Utility of Lung Clearance Index Testing as a Noninvasive Marker of Deployment-related Lung Disease

Silpa Dhoma Krefft, MD, MPH, Matthew Strand, PhD, Jennifer Smith, PA-C, Catherine Stroup, MPH, Richard Meehan, MD, and Cecile Rose, MD, MPH

Objective: The aim of this study was to determine utility and sensitivity of lung clearance index (LCI) testing as a marker of lung injury in symptomatic military deployers compared with healthy controls. **Methods:** We tested 24 healthy controls and 28 deployers with respiratory symptoms (17 of 28 with definite and 11 of 28 with probable deployment-related lung disease). We compared mean LCI scores between deployers and controls using *t* tests; adjusted tests were derived from multiple regression models. **Results:** Mean LCI scores were significantly higher ($P = 0.001$) in deployers [7.76, 95% confidence interval (95% CI) 7.34 to 8.17] than controls (6.95, 95% CI 6.73 to 7.17). Adjusting for body mass index (BMI), smoking, and age, there were no significant differences ($P = 0.10$) between mean LCI scores in deployers (7.42, 95% CI 7.13 to 7.71) and controls (7.06, 95% CI 6.74 to 7.39). **Conclusions:** The trend toward higher LCI scores in symptomatic deployers may be linked to underlying lung disease and/or BMI but requires further investigation in a larger population.

BACKGROUND

More than 2.5 million United States military personnel and civilian contractors have deployed to Iraq, Afghanistan, and other locations in southwest Asia since 2001, as a part of several military operations. The major ones include Operation Enduring Freedom (OEF, the U.S. military efforts in Afghanistan from 2001 to 2014), Operation Iraqi Freedom (OIF, the period of U.S. military involvement in Iraq between 2003 and 2009), and Operation New Dawn (OND, the post-2009 military effort in Iraq).[1,2] OEF/OIF/OND deployers report variable exposures to desert dust particulate matter, burn pit combustion emissions, diesel exhaust particulates, industrial fires, and combat dust debris as well as more conventional workplace exposures linked to their military occupational specialty (MOS).[3–5] Respirable particulate matter ($PM_{2.5}$) sampling conducted in 15 locations in southwest Asia (six in Iraq, two in Afghanistan) was notable for all sites exceeding the Military Exposure Guideline of 15 $\mu g/m^3$.[6] Prompted by concerns about exposure to burn pits, the Institute of Medicine issued a report in 2011 concluding that deployment inhalational hazards involve more than burn pit emissions alone, and recommended further research on long-term health effects.[7]

From the National Jewish Health Denver, Denver, Colorado (Dr Krefft, Dr Strand, Smith, Stroup, Dr Meehan, Dr Rose); VA Eastern Colorado Health Care System, Denver, Colorado (Dr Krefft); University of Colorado Anschutz Medical Campus, Aurora, Colorado (Drs Krefft, Strand, Rose); Colorado School of Public Health, Aurora, Colorado (Drs Krefft, Strand, Rose).

This study was supported by the grant T42OH009229, and funded by the Centers for Disease Control and Prevention and Sergeant Sullivan Center. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the Centers for Disease Controls and Prevention or the Department of Health and Human Services.

In addition to the funding sources listed above, the authors and the institution have received research funding from the Department of Defense.

The authors disclose no conflicts of interest.

Address correspondence to: Silpa Dhoma Krefft, MD, MPH, 1400 Jackson Street, DEOHS–G208, Denver, CO 80206 (KrefftS@NJHealth.org).

DOI: 10.1097/JOM.0000000000001058

An unknown number of military deployers and civilian contractors have returned from these combat zones with respiratory symptoms unexplained by pulmonary function testing (PFT) and chest computerized tomography (CT) and may be at risk for bronchiolitis that requires surgical lung biopsy for diagnosis.[8,9] In addition to bronchiolitis, we reported histopathologic findings of granulomatous pneumonitis and emphysema/hyperinflation predominantly in the distal airways of OEF/OIF/OND deployers.[10] Given the risks and costs associated with surgical lung biopsy, there is a critical need to identify noninvasive markers of deployment-related lung injury.

