Soluble Interleukin 6 Receptor
A Novel Marker of Moderate to Severe Sleep-Related Breathing Disorder

Reena Mehra, MD, MS; Amy Storfer-Isser, MS; H. Lester Kirchner, PhD; Nathan Johnson; Nancy Jenny, PhD; Russell P. Tracy, PhD; Susan Redline, MD, MPH

Background: Given the previously described association between sleep-related breathing disorder (SRBD) and markers of inflammation, we assessed the relationship of SRBD with levels of both interleukin 6 (IL-6) and soluble IL-6 receptor (sIL-6R), a marker with more expansive physiologic effects than IL-6. The objectives were to explore the relationship between moderate to severe sleep apnea with IL-6 and sIL-6R levels and to examine morning and evening variability for each cytokine.

Methods: A total of 385 adult participants (≥18 years of age) in the Cleveland Family Study, Cleveland, Ohio, underwent sleep studies and determination of IL-6 and sIL-6R levels in samples obtained in the evening and morning of polysomnography. Moderate to severe SRBD was defined as a respiratory disturbance index greater than or equal to 30.

Results: The subjects were aged 44.9±16.7 (mean±SD) years, 44% were male, and 48% were African American, with a body mass index (weight in kilograms divided by the height in meters squared) of 32.5±8.1 (mean±SD). Linear regression analysis showed that after adjustment for subject characteristics, waist circumference, and comorbidities, SRBD was not significantly associated with morning IL-6 levels. In contrast, linear regression analyses showed that, compared with the participants without SRBD, those with SRBD had significantly higher morning sIL-6R levels (mean±SD, 4.60±1.42 ng/mL [P=.001]), even after adjustment for subject characteristics, waist circumference, and comorbidities, which persisted after adjustment of evening sIL-6R levels.

Conclusions: Morning sIL-6R levels demonstrated stronger associations with moderate to severe SRBD than morning IL-6 levels. Associations with SRBD and morning sIL-6R levels persisted even after adjustment for waist circumference, cardiovascular disease, and evening sIL-6R levels, suggesting the potential utility of sIL-6R as a marker for measuring overnight SRBD stresses. Further investigation of this biomarker may provide insight into SRBD-related inflammation.

Arch Intern Med. 2006;166:1725-1731
The sIL-6R mechanistic pathway involves binding to IL-6 to form the IL-6–sIL-6R complex, which binds to membrane-bound glycoprotein 130 (gp-130) receptor in a process termed trans-signaling. The gp-130 receptor is expressed on all body cells, and because IL-6 alone cannot bind to gp-130, the IL-6–sIL-6R complex activates cells that are normally unresponsive to IL-6. In the absence of sIL-6R, IL-6 binds to the transmembrane IL-6α receptor, which is present on a limited number of cells compared with gp-130. Therefore, the impact of IL-6 may be enhanced by sIL-6R, which may augment and drive the biologic effects of IL-6 by broadening the array of potential IL-6 targets.

We examined the relationship of SRBD with IL-6 as well as with sIL-6R because the IL-6–sIL-6R complex appears to exert proinflammatory effects that are more expansive than those of the IL-6 ligand and may be more relevant to the pathogenesis of SRBD-associated inflammation or atherosclerosis. We also explored morning and evening differences (ie, morning and evening variation) of IL-6 and sIL-6R levels with the expectation that elevated morning levels compared with evening levels may reflect overnight SRBD-related physiologic stress such as hypoxia and sympathetic nervous system activation.

**METHODS**

**STUDY POPULATION**

The subjects were participants in the Cleveland Family Study (Cleveland, Ohio), a longitudinal genetic epidemiological study that was designed to investigate the causal factors and natural history of SRBD. The methods of recruitment and data collection have been previously described. Affected families were identified based on a proband with diagnosed sleep apnea, and neighborhood controls were recruited. Of 2462 cohort members, 700 were selected to undergo cardiovascular and metabolic risk factor studies based on expected informativeness for genetic analyses. At the time of this report, 465 adult subjects (≥18 years of age) participated in the study and had serum samples assayed for cytokines. Sixty participants who were receiving continuous positive airway pressure therapy were excluded, as were 20 participants with potential confounding medical conditions (eg, rheumatologic or autoimmune disorders). The final analytic sample included 385 adults who underwent either both morning and evening IL-6 measurements (n = 358) or both morning and evening sIL6-R measurements (n = 382).

