Interleukin-6 and Tumor Necrosis Factor- α Are Associated with Quality of Life–Related Symptoms in Pulmonary Arterial Hypertension

Lea Ann Matura¹, Corey E. Ventetuolo², Harold I. Palevsky³, David J. Lederer⁴, Evelyn M. Horn⁴, Stephen C. Mathai⁵, Diane Pinder³, Christine Archer-Chicko³, Emilia Bagiella⁶, Kari E. Roberts⁷, Russell P. Tracy⁸, Paul M. Hassoun⁵, Reda E. Girgis⁹, and Steven M. Kawut³

¹University of Pennsylvania School of Nursing, Philadelphia, Pennsylvania; ²Division of Pulmonary, Critical Care, and Sleep Medicine and Division of Health Services Policy and Practice, Alpert Medical School of Brown University, Providence, Rhode Island; ³Pulmonary, Allergy, and Critical Care, Perelman School of Medicine of the University of Pennsylvania, Philadelphia, Pennsylvania; ⁴Columbia University College of Physicians and Surgeons, New York, New York; ⁵Division of Pulmonary and Critical Care Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland; ⁶Mount Sinai School of Medicine, New York, New York; ⁷Department of Medicine, Pulmonary, Critical Care, and Sleep Medicine, Tufts University, Boston, Massachusetts; ⁸College of Medicine, University of Vermont, Colchester, Vermont; and ⁹Richard DeVos Heart and Lung Transplant Services, Spectrum Health Hospitals, Grand Rapids, Michigan

Abstract

Rationale: Inflammation is associated with symptoms in many chronic illnesses; however, this link has not been established in pulmonary arterial hypertension.

Objectives: The objective of this study was to investigate the association between inflammatory markers and quality of life–related symptoms in patients with pulmonary arterial hypertension. We hypothesized that higher circulating IL-6 and tumor necrosis factor- α levels would be associated with worse quality of life–related symptoms.

Methods: We performed a secondary analysis using baseline and 3-month assessments of 62 subjects in a clinical trial of aspirin and simvastatin to determine the association between plasma IL-6 and tumor necrosis factor- α levels and the Medical Outcomes Study Short Form-36 subscales (pain, vitality, mental health).

Measurements and Main Results: The mean age was 49.7 ± 13.4 years; 87% were female. Higher IL-6 levels were significantly associated with lower Medical Outcomes Study Short Form-36 subscale scores, indicating worse bodily pain, vitality, and mental health (all $P < 0.01$). Higher tumor necrosis factor- α levels were significantly associated with increased bodily pain, but better mental health scores.

Conclusions: IL-6 and tumor necrosis factor- α levels are associated with certain quality of life domains in patients with pulmonary arterial hypertension.

Clinical trial registered with www.clinicaltrials.gov (NCT00384865).

Keywords: pulmonary arterial hypertension; interleukin-6; tumor necrosis factor- α ; fatigue; pain

(Received in original form October 11, 2014; accepted in final form January 6, 2015)

Supported by National Institutes of Health grants K23 NR014885, R01 HL082895, and HL082895-S1; the American Nurses Foundation; and the Biobehavioral Research Center at the University of Pennsylvania School of Nursing. This publication was made possible by grants UL1 RR025005, UL1 RR025752, UL1 RR024156, and UL1 RR024134 from the National Center for Research Resources, the National Institutes of Health Roadmap for Medical Research.

Author Contributions: L.A.M. had full access to all the data in the study, takes responsibility for the integrity of the data and the accuracy of the data analysis, contributed to the study design and conception, and data analysis and interpretation. She drafted the first version of the manuscript and approved the final manuscript. C.E.V. contributed to the hypothesis generation, data analysis and interpretation, and manuscript preparation. H.I.P. contributed to the study design, patient recruitment, data analysis and interpretation, and manuscript preparation. D.J.L. contributed to the study design and patient recruitment, data analysis and interpretation, and manuscript preparation. E.M.H. contributed to the study design and conception, data analysis, and manuscript preparation. S.C.M. contributed to the study conception, data analysis and interpretation, and manuscript preparation. D.P. contributed to the study design and patient recruitment, acquisition of the data, and manuscript preparation. C.A.-C. contributed to the study design, acquisition of the data, and manuscript preparation. E.B. contributed to the study design, data acquisition, and manuscript preparation. K.E.R. contributed to the study design, data acquisition and analysis, and manuscript preparation. R.P.T. contributed to the study design, biospecimen analysis, and manuscript preparation. P.M.H. contributed to the study design, data analysis, and manuscript preparation. R.E.G. contributed to the study design, data analysis, and manuscript preparation. S.M.K. contributed to the study design and conception, data analysis and acquisition, and manuscript preparation.

