Standardized quantification of pulmonary fibrosis in histological samples

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The Ashcroft scale for the evaluation of bleomycin-induced lung fibrosis is the analysis of stained histological samples by visual assessment. Based on the knowledge that this procedure is not standardized in animals and results are highly variable, we hypothesized that modification of this method may improve quantification of lung fibrosis in small animals. To prove our hypothesis, we evaluated pulmonary fibrosis in Lewis rats induced by a single intratracheal injection of 0.3 mg/kg body weight bleomycin (n = 13) compared with the same amount of saline in a control group (n = 4). We modified the Ashcroft scale by precisely defining the assignment of grades from 0 to 8 for the increasing extent of fibrosis in lung histological samples. Thirty-two observers were randomly assigned to evaluate 108 photographs of slides using either the Ashcroft scale or the modified scale. Consistent with our hypothesis, there was a significant reduction in the variability of standard deviations with the modified scale compared with the Ashcroft scale (mean of variability 0.25 versus 0.62, P < 0.0001). Applying the κ index, the Ashcroft scale showed only a fair to moderate agreement (0.23–0.59) between the observers and a low intra-observer agreement (0.51–0.74) in contrast to the modified scale, which demonstrated a moderate to good agreement between the observers (0.65–0.93, P < 0.0001) and a high intra-observer agreement (0.87–0.91, P < 0.05). To test the modified scale in vivo, we compared both scales with the results of computed tomography (CT) of the lungs obtained from the same mice. In agreement, the modified scale demonstrated a better correlation to CT scans (R = 0.58) compared with the Ashcroft scale (R = 0.33). In summary, quantification of lung fibrosis in histological lung sections using the modified scale is reliable and reproducible.

INTRODUCTION

Pulmonary fibrosis is a severe disorder frequently progressing to complete loss of lung function and death (1–4). Major efforts have been undertaken to evaluate fibrotic processes in the lung to understand the pathogenesis of the disease (5). The best characterized model for pulmonary fibrosis is induced by bleomycin, which is commonly administered in chemotherapy protocols for leukemia and testicular cancer (6). Animal models using bleomycin are an important tool for the analysis of pathogenetic processes in lung fibrosis and for the evaluation of new antifibrotic drugs (6,7), although processes involved in idiopathic pulmonary fibrosis (IPF) may not be identical (8). The current gold standard for diagnosis and staging of diseases with increased fibrous tissue is histopathological analysis (9–11).

However, major obstacles hamper this quantification. The development of fibrosis is heterogeneously distributed throughout the lung with some areas of lung unaffected (3,8,12). In addition, assessment of fibrosis in microscopic samples is variable depending on the observers experience and individual judgment. Therefore, a reliable scoring system for the assessment of lung fibrosis is necessary (9,13,14).

Ashcroft et al. (15) approached this problem by assigning a numerical scale, with grades from 0 to 8, of the amount of fibrotic tissue in histological samples. This scale was originally developed in human tissue, but has been widely used in bleomycin-induced pulmonary fibrosis in animals (16–27). However, conclusions from observations applying this scale may be uncertain (12). This Ashcroft scale has never been validated for lungs from animals. In addition, a considerable degree of variability between the results obtained from different research laboratories makes comparisons difficult. This problem may be caused by the kind of definitions used in the Ashcroft scale and the omission of a description for grades 2, 4, and 6.

In the context of these observations, we hypothesized that modification of the Ashcroft scale may improve quantification of lung fibrosis in small animals. To assess this hypothesis, we modified this scale by precisely defining each grade of fibrosis and the method of microscopic scanning. We analyzed this modified scale in comparison to the Ashcroft scale in a rat model of lung fibrosis, recruiting 32 observers for the independent evaluation of 108 histological samples with one of the methods. In addition, fibrotic scores were correlated to lung densitometries obtained by

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**MATERIALS AND METHODS**

**Experimental Animal**

Lewis rats (Charles River, Sulzfeld, Germany) were 10 weeks old and weighed between 200 and 220 g. Animals were maintained on 12 h dark/light cycles and provided with water and standard rodent diet ad libitum. The experimental protocol was approved by the Ministry of Nature, Environment, and Country Development, Schleswig-Holstein, Germany.

