HIGH-THROUGHPUT, AUTOMATED ANALYSIS OF BLOOD VESSEL HISTOMORPHOMETRY IN TWO RAT MODELS OF PULMONARY ARTERIAL HYPERTENSION

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BACKROUND AND OBJECTIVES

Pulmonary arterial hypertension (PAH) is a complex and debilitating disease that causes progressive vasoconstriction and vascular remodeling of the distal pulmonary arteries. Monocrotaline (MCT) and Sugen5416-hypoxia (SuHx) induced PAH models are the most extensively used rat models to investigate the understanding of the pulmonary hypertensive process and assess drug efficacy. However, the visual pathological assessment of PAH is tedious and time-consuming, leading to non-comprehensive conclusions. In the present work, we have developed a digital pathology software to reliably and accurately assess the histomorphometric features of PAH in MCT and SuHx models.

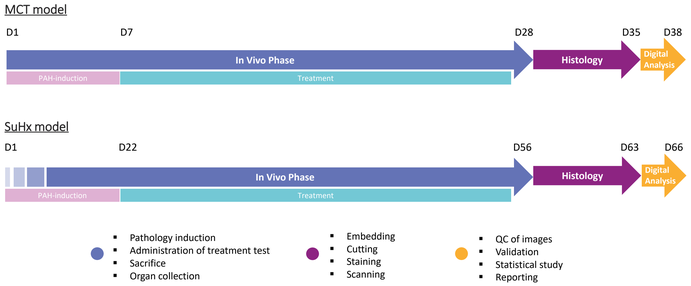
METHODS

Male Sprague Dawley rats were either injected subcutaneously with 60 mg/kg of MCT or 20 mg/kg of Sugen5416 to induce PAH. Rats receiving Sugen5416 were housed in hypoxic chambers for 21 days followed by 35 days in normoxic conditions. Rats receiving MCT were housed in normoxic conditions for 28 days. For each model, three groups were assessed; aged match control, PAH-induced +/- Sildenafil. Transversal histological sections of left lobes were labelled with α-smooth muscle actin (α-SMA) and digitized at 20x. An automated artificial intelligence morphometry-based system (MorphoQuant™) was developed to detect and perform quantitative measurements on muscularized blood vessels (MBV). After automated discarding of bronchi, on 500-1000 MBV, MorphoQuant™ assessed the lung area, α-SMA label, the number of total and obstructed MBV and their proportions, as well as the intima, media and wall thicknesses of MBV, per size category small (S), medium (M) and large (L) MBV (< 50; 50-100; 100-200 µm, respectively).

CONCLUSIONS

We have developed a digital histopathology software enabling the comprehensive assessment of MBV features to support the understanding of PAH physiopathology and reliable assessment of drug efficacy, applicable to the two more extensively used rat models.

.01 STUDY DESIGN AND TIMELINE



Group Name	Animal Model	Number of Subjects
MCT		
Sham	Control	5
MCT	MCT + Vehicle	12
Sild	MCT + Sildenafil 50 mg/kg BID	9
SuHx		
Nx	Control in normoxia condition	8
SuHx	SuHx + Vehicle	9
Sild	SuHx + Sildenafil 50 mg/kg BID	8

.02 PULMONARY AND CARDIAC FUNCTIONAL OUTPUTS

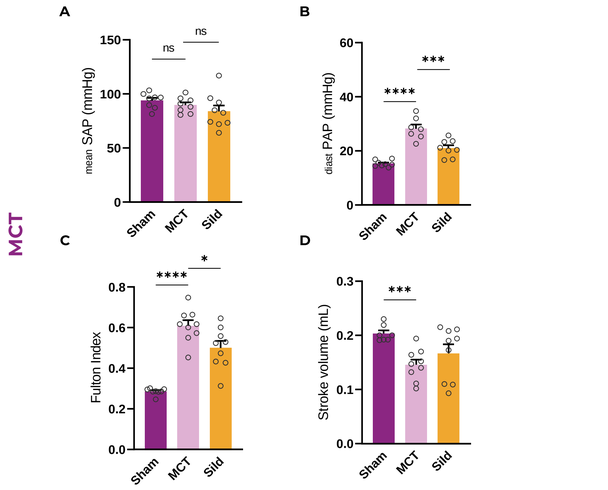


Figure 1. Pulmonary and cardiac functional outputs of MCT-induced PAH rats.
A. Mean systemic arterial pressure. **B.** Diastolic pulmonary arterial pressure. **C.** Fulton index. **D.** Stroke volume.

