

Modeling the impact of COPD on the brain

Soo Borson¹
 James Scanlan¹
 Seth Friedman²
 Elizabeth Zuhr¹
 Julie Fields³
 Elizabeth Aylward^{1,2}
 Rodney Mahurin²
 Todd Richards²
 Yoshimi Anzai²
 Michi Yukawa⁴
 Shingshing Yeh⁵

¹Department of Psychiatry and Behavioral Sciences, University of Washington, Seattle, WA, USA; ²Radiology Department, University of Washington, Seattle, Washington, USA; ³Department of Psychology (Neuropsychology), University of Texas Southwestern Medical Center, Texas, USA; ⁴Department of Medicine (Geriatrics), University of Washington, Seattle, WA, USA; ⁵Department of Medicine (Geriatrics), Veterans Affairs Medical Center, Northport, New York, USA

Abstract: Previous studies have shown that COPD adversely affects distant organs and body systems, including the brain. This pilot study aims to model the relationships between respiratory insufficiency and domains related to brain function, including low mood, subtly impaired cognition, systemic inflammation, and brain structural and neurochemical abnormalities. Nine healthy controls were compared with 18 age- and education-matched medically stable COPD patients, half of whom were oxygen-dependent. Measures included depression, anxiety, cognition, health status, spirometry, oximetry at rest and during 6-minute walk, and resting plasma cytokines and soluble receptors, brain MRI, and MR spectroscopy in regions relevant to mood and cognition. ANOVA was used to compare controls with patients and with COPD subgroups (oxygen users [$n = 9$] and nonusers [$n = 9$]), and only variables showing group differences at $p \leq 0.05$ were included in multiple regressions controlling for age, gender, and education to develop the final model. Controls and COPD patients differed significantly in global cognition and memory, mood, and soluble TNFR1 levels but not brain structural or neurochemical measures. Multiple regressions identified pathways linking disease severity with impaired performance on sensitive cognitive processing measures, mediated through oxygen dependence, and with systemic inflammation (TNFR1), related through poor 6-minute walk performance. Oxygen desaturation with activity was related to indicators of brain tissue damage (increased frontal choline, which in turn was associated with subcortical white matter attenuation). This empirically derived model provides a conceptual framework for future studies of clinical interventions to protect the brain in patients with COPD, such as earlier oxygen supplementation for patients with desaturation during everyday activities.

Keywords: oxygen desaturation, frontal choline, cognition, mood, SGRQ, cytokines

Introduction

Impaired brain function has long been recognized as a complication of COPD (see Hynninen et al 2005 for a comprehensive review). While cognitive and mood changes in COPD have generally been associated with hypoxemia, much of the variance remains unexplained, particularly in highly heterogeneous or less well characterized subject samples. This exploratory study examines in detail the pathways from lung disease to brain dysfunction in a carefully selected group of COPD patients and matched controls, using disease-specific measures of disease severity and disability, validated measures of mood and cognition, plasma measures of proinflammatory cytokines, and both structural and neurochemical measures of brain integrity. As our intent was to construct a testable working model of COPD and the brain, we used a broad conceptual framework and inclusive measurement domains. Initial hypotheses were that COPD severity and requirement for oxygen supplementation would be related to poorer cognitive processing but not premorbid intelligence; to increased proinflammatory cytokines, a possible systemic mediator between lung and brain; and to signs of structural and neurochemical damage in brain regions known to be related to mild cognitive deficits and highly sensitive to oxygen deprivation (hippocampus and frontal lobes).

Correspondence: Soo Borson
 1959 NE Pacific Street,
 Campus Box 356560, Seattle,
 WA 98195, USA
 Tel +1 206 685 9453
 Fax +1 206 685 1139
 Email soob@u.washington.edu

Methods

Subjects were 18 patients with clinically stable COPD and 9 age- and gender-matched healthy controls, carefully screened to exclude persons with major chronic medical comorbidity or use of medications known to be associated with cognitive impairment. Clinical laboratory evaluation (CBC, extended chemistry panel including albumin) was normal for all included subjects and none was clinically cachectic (ie, all BMI > 20). Pulmonary disease management had been optimized by the subject's usual pulmonary physician prior to enrollment; one subject awaiting lung transplantation was matched for surgery before completing the physiological components of the protocol. No COPD or control subject met diagnostic criteria for dementia, current major depression, primary panic disorder, or alcohol abuse, and none was a current smoker. Table 1 summarizes subject characteristics. Patients receiving supplemental oxygen used their normal prescribed daytime flow rates during all study procedures (cognitive and mood assessment, 6-minute walk, and all neuroimaging studies).

