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Unravelling a clinical paradox - why does bronchial thermoplasty work in asthma?

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Why does bronchial thermoplasty work?

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8.21 Modeling: Structure Function Relationships

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GMD and PBN designed the study, analysed the data, and wrote the manuscript. JGE, FHYG and ALJ provided the primary data and edited the manuscript. GMD constructed the mathematical model and performed the simulations.

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Abstract:

Rationale

Bronchial thermoplasty is a relatively new but seemingly effective treatment in asthmatic subjects who do not respond to conventional therapy. While the favoured mechanism is ablation of the airway smooth muscle layer, because bronchial thermoplasty treats only a small number of central airways, there is ongoing debate regarding its precise method of action.

Objectives

Elucidate the underlying method of action behind bronchial thermoplasty.

Methods

We employed a combination of extensive human lung specimens and novel computational methods. Whole left lungs were acquired from the Prairie Provinces Fatal Asthma Study. Subjects were classified as control (N=31), non-fatal asthma (N=32), or fatal asthma (N=25). Simulated lungs for each group were constructed stochastically, and flow distributions and functional indicators (e.g., resistance) were quantified both before and after a 75% reduction in airway smooth muscle in the 'thermoplasty-treated' airways.

Main Results

Bronchial thermoplasty triggers global redistribution of clustered flow patterns, wherein structural changes to the treated central airways lead to a re-opening cascade in the small airways and significant improvement in lung function via reduced spatial heterogeneity of flow patterns. This mechanism accounts for progressively greater efficacy of thermoplasty with both severity of asthma and degree of muscle activation, broadly consistent with existing clinical findings.

Conclusions

We report a probable mechanism of action for bronchial thermoplasty: alteration of lung-wide flow patterns in response to structural alteration of the treated central airways. This insight could lead to improved therapy via patient-specific, tailored versions of the treatment -- as well as implications for more conventional asthma therapies.

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At a Glance

Scientific Knowledge on the Subject

More than 200 papers concerning bronchial thermoplasty have been published in the last 10 years. It is approved for treatment and known to be safe and effective over a 5 year timescale, with modest increases in quality of life scores and more substantial decreases in severe exacerbations and emergency department visits. However, the underlying mechanism of action is unknown and controversial; the small number of large airways treated directly is thought to be insufficient to explain the

observed benefits, and several hypotheses have been advanced through which treatment effects may extend to a greater portion of the lung.

What This Study Adds to the Field

We show that the observed benefits of bronchial thermoplasty (BT) can be explained by changes in whole-lung flow patterns, induced by structural changes in the central, BT-treated airways, providing a probable mechanism of action for BT. No other non-local airway changes are needed to explain the improvement. An improved understanding of the underlying mechanism of action may allow more informed patient selection for BT, patient-specific targeted versions of BT, and other novel therapies operating through a similar method of action.

Introduction

The underlying pathophysiology of asthma is poorly understood(1), and attempts to improve our understanding of pulmonary structure and function are bedeviled by complexity in many forms; these range from the extent of the branching tree structure(2) and structural heterogeneity of the airways(3, 4) through to self-organized emergent phenomena such as spatial ventilation heterogeneity and clustered ventilation defects(5–9), all in the context of multiscale interactions(10, 11). Combined with variation in patient phenotypes, this leaves us with an insufficient understanding of the pathophysiology of asthma(12). Arguably this failing is less acute for those whose asthma is well-controlled by standard therapies, but for the minority where this is not the case, asthma can be fatal; this group accounts for the majority of the health care burden. Here especially is where our lack of a complete understanding of the complexity of the disease remains an impediment to effective therapy.

One recent addition to the arsenal of available therapies for asthma is bronchial thermoplasty (BT), approved by the US Food and Drug Administration in 2010. BT delivers targeted thermal energy to the central airways via bronchoscope and radiofrequency catheter, and is considered in the treatment of moderate-to-severe asthmatic patients who do not respond to conventional therapy. Although BT has been shown to increase quality of life scores and decrease severe exacerbations and emergency department visits(13), the mode by which these improvements occur is unclear(14, 15). The most likely direct mechanism is a reduction in airway smooth muscle (ASM) mass in the treated airways(16). Given that activation and contraction of the ASM are responsible for airway narrowing during acute events(17), this is an appealing hypothesis. However, BT treatment is restricted to a small number of large, central airways, while asthmatic pathophysiology is widely believed to have a significant contribution from smaller airways(18, 19); thus local thermal ablation of ASM in a small number of central airways is thought by many to be insufficient to explain the observed benefits of BT. As a result, several hypotheses have been advanced by which the effects of BT may extend to tissues which were not directly treated, for example via changes in immunomodulation(20, 21) or neural control(22).

