Nonreversible conductive airway ventilation heterogeneity in mild asthma

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IT HAS BEEN SUGGESTED THAT in asthma, inflammatory and chronic structural changes potentially occur down to the level of the lung periphery (23), implicating also the alveolar ducts in the inflammatory process of the early asthma disease state (7). Novel drug delivery devices of anti-inflammatory medication claim enhanced peripheral deposition, assuming that the target is "the small airway" (15). Although the need for measurement of small airway function continues to be regularly emphasized (6, 10, 23, 25, 32), in vivo measurements of small airway alteration in the asthmatic patient remain sparse. Despite radiation exposure, high-resolution computed tomography probably provides the best noninvasive measure of structural alterations in different-sized airways (1, 25). Ventilation distribution tests are an attractive noninvasive alternative, and Woolcock (32) actually suggested the use of the vital capacity single-breath washout (SBW) for monitoring small airway function in the follow-up of asthma patients and their treatment.

Since the introduction of the vital capacity SBW test for epidemiological studies after the pioneering work of Cosio et al. (3), various theoretical and experimental studies have been conducted to investigate the actual role of the small airways in ventilation distribution tests in general. Regarding the phase III slope of a vital capacity SBW maneuver, it is intrinsically impossible to distinguish between ventilation heterogeneity originating from structural change at the first and the last branching generation of the human lung, unless tracer gases with different diffusivities are used. To further complicate the issue, airway closure, which has been suggested as an integral part of asthma (14), is also shown to contribute to the vital capacity SBW phase III slope (5). In this respect, abnormal vital capacity SBW N2 phase III slopes obtained by in't Veen et al. (11) in individuals with severe asthma after inhalation of 400 μg salbutamol do point to a residual structural alteration but cannot be conclusive about the size of the airways involved. Other more sophisticated ventilation distribution studies, such as bolus studies (8) or N2-washout studies (16), also previously used to assess the asthmatic lung, essentially suffer from the same drawback that no distinction whatsoever can be made between structural changes occurring at different lung depths.

A phase III slope analysis of the multiple-breath washout (MBW), originally developed for the study of ventilation distribution in normal subjects by Crawford et al. (4), can distinguish ventilation maldistribution due to structural changes originating in the acinar vs. the conductive lung zone, i.e., with a cutoff around generation 15. The MBW phase III slope analysis is based on a model of convection and diffusion in a realistic human lung geometry (20). The conductive MBW index of ventilation heterogeneity relates to the proximal lung structure where convection dominates gas transport, and the acinar MBW index of ventilation...
heterogeneity relates to the peripheral lung where convection-diffusion interaction occurs. Experimental studies showing a differential response of proximal and peripheral MBW indexes to oleic acid-induced edema in mongrel dogs where indeed only the peripheral MBW index was affected (26), or actual correlations of the peripheral MBW index to histomorphometry in rats with induced emphysema (24), lend support to the potential of the MBW test.

A previous MBW study in 20 patients with moderate asthma indicated structural abnormality at the level of both the acinar and conductive lung zones (28). At both these levels, abnormalities were partly reversible after inhalation of 400 μg salbutamol, yet none of the spirometric nor ventilation heterogeneity indexes under study was fully reversed to normal values. Possibly, the degree of reversibility observed in that study may have been influenced by the baseline spirometry and ventilation heterogeneity of the asthma patients on the study day or by the variability of aerosol delivery characteristics. Neither source of variability had been explicitly explored in that study, and both are in fact rarely considered in any study involving asthmatic patients. In the present work, we investigated the maximum possible reversibility in an experimental setting where variability of aerosol delivery was minimal, intrasubject variability of the asthma patient’s baseline condition was taken into account, and state-of-the-art ventilation distribution tests were used. We also deliberately included individuals with mild asthma so as to maximize the chances of complete reversibility. We hypothesized that if optimal β₂-agonist agonist reversibility could be realized in asthma patients under the best of two baseline conditions, a distinctive pattern might be revealed of a residual non-β₂-agonist reversible obstruction with a predominantly acinar or conductive component that would then constitute the target for anti-inflammatory medication or any other drug aimed at prevention of remodeling of the asthmatic lung. As this study will show, it is the conductive airways’ contribution to ventilation heterogeneity that shows a distinct failure to normalize after bronchodilation even in individuals with mild asthma, its magnitude being actually similar to the postdilation conductive abnormality previously observed in individuals with moderate asthma (28).

