JOURNAL OF AEROSOL MEDICINE AND PULMONARY DRUG DELIVERY
Volume 28, Number 3, 2015

Pp. 211–218
DOI: 10.1089/jamp.2013.1095

Heterogeneity of Particle Deposition by Pixel Analysis of 2D Gamma Scintigraphy Images

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Abstract

Background: Heterogeneity of inhaled particle deposition in airways disease may be a sensitive indicator of physiologic changes in the lungs. Using planar gamma scintigraphy, we developed new methods to locate and quantify regions of high (hot) and low (cold) particle deposition in the lungs.
Methods: Initial deposition and 24 hour retention images were obtained from healthy (n=31) adult subjects and patients with mild cystic fibrosis lung disease (CF) (n=14) following inhalation of radiolabeled particles (Tc99m-sulfur colloid, 5.4 μm MMAD) under controlled breathing conditions. The initial deposition image of the right lung was normalized to (i.e., same median pixel value), and then divided by, a transmission (Tc99m) image in the same individual to obtain a pixel-by-pixel ratio image. Hot spots were defined where pixel values in the deposition image were greater than 2X those of the transmission, and cold spots as pixels where the deposition image was less than 0.5X of the transmission. The number ratio (NR) of the hot and cold pixels to total lung pixels, and the sum ratio (SR) of total counts in hot pixels to total lung counts were compared between healthy and CF subjects. Other traditional measures of regional particle deposition, nC/P and skew of the pixel count histogram distribution, were also compared.
Results: The NR of cold spots was greater in mild CF, 0.221 ± 0.047(CF) vs. 0.186 ± 0.038 (healthy) ($p < 0.005$) and was significantly correlated with FEV1 %pred in the patients ($R = -0.70$). nC/P (central to peripheral count ratio), skew of the count histogram, and hot NR or SR were not different between the healthy and mild CF patients.
Conclusions: These methods may provide more sensitive measures of airway function and localization of deposition that might be useful for assessing treatment efficacy in these patients.

Key words: gamma scintigraphy, inhaled particles, regional deposition

Introduction

Heterogeneity of particle deposition in the lung may be a sensitive indicator of non-homogeneous airway obstruction associated with airways disease. The heterogeneity of particle deposition by gamma scintigraphy has generally been assessed by partitioning lung images into multiple, but relatively large, regions of interest (ROIs).[1,2] To date, these relatively simple, but still effective, methods have been used to characterize regional deposition of radiolabeled particles in the lung. Central to peripheral, or outer to inner, ratios of deposited activity show strong correlations with particle clearance from the lungs (i.e., increasing central [inner] deposition results in increased clearance up to 24 hours post deposition).[3–5] However, for cystic fibrosis (CF), asthma, and chronic obstructive pulmonary disease (COPD), regional deposition may be quite heterogeneous throughout the entire lung (not just central vs. peripheral) and require additional, detailed analyses that might better characterize both the regional deposition and subsequent clearance of particles from the lungs.

Previous 2D scintigraphy studies have illustrated the increased ''patchiness'' of particle deposition associated with chronic or induced bronchoconstriction. For example, a study by Pellegrino et al.[6] clearly illustrated this effect in asthmatics as they were challenged with increasing doses of methacholine to compare the pattern of fine particle deposition in the lung with the occurrence of expiratory flow limitation. As maximal expiratory flow was reduced following methacholine challenge, particle deposition became

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less uniform with increasing occurrence of ''hot spots,'' presumably at sites of airway obstruction and expiratory collapse. Our recent studies showed both an increase in bronchial airway deposition and increased ''patchiness'' of particle deposition 4 hours post allergen and endotoxin challenge, despite the fact that spirometric function had returned to pre-challenge values prior to inhalation of the radiolabeled aerosol.[7,8] We assessed regional deposition heterogeneity as the skew of the histogram distribution (counts/pixel vs. #pixels)[9] within the right whole lung ROI, increasing with increased frequency of ''hot spots'' in the lung. These hot spots are presumed due to increased deposition within bronchial airways throughout the lung so that skew is independent of the specific region within the lung (e.g., central vs. peripheral).

