Inhaled aerosol dose distribution
between proximal bronchi and lung periphery.

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ABSTRACT

Modern inhaled drug discovery programs assess dose delivery to proximal and distal airways using rudimentary imaging indices, where relative deposition is estimated by generically defined ‘central’ and ‘peripheral’ lung regions. Utilizing recent data linking the proximal airway topology to a characteristic pattern of aerosol lung deposition, we provide a direct measure of dose distribution between the proximal bronchi and the distal lung. We analyzed scintigraphic lung images of twelve asthma patients following inhalation of 1.5-, 3- and 6-µm monodisperse drug particles at breathing flows of 30- and 60-L/min. We explicitly used the central hot-spots associated with each patient’s specific bronchial topology to obtain a direct measure of aerosol deposition in the proximal bronchi, rather than applying standard templates of lung boundaries. Maximum deposition in the central bronchi (as % of lung deposition) was 52±10(SD)% (6µm;60L/min). Minimum central deposition was 17±2(SD)% (1.5µm;30L/min) where the 83% aerosol ‘escaping’ deposition in the central bronchi reached 75±17(SD)% of the lung area that could be reached by Krypton gas. For all particle sizes, hot-spots appeared in the same patient-specific central airway location, with greatest intensity at 60L/min. For a range of respirable aerosol sizes and breathing flows, we have quantified deposited dose in the proximal bronchi and their distal lung reach, constituting a platform to support therapeutic inhaled aerosol drug development.

KEYWORDS: inhalation therapy, drug targeting, aerosol drug dose, central airways, hot-spots, in situ lung imaging.
LIST OF ABBREVIATIONS (in alphabetical order):

BMI : body mass index
COPD : chronic obstructive pulmonary disease
DPI : dry powder inhaler
FEV₁ : forced expiratory volume in one-second
FVC : forced vital capacity
ROI : region of interest
INTRODUCTION

Established practice in planar lung scintigraphic imaging of inhaled radiolabeled aerosols utilizes regions of interest (ROI) for analysis of aerosol distribution within the lungs\textsuperscript{1,2}. However, the terms ‘central’ and ‘peripheral’ ROI are used interchangeably to represent respectively ‘large’ and ‘small’ airways. This is an approximation, since the central ROI contains not only radioactivity emanating from aerosol deposition in the proximal bronchi, but also that from the peripheral air spaces beyond. Indeed, this intrinsic overlap in planar 2D lung imaging is the ‘Achilles heel’ of using the traditional central ROI as a basis for lung dose quantification and distribution, reported in many lung scintigraphic deposition studies of therapeutic and toxic aerosols.

*In vivo* human scintigraphic lung imaging studies with inhaled aerosols typically show high local airway deposition of aerosol doses (“hot-spots”) in the ‘central’ ROI and beyond\textsuperscript{3,4,5,6}. While hot-spots are often viewed as indicating local airway obstruction, a recent study revealed that even in the absence of airway narrowing and in case of homogeneous ventilation distribution, a few distinct central hot-spots were formed in planar images of the central airways, and that these hot-spot patterns were associated with the specific topology of the first bronchial generations\textsuperscript{7}. By considering that these proximal airways are spatially concentrated, the advantage of defining relatively small central ROIs associated with a patient’s distinct central hot-spot pattern becomes appealing. Certainly, such smaller and anatomically linked
central ROIs would be less contaminated by peripheral air spaces. The translational implication of this approach is clear for drug discovery programs. For those inhaled drugs that need to target the proximal airways, such as bronchodilators, these particular central hot-spots can be viewed as representing the therapeutic lung dose. In contrast, for drugs needing more peripheral airway targeting (e.g., anti-inflammatory agents), these central hot-spots represent loss of inhaled drug on the way to its site of therapeutic action. This approach can also help interpret toxic environmental aerosol effects in the lungs.

The aim of our study was to quantify the amount of inhaled aerosol contained in the central hot-spots of patients with mild-to-moderate asthma, for aerosol particle sizes and breathing flows covering the aerodynamic range of the common inhaler devices used in clinical practice. In addition, we examined the ability of those aerosols that escaped the filtering effect of the proximal bronchi, to reach the distal lung region.

