Cross-sectional associations of pulmonary function with systemic inflammation and oxidative stress in individuals with chronic spinal cord injury

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Context/Objective: Systemic inflammation, and to a lesser extent oxidative stress, have been associated with reduced pulmonary function. Our objective was to evaluate the associations between biomarkers of inflammation (C-reactive protein (CRP), interleukin-6 (IL-6)) and novel makers of global oxidative stress (fluorescent oxidation products (FLOx)) with spirometric and lung volume measures in individuals with chronic spinal cord injury (SCI).

Design: Cross-sectional study.

Setting: Veterans Affairs Medical Center.

Participants: One-hundred thirty-seven men with chronic SCI participating in an epidemiologic study.

Methods: Participants provided a blood sample, completed health questionnaires, and underwent pulmonary function testing, including helium dilution measurement of functional residual capacity (FRC). General linear models were used to model associations between increasing quartiles of inflammation or oxidative stress with each outcome measure, after adjustment for a number of potential confounders.

Outcome Measures: Percent-predicted forced vital capacity in one second (FEV₁), percent-predicted forced vital capacity (FVC), FEV₁/FVC, percent-predicted residual volume (RV), percent-predicted FRC, and percent-predicted total lung capacity (TLC).

Results: After adjustment for a number of confounders, participants with higher levels of CRP and IL-6 had lower percent-predicted FEV₁ and FVC measurements. There were no clear patterns of association with any of the oxidative stress biomarkers or other outcome measures.

Conclusion: Increased systemic inflammation was associated with reductions in FEV₁ and FVC independent of a number of covariates. Although the mechanism is uncertain, these results suggest that reductions in pulmonary function in SCI are associated with systemic inflammation.

Keywords: Pulmonary function, Inflammation, Oxidative stress, Spinal cord injury

Introduction

Studies have demonstrated cross-sectional inverse relationships between pulmonary function and biomarkers of

systemic inflammation among individuals with and without chronic obstructive pulmonary disease (COPD).^{1–11} Some,^{12–15} but not all,^{16,17} longitudinal studies have observed such associations. Fewer studies have demonstrated cross-sectional associations with

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biomarkers of oxidative stress.^{18–22} Similar findings in populations with and without COPD suggest that the mechanisms responsible are not likely to be solely mediated by pulmonary inflammation, but may be related to other sources of systemic inflammation and oxidative stress.

Chronic spinal cord injury (SCI) is characterized by abnormal pulmonary function as a consequence of respiratory system changes secondary to respiratory muscle weakness and paralysis, but is not known to result in pulmonary inflammation. SCI is, however, characterized by factors that promote systemic inflammation and oxidative stress, including central fat accumulation, reduced mobility, pressure ulcers, and urinary tract infections.^{23–30} We previously reported an inverse association between C-reactive protein (CRP) and interleukin-6 (IL-6) and forced expiratory volume in 1 second (FEV₁) and forced vital capacity (FVC) in a pilot study of 59 individuals with chronic SCI.³¹ In the current analysis, we examined cross-sectional associations between CRP and IL-6 and FEV₁, FVC, and FEV₁/FVC in a larger sample to validate our previous findings. In addition, we also examined relationships between lung volumes (residual volume (RV), functional residual capacity (FRC), and total lung capacity (TLC)) with CRP and IL-6, and with novel circulating biomarkers of global oxidative stress.

Methods

Study population

The Boston SCI Study began in 1994 with 556 individuals age 22 or older, at least one year post-injury, who were not-ventilator dependent and had no other neurologic condition. Participants were recruited from VA Boston and the community and at each visit completed questionnaires, performed pulmonary function testing, and, at visits between 2003 and 2007, provided a blood sample.^{32–34} For the current analysis, we included all men who provided a blood sample and performed pulmonary function testing within a 30 day period ($n = 137$, 134 provided blood and performed PFTs on the same day). Fifty-four were included in our previous report.³¹ The study was approved by the VA Boston Institutional Review Board, and participants provided informed consent prior to participating.