**DATA COLLECTION**

Testing was conducted in the General Clinical Research Laboratory at University Hospitals of Cleveland after institutional review board approval and written informed consent were obtained. Height was measured with a rigid stadiometer, and weight was measured with a calibrated digital scale using standardized methods; these measures were used to calculate body mass index (BMI). Venous blood was sampled between 10 and 11 PM with the participant in the supine position before sleep onset. Blood was again drawn, supine, between 7 and 8 AM the next morning after overnight polysomnography and an overnight fast. After the samples were centrifuged and aliquoted using standardized protocols, they were stored at −80°C until being assayed at the University of Vermont Laboratory for Clinical Biochemistry Research, Burlington. Quantitative sandwich enzyme immunoassays (Quantikine HS Human IL-6 and IL6-sR Immunoassays; R&D Systems, Minneapolis, Minn) were used to measure IL-6 and sIL-6R levels in serum samples. Intralaboratory coefficients of variation for the assays were 9.1% for IL-6 and 6.8% for sIL-6R.

**STATISTICAL ANALYSIS**

The 2 primary outcomes in this analysis were morning IL-6 (picograms per milliliter) and sIL-6R (nanograms per milliliter) levels. Exploratory analyses assessed the association of each outcome with RDI, categorized as (1) 0 through 4.9; (2) 5.0 through 9.9; (3) 10.0 through 14.9; (4) 15.0 through 29.9; and (5) 30.0 or higher, using linear mixed models. The primary exposure was moderate to severe SRBD, defined as an RDI score of 30 or more, levels shown to confer increased CVD risk. Spearman correlations assessed relationships between the 2 outcomes as well as with continuous subject characteristics. Linear mixed models assessed the association between SRBD and each outcome. Models included a random intercept to account for the intrafamilial correlation, with adjustment for subject characteristics (age, sex, and race) (model 1); subject characteristics and waist circumference (model 2); and subject characteristics, waist circumference, and CVD measures, including diabetes mellitus (use of oral hypoglycemics and/or insulin, fasting plasma glucose of ≥126 mg/dL [7.0 mmol/L], or self-reported physician’s diagnosis), hypertension (use of antihypertensive medications, average systolic blood pressure ≥140 mm Hg, and/or average diastolic pressure ≥90 mm Hg), and CVD (history of angina, coronary angioplasty, coronary artery bypass graft surgery, myocardial infarction, coronary heart disease, stroke, carotid endarterectomy, or heart failure) (model 3). Because we postulated that cytokine levels increase in response to overnight SRBD-related stresses, we performed analyses after further adjusting for the evening values of each outcome measure (model 4). Alternatively, evening values were used as outcomes in analyses exploring morning and evening differences in cytokine levels. Results of linear mixed models are summarized via β coefficients, adjusted least squares means for categorical covariates, and slopes for continuous covariates, along with standard errors. Owing to the skewed distribution of IL-6 morning and evening measures, these outcomes were transformed using the natural logarithm to achieve approximate normality; the results are back-transformed to facilitate interpretation. Analyses were performed using SAS software (Version 9.1; SAS Institute Inc, Cary, NC).

Secondary analyses explored potential physiologic pathways relating morning IL-6 or sIL-6R levels to (1) arousal index (natural log of the number of arousals per hour of sleep); (2) total sleep time less than 90% oxygen saturation (dichotomized at 2% based on its highly skewed distribution); and (3) inclusion of the Epworth Sleepiness Scale (ESS), a mea-
sure of subjective sleepiness. Models 1 through 3 were also refit excluding current asthmatic patients, defined as those using inhaled bronchodilators and/or inhaled steroids over the past 3 days.