MATERIALS AND METHODS

Spirometry and ventilation distribution testing. Baseline spirometry was obtained by means of standardized equipment (Vmax 20C, SensorMedics Bilthoven, The Netherlands) reporting forced expired volume in 1 s (FEV₁), peak expiratory flow (PEF), forced vital capacity (FVC), and forced expiratory flow after exhalation of 75% FVC (FEF₇₅). The MBW test was carried out with a computer-controlled breathing assembly in which data acquisition, pneumatic valve control, and visual feedback to the subjects are handled by Labview software (National Instruments, Austin, TX).

The MBW indexes, Scond and Sacin, were essentially the conductive and acinar components of ventilation heterogeneity, respectively, derived from the normalized phase III slope analysis. During each expiration, the N₂ phase III slope is normalized by the mean expired N₂ concentration, leading to an increasing normalized slope as a function of breath number or lung turnover; lung turnover is determined as the cumulative expired volume over functional residual capacity (FRC). In such curves, Sacin is computed as the normalized phase III slope of the first MBW expiration minus a correction term to discard any conductive lung zone contribution; this correction term equals the lung turnover corresponding to the first breath multiplied by Scond. Scond is computed as the rate of normalized phase III slope increase as a function of lung turnover, between 1.5 and 6 lung turnovers (without any correction because there is no acinar lung zone contribution in this part of the MBW). The theory of the phase III slope analysis has been previously described (29), and it implies that ventilation heterogeneity can be attributed to different lung levels and that Scond and Sacin are intrinsically independent. Because Scond and Sacin are derived from phase III slopes, their values increase when ventilation heterogeneity increases. In particular, Sacin will increase in value if ventilation heterogeneity is increased in the acinar lung zone, due to an alteration of the intra-acinar asymmetry, before the turn of flow asynchrony of flow asynchrony. On the other hand, if the conductive airways and their subtended units undergo differences in specific ventilation and deflate in asynchrony, such that the best ventilated unit empties first preferentially early in expiration.

Reversibility testing. A computer-controlled aerosol delivery setup previously used for aerosol bolus experiments (24) was adapted to incorporate a 750-ml spacer (Volumatic, GlaxoSmithKline, Research Triangle Park, NC) and a pressured metered dose inhaler (100 μg hydrofluoroalkane-Ventolin, GlaxoSmithKline, Research Triangle Park, NC). The spacer was coated with an anionic detergent, diluted in water 1:2,500 according to recommendations (21), and dried during the night preceding the day of reversibility testing (only 1 reversibility test on any given day). The timing of the salbutamol aerosol release into the spacer and inhalation with respect to the patient’s breathing pattern was strictly controlled: the residence time of the 100-μg aerosol in the spacer was invariably 3 s, and the time between onset of aerosol inspiration and end of the end-inspiratory breath hold was fixed at 20 s. The reversibility protocol started with baseline spirometry and three MBW tests, after which two 100-μg puffs were administered via the spacer-incorporated salbutamol delivery setup (1 inhalation maneuver per 100-μg puff). After exactly 10 min, only spirometry was recorded and a second batch of two 100-μg puffs was delivered in an identical fashion. After another 10-min interval, spirometry was repeated and three MBW tests were carried out.