Using planar gamma scintigraphy, we have developed new analytical methods that are independent of specific regions of interest to improve localization/quantification of high (hot) and low (cold) regions of inhaled, particle deposition. We have applied and compared these methods in a group of healthy and cystic fibrosis (CF) patients with mild airways disease to determine if they are sensitive at distinguishing regional deposition in these two cohorts and their relationship to subsequent particle clearance. We also compare these new indices to the standard ROI measures of regional deposition and the skew of the histogram distribution.

Methods

Healthy nonsmoking adult subjects ($n = 31$, 20M/11F, mean FEV1%pred = 104, mean age 26) and mild CF ($n = 14$, 9M/5F, mean FEV1%pred = 81, mean age 25) adults participated in the study. These subjects had been recruited for studies of mucociliary clearance (MC) that required 2D gamma scintigraphy of inhaled radiolabeled particles over time. The regional deposition analyses performed here were done on the initial deposition scans associated with baseline measures of MC in these subjects. Informed written consent was obtained from all subjects prior to their participation in the study that was approved by the Biomedical Institutional Review Board of the University of North Carolina at Chapel Hill.

Each subject was seated with his/her back against a large field-of-view gamma camera (Mie America BodyScan). A rectangular phantom containing the radioisotope Tc99m (2 mCi) and water was placed in front of the subject at a specified distance (5 cm) for acquisition of a transmission scan of the lungs.[10] Subject alignment in front of the camera was indicated by a laser pointer projected on a marker at the sternal notch. Approximately 4 mCi (in 2 mL) of Tc99m-sulfur colloid solution was loaded in a Devilbiss 646 nebulizer (Sunrise Medical; Somerset, PA) from which the subject inhaled according to a prescribed breathing pattern (30 breaths/min at 500 mL/sec) for less than a minute.[5] Based on the initial camera count rate from lungs, we estimated approximately 40 uCi deposited in the lungs of each subject. A 2-min deposition image was obtained from subjects (128×128) immediately following inhalation of the radioparticles. A 30-min scan was obtained 24 h (± 4 h) after initial deposition and was background/decay corrected for comparison to the initial scan (i.e., 24 h Retention). Sulfur colloid particles radiolabeled with technetium (99mTc-SC) were prepared from Sulfur Colloid Kits (CIS-Sulfur Colloid, CIS-US, Inc. Bedford, MA) following the procedure provided by the manufacturer. The binding of 99mTc to SC for these binding kits was greater than 98% determined by paper chromatography.[11] The submicrometer (0.22 μm, gsd 1.75) Tc99m SC particles[11] are insoluble and were suspended in a normal saline solution for delivery by the jet nebulizer (Devilbiss 646). A Malvern Mastersizer S (Malvern Instruments USA, Westborough, MA) was used to determine the volume median diameter (VMD) of the nebulized aerosol (5.4 μm) and the geometric standard deviation (1.8).

Regional deposition analysis of gamma camera images

For many years, our laboratory and others[3,5,12] have used the Xe133 ventilation equilibrium scintigraphy scan to both determine lung outline and to normalize particle deposition to lung volume in measures of regional lung deposition. We recently showed that normalization of regional particle deposition by the Xe133 vs. Tc99m transmission scans (i.e., nC/P) were highly correlated.[10] On the one hand, using a transmission scan instead of a ventilation scan to define the lung borders may limit problems associated with poorly ventilated lung regions in patients. However, it is also clear that a transmission scan does not provide a true volumetric correction for the 2D lung image, but rather produces a density image through the entire thickness of the chest, independent of gas volume heterogeneities, proportional only to the density of the tissue through which it attenuates.[10] Nevertheless, because it is increasingly technically difficult for many laboratories to obtain gas equilibrium scans, our goal in this study was to use the transmission scan to approximate the lung volume associated with the 2D deposition images. To determine how accurately transmission scans approximate volume, we obtained a Xe133 equilibrium scan as a gold-standard reference, and compared it to the corresponding Tc99m transmission scan, as described by Zeman et al.,[10] in our first ten healthy subjects studied (Fig. 1).

