Research Highlights

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News & Views

Standardized scoring of chest CT scans can predict future lung function in cystic fibrosis

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Evaluation of: Sanders DB, Li Z, Brody AS, Farrell PM. Chest CT scores of severity are associated with future lung disease progression in children with CF. *Am. J. Respir. Crit. Care Med.* doi: 10.1164/rccm.201105-0816OC (Epub ahead of print) (2011).

Newborn and young infants with cystic fibrosis (CF) are born with structurally normal lungs. Objective measures of the onset and progression of CF lung disease have been hampered by the low resolution of chest radiographs and the need for sedation for pulmonary function testing in infants. Chest CT scan scoring may offer a more sensitive measure of pulmonary decline. The Wisconsin CF neonatal screening cohort of 81 children underwent a standardized thoracic CT scanning protocol beginning in the year 2000 and were then evaluated in the clinic and with lung function testing every 3 months over a mean of 7.5 years or until the age of 21 years. The Brody chest CT scoring protocol was implemented. Other objective measures included FEV1 and chest radiographs. Multivariable linear regression was used to determine associations between chest CT scores and lung function. A strong association between the Brody chest CT scores in the year 2000 and most recent lung function was uncovered. The authors suggest that serial chest CT imaging offers a better prediction of the most recent assessment of lung function than spirometry or chest radiographs.

The Brody CT scores from a control cohort and the newborn screening cohort were assigned by Dr Brody and two other radiologists [1]. The most significant association with the most recent FEV1% predicted was the chest CT score. Given the average length of time between CT scan and recent FEV1 (7 years), this suggests that the score reflects a structural condition of the lung that is more predictive of lung disease association than a low resolution chest radiograph and other lung disease surrogates. A subscore called the Brody bronchiectasis score was even more strongly associated than the overall score. This suggests that quantitation from imaging of the irreversible structural changes associated with bronchiectasis is more predictive than scores that involve changes that can be reversed, such as atelectasis or air trapping.

In general, a single FEV1% predicted is less meaningful than a longitudinal measure of FEV1% predicted [2,3]. Similarly, one might assume that two or three CT scans over time might be more accurate in predicting lung disease progression than a single scan. In weighing the risk:benefit ratio of instituting chest CT scans at regular intervals, it is necessary to discuss radiation dosage. These investigators used a thin-section technique (1.25 mm section thickness) with inspiratory images at 10 mm intervals and expiratory images at 20 mm intervals. The effective dose (ED) is calculated by the number of slices and type of scans and should be considered for surrounding organs, such as breasts, bone marrow and thyroid gland. In one recent study the average ED in CF patients was 6.5 mSv [4]. Given the rising median

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survival for CF patients, the lifetime ED from serial chest CT may not be inconsequential. Standardized chest CT scores may be most beneficial when used as an outcome measure in a clinical trial during drug development.

References

1 Brody AS, Klein JS, Molina PL, Quan J, Bean JA, Wilmott RW. High-resolution computed tomography in young patients with cystic fibrosis: distribution of abnormalities and correlation with pulmonary function tests. *J. Pediatr.* 145(1), 32–38 (2004).

2 Corey M, Edwards L, Levison H, Knowles M. Longitudinal analysis of pulmonary function decline in patients with cystic fibrosis [see comments]. *J. Pediatr.* 131(6), 809–814 (1997).

3 VanDevanter DR, Wagener JS, Pasta DJ *et al.* Pulmonary outcome prediction (POP) tools for cystic fibrosis patients. *Pediatr. Pulmonol.* 45(12), 1156–1166 (2010).

4 Donadieu J, Roudier C, Saguintaah M, Maccia C, Chiron R. Estimation of the radiation dose from thoracic CT scans in a cystic fibrosis population. *Chest* 132(4), 1233–1238 (2007).

Lung clearance index and standardized scoring of chest CT scans measure different aspects of lung function in cystic fibrosis

Evaluation of: Owens CM, Aurora P, Stanojevic S *et al.* Lung Clearance Index and HRCT are complementary markers of lung abnormalities in young children with CF. *Thorax* 66, 481–488 (2011).

