Location-specific pathology analysis of monopodial airways

In a rabbit model of bronchopulmonary dysplasia

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Summary / Abstract

Comparing equal structures between groups

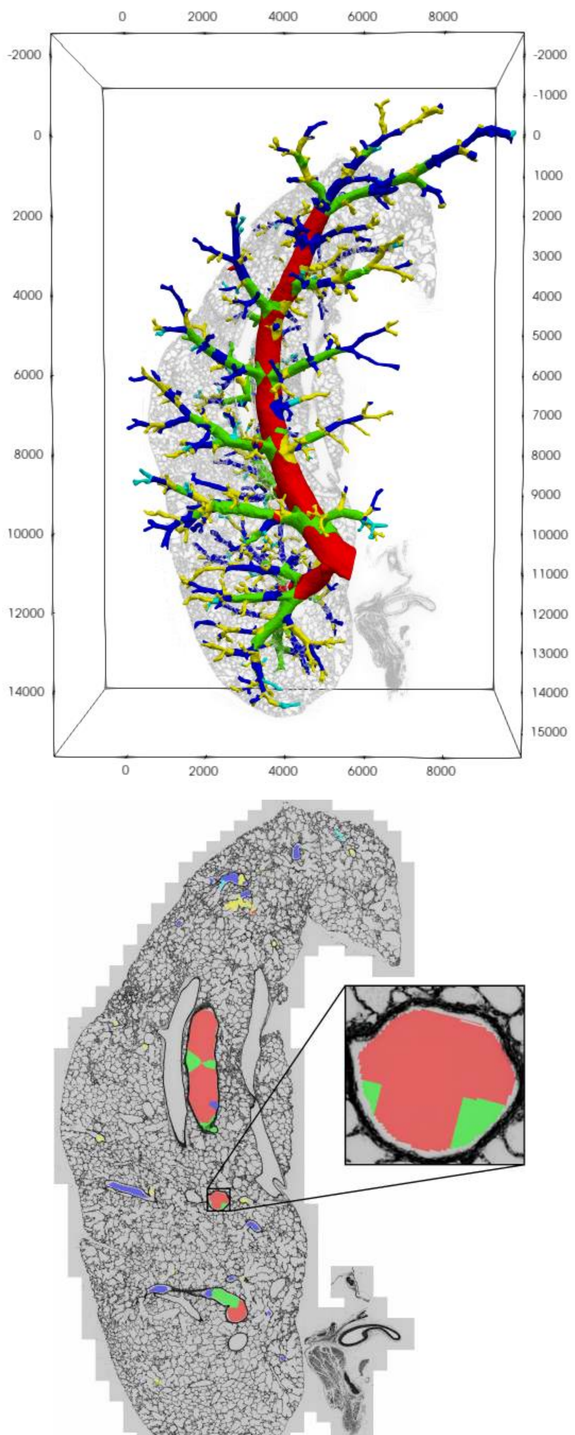
The pre-alveolar airways of the mammalian lung form a tree-like structure, that is composed of heterogeneous sub-structures or compartments. They vary in morphological characteristics such as composition of airway epithelium, presence of cartilage plates, and number of smooth muscle cell layers or lumen diameter. These compartments may vary in their reaction to different pathological stimuli. Thus, when studying a particular lung disease, compartments need to be investigated individually and not as part of a more global portmanteau compartment. In common lab animals, the airway exhibits a highly asymmetric branching pattern, that can not be subdivided in a meaningful way by common approaches like generations or orders. Therefore, a morphological clustering approach was tested for its suitability of dividing an airway into biologically meaningful sub-compartments. On this basis, an investigation of the distribution of pulmonary airway changes in a rabbit model of bronchopulmonary dysplasia (BPD) was conducted. It proved to be capable of creating meaningful airway compartments. This way, the distribution of differences between disease model and control group, that would not have been visible in a purely global comparison of morphological characteristics, could be identified.