## RESULTS

### PRIMARY ANALYSES

Table 1 shows subject characteristics for the analytic sample stratified by RDI category. Subjects were aged 44.9±16.7 (mean±SD) years, 44.4% were male, and 47.5% were African American, with a BMI (weight in kilograms divided by the height in meters squared) of 32.5±8.1 (mean±SD). Spearman correlations reflected a stronger association between BMI and IL-6 levels (\( r = 0.44 \)) compared with BMI and sIL-6R levels (\( r = 0.11 \)). Waist circumference also was more strongly associated with IL-6 compared with sIL-6R (\( r = 0.44 \) and \( r = 0.14 \) for IL-6 and sIL-6R, respectively). Interleukin 6 and sIL-6R were weakly correlated (\( r = 0.16 \) and \( r = 0.14 \) for morning and evening levels, respectively). These results suggest a differential contributory role and utility of separately exploring the relationships of SRBD relative to IL-6 and sIL-6R measures.

Overall, the mean evening IL-6 levels were higher than the morning IL-6 levels (\( P<.001 \)), and this trend persisted for each RDI category in both unadjusted and adjusted models (Figure 1). While unadjusted results show that the participants with moderate to severe SRBD (RDI≥30) had significantly higher morning and evening IL-6 levels than those without sleep apnea (RDI<5) (\( P<.001 \) for both morning and evening levels), these differences were not statistically significant after adjustment for subject characteristics (\( P=.81 \) and \( P=.56 \) for morning and evening IL-6 levels, respectively). Morning sIL-6R levels were higher than evening levels across the RDI categories (Figure 2). Also, sIL-6R values were similar across the 4 lowest RDI categories and elevated for the highest RDI category. In unadjusted and adjusted models, the highest RDI group had significantly higher mean morning sIL-6R levels than each of the other 4 groups (all \( P \) values < .05), and none of the pair-wise comparisons among the other 4 RDI categories was statistically significant (all \( P \) values > .05).

While SRBD (RDI≥30) was significantly associated with morning IL-6 levels after adjustment for age, age², sex, and race (model 1), the magnitude of the association decreased by 74% and was no longer statistically significant after further adjustment for waist circumference (model 2). This lack of statistical significance persisted after further adjustment for CVD measures and evening IL-6 levels (Table 2).

In unadjusted and all adjusted models, SRBD (RDI≥30) was significantly associated with higher morning levels of sIL-6R. The association between SRBD and sIL-6R levels was reduced by approximately 20% after waist circumference (model 2) and CVD measures (model 3) were adjusted for, but remained statistically significant. After these covariates were controlled for, on average the participants with SRBD had levels of sIL-6R

### Table 1. Subject Characteristics, by Sleep-Related Breathing Disorder Category

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Analytic Sample (n = 385)</th>
<th>RDI 0-4.9 (n = 177)</th>
<th>RDI 5.0-9.9 (n = 58)</th>
<th>RDI 10.0-14.9 (n = 39)</th>
<th>RDI 15.0-29.9 (n = 62)</th>
<th>RDI ≥30 (n = 57)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>44.9 ± 16.7</td>
<td>38.6 ± 16.1</td>
<td>45.2 ± 16.2</td>
<td>51.5 ± 14.5</td>
<td>52.4 ± 14.4</td>
<td>51.6 ± 15.8</td>
</tr>
<tr>
<td>Male sex</td>
<td>171 (44.4)</td>
<td>58 (32.8)</td>
<td>22 (44.0)</td>
<td>17 (43.6)</td>
<td>29 (46.8)</td>
<td>45 (79.0)</td>
</tr>
<tr>
<td>African American</td>
<td>183 (47.3)</td>
<td>82 (46.3)</td>
<td>26 (52.0)</td>
<td>18 (46.2)</td>
<td>29 (46.8)</td>
<td>28 (49.1)</td>
</tr>
<tr>
<td>BMI</td>
<td>32.5 ± 8.1</td>
<td>28.7 ± 8.6</td>
<td>33.0 ± 8.1</td>
<td>36.0 ± 7.3</td>
<td>38.4 ± 9.0</td>
<td></td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>95.6 ± 18.1</td>
<td>88.4 ± 14.5</td>
<td>99.2 ± 13.2</td>
<td>107.1 ± 13.7</td>
<td>105.9 ± 15.7</td>
<td>115.8 ± 16.8</td>
</tr>
<tr>
<td>Hypertension</td>
<td>126 (32.7)</td>
<td>34 (19.2)</td>
<td>17 (34.0)</td>
<td>17 (43.6)</td>
<td>28 (45.2)</td>
<td>30 (52.6)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>44 (11.5)</td>
<td>8 (4.6)</td>
<td>7 (14.0)</td>
<td>6 (15.4)</td>
<td>9 (14.5)</td>
<td>14 (24.6)</td>
</tr>
<tr>
<td>Cardiovascular disease</td>
<td>46 (12.0)</td>
<td>10 (5.7)</td>
<td>6 (12.0)</td>
<td>7 (18.4)</td>
<td>13 (21.0)</td>
<td>10 (17.5)</td>
</tr>
</tbody>
</table>