The histopathologic abnormalities in some symptomatic deployers demonstrate primarily peripheral small airways abnormalities, a feature that is shared with early lung abnormalities in cystic fibrosis (CF).[10–12] CF is a genetic condition that afflicts one out of every 3700 newborns in the United States.[13] There are an estimated 1000 new diagnoses of CF in children and adults every year. CF affects primarily the lung and pancreas. Over time, the small airways of those with CF become plugged with mucus due to diminished clearance of secretions.[14] Lung clearance index (LCI) testing has been used as a screening tool for early detection of peripheral airways disease and risk for lung function decline in the pediatric CF population.[15,16] More recently, LCI also has shown promise in detecting ventilation heterogeneity in the non-CF chronic airways diseases such as bronchiolitis obliterans.[17] LCI also is a sensitive, reproducible, noninvasive, and practical measure of airways disease in adults with CF,[18] and has a narrow range of normal over a wide age range, making it ideal for long-term follow-up studies.

The LCI is determined by the number of lung volume turnovers necessary to clear the lungs of an inert tracer gas such as nitrogen that is inhaled at the beginning of the test. The LCI score is calculated by determining the cumulative expired volume (CEV), then dividing the CEV by the functional residual capacity. Values of less than 7.0 are considered normal, while an LCI score exceeding 7.0 is associated with heterogeneity in ventilation and peripheral airway abnormalities.[15,16,18–20] The ability to detect peripheral airways disease may make LCI a better modality for detecting the lung histopathologic abnormalities in symptomatic deployers that are often missed on conventional PFT.

Spirometry has long been known as an insensitive measure of small airways disease. The small airways (those less than 2 mm in diameter) have a large combined cross-sectional surface area and therefore have low mean flow rates and combined resistance. In healthy adults, they contribute less than 10% of total airways resistance.[21] Considerable structural damage to these airways therefore can occur before there is any impairment of the forced expiratory volume in 1 second (FEV1). Using hyperpolarized helium magnetic resonance to image the distribution of inhaled helium, studies have shown that FEV1 is insensitive to disturbances in ventilation distribution.[22] It is the sensitivity to small airways dysfunction that makes LCI such a valuable measure of airway physiology.[18] As such, LCI fills an important gap in our ability to monitor airways disease noninvasively in the so-called "silent zone" between onset of injury or inflammation and its detection with standard lung function tests.

Our study examined the role of LCI testing as an early marker of lung injury in a cohort of military men and women returning from post-2001 deployment with unexplained chest symptoms who had been comprehensively characterized using conventional clinical diagnostic tools. We hypothesized that, in these symptomatic deployers, the LCI would be a useful early marker of lung injury that correlates with abnormalities found on surgical lung biopsy. Further, we hypothesized that LCI would be better at predicting lung biopsy abnormalities in symptomatic deployers than findings on either PFT or high-resolution chest CT scan.

METHODS

Study Design and Case Definitions

To explore the utility of LCI as a noninvasive marker of deployment lung disease, we conducted an observational pilot study comparing 24 healthy volunteers with 28 symptomatic deployers, 17 of whom had undergone video-assisted thoracopic surgical (VATS) lung biopsy and all meeting our case definitions for definite or probable deployment-related lung disease. Seventeen of the twenty-eight symptomatic deployers were classified as having definite deployment-related lung disease, and 11 were classified as having probable deployment-related disease. We defined definite deployment-related lung disease as the presence of chest symptoms in a deployer who, on surgical lung biopsy, had bronchiolitis, granulomatous pneumonitis, and/or emphysema/hyperinflation without other known cause.[11] Probable cases were those deployers reporting respiratory symptoms of shortness of breath, chest tightness, wheezing, persistent cough, or decreased exercise tolerance (eg, inability to pass military physical fitness test requirements) during or following Iraq and/or Afghanistan deployment and who had at least one abnormal noninvasive clinical test result on PFT (ie, abnormal spirometry, lung volumes, and/or diffusion testing as described below) or chest imaging abnormalities concerning for small airways disease such as bronchiolitis (ie, presence of centrilobular nodularity and/or air trapping/mosaicism as interpreted for clinical purposes by an experienced pulmonary radiologist). Probable cases did not undergo surgical lung biopsy for diagnosis.