**Bleomycin-induced Lung Fibrosis in Rats**

Pulmonary fibrosis was induced in 13 rats by intratracheal instillation of 0.3 mg/kg body weight (bw) bleomycin dissolved in 0.5 mL sterile saline, while control rats (n = 4) received the same amount of saline. Rats were anesthetized with chloralhydrate (40 mg/kg bw) and intubated with a plastic tube. To ensure a homogenous distribution, 0.5 mL air was injected twice after bleomycin administration. All rats survived bleomycin administration. Rats were sacrificed by exsanguination, 14 and 45 days after bleomycin instillation, under chloralhydrate anesthesia immediately following CT scan. The lungs were ligated at the trachea and removed en bloc. Lungs were inflated with 10% formalin under a constant pressure of 20 cm water until the pleural surface became smooth. Then the lungs were immersed in the same fixative and processed through a graded series of alcohols and xylene prior to paraffin embedding. Coronal sections (5 μm) of the upper, upper-mid, lower-mid, and lower part of the right lungs were deparaffinized and stained with Masson’s trichrome.

**Definition of the Ashcroft Scale and Modified Scale**

According to the scale defined by Ashcroft et al. (15), lung sections were assessed by a system of grades following the instructions of the original publication (Table 1). If there was any difficulty in deciding between two odd-numbered grades, the field would be given the intervening even-numbered score. In every field, the predominant degree of fibrosis was recorded as that occupying more than half of the field area. The modified scale was defined according to the criteria outlined in Table 2. Sample photographs were provided for both scales (Figure 1).

**Validation Based on Photographs**

More than 150 microscopic fields of upper, upper-mid, lower-mid, and lower sections of the right lungs were randomly chosen and microscopically photographed with a 20-fold magni-
fication in all 13 rats with lung fibrosis by a technician blinded to the results (three photographs per section per rat). After printing, photographs were randomly numbered in a blinded fashion. The authors chose 108 photographs according to quality criteria (sharply photographed, >95% of photographs had to be covered with lung tissue) and to ensure that the frequency of each grade of fibrosis was similar to avoid a bias to certain fibrotic grades. A certified pathologist determined the correct score of all photographs.

To test the modified scale, 32 observers were randomly assigned to evaluate photographs of fibrotic lung sections using either the Ashcroft scale \( (n = 16) \) or the modified scale \( (n = 16) \). Two observers in each group had extensive experience evaluating pulmonary fibrosis histologically, and the remaining 14 observers were medical students who had gained basic experience evaluating microscopic fields during their medical school training. The observers were provided with the descriptions of the scale and sample photographs by the authors to assist in evaluation. Sample

<table>
<thead>
<tr>
<th>Grade of Fibrosis</th>
<th>Sample Photograph</th>
<th>Ashcroft Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Figure 1A</td>
<td>Normal lung</td>
</tr>
<tr>
<td>1</td>
<td>Figure 1B</td>
<td>Minimal fibrous thickening of alveolar or bronchiolar vessels</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>None</td>
</tr>
<tr>
<td>3</td>
<td>Figure 1D</td>
<td>Moderate thickening of walls without obvious damage to lung architecture</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>None</td>
</tr>
<tr>
<td>5</td>
<td>Figure 1F</td>
<td>Increased fibrosis with definite damage to lung structure and formation of fibrous bands or small fibrous masses</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>None</td>
</tr>
<tr>
<td>7</td>
<td>Figure 1G</td>
<td>Severe distortion of structure and large fibrous areas; “honeycomb lung” is placed in this category</td>
</tr>
<tr>
<td>8</td>
<td>Figure 1I</td>
<td>Total fibrous obliteration of the field</td>
</tr>
</tbody>
</table>

Ashcroft scale and sample photographs according to Reference 15. None, no definition provided.

Figure 2. Inter-observer variability. Photographs (108) of lung fibrotic sections were evaluated by 32 observers using either the Ashcroft scale or the modified scale. For both scales, 2 observers in each group had extensive experience evaluating pulmonary fibrosis histologically, and the remaining 14 observers were medical students who had gained basic experience evaluating microscopic fields during their medical school training. Black bar indicates the Ashcroft scale, gray bar indicates the modified scale. Data shown are mean ± standard error.
photographs for the Ashcroft scale were chosen based on examples of pictures published by Ashcroft et al. (15). Three randomly chosen observers from each group evaluated the same 108 fields of different lung sections 1 month later to assess intra-observer agreement.

