Pulmonary function and disease progression in bleomycin-induced and spirometry-confirmed mouse model of IPF

Authors
Asbjørn Graver Petersen, Denise Oró, Alba Manresa Arraut, Martin Rønn Madsen, Michael Feigh

Gubra, Hørsholm, Denmark

Corresponding author
Michael Feigh - mfe@gubra.dk

BACKGROUND & AIM

Idiopathic pulmonary fibrosis (IPF) is a chronic and fatal interstitial lung disease, characterized by progressive fibrotic development within the lungs and decline in pulmonary function. Thus, preclinical models of IPF should demonstrate pulmonary dysfunction in conjunction with disease progression for translational evaluation of novel drug therapies.

The aim of the present study was to characterize pulmonary function, metabolic, biochemical, histological, and transcriptomic changes in a bleomycin-induced (BLEO) and spirometry-confirmed mouse model of IPF.

METHODS

10-12 weeks old C57BL/6JRj male mice received either a single intratracheal instillation of bleomycin (1.5 mg/kg, 50 µL) or saline (CTRL) at study day 1. BLEO-IPF animals were randomized into study groups based on body weight loss at day 6 post-BLEO and terminated at specified time points. Terminal pulmonary end-points included spirometry (Flexivent) for expiratory/inspiratory capacity, biochemical analysis for hydroxyproline (HP) content, quantitative histomorphometry for markers of inflammation and fibrosis, and whole lung RNAsequencing including bioinformatic analysis. Gubra Histopathological Objective Scoring Technique (GHOST) was used for performing pathological Ashcroft scoring.

www.gubra.dk

1 Study outline

Group	Animal model	Name	Number of animals
1	CTRL	CTRL	10
2	BLEO-IPF	D7	10
3	BLEO-IPF	D14	11
4	BLEO-IPF	D21	12
5	BLEO-IPF	D28	12
6	BLEO-IPF	D35	12
7	BLEO-IPF	D42	7

2 Metabolic and biochemical parameters

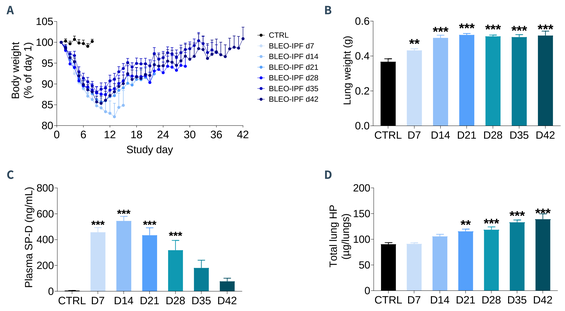

Figure 1. Metabolic and biochemical parameters in BLEO-IPF mice. (A) Body weight change relative to baseline (day 1). **(B)** Terminal lung weight (g). **(C)** Plasma surfactant protein D (SP-D). **(D)** Terminal lung total HP. **p<0.01 and ***p<0.001 compared to CTRL group (Dunnett's test one-factor linear model).

3 Pulmonary function testing

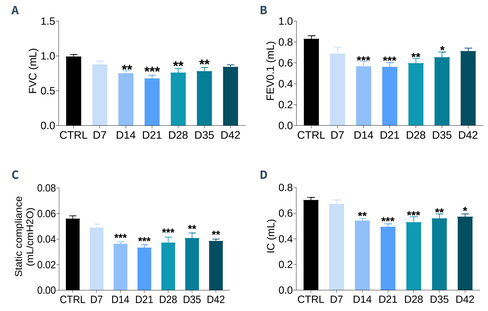

Figure 2. Pulmonary function testing in BLEO-IPF mice. (A) Forced vital capacity (FVC). **(B)** Forced expiratory volume in 0.1 seconds (FEV0.1). **(C)** Static compliance. **(D)** Inspiratory capacity (IC). *p<0.05, **p<0.01, and ***p<0.001 compared to CTRL group (Dunnett's test one-factor linear model).

4 Histopathological Ashcroft scoring

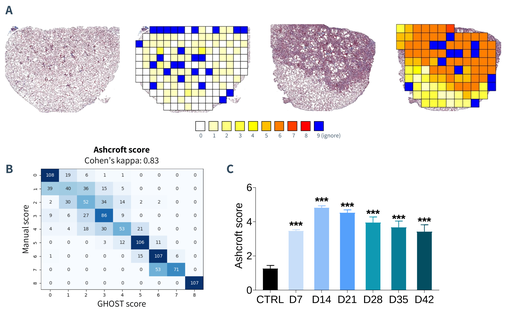

Figure 3. Ashcroft score. Histopathological Ashcroft score were determined by GHOST deep learning-based image analysis. **(A)** Representative Masson's Trichome photomicrographs used for GHOST evaluation. **(B)** Ashcroft compared by GHOST assessment and manual scoring. **(C)** Ashcroft score by GHOST. Mean ± SEM. ***p<0.001 compared to CTRL group (Dunnett's test one-factor linear model).

