DUAL HIGH-THROUGHPUT AUTOMATED ANALYSIS OF HISTOMORPHOMETRIC MARKERS OF FIBROSIS AND EMPHYSEMA IN TWO BLEOMYCIN-INDUCED MOUSE MODELS OF PULMONARY FIBROSIS

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Authors:
Cindy Serdjebi[1], Asbjørn Graver Petersen[2], Florine Chandes[1], Denise Oró[2], Henrik H Hansen[2], Bastien Lepoivre[1], Joel Berniac[1], Michael Feigh[2]

[1]Biocellvia, Marseille, France
[2]Gubra A/S, Hørsholm, Denmark

Cindy Serdjebi, R&D Director,
cindy.serdjebi@biocellvia.com

BACKGROUND AND OBJECTIVES

Emphysema coexists with idiopathic pulmonary fibrosis (IPF), characterized by expiratory flow limitation and alveolar injury, in up to one third of IPF patients. As for IPF, the prognosis of combined pulmonary fibrosis and emphysema (CPFE) is poor, and no curative treatments exists. While the bleomycin (BLEO)-induced fibrotic mouse is the most used rodent model of IPF, it is still unclear whether the BLEO-IPF mouse also shows histological features of emphysema. In the present study, we evaluated the BLEO-IPF mouse for histopathological hallmarks of IPF and emphysema at once, using a fully automated software suite. Considering that lipotoxicity is a risk factor for developing pulmonary complications, the lung histopathological phenotype was assessed in both low- and high-fat diet-fed BLEO-IPF mice.

MATERIALS AND METHODS

Twelve-weeks-old male C57BL6/JRJ mice were either fed chow or a high-fat diet (HFD, 60% kcal of fat) for 2 weeks prior to receiving a single intratracheal instillation of BLEO (1.5 mg/kg) or vehicle (sterile 0.9% saline). Mice were kept on the respective diet throughout the study. To study progression of lung injuries, mice were terminated 7-35 days after BLEO administration. Picrosirius red-stained (PSR) lung sections were evaluated using a fully automated digital pathology software (MorphoQuant™, Biocellvia) for quantitative assessment of fibrosis and emphysema histological endpoints.

CONCLUSION

Automated dual assessment of both IPF and emphysema characteristics is possible from PSR-stained slides. BLEO-IPF and HFD-BLEO-IPF mice demonstrate severe lung injury characterized by progressive fibrosis concomitant with emphysema features, i.e. alveolar loss and enlargement. The marked destruction of alveoli could translate into development of emphysema in the models. BLEO-IPF and HFD-BLEO-IPF mouse models are suitable for profiling compounds with therapeutic potential in IPF and CPFE.

.01 STUDY DESIGN AND OUTLINE

- Day 1: Intra-tracheal bleomycin (BLM) administration
- Day 6: Randomization per bodyweight
- Days 7/21/28/35: Lung function test, biochemistry and histology
- Embedding
- Cutting
- Picrosirius Red staining
- Scanning (X20)
- QC of images
- Validation
- Statistical study
- Reporting

Name	Animal Model	Number of Subjects
CTRL	Chow HFD	10 10
D7	BLEO-IPF HDF-BLEO-IPF	11 11
D21	BLEO-IPF HDF-BLEO-IPF	12 12
D28	BLEO-IPF HDF-BLEO-IPF	12 12
D35	BLEO-IPF HDF-BLEO-IPF	12 12

.02 DUAL ANALYSIS OF PULMONARY FIBROSIS AND EMPHYSEMA IN TWO BLEOMYCIN-INDUCED FIBROTIC MOUSE MODELS

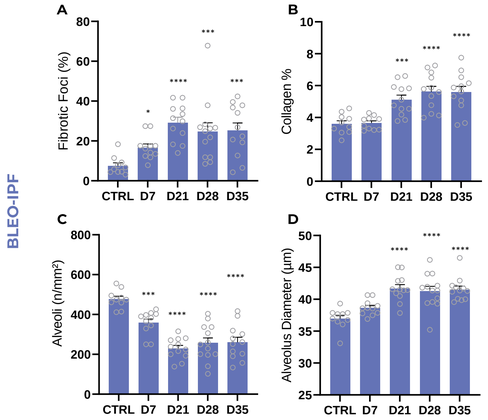

Figure 1. Digital analysis of pulmonary fibrosis and emphysema from PSR-stained sections in BLM-IPF mouse model over time. A. Fibrotic foci. **B.** Collagen. **C.** Number of alveoli. **D.** Alveolus diameter.

