussion

Our study demonstrates that dark-field CT imaging allows for the detection of radiation-induced lung injury at early stages and, in that respect, is beneficial compared with conventional CT imaging. Dark-field chest radiography has shown utility for lung imaging in animal models and recently in humans (1–3). This study shows the feasibility of dark-field CT for

6 Edits OK in sentence beginning with "For conventional scans…"?

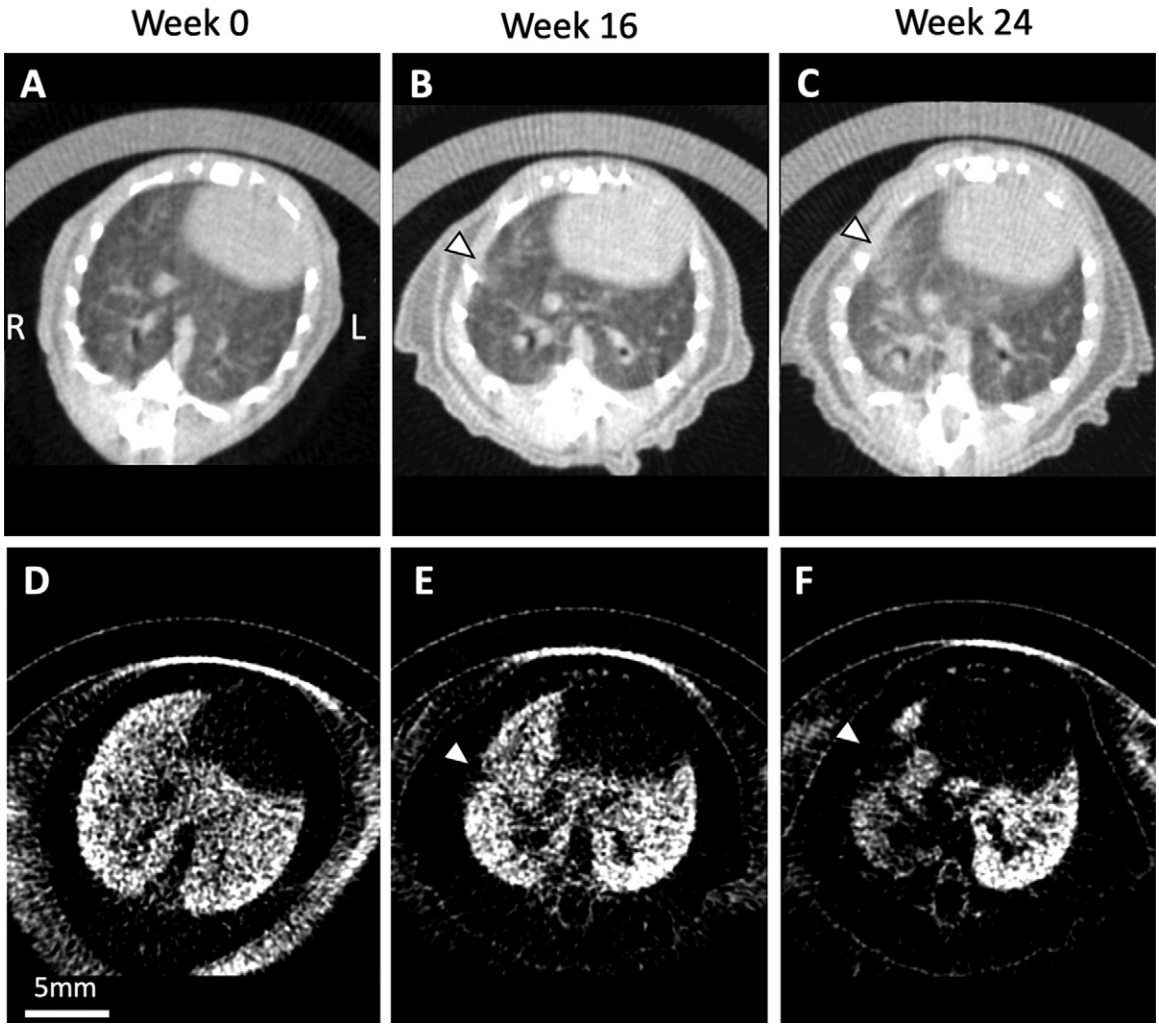

Figure 1: **(A–C)** Conventional and **(D–F)** dark-field CT images in a mouse before irradiation of the right lung (0 weeks) and 16 and 24 weeks after irradiation. Conventional images show an increase of signal intensity in the right lung, while dark-field images show a decrease of signal intensity. Both imaging techniques show typical signs of lung fibrosis (arrowheads); however, they are more subtle in conventional images[13].

13 Edits OK in the Figure 1 legend?

7 In the sentence beginning with "In humans…," please clarify "their production." Does this refer to the manufacture of dark-field CT scanners?

lung imaging in small animals. However, to be able to apply dark-field CT in humans, some important challenges must be overcome. In humans, a larger field of view is required, and the cylindrical grating geometry further complicates their production[7]. Moreover, the fast rotation times, with all associated vibrational instabilities, pose many algorithmic challenges. These obstacles will need to be solved before the use of dark-field CT for pulmonary disease in humans is possible.

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9 Please provide the name of the entity in which F.P. has stocks.

References

1. Gassert FT, Urban T, Frank M, et al. X-ray dark-field chest imaging: qualitative and quantitative results in healthy humans. Radiology 2021;301(2):389–395.
2. Willer K, Fingerle AA, Noichl W, et al. X-ray dark-field chest imaging for detection and quantification of emphysema in patients with chronic obstructive pulmonary disease: a diagnostic accuracy study. Lancet Digit Health 2021;3(11):e733–e744.