Correspondence and requests for reprints should be addressed to Lea Ann Matura, Ph.D., R.N., University of Pennsylvania School of Nursing, Claire M. Fagin Hall, 418 Curie Blvd., Room 322, Philadelphia, PA 19104-4217. E-mail: matura@nursing.upenn.edu

Ann Am Thorac Soc Vol 12, No 3, pp 370–375, Mar 2015

Copyright © 2015 by the American Thoracic Society

DOI: 10.1513/AnnalsATS.201410-463OC

Internet address: www.atsjournals.org

Pulmonary arterial hypertension (PAH) is a chronic, debilitating disease affecting primarily young to middle-aged women; the mean age is 50 years at diagnosis (1). Patients are limited in their daily activities and the risk of mortality is high (2). Elevated right ventricular afterload leads to right heart failure and, ultimately, premature death (3). Although patients with PAH report multiple symptoms, dyspnea, fatigue, anxiety, and depression are some of the most prominent and severe manifestations (4–6). These symptoms impair function (7) and negatively impact quality of life (QOL) (4–6, 8, 9). To date, the biologic mechanisms of the symptomatology and patient-reported outcomes of PAH have not been well studied.

Investigators have found a link between inflammation and symptoms in other chronic conditions. Inflammatory cytokines such as IL-6 and tumor necrosis factor (TNF)- α can produce malaise, fatigue, muscle aches, and loss of appetite (10). Inflammation has been linked to fatigue (11), sleep disturbance, pain (12–15), depression (16, 17), and anxiety (18–20). Studies have shown an increase in cytokines (21) in idiopathic and heritable PAH, and higher IL-6 levels are associated with an increased risk of death in PAH (22). Despite these observations, there are few data regarding the associations between cytokine levels and QOL in PAH.

Patient-reported outcomes have gained importance in clinical research (23). Patients are able to relay information that cannot be captured via physical or physiological data. A commonly used patient-reported outcome is the Medical Outcomes Study Short Form-36 (SF-36). The SF-36 contains eight subscales that measure distinct health concepts including physical and mental health components. Using a study sample from a randomized clinical trial of aspirin and simvastatin (24), we investigated the association of IL-6 and TNF- α levels with QOL-related symptoms on the SF-36 subscales for bodily pain, vitality (energy/fatigue), and general mental health (psychological distress and psychological well-being) in PAH. Some of the results of this study have been previously reported in the form of an abstract (25).

Methods

Study Design and Sample

This study was approved by the University of Pennsylvania (#807771) Institutional Review Board. Patients meeting inclusion criteria signed an informed consent form. The patients were participants in the ASA-STAT study, a randomized clinical trial of the safety and efficacy of aspirin and simvastatin in PAH (24, 26). The parent study was a double-blind, placebo-controlled (enteric coated aspirin, 81 mg once daily and/or simvastatin, 40 mg), 2×2 factorial multicenter trial that included patients at least 18 years of age with PAH (mean pulmonary artery pressure of >25 mm Hg at rest with a pulmonary capillary wedge pressure <16 mm Hg). The following clinical subtypes of PAH were included: idiopathic, heritable, or associated PAH with connective tissue disease, HIV infection, congenital systemic-to-pulmonary shunt, or former anorexigen use. Exclusion criteria included absolute indication for aspirin therapy (e.g., coronary artery disease), current statin therapy, peptic or duodenal ulcer, and bleeding (detailed criteria are published) (24, 26).

Procedures

After informed consent was obtained, subjects in the parent study were randomized to enteric coated aspirin (81 mg once daily), simvastatin (40 mg daily), both, or neither (placebo). The primary outcome of the parent study was 6-minute walk distance (6MWD) at 6 months. Secondary outcomes included N-terminal pro-brain natriuretic peptide (NT-proBNP) levels, C-reactive protein (CRP) levels, New York Heart Association (NYHA) functional class, and the SF-36 at baseline, 6 weeks, 3 months, and 6 months (24). Detailed methods have been published and the protocol is available (26).

In this study, we included data from the baseline and 3-month assessments. We incorporated both assessments to provide more power while minimizing the presence of missing data (which occurred for later assessments). Age, sex, race/ethnicity, PAH type, and medication use were collected via subject interviews and chart reviews. Phlebotomy was performed in the morning, using a standardized technique

after an overnight fast (except water). Blood was centrifuged and plasma samples were immediately stored at -70°C until analysis.