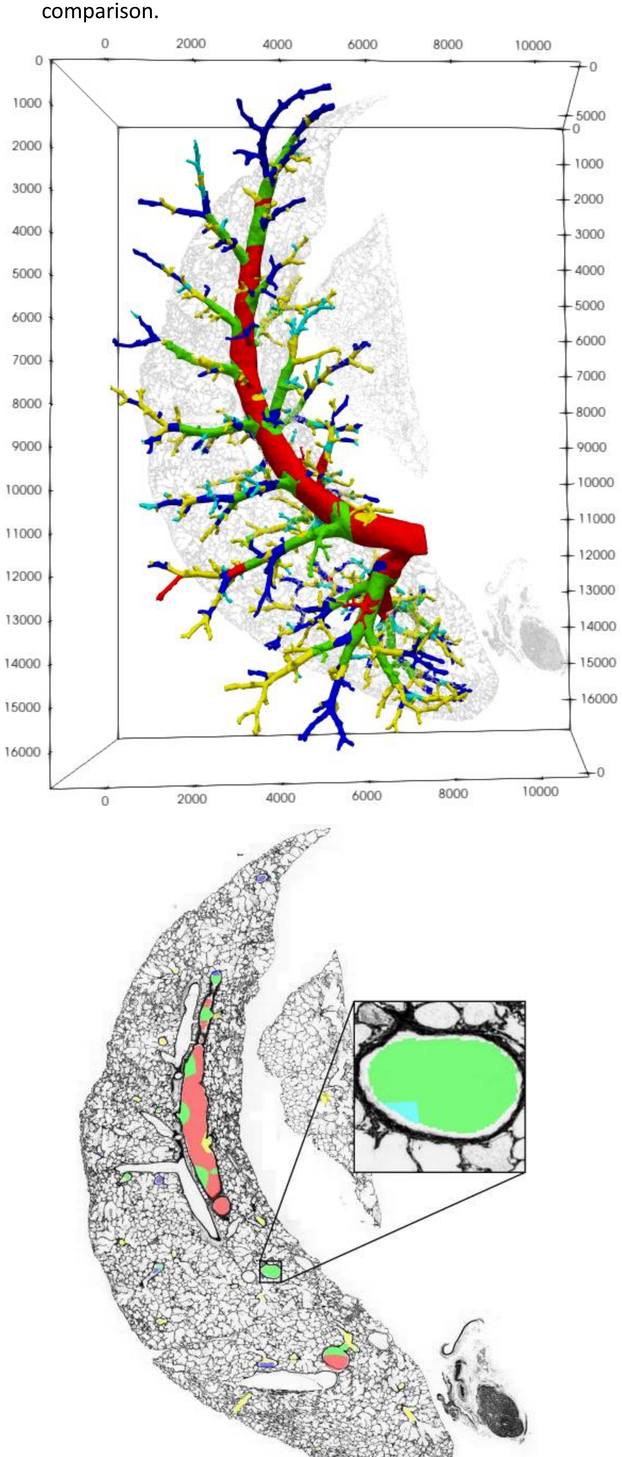
Introduction / Background

A basis for the comparison of monopodial lungs

The airways of non primate lungs branch in an asymmetrical pattern. The degrees of asymmetry differ between species. Common lab animals such as rats or rabbits exhibit a highly asymmetric monopodial branching, characterized by one long central airway with smaller lateral branches.

In contrast to the situation in the relatively symmetrical structure of the human lung, the use of generations is unable to group this airway structure into homogeneous subgroups. To address this, we used the approach of morphology based clustering, developed by Labode et al. 2022, as a basis for a location-specific comparison.





NOX Lung, Scale bar = 1 mm

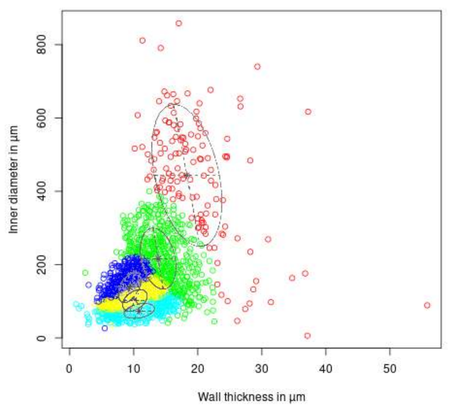
HYX Lung, Scale bar = 1 mm

Material & Methods

Finding clusters of vessels

For the grouping, the conductive airway tree was segmented in a µCT scan of each lung. Every section (segment between two bifurcations) was marked with an ID. These were then color coded and superimposed on corresponding high resolution LM sections for morphological feature assessment**.**

The measurements taken were lumen diameter and airway wall thickness. To identify homogenous clusters of airways, a Gaussian Mixture Model was employed on them. It identifies Gaussian distributions within datasets, thus dividing it into homogenous groups.