Target Population and Enrollment

With informed consent, we recruited study participants from our Occupational Lung Disease Clinic at National Jewish Health (NJH) who were referred for evaluation of unexplained respiratory symptoms with onset during or following OEF/OIF/OND deployment. The healthy control group consisted of 24 participants who were 18 years of age or older, had no history of pre-existing lung disease, and reported no respiratory illness in the 4 weeks preceding enrollment and testing. Inclusion criteria for the deployer group included presence of respiratory symptoms (cough, chest tightness, wheezing, shortness of breath, or decreased exercise tolerance) following deployment, age at least 18 years, history of post-2001 deployment exceeding 6 weeks as either military personnel or as a civilian contractor, and who met our case definitions for definite or probable deployment-related lung disease. We excluded deployers from this study if, on clinical evaluation, they were found to have other explanations for their respiratory symptoms. We enrolled study participants with Institutional Review Board approval.

Clinical and LCI Testing

LCI Using Multiple Breath Washout (MBW)

We used the EcoMedics Exhalyzer D LCI instrument and accompanying Spiroware software provided by the manufacturer Eco Physics, Inc. (Ann Arbor, MI) for all LCI testing. Study LCI technicians were experienced and proficient in the use of the LCI machine. Study participants remained seated during the test and, when instructed, breathed passively through a mouthpiece while wearing a noseclip. The test commenced with the participant taking 10 breaths of room air, *followed by a wash-in phase of breathing 100% oxygen*. After reaching equilibrium, a wash-out phase began by switching back to room air. Testing ended once the wash-out phase was complete (defined as a reduction in nitrogen gas concentration below 1/40 or 2.5% of the initial nitrogen level). Each participant completed two to three LCI tests following published LCI testing protocols.[18–20] We conducted additional tests if two LCI tests did not meet the quality criteria (for both acceptability and reproducibility). Testing typically required 30 to 45 minutes to complete.

Pulmonary Function Testing

As part of their clinical evaluation, deployers completed body plethysmographic PFT with pre- and post-bronchodilator spirometry, lung volumes, and diffusion capacity in accordance with American Thoracic Society standards.[23–25] We defined abnormal spirometry as a forced vital capacity (FVC) percent predicted below the lower limit of normal (LLN), a FEV1 percent predicted below the LLN, or an FEV1/FVC ratio below the LLN. Spirometry reference values were obtained from the National Health and Nutrition Examination Survey III.[26] Lung volume reference values were based on the 1995 ATS/ERS workshop, and reference values for carbon monoxide diffusing capacity were obtained from the Crapo prediction set.[25,27]

Cardiopulmonary Exercise Tolerance (CPET) Testing

Deployers underwent maximal cardiopulmonary exercise tolerance testing with arterial line placement. We analyzed maximum oxygen consumption (VO_2 max percent predicted) and considered less than 85% as reduced exercise tolerance.[28]

Chest Computed Tomography (CT)

All 28 symptomatic deployers had chest CT scans (22 with high-resolution images) available for review by nine experienced NJH pulmonary radiologists who were not blinded to deployer status. Three pulmonologists independently reviewed written radiology reports for parameters of small airways abnormalities, and findings were scored as present if all three were in agreement. These chest CT parameters included centrilobular nodularity and mosaic attenuation/air trapping.

Lung Biopsy

Surgical lung biopsies were available in 17 deployers. We reviewed lung biopsy pathology reports from six experienced pulmonary pathologists for findings previously described as part of the spectrum of deployment-related lung disease.[10,11] The presence of one of more of these findings (bronchiolitis, granulomatous pneumonitis, and emphysema/hyperinflation) in a deployer with respiratory symptoms met the case definition for definite deployment-related lung disease.

Data Collection and Analysis

We reported the mean LCI score of two or three tests that met published quality criteria.[18–20] We used descriptive statistics, *t* test, and Fisher exact test to compare demographic data between controls and deployers using alpha = 0.05. We compared mean LCI score between controls and deployers using unadjusted and adjusted *t* tests. The adjusted tests were derived from multiple linear regression models that included and adjusted for smoking, age, and body mass index as covariates. Due to the small sample size of deployers with surgical lung biopsy, we used descriptive statistics to characterize the potential advantage of LCI as a predictor of lung abnormalities in deployers compared with conventional lung function testing and chest CT scans.