5 Histological markers of inflammation, fibrosis, and fibrogenesis

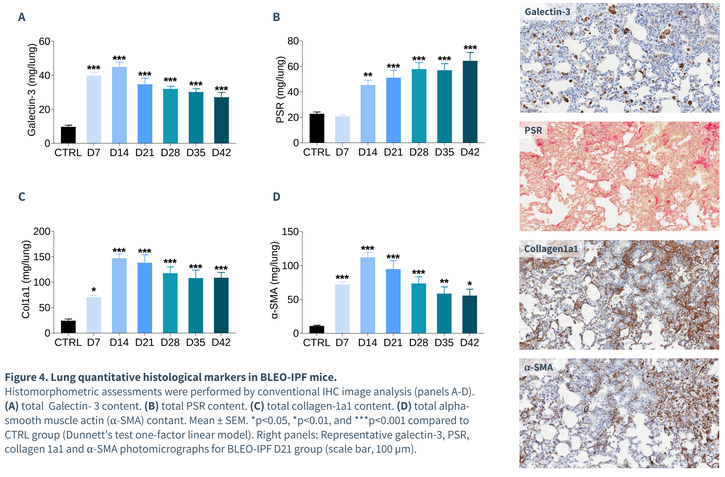

Figure 4. Lung quantitative histological markers in BLEO-IPF mice.
Histomorphometric assessments were performed by conventional IHC image analysis (panels A-D). **(A)** total Galectin- 3 content. **(B)** total PSR content. **(C)** total collagen-1a1 content. **(D)** total alpha-smooth muscle actin (α-SMA) contant. Mean ± SEM. *p<0.05, *p<0.01, and ***p<0.001 compared to CTRL group (Dunnett's test one-factor linear model). Right panels: Representative galectin-3, PSR, collagen 1a1 and α-SMA photomicrographs for BLEO-IPF D21 group (scale bar, 100 µm).

6 Transcriptomic profile for fibrosis and inflammation

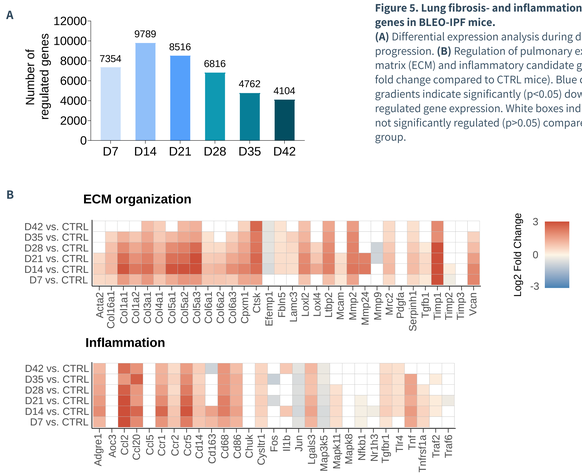

Figure 5. Lung fibrosis- and inflammation-associated genes in BLEO-IPF mice.
(A) Differential expression analysis during disease progression. **(B)** Regulation of pulmonary extracellular matrix (ECM) and inflammatory candidate genes (log2-fold change compared to CTRL mice). Blue colour gradients indicate significantly (p<0.05) down-regulated gene expression. White boxes indicate genes not significantly regulated (p>0.05) compared to CTRL group.

CONCLUSION

- BLEO-IPF mice demonstrate progressive increase in lung weight, total hydroxyproline content, and plasma SP-D.
- BLEO-IPF demonstrate reduced pulmonary expiratory and inspiratory function.
- BLEO-IPF mice demonstrate increased histopathological Ashcroft score.
- BLEO-IPF mice demonstrate increased lung levels of quantitative histological markers of fibrosis, inflammation and fibroblast cell activation.
- BLEO-IPF mice demonstrate marked lung transcriptomic regulation and increased fibrosis- and inflammation-associated gene expression.
- The BLEO-IPF mouse represent a translational preclinical model for exploring novel therapeutic agents for IPF.