Mucus Removal Is Impaired in Children with Cystic Fibrosis Who Have Been Infected by *Pseudomonas aeruginosa*

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Objective To determine if mucus removal is impaired in children with cystic fibrosis (CF) who have been recently infected with *Pseudomonas aeruginosa*.

Study design We compared mucociliary clearance (MCC), cough clearance (CC), lung morphology, and forced expiratory volume in 1 second (FEV_1) in 7- to 14-year-old children with CF and mild lung disease ($FEV_1 \geq 80\%$). Children were either *P aeruginosa* negative (n = 8), or *P aeruginosa* positive (*P aeruginosa* obtained from at least 1 airway culture in the preceding 18 months) (n = 10). MCC and CC were quantified from gamma camera imaging of the right lung immediately after inhalation of ^{99m}technetium sulfur-colloid (time 0), over the next 60 minutes (average percent clearance over the first 60 minutes [AveMCC60]), 60-90 minutes (average percent clearance between 70 and 90 minutes [AveMCC/CC90]), and after 24 hours (percent clearance after 24 hours [MCC24hrs]). Children coughed 30 times between 60 and 90 minutes. Lung morphology was assessed by high resolution computed tomography (HRCT) scores of both lungs (total score) and of the right lung, using the Brody scale. Percent AveMCC60, AveMCC/CC90, MCC24hrs, FEV_1, and HRCT scores were compared across the 2 groups using unpaired *t* tests. Associations were assessed using Spearman correlation.

Results There were no differences between the 2 groups in AveMCC60, MCC24hrs, mean HRCT total scores, right lung HRCT scores, or mean FEV_1. AveMCC/CC90 was significantly decreased in children with *P aeruginosa* compared with those without (16.2% ± 11.0% vs 28.6% ± 7.8%, respectively; $P = .016$). There was a significant negative correlation of AveMCC60 and AveMCC/CC90 with total lung HRCT score (all $P < .05$) but not with FEV_1.

Conclusions Infection with *P aeruginosa* is associated with a significant slowing of MCC/CC in children with mild CF and may be a more sensitive indicator of the effects of *P aeruginosa* than measurements of FEV_1. *(J Pediatr 2014;164:839-45)*.

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Approximately 70 000 people worldwide have cystic fibrosis (CF), an inherited, autosomal recessive disease.[1,2] Mutations in the CF transmembrane conductance regulator gene lead to loss, or misregulation, of chloride, sodium and water transport, accumulation of viscous secretions in the airways of individuals with CF[2-5] and impaired mucus removal.[3,4] In healthy lungs, mucus is removed from the lungs within a few hours by the mucociliary clearance (MCC) apparatus, an important aspect of host defense. Cough clearance (CC), which facilitates mechanical removal mucus, takes over when there is mucus overload, or when MCC becomes impaired.

Individuals with CF become infected with numerous bacteria and fungi during the course of their disease and chronic infection of the airways is the major cause of morbidity and mortality.[6] Common pathogens known to infect the airways of CF patients include *Pseudomonas aeruginosa*, *Aspergillus fumigatus, Aspergillus niger*, *Haemophilus influenzae*, *Stenotrophomonas maltophilia*, *Staphylococcus aureus,* and methicillin-resistant *Staphylococcus aureus.*

The most common bacterium affecting individuals with CF is *P aeruginosa*, with the majority being infected by the age of 18.[7] Nevertheless, there has been little, if any, research into whether infection with *P aeruginosa* affects removal of mucus from the lung in individuals with CF, or if it contributes to airway

AveMCC60	Average percent mucociliary clearance over the first 60 minutes
AveMCCCC90	Average percent clearance between 70 and 90 minutes
CC	Cough clearance
CF	Cystic fibrosis
CT	Computed tomography
FEV_1	Forced expiratory volume in 1 second
FVC	Forced vital capacity
HRCT	High resolution computed tomography
MCC	Mucociliary clearance
MCC24Hrs	Percent clearance after 24 hours
O:I	Outer/inner lung zone ratio

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damage and morphologic changes such as bronchiectasis. For this reason, we compared mucus removal in children who had been recently infected with *P aeruginosa* with children with mild CF lung disease who had not been infected with *P aeruginosa*. We hypothesized that recently infected children would demonstrate significantly slower mucus removal and greater lung morphologic abnormalities than children who had not been infected with *P aeruginosa*.