**Histological Assessment of Bleomycin-induced Lung Fibrosis**

After the validation of the modified scale based on photographs, we tested the modified photos in vivo for evaluation of lung fibrosis in small animals. One observer assessed and graded four different levels of the lung parenchyma (upper, upper-mid, lower-mid, and lower sections), first with the modified scale, and 3 months later with the Ashcroft scale. In order to prevent observer bias, all histological specimens were randomly numbered in a blinded fashion. Applying the Ashcroft scale, lungs were initially scanned microscopically with a 10-fold objective according to the criteria of Ashcroft et al. (15). We found microscopic assessment with a 10-fold objective more difficult, because the field to be analyzed was too heterogeneous, which made assessment of the sections too difficult. Therefore, we changed magnification to a 20-fold objective using the modified scale, which allowed the evaluation of fine structures as well as providing a sufficient overview. For both scales, each field was inspected following a “raster-like pattern” throughout the whole section. Areas with dominating tracheal or bronchial tissue were omitted. The grades were summarized and divided by the number of fields to obtain a fibrotic index for the lung.

**Quantification of Lung Density by CT**

Lung fibrosis of rats was evaluated using high-resolution CT. CT images were obtained with a 16-row detector CT-scaner (Somatom Sensation; Siemens Medical Systems, Erlangen, Germany). Rats were anaesthetized with chloralhydrate (40 mg/kg bw) and atropinsulfate (0.1 mg/kg bw), intubated with a plastic tube, and tracheally ventilated with a volume-cycled small animal ventilator (Harvard Rodent Ventilator; Harvard Apparatus, South Natick, MA, USA).
The animals were placed in the supine position on the CT table. During each scan (5–8 s), a constant inspiration pressure (20 cm water) was applied to suspend breathing in deep inspiration. Sequential, contiguous high-resolution CT of the whole lung was acquired with a collimation of 0.75 mm (technical parameters: 120 kV, 300 mAs, field of view 50 mm, matrix 512 × 512 pixel). Images were reconstructed at 1 mm slice-thickness using a high spatial frequency kernel and were displayed on a standard work station (Wizard; Siemens Medical Systems) at a window width of 1600 Hounsfield units (HU) and a center of -450 HU. Analysis of lung density was performed with commercially available software (Pulmo; Siemens Medical Systems). Lung parenchyma of each CT slice was labeled automatically using an algorithm based on gray-level thresholds between -1024 HU to -200 HU. Manual correction took place if the central airways were falsely included or if areas of fibrotic lung tissue were initially missed due to increased density. Means of lung density of both groups were achieved by evaluation of all CT scans acquired from the apices to the bases of the lungs. The radiologists were blinded to the results of the histological assessment.

Statistics

Ashcroft and modified scales were tested on reliability and reproducibility by evaluating 108 photographs of fibrotic sections. The χ index (28) was used to assess the agreement of a single observer (intra-observer agreement) and between the observers of one group (inter-observer agreement). A χ index greater than 0.7 indicated a good agreement, between 0.4 and 0.7 indicated a moderate agreement, and between 0.2 and 0.4 indicated a fair agreement (28). The variability of all “incorrect” grades (higher or lower) was described by the standard deviation and summarized by median values with 25th and 75th percentiles (Table 3). Wilcoxon rank sum test was used for comparisons between the modified and Ashcroft scale. Correlation analyses were performed using Spearman rank correlation analysis. P values <0.05 indicate a significant difference. The statistical analysis was carried out using the software R for Windows, Version 2.0.1.

RESULTS

Inter-Observer Variability

To assess the variability between different observers, 108 photographs were evaluated by 32 observers using either the Ashcroft scale or the modified scale.

