

Combined MRI methods enable follow-up of bleomycin-induced murine lung fibrosis

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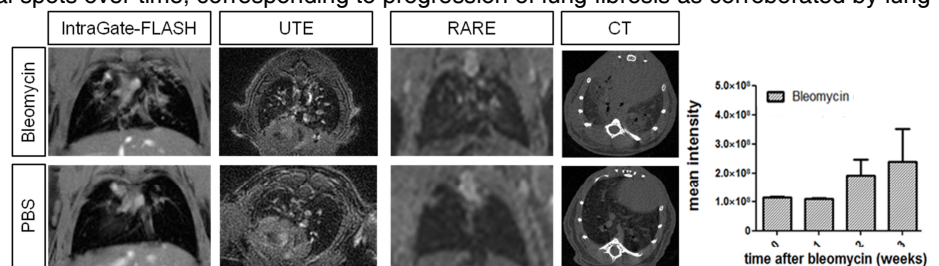
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TARGET AUDIENCE: Researchers interested in the non-invasive & longitudinal assessment of lung pathology in small animal models.

PURPOSE: Pulmonary fibrosis, either idiopathic or secondary to diseases such as systemic sclerosis, is a devastating and life threatening disorder for which effective treatment is still lacking¹. Animal models are indispensable to unravel fibrotic processes in the lung and to develop new therapeutic strategies in a preclinical setting. The bleomycin-induced pulmonary fibrosis model is well-characterized and the most widely used mouse model². The resulting fibrosis is routinely quantified by labor-intensive end-stage histological assessments, requiring many animals. Although histological techniques will remain essential to unravel pathogenesis at a molecular and cellular level, they lack the ability to follow-up on disease progression and potential therapeutic effects in the individual animal. As the course of fibrosis progression in rodent models shows substantial interindividual variation, non-invasive techniques are indispensable to dynamically monitor initial lung inflammation and fibrosis progression to establish the kinetics of pathogenic events or treatment effects for each animal individually. At present, imaging tools for the evaluation of lung disease with good temporal and spatial resolution *in vivo* are limited (e.g. radiotoxicity concerns in micro-computed tomography (CT)), advances made in lung MRI techniques to follow-up disease progression will greatly enhance this research. Therefore, we aimed to optimize and evaluate lung MRI protocols to visualize disease onset and progression in the bleomycin-induced mouse model of lung fibrosis. We compared prospectively and retrospectively gated MRI sequences and validated our results with established CT imaging of lung fibrosis³ and histochemical techniques.

METHODS: animal model: male C57Bl/6 mice were intratracheally instilled with bleomycin (0.05U in 50 μ l of PBS) or sham. The mice were scanned with MRI and CT at baseline and weekly until 3 weeks after instillation. After the last imaging time point, mice were sacrificed, *ex vivo* CT data were acquired and the lungs were isolated for histological analysis and quantification as described before³. MRI methods: images were acquired at 9.4T (Bruker Biospin, 20 cm) in combination with a 7.5cm quadrature coil, using the following sequences: (1) a respiratory triggered RARE sequence (TR 6000ms TE_{eff}=15.9ms, 50slices of 0.5mm thick, in plane resolution of 200 μ m \times 150 μ m, 2 averages), (2) a respiratory triggered ultra short echo (UTE) sequence (FID mode, TR= 20ms, TE=0.4ms, 8 slices, 0.6mm slice thickness, in plane resolution of 175 μ m and 3 averages) and (3) a retrospectively gated FLASH sequence IntraGate (TR/TE = 30/1.26 ms, 17 deg flip angle, 5 slices covering the lung, slice thickness 1 mm and gap of 0.5 mm, in plane resolution of 156 μ m, 80 repetitions resulting in a 10 min acquisition; 1 cm wide navigator slab, excited with a 0.8 ms sinc10H pulse with a 1.5 deg flip angle. For reconstruction, 70% of the respiration and ECG period was used (Paravision 5.1, Bruker)). MRI data were quantified using ImageJ. CT methods: retrospectively gated CT images were acquired on a small animal μ CT scanner (SkyScan 1076, Bruker microCT) and quantified as described before³.

RESULTS: The prospectively gated UTE and RARE protocols as well as retrospectively gated IntraGate-FLASH imaging were able to visualize an increase of hyperintense focal spots over time, corresponding to progression of lung fibrosis as corroborated by lung CT images (Figure). Quantification of the mean lung signal intensity shows an increase over time (figure: graph), which was confirmed by the decrease in aerated lung volume quantified from the CT data and by histology. UTE, RARE and IntraGate-FLASH images of control animals confirmed the absence of contrast without fibrosis induction.



DISCUSSION: The evaluated MRI protocols were all able to non-invasively visualize and quantify lung disease progression. Moreover, the IntraGate-FLASH protocol does not need setup of respiratory triggering for lung imaging, making it an easy to use and efficient alternative to more conventional sequences. Where CT provides poor soft tissue contrast, MRI has the potential to provide contrast differences between vasculature, fibrotic areas and inflammation, without concerns for radiotoxicity when scanning the same animal repeatedly.

CONCLUSION: In this study, we show that both prospective and retrospective lung MRI protocols are valuable techniques in the longitudinal follow-up of disease progression in mice with pulmonary fibrosis. This opens perspectives to monitor lung fibrosis and therapeutic response on an individual basis with high temporal resolution using MRI, without any concerns for radiation toxicity. Further MRI experiments will be performed to finetune and evaluate lung MRI sequences regarding their ability to visualize the switch from inflammatory to fibrotic processes in bleomycin-instilled lungs.

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