

Assessment of Clinically Relevant Extracellular Matrix Markers in a Bleomycin-Induced Model of Lung Fibrosis in the Mouse

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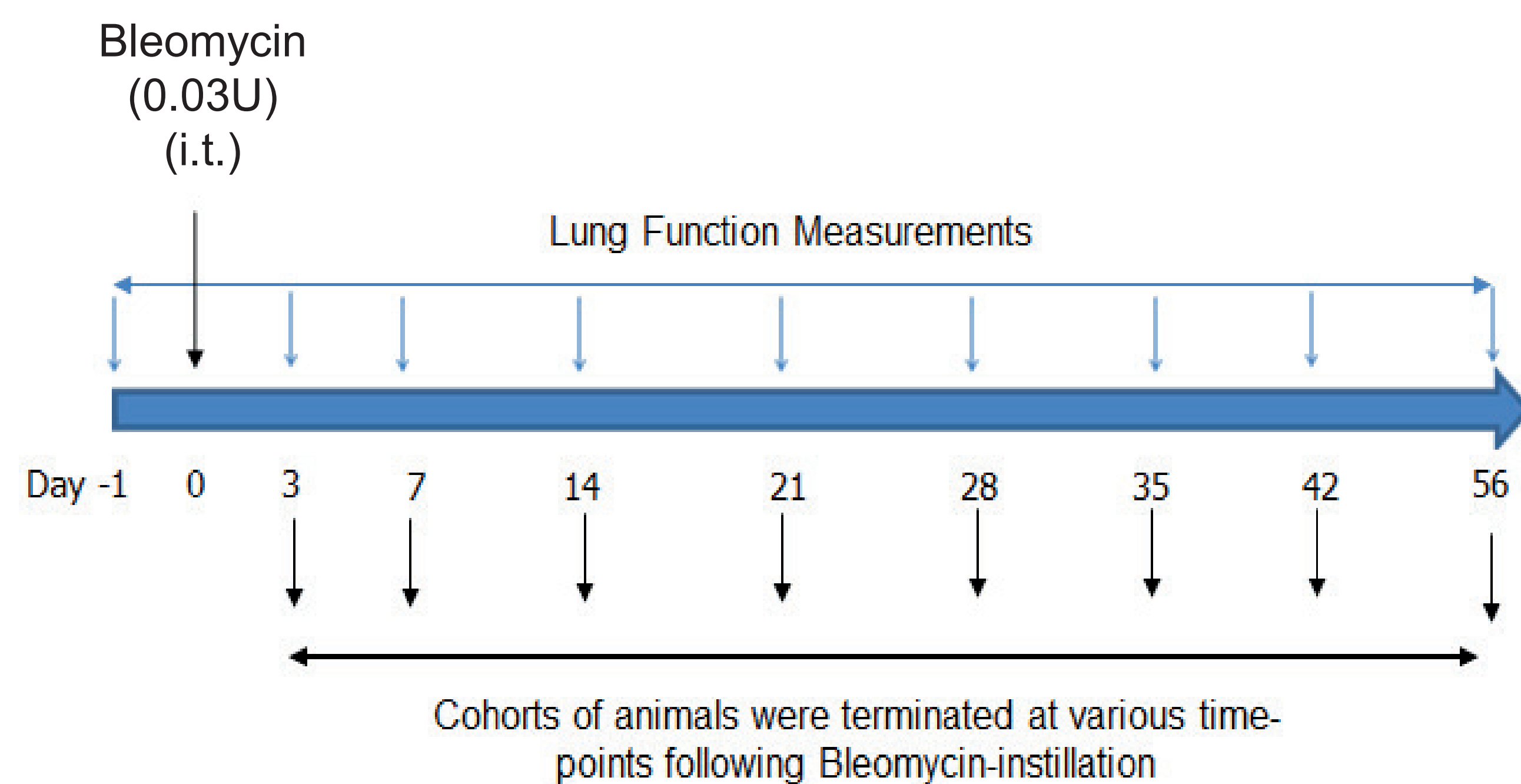
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1 Introduction & Methods

The bleomycin-induced lung fibrosis model is routinely used to investigate efficacy of potential therapies for treating idiopathic pulmonary fibrosis (IPF).

