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Blood biomarkers of endocrine, immune, inflammatory, and metabolic systems in obstructive sleep apnea

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ABSTRACT

Objective/background: Obstructive sleep apnea (OSA) is a common disorder, affecting over 100 million adults. Untreated OSA leads to serious health consequences and perturbations in endocrine, immune, inflammatory, and metabolic systems. Study objectives are to evaluate the association between OSA and biomarkers, and to test the hypothesis that a combination of markers may be useful in screening for OSA.

Patients/methods: A multicenter trial was conducted enrolling symptomatic male patients with suspected OSA. All subjects underwent in-laboratory overnight polysomnography. A non-symptomatic control group was also obtained. Eleven biomarkers were tested: HbA1c, CRP, EPO, IL-6, uric acid, cortisol, hGH, prolactin, testosterone, DHEA (Beckman Coulter UniCel DxC 600i Synchron® Access® Clinical Systems), IGF-1.

Results: 73 male subjects were enrolled; 26 had moderate/severe OSA. ROC curve analysis showed HbA1c, CRP, EPO, IL-6, and Uric Acid (AUCs: 0.76, 0.73, 0.65, 0.65, 0.61) were superior to the Epworth Sleepiness Scale (AUC: 0.52). Concurrent elevation of HbA1c and CRP provide even greater predictive power. A combination of elevated HbA1c, CRP, and EPO provided 0.08 increase in AUC (0.84 [0.75 - 0.94]) over individual markers ($p<0.05$), with high sensitivity (85%), and specificity (79%) for moderate/severe OSA.

Conclusions: OSA induces characteristic endocrine, immune, inflammatory, and metabolic disturbances that can be detected with blood biomarkers. These biomarkers are superior to standard screening questionnaires. Various clusters of these biomarkers have an even greater association with OSA and thus may represent physiologic signatures of the disorder that may have value in initial screening for OSA as well as for follow-up of therapy response.

1. Introduction

Obstructive sleep apnea (OSA) is a common disorder, characterized by repetitive episodes of complete (apnea) or partial (hypopnea) obstructions of the upper airway during sleep, with decreasing oxygen saturation and sleep fragmentation. More than 22 million American adults have OSA. In the Wisconsin Sleep Cohort Study [1], representing a large, random sample of 30 to 60 year old individuals reporting habitual snoring, 9% of women and 24% of men had OSA. The World Health Organization estimates 100 million worldwide have OSA [2], and up to 90% of individuals with OSA remain undiagnosed and untreated. OSA prevalence is increasing [3] and may soon become the most common chronic disease in industrialized countries.

Untreated OSA can lead to serious health consequences, including increased mortality [4–7]. Recurrent respiratory events and hypoxia cause sympathetic activation, hypertension, oxidative stress, and metabolic dysregulation [5,6,8–12]. Patients with OSA have an elevated risk of developing coronary artery disease, cardiac arrhythmia, myocardial infarction, heart failure, stroke, diabetes, obesity, metabolic syndrome, and memory decline [4–6,8,13–22]. OSA increases cardiovascular risks and mortality independent of factors such as age, sex, race, smoking, diabetes, obesity, dyslipidemia, and hypertension [13]. In addition, individuals with untreated OSA are more likely to be involved in work-related or driving accidents [23].

Given the significant health issues associated with untreated OSA [19,20,24,25], diagnosis and treatment of this disorder are critical. Continuous positive airway pressure (CPAP) treatment reduces the risk of

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adverse outcomes [26]. The gold standard for diagnosis is currently based upon overnight polysomnography (sleep study), and patients are often not referred for this definitive testing.

The role that biomarkers may play in the identification of patients with OSA is unknown. The association between biomarkers and OSA is an area of active investigation [27–30]. OSA-induced metabolic and endocrine dysfunction, chronic inflammation, hypoxia, sleep fragmentation, and stress cause changes in multiple markers including glycated hemoglobin (HbA1c), C-reactive protein (CRP), erythropoietin (EPO), interleukin-6 (IL-6), and uric acid [7,17,27,28,31–41].

A recent comprehensive literature review of biomarkers associated with OSA [28] noted that an ideal test for OSA should be sensitive and specific, simple, timely, inexpensive, and correlate to severity of disease. A blood test, or combination of blood tests, could potentially meet these criteria.

The objective of this multicenter prospective trial was to evaluate the association between OSA and eleven blood biomarkers in a representative population of consecutively enrolled symptomatic patients with a clinical suspicion of obstructive sleep apnea.

An additional objective was to test the hypothesis that various combinations of two to three biomarkers have an even greater association with OSA than individual biomarkers. Such clusters of OSA-induced endocrine, immune, inflammatory, and metabolic disturbances could provide a characteristic physiologic signature of OSA that may have value in screening for OSA.