After normalizing each pair of images to the same median count value, the Xenon image was divided by the transmission image. We found on average that 98% of the pixel count ratios were in the range of 0.5 to 1.6 for this xenon/transmission ratio image, indicating the range of error when using transmission scan to define volume. These limits were then used to guide selection of the thresholds that defined whether a pixel (having dimensions of 4.77 mm square) was considered ''hot'' (deposition/transmission ratio > 2) or ''cold'' (deposition/transmission ratio < 0.5) in subsequent analyses (described further below). The pixel size, 4.77 mm, is greater than the intrinsic resolution of the camera (3.5 mm) and therefore is the resolution associated with our measurements.

Only the right lung was used to analyze regional deposition because of the potential overlap of stomach and lung activity on the left side. The transmission scan was used to identify the outline of the lung field and to create regions of interest (ROI)[10] (Fig. 2). Both the transmission and initial deposition images were smoothed by Gaussian-filter to reduce image noise (i.e., convolution or smoothing of the image with a Gaussian function). The initial deposition image of the right lung was normalized to (i.e., same median pixel value) and then divided by the transmission (Tc99m) image in the same individual to obtain a pixel-by-pixel ratio

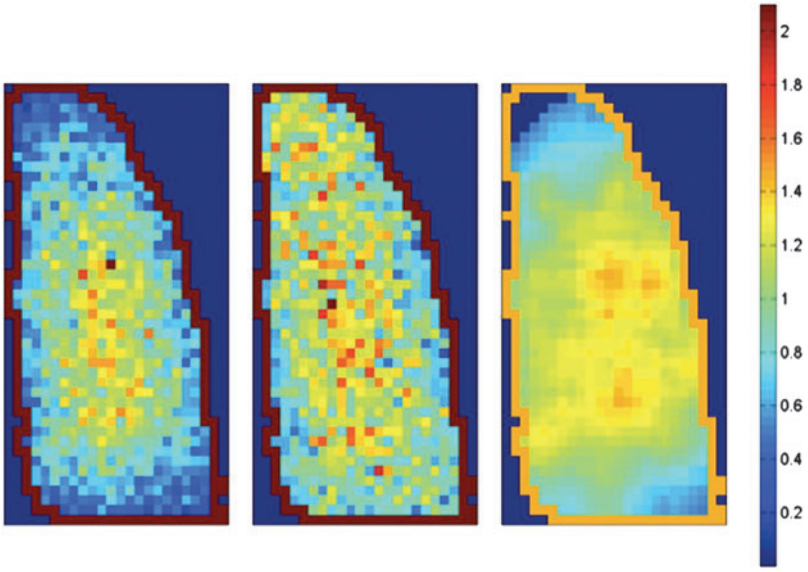

FIG. 1. *Left:* Right lung xenon equilibrium image for healthy subject; *Center:* Transmission image for same subject normalized to same median counts as xenon image; *Right:* Xenon divided by transmission image with color scale showing relative ratio for each pixel. For this subject: % of total pixels where xenon > 1.6 Trans = 0 pixels; % of total pixels where xenon < 0.5 Trans = 3.6.

image (Fig. 2a and b, left). Hot spots (H) of deposition were defined where pixel values in the deposition image were greater than 2X of transmission (Fig. 2a and b, center), and, similarly, cold spots (C) as pixels where the deposition image was less than 0.5X of transmission (Fig. 2a and b, right). In other words, hot spots were defined to be those pixels that had more than 2X the activity of that predicted by their associated volume, as determined by our estimate from transmission scans. Similarly, cold spots were defined as those pixels having less than 0.5 of their predicted activity based on their associated volume. The number ratio (NR) of hot (or cold) spots in the right lung was then determined by

