Use of mean airspace chord length to assess emphysema

Wayne Mitzner
Johns Hopkins University, Baltimore, Maryland

There is currently a great deal of interest in mechanisms underlying COPD. Although COPD is not a term commonly applied to mouse models, in mice there has been an increasing number of studies in recent years on one of the hallmarks of COPD, i.e., emphysema. Emphysema is characterized by destruction of alveolar walls, with concomitant loss of alveolar surface area, enlargement of airspaces, and increased lung volume and compliance. To assess the extent of emphysema, nearly all studies that have attempted to quantify histologic sections have used the mean linear intercept (MLI) or mean airspace chord length (Lm). The MLI and Lm differ by the mean thickness of the alveolar septa, but both are inversely proportional to the parenchymal surface/volume ratio (3). Both of these means are easy to measure from digital images, but measurement of individual airspace chords has the advantage of being able to provide statistics other than the mean (6, 8, 13). Although there often is a seductive intuition that has led many investigators (present author included) to implicitly and explicitly equate Lm with alveolar diameter, this is at best only a visual approximation, since the measurement chords traverse a complex structure of alveoli and ducts. This incorrect intuition is often compounded by commercial image analysis programs that explicitly generate ill-defined and largely uninterpretable measures of things like alveolar diameter or even their minimal and maximal values (2, 10). In any event, given the fact that the use of Lm (or MLI) has been and continues to be so ubiquitous in investigative research, it seems in stark contrast to the statement recently made in a paper in this journal that the method of Lm measurement is so fraught with danger that it should be abandoned (17). If Lm is indeed so bad that it should be discarded, then what can researchers use to quantify the pathology? The alternative is that, with proper precautions, perhaps Lm is not that bad, and it is this perspective that I will support in the remainder of this essay.

One critical concern with the use of Lm by itself to assess emphysema is that Lm is a function of lung volume (5, 18). So if there are any changes in lung elasticity that result in changes in lung volume, as would be expected with emphysema, Lm will not be able to separate the effects on tissue destruction from those on tissue distension. This fact makes it essential to measure the lung volume (V) at which the Lm is being measured, and without such a volume measurement, Lm cannot be properly interpreted. Fortunately, in mice, with proper precautions, it is relatively straightforward to measure fixed lung volume using water immersion (14), and with this reference volume, one can then get a measure of the parenchymal internal surface area (S). The quantitative link between Lm and S is independent of the shape of the airspaces, and is given by the formula, \[ S = 4V/Lm \] (3, 16). Thus, if one knows V and Lm, then changes in Lm can be linked to changes in alveolar surface area, which is more closely related to the extent of tissue destruction. Despite the ease of making this surface area measurement, it unfortunately is not routinely obtained. This may reflect the fact that histologically, very severe emphysema can be easily detected visually (7), and large increases in Lm surely can quantify such changes even when some of the increase simply reflects a larger lung volume. At the other extreme, if one observes no increase in Lm (1), one can cautiously conclude that there is little airspace destruction, as long as there are no substantive changes in lung volume. This issue of interpreting Lm changes becomes increasingly more critical as the degree of emphysema lessens, and without a measure of volume, one does not know at what level of change incorrect interpretations may begin. Furthermore, given the recently documented anisotropy and heterogeneity of mouse lung parenchyma (11), it is unlikely that a change in Lm of less than ~5 \( \mu m \) could ever be reliably detected. This anisotropy also may lessen the value of unbiased sampling, since if the pathology is not randomly located then random selection of sites to analyze may not be the best way to detect the pathology.

Another issue with Lm is how many chords should be counted. Although it is often recommended that only 100–200 chords per mouse are sufficient (4, 17), the existing anisotropy in normal mice and increasing heterogeneity in emphysematous mice make this a highly inadequate number. Without using proper uniform sampling procedures, selecting as few as 100 airspace chords for measurement (15) may introduce substantial bias into the Lm measurement. It was recently shown that a safer approach would be to sample every alveolus in each of the sections, with a total number of chords per lung in the range of 10–15,000 (11). An additional reason to sample at least this many chords is that one could then analyze different metrics from the chord length distribution. Such advanced statistical analyses may aid in the early detection of disease, but these alternatives have not yet been evaluated. Furthermore, the chord length distribution (and any metric based on it) or any alternative analytic strategies that amplify the effect of large airspaces by quantifying areas (12), must also take into consideration the potential effects of changes in the reference lung volume and other sampling biases.

In summary, it seems that measurement of Lm and parenchymal surface area will continue to be convenient and useful measurements to examine histologic changes in lung parenchyma. Another advantage of Lm is that it is directly comparable to the in vivo measurements of the ADC (apparent
diffusion coefficient of hyperpolarized $^3$He or $^{129}$Xe), which effectively measures a mean free path (analogous to chord length) in the airspaces of the acini (9). In using these measures, however, investigators need to remain aware of the potential limitations. First, it is essential that the lung volume must be measured—without this measurement, $L_m$ (or ADC) by itself may provide misleading information. Second, how many chords need to be measured for $L_m$ will depend on the pathology being studied, but since it is so easy to do with digital images, it seems quite reasonable to sample large parenchymal areas with high-resolution images to obtain relatively large numbers of chords that may be analyzed later for distributional statistics. Third, if one is not using uniform random sampling of tissue in all lobes, then one must carefully describe where the tissue blocks come from and how the sections for analysis are selected. It is worth noting that in the hundreds of published studies where $L_m$ has been measured, there is very rarely a clear description of how and where the tissue samples and “random” sections were chosen for analysis. This is another good reason to measure much more than 100–200 chords. If one effectively samples all the alveoli in a particular section, any possible selection bias in that section is thereby eliminated. Using restricted tissue sampling with consistent sectioning from every mouse (e.g., sections transverse to the cranial-caudal axis of the left lung) may be acceptable, as long as one remains cognizant of the fact that if there are gross lobar heterogeneities, the pathology might be missed entirely. Until we have additional knowledge about the nature of the heterogeneity and anisotropy in normal and pathologic lungs, more rigorous isotropic uniform random sampling may be required. Thus it would seem that for the present time, as long as lung volume is accurately measured, $L_m$ and more importantly, parenchymal surface area, should provide quite adequate measurements to use for an initial assessment of airspace enlargement and tissue destruction in animal models of emphysema.

REFERENCES