Received: 21 December 2022 | Accepted: 8 January 2023

DOI: 10.1111/resp.14454

COMMENTARY

Official Journal of the Asian Pacific Society of Respirology Respirology APSR WILEY

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.[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 indistensible membrane may not be constant with respect to fixation procedures or other factors.[1,3] As clearly stated by Chin et al.,[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 $\sqrt{ASM}/P_{bm}$).[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 $ASM \propto P_{bm}^a$ 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 $\sqrt{ASM}/P_{bm}$) is to assume that $a = 2$. Both ASM and gross wall area do follow a power law to a large degree[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).

> **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.

However, the power law exponent appears not to be constant, either with respect to airway development[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.

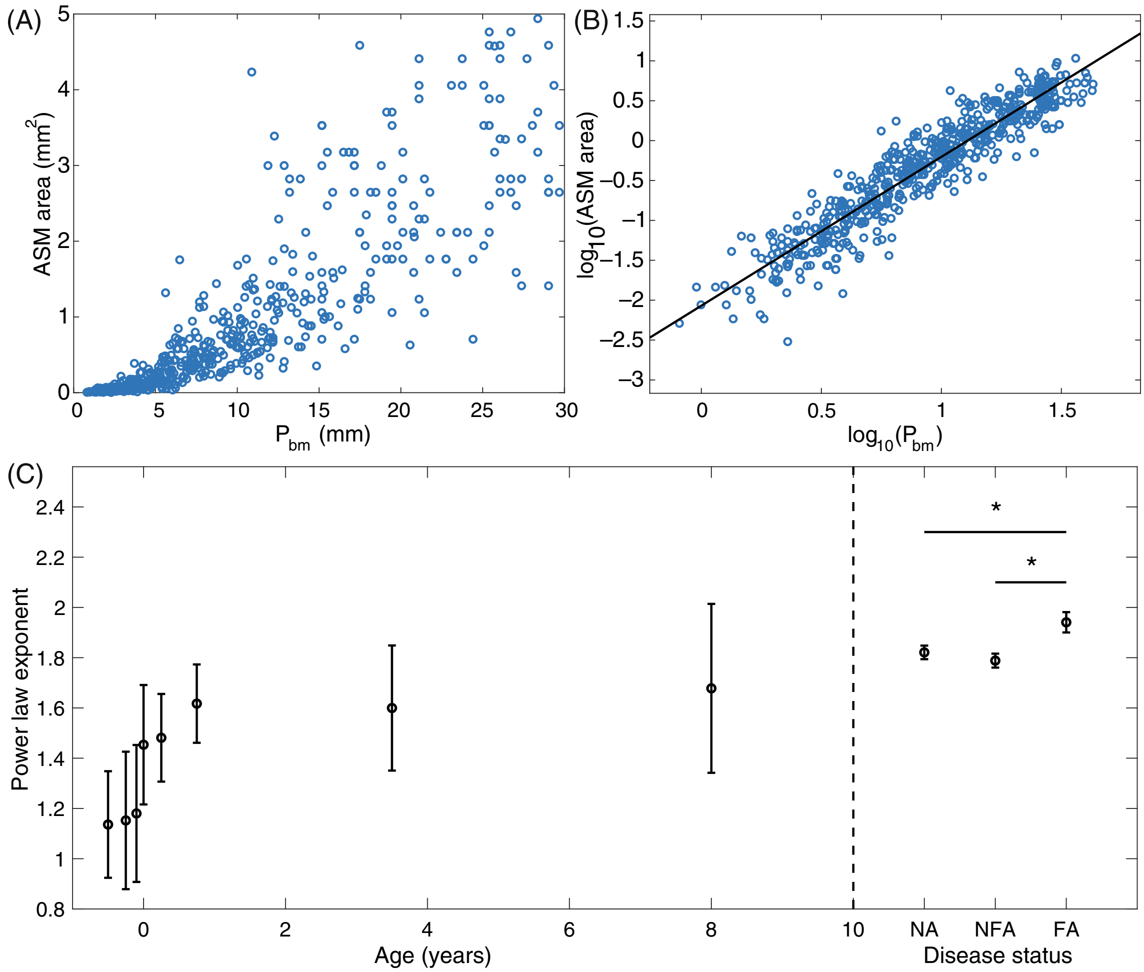

FIGURE 1 (A,B) Airway data relating ASM area and P_{bm} for non-fatal asthma[6] and power law fit, on a linear scale (A) and the same data on a log–log scale (B) with a linear regression illustrating the power-law exponent. (C) Evolution of ASM power law exponent. Age-stratified ontogeny cases from Reference 8 and disease stratified data, shown after the axis break, from Reference 6. Error bars show standard error. Age-differentiated cases, before the axis break: one-way ANOVA $p = 0.013$; disease stratification differences by two-sample t-test, '*' indicates $p < 0.05$ after Bonferroni correction. ASM, airway smooth muscle; FA, fatal asthma; NA, no asthma; NFA, non-fatal asthma; P_{bm}, perimeter of the basement membrane.

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,[9,10] optimize current approaches[11,12] and to add an additional dimension to patient phenotyping.[13] Specifically, newer in vivo approaches such as polarization-sensitive optical coherence tomography are being developed for identifying and mapping ASM remodelling,[14] and will require an effective normalization method for airway size.