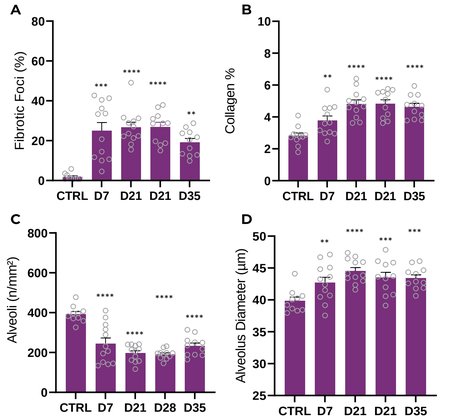

Figure 2. Digital analysis of pulmonary fibrosis and emphysema from PSR-stained sections in HFD-BLM-IPF mouse model over time. A. Fibrotic foci. **B.** Collagen. **C.** Number of alveoli. **D.** Alveolus diameter.

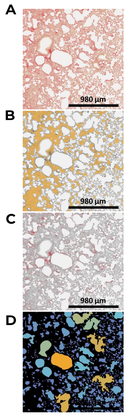

Figure 3. Mapped images. A. Original PSR-stained section. **B.** Fibrotic foci. **C.** Original scan. **D.** Emphysema.

.03 COMPARISON OF PULMONARY FIBROSIS AND EMPHYSEMA INDUCTIONS USING AUTOMATED DIGITAL ANALYSIS

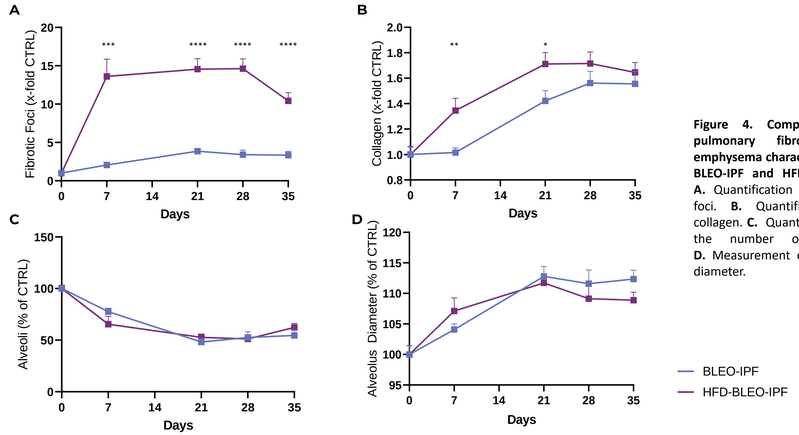

Figure 4. Comparison of pulmonary fibrosis and emphysema characteristics in BLEO-IPF and HFD-BLEO-IPF A. Quantification of fibrotic foci. **B.** Quantification of collagen. **C.** Quantification of the number of alveoli. **D.** Measurement of alveolus diameter.

.04 CORRELATION OF DIGITAL ANALYSIS WITH BIOCHEMISTRY AND SPIROMETRY

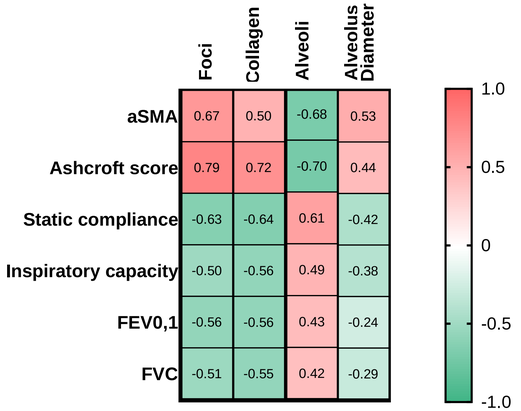

Figure 5. Heatmap of correlations between digital analysis, histopathological Ashcroft score and functional testing. Spearman correlation rates. aSMA: alpha-smooth muscle actin. FEV0.1: Forced expired volume in 0.1 second. FVC: Force vital capacity. Red means strong positive correlation while green means strong negative correlations.

AT A GLANCE

- Combined pulmonary fibrosis and emphysema (CPFE) is a serious condition that is poorly investigated.
- Pulmonary fibrosis is extensively investigated in bleomycin-induced mouse model.
- Little is known in this model regarding its potential to assess CPFE.
- A fully automated image analysis tool (MorphoQuant™) was developed to assess lung remodeling and alveolar damages from a single image.
- The software was used to investigate a BLEO-IPF and a HFD-BLEO-IPF mouse models.
- Both models showed combined emphysema and pulmonary fibrosis characteristics, making them great models for CPFE investigation.