The present study investigated the mechanism through which BT exerts its effects on respiratory function. We used extensive human lung specimens (N=2339 airways from 88 subjects) to construct structurally-heterogeneous simulated human lungs for non-asthma (NA), non-fatal asthma (NFA) and fatal asthma (FA) populations. Within these simulated lungs we employed an appropriate ventilation model and analysed the resulting lung function (flow patterns and resistance) both prior to, and after, simulated BT. Our simulated BT protocol is matched to current clinical practice, and we assume that structural changes are restricted to directly treated airways, in which ASM undergoes a sustained, long-term reduction.

Methods

Human lung specimens

Whole left lungs were acquired from the Prairie Provinces Fatal Asthma Study(23–26). Subjects were classified as: Control (n=31) - no history of asthma, wheeze or other lung disease; non-fatal asthma (n=32) - death attributed to a non-respiratory cause with a confirmed history of asthma; and fatal asthma (n=25) - death attributed to asthma with a confirmed history. The main bronchus and pulmonary artery were perfused simultaneously with glutaraldehyde (2.5% CA in 0.05 M phosphate buffer, pH 7.4, 350 mOsm with sucrose) at pressure heads of 20 and 40 cmH₂O respectively, thus maintaining a capillary-alveolar pressure difference of 15-20cmH₂O. Vascular perfusion was maintained for 2 hours and bronchial perfusion overnight.

Three segmental bronchi, two from the lower lobe (anterior and posterior basal) and one from the upper lobe (apical), were isolated from lung parenchyma. Portions of airway (with adjacent parenchyma) were acquired at nine equidistant levels from proximal to distal locations. This yielded 27 airways per subject. Samples were processed into wax blocks, sectioned (5 µm) and stained using the Elastic Trichrome technique. The area of the ASM layer and total wall area were measured by point counting using a Nikon light microscope, drawing tube and square lattice grid containing 240 points.

Mathematical Model

Here we provide an overview of the mathematical model. *Full technical details are provided in the online supplement*, including a flowchart for visualization. Below is an outline of the model process, which is repeated for each group (NA, NFA, FA).

1. **Structural airway data.** We begin with the structural data for the group, acquired from human lung specimens as above, using basement membrane perimeter (Pbm), wall area (WA), ASM area (ASM) and anatomical level(27) for each sampled airway.
2. **Statistical fit.** The structural data is used to fit distributions for Pbm, WA and ASM for each airway order (28).
3. **Simulated lung structure and function.** Steps below are repeated 25 times to generate independent simulations via Monte Carlo simulation(29).
 - a. Generate **synthetic lung and airway structure** using the fitted distributions for each airway order.
 - b. Calculate **pre-BT flow** using the generated synthetic lung and airway structure(30, 31).
 - c. Apply **simulated BT treatment** to the airway structure. Simulated BT protocols are based on current clinical practice(13), treating the lower lobe bronchus, upper lobe bronchus, superior division bronchus, and LB1-10. Treated airways undergo a 75% reduction in ASM mass(16, 22, 32); wall area is altered via ASM reduction but we assume no other changes to the airway wall. Two variations in BT were also simulated: treating the lower lobe only, or an extended BT that treats airways one further generation distally. See the online supplement for details.
 - d. Calculate **post-BT flow** using the BT-treated synthetic lung and airway structure.
 - e. Calculate **impedance** (i.e., resistance) using a standard circuit analog model(3, 4), and assess **dose-response** by repeating flow and function calculations for different levels of agonist (methacholine) stimulus, related to ASM activation using human ASM data(33).
4. **Compute statistics for the group** using function and dose-response across all simulated lungs.

Results

Dose-response curves for each group (non-asthma (NA), non-fatal asthma (NFA), fatal asthma (FA)), both pre- and post-BT, are provided in Fig. 1a wherein ASM activation increases with theoretical dose of agonist (methacholine) (34). In the absence of ASM activation no significant improvement is observed post-BT in any group; however, significant improvement is observed concomitantly with increases in either asthma severity or agonist dose.

