Optical coherence tomography to detect acute esophageal radiation-induced damage in mice: A validation study

Pouya Jelvehgaran1,2,3* | Daniel M. de Bruin1,4 | Artem Khmelinskii5 | Gerben Borst5 | Jeffrey D. Steinberg6 | Ji-Ying Song7 | Judith de Vos1 | Ton G. van Leeuwen1 | Tanja Alderliesten2 | Johannes F. de Boer3 | Marcel van Herk1,8

1Department of Biomedical Engineering and Physics, Cancer Center Amsterdam, Amsterdam UMC, University of Amsterdam, Amsterdam, the Netherlands
2Department of Radiation Oncology, Amsterdam UMC, University of Amsterdam, Amsterdam, the Netherlands
3Department of Physics and Astronomy, Institute for Laser Life and Biophotonics Amsterdam, Amsterdam, the Netherlands
4Department of Urology, Amsterdam UMC, University of Amsterdam, Amsterdam, the Netherlands
5Department of Radiation Oncology, The Netherlands Cancer Institute (NKI), Amsterdam, the Netherlands
6Mouse Clinic for Cancer and Aging (MCCA) Imaging Unit, The Netherlands Cancer Institute, Amsterdam, the Netherlands
7Department of Experimental Animal Pathology, The Netherlands Cancer Institute (NKI), Amsterdam, the Netherlands
8Manchester Cancer Research Centre, Division of Cancer Sciences, Faculty of Biology, Medicine, and Health, University of Manchester, Manchester Academic Health Sciences Centre, Manchester, UK

*Correspondence
Pouya Jelvehgaran, Department of Biomedical Engineering and Physics, Amsterdam UMC, University of Amsterdam, Meibergdreef 9, 1105 AZ Amsterdam, the Netherlands.
Email: p.jelvehgaran@amc.uva.nl

Abstract
Radiation therapy for patients with non-small-cell lung cancer is hampered by acute radiation-induced toxicity in the esophagus. This study aims to validate that optical coherence tomography (OCT), a minimally invasive imaging technique with high resolution (~10 μm), is able to visualize and monitor acute radiation-induced esophageal damage (ARIED) in mice. We compare our findings with histopathology as the gold standard. Irradiated mice receive a single dose of 40 Gy at proximal and distal spots of the esophagus of 10.0 mm in diameter. We scan mice using OCT at two, three, and seven days post-irradiation. In OCT analysis, we define ARIED as a presence of distorted esophageal layering, change in backscattering signal properties, or change in the esophageal wall thickness. The average esophageal wall thickness is 0.53 mm larger on OCT when ARIED is present based on histopathology. The overall sensitivity and specificity of OCT to detect ARIED compared to histopathology are 94% and 47%, respectively. However, the overall sensitivity of OCT to assess ARIED is 100% seven days post-irradiation. We validate the capability of OCT to detect ARIED induced by high doses in mice. Nevertheless, clinical studies are required to assess the potential role of OCT to visualize ARIED in humans.

KEYWORDS
acute radiation-induced esophageal damage, image-guided radiation therapy (IGRT), lung cancer, optical coherence tomography (OCT), small animal models
1 | INTRODUCTION

Radiation therapy (RT) for patients with non-small-cell lung cancer is hampered by acute radiation-induced toxicity in the esophagus. Esophageal toxicity arises in most patients undergoing thoracic RT.[1–4] Patients with non-small-cell lung cancer treated with neoadjuvant chemo-RT (nCRT) may suffer from complications such as reduced food intake and hospitalization, which are dose-limiting factors.[1, 2] Hence, it is crucial to optimize the balance between local tumor control and radiation-induced toxicity to nearby healthy organs during RT. Acute radiation-induced esophageal damage (ARIED) or acute esophagitis can develop within 3 months post RT.[3, 4] ARIED as a dose-limiting factor can add complications to the local tumor control during RT.[3, 5, 6] ARIED is qualitatively scored based on patient symptoms, such as dysphagia, hyperalimentation, and retrosternal pain in the current clinical practice.[1, 2, 7] Imaging modalities, such as white light endoscopy (WLE) and [18F] fluorodeoxyglucose positron emission tomography (FDG-PET), have been used to assess ARIED.[8] WLE is limited to visualizing only the superficial neoplasia and unable to provide depth information regarding the esophageal wall layering structure, FDG-PET has a low resolution that results in many false-positive and false-negative outcomes.[9]

Optical coherence tomography (OCT) is a minimally invasive imaging technique that uses near-infrared light to illuminate tissue and acquires three-dimensional (3D) cross-sectional images using back scattered light.[10–12] Even though OCT has a limited imaging depth (up to 2-3 mm), its resolution is around 10 μm and therefore OCT is capable of visualizing esophageal wall layers from the epithelium to the muscle layers in humans.[13, 14] In a previous study, we also demonstrated that OCT can potentially detect and monitor ARIED in mice, but repeated insertions of the OCT probe led to some additional damages and induced limited ARIED.[13] Further preclinical in vivo 3D OCT imaging was required to validate the visibility of ARIED in OCT images—excluding the effect of probe insertion damage by inducing more substantial ARIED cases.