Measures of pulmonary function included spirometry, pulse oximetry at rest and change during a 6-minute walk, and distance walked (Enright 2003). Health status was assessed using the St George's Respiratory Disease Questionnaire (SGRQ; Jones et al 2005).

Cognitive measures included the Dementia Rating Scale-2 (Jurica et al 2001), a measure of global cognitive functioning; the Wide Range Achievement Test-3 (Wilkinson 1993), a measure of premorbid intelligence; the logical memory

subtest of the WMS-3 (Wechsler 1997a); and a measure of moment-to-moment cognitive processing sensitive to mild brain dysfunction, the Digit Symbol coding test (Wechsler 1997b; Anger et al 2000; Kilburn 2001; Lopez et al 2003).

Depression was assessed using two validated measures, the Hamilton Depression Rating Scale (Ham-D, 17 item version [Reynolds 1995], a symptom severity scale) and the PHQ-9 (Kroenke et al 2001), which yields both symptom severity and good reliability with diagnoses of major and minor depression. Anxiety was measured with the Patient-Rated Anxiety Scale (PRAS [Sheehan 1983]) which was previously found to be sensitive to both psychological anxiety/worry and somatic symptoms of sympathetic overarousal in COPD (Borson et al 1992).

A plasma cytokine panel was assayed on flash-frozen plasma collected after completion of questionnaires and cognitive tests. IL-1b, IL-2r, IL-6, TNF α , and hsCRP were measured using immunoassay kits (Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA) on the Siemens Immulite (IL-1b, IL-2r, TNF α) and Siemens Immulite 2000 (hsCRP and IL-6). Measurement of the soluble TNFR1 (also known as TNFR-p55) receptor and TNFR2 (TNFR-p75) receptor was performed using enzyme immunoassay kits (Biosource International, Camarillo, CA, USA) run on the Roche COBAS Core II (Roche Diagnostics, Basel, Switzerland). The intra-assay and inter-assay coefficients of variation are generally <5.0% for all assays over the entire assay range, and in no case >10%, according to the reagent literature.

Neuroimaging was done on a different day separated by up to a week apart from cognitive, mood, 6-minute walk, and blood sample acquisition. Measures included structural magnetic resonance imaging (MRI) and neurochemical imaging with ¹H magnetic resonance spectroscopy. A set of 3 dimensional, high-resolution (1.5 mm) T1-weighted coronal MRI images was acquired extending through the entire brain, using, a spoiled GRASS (SPGR) sequence (TE 5 ms and TR 35 2000 ms; field of view 24 cm; matrix size 256 $\times$ 256; flip angle 45°; NEX 1, interleaved with 0 gap). A set of axial FLAIR sequences extending through the entire brain was also acquired (TR 10,000 ms, TE 130 ms; TI 220; field of view 24 cm; matrix size 256 $\times$ 192, NEX1, 3 mm thick, interleaved with 0 gap). Global ratings of brain atrophy (T1) and subcortical white matter hyperintense lesions (FLAIR) were scored by two independent raters and any discrepancies reconciled before analysis. Hippocampal volume was measured using previously published quantitative methods (Aylward et al 1999).

Table 1 Sample description

Demographics	Controls (n = 9)	COPD (n = 18)
Age, mean years (SD)	68.2 (5.8)	68.5 (8.0)
Sex (% women)	62%	64%
Education, mean years (SD)	16.8 (2.8)	17.7 (2.5)
Ethnicity (% white)	100%	100%
Pulmonary disease indicators		
GOLD stage (number $\times$ stage)		
Ila (FEV ₁ 50%–79% predicted)	na	2
Ilb (FEV ₁ 30%–49% predicted)	na	8
III (FEV ₁ < 30% predicted)	na	7 ^a
SGRQ mean total (SD)	6 (3)	56 (17)
6 MWD, mean feet (SD)	1545 (196)	922 (262)
Long-term continuous O ₂ use (n, %)	0	9 (53%)
SpO ₂ , mean resting (SD)	98.1 (1.5)	96.0 (2.2)
SpO ₂ , mean minimum during 6MW (SD)	94.6 (1.7)	88.0 (3.1)

^a1 subject dropped out for lung transplant before spirometry measures completed. Analyses by 1-way ANOVA.

