Changes in airway platelet population and morphology in a murine model of lung fibrosis

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Introduction

Idiopathic pulmonary fibrosis (IPF) is a progressive interstitial lung disease characterized by increased deposition of extra cellular matrix and scarring of lung tissue over an extended period. There are currently only two approved treatments for pulmonary fibrosis, both of which slow down the decline in lung function in some patient cohorts. There is a need to discover and evaluate new targets to develop novel treatments for IPF.

It has previously been shown that platelets play a role in the remodelling of the airways in a murine model of ovalbumin induced allergic inflammation, and it is known that platelets contain and release mediators such as platelet-derived growth factor and TGF-β, which have been shown to be important in the pathogenesis of fibrosis. It is hypothesised that platelets may be playing a role in the development of IPF. The aim of this study was to investigate whether the platelet population and/or morphology in the bronchoalveolar lavage (BAL) was altered in fibrotic mice and to determine if any changes in platelet population or morphology correlated with expression of standard preclinical fibrosis biomarkers.

Methods

Male C57BL/6J mice were administered bleomycin intraperitoneally twice a week for 4 weeks (a total of 8 injections). Mice were then terminated on Day 35. Lung function measurements were performed using an eSpira Forced Manoeuvres system (EMMS) and a forced oscillation system to measure forced vital capacity (FVC), forced expiratory volume in 50 msecs (FEV50) whole lung compliance, and elastance, and tissue elastance. White blood cells and platelets in the BAL fluid were quantified and measured using an XT2000iV haematology analyzer (Sysmex). TGF-β1 levels were also analyzed in the BAL fluid using a Quantikine ELISA in singlet. Lungs were removed and fixed in 10% neutral buffered formalin for modified Ashcroft scoring. Statistical analysis was carried out using GraphPad Prism with individual student t-tests being used to assess changes in platelet number and size and Pearson correlation coefficient being calculated for the correlation analysis.

Results

Bleomycin administration resulted in significant increases (p<0.001) in Ashcroft score, and BAL TGF-β1 levels, and significant decrease (p<0.001) in FVC compared to saline treated animals (Figure 1).

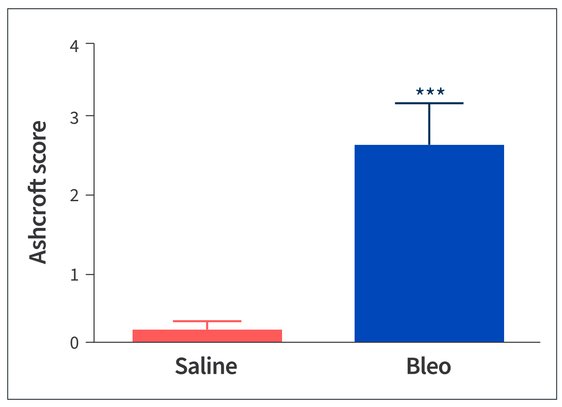

Figure 1A. Significant (p<0.001) increase in Ashcroft score of bleomycin treated animals compared to saline treated animals.

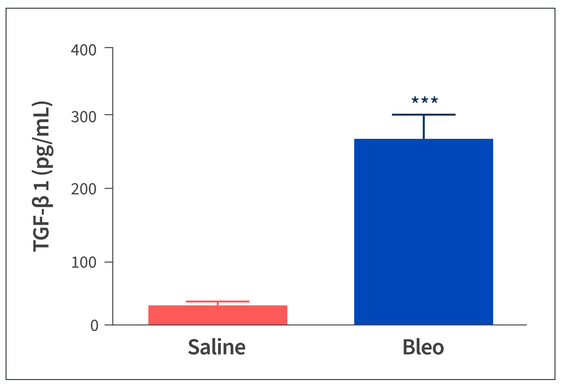

Figure 1B. Significant (p<0.001) increase in BAL TGF-β1 levels of bleomycin treated animals compared to saline treated animals.

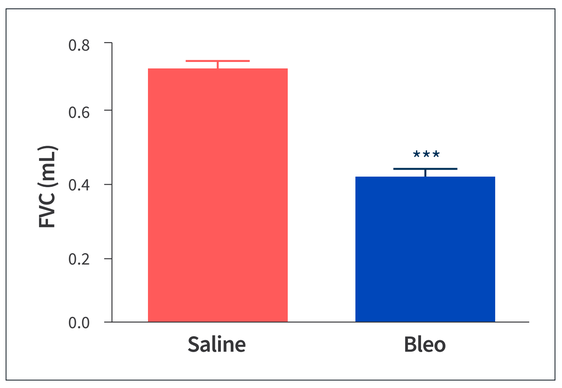

Figure 1C. Significant (p<0.001) decrease in FVC of bleomycin treated animals compared to saline treated animals.