High resolution CT (HRCT) scans of the lung are more sensitive for detecting early cystic fibrosis (CF) lung disease than spirometry or plain chest radiography. The London Cystic Fibrosis Collaboration undertook an assessment of the lung clearance index (LCI) derived from multiple breath inertgas washout and compared the LCI with HRCT, spirometry, plethysmography and multiple breath washout. The Brody II CT scoring system was used to assess the presence and extent of bronchiectasis, airway wall thickening, mucus plugging, and parenchymal opacities. In total, 60 children with CF and 54 healthy controls underwent lung function testing and HRCT on the same day. The concordance between LCI and HRCT was not as good as expected leading the authors to conclude, that while both techniques are sensitive, they are measuring different conditions.

As HRCT requires ionizing radiation, the study group hoped to identify an alternative that would be just as sensitive. They chose the LCI, a noninvasive measure of ventilation inhomogeneity derived from multiple breath inertgas washout. It has been suggested that measuring the washout of an inert gas during tidal breathing would be a sensitive marker of early CF lung disease. A previous study in 44 children with CF found LCI to be more sensitive than FEV1 and HRCT since there were children with abnormal LCI and normal HRCT [1]. Others have studied LCI in sleeping infants with CF and detected abnormalities relatively early as well [2]. In the current manuscript, children 6–10 years of age, with or without *Pseudomonas aeruginosa* colonization, were enrolled. The HRCT were performed at a single site with a Siemens Sensation 16 detector unit and isotropic volumetric acquisition during inspiration plus three 1 mm slices on expiration. They estimated the ED to be from 1.2 to 2.0 mSv depending on the size of the subject. Two radiologists, blinded to cohort or clinical status scored the study for gas trapping, bronchiectasis and total score.

The lung function tests were also performed that same morning during a period of clinical stability and after standard airway clearance at home. The MBW, spirometry and plethysmography protocols adhered closely to American Thoracic Society (ATS)/European Respiratory Society (ERS) recommendations. The bottom line after attempting to correlate different lung function tests with HRCT or with LCI, was that healthy children have similar LCI, but CF children, even during a period of stability, display a wide range of values. Moreover, more abnormalities are measurable on LCI than on HRCT performed on the same morning. CF children with normal LCI can have abnormalities on HRCT and children with normal HRCT can have abnormal LCI. This suggests there is not a tight association between bronchiectasis and LCI early in the disease. If LCI and HRCT are being considered as outcome measures in a clinical trial, it would be prudent to determine whether the study drug or protocol is more likely to affect function versus slowing structural damage.

References

1 Gustafsson PM, De Jong PA, Tiddens HA, Lindblad A. Multiple-breath inert gas washout and spirometry versus structural lung disease in cystic fibrosis. *Thorax* 63(2), 129–134 (2008).

2 Kieninger E, Singer F, Fuchs O *et al.* Long-term course of lung clearance index between infancy and school-age in cystic fibrosis subjects. *J. Cyst. Fib.* (2011).

No advantage to bronchoalveolar lavage cultures over oropharyngeal cultures in cystic fibrosis early stage lung infections

Evaluation of: Wainwright CE, Vidmar S, Armstrong DS *et al.* Effect of bronchoalveolar lavage-directed therapy on *Pseudomonas aeruginosa* infection and structural lung injury in children with cystic fibrosis: a randomized trial. *JAMA* 306(2), 163–171 (2011).

Given the importance of *Pseudomonas aeruginosa* infections and development of subsequent bronchiectasis to progress of lung damage in cystic fibrosis (CF), it is reasonable to hypothesize that direct culture of the airways from bronchoalveolar lavage will offer an advantage in the early detection and treatment of lung infections in CF. The authors undertook a multicenter, two nation, randomized, parallel two group study, the Australasian Cystic Fibrosis Bronchoalveolar Lavage (ACFBL) in which standard management was compared with bronchoalveolar lavage (BAL)-directed therapy over 5 years following diagnosis. The primary outcomes were *P. aeruginosa* infections and structural damage as quantified by the Brody II score of a low dose, high resolution chest CT scan. The study ran between 1999 and 2009 and 157 patients completed the study. The BAL group underwent the procedure before the age of 6 months, during hospitalizations for pulmonary exacerbation, when *P. aeruginosa* was isolated from an oropharyngeal culture, and after eradication therapy. Standard of care was to monitor oropharyngeal cultures. Both groups had a final BAL to quantify the *P. aeruginosa* and a high resolution chest CT scan.