Abbreviations: SGRQ, St. George's Respiratory Disease Questionnaire total score (% possible disability points); 6 MWD, 6-minute walk distance; SpO₂, O₂ saturation by pulse oximeter.

Neurochemical measures were collected in left frontal lobe white matter and left hippocampus using ^1H MRS (voxel size $15 \times 15 \times 12$ mm and TR/TE 2000/144 ms. MRS data were fit using custom basis sets in LCModel (Provencher 1993) and quantified using a partial volume (tissue) corrected unsuppressed water signal. These methods yielded millimolar concentrations of N-acetylaspartate + N-acetylaspartyl-glutamate (NAA+), creatine (Cre, the sum of phosphocreatine and creatine), and choline-containing compounds (Cho). The neurochemical measures have coefficients of variation in our hands ranging from 3.3% to 5.3% for NAA, Cre, and Cho respectively, and reproducibility is equivalent within and between sessions (Brooks et al 1999).

Analytic approach and statistical methods

All dependent measures (mood, cognition, cytokines, imaging variables) were first subjected to one-way ANOVAs comparing COPD and control subjects, and COPD oxygen users with non-users and controls. After identifying dependent variables associated with COPD group membership and/or oxygen use at the $p < 0.05$ level (see Table 2), stepwise multiple regression was used to determine which of the COPD variables were uniquely associated with observed group differences. Major variables describing the presence and severity of COPD were used to construct a “backbone” comprised of the following: presence or absence of COPD; SGRQ total score; $\text{FEV}_1\%$ predicted; oxygen use; and 6-minute walk distance and maximum walking-related change in oxygen saturation (see Figure 1). The criteria for including a variable in the “backbone” were primarily clinical (based on the core measures used in COPD assessment and staging, eg, SGRQ, FEV_1 , oxygen dependence), while the sequential ordering of the backbone variables was statistical (see Figure 1). Variables were placed according to their strongest “near-neighbor” relationships in regressions. In regressions predicting mood, cognition, and brain imaging outcomes, in addition to the COPD backbone variables we also allowed stepwise entry of age, education and gender to control their potential confounding effects. The end result of all regressions is summarized in a final model showing R^2 relationships between the relevant variables (see Figure 1). In the model, “mood” is a z-score composite of three highly intercorrelated scales (Ham-D, PHQ-9, and PRAS; all $r = 0.7\text{--}0.8$). To limit Type I statistical errors, and to increase clarity and robustness in the final model, only relationships with $p \leq 0.01$ were included. This analytic approach simulates structural equation modeling, which is precluded here by small sample size.

Table 2 Neuropsychiatric, neuroanatomical, and neurochemical measures (means and standard deviations)

Domain	Controls	COPD		P control vs COPD
	n = 9	No O ₂ (n = 9)	O ₂ dependent (n = 9)	
Cognition				
WRAT-3	109.1 (9.8)	109.1 (5.9)	103.7 (8.3)	ns
DRS-2 Total	12.3 (3.2)	9.0 (3.3)	9.9 (2.2)	0.019
Memory	11.9 (1.7)	10.3 (2.7)	9.6 (2.8)	0.049
WAIS-3				
Digit symbol	12.7 (1.5)	12.1 (1.5)	8.4 (3.3)	0.034 ^a
WMS-3				
Logical memory	13.7 (2.1)	12.6 (2.1)	10.7 (3.1)	0.049 ^b
Mood (depression, anxiety)				
Ham-D 17	3.4 (2.9)	8.4 (3.3)	12.2 (5.7)	0.001 ^c
PHQ-9	1.3 (1.2)	6.1 (6.2)	6.1 (4.1)	0.005
PRAS	2.8 (3.0)	22.0 (20.7)	29.3 (19.2)	0.002
Brain structure				
Hippocampal volume	5.4 (0.9)	5.2 (0.8)	4.8 (0.8)	ns
White matter lesions	2.0 (0.0)	2.7 (0.8)	2.0 (0.9)	ns
Neurochemistry				
Frontal choline	3.96 (0.38)	3.30 (0.46)	4.50 (0.71)	ns ^d
Hippocampal NAA+	12.6 (1.8)	11.9 (2.0)	13.0 (1.7)	ns
Inflammation				
Soluble TNFRI	2.14 (0.50)	2.37 (0.97)	2.71 (0.62)	0.03