SF-36. While the SF-36 overall measures generic QOL, the subscales cover areas relating to physical and mental health concepts. The eight subscales of the SF-36 are combined to form the mental and physical health composite summary scores. These subscales include QOL-related symptoms. *A priori*, we selected three subscales of the SF-36 as our primary outcomes. The “pain” subscale asks patients how much pain they have had over the past 4 weeks and how much the pain has interfered with their lives. The “vitality” subscale asks questions regarding how much energy patients have and how “worn out” or tired they feel. The “mental health” subscale asks questions about whether patients feel nervous or depressed. Subscales and the composite summary scores are reported on a scale of 0–100 (normalized to the U.S. population), with higher scores indicating better QOL. For normative data, scores higher than 50 are considered to indicate QOL above average (27, 28). Psychometric properties have been established (29); Cronbach α ranges between 0.73 and 0.96.

Laboratory Analyses

Human plasma IL-6 was measured with a high-sensitivity solid-phase quantitative sandwich ELISA provided by R&D Systems, Inc. (Minneapolis, MN) (catalog no. HS600B). The assay has a detection range of 0.156–10.0 pg/ml. The interassay coefficient of variation in the laboratory is 14%. TNF- α plasma was measured with a sandwich ELISA provided by Millipore Corporation (Billerica, MA) (catalog no. EZHTNFA). The assay has a dynamic range of 15.6–1,000 pg/ml. The interassay coefficient of variation is 15%. Detailed analytic methods for other biomarkers have been published (24).

Data Analysis

Continuous variables are shown as means $\pm$ SD or medians (interquartile range) and categorical variables as n (percentage). Comparisons between SF-36 baseline and 3-month data points were analyzed using paired t tests. Wilcoxon signed-rank tests were used for IL-6 and TNF- α levels. Generalized estimating equations were used to assess the

Table 1. Baseline characteristics of 62 patients with pulmonary arterial hypertension

Characteristic	Value
Age, yr	49.7 ± 13.4
Female sex	54 (87)
Body mass index, kg/m ²	27.9 ± 6.9
Race/ethnicity	
White (non-Hispanic)	42 (68)
Black	13 (21)
Other	7 (11)
PAH etiology	
Idiopathic	32 (51)
Heritable	3 (5)
Congenital heart disease	6 (10)
Connective tissue disease	18 (29)
Drugs/toxins	2 (3)
Overlap syndrome	1 (2)
Concomitant medications	
Sildenafil	39 (63)
Ambrisentan	18 (29)
Bosentan	17 (27)
Epoprostenol	15 (24)
Iloprost (inhaled)	10 (16)
Treprostinil (intravenous)	5 (8)
Warfarin	50 (81)
NYHA functional class	
Class I	5 (8)
Class II	39 (63)
Class III	18 (29)
6MWD, m	434.5 ± 113.1
NT-proBNP, pg/ml	1,100.3 ± 1,919.6
CRP, mg/L	5.4 ± 9.0

Definition of abbreviations: 6MWD = 6-minute-walk distance; CRP = C-reactive protein; NT-proBNP = N-terminal pro-brain natriuretic peptide; NYHA = New York Heart Association; PAH = pulmonary arterial hypertension. Data are shown as means ± SD or as n (%).

association between IL-6 and TNF- α (independent variables) and the SF-36 subscales (bodily pain, vitality, and mental health; dependent variables) at baseline and 3 months. Combining baseline and 3-month data points increases the power of our analyses. Generalized estimating equation models were adjusted for those variables thought potentially related to inflammation and QOL: age, sex, race, body mass index (BMI) (30), PAH type, NYHA functional class, 6MWD, NT-proBNP, and randomization to study drug (31). We also adjusted for CRP to account for nonspecific inflammation and subgroup analyses based on the use of medications (prostacyclin analogs or endothelin receptor antagonists). *P* values less than 0.05 were considered statistically

significant. SAS version 9.3 (Cary, NC) (32) was used for all analyses.

Results

ASA-STAT included 65 subjects. Sixty-two subjects had available biomarker analyses and composed the study sample for this analysis (Table 1). The mean age of the participants was 49.7 ± 13.4 years. The majority were female (87%), white (68%), and carried a diagnosis of idiopathic/heritable PAH (56%). More than half were taking phosphodiesterase type 5 inhibitors (63%) and/or endothelin receptor antagonists (56%); 48% received a prostacyclin analog (inhaled, continuous intravenous or subcutaneous); and 81% were taking warfarin. Thirty-nine (63%) of the subjects were NYHA functional class II. The mean 6MWD at baseline was 434.5 ± 113.1 m. NYHA functional class and 6MWD did not change over the 3-month follow-up period. Plasma IL-6 and TNF- α levels did not differ between baseline (3.3 and 3.6 pg/ml, respectively) and 3 months (3.3 and 3.6 pg/ml; *P* ≥ 0.59 for both). NT-proBNP and CRP levels were also unchanged over 3 months (*P* ≥ 0.70 for both). SF-36 scores on both sub- and summary scores also remained stable over 3 months (Table 2).