**MATERIALS AND METHODS**

We applied our recently described image analysis technique, in order to identify the characteristic pattern of localized “hot-spots” associated with the particular anatomy of the proximal bronchi (up to generation 5) (Figure 1, inset). The distinct pattern of a large single hot-spot originating from aerosols deposited in the left bronchi and two smaller hot-spots from deposition in the right bronchi was also seen in the *in vivo* planar lung images (Figure 1,
excluding oropharyngeal deposition which was quantified before\(^\text{10}\)). This offered an opportunity to quantify the aerosol lung dose in the proximal bronchi from posterior lung images of aerosol deposition in upright sitting asthma patients\(^\text{10}\): \(n=12\) (6 male; \(33\pm11\) (SD) years; BMI=24\(\pm3\) (SD); mean forced expiratory volume in one-second (FEV\(_1\)) 78 \(\pm13\) (SD)%predicted; mean forced vital capacity (FVC) 100\(\pm10\) (SD)%predicted (Ethics Committee reference number 99-237). For each subject, seven lung images were constructed: one Krypton ventilation-scan, and six aerosol-deposition scans (on six different days) of monodisperse albuterol aerosols of particle size 1.5-, 3-, 6-\(\mu\)m each inhaled at two breathing flows of 30- and 60-L/min\(^\text{11}\).

**Central hot-spot deposition**

After having determined the relative left-to-right lung intensity on the ventilation and aerosol images, central and peripheral aerosol deposition patterns were systematically analyzed in the right lung (as per scintigraphy guidelines\(^\text{2}\)). Aerosol deposition contained in the central hot-spots was calculated as the sum of activity from the two ROIs per right lung (black squares in Figure 1) delimiting a 2-pixel-wide ring around the two pixels with maximum activity (2 solid black pixels per right lung in Figure 1); in these 64x64 planar lung images, each hot-spot ROI covered an area of approximately 20cm\(^2\). The summed activity from both right lung hot-spots was expressed as a percentage of total right lung dose, here referred to as “central hot-spot deposition”.

**Central hot-spot location**

To appreciate the respective location of the hot-spots across the different aerosol particle sizes and inhalation breathing flows for any given patient, we constructed a vertical profile of right lung activity by summing all activity counts for any given lung height, normalized to total right lung activity (Figure 2). For each patient, the vertical profiles for all aerosol images were superimposed for all flow and particle size combinations. Note that the peaks in these vertical right lung profiles include the activity of the pixel with maximum local activity (black pixel in Figure 1) as well as the activity of all pixels at the same vertical lung height. This contrasts with the ROI centered around the pixel with maximal activity (black squares in Figure 1) which is used for computation of central hot-spot deposition as described in the previous section.

**Quantification of total lung penetration**

Within the right lung ROI, an additional criterion was applied to identify which pixels should be included to estimate lung penetration or lung reach; that is, the lung area reached by aerosols that escape the generation 5 bronchi. In order to avoid overestimation of lung reach (by including lung pixels with low activity), only those pixels with an activity greater than 10% median lung activity were included. Others have used 20% peak activity to delimit isocontour lung ROIs\(^{12}\), but here we wanted to avoid a bias by local hot-spot intensity, so we used median activity as a reference and hence a lower threshold. The resulting number of pixels within this isocontour ROI were multiplied by image pixel size to obtain the right lung area (cm\(^2\)) reached.
by the three particle sizes at both breathing flows. For Xenon scans, this lung area has been shown to relate to lung volume\textsuperscript{12}. After verifying that such a relationship can also be obtained for Krypton, the 2D lung area can be used as a surrogate measure to determine which proportion of the lung zones can be reached by the aerosol particles, as a percentage of the lung zones that can be reached by Krypton.

Statistical analysis was done using Medcalc (v18; Mariakerke, Belgium) for correlations and repeated ANOVA (with Bonferroni-correction). Normality of residuals was tested by Wilk-Shapiro test.

RESULTS

Validation of lung image contours

We validated our image analysis by comparing the left lung ventilation (typically $\sim 45\%$ total ventilation in the literature)$^{13}$, with left lung intensity obtained here for the Krypton and aerosols images. Left lung intensity (as a $\%$ total lung intensity) was similar for Krypton gas and aerosols: $45\pm 4$(SD)$\%$(Krypton); $46\pm 3$(SD)$\%$(1.5µm); $45\pm 3$(SD)$\%$(3µm); $46\pm 3$(SD)$\%$(6µm). The isocontour ROI method also behaved as predicted\textsuperscript{12}, in that it showed a direct correlation between the right lung isocontour ROI area for Krypton (in cm$^2$) and FVC (in L) ($r=+0.68$; $P=0.015$). The Krypton gas right lung ROI area (ranging 350-550 cm$^2$), encompasses all ventilated airspaces and can be considered as the maximum outermost contour that can be reached by
aerosols. Hence, the area of this Krypton ROI can reliably be used as a reference to evaluate lung penetration of the aerosols.