All comparisons by 1-way ANOVA with post-hoc tests; $p > 0.05$ reported as ns. For cognitive tests, age/education scaled scores reported. For mood scales, total scores reported. Hippocampal volume reported as cubic centimeters for right and left hippocampus combined. White matter lesions reported as relative predominance of subcortical vs periventricular hyperintensities. Soluble TNFRI reported as ng/mL.

^a O₂ users worse than both other groups ($p \leq 0.005$).

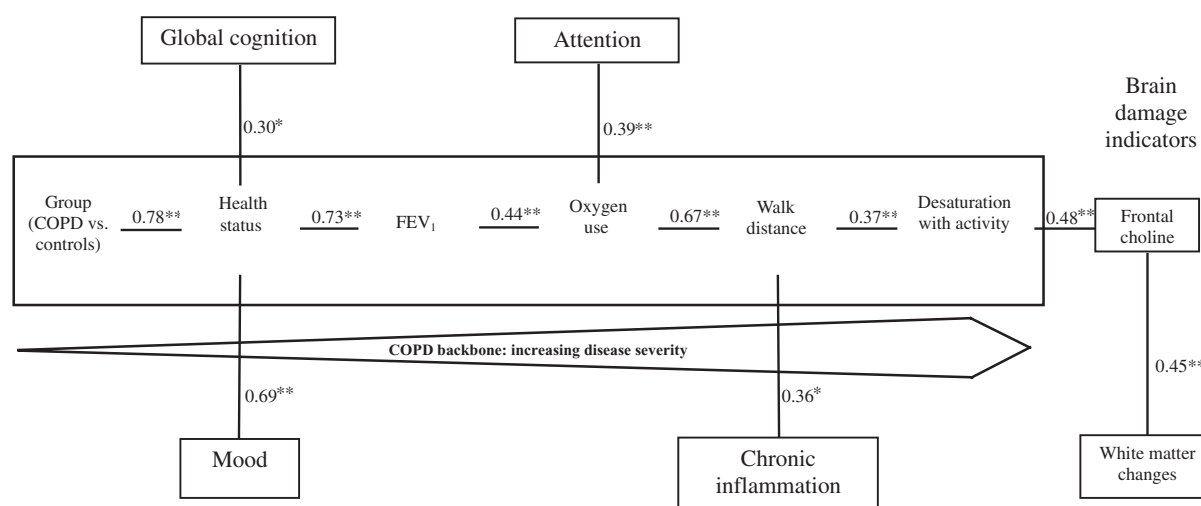
^b O₂ users borderline worse than both other groups ($p = 0.05\text{--}0.10$).

^c All groups significantly different ($p = 0.001$).

^d O₂ users worse than nonusers and controls ($p \leq 0.005$).

Results

Descriptive data for the sample are shown in Table 1. Groups were well matched across demographic characteristics and showed the expected differences in the main lung disease related predictor (independent) variables. Group comparisons for key outcome (dependent) variables are shown in Table 2. Statistically significant differences between controls and the whole COPD group were found for several cognitive and mood measures but not for brain structural or neurochemical measures. Both depression measures, the Ham-D and PHQ-9, showed group differences. PHQ-9 differed between control and combined COPD groups, whereas



* = $p < 0.01$
 ** = $p < 0.001$

Figure 1 Modeling the impact of COPD on brain function (R^2 shown).

Stepwise multiple regressions with "backbone" variables used as predictors of outcome variables already showing COPD group differences (see text). Numbers next to lines connecting domains indicate R^2 for the relevant relationship. All effects shown are independent of age, education, and gender.

