

Quantitative Image Analysis of Fibrotic Lesion Progression in the Mouse Bleomycin Model of Pulmonary Fibrosis

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1 INTRODUCTION

Evaluation of therapeutic interventions in the bleomycin (Bleo) model of lung fibrosis is challenging due to the presence of complex pathological changes and the variable distribution of lesions within the lung. The extent and severity of fibrosis is routinely assigned by direct observation using the Modified Ashcroft score (Fig 1.). The Modified Ashcroft score for a given lung sample is the mean of the grades (1 to 8) assigned to each of a subset of x20 fields (Fig 1.) within the section. However, an image analysis method able to identify different severities of fibrosis would provide rapid and greater objectivity to the assessment of fibrosis across the complete lung section.

Our objective was to classify areas of distinct pathology in lungs from Bleo-challenged mice over a 56 day time course. To accurately assess the degree of pulmonary injury, we utilised whole slide digital image analysis of picosirius red (PSR) stained lung sections. Together with pathology review, we classified and objectively quantified two types of Bleo-induced pulmonary lesion with distinct histo-morphological characteristics.

2 MATERIALS AND METHODS

Mice received a single dose of Bleo (0.03u, i.t.) on Day 0. Separate groups were examined at Day 3, Day 7 and then at weekly intervals until Day 56. Inflammatory cells were quantified in the BALF and hydroxyproline measured in the tissue. Lung fibrosis was assessed using a modified Ashcroft score. Additionally, an algorithm was developed using Halo[®] image analysis software to classify specific regions of interest (Fig 2. & 3.) within PSR stained lung sections (7 per animal, total = 1,001 sections) identified by a Pathologist as:

- Type I Lesion weakly PSR positive, individual & irregularly arranged collagen fibrils, with cellular infiltrates within the alveoli.
- Type II Lesion - strongly PSR positive, dense bundles of extracellular collagen fibrils within or closely associated with Type I lesion.
- Normal parenchyma - alveolar ducts and alveoli free of cellular or extracellular matrix infiltrates, walls of normal thickness.
- Basal Collagen - prominent bronchial and vascular structures
- Large White Space areas – airway and vessel lumen etc

3 RESULTS

Bleo induced fibrotic areas increase in both lung HP (data not shown) and modified Ashcroft score. Detailed review showed the presence of Type I & Type II lesions across the time course. Increased levels of Type I lesions compared to Type II lesions were seen at all time points, with Type I & Type II lesions peaking at Day 14, and regressing at later time points. However, the Type I lesion appeared at Day 7 whereas the Type II was only evident from Day 14 (Fig 4. & 5.).

4 CONCLUSION

Digital image analysis together with pathology review may provide a more accurate interpretation of the development of the bleo-induced fibrotic lesion. This may provide a new tool to pre-clinically investigate the effect of novel therapies on more refined lesion phenotypes, which may align closer to fibrotic disease development and therapeutic responsive pathology observed in clinical fibrotic lung tissue.