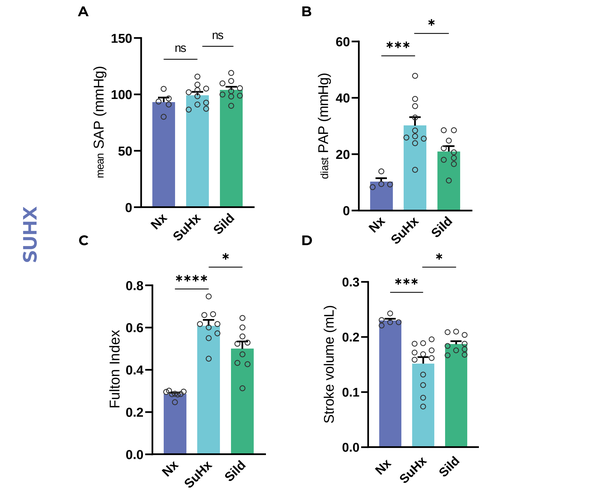


Figure 2. Pulmonary and cardiac functional outputs of SuHx-induced PAH rats.
A. Mean systemic arterial pressure. **B.** Diastolic pulmonary arterial pressure. **C.** Fulton index. **D.** Stroke volume.

.03 AUTOMATED HISTOMORPHOMETRIC ANALYSIS OF MUSCULARIZED BLOOD VESSELS

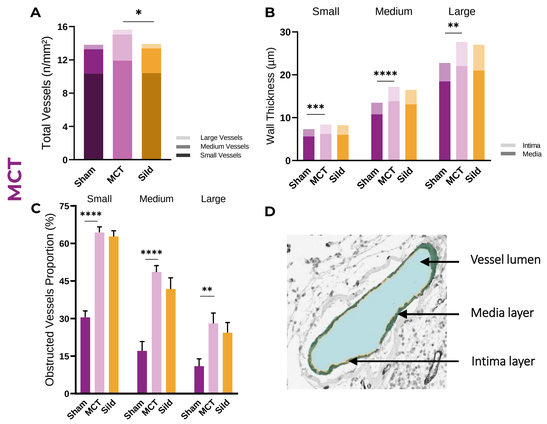


Figure 3. Automated histomorphometric analysis of muscularized blood vessels in MCT rat model. A. Total vessels. **B.** Wall thickness. **C.** Obstructed vessels proportion **D.** MorphoQuant recognition of large vessels.

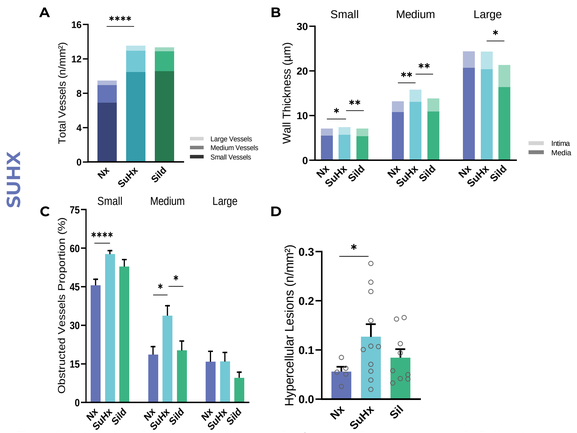


Figure 4. Automated histomorphometric analysis of muscularized blood vessels in SuHx rat model. A. Total vessels. **B.** Wall thickness. **C.** Obstructed vessels Proportion **D.** Hypercellular lesions.

.04 COMPARISON BETWEEN FUNCTIONAL AND HISTOLOGICAL DATA

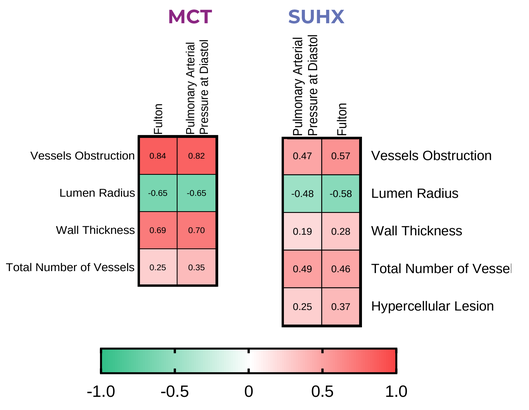


Figure 5. Heatmap of correlations between digital analysis and functional testing for MCT (right) and SuHx (left) rat models. Spearman correlation rates. Red means strong positive correlation while green means strong negative correlations.

AT A GLANCE

- PAH is a complex and debilitating disease.
- MCT and SuHx are the most extensively rat models used to investigate PAH.
- Visual histopathological assessment of PAH is tedious and time-consuming, leading to non-comprehensive conclusions.
- We have developed a fully automated image analysis tool (MorphoQuant™) enabling the comprehensive assessment of features of muscularized blood vessel, including intima, media and wall thicknesses, leading to a better understanding of PAH physiopathology, applicable to the two most extensively used rat models.