**Location and quantification of hot-spot deposition**

The color grading radioactivity maps in Figure 1 clearly showed characteristic patterns of two distinct hot-spots in the right lung and a bigger one in the left lung. The vertical right lung profiles in Figure 2 illustrate that the 2 characteristic peaks occurred at the same airway location across particle sizes and inhalation flows. This was the case for all patients (see Online Repository). When considering hot-spot intensity, defined as the combined activity of the two right lung hot-spot ROI as a percentage of total right lung activity; Figure 1), this consistently increased with increasing particle size and faster breathing flows (Figure 3A), such that approximately half of the lung dose was retained in the central airways when 6-µm particles were inhaled at 60L/min. Figure 3A represents hot-spot intensity, defined as the combined activity of the two right lung hot-spot ROI (Figure 1), and expressed as a percentage of total right lung activity. Hot-spot intensity consistently increased with increasing particle size and faster breathing flows, and for 6-µm particles inhaled at 60L/min, approximately half of the lung dose was retained in the central airway hot-spots. At 60L/min this flow, central hot-spot deposition for 3-µm and 1.5-µm particles was respectively 34% and 24% of the lung dose. Central hot-spot intensity for 6µm at 60L/min also showed a significant inverse correlation with the patient’s physiological (central) airway function (FEV₁: r=-0.62; P=0.031), implying that more bronchial airway narrowing is associated with greater hot-spot intensity. At 30L/min, central
hot-spot deposition was generally lower, but even for the smallest particle size (1.5µm) it still amounted to 17% lung dose, or 9.5% delivered dose (=17%*56%).
**Total lung penetration**

The portion of the right lung reached by the inhaled particles (as a percentage of that reached by Krypton gas) consistently decreased with increasing particle size and higher breathing flows (Figure 3B). Combined with the central hot-spot deposition data, it can be inferred how deep the amount of aerosol that has not been filtered out of the proximal bronchi, is able to spread across the lungs. For instance at 30L/min, 66% of the 6µm aerosol not deposited in the proximal bronchi (=100-34%; Figure 3A), was spread over almost half the ventilated (Krypton) lung area (48%; Figure 3B). In addition, there was a direct correlation between %area that could be reached by these particles and the patient’s FEV₁ (r=+0.63; P=0.027). For the asthma group as a whole, the best lung penetration was for 1.5µm at 30L/min, where 83% of lung dose reached 75% of the ventilated lung area.

**DISCUSSION**

We describe a unique quantitative analysis of lung aerosol images in asthma patients that provides a realistic determination of drug dose deposited in the proximal bronchi for various combinations of particle size and breathing flows (Figure 3). This data set is more readily interpretable than standard lung scintigraphy indices such as penetration index, derived from central and peripheral ROIs that are based on rectangles constructed from the outer lung boundaries. It is recognized that depending on each patient’s particular bronchial topology, hot-spots may well be located near the boundaries between both ROIs and introduce considerable
noise to the penetration index\(^1\). In contrast, our technique utilizes the central hot-spots themselves as a patient-specific anatomical reference point to determine the aerosol dose in the proximal bronchi.

We previously demonstrated in healthy tracheobronchial airways that the central hot-spots were a natural consequence of aerosol aerodynamics within the geometrical array of the proximal bronchi\(^7\) (Figure 1, inset). This is further confirmed here by the fact that the central hot-spots occurred at the same anatomical airway location for both 6\(\mu\)m and 3\(\mu\)m particles for any given breathing flow (Figure 2), where only the intensity of the radioaerosol hot-spots differed between the particle sizes. This supports the inference that the central hot-spots observed in this present study do not \textit{a priori} reflect heightened aerosol trapping typically associated with airway narrowing. There may however be a superimposed effect of airway constriction, as exemplified here by the correlation between hot-spot intensity and the patient’s severity of airway narrowing. This implies that with worsening disease (lower FEV\(_1\)), central hot-spots will become more intense and hence, relatively less aerosol dose is delivered to the distal lung regions beyond these airways. More severe central airway narrowing can render central hot-spots more prominent even for small particle sizes, while more peripheral airway obstruction may lead to additional hot-spots emerging in more distal airway locations\(^4,6,14\).

Our findings have important translational relevance into daily clinical practice, implying that a substantial portion of aerosol lung dose out of current therapeutic inhaler devices, will be deposited in the proximal bronchi. Consequentially, the potential lung area reached by the
remainder of the aerosol will be considerably reduced, and depending on the therapeutic target this may or may not be an advantage. Indeed, clinical efficacy of the different inhaled drug molecules is determined by the physical location of corresponding lung receptors, where bronchodilators act on airway smooth muscle which is predominant in central airways, while receptors for anti-inflammatory therapy are in both central and distal air spaces\textsuperscript{8,9}. It is increasingly being recognized that the small airways are also an important therapeutic target in patients with asthma and COPD\textsuperscript{15}, which would require small particles inhaled at low flows. Our deposition data (Figure 3) provide an estimate of drug dose and lung reach that can effectively be achieved by such aerosols.