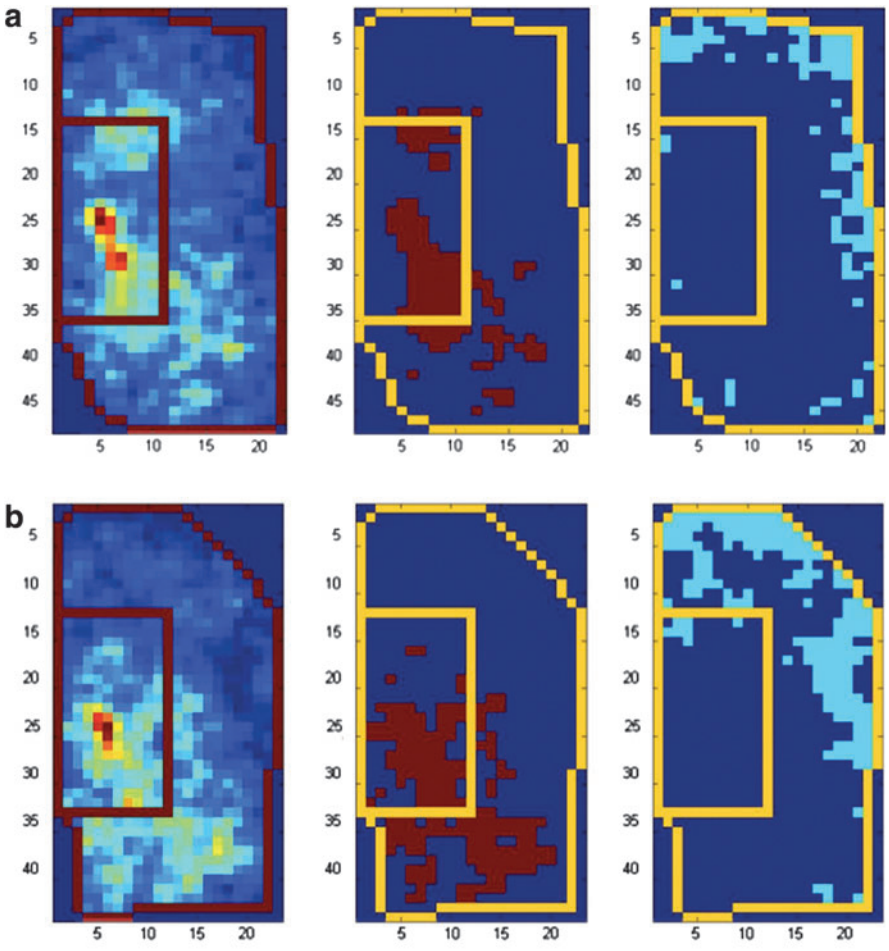

FIG. 2. **(a)** *Left:* Ratio image for particle deposition in right lung of healthy subject; *Center:* Deposition hot spots (NR = 0.144) (*red* = hot); *Right:* Deposition cold spots (NR = 0.162) (*light blue* = cold); nC/P = 1.752, C/WL = 0.408, and skew = 1.481. **(b)** *Left:* Ratio image for particle deposition in right lung of CF patient; *Center:* Deposition hot spots (NR = 0.221) (*red* = hot); *Right:* Deposition cold spots (NR = 0.239) (*light blue* = cold); nC/P = 1.594, C/WL = 0.410, and skew = 1.288.

counting the number of hot (or cold) pixels and dividing by the total number of pixels in the whole lung region (Fig. 2). The sum ratio (SR) of hot pixels was determined by dividing the total number of counts in hot pixels by the total counts in the whole right lung region.

These hot and cold deposition metrics were compared to the more traditional measures of central to peripheral ratio (nC/P) of deposition[2,3,5,12] and the skew of the histogram distribution (counts/pixel vs. #pixels) in the whole right lung.[9] To assess central (C) vs. peripheral (P) deposition, two regions of interest (ROI) were created over the right transmission lung image (Fig. 2); 1) the same outline region created for the whole lung as described above, and 2) a central (C) ROI, created by first circumscribing a rectangular whole lung region on the edges of the whole lung outline region and then creating a C region with dimensions equal to half the whole lung rectangular ROI's width and one-half its height.[10] The C region was positioned on the medial boundary of the lung, centered by height, 25% of the area of the whole lung rectangular ROI. The peripheral region (P) was defined as the area lying between the rectangular central and whole lung outline ROI (Fig. 2). These regions were overlaid onto the initial aerosol deposition scans to determine the initial counts in each region (Fig. 2). We then calculated the ratio of central to peripheral counts, (C/P)Dep, and normalized this ratio by dividing by the central-to-peripheral ratio for the transmission scan,

$$(C/P)Dep/(C/P)Trans = nC/P$$

This normalization was done to account for the difference in relative lung areas and thickness between the central and peripheral regions. nC/P provides an index of relative deposition between the two regions. A nC/P of 1.0 reflects equal deposition in each region. However, because the central region outlines both bronchial airways and lung parenchyma surrounding these airways, a nC/P of near unity reflects primarily deposition in the pulmonary airspaces distal to anatomic dead space. Increases in nC/P to values greater than unity reflect an increase in central vs. peripheral deposition primarily as a result of increased bronchial deposition. To determine skew, frequency distribution histograms were constructed from the right lung deposition images and whole lung outline ROI created from the transmission scan, with the number of pixels with a given count value (expressed as a fraction of total pixels) on the y axis and the count values on the x axis.[9] These histograms were analyzed for skew (a measure of histogram symmetry, the third moment about the mean of the histogram),[9] with increasing skew reflecting deposition heterogeneity.