Analyses using generalized estimating equation models showed that higher IL-6 levels were significantly associated with lower SF-36 subscale scores, indicating worse bodily pain, vitality, and mental health after adjustment for age, sex, race, BMI, PAH type, NYHA functional class, 6MWD, CRP, NT-proBNP, and randomization to treatment group

(Table 3). Higher TNF- α levels were significantly associated with lower bodily pain scores, consistent with worse pain-related QOL. Unexpectedly, higher TNF- α levels were associated with better mental health on the SF-36 subscales.

Adjusted models stratified by diagnosis showed that limiting the analysis to those with idiopathic/heritable PAH also demonstrated a significant association between IL-6 and bodily pain, vitality, and mental health (Table 4). In contrast, only TNF- α and vitality levels were significantly associated. Unexpectedly, as TNF- α levels increased vitality was improved. Analyses for connective tissue disease were similar (data not shown). There was no significant interaction between sex or diagnosis and IL-6 or TNF- α . The results of the subgroup analyses based on the use of medications (prostacyclin analogs or endothelin receptor antagonists) were consistent with the overall results.

Discussion

This study aimed to investigate the association between circulating inflammatory cytokines and QOL-related symptoms (bodily pain, vitality, and mental health) in patients with PAH enrolled in a clinical trial. To our knowledge, this is the first study to demonstrate an association between IL-6 and TNF- α levels and certain QOL domains in PAH. As IL-6 levels increased, reported levels of bodily pain, vitality (fatigue), and mental (anxiety/depressions) symptoms also increased. As TNF- α levels increased bodily pain increased. In contrast, as TNF- α levels

Table 2. Medical Outcomes Study Short Form-36 at baseline and 3 months: norm-based scoring

	Baseline	3 Months	<i>P</i> Value
Physical functioning	37.3 ± 10.2	37.5 ± 10.0	0.94
Role-physical	42.6 ± 9.9	42.1 ± 10.6	0.78
Bodily pain	50.2 ± 8.2	49.0 ± 9.0	0.44
General health	41.0 ± 11.2	39.6 ± 9.7	0.46
Vitality	49.4 ± 8.9	48.4 ± 9.2	0.58
Social functioning	47.3 ± 9.7	46.9 ± 9.9	0.78
Role-emotional	48.4 ± 10.2	48.5 ± 11.0	0.94
Mental health	51.3 ± 8.8	51.6 ± 8.9	0.86
Physical component score	42.8 ± 7.5	42.1 ± 7.7	0.59
Mental component score	49.1 ± 7.6	48.9 ± 7.8	0.86

P value for difference between baseline and 3-month assessments; mean ± SD; paired *t* test.

Table 3. Adjusted general estimating equation models for IL-6 and tumor necrosis factor- α and symptoms

	Estimate	SE	P Value
IL-6 (per SD)			
Bodily pain	-1.63	0.01	<0.001
Vitality (fatigue)	-1.90	0.29	<0.001
Mental health (anxiety/depression)	-2.07	0.72	0.004
TNF- α (per SD)			
Bodily pain	-0.93	0.04	<0.001
Vitality (fatigue)	1.99	1.08	0.07
Mental health (anxiety/depression)	1.18	0.49	0.02

Definition of abbreviation: TNF- α = tumor necrosis factor- α .

Adjusted for age, sex, race, body mass index, pulmonary arterial hypertension (PAH) type, functional class, 6-minute-walk distance, C-reactive protein, N-terminal pro-brain natriuretic peptide, and randomized PAH treatment group.

increased mental health QOL-related symptoms actually decreased.

Higher circulating IL-6 levels are associated with greater self-reported pain affecting QOL in other conditions. Patients reporting increased depressive symptoms associated with greater pain intensity have higher IL-6 levels (13). In older adults, higher IL-6 levels are associated with more pain over a 6-year period after controlling for depression, age, sex, BMI, medication use, and health behaviors (sleep, exercise, and smoking) (15).

