Automated AI-assisted Ashcroft scoring of lung fibrosis in spirometry-confirmed and bleomycin-induced mouse model of IPF

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Background & Aim

Clinically derived Ashcroft scoring of lung fibrosis is commonly applied to preclinical models of idiopathic pulmonary fibrosis (IPF). As for any manual, semiquantitative histopathological scoring system, Ashcroft scoring is prone to inter- and intra-observer variability which influences accuracy and reproducibility of study results. The present study aimed to develop and validate an automated deep learning-assisted digital imaging analysis pipeline, termed GHOST (Gubra Histopathological Objective Scoring Technology) for objective assessment of Ashcroft score in the spirometry-confirmed and bleomycin-induced mouse model of IPF (BLEO-IPF).

Methods

Male C57/BL6 mice received a single intratracheal installation (II) of bleomycin (BLEO, 1.5 mg/kg) or saline. Mice were terminated 14 or 21 days after dosing and the lungs were collected, perfusion-fixed and paraffin embedded. Lung samples were sectioned (4 µm thickness), stained with Masson's trichrome (MT) and digitally scanned. Images were downscaled and split into tiles of 512x512 pixels. An expert histopathologist scored all tiles according to the Ashcroft criteria from grade 0 to 8 (normal lung tissue to total fibrous obliteration). An additional class was used for tiles showing non-alveolar tissue and therefore excluded. AI-assisted pathology was developed in Python 3.7 and trained to reproduce the Ashcroft grading system and then used to assign each tile a score based on a convolutional neural network (CNN). A composite Ashcroft score, expressed as whole-section score, was calculated as average score of all tiles in the individual lung section.

1 Sample collection for Ashcroft GHOST app development

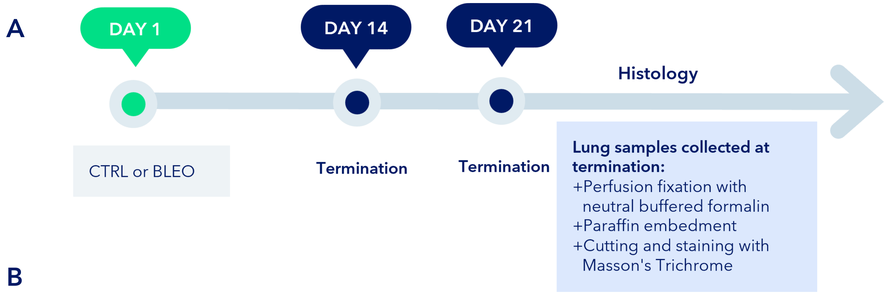

Figure 1. Lung sample collection.
A. Study outline. CTRL: Control (saline) . BLEO: bleomycin (2 mg/kg – 3 U/kg) , II: Intratracheal installation. **B.** Representative images of lung sections stained with Masson's Trichrome from control (saline treated group), at day 14 and day 21 after bleomycin installation. Scalebar=100 µm.