Fig. 1 -- (a)-(e)

- Dose-response curves for respiratory resistance (R , normalized) as a function of agonist dose (log scale). Pre-BT control simulations (dashed line) and post-BT simulations (solid line) are provided for each group (FA, red; NFA, blue; NA, green). $N=25$ simulations per group, error bars are standard deviation, and * indicates statistical significance (post-BT vs pre-BT) at $p<0.05$ via two-way ANOVA.
- Spatial heterogeneity index (see online supplement) as a function of agonist dose. Pre-BT control simulations (dashed line) and post-BT simulations (solid line) are provided for each group (FA, red; NFA, blue; NA, green). $N=25$ simulations per group, error bars are standard deviation, and * indicates statistical significance (post-BT vs pre-BT) at $p<0.05$ via two-way ANOVA.
- Pre-BT flow pattern for typical simulation (NFA at $\log(\text{dose}) -4.4$). The color bar to the right of (f) indicates the scale for both (e) and (f), showing flow through each airway (normalized to nominal flow; see online supplement).
- Post-BT flow pattern for typical simulation (NFA at $\log(\text{dose}) -4.4$). Details as in (c).

- (e) BT-induced flow pattern changes for typical simulation (NFA at log(dose) -4.4). Color bar indicates the difference in flow (post-BT less pre-BT, normalized to nominal; see online supplement).

Since the effect of our simulated BT is solely limited to changes in the ASM of the BT-treated (large) airways, the observed global improvement cannot be explained by more widely disseminated structural effects on other tissue types or in smaller airways, as has been postulated(35). Indeed, further analysis demonstrates the method of action. While Fig 1a shows statistics for each group with 25 simulations per group, it is illustrative to examine individual simulated lungs more closely. Typical flow distributions are provided in Fig 1 (c, pre-BT) and (d, post-BT); visually, recruited areas are apparent, along with a decrease in spatial heterogeneity. The BT-induced change is quantified explicitly in Fig. 1(e), showing both the expected regions of flow increase, but also paradoxical regions exhibiting decreased flow (a video of the evolution of the flow states for the simulation is included with the Online Supplement).

A similar pattern of change with BT is demonstrated when assessing the relationship between BT-treated airways and their subtended airways, with respect to both flow and lumen radius (Fig. 2). Broadly, recruitment of new regions allows modest reduction in flow in previously hyper-inflated areas. Further, while BT-treated airways do dilate, untreated airways show a mixed contraction-dilation pattern, an effect recently demonstrated with synchrotron phase-contrast CT(36). These results are typical and other simulations show similar results.

To quantify this explicitly we compute a spatial heterogeneity index which captures both overall heterogeneity, and the extent to which flow is clustered (see online supplement). We calculate this spatial heterogeneity index for all simulations, shown in Fig. 1b, indicating a systematic reduction in spatial heterogeneity due to BT which closely mirrors the dose-response curves. Thus the central mechanism behind the effectiveness of BT as a treatment for asthma appears to be that central airway structural changes trigger global flow redistribution toward a more homogeneous and efficient flow configuration, e.g. reducing ventilation heterogeneity.

Fig. 2

- (a) Change in flow through individual airways for an typical simulation (NFA at $\log(\text{dose}) = -4.4$) as a function of airway order⁶(28). Red airways are BT-treated; tree connectivity is indicated by grey lines, and orange back-shading indicates point-density.
- (b) Change in airway radius for individual airways for a typical simulation; details as in (a).

We also consider two BT variation therapies: treating the lower lobe (LL) only, or an extended BT in which one additional generation of airways is treated, beyond current practice. These treatment regimes are illustrated in Fig. 3a. Quantification of flow pattern differences for the typical simulation are provided in 3 (b) and (c) for LL-only and extended-BT treatment, respectively. Unsurprisingly the lower lobe treatment shows flow increases exclusively within the treated lobe, while the extended treatment shows effects across the entire lung. Dose-response curves are shown for LL-only and extended BT in 3(d,e,f) for NA, NFA, and FA groups respectively. These data indicate that if BT were limited to LL-only treatment there would still be significant improvement in most situations, though not as effective as current clinical protocol. The extended BT shows improvements in function, increasing with severity of both disease and dose.