IL-6 was also associated with the vitality subscale in our study. Similar to our findings, patients with rheumatoid arthritis with higher levels of IL-6 levels also report increased levels of fatigue (33). In the Whitehall II study, investigators found an association between higher IL-6 and CRP levels and an increase in the incidence of self-reported fatigue (34). IL-6 was also significantly associated with all dimensions of fatigue (general fatigue, physical fatigue, reduced activity, mental fatigue, reduced motivation) in patients with diabetes mellitus type 2 (35). Our analyses were adjusted for CRP, suggesting that IL-6 has a specific biologic effect, rather than just indicating greater inflammation. Our study supports the possible mechanistic role of IL-6 in worsening symptoms and QOL in PAH. In contrast to our findings, a study including patients with heart failure with reduced ejection fraction found no association between IL-6 and self-reported fatigue levels (36).

We demonstrated an association between higher levels of IL-6 and lower

SF-36 mental health scores reflecting anxiety and depressive symptoms. Anxiety has been linked to higher IL-6 levels independent of age, sex, and depressive symptoms (37). Higher IL-6 levels were also associated with anxiety and depression in patients with cancer and in those experiencing trauma (38, 39). Omega-3 supplementation significantly reduced IL-6 and TNF- α levels in addition to anxiety levels in a clinical trial (40). A paradigm for this link between inflammation and anxiety has been established in studies of patients with cancer (38).

Higher TNF- α levels were also associated with worse QOL-related pain levels, an association that has been made in patients with rheumatoid arthritis (41). Although unexpected, our study also demonstrated that higher TNF- α levels were associated with lower levels of anxiety and depression. However, others have

reported similar findings (42), and TNF- α was not associated with depressive symptoms (43) or anxiety (44) in other studies. We also found that higher TNF- α levels were associated with higher vitality levels in this cohort of patients with PAH. This contradicts literature on other diseases (45, 46). It is possible that the treatments used in PAH might affect TNF levels, accounting for these unexpected results. Current PAH therapies targeting the underlying pathobiology of PAH such as endothelin receptor antagonists (bosentan) have been shown to decrease IL-6 in systemic sclerosis (47) and TNF- α in a murine model (48). Prostacyclin (epoprostenol) has also been shown to reduce IL-6 in individuals with traumatic brain injury (49) and TNF- α (50). A phosphodiesterase type 5 inhibitor (sildenafil) is also associated with lower IL-6 levels in men with diabetes type 2 (51). Physical activity can also lower TNF- α levels in chronic heart failure (52). We did not collect data on physical activity or exercise patterns in the current study.

There are several limitations to this study. First, the small sample size derived from a clinical trial could limit the generalizability of these observations. Despite this, the cohort is demographically and therapeutically comparable to the larger population with PAH (7). Second, although the SF-36 provides insight into how patients feel regarding their physical and mental health including QOL-related symptoms (pain, vitality, and mental health), it does not account for the

Table 4. Adjusted general estimating equation models for IL-6 and tumor necrosis factor- α and symptoms in idiopathic and heritable pulmonary arterial hypertension

	Estimate	SE	P Value
IL-6 (per SD)			
Bodily pain	-2.06	0.38	<0.001
Vitality (fatigue)	-2.26	0.27	<0.001
Mental health (anxiety/depression)	-2.85	0.98	0.004
TNF- α (per SD)			
Bodily pain	1.67	1.61	0.30
Vitality (fatigue)	2.24	0.23	<0.001
Mental health (anxiety/depression)	0.75	0.63	0.24

Definition of abbreviation: TNF- α = tumor necrosis factor- α .

Adjusted for age, sex, race, body mass index, functional class, 6-minute-walk distance, C-reactive protein, N-terminal pro-brain natriuretic peptide, and randomized pulmonary arterial hypertension treatment group.

multidimensionality of symptoms such as fatigue, anxiety, and depression. Another QOL measure, the U.S. Cambridge Pulmonary Hypertension Outcome Review, was not available before the initiation of the original ASA-STAT trial (53). Third, there may be other factors influencing inflammatory biomarkers that were not collected, such as exercise and alcohol intake. Additional inflammatory biomarkers may reveal other mechanisms of symptoms in PAH. Furthermore, the addition of other symptoms that are

important, severe, and interfere with the lives of patients with PAH need to be included in future studies.

Conclusions

Ours is the first study to describe the association between the inflammatory biomarkers IL-6 and TNF- α and QOL-related symptoms of pain, fatigue, anxiety, and depression in PAH measured by the SF-36 subscales (bodily pain, vitality, mental health). Although this gives us preliminary evidence of the

association between inflammation and symptoms in PAH, more research is needed to further define those possible mechanisms that may lead to targeted interventions to improve symptoms and QOL. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

Acknowledgment: The authors thank Amy Praestgaard, M.S., for providing the statistical analyses.