There was a 113% increase (p<0.001) in the number of platelets measured in the BAL of animals administered bleomycin compared to animals receiving saline, and also a 14% decrease (p<0.001) in the mean platelet volume (MPV) of the platelets detected in the BAL fluid of bleomycin challenged animals compared to saline treated animals (Figure 2).

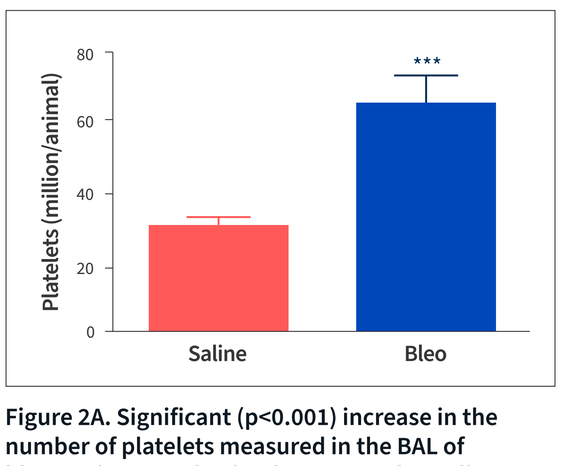

Figure 2A. Significant (p<0.001) increase in the number of platelets measured in the BAL of bleomycin treated animals compared to saline treated animals.

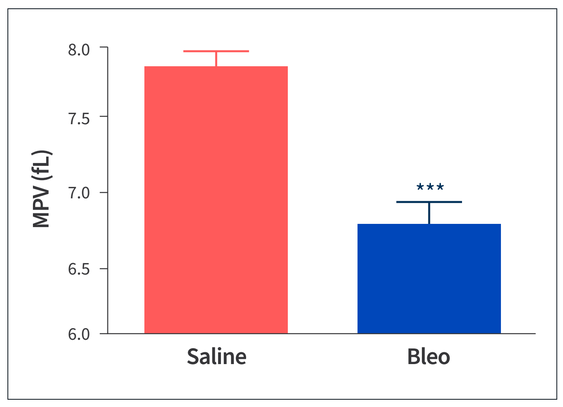

Figure 2B. Significant (p<0.001) decrease in the volume of the platelets in the BAL of bleomycin treated animals compared to saline treated animals.

There were significant correlations between the number of platelets in the BAL and the Ashcroft score, BAL TGF-β1 levels and the decline in FVC (Figure 3).

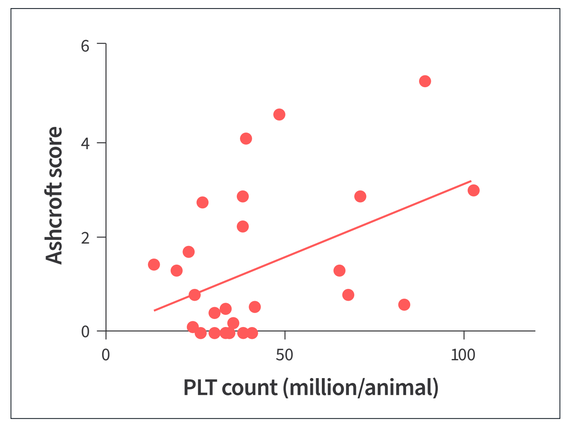

Figure 3A. Significant (p<0.05) correlation between Ashcroft score and platelet count in the BAL.

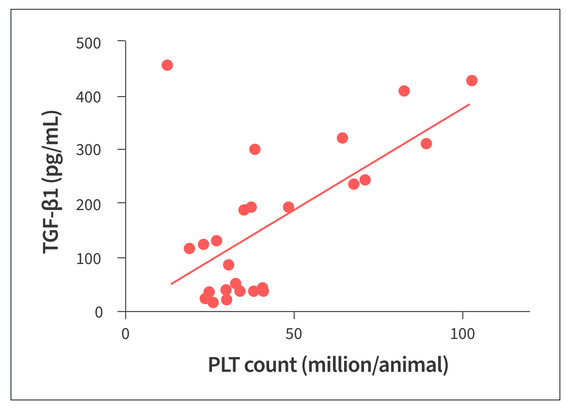

Figure 3B. Significant (p<0.001) correlation between TGF-β1 content in the BAL and platelet count in the BAL.