This study explored whether OCT is able to visualize and monitor ARIED. We performed in this study qualitative and quantitative analysis on OCT images and compared it with histopathology.

2 | MATERIALS AND METHODS

The local ethics committee approved our protocol in compliance with the guidelines of the European community (EUVD 86/609/EEC). Forty-five specific pathogen-free FVB female mice bought from a commercial vendor (Janvier, Le Genest-Saint-Isle, France) were randomly assigned into five study groups (one test group, three control groups and a sham group) as shown in Figure 1. While mice in the test group underwent irradiation and OCT imaging, radiation-only and OCT-only control groups received only irradiation or OCT imaging, respectively. Based on a previous study,[13] where limited ARIED was detected, we chose a single-fraction dose level of 40 Gy on two 10.0-mm-diameter spots spaced about 10.0 mm apart and irradiated with two orthogonal beams to the esophagi of mice in the test group. We performed OCT imaging at two, three, and seven days after irradiation for the test and OCT-only groups. The experimental protocol has been described previously in a paper presenting the magnetic resonance imaging (MRI) components of the study.[15] We irradiated the proximal and the distal esophagus leaving a portion of tissue in between intact as a reference in our OCT analysis. This allowed us to explore if there is a difference in radiobiological sensitivity between the proximal and distal portions of the esophagus.[15] However, we recognize that irradiation of two portions with such a high dose was a radiation burden to the mice. We estimate that in total 1.3 cm³ received 40 Gy while 20 cm³ received negligible dose.

2.1 | OCT imaging

We used a commercially available C7-XR intravascular imaging system (St. Jude Medical, St. Paul, Minnesota) for our endoscopic in vivo 3D OCT imaging. The system uses commercially available C7 Dragonfly intravascular imaging probes (St. Jude Medical) designed for percutaneous coronary intervention. The OCT probe had an outer diameter of 0.9 mm with a rotating fiber and utilized a longitudinal pull-back to obtain 3D imaging.[13] The pullback length was about 6 cm and the axial and lateral resolutions were 15.0 and 30.0 μm, respectively.[13] All mice were anesthetized and received probe insertion with one hand while the animals were being held by the scruff of the neck with the other hand.[13] During the probe insertion, it was advanced carefully while the tongue was visible to avoid damage.[13] The probe was inserted until we felt resistance by the stomach valve.

2.2 | Cone-beam CT imaging and irradiation

We performed cone-beam CT imaging and dose delivery using the commercially available image-guided small animal irradiation device (X-RAD 225Cx; Precision X-ray Inc., North Branford, Connecticut).[13, 15] We ensured the visibility of the esophagus during RT planning with insertion of an OCT probe (C7 Dragonfly OCT imaging catheter;
St. Jude Medical) filled with diluted CT contrast (Telebrix Gastro; Guerbet, cedex, France). We irradiated the target location centered on the esophagus with a single fraction dose on a 10.0 mm spot using two orthogonal circular beams for both the proximal and distal portions of the esophagus (Figure 2). We chose the proximal target regions close to the sixth vertebra of the spinal cord. The same procedure was repeated for the distal portion of the esophagus. After irradiation, the probe was retracted, and the animal was placed on a heating mat to recover.

Cone-beam CT imaging was performed with a 2.0 mm Al filter using the following scanning protocol: 40 kV, 0.5 mA, mid gain, 5.0 fps. Irradiation was performed with a 0.3 mm Cu filter with 225 KVp, 13.0 mA, delivering 10 Gy in 255.0 seconds.