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by the height in meters squared); RDI, respiratory disturbance index.

*Continuous variables are expressed as mean ± SD; categorical variables, as number (percentage).
that were 4.60±1.42 (mean±SD) ng/mL higher than those without SRBD. When the evening sIL-6R values were added (model 4), the association between SRBD and sIL-6R levels was further attenuated but remained statistically significant (1.31±0.58 ng/mL; P = .02) (Table 3).

Compared with the morning IL-6 values, the evening IL-6 values had a stronger relationship with SRBD. Unlike the results of morning IL-6 analysis, those of the evening IL-6 analysis showed a marginally significant association between SRBD and evening IL-6 levels after adjustment for waist circumference and CVD measures. In contrast, both morning and evening adjusted sIL-6R levels were associated with SRBD, with stronger associations noted with morning levels than with evening levels (Table 4).

SECONDARY ANALYSES

The association of arousal index and IL-6 levels was not statistically significant in all models. After subject characteristics, waist circumference, and CVD measures were adjusted for, subjects with nocturnal hypoxemia (ie, with ≥2% sleep time spent at <90% oxygen saturation) had marginally significantly higher morning IL-6 levels than those with less overnight hypoxemia (2.31±0.20 pg/mL vs 1.96±0.10 pg/mL; P = .10). After subject characteristics, waist circumference, and CVD measures were controlled for, the sIL-6R levels were positively associated with the arousal index (1.94±0.91 ng/mL per 1-U increase; P = .03) and tended to be associated with nocturnal hypoxemia (33.0±1.1 ng/mL vs 30.8±0.6 ng/mL; P = .07, for participants with ≥2% time at <90% oxygen saturation compared with others, respectively). Sensitivity analyses were performed with models 1 through 3 refitted for both outcomes (IL-6 and sIL-6R) after excluding current asthmatic subjects (n=15 without SRBD; n=3 with SRBD). The sensitivity analyses yielded similar results.

Correlations with the levels of cytokines and the ESS scores were low (morning IL-6 levels and ESS score, r = 0.02; morning sIL-6R levels and ESS score, r = −0.10). Inclusion of the ESS scores, as in models relating SRBD and IL-6 and sIL-6R levels, did not appreciably change the prior associations. Inclusion of BMI rather than waist circumference also did not lead to differences in model estimates.

The present study demonstrated significant associations between SRBD and morning IL-6 levels in unadjusted analyses and in analyses adjusted for age, sex, and race. However, in our sample, which had a wide range of age and obesity, this relationship did not persist after waist circumference was taken into account or after additional consideration of CVD risk factors, CVD, and evening IL-6 levels. In contrast, a significant association between moderate to severe SRBD (RDI≥30) and morning sIL-6R levels was noted, which persisted after these same covariates were taken into account. Adjustment for waist circumference and CVD measures did not lead to substantive attenuation of the association between sIL-6R levels and SRBD. The strength of the association between SRBD was higher for morning than for evening sIL-6R levels, and the relationship between SRBD and morning sIL-6R levels persisted after additional adjustment for evening sIL-6R levels. These findings suggest that morning sIL-6R levels may provide a more sensitive reflection of the acute pathophysiologic effects accompanying SRBD than morning IL-6 levels. Morning sIL-6R levels may therefore be a potentially useful marker for evaluating the overnight stresses of SRBD and may be an especially useful marker for morning and evening variability in SRBD-related inflammatory stress.