TABLE 1. Demographics of Study Subjects

	Controls ($n=24$)	Deployers ($n=28$)	P *
Mean age in years (range)	37.8 (26–62)	41.2 (25–59)	0.18
Gender (%)			
Female	4	11	0.61
Male	96	89	
Ethnicity (%)			
Hispanic/Latino	4	14	0.36
Not Hispanic/Latino	96	86	
Race (%)			
African–American or Black	4	4	0.45
White	88	96	
More than one race	8	—	
Smoking status (%)			
Current smoker	8	4	0.23
Former smoker	13	25	
Never smoker	79	71	
Mean BMI in kg/m^2 (range)	25.6 (19.9–42.8)	30.1 (20.5–39.8)	0.0014

BMI, body mass index.
*Note: $P=0.05$, analysis with t test for age and BMI, Fisher exact test for gender, ethnicity, race, and smoking status.

RESULTS

Table 1 summarizes the demographics of study participants. We found no significant differences between controls and deployers in age, gender, race, ethnicity, and smoking status, but observed a significant difference in BMI between controls and deployers, with deployers having higher BMI scores.

Figure 1 and Table 2 show the unadjusted and adjusted mean LCI scores in controls compared with deployers. LCI scores were significantly higher (more abnormal) in deployers than in controls. After adjusting for age, smoking, and BMI, we noted a trend toward a higher LCI score in deployers, but this was not statistically significant. LCI score greater than 7.0 was present in over half of healthy controls without a history of lung disease and in 82% of deployers with probable or definite deployment-related lung disease.

Table 3 summarizes clinical findings in symptomatic deployers and demonstrates that LCI score greater than 7.0 was the most common abnormality, followed by abnormal chest CT findings of centrilobular nodularity and/or air trapping/mosaicism. Both tests appeared to be substantially better markers of lung abnormalities than were any of the parameters on resting or exercise lung function testing. Not surprisingly, diminished exercise tolerance was more sensitive than spirometry in predicting those with a clinical diagnosis of deployment-related lung disease, though both were inferior to LCI testing in our study population.

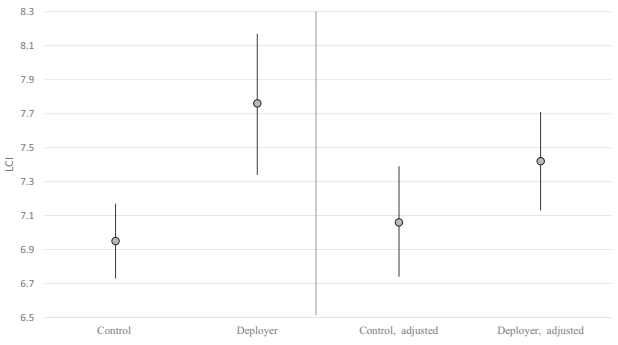

FIGURE 1. Both unadjusted and adjusted (age, smoking status, body mass index) LCI means with 95% confidence intervals are shown for healthy adult controls and symptomatic deployers.

TABLE 2. LCI Results in Controls and Deployers

	Controls ($n=24$)	Deployers ($n=28$)	P *
Mean LCI, unadjusted (95% CI)	6.95 (6.73–7.17)	7.76 (7.34–8.17)	0.001
Mean LCI, adjusted (95% CI)	7.06 (6.74–7.39)	7.42 (7.13–7.71)	0.10
Percent LCI >7	54%	82%	

95 CI, 95% confidence interval; LCI, lung clearance index.
*Note: P value of 0.001 reported is unadjusted; t test adjusted for covariates of age, smoking status, and BMI demonstrates no significant difference ($P=0.10$) between mean LCI of controls (7.06) and deployers (7.42).

TABLE 3. Number and Percent of Symptomatic Deployers With Abnormal Clinical Testing ($n=28$)

Test	Number/Percent With Abnormal Test
LCI >7	23/28 (82%)
Chest computerized tomography (CT) findings of CLN and/or AT/mosaicism	19/28 (68%)
Cardiopulmonary exercise tolerance test (VO_2 max <85% predicted)*	14/27 (52%)
Abnormally elevated residual volume (RV) >120%	7/28 (25%)
Diffusion capacity (DLCO) <80% predicted	6/28 (21%)
Spirometry†	5/28 (18%)
FEF25–75% <lower limit of normal (LLN)‡	5/28 (18%)

AT, air trapping; CLN, centrilobular nodularity; LCI, lung clearance index.
*Note: Cardiopulmonary exercise tolerance test data available in 27 deployers (percent abnormal calculated for $n=27$).
†Abnormal spirometry definition: FVC percent predicted (PP) below the lower limit of normal (LLN), FEV1 PP <LLN, or FEV1/FVC ratio <LLN.
‡FEF25–75% <LLN defined as abnormal.