KEYWORDS

asthma, pathology, respiratory structure and function

ACKNOWLEDGEMENT

Open access publishing facilitated by The University of Western Australia, as part of the Wiley - The University of Melbourne agreement via the Council of Australian University Librarians.

CONFLICT OF INTEREST

None declared.

Graham M. Donovan PhD[1]
Kimberley C. W. Wang PhD[2,3]
John G. Elliot MSc[3,4]
Alan L. James MBBS, FRACP, MD[4,5]
Peter B. Noble PhD[3]

[1]*Department of Mathematics, University of Auckland, Auckland, New Zealand*
[2]*Telethon Kids Institute, The University of Western Australia, Nedlands, Western Australia, Australia*
[3]*School of Human Sciences, The University of Western Australia, Crawley, Western Australia, Australia*
[4]*Department of Pulmonary Physiology and Sleep Medicine, West Australian Sleep Disorders Research Institute, Sir Charles Gairdner Hospital, Nedlands, Western Australia, Australia*
[5]*Medical School, The University of Western Australia, Nedlands, Western Australia, Australia*

Correspondence
Peter B. Noble
Email: peter.noble@uwa.edu.au

ORCID

Graham M. Donovan https://orcid.org/0000-0001-5903-5484
Kimberley C. W. Wang https://orcid.org/0000-0002-8604-5909
John G. Elliot https://orcid.org/0000-0002-4899-2948
Alan L. James https://orcid.org/0000-0002-6018-0547
Peter B. Noble https://orcid.org/0000-0001-9028-7751

TWITTER

Kimberley C. W. Wang *@DrKimberleyWang*

REFERENCES

1. James AL, Green FH, Abramson MJ, Bai TR, Dolhnikoff M, Mauad T, et al. Airway basement membrane perimeter distensibility and airway smooth muscle area in asthma. J Appl Physiol. 2008;104:1703–8.
2. James AL, Pare P, Hogg JC. Effects of lung volume, bronchoconstriction, and cigarette smoke on morphometric airway dimensions. J Appl Physiol. 1988;64:913–9.
3. Noble P, Sharma A, McFawn P, Mitchell H. Elastic properties of the bronchial mucosa: epithelial unfolding and stretch in response to airway inflation. J Appl Physiol. 2005;99:2061–6.
4. Chin LY, Bossé Y, Pascoe C, Hackett TL, Seow CY, Paré PD. Mechanical properties of asthmatic airway smooth muscle. Eur Respir J. 2012;40:45–54.
5. Kuwano K, Bosken CH, Paré PD, Bai TR, Wiggs BR, Hogg JC. Small airways dimensions in asthma and in chronic obstructive pulmonary disease. Am Rev Respir Dis. 1993;148:1220–5.
6. Donovan GM, Elliot JG, Green FH, James AL, Noble PB. Unraveling a clinical paradox: why does bronchial thermoplasty work in asthma? Am J Respir Cell Mol Biol. 2018;59:355–62.
7. Pascoe CD, Seow CY, Hackett TL, Paré PD, Donovan GM. Heterogeneity of airway wall dimensions in humans: a critical determinant of lung function in asthmatics and nonasthmatics. Am J Physiol Lung Cell Mol Physiol. 2017;312:L425–L31.
8. Wang KC, Donovan GM, Saglani S, Mauad T, James AL, Elliot JG, et al. Growth of the airway smooth muscle layer from late gestation to childhood is mediated initially by hypertrophy and subsequently hyperplasia. Respirology. 2022;27:493–500.
9. Donovan GM, Wang KC, Shamsuddin D, Mann TS, Henry PJ, Larcombe AN, et al. Pharmacological ablation of the airway smooth muscle layer—mathematical predictions of functional improvement in asthma. Physiol Rep. 2020;8:e14451.
10. Wang KC, Donovan GM, James AL, Noble PB. Asthma: pharmacological degradation of the airway smooth muscle layer. Int J Biochem Cell Biol. 2020;126:105818.
11. Donovan GM, Elliot JG, Boser SR, Green FH, James AL, Noble PB. Patient-specific targeted bronchial thermoplasty: predictions of improved outcomes with structure-guided treatment. J Appl Physiol. 2019;126:599–606.
12. Donovan GM, Noble PB, Langton D. Therapeutic response to bronchial thermoplasty—toward feasibility of patient selection based on modelling predictions. J Appl Physiol. 2022;133:1341–8.
13. James AL, Donovan GM, Green FH, Mauad T, Abramson MJ, Cairncross A, et al. Heterogeneity of airway smooth muscle remodelling in asthma. Am J Respir Crit Care Med. 2022. https://doi.org/101164/rccm.201110-1849OC
14. Hackmann MJ, Elliot JG, Green FH, Cairncross A, Cense B, McLaughlin RA, et al. Requirements and limitations of imaging airway smooth muscle throughout the lung in vivo. Respir Physiol Neurobiol. 2022;301:103884.

How to cite this article: Donovan GM, Wang KCW, Elliot JG, James AL, Noble PB. Quantifying airway remodelling for research or clinical purposes: How should we normalize for airway size? Respirology. 2023. https://doi.org/10.1111/resp.14454