In total 93%, or 157 children, completed the study. There was no significant difference in detection of *P. aeruginosa* between the two groups, 10% in the BAL-directed group and 12% in the standard treatment group. There was no difference in any of the CT scores between the groups. The prevalence of other pathogens in the BAL was no different at the end of the study. The authors believe that the unexpectedly low prevalence of *P. aeruginosa* at the end of the study explains the inability to detect a meaningful difference in outcomes. However this result is similar to that in the Early Inhaled Tobramycin for Eradication (ELITE) trial in the USA, where there was no difference in outcomes between treating *P. aeruginosa* on a regular interval or by culture directed therapies [1]. Any early and aggressive therapy seems to afford long-term benefit, which is good news overall. Yet, in the ACFBAL study, the low prevalence rate of BAL-positive cultures for *P. aeruginosa* was accompanied by discouragingly high CT scan scores indicative of air trapping and bronchiectasis. Perhaps inflammation remains unaddressed by anti-infective therapies, and/or CFTR dysfunction has additional secondary consequences that fail to improve with control of *P. aeruginosa*. A recent study of the distribution of microbes within different regions of the CF lung suggests that there is significant heterogeneity [2]. Not only is the airway polymicrobial, but if the BAL fails to sample a specific region with an important pathogen, the antibiotic profile may be incomplete. At the very least, BAL on a routine basis is unlikely to afford enough benefit to become part of the guidelines for the management of early CF.

References

1 Ratjen F, Munck A, Kho P, Angyalosi G. Treatment of early Pseudomonas aeruginosa infection in patients with cystic fibrosis: the ELITE trial. *Thorax* 65(4), 286–291 (2010).

2 Willner D, Haynes MR, Furlan M *et al.* Spatial distribution of microbial communities in the cystic fibrosis lung. *ISME J.* doi: 10.1038/ismej.2011.104 (2011) (Epub ahead of print).

Clinical trial of inhaled levofloxacin in cystic fibrosis demonstrates a reduction in concentration of *Pseudomonas aeruginosa* in the airways of cystic fibrosis subjects

Evaluation of: Geller DE, Flume PA, Staab D *et al.* Levofloxacin inhalation solution (MP376) in patients with cystic fibrosis with *Pseudomonas aeruginosa*. *Am. J. Respir. Crit. Care Med.* 183(11), 1510–1516 (2011).

There is a continued need for antibiotics directed at *Pseudomonas aeruginosa* in the cystic fibrosis (CF) airways. A novel formulation of levofloxacin, (MP376) is being developed for patients colonized with *P. aeruginosa*. Levofloxacin is available and used as an oral formulation in CF, but the inhaled route offers a number of advantages including the ability to achieve a higher concentration in the airways lining fluids than in the bloodstream while avoiding toxicities of systemic administration [1,2]. Levofloxacin is a fluoroquinolone that is active against *P. aeruginosa* and other Gram-negative bacteria and is not inactivated in CF sputum. It may even be

immunomodulatory [2]. MP376 is formulated with the eFlow delivery device (Pari Pharma, Munich, Germany).

The clinical trial was conducted as a randomized, placebo controlled, multinational, multicenter, multidose study in CF volunteers over 16 years with FEV1 between 25 and 85% predicted, evidence of *P. aeruginosa* infections previously, and history of regular cycling inhaled antibiotics. Patients were assigned to placebo, 120 mg/day, 240 mg/day, or 240 mg twice daily. The primary end point was change in *P. aeruginosa* concentration in sputum between day 28 and day 0. Secondary end points included changes in pulmonary function, time to need for additional antipseudomonal antibiotics, and the change in respiratory portion of the Cystic Fibrosis Questionnaire-Revised (CFQR). Standard safety monitoring was incorporated. Analyses were reported as a modified intent to treat (any randomized patient who received any study drug) for safety. A sample size of 128 subjects provided at least 80% power to detect a difference between treatment arms with respect to the primary end point.

All doses of MP376 effectively reduced concentration of *P. aeruginosa*, and the highest dose, twice daily, reduced the concentration by nearly one log when compared with placebo. This dose regimen led to an 8.7% improvement in FEV1 and reduced the need for antipseudomonal antibiotics. Tolerance of the drug was sufficient. The results are promising and levofloxacin has theoretical advantages over existing approved drugs with respect to *in vitro* activity against other pathogens commonly cultured in CF sputum, such as *Burkholderia cepacia* or *Stenotrophomonas maltophilia*. Levofloxacin shows superior activity in anaerobic conditions and the activity is not affected by biofilms. Further development is anticipated.