Variable definitions

Independent variables – COPD backbone:

Group: COPD vs control; Health status: total score, St. Georges Respiratory Questionnaire; FEV₁: forced expiratory volume % predicted; Oxygen use: use/nonuse of supplemental oxygen; Walk distance: distance (feet) walked in 6 minutes (or to maximum tolerated, if less than 6 minutes); Desaturation with activity: resting SpO₂ minus minimum SpO₂ during walk.

Dependent variables:

Global cognition: total score, Dementia Rating Scale; Mood: z score combination of 17-item Hamilton Depression Scale, Patient Rated Anxiety Scale, and Patient Health Questionnaire-9; Attention: digit symbol substitution (WAIS); Chronic inflammation: soluble tumor necrosis factor receptor 1 (TNFR1); Frontal choline measured in frontal white matter by ¹H MRS; White matter changes: subcortical white matter hyperintensities on MRI FLAIR images.

Ham-D scores increased linearly across the three groups from control (lowest) to hypoxemic/oxygen dependent (highest), an increase explained by items rating symptoms of physical disability rather than depressed mood (data not shown). Oxygen-dependent COPD patients fared worse than controls and nonoxygen-dependent COPD patients on cognitive as well as mood measures.

Brain atrophy, number and estimated volume of white matter hyperintensities, and frontal and hippocampal neurochemical indices, did not differ between COPD and control groups (not all data shown). Mean hippocampal volume was smaller in COPD subjects than controls, with oxygen-dependent patients having an 11% smaller volume, but these differences were not statistically significant in analyses across all subjects. On the other hand, frontal choline was significantly higher in oxygen dependent subjects, consistent with evidence of brain damage in this most impaired group.

Circulating proinflammatory cytokine levels were highly skewed and were therefore rank-transformed before analysis (data not shown). Only soluble TNFR1 showed significant

group differences, with the highest levels in oxygen-dependent COPD subjects.

The working model constructed by stepwise regressions relating primary COPD variables with multidimensional outcome measures (Figure 1) shows the unique variance accounted for (R^2) at each step.

Discussion

This study replicates known relationships between measures of COPD disease severity, general cognition, and mood (Grant et al 1987) in a group of subjects with normal premorbid intelligence and good educational attainment, selected for minimal medical comorbidity. It extends earlier findings on cognition using measures sensitive to mild cognitive impairment (digit symbol substitution). It also provides new insights into the mechanisms by which severe ambulatory stages of oxygen-dependent COPD may produce cumulative damaging effects on the brain. The chain linking oxygen desaturation during relatively low-intensity exercise (6-minute walk) to elevations in frontal choline, and then to changes in brain

white matter, appears to be robust ($R^2 = 0.45\text{--}0.48$). This elevation in brain choline levels cannot be attributed to acute oxygen desaturation during the 6-minute walk test, as spectroscopy was performed on a different day, and oxygen-dependent patients were transported to the scanner by wheelchair.

We propose that frequent oxygen desaturation during everyday activity may be a key mechanism underlying the damage to brain tissue reflected in elevated brain choline. Similar choline elevations are observed in systemic diseases secondarily associated with brain tissue breakdown and cognitive impairment (Friedman et al 1998, 1999; Forton et al 2005), and appear to reflect damage to myelin and increased turnover of neuronal membrane precursors (Ross and Michaelis 1994). It is likely that increases in choline in advanced hypoxemic COPD reflect such a combination of membrane breakdown and turnover changes in the brain, and that these are eventually manifested as white matter hyperintensities on structural imaging. Other spectroscopy approaches (eg, decoupled ^{31}P MRS) to measure individual constituents of the choline peak could be helpful in refining and further interpreting these results.

Elevation of frontal choline in oxygen-dependent COPD patients is consistent with the work of Incalzi et al (2003) using a different (perfusion) imaging approach. In that comprehensive mapping study, substantial perfusion deficits were identified in frontal lobes and were most marked in oxygen-dependent subjects. Only one previous study used ^1H MRS to measure brain neurochemical species in patients with COPD (Shim et al 2001). Shim et al reported decreases in NAA, creatine, and choline in COPD subjects relative to controls, whereas we found no differences in NAA or creatine and an *elevation* in choline, and this was limited to oxygen-dependent subjects. However, in the Shim study, oxygen dependent subjects were excluded, participants were younger, analyses were not adjusted for age and education, and the brain regions sampled were different from those used in the present study and possibly less likely to be related to changes in mood and cognition.