Pulmonary function testing

Spirometry was based on the 1994 American Thoracic Society (ATS) standards³⁵ modified for use in SCI as described previously.³⁶ Briefly, participants were instructed to exhale maximally and to sustain the effort for at least 6 seconds, or for as long as possible. Efforts were made to obtain at least three acceptable

efforts from each participant. Short expiratory efforts (less than 6 seconds) and excessive back extrapolation are common in SCI, but we have demonstrated that FVC and FEV₁ are reproducible in this population. Therefore, we accepted excessive back extrapolation and efforts less than six seconds if the effort was maximal with an acceptable flow-volume loop, and there was at least a 0.5-second plateau at RV. Spirometry was performed using a 10-L water-seal (Warren E. Collins DSII) ($n = 9$), an 8-L portable spirometer (Warren E. Collins Health Survey III) ($n = 6$), or a dry-rolling seal spirometer (CPL, nSpire Health) ($n = 122$). The highest values of FEV₁ and FVC from acceptable efforts were used.

Lung volume measurements ($n = 120$) were collected using helium dilution based on standards suggested by a European Respiratory Society/ATS Workshop.^{37,38} At least 5 minutes elapsed between measurements, and efforts were made to obtain at least 2 acceptable values. FRC was calculated as the mean of the 2 closest values, and ERV was calculated as the average of the 2 highest SVCs. TLC was calculated by adding RV (FRC-ERV) to the greater of SVC or FVC. Percent-predicted FEV₁ and FVC were calculated based on NHANES race specific equations and percent-predicted values of TLC, FRC, and RV were based on Crapo *et al.* with race adjustment.^{39–41}

Inflammation and oxidative stress biomarkers

Blood samples were drawn using an ethylenediaminetetraacetic acid tube, shipped overnight to a core blood laboratory with a freeze pack in an insulated cooler, and processed and stored as previously described.³¹ Plasma CRP was determined by a high-sensitivity immunoturbidimetric assay and IL-6 was determined by an ultra-sensitive enzyme linked immunosorbent assay at the Clinical and Epidemiologic Research Laboratory, Children's Hospital, Boston. Measurements of fluorescent oxidation products (FLOx) were performed in the laboratory of Dr Wu at the University of Cincinnati. FLOx reflects lipid oxidation products from reactions amongst proteins, DNA, and carbohydrates, were measured using the method developed by Dillard and Tappel in the 1970s, later modified by Shimasaki, and described in detail previously.^{42–44} Briefly, plasma was extracted with diethylether (3/1, v/v) and was measured by a spectrofluorometer at the wave lengths of 360/430 nm, 320/420 nm and 400/475 nm (excitation/emission wavelength) for FLOx1, FLOx2 and FLOx3, respectively. The fluorescence was determined as relative fluorescent intensity (FI) units per milliliter of plasma.

The majority of products are FLOx1, but FLOx2 is generated when linoleate oxidizes with DNA in the presence of metals, and FLOx3 is generated from the reactions of malondialdehyde with proteins and phospholipids.

Potential confounders

Potential confounders selected *a priori* included age, race, body mass index (BMI), cigarette smoking status (current, former, never) and pack-years of smoking, SCI duration, level, and severity, previous diagnosis of COPD, and current use of aspirin, non-steroidals, statins, and pulmonary medications. Each participant completed the ATS respiratory questionnaire,⁴⁵ was weighed, and had supine length measured. Self-reported height and weight were used for 10 and 6 participants, respectively. COPD was defined based on report of doctor-diagnosed chronic bronchitis, emphysema, or COPD. SCI level and severity were assessed by exam and record review. Motor level and completeness of injury was categorized according to the American Spinal Injury Association Impairment Scale (AIS).⁴⁶ Participants were classified as motor complete, no motor function below the neurological level (AIS A or B); AIS C (motor incomplete, motor function preserved below the neurological level, and more than half the key muscles below the neurological level not strong enough to overcome gravity); or AIS D (motor incomplete, motor function preserved below the neurological level, and half or more of key muscles below the neurological level strong enough to overcome gravity). Participants were grouped into cervical motor complete and AIS C, non-cervical motor complete and AIS C, and all others (AIS D). In sensitivity analyses we also considered an alternative classification, all motor complete (AIS A and B), AIS C, and all others (AIS D).