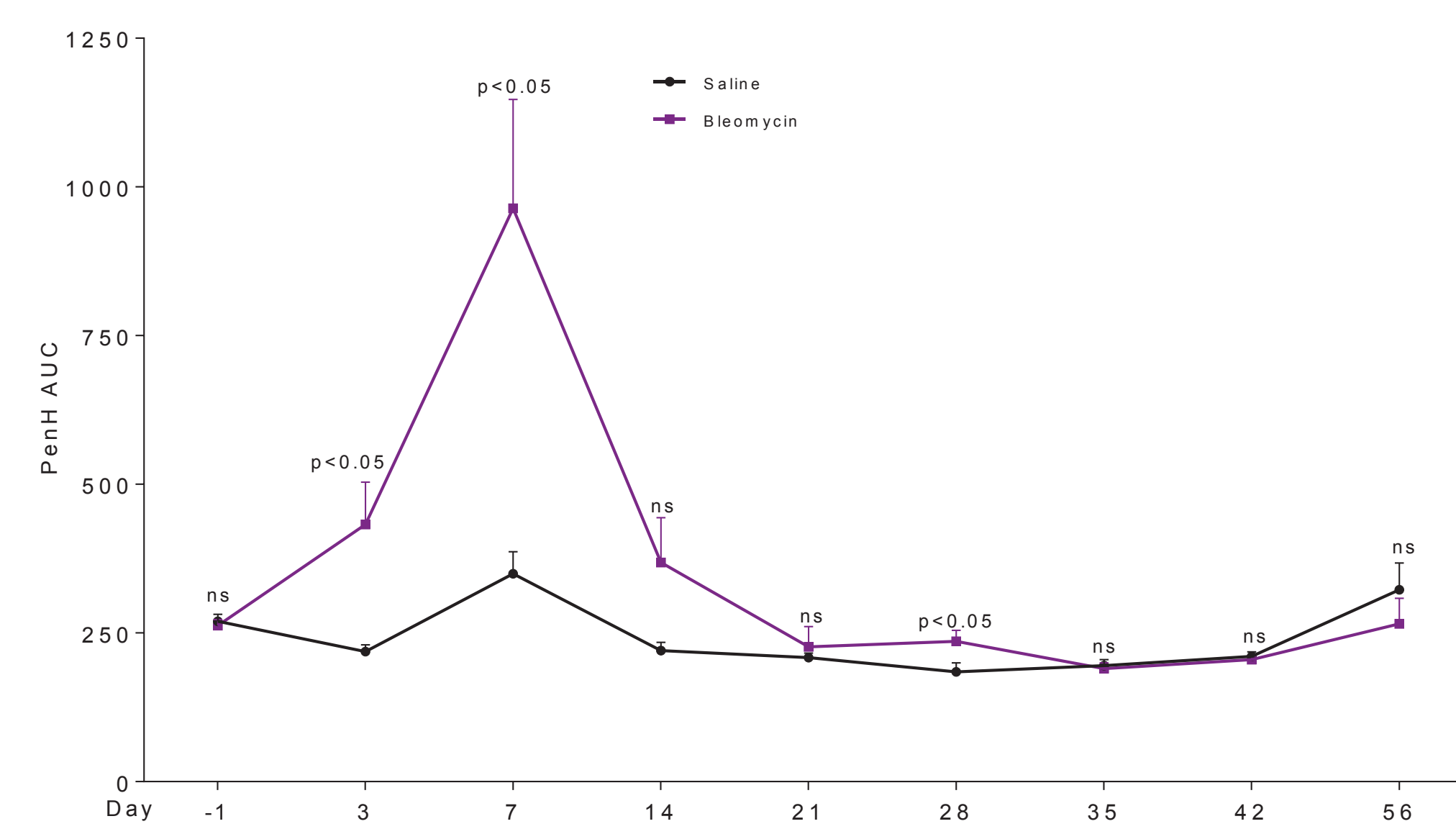
Our aim was to investigate the effects of bleomycin over a two month time course post-dosing to characterise changes in lung inflammation, lung function, collagen deposition, pathology and clinically validated serum extracellular matrix (ECM) biomarkers (C1M, C3M and P4NP7S). These markers have been associated with the diagnosis and prognosis of human IPF (Jenkins *et al. Lancet Respir Med* 2015).