Patients. The study protocol was approved by the local ethics committee. The 15 patients participating in this study had a clinical history of asthma (>2 yr) and had shown, in the 2-yr period before testing, a positive histamine provocation test or a baseline obstruction with >10% of predicted FEV₁ reversibility. Five patients were receiving maintenance treatment (inhaled corticosteroids with or without long-acting bronchodilators), whereas the remaining ten only used salbutamol as rescue medication. All asthma patients were never-smokers. After having obtained informed consent, we requested all patients to withdraw from any medication 12 h before the time of study, which patient was asked to come to the laboratory for up to 3 separate days at the same time of the day to study the influence of intrasubject baseline variability on reversibility, under experimental conditions that guaranteed minimal variability of the salbutamol aerosol delivery itself. Visit 1 included baseline spirometry and venti-
Spirometry and ventilation distribution data obtained on all asthma patients on two visits each, with visits classified as “best” or “worst” according to baseline FEV1 and corresponding dilation

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<th>Baseline Value</th>
<th>Postdilation Value</th>
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<tr>
<td></td>
<td>Best visit</td>
<td>Worst visit</td>
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<tr>
<td>FEV1, %pred</td>
<td>87.1±6.2</td>
<td>96.6±4.9</td>
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<td>FVC, %pred</td>
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<td>112.9±3.4</td>
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<td>PEF, %pred</td>
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<tr>
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<td>58.4±10.3</td>
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<tr>
<td>Scond, liter⁻¹</td>
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<td>0.053±0.006</td>
</tr>
<tr>
<td>Sacin, liter⁻¹</td>
<td>0.113±0.019</td>
<td>0.089±0.013</td>
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Values are means ± SE for 13 asthma patients. Best and worst visit, visit corresponding to respectively best and worst baseline FEV1 for each patient (see text for details). FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; PEF, peak expiratory flow; FEF75, forced expiratory flow after exhalation of 75% of FVC; Scond, Sacin, multiple-breath washout indexes of acinar and conductive lung zone ventilation heterogeneity, respectively (see text for details); P values indicate the significance of differences between each patient’s worst and best baseline, or between corresponding dilation values. *Significant difference between baseline and dilation on worst visit, P < 0.05.
classifying the visits. The parameters in Table 1 other than FEV\textsubscript{1} showed a consistent picture of generally higher PEF, FVC, and FEF\textsubscript{75} values and lower $S_{\text{cond}}$ and $S_{\text{acin}}$ values on the best visit, with a difference between best and worst visits still reaching significance on all five parameters at baseline (baseline values of Table 1). After bronchodilation (postdilation values in Table 1), the visit corresponding to the best baseline FEV\textsubscript{1} also led to the best postdilation FEV\textsubscript{1}, with a significant intrasubject intervisit average difference still amounting to 6.6\% of predicted ($P = 0.01$). The same tendency was seen for postdilation FVC ($P = 0.02$), FEF\textsubscript{75} ($P = 0.02$), and $S_{\text{cond}}$ ($P = 0.03$), maintaining significant intrasubject intervisit differences. By contrast, the intrasubject intervisit baseline PEF and $S_{\text{acin}}$ differences completely disappeared after bronchodilation.

Despite the inability of the $\beta_2$-agonist to completely abolish residual intrasubject intervisit variability in FEV\textsubscript{1} and $S_{\text{cond}}$ (postdilation values in Table 1), it did elicit FEV\textsubscript{1} and $S_{\text{cond}}$ responses that depended on baseline smooth muscle tone, with the greatest dilation obtained for the worst baseline FEV\textsubscript{1} or $S_{\text{cond}}$ value. Indeed, by using two data points per patient on all 13 patients, significant correlations were found between $S_{\text{cond}}$ changes (i.e., $S_{\text{cond}}$ after 400 $\mu$g minus $S_{\text{cond}}$ at baseline) and baseline $S_{\text{cond}}$ ($r = -0.81; P < 0.001$); the corresponding correlation for FEV\textsubscript{1} was $r = -0.65$ with $P < 0.001$. By contrast, bronchodilator $S_{\text{cond}}$ or FEV\textsubscript{1} response did not correlate significantly with postdilation $S_{\text{cond}}$ or FEV\textsubscript{1}. Finally, the $\beta_2$-agonist also elicited greater $S_{\text{acin}}$ reversibility when baseline $S_{\text{acin}}$ was greater ($r = -0.81; P < 0.001$).