In the present study, we also found evidence of chronic systemic inflammation, measured as an elevation of TNFR1, in chronically hypoxemic patients, an effect associated with exercise limitation (impaired 6-minute walk distance). We did not examine acute cytokine responses to exercise in this study; a previous study in 11 GOLD Stage IIb patients found an abnormally elevated systemic TNF α response to a single session of moderate acute exercise, but no acute effect of exercise on TNFR1 (Rabinovich et al 2003). TNFR1,

in contrast to TNF α , most likely reflects the presence of a chronic, but not acute, systemic inflammatory milieu. Such a milieu might be promoted by repeated inflammatory responses to everyday exertion in oxygen-dependent patients. This theory is consistent with data showing elevated TNFR1 levels in both typical inflammatory diseases (rheumatoid arthritis [Gattorno et al 1996; Maury et al 2003]) and diseases in which the role of chronic systemic inflammation has only recently emerged (eg, ischemic cardiovascular disease [Cesari et al 2003]). These studies suggest that TNFR1 may be among the most sensitive indicators of long-term systemic inflammation in many different chronic diseases, now including COPD.

Implications

This study was designed to develop a testable multidimensional model of the emergence of brain dysfunction in patients living with COPD. Although many studies have found relationships between depression, anxiety, and cognition in COPD patients that are generally more apparent with greater disease severity, the present study explicitly traces a pathway leading from symptoms through objective measures of pulmonary disease to brain dysfunction that may occur with disease progression. We confirmed that the presence of COPD is associated with mild decrements in mood and cognition. Severe disease is associated with evidence of chronic systemic inflammation (elevated TNFR1) and subtle cognitive deficits (digit symbol). Levels of oxygen desaturation that are likely to occur with everyday activities in severe COPD appear to mediate specific changes in brain neurochemistry and structure that suggest sustained brain damage. The strong relationships we report in the working model presented here are likely to survive replication in larger samples, as the effect sizes we found for 14 of 15 R^2 relationships qualify as large (Cohen and Cohen 1983).

The cross sectional model presented here would require testing in prospective research designs to evaluate its ability to predict longitudinal decline in cognition and incident damage to brain tissue integrity. However, such damage, once it has occurred, may not be reversible. While the evidence is limited, it appears that the cognitive impairment reported in advanced COPD is only partially reversible with oxygen therapy started late in the disease course (Heaton et al 1983). If our model is correct, routine identification of episodic desaturation with everyday activity, and/or sleep, and earlier oxygen supplementation, could help to prevent irreversible brain damage in COPD patients.

Acknowledgments

This study was supported in part by pilot funding from the University of Washington ADRC/LEAD program.

Disclosures

None of the authors has any conflicts of interest to disclose.