2. Material and methods

2.1. Patient population

A multicenter prospective trial was conducted to evaluate the association between OSA and endocrine, immune, inflammatory, and metabolic biomarkers in a representative population of consecutively enrolled symptomatic male patients with a clinical suspicion of sleep apnea; subjects were followed from a general medical clinic population (general practitioners, internists, cardiologists) to a sleep clinic after referral for further evaluation. Subjects with previously diagnosed or treated OSA were excluded, as were those with predominant central sleep apnea, and those currently using steroids, testosterone supplements, DHEA, or opioid pain medications. All subjects underwent polysomnography.

Patients with an apnea-hypopnea index (AHI) ≥5 were considered to have OSA, and patients with an AHI < 5 were enrolled as the Non-OSA control group.

An asymptomatic healthy control group, in whom occult OSA was excluded, was also enrolled. These subjects were obtained from a separate Healthy Controls study conducted at two participating centers.

The trial was approved by the institutional review boards at participating centers. Written informed consent was obtained from all subjects.

2.2. Clinical assessment, sleep measures, polysomnography

Questionnaires were completed and general physical measurements were taken before the subjects were prepared for polysomnography. Measurements included blood pressure, body weight, height, and calculation of body mass index (BMI). Daytime sleepiness was assessed by the Epworth Sleepiness Scale (ESS). ESS scores range from 0 to 24, with higher scores representing greater daytime sleepiness.

The presence and severity of sleep disordered breathing was evaluated using in-laboratory overnight polysomnography. The apnea-hypopnea index (AHI), used in the diagnosis and assessment of severity, is the average number of apneas and hypopneas per hour of sleep, with apnea defined as absence of airflow for ≥10 s, and hypopnea defined as a reduction in airflow for ≥10 s in association with either ≥4% arterial oxygen desaturation or an arousal.

In addition to AHI, polysomnography included measurement of other hypoxia-related parameters such as oxygen saturation (percentage of available hemoglobin that is saturated with oxygen estimated by pulse oximetry).

2.3. Sample collection and biomarker testing

Eleven biomarkers were tested by personnel blinded to patient characteristics, results of the sleep studies and diagnosis: HbA1c, high sensitivity CRP, IL-6, uric acid, EPO, cortisol, hGH, prolactin, testosterone, DHEA (Beckman Coulter UniCel DxC 600i Synchron® Access® Clinical Systems), and IGF-1. Clinicians were not provided with biomarker results prior to patient diagnosis.

Blood samples were collected and processed prior to initiation of treatment. Whole blood samples were shipped at 4 °C to a central laboratory for HbA1c testing. Serum for all other testing was separated from cells, dispensed into cryo-tubes, stored at −70 °C, and shipped to a central laboratory for testing.

2.4. Statistical methods

Descriptive statistics for each variable were two-tailed, and performed using Student's *t*-test, ANOVA, or Mann-Whitney test for continuous variables depending upon data distribution and normality, and Fisher's Exact test or Chi-square test for dichotomous variables. Clinical characteristics, biomarker concentrations, and sleep study results were compared between patients with moderate/severe OSA and those with mild/no OSA. Pearson (r) and Spearman (ρ) correlation coefficients were used for Gaussian and skewed variables, respectively. Statistical analyses were performed using SAS System software (version 9.3, SAS Institute Inc., Cary, NC, USA) and Analyse-it (version 2.26, Analyse-it Software, Ltd., Leeds, United Kingdom), with a p value <0.05 indicating statistical significance.

The primary outcome variable was AHI. OSA was graded according to AHI as mild (5–14.9), moderate (15–29.9), or severe (≥30). Mean and minimum oxygen saturation, two additional measures of OSA severity, were also evaluated.

To evaluate clinical performance for detection of moderate/severe OSA, a series of potential cutoffs were assessed using Receiver Operating Characteristic (ROC) curves [42,43]. Sensitivity and specificity results for each of these cutoffs were calculated. ROC curves are graphic plots of the true positive rate (sensitivity) against the false positive rate (1 minus specificity) for different possible cutoffs, and demonstrate the tradeoff between sensitivity and specificity (any increase in sensitivity will be accompanied by a decrease in specificity). The closer the curve to the upper left corner of the plot, the more accurate the test. The Area Under the Curve (AUC) and 95% confidence intervals were determined. The AUC is a measure of test accuracy, with higher values indicating greater accuracy. The Delong Delong Clarke-Pearson method was used to compare areas under the ROC curve.

2.5. Biomarker combinations

Algorithm development investigated the discriminative power of 11 biomarkers in the following manner: 2- and 3-biomarker combinations were examined with 9 mathematical models of classification and 4 optimization methods (linear model - linear value, linear model - log value, non-linear model - linear value, non-linear model - log value). Either the original values or the log values of the biomarkers were treated as independent variables of a predictive model and the output of the model was a numerical score indicating the severity of OSA.