TABLE 4. Comparison of Abnormal Noninvasive Test Results in Deployers With Biopsy-proven Lung Disease ($n=17$)

	LCI (Score >7.0)	Chest CT*	CPET†	PFT‡	FEF 25–75%§
Number and percentage of deployers with abnormal test	14/17 (82%)	13/17 (76%)	8/16 (50%)	7/17 (41%)	5/17 (29%)

CPET, cardiopulmonary exercise tolerance; CT, computerized tomography; LCI, lung clearance index; PFT, pulmonary function testing.
*Chest CT scan considered abnormal if presence of centrilobular nodularity (CLN) or air trapping (AT)/mosaicism.
†Cardiopulmonary exercise tolerance test data available in 16 deployers (percent abnormal calculated for $n=16$); VO_2 max <85 PP considered abnormal.
‡Abnormal PFT definition: FVC percent predicted (PP) below the lower limit of normal (LLN), FEV1 PP <LLN, or FEV1/FVC ratio <LLN, lung volume (TLC, TGV, or RV <80 PP or >120 PP), or diffusion capacity (DLCO) <80 PP.
§FEF25–75% <LLN defined as abnormal.

Table 4 summarizes findings in the 17 deployers with definite (ie, biopsy-proven) deployment-related lung disease. We found the same trend in higher sensitivity of LCI score as that described in the total population of symptomatic deployers, followed by abnormal

chest CT scan. Abnormal FEF25–75% was the least sensitive of all the parameters examined.

CONCLUSIONS

There is a compelling need to identify methods to detect early small airways abnormalities that may not be readily apparent in clinical testing without invasive lung biopsy. LCI is a promising noninvasive test with a very low-risk profile that is being explored increasingly in a variety of settings wherein early disease detection would be important for treatment and longitudinal follow-up.[15–18]

To validate the sensitivity of such tools, biopsy findings remain important. A significant strength of our study was information on the histopathologic confirmation of lung disease from surgical lung biopsies in the majority of the symptomatic deployer group. Access to comprehensive clinical profiles that included resting and exercise lung function and high-resolution chest CT scans in nearly all of the symptomatic deployer groups for comparison with LCI scores was an additional strength.

We anticipated that mean LCI scores would be higher (ie, more abnormal) in symptomatic deployers with deployment-related lung disease than in adjusted controls, and this trend was observed in our study despite small numbers of participants. We also found that abnormal LCI scores were more sensitive than both resting and exercise parameters of lung function and CT imaging in identifying those with deployment lung disease. This study demonstrates a trend toward higher LCI scores in deployers with respiratory symptoms than healthy controls. We also found higher agreement between elevated LCI score (>7.0) and abnormal lung biopsy than rest and exercise lung function testing.

BMI appeared to account for some of the difference in LCI between the deployers and controls. However, the influence of elevated BMI (>25 kg/m^2) on LCI score remains uncertain, as we did not observe a linear relationship between increasing BMI and higher LCI scores in controls, and previous studies have not recommended correction for BMI.[29] To our knowledge, an association between BMI and LCI score has not been reported previously and warrants further evaluation of populations with a wider range of BMI.

We also observed that the average mean LCI score in our control group was greater than 7.0, raising questions about misclassification of healthy controls who may have had subtle or undiagnosed subclinical respiratory disease. More likely, this finding reflects the limited normative data on older adult controls with higher BMIs, as the majority of LCI research has been conducted in relatively young cohorts of CF patients who have low to average BMIs. Our study findings identify areas for future research in how age and BMI may affect LCI score.

Although there were no significant differences in age and smoking status between controls and deployers, age and smoking status may have an impact on LCI scores, and were therefore included in our adjusted analysis. Lung function measured by spirometry is adjusted for age based on an expected decrease in lung function with increasing age.[30,31] Similarly, there is some evidence that LCI scores would be expected to increase with age, but studies with larger sample sizes and other potential contributing or confounding factors such as smoking history are needed to verify these emerging findings.[32,33]

An abnormally increased LCI may be due to ventilation heterogeneity between relatively large lung units subtended by proximal conducting airways or by increased dead space abnormalities related to diffusion capacity at the acinar level (distal to the terminal bronchiole and the area where most gas exchange occurs in the lungs).[34] Verbanck et al[35] described a method that may be able to differentiate these lung zone effects on LCI (using the variable S_{cond} for conductive ventilation heterogeneity index and S_{acin} for acinar ventilation heterogeneity index). We did not analyze either S_{cond} or S_{acin} variables in this pilot study, but plan to systematically measure this in an expanded LCI study to add to the growing literature examining potential mechanisms related to abnormally increased LCI measurements.