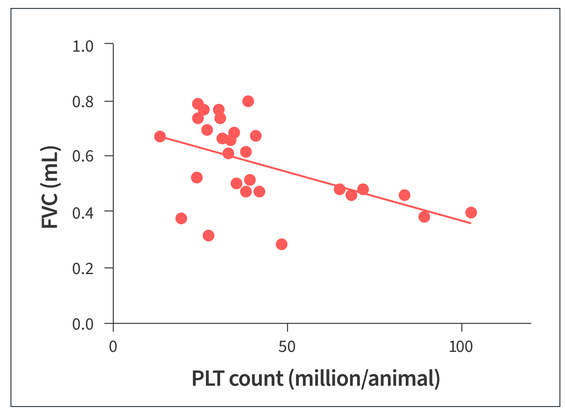

Figure 3C. Significant (p<0.01) correlation between FVC and platelet count in the BAL.

There were also significant correlations between the mean volume of the platelets in the BAL and the Ashcroft score, BAL TGF-β1 and the decrease in FVC (Figure 4).

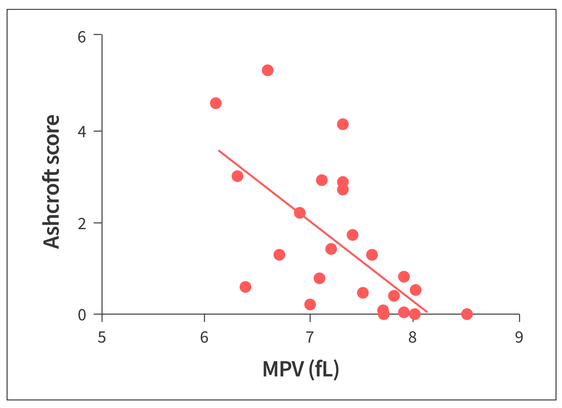

Figure 4A. Significant (p<0.001) correlation between Ashcroft score and MPV in the BAL.

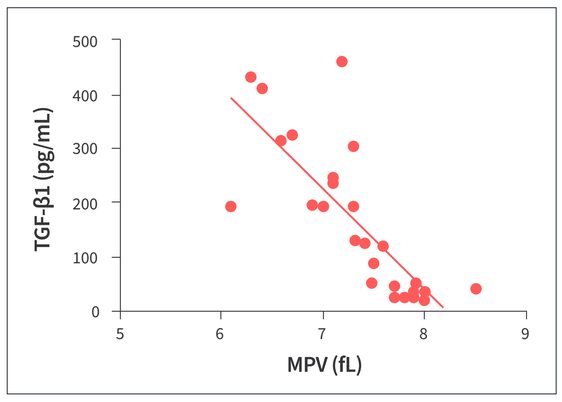

Figure 4B. Significant (p<0.001) correlation between TGF-β1 levels in the BAL and MPV in the BAL.

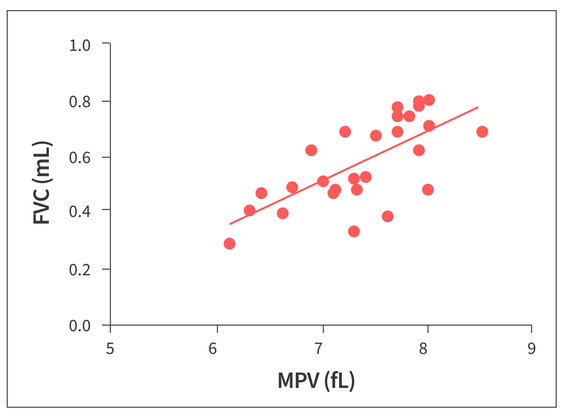

Figure 4C. Significant (p<0.001) correlation between FVC and MPV in the BAL.

It was confirmed that the increase in platelet number was not associated with haemorrhage in the lungs as there was no correlation between red blood cell count and platelet count in the BAL of the animals.

Conclusion

This study has shown that there is an increase in the number of platelets in the BAL of bleomycin treated animals and that the platelets that are in the BAL are significantly smaller in bleomycin treated animals. These changes in the numbers and size of the platelets in the BAL correlate with changes in fibrotic biomarkers.

This suggests that platelets may have a role in the fibrotic process and further research is warranted to elucidate the exact role that platelets may be playing and to uncover potential new targets for the treatment of IPF.

Animal experiments were conducted according to the Animals (Scientific Procedures) Act, 1986, and 2012 amendments following local ethical approval. Work was conducted in an AAALAC-accredited facility.