Using *in vivo* µCT for longitudinal evaluation of silica-induced pulmonary fibrosis in mouse lungs

Kaat Dekoster[1], Sofie Van Den Broucke[2], Tatjana Decaesteker[3], Nathalie Berghen[4], Anton Krouglov[1], Jens Wouters[1], Rik Lories[4], Peter Hoet[3], Jeroen Vanoirbeek[2], Greetje Vande Velde[1]

[1]Biomedical MRI unit/MoSAIC, Department of Imaging and Pathology, KU Leuven, Leuven, Belgium
[2]Centre for Environment and Health, Department of Public Health and Primary Care, KU Leuven, Leuven, Belgium;
[3]Pneumology, Department of Public Health and Primary Care, KU Leuven, Belgium;
[4]Skeletal Biology and Engineering Research Center, Department of Development and Regeneration, University Hospitals Leuven, Leuven, Belgium.

Aims

Pulmonary fibrosis is a respiratory disease often referred to as 'scarring' of the lungs. It is characterized by an excessive formation of connective tissue in the lungs. Patients suffering from this disease show symptomps such as shortness of breath and coughing. In the case of idiopathic pulmonary fibrosis, the median survival time after diagnosis is only 2-4 years[1].

Although it is a major-life threatening condition and perspectives are often bad, effective treatment is still lacking[2,3]. In the past years, only two of the many drugs that were successful in a preclinical context, were made commercially available for treatment of idiopathic lung fibrosis, indicating that there is a gap in our knowledge regarding the disease mechanism and potential treatment targets in lung fibrosis.

Researchers often use single-end point measurements to evaluate a pulmonary disease process. These *ex vivo* measurements are really usefull to give detailed cellular and molecular information about the disease process. However, they are intrinsically unable to follow the kinetics of disease and host response processes, which are dynamic in space and time. As we need to rethink the methodology and disease models used to evaluate lung fibrosis and its therapy in the lung research field, we introduced an *in vivo* 4D-respiratory-gated low dose µCT protocol. The fact that we use a *low dose* µCT scanner is essential since we want to follow-up a disease model in a longitudinal way, hereby taking several scans and exposing the animal to a certain accumulated radiation dose. Our *in vivo* 4D-respiratory-gated low dose µCT protocol allows longitudinal assessment of an improved silica-induced lung fibrosis model in *free-breathing* mice, and offers ideal opportunities for efficient antifibrotic therapy evaluation.

Method

C57BL/6 mice were instilled endotracheally with either crystalline silica (to induce lung fibrosis, n=6, MIN-U-SIL) or saline (control, n=4). After instillation, animals were scanned weekly for a period of 5 weeks. Respiratory gated µCT images of free-breathing, anesthetized mice (1.5-2% isoflurane in 100% oxygen) were obtained using a small-animal µCT scanner (SkyScan 1278, Bruker microCT, Belgium) with the following parameters: 50 kVp X-ray source voltage, 1 mm Al filter, 918 µA source current, 55 ms exposure time, 9 projection images per 0.9° rotation step over a total angle of 180° in list mode and retrospectively gated. This resulted in four reconstructed 3D datasets with 50 µm isotropic reconstructed voxel size corresponding to four different phases of the breathing cycle (4D). Data reported here is at the end of expiration.

Software provided by the manufacturer (TSort, NRecon, DataViewer and CTan) was used to retrospectively gate, reconstruct, visualize and process µCT data[4].

To assess whether inflammation and fibrosis occurred in the silica-induced mice, we evaluated three different lung biomarkers derived from the scans: total lung volume, lung tissue volume and aerated lung volume[5]. Quantification of these biomarkers was done for a manually delineated volume-of-interest covering the lung, thereby avoiding the heart. The treshhold used to distinguish aerated from lung tissue volume was manually set at 105 and kept constant for all datasets.

After the last scan, at day 35, lung function measurements were obtained (FlexiVent 7.3, SCIREQ) and data was cross-validated with *ex vivo* gold standard techniques (OH-proline content and histology).

Results

Silica instillation resulted in a significant increase of tissue volume, corresponding with tissue fibrosis, already visible after 7 days on the µCT scans and remaining stable up until 5 weeks (Fig. 1).