References

- Badesch DB, Raskob GE, Elliott CG, Krichman AM, Farber HW, Frost AE, Barst RJ, Benza RL, Liou TG, Turner M, *et al.* Pulmonary arterial hypertension: baseline characteristics from the REVEAL Registry. *Chest* 2010;137:376–387.
- Wu WH, Yang L, Peng FH, Yao J, Zou LL, Liu D, Jiang X, Li J, Gao I, Qu JM, *et al.* Lower socioeconomic status is associated with worse outcomes in pulmonary arterial hypertension. *Am J Respir Crit Care Med* 2013;187:303–310.
- Galiè N, Hoeper MM, Humbert M, Torbicki A, Bachieri JL, Barbera JA, Beghetti M, Corris P, Gaine S, Gibbs JS, *et al.* Guidelines for the diagnosis and treatment of pulmonary hypertension. *Eur Heart J* 2009;30:2493–2537.
- Matura L, McDonough A, Carroll D. Cluster analysis of symptoms in pulmonary arterial hypertension: a pilot study. *Eur J Cardiovasc Nurs* 2012;11:51–61.
- Matura LA, McDonough A, Carroll DL. Health-related quality of life and psychological states in patients with pulmonary arterial hypertension. *J Cardiovasc Nurs* 2014;29:178–184.
- McDonough A, Matura L, Carroll D. Symptom experience of pulmonary arterial hypertension patients. *Clin Nurs Res* 2011;20:120–134.
- Barst RJ, Chung L, Zamanian RT, Turner M, McGoon MD. Functional class improvement and 3-year survival outcomes in patients with pulmonary arterial hypertension in the REVEAL Registry. *Chest* 2013;144:160–168.
- McCollister DH, Beutz M, McLaughlin V, Rumsfeld J, Masoudi FA, Tripputi M, Yaeger T, Weintraub P, Badesch DB. Depressive symptoms in pulmonary arterial hypertension: prevalence and association with functional status. *Psychosomatics* 2010;51:339–339.e8.
- Lowe B, Grafe K, Ufer C, Kroenke K, Grünig E, Herzog W, Borst MM. Anxiety and depression in patients with pulmonary hypertension. *Psychosom Med* 2004;66:831–836.
- Dantzer R, Bluthé RM, Layé S, Bret-Dibat J-L, Parnet P, Kelley KW. Cytokines and sickness behavior. *Ann N Y Acad Sci* 1998;840:586–590.
- Miller AH. Norman Cousins Lecture: Mechanisms of cytokine-induced behavioral changes: psychoneuroimmunology at the translational interface. *Brain Behav Immun* 2009;23:149–158.
- Heffner KL, France CR, Trost Z, Ng HM, Pigeon WR. Chronic low back pain, sleep disturbance, and interleukin-6. *Clin J Pain* 2011;27:35–41.
- Poleshuck EL, Talbot NL, Moynihan JA, Chapman BP, Heffner KL. Depressive symptoms, pain, chronic medical morbidity, and interleukin-6 among primary care patients. *Pain Med* 2013;14:686–691.
- Watkins LR, Maier SF, Goehler LE. Immune activation: the role of pro-inflammatory cytokines in inflammation, illness responses and pathological pain states. *Pain* 1995;63:289–302.
- Graham JE, Robles TF, Kiecolt-Glaser JK, Malarkey WB, Bissell MG, Glaser R. Hostility and pain are related to inflammation in older adults. *Brain Behav Immun* 2006;20:389–400.
- Raison CL, Capuron L, Miller AH. Cytokines sing the blues: inflammation and the pathogenesis of depression. *Trends Immunol* 2006;27:24–31.