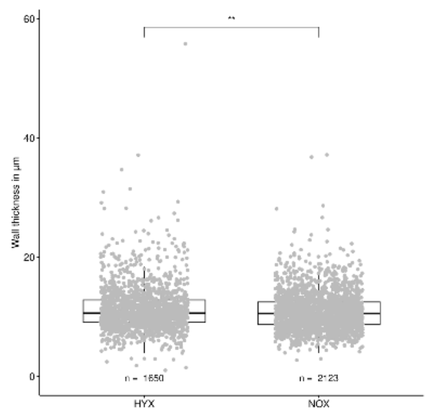


Cluster identification by looking for Gaussian distributions

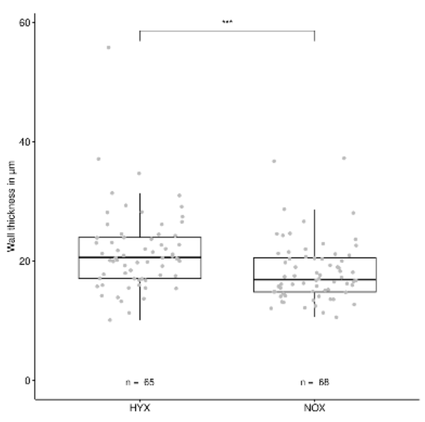
Results

Pinpointing and uncovering differences

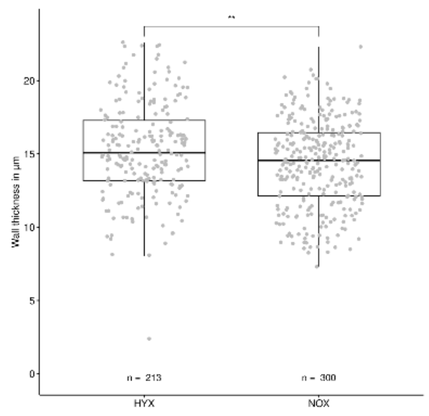
The samples from the BPD model contain a normoxic (NOX, 21% O_2) and a hyperoxic (HYX, ≥95% O_2) group (n = 2 each). When a global comparison of the airway wall thickness is performed, a significant difference can be identified, with the HYX group exhibiting a higher wall thickness overall. Once the cluster based analysis is applied, a higher data resolution is reached. With it, the significant differences can be pinpointed to the first three clusters. In the fourth cluster, this difference has disappeared, while in the fifth cluster it has flipped.



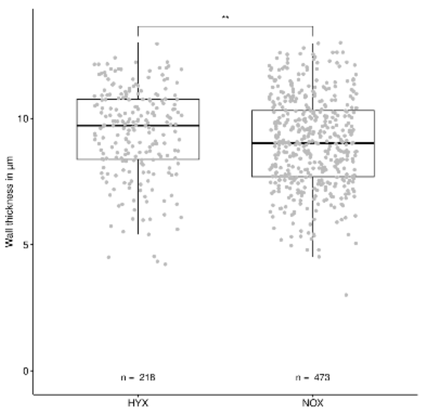
Global comparison of all airway sections



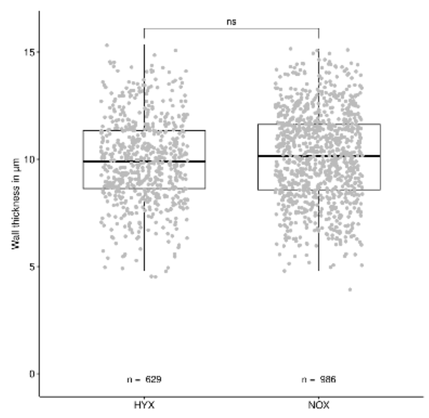
1. Red cluster: Large airways



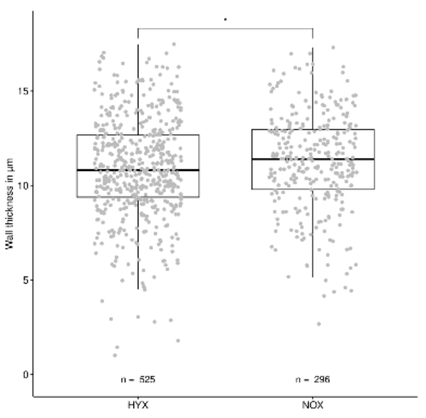
2. Green cluster: Medium airways



3. Blue cluster: Small airways, large diameter & thin walls



4. Yellow cluster: Small airways, medium diameter & medium walls



5. Cyan cluster: Small airways, small diameter & thick walls

Discussion

Workflow design and future usage scenarios

This study showed that the employed clustering model is applicable to study the effect of a pulmonary disease on different airway sub-compartments. It concludes a series of investigations in monopodial lung branching. First, a basic comparison of grouping methods and the identification of the most applicable one for monopodial structures was performed. Second, a pilot study employing that method to the pulmonary vasculature in a BPD disease model followed. Now, its application to the conducting airways in the same disease context has been tested. Its suitability for the analysis task on hand could be proven. Over these investigations, computational tools and workflows were developed and optimized. For the future, we now plan its usage in full scale comparative disease studies, where sample groups of representative size will be compared to uncover both the manifestation of a disease in the lung, as well as the effects of treatments.

While the basic clustering approach is a sound mathematical concept with little room for improvement, the larger workflow can still profit from future developments. Deep learning based image segmentation of blood vessels and airways would be an improvement over current threshold based approaches that necessitate manual involvement. The same applies to the division of the airway tree into conductive- and gas exchange regions. These developments have come within reach in the last years and would allow the analysis of larger sample sizes with manageable manual involvement.

References

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2 . Labode, J.; Dullin, C.; Wagner, W. L.; Myti, D.; Morty, R. E. & Mühlfeld, C. Evaluation of classifications of the monopodial bronchopulmonary vasculature using clustering methods, Histochemistry and Cell Biology, 2022 DOI: 10.1007/s00418-022-02116-x

Disclosure of Conflicts of Interest

☒ I have no financial or non-financial conflicts of interest to declare

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