To assess the apical to basal lung distribution of particle deposition and make comparisons with the new indices of hot and cold deposition, we calculated upper to lower (U/L) ratio of deposition by dividing the right whole lung rectangular region into three equal rectangles (upper, mid, and lower).[13] The U/L ratio of deposition activity was determined and normalized by the U/L for the transmission scan in the same fashion as nC/P described above to provide nU/L.

Finally, because the various deposition indices have been shown to correlate with particle retention in the lung, we compared these indices to the 24-h retention of lung activity in each subject. Again 24 h retention was only determined from the right lung rectangular ROI and was background/decay corrected to the same time as the deposition image.

Table 1. Deposition Heterogeneity for the Whole Lung

	NR of Hot	*NR of Cold*	*SR of Hot*	*nC/P*	*C/WL*	*Skew*
Healthy ($n=31$)	**0.144**	**0.186**	**0.306**	**1.612**	**0.409**	**1.510**
SD	0.059	0.038	0.121	0.323	0.053	0.559
Mild CF ($n=14$)	**0.157**	**0.221**	**0.338**	**1.594**	**0.403**	**1.669**
SD	0.036	0.047	0.090	0.345	0.053	0.748
P value	0.44	**0.01**	0.38	0.87	0.76	0.43

Statistical methods

Comparisons of indices between healthy and CF subjects were analyzed using Student's *t*-test (Excel for MacIntosh). The significance of relationships between individual variables was tested using linear regression analysis (Stata for

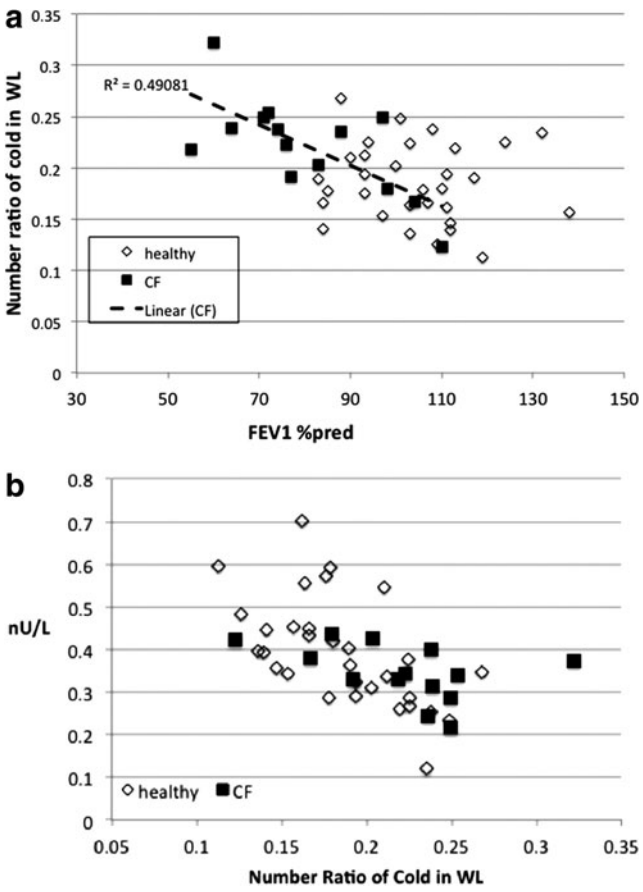

FIG. 3. **(a)** Number ratio (NR) of cold deposition in whole lung (WL) for CF and healthy subjects as a function of FEV1 %pred. Regression line is shown for CF ($R=-0.70$, $p<0.05$ for CF, NS for healthy). **(b)** Normalized ratio of deposition in upper/lower (nU/L) regions of the lung vs. number ratio of cold deposition in whole lung (WL) for CF and healthy subjects. ($R=-0.44$, NS, and $R=-0.58$, $p<0.005$, for CF and healthy, respectively).