Figure 1. Example Stained Images from Saline and Bleomycin Treated Mice

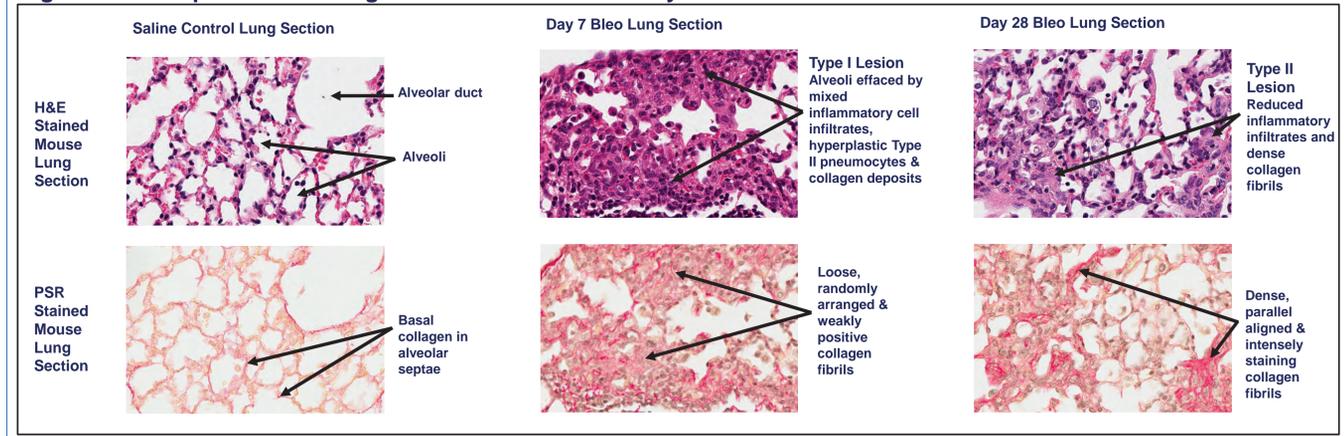


Figure 2. Example Analysis from Saline and Bleomycin Treated Groups

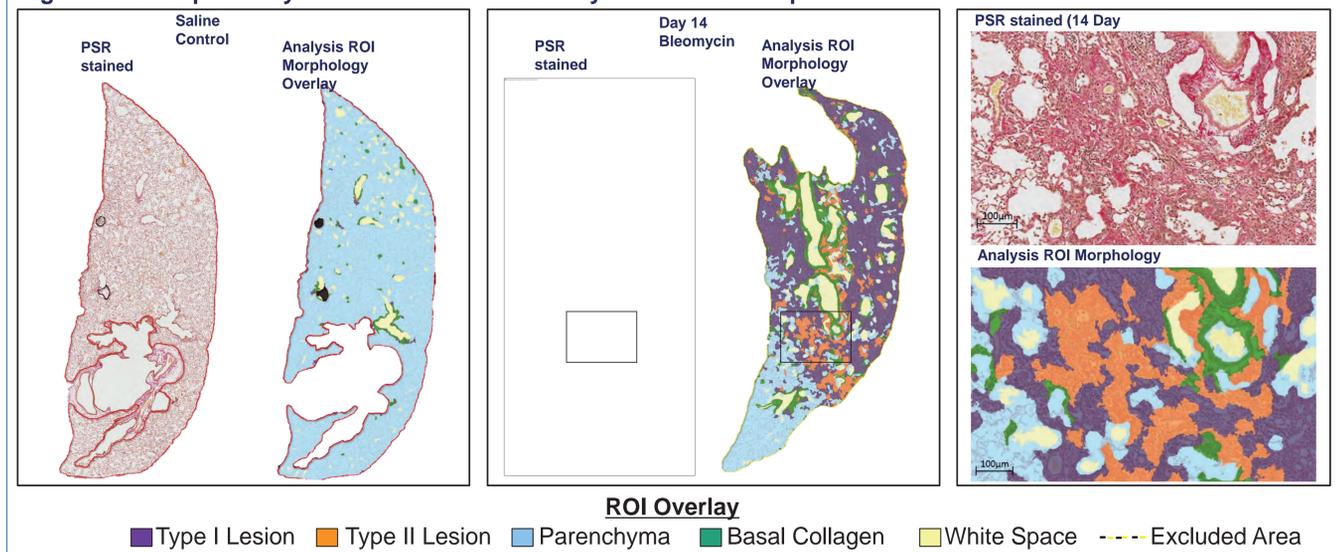


Figure 3. Morphology Data

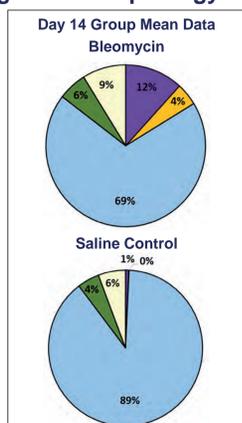


Figure 4. Type I and Type II Lesion Content Image Analysis Data

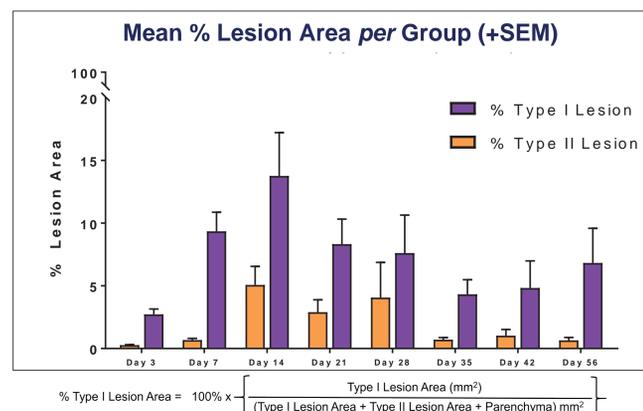
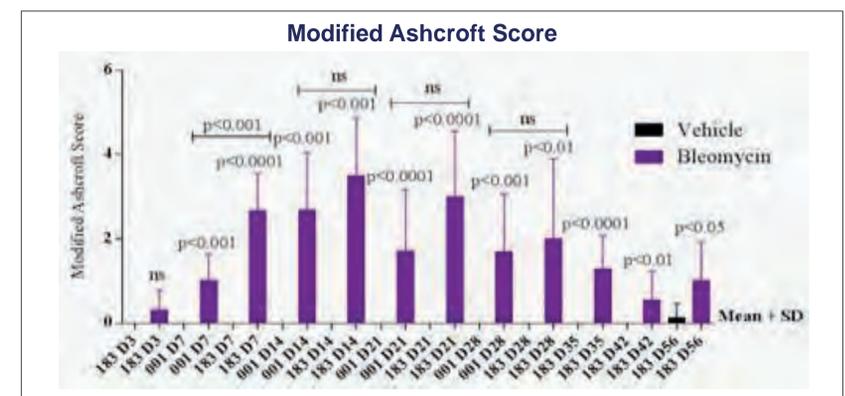


Figure 5. Modified Ashcroft Score Data



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