References

- Anger WK, Liang YX, Nell V, et al. 2000. Lessons learned – 15 years of the WHO-NCTB: a review. *Neurotoxicology*, 21:837–46.
- Aylward EH, Li Q, Honeycutt NA, et al. 1999. MRI volumes of the hippocampus and amygdala in adults with Down's Syndrome with and without dementia. *Am J Psychiatry*, 156:564–8.
- Borson S, McDonald GJ, Gayle T, et al. 1992. Improvement in mood, physical symptoms, and function with nortriptyline for depression in patients with chronic obstructive pulmonary disease. *Psychosomatics*, 33:190–201.
- Brooks WM, Friedman SD, Stidley CA. 1999. Reproducibility of 1H-MRS in vivo. *Magn Reson Med*, 41:193–7.
- Cesari M, Penninx BW, Newman AB, et al. 2003. Inflammatory markers and cardiovascular disease (The Health, Aging and Body Composition [Health ABC] Study). *Am J Cardiol*, 92:522–8.
- Cohen J, Cohen P. 1983. *Applied Multiple Regression/Correlation Analysis for the Behavioral Sciences* (2nd ed.). Hillsdale: Laurence Erlbaum Associates.
- Enright PL. 2003. The six-minute walk test. *Respir Care*, 48:783–5.
- Forton DM, Allsop JM, Cox IJ, et al. 2005. A review of cognitive impairment and cerebral metabolite abnormalities in patients with hepatitis C infection. *AIDS*, Suppl 3:S53–63.
- Friedman SD, Brooks WM, Jung RE, et al. 1999. Quantitative proton MRS predicts outcome after traumatic brain injury. *Neurology*, 52:1384–91.
- Friedman SD, Stidley CA, Brooks WM, et al. 1998. Brain injury and neurometabolic abnormalities in systemic lupus erythematosus. *Radiology*, 209:79–84.
- Gattorno M, Picco P, Buoncompagni A, et al. 1996. Serum p55 and p75 tumour necrosis factor receptors as markers of disease activity in juvenile chronic arthritis. *Ann Rheum Dis*, 55:243–7.
- Heaton RK, Grant I, McSweeney AJ, et al. 1983. Psychologic effects of continuous and nocturnal oxygen therapy in hypoxemic chronic obstructive pulmonary disease. *Arch Intern Med*, 143:1941–7.
- Hynninen KMJ, Breivte MH, Wiborg AB, et al. 2005. Psychological characteristics of patients with chronic obstructive pulmonary disease: A review. *J Psychosomatic Res*, 59:429–43.
- Incalzi RA, Marra C, Giordano A, et al. Cognitive impairment in chronic obstructive pulmonary disease: A neuropsychological and SPECT study. *J Neurol*, 250:325–32.
- Jones P, Lareau S, Mahler DA. 2005. Measuring the effects of COPD on the patient. *Respir Med*, 99:S11–18.
- Jurica PJ, Leitten CL, Mattis S. 2001. DRS-2: Dementia Rating Scale-2 Professional Manual. Lutz, FL: Psychological Assessment Resources, Inc.
- Kilbourn KH. 2001. Function testing for chemical brain damage: a review. *Arch Environ Health*, 56:132–7.
- Kroenke K, Spitzer RL, Williams JB. 2001. The PHQ-9: validity of a brief depression severity measure. *J Gen Intern Med*, 16:606–13.
- Lopez OL, Jagust WJ, Dulberg C, et al. 2003. Risk factors for mild cognitive impairment in the Cardiovascular Health Study Cognition Study: part 2. *Arch Neurol*, 60:1394–9.
- Maury CP, Liljestrom M, Laiho K, et al. 2003. Tumour necrosis factor alpha, its soluble receptor I, and -308 gene promoter polymorphism in patients with rheumatoid arthritis with or without amyloidosis: implications for the pathogenesis of nephropathy and anemia of chronic disease in reactive amyloidosis. *Arthritis Rheum*, 48:3068–76.
- Provencher SW. 1993. Estimation of metabolite concentrations from localized in vivo proton NMR spectra. *Magn Reson Med*, 30:672–9.
- Rabinovich RA, Figueras M, Ardite E, et al. 2003. Increased tumour necrosis factor-alpha plasma levels during moderate-intensity exercise in COPD patients. *Eur Respir J*, 21:789–94.
- Reynolds WM, PAR Staff. 1995. Hamilton Depression Inventory Scoring Program Version 1.0, Psychological Assessment Resources.
- Ross B, Michaelis T. 1994. Clinical applications of magnetic resonance. *Magn Reson Q*, 10:191–247.
- Sheehan DV. 1983. *The Anxiety Disease*. New York: Charles Scribner and Sons.
- Shim TS, Lee JH, Kim SY, et al. 2001. Cerebral metabolic abnormalities in COPD patients detected by localized proton magnetic resonance spectroscopy. *Chest*, 120:1506–13.
- Wechsler D. 1997a. Wechsler Memory Scale. Third Edition Manual. San Antonio: The Psychological Corporation.
- Wechsler D. 1997b. Wechsler Adult Intelligence Scale-III. San Antonio: The Psychological Corporation.
- Wilkinson GS. 1993. WRAT3 Administration Manual. Delaware: Wide Range.