Table 2. Central (C) Region Heterogeneity

	NR of Hot in C relative to WL	*NR of Hot in C relative to C*	*SR of Hot in C relative to WL*	*SR of Hot in C relative to C*	*NR of Cold in C relative to WL*	*NR of Cold in C relative to C*
Healthy ($n=31$)	**0.074**	**0.261**	**0.182**	**0.430**	**0.011**	**0.039**
SD	0.032	0.112	0.089	0.170	0.009	0.031
Mild CF ($n=14$)	**0.079**	**0.277**	**0.195**	**0.469**	**0.018**	**0.063**
SD	0.026	0.091	0.081	0.149	0.012	0.043
P value	0.60	0.65	0.65	0.47	**0.04**	**0.04**

MacIntosh). An overall significance level of $p \leq 0.05$ was considered to be significant. All values are expressed as the mean ($\pm$standard deviation).

Results

CF patients had relatively mild obstruction but had significantly different spirometric values than the healthy subjects (FEV1% pred = 81 ± 17 vs. 104 ± 14, respectively, $p < 0.001$)). Table 1 provides a summary of deposition heterogeneity for the two groups of subjects. We found no differences between these groups in traditional measures of regional deposition, as described by nC/P or skew. Only the NR of cold spots was significantly greater in CF vs. healthy subjects, and was inversely correlated with FEV1 %pred in the CF patients ($R = -0.70$) (Fig. 3a).

Tables 2 and 3 summarize hot and cold spot analysis as applied to both the C and P lung regions. These data show that for either group the NR and SR of hot spots for the whole lung (WL) were evenly distributed between the C and P regions. On the other hand, the cold spots were almost entirely associated with the P region in both groups. As with the whole lung (Table 1), the difference in NR of cold spots between the two groups was also statistically significant within both the C and P regions.

The NR of cold spots was inversely correlated with the nU/L ratio, Figure 3b, in the healthy subjects, suggesting that most sites of little or no particle deposition occurred predominantly in the apices of the lungs (as illustrated in Fig. 2). There was a trend towards lower nU/L in the mild CF patients when compared to healthy subjects (mean nU/L = 0.346 ± 0.066 and 0.393 ± 0.127, respectively) but the difference was not statistically significant ($p = 0.20$).

Table 4 provides a summary of correlations (R values) between traditional deposition descriptors (nC/P, C/WL, and skew) and the hot and cold spot indices. The highest correlations in either study group occurred between the SR of hot spots and skew and nC/P. There was a significant inverse relationship between 24 h retention and both nC/P and SR of hot spots (Fig. 4a and b, respectively) in each group of subjects ($p < 0.05$). CF patients had significantly higher 24 h retentions than healthy subjects (0.74 ± 0.15 vs. 0.58 ± 0.15 respectively, $p = 0.002$) suggesting slowed clearance of particles from the bronchial airways in CF.

Discussion

The heterogeneity of inhaled particle deposition in CF patients is likely due to airway obstruction and the associated inhomogeneity in ventilation.[13,14] We chose to study CF patients with relatively mild obstruction (mean FEV1 = 81%pred) to assess the sensitivity of the various indices of regional deposition by 2D gamma scintigraphy for differentiating between healthy and CF patients. We found no difference in nC/P and skew between these CF patients with mild disease and our healthy controls. Previous studies assessing regional deposition by nC/P and/or skew in CF patients have also found no difference when the patients had mild disease,[4] but as their reduction in FEV1 becomes more moderate-severe, both nC/P[13] and skew[15] are significantly increased from normal. While we found no difference in these traditional indices of regional deposition (i.e., nC/P and skew) between healthy and mild CF subjects, our new approach revealed more cold deposition spots in CF (i.e., areas of little or no particle deposition as predicted by the volume in those areas). The number of deposition cold spots increased with decreasing FEV1 in the CF patients. Others have analyzed heterogeneity of ventilation,[16] or ventilation relative to perfusion[17] by dividing the lung into smaller regions of interest but to our knowledge we are the first to perform 2D ''pixel'' analysis on regional particle deposition normalized to lung volume as approximated by transmission scans in the same individual. Similar analysis in 3D should be possible by comparing the deposition within