The Ashcroft scale demonstrated a significantly higher variability of standard deviations of one photograph evaluated by 16 observers (intra-observer variability; P < 0.0001, Figure 2). Two observers in each group had extensive experience evaluating pulmonary fibrosis histologically, and the remaining 14 observers were medical students who gained basic experiences evaluating microscopic fields during their medical school training. The modified scale revealed for both groups highly less intra-observer variability than the Ashcroft scale (P < 0.0001, Figure 2).

Interestingly, when only applying the modified scale, observers with extensive experience revealed even less variability than observers with basic experiences (P < 0.001, Figure 2), suggesting the modified scale as a powerful tool for researchers with more extensive experiences. For both methods, variability was higher for the lower grades such as 0, 1, or 2 (Table 3). However, direct comparison within each grade showed a lower variability for the modified scale than the Ashcroft scale.

Inter- and Intra-Observer Agreement

Using the χ index, the Ashcroft scale demonstrated a less inter-observer agreement than the modified scale (P < 0.0001, Figure 3A). For each grade from 0 to 7, the Ashcroft scale showed only a fair to moderate agreement between the observers, but the modified scale demonstrated a moderate to good agreement. Only in cases with complete fibrotic change of the lung (grade 8 in both scales) did both scoring systems show a good

Table 3. Inter-Observer Variability and Agreement of Grading Comparing Ashcroft Scale and Modified Scale According to Different Histological Grades

<table>
<thead>
<tr>
<th>Histological Grade</th>
<th>Variability Median (25th–75th)</th>
<th>Agreement (χ index)</th>
<th>Variability Median (25th–75th)</th>
<th>Agreement (χ index)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.79 (0.72–0.81)</td>
<td>0.34</td>
<td>0.47 (0.37–0.60)</td>
<td>0.65</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>1</td>
<td>0.73 (0.65–0.81)</td>
<td>0.34</td>
<td>0.49 (0.40–0.50)</td>
<td>0.53</td>
<td>P &lt; 0.0001</td>
</tr>
<tr>
<td>2</td>
<td>0.76 (0.65–0.85)</td>
<td>0.23</td>
<td>0.37 (0.25–0.40)</td>
<td>0.66</td>
<td>P &lt; 0.0001</td>
</tr>
<tr>
<td>3</td>
<td>0.53 (0.50–0.80)</td>
<td>0.37</td>
<td>0.25 (0.00–0.34)</td>
<td>0.81</td>
<td>P &lt; 0.0001</td>
</tr>
<tr>
<td>4</td>
<td>0.76 (0.52–0.89)</td>
<td>0.32</td>
<td>0.00 (0.00–0.25)</td>
<td>0.90</td>
<td>P &lt; 0.0001</td>
</tr>
<tr>
<td>5</td>
<td>0.54 (0.50–0.63)</td>
<td>0.44</td>
<td>0.25 (0.00–0.34)</td>
<td>0.85</td>
<td>P &lt; 0.0001</td>
</tr>
<tr>
<td>6</td>
<td>0.63 (0.63–0.65)</td>
<td>0.33</td>
<td>0.29 (0.25–0.39)</td>
<td>0.87</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>7</td>
<td>0.48 (0.45–0.52)</td>
<td>0.59</td>
<td>0.12 (0.00–0.25)</td>
<td>0.93</td>
<td>P &lt; 0.0001</td>
</tr>
<tr>
<td>8</td>
<td>0.00 (0.00–0.00)</td>
<td>0.78</td>
<td>0.00 (0.00–0.00)</td>
<td>0.97</td>
<td>P = n.s.</td>
</tr>
</tbody>
</table>

Variability values are shown as median values (25th percentile–75th percentile). Agreements between observers are shown as χ indices (higher values represent higher agreement). P value represents significance level of difference in variability between the Ashcroft scale and the modified scale. n.s., not significant.
agreement (Table 3). Three observers of each group repeated analysis of the same panel of 108 pictures following 1 month to test intra-observer variability. In each case, the $\kappa$ index showed a better intra-observer agreement for the modified scale compared with the Ashcroft scale ($P < 0.05$, Figure 3B).