2.3 | Co-localization of OCT images with histopathology

The two irradiation spots were located in the proximal and distal portions of the esophagus. We centered the proximal irradiation beam on portion of the esophagus adjacent to the sixth vertebra of the spine. The tracheal bifurcation was used as an anatomical landmark on the OCT images; irradiated spots are found anterior and posterior of the tracheal bifurcation. We analyzed cross-sectional OCT images axially because of its superior resolution compared with sagittal images. In histopathology, we embedded specimens of the esophagus in the sagittal orientation according to the general procedure and divided each specimen into two regions for proximal and distal histological analysis. Here, the position of the stomach was used for orientation.
2.4 | Data analysis

Prior to double-blind histopathology and OCT analysis, we trained ourselves for these data by inspecting OCT for one case where mild ARIED and severe ARIED were observed in histopathology. In order to maintain a sufficient number of mice per group, the training case was not excluded from data analysis. In histopathological analysis, we evaluate the severity of the ARIED based on the extent of the damage. Severe ARIED was diagnosed when massive inflammatory infiltrations in submucosa with extension to muscularis externa and adventitia and/or necrosis took place. In the OCT images, we determined an increase in tissue scattering, increased thickness of the esophagus wall, and the presence of distortion of esophageal layering structure. These correspond to inflammatory infiltration and edema—as confirmed histopathologically in our previous small animal study [13]—and necrosis. Our previous clinical study also showed ARIED (radiation-induced fibrosis) as distorted esophageal wall-layering structure in humans.[14] Hence, we defined ARIED as the presence of one or more of these features. We next scored proximal and distal esophagi for all cases in three categories: no ARIED, mild ARIED, and severe ARIED in both OCT and histopathological images based on visible defects. In OCT results, very mild ARIED was excluded to reduce the impact of image artifacts that can mislead outcomes.

Our quantitative evaluation of OCT consisted of measuring the thickness of the esophageal wall on cross-sectional OCT images from the beginning of the epithelium to the ending of the muscle layers. We used a repeated measured analysis of variance to test for statistical analysis—results were considered statistically significant at \( p < .05 \).

3 | RESULTS AND DISCUSSION

3.1 | Results

We excluded three mice from our study—one in the 3-day test group, one in the 3-day irradiation-only group and one in the 7-day irradiation-only group—because of technical issues with the irradiator and inappropriate dose delivery. We summarize histopathology and OCT findings in Table S1 (supporting materials).

3.1.1 | Histopathology

Histopathological analysis revealed inflammatory infiltration, edema, and necrosis ARIEDs in either proximal or distal esophagus, in 75% of all mice (24 cases) that underwent radiation, where 33% showed severe ARIED. All cases with severe ARIED were either from the test or irradiation-only control groups seven days post-irradiation. Results confirmed the absence of ARIED in un-irradiated mice. Inflammatory infiltration, edema, and necrosis were found only in the test and the radiation-only control groups, showing proximal or distal ARIED in 75% (79% of the test group and 70% of the radiation-only group), 42% (43% of the test group and 40% of the irradiation-only control group) and 58% (57% of the test group and 60% of the irradiation-only group) of all mice.

3.1.2 | OCT

Qualitative analysis

Our OCT images showed distorted esophageal wall layering, as well as an increase in esophageal wall thickness in the case of histopathology proven ARIED. Moreover, we observed high scattering properties in OCT images at certain depths that infiltrated into the upper esophageal layers such as mucosa and low scattering regions. Figure 3 shows in vivo endoscopic OCT images of healthy and various ARIED—inflammatory infiltration, edema, and necrosis, as the major histopathology reported damages—with corresponding histopathology images. All cases with necrosis in histopathology corresponded to distortion or loss of esophageal wall layering structure in OCT images. However, in some cases distorted layering was visible in the OCT images, while necrosis was absent in the histopathological analysis. Compared with histopathology, the overall sensitivity and specificity of OCT to detect ARIED for 28 mice—proximal and distal (56 cases)—was 94% and 47%, respectively. The overall sensitivity of OCT to detect ARIED seven days post-irradiation was 100%—at that time point we had zero true-negative and false-positive cases.

Quantitative analysis

We next measured the proximal and distal esophageal wall thicknesses on OCT (Table S2, supporting materials). The esophageal wall thickness showed significant differences between the three time points in the test group (\( p = .001 \)), whereas the difference for the OCT-only group was insignificant. The average thickness of the esophageal wall on OCT at two, three, and seven days post-irradiation of the test group were 0.80 ± 0.22 mm (range: 0.43-1.13 mm, median 0.81 mm), 0.95 ± 0.31 mm (range: 0.64-1.54 mm, median 0.83 mm) and 1.29 ± 0.26 mm (range: 0.94-1.67 mm, median 1.27 mm), respectively.