- Parissis JT, Adamopoulos S, Rigas A, Kostakis G, Karatzas D, Venetsanou K, Kremastinos DT. Comparison of circulating proinflammatory cytokines and soluble apoptosis mediators in patients with chronic heart failure with versus without symptoms of depression. *Am J Cardiol* 2004;94:1326–1328.
- Vogelzangs N, Beekman ATF, de Jonge P, Penninx BWJH. Anxiety disorders and inflammation in a large adult cohort. *Transl Psychiatry* 2013;3:e249.
- Brouwers C, Mommersteeg PMC, Nyklíček I, Pelle AJ, Westerhuis BL, Szabó BM, Denollet J. Positive affect dimensions and their association with inflammatory biomarkers in patients with chronic heart failure. *Biol Psychol* 2013;92:220–226.
- Jehn CF, Flath B, Strux A, Krebs M, Possinger K, Pezzutto A, Lüftner D. Influence of age, performance status, cancer activity, and IL-6 on anxiety and depression in patients with metastatic breast cancer. *Breast Cancer Res Treat* 2012;136:789–794.
- Dorfmüller P, Perros F, Balabanian K, Humbert M. Inflammation in pulmonary arterial hypertension. *Eur Respir J* 2003;22:358–363.
- Soon E, Holmes AM, Treacy CM, Doughty NJ, Southgate L, Machado RD, Trembath RC, Jennings S, Barker L, Nicklin P, *et al.* Elevated levels of inflammatory cytokines predict survival in idiopathic and familial pulmonary arterial hypertension. *Circulation* 2010;122:920–927.
- Deshpande P, Rajan S, Sudeepthi B, Abdul Nazir C. Patient-reported outcomes: a new era in clinical research. *Perspect Clin Res* 2011;2:137–144.
- Kawut SM, Bagiella E, Lederer DJ, Shimbo D, Horn EM, Roberts KE, Hill NS, Barr RG, Rosenzweig EB, Post W, *et al.* Randomized clinical trial of aspirin and simvastatin for pulmonary arterial hypertension/clinical perspective. *Circulation* 2011;123:2985–2993.
- Matura L, Palevsky H, Lederer D, Horn EM, Pinder D, Archer-Chicko C, Roberts KE, Tracy RP, Girgis RE, Hassound PM, *et al.* Plasma interleukin-6 is associated with symptoms in pulmonary arterial hypertension [abstract]. *Am J Respir Crit Care Med* 2013;187:A4012.
- Kawut SM, Bagiella E, Shimbo D, Lederer DJ, Al-Naamani N, Roberts KE, Barr RG, Post W, Horn EM, Tracy R, *et al.* ASA-STAT Study Group. Rationale and design of a phase II clinical trial of aspirin and simvastatin for the treatment of pulmonary arterial hypertension: ASA-STAT. *Contemp Clin Trials* 2011;32:280–287.
- Brazier JE, Harper R, Jones NM, O’Cathain A, Thomas KJ, Usherwood T, Westlake L. Validating the SF-36 Health Survey questionnaire: new outcome measure for primary care. *BMJ* 1992;305:160–164.
- Achat H, Kawachi I, Spiro A, DemOlles DA, Sparrow D. Optimism and depression as predictors of physical and mental health functioning: The Normative Aging Study. *Ann Behav Med* 2000;22:127–130.
- McHorney CA, Ware JE Jr, Raczek AE. The MOS 36-Item Short-Form Health Survey (SF-36). II. Psychometric and clinical tests of validity in measuring physical and mental health constructs. *Med Care* 1993;31:247–263.