**Deviation from Correct Score**

A certified pathologist determined the correct score of each photograph. The Ashcroft scale demonstrated higher deviation from the correct score among the observers than the modified scale (Figure 4A). To rule out that lower performance of the Ashcroft scale cannot be attributed to a few careless observers, we looked at all 16 observers individually (Figure 4B). All 16 observers applying the Ashcroft scale judged at least one photograph with a deviation of more than one grade from the correct score. In contrast, only six observers using the modified scale evaluated one or more photographs deviating more than one grade from the correct one.

**Histological Assessment of Bleomycin-induced Lung Fibrosis**

The severity of the lesions varied from one region to another with the spectrum of microscopic fields ranging from normal lung to complete fibrosis. Lung sections from control animals revealed predominately normal lung architecture. Lung fibrosis was most prominent between 14 and 21 days after bleomycin administration and less prominent at 42 and 45 days after bleomycin administration (data not shown).

**Correlation Analyses to Lung Densities on CT**

CT scanning is a well-described method of quantifying fibrotic lesions in small animals (29–33). CT scanning of control groups ($n = 4$) revealed intact lung architecture with only occasional branching structures in the lungs (Figure 5A). After bleomycin treatment ($n = 13$), rats developed subpleural and peribronchovascular patchy infiltrates and areas of consolidation, interspersed with areas of normal lung. Irregular peripheral lines arose from the pleura in the lung and partly merged to form fibrotic masses (Figure 5B). Fibrotic lesions were assessed by quantifying global lung density in HUs by marking the lung parenchyma of every CT slice and calculating total arithmetic mean of HU. Consistent with histological assessment, fibrotic lesions were more prominent between 14 and 21 days after instillation of bleomycin compared with control lungs and improved at 42 to 45 days after instillation (data not shown). Correlating lung densities with histological scores, the Ashcroft scale showed only a faint nonsignificant correlation ($P = 0.2$, Figure 5C). In contrast, when applying the modified scale, there was a stronger and significant correlation between lung density and histological score ($P = 0.04$, Figure 5D). Histological assessment with the modified scale revealed higher fibrotic scores due to increased magnification of microscopic scanning with the modified scale compared with the Ashcroft scale.

**DISCUSSION**

Ashcroft et al. (15) developed a numerical scale for determining the degree of fibrosis in lung specimens. Since this scale is frequently used (16–27), we applied it in bleomycin-induced lung fibrosis of rats and found a high variability in the scores and only fair to moderate agreement between the observers.

To our knowledge, this is the first study assessing the Ashcroft scale in an animal model of bleomycin-induced lung fibrosis. This animal model is well defined with regard to histological and biochemical changes, and results are often transferred to humans with pulmonary fibrosis, although mechanisms may be different in IPF than in bleomycin-induced lung fibrosis (8). Consistent with what we observed, animals develop pulmonary fibrosis when receiving bleomycin intratracheally or intravenously or by continuous subcutaneous infusion over 1 week. After an acute inflammatory reaction with a neutrophilic and lymphocytic alveolitis in the first week, the following
weeks are characterized by diminishing inflammatory cells, proliferation of fibroblasts, and synthesis of extracellular matrix proteins leading to perivascular, peribronchial, and subpleural fibrosis (3). The exact quantification of the fibrotic burden in the lung remains difficult, but this is essential for the assessment of antifibrotic treatments.

The current gold standard for diagnosis and staging of diseases with increased fibrous tissue is histopathological analysis (9–11). Similar to other results (12), our study demonstrates that the Ashcroft scale has limitations in quantifying fibrous tissue in bleomycin-induced lung damage. Criticisms regarding the inability of the Ashcroft scale to discriminate between established fibrosis and pneumonitis exist. In addition, we noticed a high variability in fibrosis scores obtained in different research laboratories with the Ashcroft scale, so that comparisons of results were difficult. Differences in route of administration and dose of bleomycin, time points of analyses after instillation, and genetic background of animals must also be taken into account, since the degree and intensity of lung injury may vary according to these confounders (34–37). Some strains, such as C57BL/6 mice, develop high grades of pulmonary fibrosis after application of bleomycin in a short time, while in Balb/c mice and some other murine strains, the fibrous response is either absent or markedly reduced (7,38,39).