It is also interesting to examine the population-level distribution of responses. Previous work has suggested that BT response is related to the number of radiofrequency (RF) activations during BT treatment(37), and that non-responders may not exhibit the same degree of ASM reduction(38). In our simulations, we found that 65% of FA displayed a significant response to BT, with the remainder showing essentially no change. In the other groups, 70% of NFAs and 45% NAs also demonstrated a response. For LL-only BT, positive responders reduced to 59% (FA), 45% (NFA), and 25% (NA), while extended BT would increase the response rate to 71% (FA), 80% (NFA), and 50% (NA). In all simulations the proportion of ASM ablated in the treated airways is fixed, suggesting that while inadequate RF activation during treatment would certainly contribute to non-response rates, there are also sub-populations which are intrinsically less susceptible to BT treatment due to the structure of their airways.

⁶ Horsfield order begins at the respiratory bronchioles and increases progressively moving up the airway tree.

Fig. 3 -- (a)-(f)

- (a) Illustration of three BT protocols in a simulated human lung: lower lobe only (red); current clinical practice (red + green); hypothetical "extended" BT (red + green + blue).
- (b) Lower lobe (LL)-only BT induced flow pattern changes for typical simulation (NFA at log(dose) -4.4), normalized to nominal (see online supplement).
- (c) Extended BT-induced flow pattern changes for typical simulation. Details as panel (b).
- (d) Dose-response curves for respiratory resistance (R, normalized) in non-asthma group (NA) as a function of agonist dose (log scale). LL-only BT is indicated in orange, and extended BT in green, with control (grey, dashed) and standard BT (grey, solid) repeated from Fig. 1 (mean only) for reference. For each group, N=25 simulations, error bars are standard deviation, and * indicates statistical significance (post-BT vs pre-BT) at $p < 0.05$ via two-way ANOVA.
- (e) Dose-response curve for non-fatal asthma (NFA) group; details as panel (d).
- (f) Dose-response curve for fatal asthma (FA) group; details as panel (d).

Discussion

Our principal finding is the probable underlying mechanism for bronchial thermoplasty: structural changes to the ASM in the BT-treated central airways lead to a re-opening cascade in the smaller airways, in turn creating more homogeneous and efficient flow patterns. This mechanism predicts that while little functional difference is found in the absence of agonist challenge, the effect of BT is progressively greater with increasing agonist stimulation and asthma severity. These predictions are consistent with a suite of existing empirical results. Perhaps most important are the recent 3He MRI and CT results showing mixed post-BT regional ventilation changes, i.e. post-BT regional ventilation shows both increases and decreases(39), exactly as predicted by the model.

Clinical trials vary in terms of the health parameters that are reported to improve post-BT. The AIR trial(40) showed significant improvement in quality-of-life questionnaire scores, symptom free days, symptom scores, rescue medication use, mean rate of exacerbations and morning peak expiratory flow (PEF), but showed no differences in the key functional measures: airway responsiveness (PC20) and FEV1. The RISA trial(41) in severe asthma similarly showed improvements in quality-of-life questionnaire scores and rescue medication use, and here also pre-bronchodilator FEV1 was significantly improved. Because of the potential for a large placebo effect, the sham-controlled AIR2 trial(42) is arguably the most authoritative; significant improvements were found in quality-of-life questionnaire scores, severe exacerbations, emergency visits and missed days of school/work, but without any significant improvement in FEV1 or morning PEF.

We argue that the nature of our predicted functional improvements is in fact consistent with the above, albeit mixed findings, and indeed helps to explain their apparent inconsistency. The key aspect is that we predict that functional improvements are almost non-existent at baseline, but increase with both asthma severity and degree of stimulus. Such changes will be difficult to detect in a clinical setting because it is only in severe situations, where function tests are not well-tolerated by patients, that these functional differences become large. For example, in the AIR trial (40), long-acting β adrenoceptor agonists and short-acting β_2 agonists were withdrawn prior to spirometry only if tolerated. It is perhaps not surprising, then, that the clinical trials have failed to show consistent functional improvements. On the other hand, such changes would still be expected to manifest in indirect measures such as quality-of-life questionnaire scores, severe exacerbations, emergency visits and missed days of school/work -- exactly as shown by AIR2. Evidence for BT-induced impairment of airway closure(43), demonstration of mixed contraction-dilation patterns in vivo(36), and the inability, so far, to find consistent non-local structural changes resulting from BT also lend support to this global flow redistribution mechanism.