Figure 1 represents all individual data points obtained for the five parameters of Table 1 (except for FVC). Data were again classified in terms of worst (○) and best (●) visit. The most relevant comparison in Fig. 1 was between the asthmatic individuals on their best visit after optimal $\beta_2$-agonist reversibility (individual data: ●; corresponding average: ● ± SD) and the reference values from the matched controls (horizontal bar ± SD). This comparison revealed that after maximal $\beta_2$-agonist reversibility, the asthmatic individuals showed significantly lower FEF\textsubscript{75} [78.0 ± 11.2 (SE) vs. 102.7 ± 6.2\% of predicted; $P = 0.005$] and significantly higher $S_{\text{cond}}$ [0.043 ± 0.003 (SE) vs. 0.030 ± 0.001 liter\textsuperscript{-1}; $P < 0.001$] than matched controls. By contrast, postdilation FEV\textsubscript{1}, PEF, and $S_{\text{acin}}$ values in the asthmatic individuals were indistinguishable from the matched controls ($P > 0.1$ for all). Fi-

![Fig. 1](https://i.imgur.com/3Q5Q5Q.png)

Fig. 1. Individual forced expiratory volume in 1 s (FEV\textsubscript{1}), peak expiratory flow (PEF), forced expiratory flow after exhalation of 75\% FVC (FEF\textsubscript{75}), conductive component of ventilation heterogeneity ($S_{\text{cond}}$), and acinar conductive component of ventilation heterogeneity ($S_{\text{acin}}$) values obtained in 13 asthmatic individuals before and after 400 $\mu$g salbutamol on "worst" (○) and "best" (●) visit, where the best visit and worst visit are determined on the basis of baseline FEV\textsubscript{1} (see text for details). Also represented is the average value (● ± SD) corresponding to the postdilation data from the 13 asthmatic individuals on their best visit, for comparison with the reference values obtained from 13 matched control subjects (horizontal bar ± SD). %pred, Percentage of predicted value.
nally, we repeated the same comparison after removing the three patients from the asthma group who were receiving maintenance steroid treatment. The comparison of the remaining 10 asthmatic subjects with the corresponding 10 matched controls led to the same conclusions: normal postdistillation values with respect to controls for FEV$_1$ [110.1 ± 4.0 (SE) vs. 113.3 ± 3.3% of predicted; $P > 0.1$], PEF [102.3 ± 3.8 (SE) vs. 105.0 ± 5.2% of predicted; $P > 0.1$], and $S_{\text{acin}}$ [0.065 ± 0.009 (SE) vs. 0.070 ± 0.009 liter$^{-1}$; $P > 0.1$], and abnormal with respect to control values for FEF$_{75}$ [86.0 ± 13.0 (SE) vs. 104.1 ± 7.8% predicted; $P = 0.04$] and $S_{\text{cond}}$ [0.040 ± 0.002 (SE) vs. 0.029 ± 0.002 liter$^{-1}$; $P = 0.003$].

**DISCUSSION**

The foremost important finding of the present study is the considerable abnormality of ventilatory heterogeneity in the conductive airways of asthmatic patients, persisting after bronchodilation with a β$_2$-agonist (Fig. 1). This was the case, despite a relatively mild baseline obstruction in these patients and despite a mode of salbutamol aerosol administration designed to maximize any possible β$_2$-agonist reversibility. The fact that $S_{\text{cond}}$ had been previously shown to return to normal values with 200 μg salbutamol after a twofold increase after a histamine challenge in otherwise normal nonhyperresponsive subjects (29) indicates that $S_{\text{cond}}$ is able to respond to such stimuli. The incomplete reversal of $S_{\text{cond}}$, even in this group of relatively mildly asthmatic subjects in whom $S_{\text{acin}}$ could be completely recovered, is therefore thought to be a true effect. Although the FEF$_{75}$ deficit after salbutamol suggests that there is a non-β$_2$-agonist-reversible component of abnormality in the small airways, the abnormal $S_{\text{cond}}$ locates this abnormality within the conductive zone of the small airways. Such an observation implies that drugs aimed at the relief of the non-β$_2$-agonist-reversible component in mild asthma should be preferentially targeted to the small conductive airways. This corresponds to a volumetric lung depth for drug delivery of no more than ~200 ml, with all the more peripherally located air spaces being particularly prone to unwanted side effects.