Briefly, female C57BL/6J mice were administered bleomycin or saline intratracheally (i.t.) on day 0. Lung function was assessed throughout the study. Cohorts were sacrificed during the course of the study and MMP-generated fragment of collagen type 1 (C1M), collagen type 3 (C3M) and the formation marker of collagen type IV (P4NP7S), along with lung hydroxyproline levels, inflammation and histopathology were assessed.



2 Results

Figure 1: Lung Function Changes Following Bleomycin-instillation



Lung function parameters were measured in conscious animals prior to and following bleomycin administration. PenH AUC peaked 7 days following bleomycin-instillation ($p < 0.05$). The peak in PenH AUC coincided with increased lung inflammation at day 7, which was largely comprised of lymphocytes ($p < 0.001$) and neutrophils ($p < 0.05$).

Figure 2: Lymphocytes in the BAL Fluid

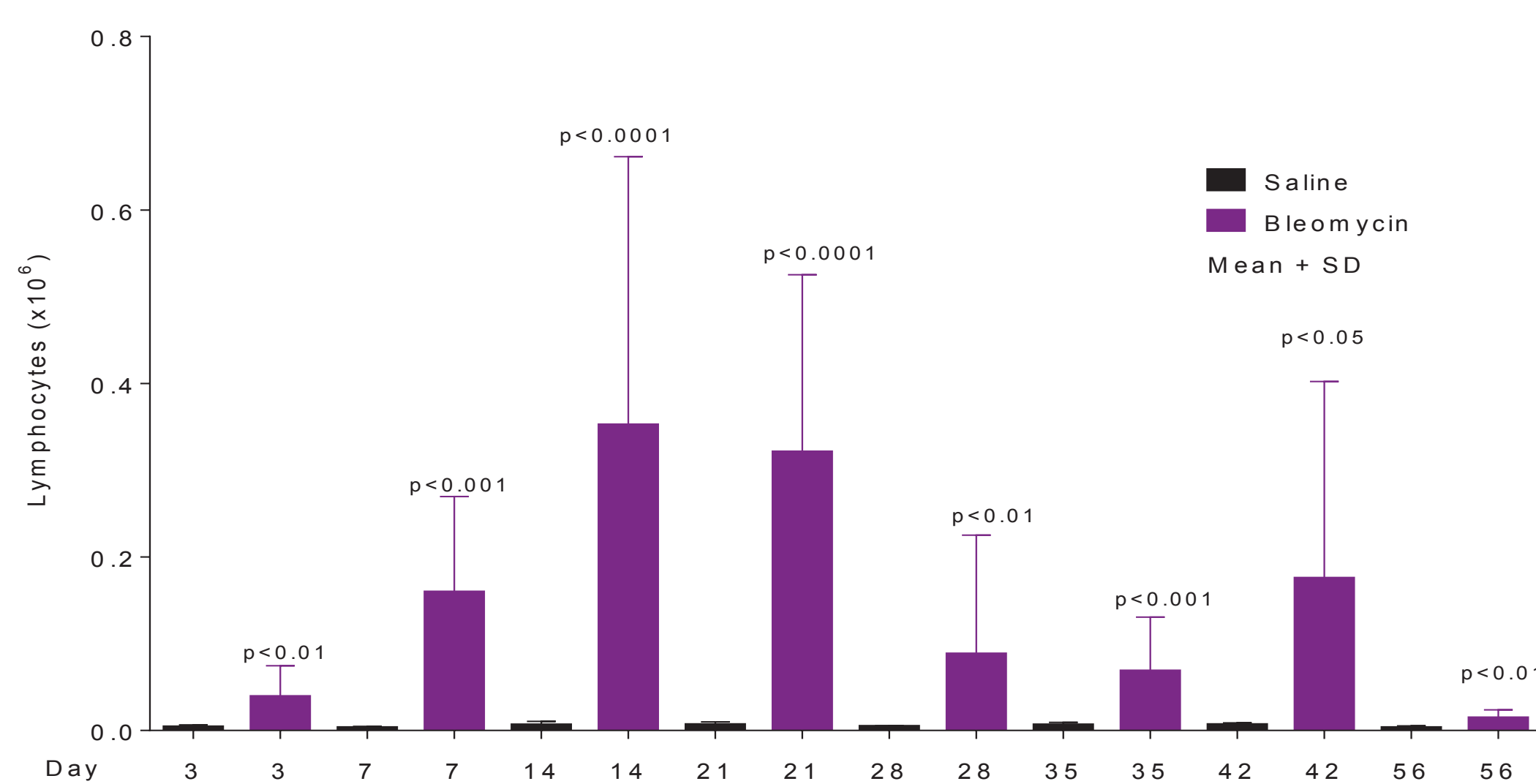
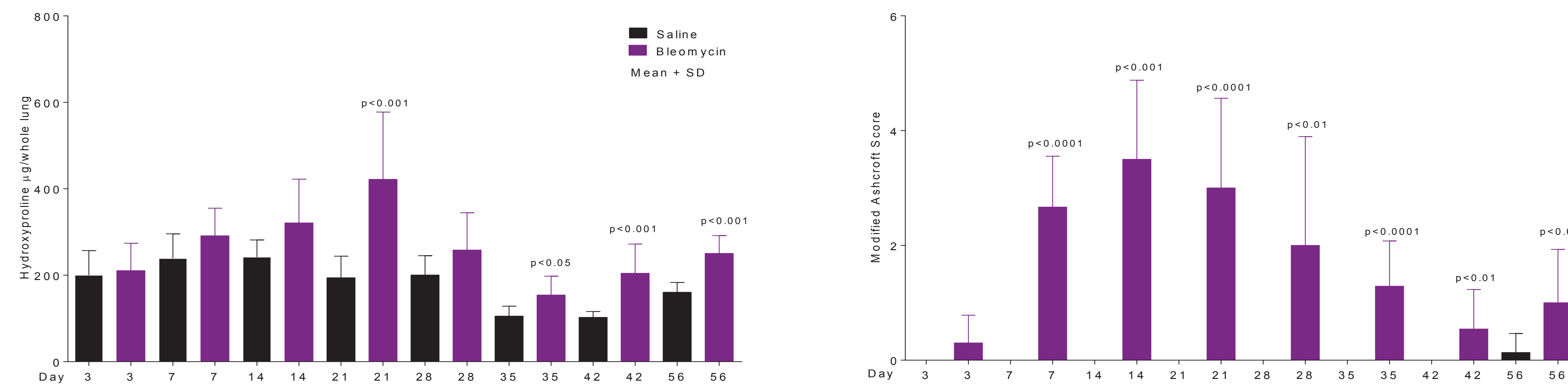