- 30 Panagiotakos DB, Pitsavos C, Yannakoulia M, Chrysoshoou C, Stefanadis C. The implication of obesity and central fat on markers of chronic inflammation: The ATTICA Study. *Atherosclerosis* 2005;183: 308–315.
- 31 Ikonomidis I, Andreotti F, Economou E, Stefanadis C, Toutouzas P, Nihoyannopoulos P. Increased proinflammatory cytokines in patients with chronic stable angina and their reduction by aspirin. *Circulation* 1999;100:793–798.
- 32 SAS Institute Inc. SAS 9.1.3. Cary, NC: SAS Institute Inc.; 2000–2004.
- 33 Davis MC, Zautra AJ, Younger J, Motivala SJ, Attrep J, Irwin MR. Chronic stress and regulation of cellular markers of inflammation in rheumatoid arthritis: implications for fatigue. *Brain Behav Immun* 2008;22:24–32.
- 34 Cho HJ, Kivimäki M, Bower JE, Irwin MR. Association of C-reactive protein and interleukin-6 with new-onset fatigue in the Whitehall II prospective cohort study. *Psychol Med* 2013;43:1773–1783.
- 35 Lasselin J, Layé S, Dexpert S, Aubert A, Gonzalez C, Gin H, Capuron L. Fatigue symptoms relate to systemic inflammation in patients with type 2 diabetes. *Brain Behav Immun* 2012;26:1211–1219.
- 36 Fink AM, Gonzalez RC, Lisowski T, Pini M, Fantuzzi G, Levy WC, Piano MR. Fatigue, inflammation, and projected mortality in heart failure. *J Card Fail* 2012;18:711–716.
- 37 O'Donovan A, Hughes BM, Slavich GM, Lynch L, Cronin MT, O'Farrelly C, Malone KM. Clinical anxiety, cortisol and interleukin-6: evidence for specificity in emotion–biology relationships. *Brain Behav Immun* 2010;24:1074–1077.
- 38 Jehn C, Kühnhardt D, Possinger K, Flath B. Plasma IL-6 and HPA-axis function in depression and anxiety in patients with cancer. *J Clin Oncol (Meeting Abstracts)* 2005;23(Suppl 16):8033.
- 39 Bob P, Raboch J, Maes M, Susta M, Pavlat J, Jasova D, Vevera J, Uhrova J, Benakova H, Zima T. Depression, traumatic stress and interleukin-6. *J Affect Disord* 2010;120:231–234.
- 40 Kiecolt-Glaser JK, Belury MA, Andridge R, Malarkey WB, Glaser R. Omega-3 supplementation lowers inflammation and anxiety in medical students: a randomized controlled trial. *Brain Behav Immun* 2011;25:1725–1734.
- 41 Alstergren P, Kopp S. Insufficient endogenous control of tumor necrosis factor- α contributes to temporomandibular joint pain and tissue destruction in rheumatoid arthritis. *J Rheumatol* 2006;33: 1734–1739.
- 42 ter Wolbeek M, van Doornen LJP, Kavelaars A, Tersteeg-Kamperman MDJ, Heijnen CJ. Fatigue, depressive symptoms, and anxiety from adolescence up to young adulthood: a longitudinal study. *Brain Behav Immun* 2011;25:1249–1255.
- 43 Karlović D, Serretti A, Vrkic N, Martinac M, Marčinko D. Serum concentrations of CRP, IL-6, TNF- α and cortisol in major depressive disorder with melancholic or atypical features. *Psychiatry Res* 2012; 198:74–80.
- 44 Blackmore ER, Moynihan JA, Rubinow DR, Pressman EK, Gilchrist M, O'Connor TG. Psychiatric symptoms and proinflammatory cytokines in pregnancy. *Psychosom Med* 2011;73:656–663.
- 45 Kwak SM, Choi YS, Yoon HM, Kim DG, Song SH, Lee YJ, Yeom CH, Koh SJ, Park J, Lee MA, *et al.* The relationship between interleukin-6, tumor necrosis factor- α , and fatigue in terminally ill cancer patients. *Palliat Med* 2012;26:275–282.
- 46 Schubert C, Hong S, Natarajan L, Mills PJ, Dimsdale JE. The association between fatigue and inflammatory marker levels in cancer patients: a quantitative review. *Brain Behav Immun* 2007;21: 413–427.
- 47 Bellisai F, Morozzi G, Scaccia F, Chellini F, Simpatico A, Pecetti G, Galeazzi M. Evaluation of the effect of bosentan treatment on proinflammatory cytokine serum levels in patients affected by systemic sclerosis. *Int J Immunopathol Pharmacol* 2011;24:261–264.
- 48 Bien S, Riad A, Ritter CA, Gratz M, Oishausen G, Wetermann D, Grube M, Krieg T, Ciecholewski S, Felix SB, *et al.* The endothelin receptor blocker bosentan inhibits doxorubicin-induced cardiomyopathy. *Cancer Res* 2007;67:10428–10435.
- 49 Wahlström MR, Olivecrona M, Ahlm C, Bengtsson A, Koskinen LO, Naredi S, Hultin M. Effects of prostacyclin on the early inflammatory response in patients with traumatic brain injury: a randomised clinical study. *Springerplus* 2014;3:98.
- 50 Eisenhut T, Sinha B, Gröttrup-Wolfers E, Semmler J, Siess W, Endres S. Prostacyclin analogs suppress the synthesis of tumor necrosis factor- α in LPS-stimulated human peripheral blood mononuclear cells. *Immunopharmacology* 1993;26:259–264.
- 51 Aversa A, Vitale C, Volterrani M, Fabbri A, Spera G, Fini M, Rosano GM. Chronic administration of sildenafil improves markers of endothelial function in men with type 2 diabetes. *Diabet Med* 2008;25:37–44.
- 52 Smart NA, Larsen AI, Le Maitre JP, Ferraz AS. Effect of exercise training on interleukin-6, tumour necrosis factor α and functional capacity in heart failure. *Cardiol Res Pract* 2011;2011:532620.
- 53 Gombert-Maitland M, Thenappan T, Rizvi K, Chandra S, Meads DM, McKenna SP. United States validation of the Cambridge Pulmonary Hypertension Outcome Review (CAMPHOR). *J Heart Lung Transplant* 2008;27:124–130.