Study limitations included small sample size, nonmatched controls, and absence of spirometry data in the healthy control group. We plan to address these limitations by expanding the number of study participants and by adding spirometric testing for controls to further verify reportedly normal lung function. We also hope to enrich our control population with healthy nondeployed military personnel.

Our findings support the importance of future research exploring LCI and other markers of small airways abnormalities in larger working populations (such as those at risk for pneumoconiosis or hypersensitivity pneumonitis); expanding normative data for LCI scores in adults (with variable smoking, BMI and age factors); and further examining the relationships between LCI findings and more conventional clinical lung diagnostic tests in symptomatic military deployers. Future research will also examine the utility of longitudinal LCI testing in monitoring for stability or progression of deployment-related lung disease.

ACKNOWLEDGMENT

We would like to thank Kenneth Hunt, Dr Jerry Nick, and Connie St. Clair for their support in providing training and oversight for Lung Clearance Index testing.

REFERENCES

1. Watson Institute of International and Public Affairs. Costs of War [Internet]. Providence, RI: Brown University; 2015 [cited March 28, 2016]. Available at: http://watson.brown.edu/costsofwar/costs/human/veterans. Accessed March 24, 2017.
2. Fischer H. A Guide to U.S. Military Casualty Statistics: Operation Freedom's Sentinel, Operation Inherent Resolve, Operation New Dawn, Operation Iraqi Freedom, and Operation Enduring Freedom. Congressional Research Service; 2015 [cited February 29, 2016]. Available at: https://www.fas.org/sgp/crs/natsec/RS22452.pdf. Accessed March 24, 2017.
3. Helmer DA, Rossignol M, Blatt M, Agarwal R, Teichman R, Lange G. Health and exposure concerns of veterans deployed to Iraq and Afghanistan. *J Occup Environ Med*. 2007;49:475–480.
4. Teichman R. Exposures of concern to veterans returning from Afghanistan and Iraq. *J Occup Environ Med*. 2012;54:677–681.
5. McAndrew LM, Teichman RF, Osinubi OY, Jasien JV, Quigley KS. Environmental exposure and health of Operation Enduring Freedom/Operation Iraqi Freedom Veterans. *J Occup Environ Med*. 2012;54:665–669.
6. Engelbrecht JP, McDonald EV, Gillies JA, Jayanty RK, Casuccio G, Gertler AW. Characterizing mineral dusts and other aerosols from the Middle East – Part 1: ambient sampling. *Inhal Toxicol*. 2009;21:297–326.
7. National Research Council. *Long-Term Health Consequences of Exposure to Burn Pits in Iraq and Afghanistan*. Washington, DC: The National Academies Press; 2011, 109–115.
8. Smith B, Wong CA, Smith TC, Boyko EJ, Gackstetter GD, Ryan MAK. Newly reported respiratory symptoms and conditions among military personnel deployed to Iraq and Afghanistan: a prospective population-based study. *Am J Epidemiol*. 2009;170:1433–1442.
9. King MS, Eisenberg R, Newman JH, et al. Constrictive bronchiolitis in soldiers returning from Iraq and Afghanistan. *N Engl J Med*. 2011;365:222–230.
10. Dhoma S, Gottschall B, Robinson M, Williams A, Meehan R, Groshong S. Lung disease in deployers returning from Afghanistan and Iraq. *Am J Respir Crit Care Med*. 2013;187:A3669.
11. Krefft SD, Meehan R, Rose C. The emerging spectrum of post-deployment respiratory diseases. *Curr Opin Pulm Med*. 2015;21:185–192.
12. Hogg JC, Macklem PT, Thurlbeck WM. Site and nature of airway obstruction in chronic obstructive lung disease. *N Engl J Med*. 1968;278:1355–1360.
13. Grosse SD, Boyle CA, Botkin JR, et al. Newborn screening for cystic fibrosis: evaluation of benefits and risks and recommendations for state newborn screening programs. *MMWR*. 2004;53:1–36.
14. Cystic Fibrosis Foundation. About Cystic Fibrosis: What you Need to Know. Available at: http://www.cff.org/AboutCF/. Accessed December 17, 2015.
15. Aurora P, Stanojevic S, Wade A, et al. Lung clearance index at 4 years predicts subsequent lung function in children with cystic fibrosis. *Am J Respir Crit Care Med*. 2011;183:752–758.