Our methods account for both heterogeneity of lung structure as well as intrinsic instability on the scale of a full human lung; this crucially allows propagation of effects elicited by reduction of ASM in BT-treated airways out to the periphery. Thus is it possible for changes in central airway structure after BT to trigger global redistribution of clustered flow patterns in the lung, reducing spatial heterogeneity, and furthermore that these changes account for significant improvements in lung function during bronchial provocation, in line with observed clinical response to BT. Importantly, this BT-induced improvement is an emergent phenomenon in a complex system; that is, feedback mechanisms (e.g. redirection of airflow) at the level of individual elements (airways) give rise to ordered behavior on a larger scale (lung-wide flow patterns). It arises from the structural alteration of a small number of BT-treated central airways only -- no other mechanisms are required, and this effect would be difficult to predict *a priori* from the structural data alone.

Closer examination of the data suggest an explanation for the above changes, namely the way in which structural changes in the large airways induce functional changes in the small airways. It appears that the BT-induced global flow redistribution arises because, although the changes in the BT-treated large airways can be relatively small, even small improvements "upstream" are sufficient to allow re-opening, at the margin, of some subtended regions. Any such re-opening allows partial reversal of some hyperinflation regions, otherwise developed in compensation for poor ventilation in other locations. Thus the overall effect is toward less heterogeneous (and more functionally efficient) flow patterns.

On the basis of this central finding, we also examined two other questions of interest. We considered variations of the existing BT treatment protocol both in terms of single-lobe treatment (LL-only) and a

hypothetical extended BT (treating one additional airway generation.) The extended BT proposal shows increased treatment effectiveness and may be promising, especially for more severe cases(42). The results from the LL-only case provide a plausible set of testable hypotheses, given that current treatment practice involves sequential lobar treatments(13); in depth examination of in vivo lobar treatment effects could be assessed between treatments, provided that sufficient time is allowed for acute inflammation to resolve. Similarly, the extended BT protocol is a natural extension requiring only an incremental decrease in catheter size and somewhat increased procedural complexity.

We also propose a more ambitious therapeutic advancement, namely patient-specific approaches to both patient selection and treatment design. The prospect that there are asthma subpopulations which are intrinsically less susceptible to BT raises two tantalising prospects. One is that it might be possible to classify BT response phenotypes based on *pre-treatment* airway structure. The current data set is consistent with the hypothesis that the patients who go on to show a BT response are those with greater (pre-treatment) ASM in the airways targeted by BT; however, we do not have the statistical power required to draw that inference with any significance. A second option would be to take a more direct, precision-medicine approach: use patient-specific structural airway data from central airways, perhaps in combination with CT and polarization sensitive optical coherence tomography (PS-OCT)(44), to design an optimal BT treatment package for that patient, which optimally reduces ventilation heterogeneity and improves functional response.

There are several methodological assumptions and limitations of this study which merit discussion. It is important to note that we are considering only the long-term effects of BT which arise from reduction of ASM in the treated airways, and we do not consider any short term effects such as inflammation or exacerbation either during or shortly after the treatment. This is especially pertinent in considering extended BT for severe cases, where the consequences of acute responses and complications would require careful assessment. In considering the effects of BT on the FA group near the plateau (e.g. at maximal agonist dose) it is worth noting that a significant portion of simulations in the control FA group at maximal activation were discarded for exceeding pre-set intrapleural pressure limits, and so the statistical power for this point is reduced. It is thus likely that the results actually *understate* the effect of BT for fatal asthmatics at or near maximal dose. For additional details see the online supplement. Finally, the proposed mechanism of BT in the present study is theoretical and requires experimental validation. Imaging technology has however advanced to the point where it should be possible to detect changes in ventilation of the magnitude observed in the present study. BT has already been shown, via ³He MRI and CT, to exhibit mixed ventilation responses on a regional basis(39), and more recent advances(45) suggest that capabilities now exist to demonstrate this effect with greater spatial resolution.