Based on histopathology, the esophageal thickness on OCT (test, OCT-only, and single-OCT groups) differed significantly between no ARIED, necrosis, inflammatory infiltration, and edema cases (\( p = .01 \)) (Figure 4A). The average esophageal wall thickness in case of ARIED was on average 0.26 mm larger than that in case of the healthy/no ARIED. The average esophageal wall thickness in case of edema was
on average 0.38 mm larger than that in case of the healthy/no ARIED. Similarly, we also found a significant difference between the esophageal thickness measurements of no ARIED, mild, and severe ARIED cases based on histopathology \((p < .0001)\). Also when basing the measurements on OCT results, the esophageal thickness measurements (test group) of healthy/no ARIED was significantly lower compared to both mild and severe ARIED cases \((p < .00001)\), (Figure 4B). Figure 4C represents the average thicknesses of the esophageal wall for three time points based on the OCT findings. We found no significant difference between severe and mild ARIED cases \((p = .97)\). Figures 4D,E represent the mean esophageal thickness measurements for different time points and severity level of ARIED and occurrence percentage of ARIED as a function of time, respectively.

### 3.2 Discussion

In this study, we modified the experimental protocol of our previous study on the feasibility of using OCT to detect ARIED in mice,[13] thereby aiming to induce substantial damage with while excluding probe-insertion-induced edema. We validated that OCT can indeed detect ARIED in mice esophagi irradiated with a single fraction dose of 40 Gy within one week after irradiation. We restricted our OCT and histopathological analyses based on the following predominant types of ARIED reported by histopathology— inflammatory infiltration, edema, and necrosis. OCT analysis showed ARIED damages as esophageal wall thickening, changes in back scattering signal properties and a distorted esophageal wall layering respectively, depending on the type of ARIED.

Clinical studies showed that visualization of esophageal wall layers, determination of healthy, radiation–induced fibrosis, and detection of residual cancer on OCT are feasible.[14, 16] Radiation-induced damage detection using OCT has been studied for various body organs, such as the eye, retinal microvasculature, oral cavity, skin tissue vascularity, and esophagus.[13, 17–22] However, there was a lack of OCT validation to detect ARIED, which we addressed in this study. Observed substantial ARIED in our results in both OCT and histopathology, and necrosis corresponded to a loss of esophageal wall layering. However, our results showed that ARIED distorted esophageal wall layering, in particular severe ARIED—this is in correspondence with our clinical study that showed this phenomena in case of radiation-induced fibrosis.[14]

One-week after irradiation, ARIED in mice was detected at a dose level of 30 Gy.[23] Our previous study showed that 20 Gy on a 5.0-mm spot does not induce substantial damage.[13] Moreover in similar protocol, we investigated the dose levels and time points in which severe ARIED may occur in a pilot study using 20 and 40 Gy dose.[15] That study showed that single 20 Gy dose was inadequate to

---

**FIGURE 3** A, Corresponding in vivo optical coherence tomography (OCT) (axial image in polar coordinates, horizontal axis spans 0-360 degrees) and histopathology images (sagittal) of B, healthy part of the esophagus—(epithelium [ep], lamina propria [lp], muscularis mucosa [mm], submucosa [sm] and muscle layers [ml])- on OCT and histopathology from mouse 11. C, Mainly Inflammatory infiltration accompanied by edema in mouse 11. D, Mainly edema accompanied by inflammatory infiltrations in mouse 11. E, Layering distortion due to necrosis in mouse 13 with the blue dashed-line indicating the region that shows layering distortion. Those areas of high scattering that indicate inflammatory infiltration are identified with black arrows. Red arrows point to those low scattering regions that indicate edema. The vertical line in OCT and histopathological images indicate the thickness of the edema. Stars indicate wall layering distortion. The OCT images were averaged over around 15 slices perpendicular to the view direction. Images may contain healthy portions, but only regions affected by acute radiation-induced esophageal damage (ARIED) were annotated.
induce severe ARIED. Substantial ARIED was, however, induced 1 week after delivery of a single dose of 40 Gy on a 10.0 mm spot.[15] This is the reason we chose to use this dose level. Most of the 40 Gy dose was delivered to the esophagus and tissue close to it (total 0.65 cm³), corresponding to the dose in the intersection of two beams. The reported human esophageal damage tolerance is 27 Gy with $\alpha/\beta$ ratio at 3 Gy$^{-1}$ for a single fraction dose delivery.[24] Although mice and humans have a similar esophageal wall structure, insufficient data exists to suggest that the esophagus of both organisms have the same radiobiological sensitivity.[15] Damage to a single functional subunit can progress to the whole organ since the esophagus is a serial organ.[13, 25] Such a serial effect could not explicitly be studied in our experimental protocol, because multiple spots were irradiated per esophagus.[13, 25] However, most observed damage was restricted to the spots. We used a new generation of dragonfly OCT probes—commercially available as a part of percutaneous coronary intervention procedure, which seemed a bit more delicate than the previous generation used in our feasibility study.[13] We believe that this new probe was more sensitive to movements from respiration and the rotating optical head. Thus, we had more artifacts in our OCT images, which may explain the relatively high false-positive rates. Better probes and further image reprocessing may reduce the false positive rate in the future. Also for our previous study we reported that non-uniform rotational distortion (NURD) artifact hampered the OCT imaging quality.[13] Mice are similar to humans in terms of the esophageal wall layering structure, and therefore suitable for such preclinical studies. We chose FVB mice to be consistent with our previous feasibility study.[13] We chose the number of mice, $n = 45$, by using our previous study[13] for the power estimation. The average weight of our FVB mice was 23 g (18 mice), roughly corresponding to a volume of 23 cm³ per mouse. The irradiation spots were 10.0 mm in diameter delivered using two perpendicular cylindrical beams. The high dose radiation field will have