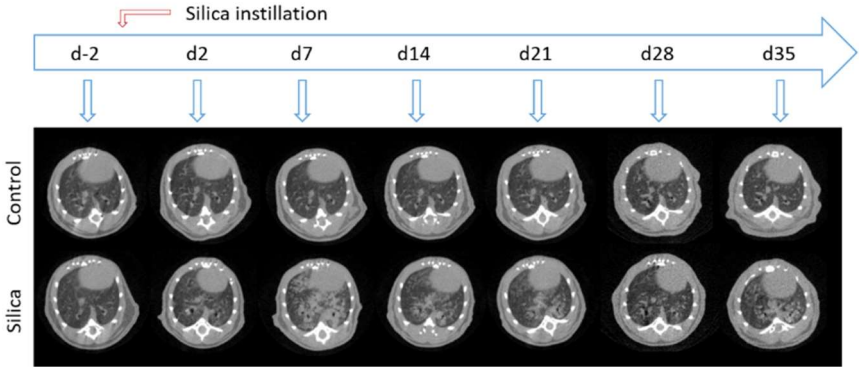

Figure 1: Longitudinal µCT of lung fibrosis. Tomographic images of longitudinal evaluation of silica-induced pulmonary fibrosis from a representative mouse. Endotracheal instillation of crystalline silica (5mg in 40 µL) resulted in persistent, diffuse inflammation and fibrosis that is sensitively detected by low-dose µCT.

In agreement with the visual aspect of our µCT scans, lung tissue volume increased significantly starting from day 7 after silica instillation (Fig. 2B). Moreover, total lung volume increased after 7 days (Fig. 2C). Remarkably, the aerated lung volume dropped after 7 days to then gradually increase again (Fig. 2A). At day 35, hydroxyproline content was significantly increased indicating that the collagen content in the lungs was elevated after silica exposure. In correspondence with this finding, we saw granuloma formation (Fig. 2E).

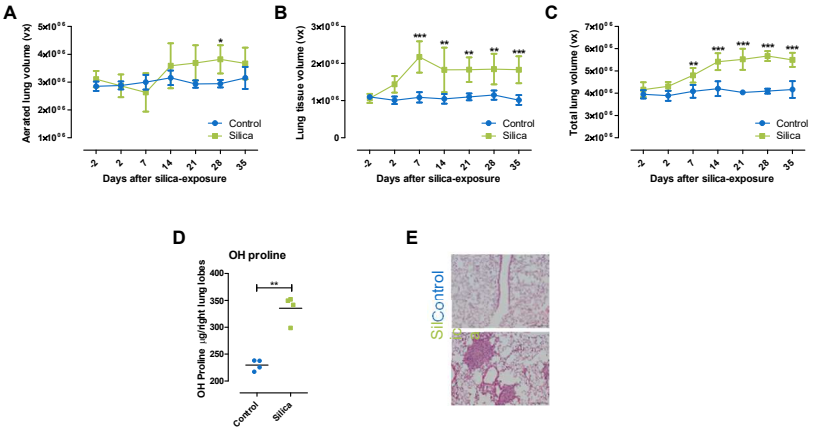

Figure 2: Longitudinal *in vivo* lung µCT derived biomarkers and conventional *ex-vivo* read-outs to monitor pulmonary fibrosis. Graphs (A, B, C) represent longitudinal changes of three lung biomarkers quantified from µCT images. Graph D represents hydroxyproline content, which is an indication of the amount of collagen in the lungs. Graph E shows histological assessment of the lungs (H&E staining). Results are representative images of 4 control and 6 silica mice; endotracheal instilled with saline or 5 mg per 40 µL silica. Data represent mean (n=4-6) ± standard deviation. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; vx = voxels

Conclusion

Our findings indicate that low-dose µCT is an excellent technique to longitudinally evaluate silica-induced pulmonary fibrosis in mice, a novel approach that allows evaluating host response, disease progression and stabilization over several weeks. Imaging results were confirmed by *ex vivo* histology and hydroxyproline assay. Pulmonary fibrosis in the silica model is persistent over the investigated time frame of 5 weeks; this is an advantage over the currently most used fibrotic animal model (bleomycin). The combination of imaging and a fibrotic model is ideal for evaluating the therapeutic efficiency of novel antifibrotic strategies.

Acknowledgement

This research was supported by KU Leuven Internal Funds (C24/17/061).

References:

1. Richeldi, L., Collard, H. R. & Jones, M. G. Idiopathic pulmonary fibrosis. *Lancet* **389,** 1941–1952 (2017).
2. Gabrielli, A., Avvedimento, E. V & Krieg, T. Scleroderma. *N. Engl. J. Med.* **360,** 1989–2003 (2009).
3. Volkmann, E. R. & Tashkin, D. P. Treatment of systemic sclerosis-related interstitial lung disease: A review of existing and emerging therapies. *Ann. Am. Thorac. Soc.* **13,** 2045–2056 (2016).
4. Vande Velde, G. *et al.* Magnetic Resonance Imaging for Noninvasive Assessment of Lung Fibrosis Onset and Progression: Cross-Validation and Comparison of Different Magnetic Resonance Imaging Protocols With Micro-Computed Tomography and Histology in the Bleomycin-Induced Mouse Mod. *Invest. Radiol.* **0,** 1–8 (2014).
5. Vande Velde, G. *et al.* Longitudinal micro-CT provides biomarkers of lung disease and therapy in preclinical models, thereby revealing compensatory changes in lung volume. *Dis Model Mech* **9,** 91–98 (2016).