Taken together, the results of the present study suggest that the primary method of action for BT in asthma is non-local alteration of flow patterns toward decreased spatial ventilation heterogeneity, caused by local thermal ablation of ASM in the treated, central airways. This is an emergent phenomenon in a complex system which cannot be easily predicted from changes in airway structure alone. Non-local structural effects may still contribute, but are likely to be secondary phenomena. By providing a more basic understanding of both the underlying pathophysiology of asthma, and the central method of action of BT, this interdisciplinary solution to a clinical puzzle provides possible routes to both short-term improvements of existing therapies, and longer-term development of novel therapies. Both possibilities hinge on a key insight: that significant functional improvements can arise from reductions in spatial flow heterogeneity, and that this can be achieved by the direct targeting of large airways alone. This central idea has potential implications beyond BT; for example, the effectiveness of aerosolised pharmacological treatments depends on particle deposition patterns, which are in turn strongly influenced by aerosol

particle size (46–48). While much effort has been dedicated to reducing particle size in order to increase deposition in the smaller airways, present results suggest that a more subtle approach may be warranted. Given the effectiveness of large-airway only treatment in BT via global flow pattern alteration, optimizing particle deposition patterns to target the smaller airways may not be the best approach, if this compromises effective treatment of the larger airways.

References

1. Wenzel SE. Asthma phenotypes: the evolution from clinical to molecular approaches. *Nat Med* 2012;18:716–725.
2. Weibel ER, Gomez DM. Architecture of the Human Lung: Use of quantitative methods establishes fundamental relations between size and number of lung structures. *Science* 1962;137:577–585.
3. Thorpe CW, Bates JH. Effect of stochastic heterogeneity on lung impedance during acute bronchoconstriction: a model analysis. *J Appl Physiol* 1997;82:1616–1625.
4. Lutchen KR, Gillis H. Relationship between heterogeneous changes in airway morphometry and lung resistance and elastance. *J Appl Physiol* 1997;83:1192–1201.
5. Venegas JG, Winkler T, Musch G, Vidal Melo MF, Layfield D, Tgavalekos N, Fischman AJ, Callahan RJ, Bellani G, Harris RS. Self-organized patchiness in asthma as a prelude to catastrophic shifts. *Nature* 2005;434:777–782.
6. Anafi RC, Wilson TA. Airway stability and heterogeneity in the constricted lung. *J Appl Physiol* 2001;91:1185–1192.
7. Tgavalekos NT, Musch G, Harris RS, Vidal Melo MF, Winkler T, Schroeder T, Callahan R, Lutchen KR, Venegas JG. Relationship between airway narrowing, patchy ventilation and lung mechanics in asthmatics. *Eur Respir J* 2007;29:1174–1181.
8. Campana L, Kenyon J, Zhalehdoust-Sani S, Tzeng Y-S, Sun Y, Albert M, Lutchen KR. Probing airway conditions governing ventilation defects in asthma via hyperpolarized MRI image functional modeling. *J Appl Physiol* 2009;106:1293–1300.
9. Svenningsen S, Kirby M, Starr D, Coxson HO, Paterson NAM, McCormack DG, Parraga G. What are ventilation defects in asthma? *Thorax* 2013;69:63–71.
10. Tawhai MH, Bates JHT. Multi-scale lung modeling. *J Appl Physiol* 2011;110:1466–1472.