Deposition in the proximal bronchi has been shown to be greater for a dry powder inhaler (DPI) aerosol than for a nebulized aerosol\textsuperscript{16}. Using a DPI inhaled at 80L/min\textsuperscript{17}, healthy males achieved 30% lung deposition, with 33% of lung dose in the large bronchi (1st shell). This is in agreement with our fast flow data (Figure 3A), where central (hot-spot) deposition for 1.5-, 3- and 6-\(\mu\)m particles (60L/min) averages respectively 24%, 34% and 52% lung deposition. Considering in a first approximation, a mix of these particle sizes in equal amounts, this would imply that the large bronchi receive 36% of right lung dose. Similar calculations can be made from Figure 3A for other therapeutic inhaler devices with different particle size and breathing flow characteristics.

Monodisperse aerosols are specifically suited for accurate assessment of lung deposition\textsuperscript{18}, showing here that central hot-spots became systematically more prominent as
particle size and flow rate increased, and intensified with worsening patient severity (decreasing FEV$_1$). A secondary aspect of our analysis concerned the distal lung reach of those aerosols that escape the proximal bronchi. If for instance, central hot-spot deposition is 30%, it follows that 70% will be spread out more peripherally, however no inference can be made about how peripheral that is. This is exactly what is quantified by the aerosol lung area (Figure 3B) independent of how hot the central hot-spots are.

**Limitation of our study**

Our hot-spot quantification of proximal airway deposition on planar images can be viewed as a surrogate for 3D imaging of inhaled aerosol deposition, which would enable a more accurate identification of proximal bronchi$^{19,20}$. However, the radioactive burden and computational complexity of 3D approaches precludes their widespread application in patient studies. Despite its limitations, analysis of planar lung scintigraphy has the advantage that the aerosol can be inhaled and imaged with the patient in a natural upright posture. We used Krypton gas, which is one of the 4 methods to delineate the lung boundary$^2$, to serve as the maximum lung reach an aerosol could achieve. While our data will apply to asthma patients within the range of disease severity considered here (mild-to-moderate), this may be different for more severe asthma, COPD or cystic fibrosis patients. In those cases, a quantitative evaluation of hot and cold spots that do not have a specific anatomical link may be more appropriate$^{12}$. 
In conclusion, we have quantified here the extent of central drug deposition with increasing particle size in upright sitting asthma patients. This was also done for inhaled flows over 30L/min that are pertinent to a common class of clinical inhalers, but for which little quantitative data on central airway deposition exist. These quantitative data are important to guide future inhaled drug development, not only next-generation bronchodilators and anti-inflammatory treatments, but also inhaled biotherapeutics\textsuperscript{21} where the therapeutic index will be critical.
ACKNOWLEDGEMENTS: SV had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. M.F.B. obtained and formatted the images. S.V. and O.S.U. designed the study, performed analyses and wrote the manuscript. The authors would like to thank Senior Research Nurse Sally Meah for her assistance during this study.

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REFERENCES


FIGURE LEGENDS

Figure 1
Posterior lung images after inhalation of Krypton gas and six combinations of aerosol particle size (1.5-, 3-, 6-µm) and breathing flows (30-, 60-L/min) for one asthma patient (FEV₁=79%pred). Centered around the two local peaks of maximum activity of the right lung (black single pixels), two hot-spot ROIs are defined (thin black squares). Thick black line: isocontour ROI for determination of lung reach. Red square: ROI corresponding to swallowed particles in the stomach.

Figure 1, Inset: example of aerosol deposition in a 3D print cast to illustrate the expected hot-spots in the proximal bronchi up to generation 5 (1 big one in the left lung and 2 smaller ones in the right lung) on which the in vivo hot-spot ROIs are based.

Figure 2
Vertical activity profiles of the right lung corresponding to the images in Figure 1. The vertical dashed line is drawn in between the two peaks for 6µm and 60L/min to serve as a reference for all profiles. The Krypton gas profile does not show any peaks in activity, in contrast to 6- and 3-µm (and sometimes 1.5µm) aerosols showing peak activity at similar vertical locations across particle size and breathing flow (see vertical profiles of all patients in the Online Repository).
Figure 3

Panel A: Hotspot intensity, computed from the two hot-spot ROIs as illustrated in Figure 1, after inhalation of six combinations of aerosol particle size (1.5-, 3-, 6-µm) and breathing flow (30-, 60-L/min) for all asthma patients. Superimposed at the base of the vertical bars are the average values for total lung deposition (as a % delivered dose) in these patients (retrieved from Usmani et al\textsuperscript{10}).