Statistical analysis

General linear models (PROC GLM, SAS 9.2; SAS Institute Inc, Cary, NC) were used to model associations between CRP, IL-6, FLOx1, FLOx2, and FLOx3 quartiles and each pulmonary function measure. The significance of the trends across quartiles was assessed using the median value of each exposure quartile. Basic models for FEV₁/FVC included adjustment for age and race. All other potential confounders were included in final multivariable models if they changed at least one biomarker/outcome association by more than 10%.⁴⁷

Results

Participants were mostly Caucasian (93%) veterans (72%), with a mean age of 55 years, had a wide range of injury levels, and on average had been injured

20 years (Table 1). Most were current or former smokers with an average of 33 pack-years and were overweight (BMI ≥ 25 , 34%) or obese (BMI ≥ 30 , 26%).

The majority of participants ($n = 127$ (92.7%)) had at least two or three acceptable expiratory efforts with the best two values of FEV₁ and FVC each within 200 mL, six (4.4%) had reproducible values of either FEV₁ or FVC, and others had at least one acceptable effort. A total of 112 (93.3%) participants with lung volume data available had at least two FRC values (98 within 200 ml), and eight had one acceptable measurement. In 118 (98.3%) participants, two SVC values were available. Distributions of pulmonary function outcomes and biomarkers of inflammation and oxidative stress (Table 2) show a wide range of values for both outcome and exposure measures.

Years since injury and use of pulmonary medication, aspirin, or NSAIDs did not appear to confound any of the associations.⁴⁷ Level/severity of injury, BMI, cigarette smoking status and pack-years, current use of statins, and COPD were all associated with changes of more than 10% in at least a single univariate model, and were therefore included in all final multivariable models. Results were similar from models with the alternative classification of level/severity of injury (data not shown). Therefore we used our primary grouping in all multivariable models.

The basic and multivariable adjusted means (and 95% confidence intervals (CI)) of percent-predicted FEV₁, percent-predicted FVC, and FEV₁/FVC are shown in Figure 1. In general, higher levels of systemic inflammation were associated with reduced pulmonary function. In basic models, associations with FEV₁ (P-for-trend = 0.001 for CRP and 0.0001 for IL-6) and FVC (P-for-trend = 0.0006 for CRP and 0.002 for IL-6) reached statistical significance, but associations with FEV₁/FVC did not. These associations were attenuated in multivariable models. The P-for-trend for FEV₁ and CRP was increased to 0.17, for FEV₁ and IL-6 was 0.02, for FVC and CRP was 0.09 and for FVC and IL-6 was 0.03. Overall, there were no clear patterns with the oxidative stress biomarkers, although FLOx3 was associated with a statistically significant decrease in FEV₁/FVC in basic (P-for-trend = 0.02) and multivariable (P-for-trend = 0.03) models.

Adjusted means (and 95% CIs) of percent-predicted RV, percent-predicted FRC, and percent-predicted TLC are shown in Figure 2. Overall, there were no associations observed between RV, FRC, and TLC and CRP, IL-6, and the FLOx markers, although the lowest levels were typically observed in the highest quartiles of FLOx exposure. In basic models, statistically

Table 1 Characteristics of the study participants (n = 137)

Characteristics	
Age (yrs), mean (SD)	55.1 (14.9)
Years since injury, mean (SD)	20.3 (13.8)
Pack-Years of Cigarettes, mean (SD) ¹	33.1 (29.4)
Race, N (%)	
Caucasian	127 (92.7)
African American	10 (7.3)
US Veteran, N (%)	98 (71.5)
Smoking Status, N (%)	
Never	55 (40.2)
Former	55 (40.2)
Current	27 (19.7)
BMI (kg/m ²), N (%)	
Underweight (≤ 20)	5 (3.7)
Normal weight (20–24.9)	50 (36.5)
Overweight (25.0–29.9)	46 (33.6)
Obese (≥ 30)	36 (26.3)
Level of SCI, N (%)	
Cervical motor complete and AIS C ²	21 (15.3)
Other motor complete and AIS C ³	57 (41.6)
AIS D	59 (43.1)
Mobility, N (%)	
Motorized Wheelchair	18 (13.1)
Hand Wheelchair	61 (44.5)
Walk with Aid	31 (22.6)
Walk Unassisted	27 (19.7)
Current Medication Use, N (%)	
Pulmonary Medications ⁴	7 (5.1)
Statins	29 (21.2)
Aspirin	34 (25.0)
NSAIDs	14 (10.2)
Doctor Diagnosed COPD, N (%)	21 (15.3)

¹Among ever smokers.