The decreased intrasubject variability after dilation (Table 1) makes it more meaningful to compare our data with those previously obtained after dilation. In doing so, it is interesting to note that the average $S_{\text{cond}}$ value after 400 μg salbutamol obtained here [0.050 ± 0.004 (SE) liter$^{-1}$] is almost identical to that previously obtained after 400 μg salbutamol [0.049 ± 0.004 (SE) liter$^{-1}$] in a group of moderate-asthma patients characterized by an 19% of predicted lower baseline FEV$_1$ (28). This suggests a pathophysiological picture of the mild-to-moderate asthmatic lung with a persistent structural alteration in the conductive zone of the small airways that could be present from very early on in the disease process. A large cohort study of children in US cities followed up between the ages of 6 and 18 yr is consistent with early involvement of the small airways in asthma because a clear-cut forced expiratory flow after exhalation of 25–75% FVC deficit (15–20% lower at age 18 yr) in asthmatic vs. nonasthmatic children (FEV$_1$ deficit was 5–7% at age 18 yr) is shown (9).

It had been suggested that, even in the early stages of asthma, structural abnormality may be present as peripherally as the alveolated air spaces (6). The $S_{\text{acin}}$ data in Fig. 1 indicate that acinar ventilation heterogeneity can be fully reversible by a β-agonist in mild asthma. This indicates that smooth muscle constriction in the first acinar airway generations (terminal, respiratory bronchioles) was the major determinant of $S_{\text{acin}}$. In our patients, further supported by a consistent pattern of larger $S_{\text{acin}}$ reversibility for a greater baseline $S_{\text{acin}}$. By contrast, a previous study with subjects with moderate asthma (with a 19% lower baseline FEV$_1$) showed a greater acinar abnormality (on average $S_{\text{acin}}$ was double that obtained in the present group) that was only partly reversible (28). Hence, the appearance of non-β$_2$-agonist-reversible structural impairment at the acinar level of the lung periphery may just depend on the definition of “early disease.”

An interesting point of comparison with respect to $S_{\text{acin}}$ obtained in asthmatic individuals can be found in a ventilation distribution study in asthmatic children by Cooper et al. (2). Because these authors used a N$_2$ SBW maneuver that did not include volumes below FRC at inhalation, their phase III slopes can be roughly considered as a surrogate for $S_{\text{acin}}$. Cooper et al. showed that, of all the asthmatic children under study, the patients with abnormally steep N$_2$ phase III slopes were also those patients having shown the most severe clinical course over the year preceding the study. This finding at least points to $S_{\text{acin}}$ as one major determinant of disease severity (the contribution from $S_{\text{cond}}$ to those single-breath phase III slopes being negligible). Taken together with our observation that a previous group of asthma patients with a 19% lower FEV$_1$ were characterized by a twofold greater $S_{\text{acin}}$ but similar $S_{\text{cond}}$ (28) with respect to the present group of mildly asthmatic individuals, this leads to the intriguing hypothesis that $S_{\text{acin}}$ may be related to disease severity, whereas $S_{\text{cond}}$ appears to result from a more consistent abnormality, present even in mild degrees of asthma.

Studies of lung mechanics with different degrees of invasiveness and sophistication have reached conclusions that concur, at least in part, with our ventilation distribution findings in mildly asthmatic individuals during near-tidal breathing. Yanai et al. (33) used a catheter-tipped micromanometer wedged into the right upper lobe of patients breathing normally at FRC in the sitting position. Although they found no changes in central resistance, significant increases in peripheral resistance were present, but only in individuals with moderate-to-severe asthma (average FEV$_1$: 54% of predicted). The authors conceded that their technique may have been less sensitive to subtle peripheral changes than the wedged-bronchoscope technique used by Wagner et al. (31). With subjects supine and breath holding
at FRC, these authors found a sevenfold increase in peripheral lung resistance in individuals with mild asthma (mean FEV₁: 95% of predicted), part of which could have been exaggerated by the supine posture, but that was essentially attributed to peripheral airway narrowing and/or closure (derecruitment). With a slightly modified technique, Kaminsky et al. (13) found a higher peripheral resistance and lower peripheral compliance in asthmatic vs. normal individuals. These observations were interpreted to reflect narrowing and/or closure of peripheral collateral airways, thought to be located in the bronchioles and alveolar ducts, and a stiffening or derecruitment of distal parenchymal units. Finally, lung impedance measurements by Kaczk a et al. (12) showed that in mildly asthmatic subjects (mean FEV₁ = 97% of predicted) both airway and tissue properties can respond to salbutamol.