Panel B: Lung area reached by six combinations of particle size (1.5-, 3-, 6-µm) and breathing flow (30-, 60-L/min) for all asthma patients (as a % of lung area reached by Krypton gas); lung areas correspond to all pixels within the isocontour ROI as illustrated in Figure 1. Asterisks (*) indicate significant differences (P<0.05) in comparison to 1.5 µm.
Figure 2
Figure 3

A

Hot Spot Intensity (% of Lung)

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<th>Size (μm)</th>
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<td>56</td>
<td>51</td>
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B

Aerosp. Lung Area (% of Lung)

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<th>Size (μm)</th>
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Inhaled aerosol dose distribution
between proximal bronchi and lung periphery.

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ONLINE REPOSITORY
1/ Methodological details of aerosol generation and delivery

The monodisperse albuterol aerosols were generated using our aerosol generation and delivery system. This comprised a spinning top aerosol generator (STAG; Mark II; Research Engineers Ltd.), aerosol chamber for accumulation of aerosol prior to inhalation and an inhalation control unit for regulating aerosol delivery to the patient. The primary aerosol droplet diameter generated by the STAG is inversely proportional to the rotational speed of a disc spinning at high speed. This rotational speed can be varied to produce the three particle sizes with smallest sizes generated at higher rotational speeds than the larger ones. The STAG was calibrated to generate the three aerosol sizes (1.5, 3, 6 um) reproducibly.

The radioisotope used was technetium-99m (99mTc). We verified that this was fixed to the drug itself using an Andersen Cascade impactor (ACI, Graseby-Andersen, Smyrna, GA, USA) which showed the same proportion of isotope, radiolabelled albuterol and unlabelled albuterol were deposited on the ACI plates. The particle size distributions of the ACI were also compared with those determined by an aerodynamic particle sizer (APS, model 3310 with 3302 100:1 Diluter, TSI Inc., St. Paul, MN, USA). As a quality control, the generated aerosols were sampled by the APS immediately before inhalation to verify the particle size and drug concentrations were correct. A Fleisch flow transducer head and pneumotachograph were used to measure the patient's inhalation flow. Patients were allowed to practice the inhalation manoeuvre several times without aerosol prior to inhaling aerosol.
2/ Equivalent of Figure 2 for all patients (n=12)

Patient 1

Patient 2

Patient 3
Patient 7

(missing image for 3µm; 30 L/min)

Patient 8

Patient 9

(missing image for 3µm; 30 L/min)
Patient 10

30 L/min

Normalized Counts

vertical distance from bottom (cm)

Patient 11

30 L/min

Normalized Counts

vertical distance from bottom (cm)

Patient 12

30 L/min

Normalized Counts

vertical distance from bottom (cm)

30 L/min

Normalized Counts

60 L/min

vertical distance from bottom (cm)

60 L/min

Normalized Counts

60 L/min

vertical distance from bottom (cm)
3/ Equivalent of Figure 3 for 12 individual patients

Patient 1
Patient 2
Patient 3

![Bar chart showing Hot Spot Intensity (%) and Aerosol Lung Area (%) for 1.5μm, 3μm, and 6μm particles in patient 3.]

- **Hot Spot Intensity (%)**
  - 1.5μm: 10%
  - 3μm: 20%
  - 6μm: 60%

- **Aerosol Lung Area (%)**
  - 1.5μm: 80%
  - 3μm: 60%
  - 6μm: 40%
Patient 4

![Graph showing aerosol lung area and hot spot intensity for different particle sizes.](image)
Patient 5
Patient 6

![Graphs showing aerosol lung area and hot spot intensity for different particle sizes.](image-url)
Patient 7

(missing image for 3µm; 30 L/min)
Patient 8

[Bar charts showing aerosol lung area and hot spot intensity for three different particle sizes (1.5µm, 3µm, 6µm).]
Patient 9

![Graph showing aerosol lung area and hot spot intensity for different aerosol sizes (1.5µm, 3µm, 6µm).]
Patient 10

![Graph showing aerosol lung area and hot spot intensity for patients at different particle sizes.](image-url)
Patient 11

- Hot Spot Intensity (%lung)
- Aerosol Lung Area (%lung)
**Patient 12**

![Bar Charts]

- **Hot Spot Intensity (%lung)**
  - 1.5µm, 3µm, 6µm

- **Aerosol Lung Area (%lung)**
  - 1.5µm, 3µm, 6µm