16. Aurora P. Multiple-breath inert gas washout test and early cystic fibrosis lung disease. *Thorax*. 2010;65:373–374.
17. Gur M, Yaacoby-Bianu K, Ilivitzki A, et al. Lung clearance index (LCI) in patients with bronchiolitis obliterans: a preliminary report and comparison to cystic fibrosis patients. *Lung*. 2016;194:1007–1013.
18. Horsley AR, Gustafsson P, Macleod KA, et al. Lung clearance index is a sensitive, repeatable and practical measure of airways disease in adults with cystic fibrosis. *Thorax*. 2008;63:135–140.
19. Fuchs SI, Eder J, Ellemunter H, Gappa M. Lung clearance index: normal values, repeatability, and reproducibility in healthy children and adolescents. *Pediatr Pulmonol*. 2009;44:1180–1185.
20. Horsley AR. Lung clearance index in the assessment of airways disease. *Respir Med*. 2009;103:793–799.
21. McNulty W, Usmani OS. Techniques of assessing small airways dysfunction. *Eur Clin Respir J*. 2014. doi: 10.3402/ecrj.v1.25898.
22. Fain S, Schiebler ML, McCormack DG, Parraga G. Imaging of lung function using hyperpolarized helium-3 magnetic resonance imaging: review of current and emerging translational methods and applications. *J Magn Reson Imaging*. 2010;32:1398–1408.
23. Miller MR, Hankinson J, Brusasco V, et al. ATS/ERS Task Force. Standardisation of spirometry. *Eur Respir J*. 2005;26:319–338.
24. Wanger J, Clausen JL, Coates A, et al. ATS/ERS Task Force. Standardisation of the measurement of lung volumes. *Eur Respir J*. 2005;26:511–522.
25. Macintyre N, Crapo RO, Viegi G, et al. ATS/ERS Task Force. Standardisation of the single-breath determination of carbon monoxide uptake in the lung. *Eur Respir J*. 2005;26:720–735.
26. Hankinson JL, Odencrantz JR, Fedan KB. Spirometric reference values from a sample of the general U.S. population. *Am J Respir Crit Care Med*. 1999;159:179–187.
27. Stocks J, Quanjer PH. Reference values for residual volume, functional residual capacity, and total lung capacity. *Eur Respir J*. 1995;8:492–506.
28. Weisman IM, Beck KC, Casaburi R, et al. ATS/ACCP statement on cardiopulmonary exercise testing. *Am J Respir Crit Care Med*. 2003;167:211–277.
29. Verbanck S, Van Muylem A, Schuermans D, Bautmans I, Thompson B, Vincken W. Transfer factor, lung volumes, resistance and ventilation distribution in healthy adults. *Eur Respir J*. 2016;47:166–176.
30. Ware JH, Dockery DW, Louis TA, et al. Longitudinal and cross-sectional estimates of pulmonary function decline in never-smoking adults. *Am J Epidemiol*. 1990;132:685–700.
31. Sharma G, Goodwin J. Effect of aging on respiratory system physiology and immunology. *Clin Interv Aging*. 2006;1:253–260.
32. Verbanck S, Thompson BR, Schuermans D, et al. Ventilation heterogeneity in the acinar and conductive zones of the normal ageing lung. *Thorax*. 2012;67:789–795.
33. Htun C, Lahzami S, Luo D, Schoeffel R, King G. Normal age-dependent changes in peripheral airway function in the elderly. *Eur Respir J*. 2015;46:A2293.
34. Gonem S, Scadding A, Soares M, Singapuri A, Gustafsson P, Ohri C. Lung clearance index in adults with non-cystic fibrosis bronchiectasis. *Respir Res*. 2014;15:59. 1–9.
35. Verbanck S, Paiva M, Schuermans D, Hanon S, Vincken W, Van Muylem A. Relationshiops between the lung clearance index and conductive and acinar ventilation heterogeneity. *J Appl Physiol*. 2012;112:782–790.