2 Workflow for GHOST development

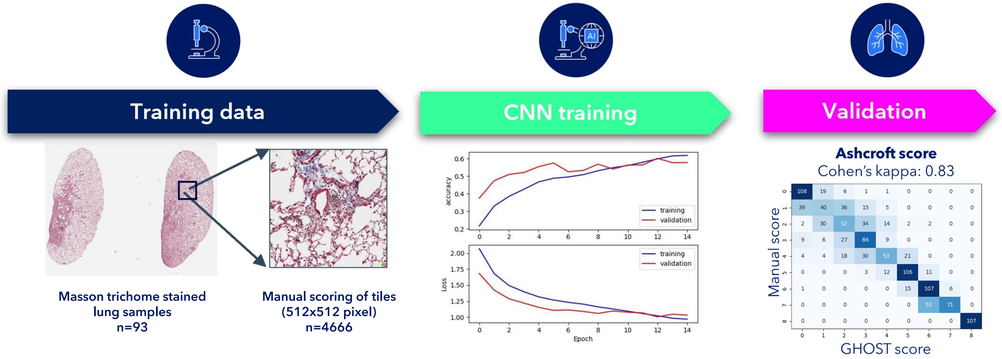

Figure 2. Deep learning-assisted digital imaging analysis pipeline for automated histopathological scoring of lung fibrosis.
The convolutional neural network (CNN) model was trained based on the Inception-v3 network architecture using the Keras library to predict the tile score. The training was performed for 25 epochs, and the accuracy was computed at every iteration. The Adam optimizer was used during training, and data augmentation was applied in the form of rotations, flips, and brightness. The CNN trained model was used to compute the Ashcroft score in lung samples of healthy controls and BLEO-IPF mice using the test set. Ashcroft score was computed and validated using a test set of lung samples from a total of 93 mice. There was a high concordance between manual and automated (GHOST) scoring (kappa value of 0.83).

3 Deep learning-based Ashcroft scoring

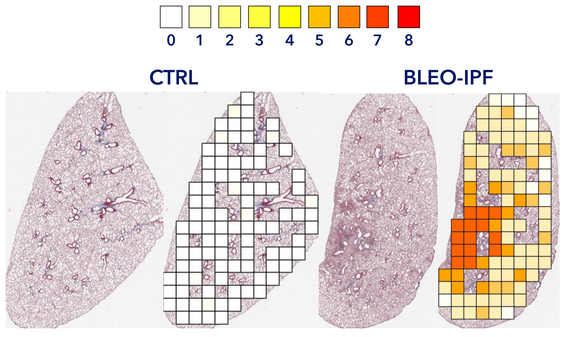

Figure 3. Automated deep learning-based Ashcroft scoring of lung fibrosis in BLEO-IPF mice. Deep learning-based Ashcroft scoring, using Gubra Histopathological Objective Scoring Technology (GHOST), applied to the entire left lung at 10x magnification. Representative Masson's trichome staining used for image analysis. Heatmaps depict Ashcroft scores (score 0-8; i.e., normal lung tissue architecture to total fibrous obliteration) in individual lung image tiles of 512×512 pixels.

4 Disease progression in BLEO-IPF mice using GHOST-based Ashcroft scoring

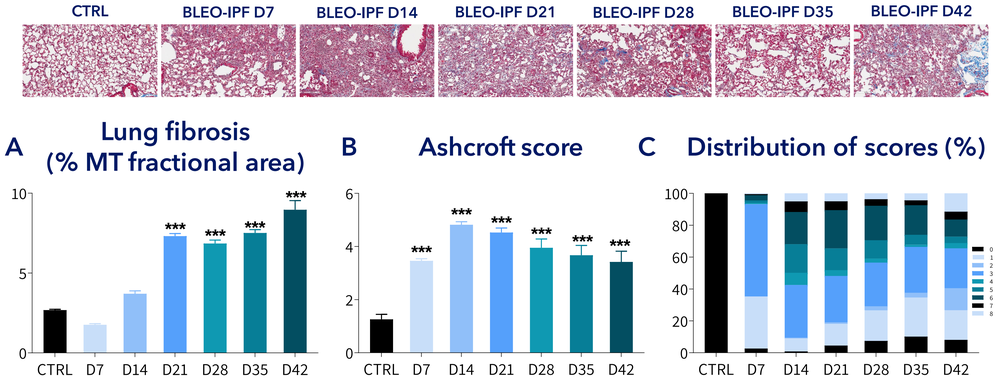

Figure 4. Evaluation of disease progression in BLEO-IPF mice using GHOST-based fibrosis scoring.
Top panel: Representative images of lung sections stained with Masson's Trichrome from control (saline treated group) at day 7,14, 21, 28, 35 and 42 after bleomycin installation. Scalebar=100 µm.
A. Quantitative fibrosis measurement using image analysis **B.** Ashcroft score **C.** Distribution of scores in groups. ***p=0.001.

Conclusion

- GHOST automated Ashcroft scoring of fibrosis demonstrates strong concordance with expert histopathologist evaluations in the BLEO-IPF mouse model
- GHOST provides unbiased, fast, accurate and reproducible Ashcroft scoring
- GHOST offers a robust, AI-based solution for automated Ashcroft scoring in BLEO-IPF mice

Scan the QR code to learn more about Gubra's IPF model