Interleukin 6 is an inflammatory cytokine that has been identified as playing a central role in inflammatory processes, including atherogenesis. It is a primary determinant of hepatic C-reactive protein production, which has been independently associated with incident CVD, progressive atherosclerosis, and CVD outcomes. Increased IL-6 levels have been associated with risk of myocardial infarction, and IL-6 is thought to be both a marker for atherosclerosis and a contributor to atherogenesis.

The levels of IL-6 have been postulated to be increased in SRBD owing to hypoxemia and sympathetic nervous system–mediated stresses, with increased levels of IL-6, possibly acting synergistically with high C-reactive protein levels, contributing to cardiovascular comorbidity. It has been suggested that elevated IL-6 levels contribute to the excessive sleepiness that has been observed in subjects with SRBD. However, the research supporting an increase in IL-6 levels in SRBD has provided inconsistent results or has been based on highly
The levels of IL-6 have been shown to be elevated in subjects with sleep apnea compared with normal control subjects \((P = .03)\); however, the primary determinant for IL-6 levels was the BMI.\(^{14,37}\) Our findings are consistent with this literature, showing strong relationship indices of SRBD and IL-6 levels, particularly evening IL-6 levels, in unadjusted analyses, with marked attenuation of these associations after consideration of waist circumference.

Our findings of somewhat stronger associations between SRBD and evening compared with morning IL-6 levels are also consistent with a possibly less specific increase in IL-6 levels occurring in SRBD, possibly secondary to the higher level of visceral obesity or to the CVD risk factors in this population. Interleukin 6 is produced by adipose tissue, with serum IL-6 levels positively correlated with adipose tissue mass, and adipose tissue–derived IL-6 accounts for 20% to 30% of total IL-6 serum levels.\(^{38-40}\) Thus, the aggregate evidence is consistent with IL-6 playing a role in SRBD-related inflammation; however, the specificity of IL-6 for SRBD stresses, and its strong association with obesity, may limit its utility as a marker of SRBD-related stress. Because sIL-6R is less strongly associated with obesity than IL-6, it may provide a more specific marker of SRBD.

Recent evidence indicates that the pathophysiologic effects of IL-6 may depend strongly on a soluble form of the receptor involved with trans-signaling pathways.\(^{21}\) Soluble IL-6 receptor is the cleaved extracellular component of the \(\alpha\) subunit of the IL-6 receptor complex and is still able to bind to the IL-6 ligand. The IL-6–sIL-6R complex activates target cells expressing gp-130, such as smooth muscle cells,\(^{19}\) resulting in vascular smooth muscle proliferation and induction of a proinflammatory and proatherogenic state.\(^{41,42}\) Although endothelial cells do not express the IL-6 receptor, they may be stimulated by the IL-6–sIL-6R complex to produce leukocyte recruitment and adhesion proteins such as vascular cell...
adhesion molecule 1 and intracellular adhesion molecule 1,43,44 which have been implicated in the SRBD-CVD pathway.19 These characteristics lend support for sIL-6R playing a role in pathways that result from SRBD-induced physiologic stresses, leading to CVD.

Clinical studies have examined the association of sIL-6R and bronchial asthma, interstitial lung disease (ie, sarcoidosis or interstitial pneumonia), and pulmonary fibrosis. These studies have revealed significantly higher levels of sIL-6R (1) in patients with asthma compared with control subjects, coinciding with the degree of airway inflammation17; and (2) in serum and bronchoalveolar fluid samples from patients with interstitial lung disease compared with those from normal subjects.18 They also demonstrated that sIL-6R levels significantly correlated with the severity of pulmonary fibrosis in systemic sclerosis.46 Although these studies support significant associations with sIL-6R levels and pulmonary disease, the role of hypoxia and relationships with oxygen desaturation and sIL-6R levels were not explored. However, biologic plausibility for hypoxia-induced increases in sIL-6R levels is supported by data showing relationships between hypoxia inducible factor 1 and sIL-6R levels in the setting of vascular endothelial growth factor up-regulation.47