²16 cervical motor complete.

³49 non-cervical motor complete.

⁴Pulmonary medications include bronchodilators and inhaled steroids.

significant trends were only observed for decreases in TLC with increasing quartiles of CRP (P-for-trend = 0.009), and decreases in RV with increases in FLOx2 (P-for-trend = 0.01). No statistically significant patterns were observed in the multivariable adjusted models, although the patterns remained similar.

Discussion

In this cohort of individuals with chronic SCI, higher levels of systemic inflammation, as measured by CRP and IL-6, were associated with lower levels of FEV₁ and FVC, although not all of the associations reached statistical significance after adjustment for multiple

Table 2 Distributions of outcomes and biomarkers among the 137 participants

	Mean	Standard Deviation	Median	25th Percentile	75th Percentile
Outcomes					
% Predicted FEV ₁	82.4	20.5	83.7	69.5	95.3
% Predicted FVC	81.5	18.5	83.1	71.3	91.5
FEV ₁ /FVC	0.8	0.1	0.8	0.7	0.8
% Predicted RV	100.5	32.2	97.3	77.6	116.9
% Predicted FRC	87.4	22.2	87.2	70.5	102.4
% Predicted TLC	89.2	14.0	89.5	80.2	97.4
Biomarkers					
hsCRP (mg/mL)	5.5	19.6	1.8	0.9	3.9
IL-6 (pg/mL)	3.2	4.3	1.9	1.2	3.4
FLOP1 (Fl/ml)	2.6	4.4	2.1	1.7	2.4
FLOP2 (Fl/ml)	1467.2	3650.6	424.4	320.8	932.5
FLOP3 (Fl/ml)	70.4	70.0	59.3	49.4	72.4
	82.4	20.5	83.7	69.5	95.3

137 participants had information on FEV₁ and FVC; 120 had information on RV, FRC, and TLC.

Figure 1 Mean (95% confidence interval) levels of each pulmonary function outcome (top to bottom: % predicted FEV₁, % predicted FVC, and FEV₁/FVC) by quartile of inflammatory or oxidative stress biomarker. P-for-trend values (based on the medians of each category) are presented for basic (top p-values, diamonds) and fully adjusted (bottom p-values, squares) models.

confounders. Global markers of oxidative stress did not appear to be consistently related to measures of pulmonary function.

In our earlier pilot study of 59 individuals (54 of whom met inclusion criteria for the current analysis), IL-6 was inversely associated with percent-predicted

FEV₁ and percent-predicted FVC in unadjusted models as well as in univariate models adjusted for either SCI level and severity, COPD, smoking, or BMI.³¹ The associations with CRP were similar, but were not as consistent. In the current study, where we were able to adjust for multiple confounders

Figure 2 Mean (95% confidence interval) levels of each pulmonary function outcome (top to bottom: % predicted RV, % predicted FRC, and % predicted TLC) by quartile of inflammatory or oxidative stress biomarker. P-for-trend values (based on the medians of each category) are presented for basic (top p-values, diamonds) and fully adjusted (bottom p-values, squares) models.

simultaneously (unlike our previous study where only one confounder at a time could be adjusted for), we found similar inverse associations between CRP and IL-6 with FEV₁ and FVC, but not with FEV₁/FVC.

Our findings of inverse associations of FEV₁ and FVC with systemic inflammation are consistent with findings from the majority of other population-based cross-sectional studies,¹⁻¹⁰ particularly those among

males.^{6,11,48} This association has been demonstrated in a number of populations, including young, disease-free individuals, the elderly, and populations with and without COPD, but other than in this and our previous report, SCI has not been studied previously. The specific inflammatory biomarkers examined have varied across these studies, but CRP and IL-6 are the most consistently examined.