Overall, these findings of abnormal peripheral lung mechanics and β₂-agonist reversibility elicited down to the lung parenchyma in asthmatic individuals with close to normal FEV₁ can be brought into agreement with our findings if we consider that, whatever the exact nature of the structural changes, they will probably occur in a heterogeneous way across the lung. At the level of the acinar lung zone, this will effectively change the asymmetry of subtended intra-acinar units, which would affect S_acin. At the level of the conductive lung zone, possibly small airways just proximal to the acinar entrance, heterogeneity would result in unequal narrowing of parallel units and affect S_cond. Although the non-β₂-agonist-reversible S_cond could potentially be increased by heterogeneity originating in large and small conductive airways, the combination of an abnormal S_cond with abnormal FEF75 but normal PEF (Fig. 1) does suggest the predominant involvement of the smaller conductive airways. However, conclusions about the exact airway size within the conductive airway tree based on comparison of indexes of heterogeneity, such as S_cond, and spirometric indexes reflecting overall change, such as FEF75, may not be unequivocal. Possibly, the additional measurement of frequency dependence of dynamic lung function, which is thought to be particularly sensitive to structural heterogeneity at different lung depths (17), could confirm the involvement of the small conductive airways in the mildly asthmatic lung.

Considering that asthma is tagged as variable airways obstruction, we also wanted to consider the variability of intra-subject reversibility in this experimental setting with minimal instrumental variability in terms of aerosol delivery. If, for instance, intra-subject intervisit differences were solely due to variability in reversible smooth muscle constriction, these differences should be entirely abolished by optimal β₂-agonist delivery. This was true for S_acin, showing an association between baseline and dilation change and disappearance of intervisit S_acin differences after dilation. In fact, not only were intervisit S_acin differences abolished but also S_acin was normalized after dilation, suggesting that reversible smooth muscle constriction was the main determinant of structural alterations in the first acinar generations of these asthmatic patients. The association between baseline S_cond and dilation change indicates that S_cond behavior was also partly determined by variable smooth muscle tone in the conductive airways. However, the abnormal post-dilation S_cond (Fig. 1) with residual intervisit intra-subject variability (postdilation values in Table 1) points to another source of variable conductive airways malfunction.

Richmond et al. (22) observed considerable intra-subject intervisit variability of inflammatory cells retrieved from endobronchial biopsies on two visits in 12 mildly asthmatic patients (mean post-salbutamol FEV₁: 92% of predicted). Although the actual correlation between FEV₁ and inflammatory cells was not addressed in that study, others have demonstrated the potential for an association between wall thickness of large airways (the apical bronchus) and FEV₁, even in mildly asthmatic individuals (19). In fact, the 7% of predicted difference between best and worst postsalbutamol FEV₁ computed from the data provided in Richmond et al. compares well with the 6.6% of predicted difference obtained here (Table 1). The residual intervisit intra-subject variability in S_cond (and PEF75), paralleling FEV₁ variability in the present study, suggests that inflammation as a source of non-β₂-agonist-reversible variability could also be present in the smaller airways of the conductive airway tree.

In summary, the present study used the noninvasive MBW test to demonstrate that the most consistent structural impairment encountered in asthma, however mild, is located in the conductive airways. In this respect, the main target for other bronchodilator drugs (e.g., anticholinergics or methylxanthines), anti-inflammatory medication (e.g., inhaled corticosteroids or antileukotrienes), or any other drug aimed at prevention of remodeling of the asthmatic lung from its earliest stages on should not include the acinar lung zone. In more advanced, moderate asthma, an additional benefit could be gained from a MBW assessment of possible abnormality in the acinar lung zone before adding it to the target zone for drug delivery.

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