Table 1: Fold-Increase of Inflammatory Cells

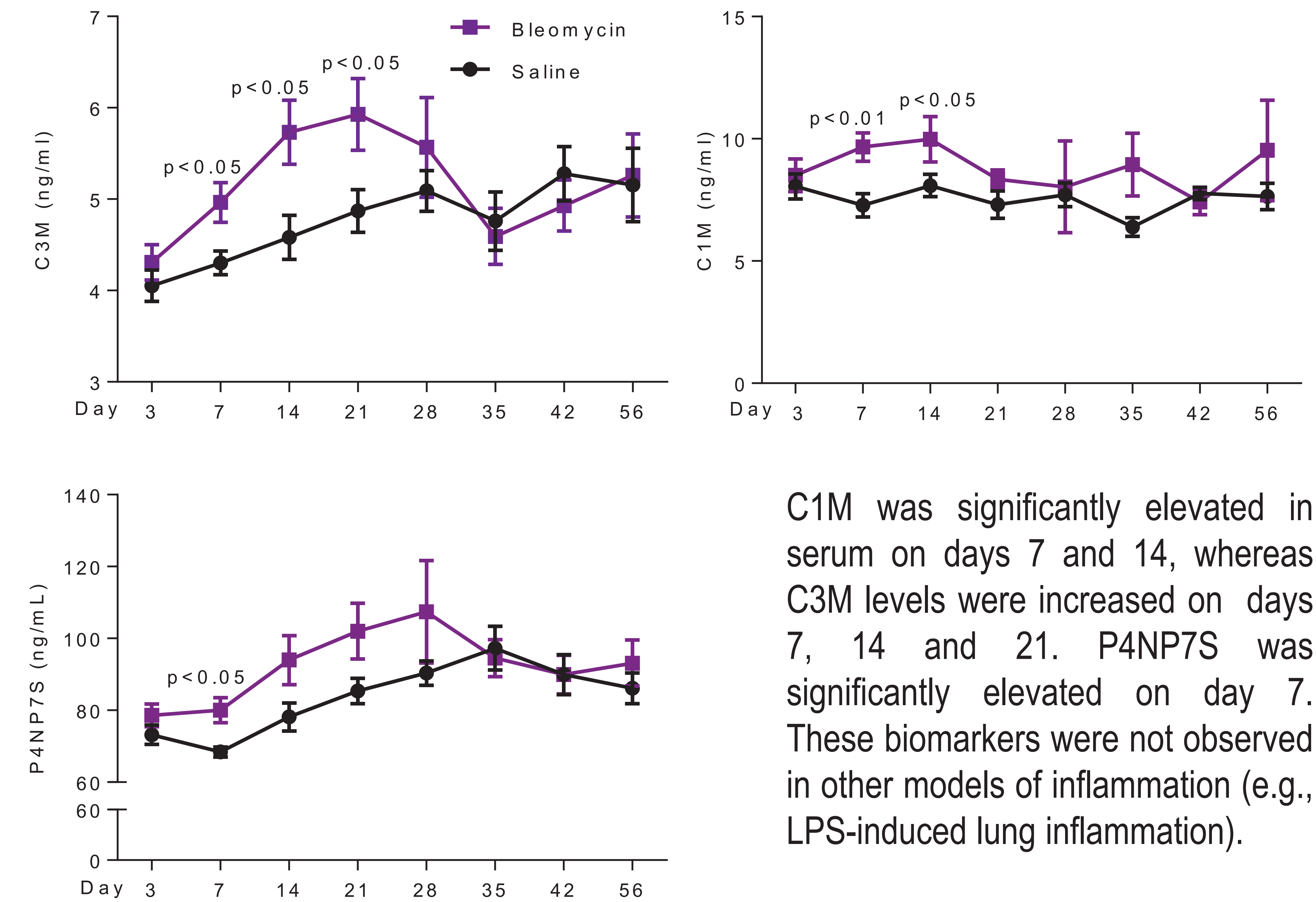
Inflammatory Cells (BAL)	Day 3	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42	Day 56
Total Cells	0.9 (ns)	2.0 ($p < 0.05$)	4.8 ($p < 0.01$)	5.2 ($p < 0.01$)	2.1 (ns)	2.2 ($p < 0.05$)	2.9 (ns)	1.9 ($p < 0.05$)
Macrophages	0.5 ($p < 0.0001$)	0.9 (ns)	1.7 ($p < 0.05$)	2.3 ($p < 0.05$)	1.5 (ns)	2.0 ($p < 0.05$)	2.1 (ns)	1.8 (ns)
Neutrophils	3.0 ($p < 0.05$)	2.5 ($p < 0.05$)	1.3 (ns)	3.0 ($p < 0.01$)	1.7 (ns)	1.6 ($p < 0.01$)	1.9 (ns)	2.0 ($p < 0.05$)

Figure 3: Hydroxyproline Levels and Modified Ashcroft Score



Hydroxyproline (HP) levels were measured as a marker of collagen deposition. HP levels were significantly elevated in the lung from day 21 onwards ($p < 0.05$). Histopathological evaluation of the lung revealed an increase in the Modified Ashcroft Score as early as day 7 ($p < 0.001$). The increased score was sustained until day 56 ($p < 0.05$). A clear inflammatory response was observed at days 7 and 14 and fibrotic lesions increasing in severity and prevalence were detected from day 21.

Figure 4: Clinically Validated ECM Biomarkers



C1M was significantly elevated in serum on days 7 and 14, whereas C3M levels were increased on days 7, 14 and 21. P4NP7S was significantly elevated on day 7. These biomarkers were not observed in other models of inflammation (e.g., LPS-induced lung inflammation).

3 Summary

These data suggest that in this lung fibrosis model there is an early inflammatory phase, followed by increased collagen deposition and pulmonary fibrosis. Clinically relevant biomarkers associated with diagnosis and progression of IPF could be measured at early time-points indicating that these may be predictive of fibrosis in this model. Interestingly, these biomarkers were not increased in other inflammatory models. Taken together this model may be used as a tool to improve the predictability and translatability of novel mechanisms and compounds for the treatment of IPF.