The Ashcroft score has never been validated in bleomycin-induced lung fibrosis before. We believe the high variability of the Ashcroft scale is caused by several factors. Instillation of bleomycin can result in extreme patchy fibrosis with all grades (3,8,12) and is therefore sensitive to sampling errors with regard to the selection and number of fields to be analyzed. The Ashcroft scale is characterized by the omission of descriptions for grade 2, 4, and 6, which leads to an underestimation of these grades as shown in our study, and the Ashcroft scale suggests using 10-fold magnification objective for microscope scanning, which makes assessment more difficult because of greater heterogeneity of fibrotic changes in each microscopic field.

Computer-assisted evaluation of fibrotic sections may be another option for the grading of bleomycin-induced lung fibrosis (9,35,36,40). However, current systems are more laborious and time consuming and not available in each laboratory. These systems rely on the quantification of colored areas after staining with a modified trichrome stain, do not include the evaluation of structural changes, and are susceptible to changes in colors. Moreover, these systems still require careful preselection of fields to be analyzed and exclusion of areas with trachea or blood vessels.

The Wagner grading system is another qualitative, commonly used method assessing fibrosis morphologically, although it was also found that it did not adequately differentiate the magnitude of early fibrosis (31). Interestingly, a simple modification of the Wagner grading system was recently published by McConnell and Davis (31). It demonstrated a clear improved distinction between different levels of fibrosis by just calculating Wagner Grade 4 lesions as a percentage of the total lung parenchyma in one section, suggesting this grading system is another useful tool to assess fibrotic burden in lung tissue.

Since 1980, CT has become the standard for noninvasive diagnosis of fibrotic lung diseases in humans (1,41). In the past, the CT technique has been problematic in small animals because of limited resolution relative to animal size (32). However, with improvements in resolution, CT scans are more frequently used to identify fibrotic lesions in lungs. Mainly larger animals like dogs, pigs, or rabbits have been examined before, but recently, lungs of smaller animals like rats or mice have been scanned (29–33). In agreement to these data, we demonstrate that CT is suitable for the

Figure 5. Correlation analyses to lung densities of computed tomography (CT) scan. CT scan of whole lung was performed in rats (n = 13) between 14 and 45 days after intratracheal administration of bleomycin and after saline administration (control group, not used for correlation analysis). (A) Representative coronal section of CT scan of lung after administration of saline (control group). (B) Representative coronal section of CT scan of lung 14 days after administration of bleomycin. A, fibrotic nodules. (C) Correlation of the Ashcroft scale to CT (R = 0.33; P = 0.2). (D) Correlation of the modified scale to CT (R = 0.58; P = 0.04). Black points indicate the Ashcroft scale, gray points indicate the modified scale.
exact assessment of fibrotic lesions after bleomycin instillation in small animals.

Another important tool to quantify the extent of fibrosis includes surrogate markers such as hydroxyproline, which is known to correlate well with the matrix content in the lung. However, for appropriate standardization, whole lobes/lungs need to be analyzed, and investigators must be aware that fibro-lytic processes may overestimate the fibrotic content in the lung. Correlation analysis between biochemical parameter and histopathological scales may be interesting, however, it may be less useful comparing the extent of fibrosis in different lobes/lungs, as the bleomycin model is highly variable in the extent of fibrosis (3,8,12).

One limitation of this study is that direct comparison of the methods for histological analysis was performed by only one observer. This was necessary to exclude variation due to different levels of experience in evaluating whole lungs. Another limitation is that the observers recruited for this study were mostly medical students, and only four observers had extensive experience evaluating the histology of pulmonary fibrosis. However, although all observers reached similar results regardless of their experiences in histopathological techniques, more experienced observers (like pathologists) should obtain results with even smaller variations. Further improvement may be reached when a scale would include differentiation between inflammation and fibrosis. However, such a scale may also be more complicated and difficult to apply.

In conclusion, the modified scale of quantification is reliable and reproducible. It requires no special equipment, and results from different laboratories should be comparable as long as a similar standard procedure is used.

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COMPETING INTERESTS STATEMENT

The authors declare no competing interests.

REFERENCES


the endothelium attenuates bleomycin-induced lung fibrosis and impairs MMP-9/TIMP-1 balance. Respiration 71:546-556.

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