References

1 Lee CK, Boyle MP, Diener-West M, Brass-Ernst L, Noschese M, Zeitlin PL. Levofloxacin pharmacokinetics in adult cystic fibrosis. *Chest* 131(3), 796–802 (2007).

2 Tsivkovskii R, Sabet M, Tarazi Z, Griffith DC, Lomovskaya O, Dudley MN. Levofloxacin reduces inflammatory cytokine levels in human bronchial epithelia cells: implications for aerosol MP-376 (levofloxacin solution for inhalation) treatment of chronic pulmonary infections. *FEMS Immunol. Med. Microbiol.* 61(2), 141–146 (2011).

Lack of common proteostatic patterns of gene expression between correctors of F508del-CFTR trafficking suggests multiple therapeutic targets may be required for effective restoration of CFTR

Evaluation of: Sondo E, Tomati V, Caci E *et al.* Rescue of the mutant CFTR chloride channel by pharmacological correctors and low temperature analyzed by gene expression profiling. *Am. J. Physiol. Cell Physiol.* 301(4), C872–C885 (2011).

Control of airways infection in cystic fibrosis (CF) is unlikely to be sufficient by itself to prevent progression of lung disease and overall morbidity and mortality. CF is a systemic disorder affecting organs with secretory function dependent on chloride secretion. The possibility of CFTR repair therapy is promising if it can be delivered systemically. There is much excitement for the potential of CFTR-based therapies for prevention and possibly even repair of organ damage. The Vertex VX-770 (Cambridge, MA, USA) for G551D CFTR and the Ataluren (PTC Therapeutics, South Plainfield, NJ, USA) for stop codon CFTR mutations are furthest along. The most common mutation, F508del CFTR is especially challenging. F508del CFTR has a reduced open time for chloride transport and is prematurely degraded within the cell so as to leave the subject with very little CFTR protein from the allele. No single compound has been developed that can both rescue CFTR from degradation and open the channel to normal levels. This paper is an *in vitro* exploration of the effects of F508del trafficking correctors on gene expression. Amongst the tested compounds are correctors 4a [1], VRT 325, and two newly identified molecules, 9-aminoacridine and cyclopirox. The outcomes – proteostasis patterns of gene expression – are not the traditional outcomes for drug development, but may be an additional measure for assessing efficacy and safety if applicable in a subject. Proteostasis commonly means the genes or proteins involved in maintaining protein folding and maturation.

The authors studied the genomic response by microarray technology in a commonly used F508del expressing cell line CFBE41o- and in primary CF bronchial epithelial cells from patients. The cell line is highly responsive to corrector therapy including induction of CFTR-mediated chloride transport without a concomitant potentiator to open the CFTR chloride channel more often. Primary bronchial epithelial cells, like the CF human subject, need both a corrector and a potentiator [2]. The experiments

reported in this paper find a discrepancy between the different putative correctors; some of them repair proteostasis, but leave chloride secretion untouched, and others have little effect on the transcriptome. However, the authors did not combine a corrector with a potentiator, which would mimic the current combination trial led by Vertex Corporation (VX809 and VX770). This approach may correct proteostasis and chloride secretion if nontoxic and tolerable.

Inspection of the identity of the genes most highly up- or down-regulated by the correctors does not yield any critical insights translatable to the clinic, with one exception. 9-aminoacridine greatly upregulates CFTR transcript (60-fold), which might be beneficial in class IV and V mutations where limited amounts of functional CFTR are present. A second interesting result was that the compounds did not behave similarly with respect to gene expression between the cell line and primary cells, but growth at a permissive low temperature led to similar genomic patterns, particularly with respect to enzymes involved in ubiquitination and quality control. Low temperature is one of the most powerful correctors and often used as the positive control for F508del. Finding an authentic chemical mimic for low temperature correction of misfolded CFTR would be a significant advance.

References

1 Grove DE, Rosser MF, Ren HY, Naren AP, Cyr DM. Mechanisms for rescue of correctable folding defects in CFTRδ F508. *Mol. Biol. Cell* 20(18), 4059–4069 (2009).

2 Phuan PW, Yang B, Knapp J *et al*. Cyanoquinolines with independent corrector and potentiator activities restore Δphe508-cystic fibrosis transmembrane conductance regulator chloride channel function in cystic fibrosis. *Mol. Pharmacol.* 80(4), 683–693 (2011).