Table 3. Peripheral (P) Region Heterogeneity

	NR of Hot in P relative to WL	*NR of Hot in P relative to P*	*SR of Hot in P relative to WL*	*SR of Hot in P relative to P*	*NR of Cold in P relative to WL*	*NR of Cold in P relative to P*
Healthy ($n=31$)	**0.069**	**0.084**	**0.124**	**0.203**	**0.174**	**0.211**
SD	0.059	0.047	0.064	0.106	0.038	0.044
Mild CF ($n=14$)	**0.078**	**0.095**	**0.143**	**0.229**	**0.203**	**0.249**
SD	0.031	0.038	0.058	0.083	0.046	0.060
P value	0.50	0.44	0.33	0.42	**0.04**	**0.02**

Table 4. Correlation Index (R) Table for New vs. Old Deposition Indices

	NR of hot		*SR of hot*		*NR of cold*	
	Healthy	*CF*	*Healthy*	*CF*	*Healthy*	*CF*
nC/P	0.42	0.31	0.53^	0.60*	−0.19	−0.32
C/WL	0.45	0.20	0.55^	0.50	−0.16	−0.14
Skew	0.41	0.16	0.61^	0.51	−0.14	−0.13

*$p < 0.05$, ^$p < 0.005$.

a voxel to the lung volume associated with that voxel (obtained by either a transmission or equilibrium gas scan) to obtain a relative deposition per unit volume for which thresholds of hot and cold deposition might be applied.

While the hot spot indices, either number or sum ratio, were not different between the two groups, the sum ratio significantly correlated with both nC/P and the skew of counts/pixel histogram (Table 4). Nevertheless, the correlations between SR of hot spots and nC/P were not high (R = 0.53 and 0.60 for healthy and CF, respectively) reflecting the fact that the location of these hot regions was not necessarily associated with the central lung ROI. While clearly most of the cold spots were located in the P region for each group (Table 3), the hot spots (both by number and sum) were distributed nearly equally between the C and P regions (as a fraction of the whole lung) (Tables 2 and 3, and Fig. 2), suggesting that the P region clearly includes bronchial airways where particles may deposit in hot spots and clear the lung through 24 hours (Fig. 4b). Likewise the C region, on a volumetric basis, is primarily made up of small airways and alveolated airspaces. Thus, the nC/P ratio, while useful as an index of changes in bronchial airway deposition, does not necessarily provide a true reflection of airway vs. alveolar deposition throughout the whole lung.