Methods

This study consisted of a screening visit and 2 study visits. Most screening visits and study visits took place over a 4-week period. However, because of scheduling conflicts and pulmonary exacerbations, some screening visits occurred between 6 and 19 weeks before the study visits. The protocol was approved by the Johns Hopkins Medicine Institutional Review Board. Written informed consent/assent was obtained from parents and participants.

Children with or without *P aeruginosa* through review of their medical records in the preceding 18 months and who met all of the eligiblity criteria were referred for recruitment by their treating physicians. Eligibility criteria included males and nonpregnant females, age 7-14 years with a diagnosis of CF by sweat chloride ≥60 meq/L, or the presence of 2 disease-causing CF transmembrane conductance regulator mutations, and a forced expiratory volume in 1 second (FEV_1) ≥80% of predicted values. Children were grouped as *P aeruginosa* positive if *P aeruginosa* had been obtained from at least 1 airway culture positive for *P aeruginosa* in the preceding 18 months, or *P aeruginosa* grew from the culture obtained at the screening visit. They were grouped as *P aeruginosa* negative if *P aeruginosa* had not been obtained from any airway culture in the preceding 18 months, or from the culture obtained at the screening visit. We chose to review each child's chart over the preceding 18 months because it is unknown how long 1 infection with *P aeruginosa* can affect MCC.

Concomitant Medications

Children who were taking inhaled tobramycin or aztreonam were studied during their "off" month of treatment. Children discontinued the use of bronchodilators, anticholinergic drugs, fluticasone propionate, recombinant human DNase, and airway clearance therapy for 12 hours before the study visit and hypertonic saline for 3 days before the study visit. All medications were discontinued for 36 hours thereafter. Our goal in withholding these medications was to minimize their possible effects on MCC measurements. The time period for withholding each medication was somewhat arbitrary because there is little, if any, information in the literature as to how long a specific drug affects MCC once the drug is stopped. Thus, although the bronchodilator effect of albuterol is thought to last only for 2 hours,[8] it is unclear how long albuterol continues to affect MCC once it is stopped. In general, we asked patients to withhold medications for as long a time as we thought was medically advisable, while also reducing possible effects on MCC. Children who were treated with oral, or intravenous, antibiotics for a pulmonary exacerbation completed those medications at least 2 weeks before the screening and study visits. Children who were being chronically treated with oral antibacterials such as sulfamethoxazole/trimethoprim were excluded from the study. Children who were being treated with azithromycin continued with their treatment before and throughout the study visits.

Screening Visit

Children underwent a focused medical history and physical examination and routine pulmonary function measurements of FEV_1 and forced vital capacity (FVC). They also underwent an induced sputum test to determine their airway microbiology and a high resolution computed tomography (HRCT) scan to assess lung morphology.

Induced Sputum Test. Children underwent the standardized sputum induction procedure that is utilized by the CF Therapeutic Development Network.[9] Sputum expectorated during this procedure was cultured for bacteria and fungi.

HRCT Scan. Children underwent an HRCT scan in the Johns Hopkins Radiology Department. Computed tomography (CT) scanning was performed as described by Brody et al.[10]

Scoring Lung HRCT Scans. HRCT scans were read by an experienced pediatric radiologist with no clinical information. Each CT scan was scored using a scoring system developed by Dr Alan Brody that evaluates the severity of lung disease in each lobe, with the lingula considered a lobe.[10] The severity of bronchiectasis, mucous plugging, peribronchial thickening, air trapping, parenchymal opacities, and overall disease severity was scored for each lung separately. Overall disease severity score for the right lung and both lungs combined was averaged for each group.

Pulmonary Function Measurements

FVC and FEV_1 were measured by a computerized 10-liter Survey III spirometer (Warren E. Collins, Inc, Braintree, Massachusetts). Spirometry was performed in accordance with American Thoracic Society/European Respiratory Society guidelines,[11] using equations from Hankinson et al[12] and Wang et al[13] and a protocol based on the CF Foundation's recommendations[14] to determine percent predicted values.

Study Visit 1A

Children underwent the following procedures: (1) a focused medical history and physical exam; (2) pulmonary function measurements of FEV_1 and FVC; (3) a ^{133}xenon-ventilation scan; (4) inhalation of aerosol generated from a solution of ^{99m}technetium sulfur-colloid; and (5) gamma camera imaging of the lungs for 90 minutes.

Ventilation Scan. Children inhaled ^{133}xenon while sitting with their back to a large-field-of-view 2-dimensional Siemens Orbiter gamma camera (Gammasonics, Des Plains, Illinois). This procedure produced a ventilation image that