Commentaries on Viewpoint: Standards for quantitative assessment of lung structure

INSTALLATION FIXATION AND OVERINFLATION OF THE MOUSE LUNG

TO THE EDITOR: We read with interest the ATS/ERS Official Research Policy Statement on the “Standards for Quantitative Assessment of Lung Structure” (2). This is an issue that has long been discussed but until now has not been formally addressed, and we support the recommendation of Mitzner and Weibel. Importantly, it is recognized that this document is a guide and it is up to the researchers to choose the appropriate metric for their study/circumstance. We would, however, like to point out the following issue that should be considered.

The use of instalation fixation is one of the most common means of fixation, particularly in studies using mouse models of disease. The well known shift in the P-V curve in liquid-filled compared to air-filled lungs raises some concerns regarding the recommended 20–25 cmH₂O instillation pressures. No study has addressed how the final liquid-filled fixed lung reflects the in vivo air-filled situation. Indeed, the known P-V relationship in the liquid filled lung (1) would suggest that instillation fixation at these pressures, in mice at least (3), would expand the lung well beyond its normal physiological range. Additionally, given that most functional measures are made close to FRC, or at least in the tidal breathing range, the range. Additionally, given that most functional measures are made close to FRC, or at least in the tidal breathing range, the value of only examining the structure of the lung at such high volumes is questionable, particularly if these structural measurements are to be related back to functional outcomes.

REFERENCES

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AIR SPACE CONNECTIVITY

TO THE EDITOR: The present Viewpoint (2) is from the functional side of the structure-function relationship. We contend that a meaningful interpretation of correlations between in situ measurements of selected lung samples (e.g., by biopsy or imaging) and tests of airway function (e.g., by gas sampling at the mouth or imaging) crucially depends on the connectivity of the measured air spaces to the rest of the lung. The amount of detail or the lung depth at which connectivity needs to be characterized depends on the physiological test and the species under study, as illustrated by the following two examples. It was only via a quantification of the particular arrangement of acinar units along the entire rat bronchial tree that it was possible to explain the greater He than SF₆ phase III slope observed in rat lungs (ref1); in this case intra-acinar branching asymmetry in fact played a negligible role. By contrast, in the human lung it is intra-acinar connectivity of air spaces that has been pivotal to obtain realistic simulations of greater SF₆ than He phase III slopes in humans (1) and which has enabled the simulation of the apparent diffusion coefficient as obtained by MRI (3). With the generalized recognition that lung function is crucially affected by structural heterogeneity, and even more so in diseased lungs, we would like to emphasize that mapping and reporting the connectivity of affected and unaffected air spaces to the rest of the lung is at least as important as the morphometry of carefully selected airway samples.

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TO THE EDITOR: We wish to emphasize that there is a need to understand alveolar structure under dynamic conditions. In this regard, our novel method for real-time quantification of alveolar structure using laser-scanning, optical-sectioning microscopy (LSOSM) (2, 3) requires consideration. LSOSM combines the subcellular resolution of light microscopy with optical sectioning, thereby affording dimensional analyses at a spatial resolution at least an order of magnitude greater than that of other in situ imaging modalities such as optical coherence tomography and magnetic resonance imaging. Optical sectioning coupled with the use of intracellular fluorescein provides accuracy of structural determinations, improving on traditional bright-field, wide-angle microscopy that relies on light reflections from the mobile air-liquid interface. As different from fixed tissue histology, LSOSM enables studies of single alveoli at multiple depths and at different transpulmonary pressures, providing a basis for dynamic 3-D interpretations. Our application of these methods to determine compliance of individual alveolar septa revealed the novel finding that lung inflation causes nonuniform alveolar expansion, with type 2 cells protected from excessive stretch (2). We also found that edema shrinks an alveolus while stretching its air-filled neighbor (3). With the unique ability to make repeat, high-precision measurements of the same structures under varying conditions, these studies shed new light on alveolar structural responses likely to be relevant to mechanical ventilation of patients with lung injury. Furthermore, LSOSM potentially provides a means for combining structural studies of the alveolus with studies of second messenger signaling (1) to enable novel under-
standing of structure-function coupling in alveolar mechanobiology.

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QUANTIFYING LUNG MICROSTRUCTURE BY MRI WITH HYPERPOLARIZED GASES

TO THE EDITOR: The Viewpoint by Mitzner and Weibel (2) draws attention to a document defining Standards for the Quantitative Assessment of Lung Structure; it focuses mostly on direct stereological methods for quantitative assessment of lung structure in microscopic studies where serious problems occur, particularly because of the very small samples that can be measured. It is also important to address “standardization” issues when quantifying lung microstructure by MRI; the problems are in part different but just as critical and are only briefly addressed in the present document.

Diffusion MRI with hyperpolarized gases has demonstrated a great potential to identifying changes in lung microstructure related to lung diseases. Substantial increases in 3He gas apparent diffusion coefficient (ADC) in lung airspaces have been reported in emphysema (1, 4, 5), suggesting use of ADC as a biomarker for disease progression. However, ADC is not a universal factor (like Lm) and depends on the details of MRI protocol that are not always described in scientific publications, making it impossible comparing data from different laboratories. Importantly, relationships between ADC and lung microstructure parameters (e.g., Lm) are not unambiguous.

MRI-based 3He lung morphometry technique (6) avoids these problems, providing quantitative information on the value and spatial distribution of lung parenchyma surface-to-volume ratio, alveolar density, and Lm—parameters traditionally used by lung physiologists. Moreover, it provides information on acinar airways radii that are exceedingly difficult to measure by conventional techniques (3).

Establishing guidelines for studying lung with hyperpolarized gases, and for the correlation with microscopic methods, would be of great value.

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TO THE EDITOR: The 2010 Official Research Policy Statement on quantitative assessment of lung structure (1) is a timely, extensive response to the fact that many investigators are performing and publishing studies based on inappropriate analysis. There is agreement that no single tissue fixation method can be declared a “Gold Standard” and that uniform guidelines and recommendations were sorely needed to avoid bias and promote consistency. In the Viewpoint (4), Mitzner and Weibel summarize and emphasize the importance of specimen fixation, tissue sampling, lung inflation, rigorous study design, and choice of analytical methodology to promote standard, uniform preparation, and proper comparisons among experimental groups and different laboratories while recognizing the variations in experimental situations and prerogatives of investigators. Likewise, Matthay (3) and Hsia et al. (2) acknowledge quantitative methods need to be accurate, reasonably efficient, have adequate statistical power, and sources of bias are identified and minimized. Although stereology of the lung has been used for over 50 years and requires no sophisticated equipment, methodological standards have not been adopted. The guiding principles outlined in Policy Statement (1) foster stereological sampling and morphometric analysis of structural data acquired by various multidimensional imaging modalities, such as CT, MRI, SPECT, and PET, allowing for more complex and efficient study design and facilitating new approaches for existing techniques (5). The Policy Statement (1), along with a general awareness and adoption of the principles, will propitiously move quantitative assessment of lung structure and the subsequent published data toward standardization and more accurate interpretation.

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