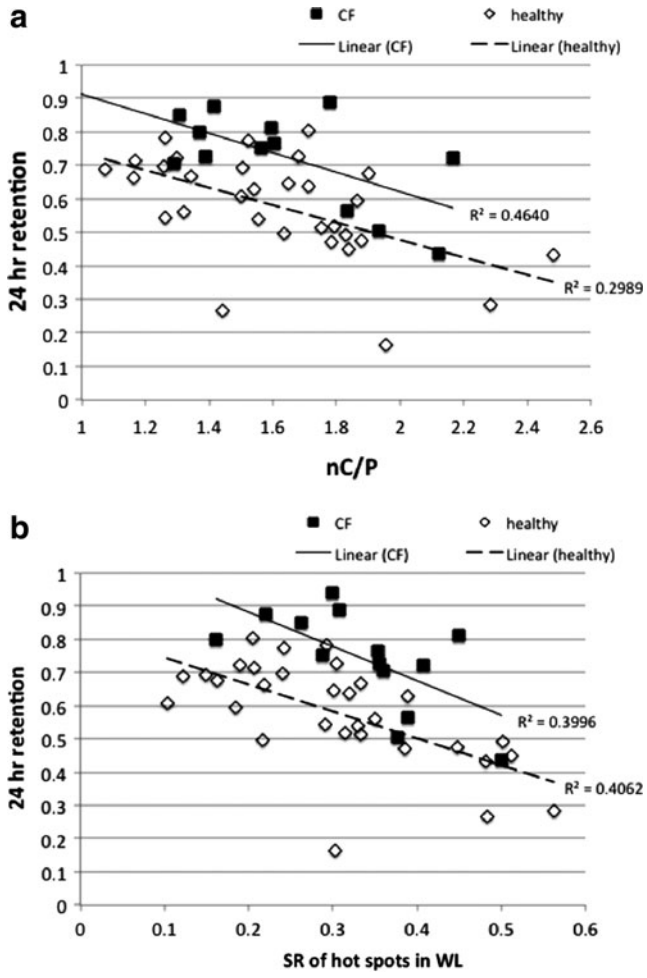

FIG. 4. **(a)** 24-hour retention as a function of nC/P in healthy and mild CF patients (R = −0.55 and −0.68, respectively, $p < 0.01$ and 0.005). **(b)** 24-hour retention as a function of sum ratio (SR) of hot spots in healthy and mild CF patients (R = −0.63 and −0.64, respectively, $p < 0.005$ and 0.02).

As discussed in Newman et al.,[18] we should consider using ''outer'' (instead of peripheral) vs. ''inner'' (instead of central) as standard nomenclature for describing these regions in the future. Since skew is primarily dependent on the presence of hot spots in the lung independent of region, the skew vs. SR of hot relationship is not all that surprising. The advantage of the sum ratio of hot spots to skew for assessing regional deposition is that while both provide a metric of regional deposition, the hot spot analysis also provides a mapping of hot and cold pixels that might be related to specific lung regions or anatomy. For example, it may be possible to compress 3D CT images into a 2D format that maintains airway vs. parenchymal differentiation and can be co-registered and compared with the 2D mapping of hot and cold spot deposition. As discussed above, application of our methods to 3D SPECT deposition images should also be possible and make comparison to 3D CT images in the same individual even easier.

The fact that the sum ratio correlates with 24-hour retention (Fig. 4b) suggests that, like the nC/P ratio (Fig. 4a), deposition hot spots are generally associated with bronchial airways that clear by mucociliary function over the 24-h period post-deposition. The 24-h retention was also higher in CF for both a given sum ratio of hot spots and nC/P (Fig. 4). The decreased clearance of particles through 24 hours in CF patients compared to healthy subjects is consistent with our previous findings[4,19] and suggests impaired small airway clearance in CF, even mildly impaired patients such as those studied here. In considering future therapeutic studies, characterization of the hot SR may provide a useful covariate that aids the interpretation of changes in mucociliary clearance over time.

Cold spots tended to occur primarily in the apices of the lung (Fig. 3b) in all subjects, with a trend towards lesser apical particle deposition in CF vs. healthy subjects. It has been shown that the apical lung region of CF patients is disproportionately affected by bronchiectasis[20,21] and is less well ventilated than the base of the lung.[13,22] We showed previously in more severe CF patients that regional deposition and ventilation were compromised in the apical regions of the lung.[13] The lesser apical deposition in both groups studied here may also have been due to the controlled breathing pattern used for these experiments. While the controlled tidal volume (400–500 mL) in the current study was typical of resting adult breathing,[23] it may be less than that employed by patients during inhalation of

aerosolized medications via a mouthpiece. Shallow tidal volumes breathed from FRC in the upright position tend to distribute preferentially to the base of the lung.[24] In fact, it should be emphasized that the results reported here are specific to the particle size (app. 5 μm MMAD) and controlled breathing pattern we employed. Nevertheless, the analytical methods we describe here can be used in future studies to assess regional deposition of a variety of particle sizes and types along with their associated breathing conditions.

Conclusions

These preliminary data suggest that this new method for assessing heterogeneity in particle deposition may provide more sensitive measures of regional particle deposition associated with airway obstruction. In addition, these tools may be useful for characterizing drug delivery to the lungs of patients with airways disease. We are currently applying these methods to assess regional deposition in other patient groups, COPD, and asthma, and under a variety of particle size/breathing conditions.

Acknowledgments

The authors are grateful to Jihong Wu and Heather Duckworth for their assistance in acquisition of the gamma scintigraphy data. The project was supported by a grant from the Cystic Fibrosis Foundation (DONALD07XX0) and National Institutes for Health SCCOR grant (Grant Number 5P50HL084934).

Author Disclosure Statement

The authors declare that there are no conflicting financial interests.

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Received on September 9, 2013
in final form, August 4, 2014

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