Quantifying airway remodelling for research or clinical purposes: How should we normalize for airway size?

The perimeter of the basement membrane (P_{bm}), as seen on whole cross-sections of airways, has become a standard index of airway size since it is independent of the effects of lung inflation, bronchoconstriction and the presence of asthma.\textsuperscript{1,2} This has allowed the comparison of airway wall dimensions, especially airway smooth muscle (ASM), between airways of different size within individuals (human or animal) and airways of the same size between individuals with and without disease. However, the relationship between ASM (or gross airway wall area) and P_{bm} is not necessarily a simple one and the question arises - how should we normalize measures of airway wall components with respect to airway size (as measured by P_{bm})?

The above question has far-reaching implications for both basic science and respiratory medicine. ‘Over-normalizing’ might reduce the apparent differences in the large airways between experimental groups or clinical cases, relative to the small airways. Conversely, ‘under-normalizing’ may introduce the opposite effect. These errors in turn affect conclusions drawn from morphological data such as, in an animal model, does a particular allergen exposure produce ASM remodelling in both small and large airways? Does a patient with asthma, who may be short or tall, with an airway diameter that will scale accordingly, exhibit clinically significant ASM remodelling? Since sampled or diagnostically examined airways will always vary in size (between and within an organism), the correct form of normalization is therefore necessary. This issue of normalization is separate from any concerns that the length of an apparently indistinguishable membrane may not be constant with respect to fixation procedures or other factors.\textsuperscript{1,3} As clearly stated by Chin et al.,\textsuperscript{4} regarding measurements of ASM, ‘(the results) could have been confounded if we were comparing airways of different size since the ratio of airway wall area/P_{bm} increases as airways get smaller’.

The uncertain relationship between areas of wall components and P_{bm} has long been acknowledged and many authors prefer to assume that ASM varies as P_{bm}^2, thus normalizing as ASM/P_{bm}^2 (or equivalently √(ASM/P_{bm})).\textsuperscript{4,5} This has the added advantage that the normalized quantity is dimensionless. Both this approach, and a simple ASM/P_{bm} ratio, can be thought of as special cases of assuming that ASM is related to P_{bm}^a by a so-called power law, where ASM ∝ P_{bm}^a for some constant ‘a’. The simple ratio ASM/P_{bm} is equivalent to taking a = 1, while the use of ASM/P_{bm}^2 (or √(ASM/P_{bm})) is to assume that a = 2. Both ASM and gross wall area do follow a power law to a large degree\textsuperscript{6,7} (Figure 1). Recall that the usual approach to visualizing a power law is to use logarithmic axes (Figure 1B), in which case the power law relationship becomes a straight line with slope ‘a’ (the power law exponent).

However, the power law exponent appears not to be constant, either with respect to airway development\textsuperscript{8} or disease. Take ASM for example, which has a power law exponent slightly above 1.0 just before birth, rising throughout early childhood to a value of approximately 1.8 in a non-asthma adult population; the exponent in fatal asthma is higher still, approaching 2 (Figure 1C). A similar trend occurs for gross wall area, though the exponent values are not identical.

Healthy adult power-law exponents are relatively close to 2 (though not exactly), providing good support for the use of ASM/P_{bm}^2 as the conventional normalization approach. Given the choice between ASM/P_{bm} and ASM/P_{bm}^2, the latter is the better option in almost all situations (pre-natal and very early childhood being the exceptions in this dataset). However, it is worth noting that the normalization is imperfect: in most cases, ASM/P_{bm}^2 will overcorrect slightly, meaning that a sample skewed towards larger airways would be biased lower.

Key points

- Measurements of airway wall dimensions are normalized to perimeter of basement membrane (P_{bm}) that is, airway size.
- The relationship between wall area and P_{bm} varies with disease and age.
- Consideration of the above issues of normalization to airway size is important as we head towards quantification of airway smooth muscle in patients using polarization-optical coherence tomography.

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With the problem now apparent, the solution is less definitive. Assuming an exponent of 1.8 might be closer in some situations, but still biased in others. In some cases, a more rigorous approach to fit the power law parameters directly$^8$ may be justified, particularly since factors specific to a certain experimental design or clinical scenario will alter the power law exponent. These include, but are not limited to, age, disease and choice of animal model. At a minimum, when using $ASM/P_{bm}^2$ (or $\sqrt{ASM/P_{bm}}$) one must be aware that there will be some over-correction and that the composition of airway sizes in the sample influences the results.

The question of how best to normalize airway measurements, and accompanying analysis, is far from a scientific niche. The need for direct and accurate measurement of $ASM$ dimensions has been proposed to expand treatment of asthma,\textsuperscript{9,10} optimize current approaches\textsuperscript{11,12} and to add an additional dimension to patient phenotyping.\textsuperscript{13} Specifically, newer in vivo approaches such as polarization-sensitive optical coherence tomography are being developed for identifying and mapping $ASM$ remodelling,\textsuperscript{14} and will require an effective normalization method for airway size.

**KEYWORDS**
asthma, pathology, respiratory structure and function

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**CONFLICT OF INTEREST**
None declared.
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