To the best of our knowledge, only one other manuscript has examined the impact of markers of global oxidative stress on pulmonary function.²¹ In 88 Dutch patients with moderate-to-severe COPD and 55 controls, the associations of percent-predicted FEV₁ with a number of measures of advanced glycation end-products were examined, with mixed results. In models adjusted for age, sex, person-years of smoking, BMI, HDL cholesterol, triglycerides, glomerular filtration rate, and CRP, N⁶-(carboxyethyl)lysine and skin autofluorescence were negatively associated and N⁶-(carboxymethyl)lysine was positively associated with percent-predicted FEV₁, and the authors concluded that the exact role of these glycation end products remains to be determined. In another study, higher levels of serum malondialdehyde, reflecting lipid peroxidation, were reported in 56 persons with severe COPD compared to 23 individuals with moderate COPD.²⁰ Others have reported an inverse association between circulating measures of lipid peroxidation and FEV₁ in general population samples.^{18,19}

The biologic mechanisms responsible for the inverse associations with FVC and FEV₁ are uncertain, particularly in conditions such as SCI that are not known to be associated with pulmonary inflammation. However, it has been postulated that systemic inflammation attributable to adipose tissue is a risk factor for asthma, contributes to COPD severity, and may affect the airway.^{49,50} In addition, functional inspiratory muscles are needed to achieve a normal TLC and functional inspiratory and expiratory muscles are needed to achieve maximal values of FVC and FEV₁. Systemic inflammation is thought to promote skeletal muscle dysfunction in COPD,^{51–53} and recent studies of effects of inflammation on muscle strength and physical performance in other populations also suggest a deleterious effect.^{2,54–57} In the high ranges of percent predicted TLC (such as in our subjects (Table 2)), the volume-pressure characteristics of the lung and inspiratory muscles are flat. In such ranges, then, TLC is relatively insensitive to muscle pressures.⁵⁸ Therefore, it is likely that our study is not sufficiently powered to detect significant associations with TLC. We did not observe consistent associations between FRC and biomarkers of

inflammation or oxidative stress. FRC is not dependent on muscle function, so this finding also is consistent with our hypothesis of inspiratory and expiratory muscle weakness as a potential mechanism for our observed findings with FVC and FEV₁.

An expected additional finding would be an association between higher inflammatory biomarker and higher RV values due to expiratory muscle weakness. However, RV is determined by a complex interaction amongst several factors including expiratory muscle strength, lung and chest wall compliance both of which are reduced in SCI,^{59,60} airway closure, flow limitation, and duration of exhalation in those with airways obstruction who do not reach a plateau even after prolonged effort.⁵⁸ It is possible that the complexity of these interactions also contributed to excessive variability in the determination of RV, and also led to our inability to detect relationships between RV and systemic inflammation.

This study has several limitations. First, due its cross-sectional nature, we are unable to determine the temporality of the associations of inflammation and oxidative stress with pulmonary function. However, recent studies suggest that it is plausible that greater levels of systemic inflammation are temporally related to a decline in pulmonary function.^{12–14} Second, with only a total of 137 participants in our sample it is possible we do not have sufficient power to detect statistically significant patterns, which would partially explain the many borderline statistically significant and other suggestive associations. Third, our use of a set of global markers of oxidative stress, FLOx, may have introduced measurement error if only specific (such as measures of lipid peroxidation), and not overall, oxidative processes are associated with decreases in pulmonary function. Lastly, these results may not be generalizable to the total SCI population since only individuals healthy enough to participate and perform pulmonary function testing were included in our study.

Conclusions

Among individuals with chronic SCI, increased levels of systemic inflammation were associated with decreases in FEV₁ and FVC, independent of SCI level and severity, doctor-diagnosed COPD, BMI, cigarette smoking, and statin use. However, there were no consistent associations between pulmonary function measures and biomarkers of global oxidative stress. These small decrements in function attributable to systemic inflammation could be particularly important in SCI populations given already impaired respiratory muscles and pulmonary function.

Disclaimer statements

Contributors JEH was involved in data analysis and interpretation, drafted the article, had full access to the data, and takes responsibility for the integrity of the data and the accuracy of the data analysis. LM, CGT and RB were involved in the design of the study, acquisition of data, provided comments on the data analysis, and revised the manuscript critically. EG was involved in the design of the study, acquisition of data, provided comments on the data analysis, revised the manuscript critically, and has agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All authors have approved the final version of the manuscript and approve its submission.

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Conflicts of interest The authors declare they have no conflicts of interest.

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