

## In vivo MRI effectively monitors onset and progression of bleomycin-induced lung fibrosis in free-breathing mice

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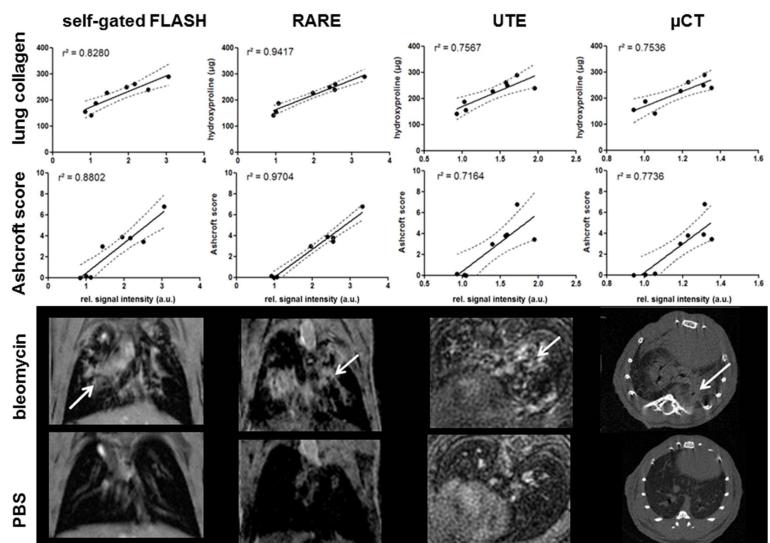
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**TARGET AUDIENCE** Researchers interested in the non-invasive and longitudinal assessment of lung diseases and their experimental treatment outcome in small animal models.

**PURPOSE** Lung fibrosis, either idiopathic or secondary to diseases such as systemic sclerosis, is a devastating and life threatening disorder for which effective treatment is still lacking<sup>1</sup>. 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 progression of fibrosis to establish the appropriate time window for anti-fibrotic treatment and its effects for each animal individually. This is important because improvement of our understanding of pathological mechanisms and treatment effects in preclinical models is likely to improve predictability of clinical outcome of experimental treatment, where real anti-fibrotic treatments will be most relevant for clinical applicability. Non-invasive MRI of progression of lung disease in bleomycin-instilled mice may provide a sensitive and valuable tool to assess onset and progression of lung fibrosis in free-breathing mice, without radiotoxicity concerns or elaborate invasive endpoint measurements. Therefore, we evaluated and compared the potential of respiratory gated ultra short echo time (UTE) and self-gated MRI approaches with a conventional respiratory triggered pulse sequence in the bleomycin-induced lung fibrosis mouse model. We validated the MRI results with *in vivo* micro-computed tomography ( $\mu$ CT)<sup>2</sup> and *ex vivo* histology as golden standard histochemical readouts for lung fibrosis.

**METHODS** *Animal model*: male C57Bl/6 mice were intratracheally instilled with bleomycin ( $n = 7$ , 0.05 U in 50  $\mu$ l PBS) or sham ( $n = 3$ , 50  $\mu$ l PBS). The mice were scanned with MRI and  $\mu$ CT at baseline and weekly until 28 days after instillation. After the last imaging time point, mice were sacrificed, *ex vivo*  $\mu$ CT data were acquired and the lungs were isolated for histological analysis and quantification as described<sup>2</sup>. *MRI methods*: MR images were acquired at 9.4 T (Bruker Biospin, 20 cm) using a 7.5 cm quadrature transmit/ receive coil and the following sequences: (1) a respiratory triggered RARE sequence (TR 6000 ms TEff = 15.9 ms, 50 slices of 0.5 mm thick, in plane resolution of 200  $\mu$ m x 150  $\mu$ m, 2 averages), (2) a respiratory triggered UTE sequence (FID mode, TR = 20 ms, TE = 0.4 ms, 8 slices, 0.6 mm slice thickness, in plane resolution of 175  $\mu$ m and 3 averages) and (3) a retrospectively gated FLASH sequence IntraGate<sup>®</sup> (TR/TE = 30/1.26 ms, 17° flip angle, 5 slices covering the lung, slice thickness 1 mm and gap of 0.5 mm, in plane resolution of 156  $\mu$ 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° flip angle. For reconstruction, 70% of the respiration and ECG period was used (Paravision 5.1, Bruker)). MRI data were quantified using ImageJ and represented as relative to baseline. *CT methods*: retrospectively gated  $\mu$ CT images were acquired on a small animal  $\mu$ CT scanner (SkyScan 1076, Bruker microCT) and quantified as described<sup>2</sup>.

**RESULTS** Prospectively gated UTE and RARE as well as self-gated FLASH sequences were able to visualize an increase of hyperintense focal spots over time, corresponding to progression of lung inflammation and fibrosis as corroborated by lung  $\mu$ CT images (Figure 1, bottom panels, 'bleomycin'). UTE, RARE and self-gated FLASH MR images of control animals confirmed the absence of contrast without fibrosis induction (Figure 1, bottom panels, 'PBS'). Quantification of the mean lung signal intensity showed an increase over time which was confirmed by the increase in mean lung signal intensity and the decrease in aerated lung volume quantified from the  $\mu$ CT data. Its strong agreement with histological measures of established lung fibrosis identifies MRI as a sensitive and accurate non-invasive readout for pulmonary lung fibrosis (Figure 1, top panels).



**DISCUSSION** The evaluated MRI protocols were all able to non-invasively visualize and quantify progression of lung disease. Their agreement with histological measures of fibrosis makes MRI a rapid alternative readout for lung fibrosis with minimal scan and anesthesia time (<15 minutes per mouse). Moreover, the self-gated 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. The availability of these three validated MRI protocols will allow the lung researcher to consider one of them as a readout method for lung fibrosis with great flexibility towards implementation depending on the available MRI setup and expertise.

**CONCLUSION** 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 rapidly and frequently monitor lung fibrosis and therapeutic response on an individual basis using MRI, without any concerns for radiation toxicity as is the case for  $\mu$ CT. Further experiments will be performed to fine-tune and evaluate lung MRI sequences regarding their ability to visualize the switch from inflammatory to fibrotic processes in bleomycin-instilled lungs. Identification of the relevant time frame for anti-fibrotic therapeutic intervention in mouse models of pulmonary fibrosis would eventually enhance pathogenesis and therapy research.

**REFERENCES** <sup>1</sup> Ley B, Collard HR, King TE, Jr. (2011), Am J Respir Crit Care Med 183 (4):431-440; <sup>2</sup> De Langhe E, Vande Velde G, et al. (2012), PLoS ONE 7 (8):e43123