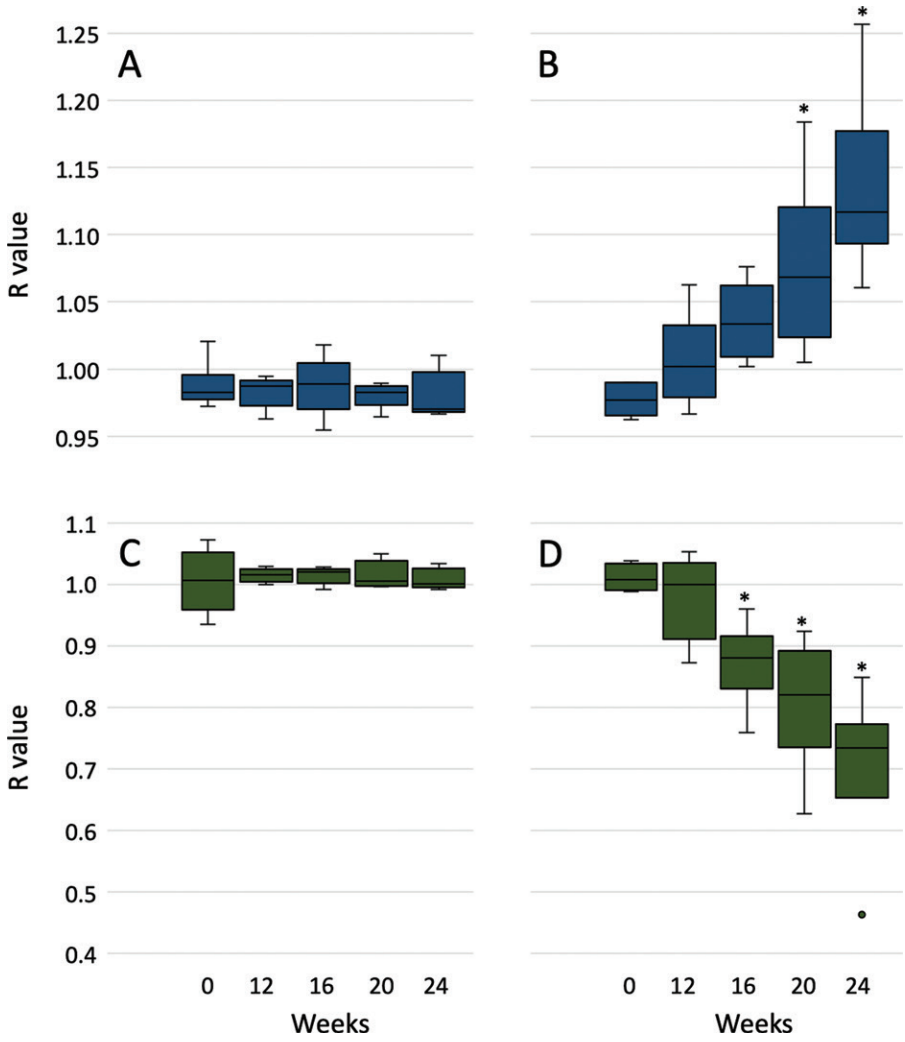

Figure 2: **(A–D)** Box and whisker plots show results from the quantitative analysis. The ratio of the mean pixel value of the right lung to that of the left lung (R) in the control group did not show any significant changes over time for conventional CT (**A**) or dark-field CT (**C**). Compared with the control group at the respective time point, the attenuation-based mean ratio of the irradiated group is higher only 20 weeks after irradiation (**B**; *P* = .011[14]). After 24 weeks, the difference remains significant (*P* = .004). The difference between the control group and the irradiated group for dark-field imaging (**D**) was significant at 16 weeks after irradiation (*P* = .003) and the subsequent time points[15] (20 weeks: *P* = .006; 24 weeks: *P* = .005[16]). * = ??.

15 In the Figure 2 legend, please confirm or amend the image type (ie, "box and whisker plots" OK?)

16 In the Figure 2 legend, please define the following: elements of the box and whisker plots (boxes/borders, whiskers, midlines), asterisks in B and D, point at 24 weeks in D.

14 Per Radiology guidelines, P values should be expressed to 2 digits for P ≥.01 (except when rounding to 3 digits would make the P value nonsignificant, eg .049 rounded to .05). If P < .01, it should be expressed to 3 digits. Please check in the Figure 2 legend; OK to round .011 to .01?

3. Urban T, Gassert FT, Frank M, et al. Qualitative and quantitative assessment of emphysema using dark-field chest radiography. Radiology 2022. 10.1148/radiol.212025. Published online January 11, 2022.
4. Dabjan MB, Buck CM, Jackson IL, Vujaskovic Z, Marples B, Down JD. A survey of changing trends in modelling radiation lung injury in mice: bringing out the good, the bad, and the uncertain. Lab Invest 2016;96(9):936–949.
5. Burkhardt R, Gora T, Fingerle AA, et al. Early detection of radiation-induced lung damage with x-ray dark-field radiography in mice. Eur Radiol 2021;31(6):4175–4183.
6. Burkhardt R, Gora T, Fingerle AA, et al. In-vivo x-ray dark-field computed tomography for the detection of radiation-induced lung damage in mice. Phys Imaging Radiat Oncol 2021;20:11–16.