Of interest, there appeared to be a threshold association between the level of SRBD and sIL-6R elevations, with little evidence for increased levels until an RDI of 30 was reached. This is a level of SRBD that is consistent with the threshold level recently implicated as a risk factor for CVD and CVD-related mortality.3 Secondary unadjusted and adjusted analyses also showed that sIL-6R levels were significantly associated with overnight hypoxemia and the arousal index, a measure of sleep fragmentation. The stronger findings in adjusted analyses between SRBD and sIL-6R levels compared with IL-6 levels suggest that sIL-6R levels may be more specifically responsive to SRBD-related stress, such as overnight hypoxemia or sympathetic activation, than IL-6 levels. Also, literature indicates that the half-life of IL-6 appears to be approximately 1 hour,48 compared with 2 to 3 hours for sIL-6R,49 suggesting that sIL-6R, with its slightly longer half-life, may better capture the cumulative overnight physiologic stresses associated with SRBD. Experimental studies are needed to further identify the extent to which SRBD-associated elevations in sIL-6R levels may reflect responses to hypoxemia, sympathetic stimulation, other influences, or combinations of exposures.

The limitations of the current study include the potential for analytic variability of measurements; however, strict quality assurance standards are followed in our core biochemistry laboratory. Standardized protocols are also followed for phlebotomy and processing procedures as well as for assays, including monitoring for assay drift and use of blind duplicate controls. Assessing the variation of a putative outcome measure, such as a cytokine with the level of SRBD, is limited by the intrinsic differences in age, sex, and obesity that are typical of patients with SRBD compared with unaffected groups. However, our analyses adjusted not only for covariate differences but also for differences in evening cytokine levels, which also vary with subject characteristics and health parameters, providing a robust means for adjusting for a variety of unmeasured confounders. Identifying variation of a cytokine with the level of SRBD, manifest even after multiple adjustments are considered, may indicate its particular utility for gauging SRBD-related stresses in typical clinical practice, where patients with SRBD are heavier and older than other groups.

The strengths of our study include the use of state-of-the-art polysomnography with several SRBD severity measures. To our knowledge, this is the largest study that has been conducted to examine the associations of IL-6 levels and SRBD and to assess the role of sIL-6R levels in SRBD, with a specific focus on day and evening differences. The sample was derived from a well-characterized cohort as opposed to a referral center, which may be more prone to selection bias. The large range of RDI values in this sample also allowed us to assess dose-response relationships and threshold effects, suggesting that elevations in the levels of these cytokines were most pronounced above a RDI of 30 and that they were associated with overnight hypoxemia and cortical arousals.

Because morning levels of sIL-6R, a factor involved in trans-signaling pathways and inflammation, exhibit a stronger association with SRBD than IL-6 levels, even after adjustment for potential confounders, measurement of morning sIL-6R levels may be particularly useful for exploring SRBD-associated inflammatory pathways and possibly for identifying those patients with SRBD who are at higher risk for cardiovascular comorbidity. Although the processes that determine receptor shedding and sIL-6R levels and downstream sIL-6R effects are not well described, they may be critical in determining the far-ranging systemic effects of IL-6 and inflammatory cascades. Our data suggest that overnight apneas, arousals, and hypoxia, as occur in moderate to severe SRBD, may effect the shedding of sIL-6R, which may have a critical impact in influencing expression of autoimmune and inflammatory processes.

Accepted for Publication: June 1, 2006.

Correspondence: Reena Mehra, MD, MS, Department of Medicine, Case Western Reserve University, 11100 Euclid Ave, Cleveland, OH 44106 (mehrar@ameritech.net).

Author Contributions: The authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Financial Disclosure: None reported.

Funding/Support: This study was supported in part by grants NIH 46380 and NIH M01 RR00080 from the National Heart Lung Blood Institute and by grant 0530188N from the American Heart Association.

Acknowledgment: We are indebted to the dedicated staff of the Cleveland Family Study, including Joan Aylor, Kathryn Clark, Rawan Nawabit, Jennifer Frame, and Heather Rogers, as well as to the nurses of the University Hospitals of Cleveland General Clinical Research Center. We are particularly grateful for the participation of the members of the Cleveland Family Study, whose continuing enthusiasm has made this study possible.


18. Teramoto S, Yamamoto H, Ouchi Y. Increased C-reactive protein and increased plasma interleukin-6 may synergistically affect the progression of coronary ath- erosclerosis in obstructive sleep apnea syndrome. Circulation 2003;107:40-7.