**FIGURE 4**

A. Average optical coherence tomography (OCT) measured thickness as a function of pathology graded ARIED. B. Average OCT measured thickness as a function of OCT graded acute radiation-induced esophageal damage (ARIED). C. Average thickness of the esophageal wall on OCT for different time points of the test group, single-OCT imaging group consists of all time points. Boxes indicate interquartile ranges, horizontal lines illustrate the median values while error bars show the range. D. Average OCT measured thickness for the test group, split according to qualitative ARIED severity scoring as a function of time. E. Percentage of OCT-graded (qualitative) ARIED severity as function of time in the test group.
We showed that ARIED differentiation between damage types on OCT was difficult. However, if the objective is to identify ARIED in general, our results showed that OCT performs well both qualitatively and quantitatively. Dedicated endoscopic OCT imaging systems for the esophagus exist,[12, 13] which may facilitate future in vivo clinical studies on the feasibility of OCT to detect ARIED. Clinical feasibility and validation investigations are necessary to study whether our findings can be translated to humans. Automated ARIED detection can be investigated based on quantification characteristics of damage types. Future research may also include OCT signal preprocessing and correction to reduce the NURD and movements due to catheter and respiration.

4 | CONCLUSION

In conclusion, we validated the feasibility of OCT to detect and monitor ARIED in the proximal and distal portions of the esophagus in 45 mice after irradiating with a single dose of 40 Gy on two spots of 10.0 mm. The overall sensitivity and specificity of OCT compared to histopathology to detect ARIED were 94% and 47%, respectively. We detected ARIED in mice as a presence of distorted esophageal layering, change of light scattering properties of OCT images and the thickness of the esophageal wall. Our results validated the role of OCT to visualize and monitor ARIED induced by high-dose radiation. However, feasibility and validation investigations are necessary to study whether our findings can be translated to clinical practice.

ACKNOWLEDGMENTS

This work was supported by The Netherlands Organization for Health and Development (ZonMw), Elekta Ltd, and NinePoint Medical, Inc. The authors would like to thank Marco Breuer, Koen van der Mark, Roel Sneepers, and Niels de Wit for their help with animal facility related preparation.

ORCID

Pouya Jelvehgaran https://orcid.org/0000-0003-0721-0572

REFERENCES

[1] M. Werner-Wasik, E. Yorke, J. Deasy, J. Nam, L. B. Marks, Int. J. Radiat. Oncol. Biol. Phys. 2010, 76, 86.
[2] L. E. Court, S. L. Tucker, D. Gomez, Z. Liao, J. Zhang, S. Kry, L. Dong, M. K. Martel, J. Appl. Clin. Med. Phys. 2013, 14, 4195.
[3] S. J. Ahn, D. Kahn, S. Zhou, X. Yu, D. Hollis, T. D. Shafman, L. B. Marks, Int. J. Radiat. Oncol. Biol. Phys. 2005, 61, 335.
SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Jelvehgaran P, de Bruin DM, Khmelinskii A, et al. Optical coherence tomography to detect acute esophageal radiation-induced damage in mice: A validation study. J. Biophotonics. 2019;12:e201800440. https://doi.org/10.1002/jbio.201800440