

# Quantification of bleomycin induced lung injury by means of <sup>1</sup>H magnetic resonance elastography

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**Introduction:** Interstitial lung disease (ILD) induced end-stage fibrosis is a multiphase process that includes: 1) an initial or exposure phase; 2) an exudative phase; and 3) a final stage with one of two outcomes; a) edema clearance associated with alveolar wound healing and recovery, or b) organization of the alveolar space filling material followed by scar formation associated with loss of normal structure and function. It has previously been demonstrated that an MR-based method of quantitating shear modulus ( $\mu$ ) known as magnetic resonance elastography (MRE) can be used to assess normal lung [1]. However, the ability to quantitate lung injury with MRE and whether or not these changes differ from normal lung remains unknown. The purpose of this work is to investigate whether or not MRE-based estimates of  $\mu$  reflect changes to normal rat lung parenchyma following administration of bleomycin, a known agent for regulation of pulmonary fibrogenesis.

**Methods:** Five adult female Sprague-Dawley rats were imaged on a 1.5T MR scanner with Twinspeed gradients (Signa Excite, GE Healthcare, Milwaukee, WI) using a previously described 10 cm diameter transmit-receive birdcage RF coil [1]. The modified coil included a pneumatic needle driver system that allowed shear waves to be generated at the point of contact between the needle and the tissue of interest. Before imaging, bleomycin was administered to four of the five animals at a dose of 0.5 U / 100 g body weight in 0.3 ml 0.15 M sterile saline using the protocol described by Bivas-Benita et al [2]. The remaining animal did not receive bleomycin and was considered a normal control (annotated as control). This protocol was approved by our Institutional Animal Care and Use Committee (IACUC) which included administration of food and water ad libitum. The four bleomycin administered animals were imaged at four (b4), seven (b7), 14 (b14) and 22 (b22) days post administration. Before imaging, each animal was sacrificed by administration of a fatal dose of anesthesia (100 mg/kg of pentobarbital sodium) after which the trachea was severed and an air-line was placed to enable inflation of the lungs. The anterior rib cage was then removed, exposing the thoracic cavity. The drum driver and needle was then positioned so that the needle inserted into the right middle or lower lobe of the lung. With the exception of the air-filled lungs, all lungs were inflated to a pressure of 3 cm H<sub>2</sub>O using a water column and pump system [1]. Visualization of shear waves was performed by acquiring coronal spin echo-based MRE images with the following parameters: field of view = 7 cm, slice thickness = 4 mm, TE/TR = 21/200 msec, mechanical excitation frequency = 200 Hz, motion sensitivity direction = slice,  $k_x/k_y = 128/64$ , bandwidth =  $\pm 15.6$  kHz, MRE phase offsets = 8. After imaging at 3 cm H<sub>2</sub>O, the lungs were inflated to 6 cm and 10 cm H<sub>2</sub>O and the acquisition repeated. For the air-filled lungs, inflation pressures of five, 10 and 15 cm were applied. Estimates of shear modulus were obtained by means of the phase-gradient shear wave inversion method [3] and were averaged over a region of interest that included the majority of the right lung visible within the imaging slice. To correct for the difference in density between lung and solid organs, the ratio of the signal within the lung and liver was calculated and applied to the MRE shear modulus estimates. Following completion of the MRE experiment, the injured lungs were excised and sent for whole mount pathology which included hematoxylin and eosin (HE) and trichrome staining.

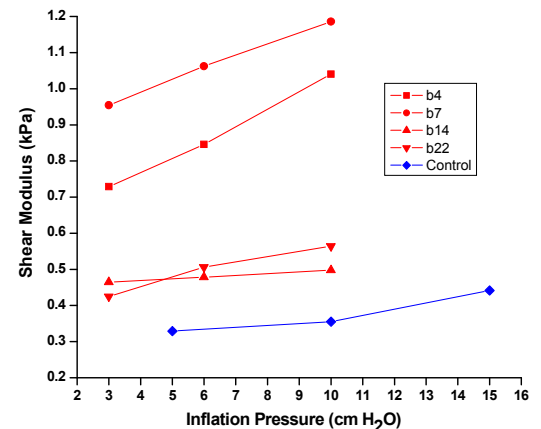
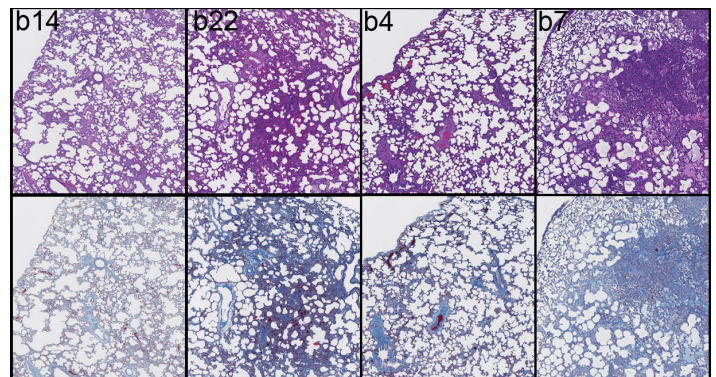


Figure 1:  $\mu$  versus inflation pressure for the air-filled (control) and bleomycin injured lungs (b4-b22).

**Results:** Figure 1 shows a plot of shear modulus versus inflation pressure for all five data sets. All data show an increase in shear modulus with inflation pressure which is consistent with punch indentation mechanical testing based measurements [4]. A clear demarcation between the control (i.e., air-filled) and injured data sets can be seen with the control having a lower overall shear modulus at all inflation pressures suggesting that lung injury results in change to the intrinsic mechanical properties of the parenchyma itself. The distribution of shear modulus versus inflation pressure for bleomycin data is somewhat heterogeneous. However this is also consistent with the relative diffuse nature of bleomycin induced lung injury and the method of administration which involves delivery by way of an aerosol spray within the trachea. Figure 2 shows HE and trichrome stained lung sections that include regions representative of the type of injury observed.

Figure 2: HE (top row) and trichrome (bottom row) stained sections of bleomycin injured lungs (b4, b7, b14, b22) in rank order of shear modulus as shown in figure 1. All sections show various amounts of abnormalities including cystic spaces, increased cellularity and injury on the HE images while the trichrome sections show areas of increased collagen deposition (dark blue).



**Conclusion:** This pilot study provides evidence to support the hypothesis that ILD injury results in changes to the intrinsic mechanical properties of the lung which are similarly reflected in MRE-based estimates of  $\mu$ .

**Acknowledgements:** This work is supported by NIH Grant EB07593

## References:

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