11. Latourelle J, Fabry B, Fredberg JJ. Dynamic equilibration of airway smooth muscle contraction during physiological loading. *J Appl Physiol* 2002;92:771–779.
12. Anderson GP. Endotyping asthma: new insights into key pathogenic mechanisms in a complex, heterogeneous disease. *Lancet* 2008;372:1107–1119.
13. Bicknell S, Chaudhuri R, Thomson NC. How to: Bronchial thermoplasty in asthma. *Breathe* 2014;10:48–59.
14. d'Hooghe JNS, ten Hacken NH, Weersink EJM, Sterk PJ, Annema JT, Bonta PI. Emerging understanding of the mechanism of action of Bronchial Thermoplasty in asthma. *Pharmacol Ther* 2017;doi:10.1016/j.pharmthera.2017.07.015.
15. Bonta PI, d'Hooghe J, Sterk PJ, Bel EH, Annema JT. Reduction of Airway Smooth Muscle Mass after Bronchial Thermoplasty: Are We There Yet? *Am J Respir Crit Care Med* 2015;191:1207–1208.
16. Pretolani M, Dombret M-C, Thabut G, Knap D, Hamidi F, Debray M-P, Taille C, Chanez P, Aubier M. Reduction of airway smooth muscle mass by bronchial thermoplasty in patients with severe asthma. *Am J Respir Crit Care Med* 2014;190:1452–1454.
17. Fredberg JJ, Inouye D, Miller B, Nathan M, Jafari S, Raboudi SH, Butler JP, Shore SA. Airway smooth muscle, tidal stretches, and dynamically determined contractile states. *Am J Respir Crit Care Med* 1997;156:1752–1759.
18. Carroll N, Elliot J, Morton A, James A. The structure of large and small airways in nonfatal and fatal asthma. *Am Rev Respir Dis* 1993;147:405–410.
19. Ueda T, Niimi A, Matsumoto H, Takemura M, Hirai T, Yamaguchi M, Matsuoka H, Jinnai M, Muro S, Chin K, Mishima M. Role of small airways in asthma: investigation using high-resolution computed tomography. *J Allergy Clin Immunol* 2006;118:1019–1025.
20. Denner DR, Doeing DC, Hogarth DK, Dugan K, Naureckas ET, White SR. Airway Inflammation after Bronchial Thermoplasty for Severe Asthma. *Ann Am Thorac Soc* 2015;12:1302–1309.
21. Marc Malovrh M, Rozman A, Škrjat S, Šilar M, Šelb J, Fležar M, Korošec P. Bronchial thermoplasty induces immunomodulation with a significant increase in pulmonary CD4(+)25(+) regulatory T cells. *Ann Allergy Asthma Immunol* 2017;doi:10.1016/j.anai.2017.06.019.

22. Pretolani M, Bergqvist A, Thabut G, Dombret M-C, Knapp D, Hamidi F, Alavoine L, Taillé C, Chanez P, Erjefält JS, Aubier M. Effectiveness of bronchial thermoplasty in patients with severe refractory asthma: Clinical and histopathologic correlations. *J Allergy Clin Immunol* 2017;139:1176–1185.
23. Hessel PA, Mitchell I, Tough S, Green FHY, Cockcroft D, Kepron W, Butt JC. Risk factors for death from asthma. *Ann Allergy Asthma Immunol* 1999;83:362–368.
24. Salkie ML, Mitchell I, Revers CW, Karkhanis A, Butt J, Tough S, Green FH. Postmortem serum levels of tryptase and total and specific IgE in fatal asthma. *Allergy Asthma Proc* 1998;19:131–133.
25. Green FHY, Williams DJ, James A, McPhee LJ, Mitchell I, Mauad T. Increased myoepithelial cells of bronchial submucosal glands in fatal asthma. *Thorax* 2010;65:32–38.
26. Tough SC, Green FHY, Paul JE, Wigle DT, Butt JC. Sudden Death from Asthma in 108 Children and Young Adults. *J Asthma Allergy* 1996;33:179–188.
27. Elliot JG, Budgeon CA, Harji S, Jones RL, James AL, Green FH. The effect of asthma on the perimeter of the airway basement membrane. *J Appl Physiol* 2015;119:1114–1117.
28. Horsfield K. Diameters, generations, and orders of branches in the bronchial tree. *J Appl Physiol* 1990;68:457–461.
29. Rubinstein RY, Kroese DP. *Simulation and the Monte Carlo Method*. John Wiley & Sons; 2011.
30. Donovan GM. Inter-airway structural heterogeneity interacts with dynamic heterogeneity to determine lung function and flow patterns in both asthmatic and control simulated lungs. *J Theor Biol* 2017;435:98–105.
31. Donovan GM. Clustered ventilation defects and bilinear respiratory reactance in asthma. *J Theor Biol* 2016;406:166–175.
32. Brown RH, Wizeman W, Danek C, Mitzner W. In vivo evaluation of the effectiveness of bronchial thermoplasty with computed tomography. *J Appl Physiol* 2005;98:1603–1606.
33. Ijpma G, Kachmar L, Matusovsky OS, Bates JHT, Benedetti A, Martin JG, Lauzon A-M. Human trachealis and main bronchi smooth muscle are normoresponsive in asthma. *Am J Respir Crit Care Med* 2015;191:884–893.
34. Ijpma G, Kachmar L, Matusovsky OS, Bates JHT, Benedetti A, Martin JG, Lauzon A-M. Human

trachealis and main bronchi smooth muscle are normoresponsive in asthma. *Am J Respir Crit Care Med* 2015;191:884–893.

35. Boulet L-P, Laviolette M. Acute effects of bronchial thermoplasty: a matter of concern or an indicator of possible benefit to small airways? *Eur Respir J* 2017;49.:
36. Dubsky S, Zosky GR, Perks K, Samarage CR, Henon Y, Hooper SB, Fouras A. Assessment of airway response distribution and paradoxical airway dilation in mice during methacholine challenge. *J Appl Physiol* 2017;122:503–510.
37. Langton D, Sha J, Ing A, Fielding D, Thien F, Plummer V. Bronchial thermoplasty: activations predict response. *Respir Res* 2017;18:134.
38. Kirby M, Ohtani K, Lopez Lisbona RM, Lee AMD, Zhang W, Lane P, Varfolomeva N, Hui L, Ionescu D, Coxson HO, MacAulay C, FitzGerald JM, Lam S. Bronchial thermoplasty in asthma: 2-year follow-up using optical coherence tomography. *Eur Respir J* 2015;46:859–862.
39. Thomen RP, Sheshadri A, Quirk JD, Kozlowski J, Ellison HD, Szczesniak RD, Castro M, Woods JC. Regional Ventilation Changes in Severe Asthma after Bronchial Thermoplasty with ³He MR Imaging and CT. *Radiology* 2015;274:250–259.
40. Cox G, Thomson NC, Rubin AS, Niven RM, Corris PA, Siersted HC, Olivenstein R, Pavord ID, McCormack D, Chaudhuri R, Miller JD, Laviolette M, AIR Trial Study Group. Asthma control during the year after bronchial thermoplasty. *N Engl J Med* 2007;356:1327–1337.
41. Pavord ID, Cox G, Thomson NC, Rubin AS, Corris PA, Niven RM, Chung KF, Laviolette M, RISA Trial Study Group. Safety and efficacy of bronchial thermoplasty in symptomatic, severe asthma. *Am J Respir Crit Care Med* 2007;176:1185–1191.
42. Castro M, Rubin AS, Laviolette M, Fiterman J, De Andrade Lima M, Shah PL, Fiss E, Olivenstein R, Thomson NC, Niven RM, Pavord ID, Simoff M, Duhamel DR, McEvoy C, Barbers R, Ten Hacken NHT, Wechsler ME, Holmes M, Phillips MJ, Erzurum S, Lunn W, Israel E, Jarjour N, Kraft M, Shargill NS, Quiring J, Berry SM, Cox G, AIR2 Trial Study Group. Effectiveness and safety of bronchial thermoplasty in the treatment of severe asthma: a multicenter, randomized, double-blind, sham-controlled clinical trial. *Am J Respir Crit Care Med* 2010;181:116–124.

43. Brown R, Wizeman W, Danek C, Mitzner W. Effect of Bronchial Thermoplasty on Airway Closure. *Clin Med Circ Respirat Pulm Med* 2007;1:1–6.
44. Adams DC, Hariri LP, Miller AJ, Wang Y, Cho JL, Villiger M, Holz JA, Szabari MV, Hamilos DL, Scott Harris R, Griffith JW, Bouma BE, Luster AD, Medoff BD, Suter MJ. Birefringence microscopy platform for assessing airway smooth muscle structure and function in vivo. *Sci Transl Med* 2016;8:359ra131.
45. Horn FC, Marshall H, Collier GJ, Kay R, Siddiqui S, Brightling CE, Parra-Robles J, Wild JM. Regional Ventilation Changes in the Lung: Treatment Response Mapping by Using Hyperpolarized Gas MR Imaging as a Quantitative Biomarker. *Radiology* 2017;284:854–861.
46. Usmani OS, Biddiscombe MF, Barnes PJ. Regional Lung Deposition and Bronchodilator Response as a Function of β_2 -Agonist Particle Size. *Am J Respir Crit Care Med* 2005;172:1497–1504.
47. Carvalho TC, Peters JI, Williams RO 3rd. Influence of particle size on regional lung deposition--what evidence is there? *Int J Pharm* 2011;406:1–10.
48. Howarth PH. Why particle size should affect clinical response to inhaled therapy. *J Aerosol Med* 2001;14 Suppl 1:S27–34.