

ARIC MS#S1047

SHHS Manuscript / Abstract Proposal

1. a. Full Title:

The Association of Sleep-Disordered Breathing with vasoconstrictive and procoagulant state.

b. Abbreviated Title:

OSA, Coagulability and Vasoconstriction

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Co-authors:

3. Timeline: Analyses can be initiated immediately

4. Analysis type: Local paper (data limited to one study site)

5. Rationale

Obstructive sleep apnea (OSA) is a widely prevalent disorder characterized by episodes of partial or complete upper airway obstruction during sleep. Emerging data point towards OSA as a risk factor for cardiovascular events. However, the mechanisms by which OSA may play a pathogenetic role in cardiovascular disorders are yet to be fully elucidated. One possible mechanism may involve OSA causing vascular endothelial dysfunction, which may lead to vasoconstriction, vascular smooth muscle proliferation, hypercoagulability, thrombosis and eventually, adverse cardiovascular events.

The normal endothelium is considered a genetically stable, "quiescent" cell line. Once "activated" by disease states, the endothelial cells may express specific markers and proteins, such as E-selectin and intercellular adhesion molecule (ICAM)-1.¹ Plasma levels of Von Willebrand Factor (vWF) and P-selectin, glycoproteins synthesized by endothelium and megakaryocytes, also increase with endothelial injury and suggest the extent of endothelial damage as well as predict future cardiovascular events and mortality ^{2,3}. Thrombin or histamine stimulation selectively induces endothelial cell P-selectin ⁴. Activated endothelial cells synthesize platelet activating factor (PAF), which acts with P-selectin in the adhesion of platelets and neutrophils to endothelium, promoting thrombosis. Endothelium releases tissue type plasminogen activator (tPA) that converts plasminogen to plasmin resulting in fibrinolysis. Plasminogen activator inhibitor type 1 (PAI-1) is constitutively produced by endothelial cells and inhibits the action of tPA ⁴. This dynamic aspect of endothelial function and fibrinolytic balance may be directly relevant to the pathogenesis of atherothrombosis.

Small studies have endeavored to assess the endothelial function in patients with OSA. Such assessment has included evaluation of vasomotor responses to endothelium-dependent vasodilators (such as acetylcholine and sodium nitroprusside) and determination of levels of diverse vasoactive, inflammatory and hemostatic mediators as well as biochemical markers that characterize the interaction of the endothelium with platelets

Plasma viscosity ⁵ and spontaneous platelet aggregation ⁶ are higher in patients with OSA and revert to normal with nCPAP therapy. One study found faster clotting times in untreated OSA patients compared to OSA patients on chronic nCPAP therapy ⁷. A modest correlation exists between fibrinogen and RDI in patients with recent stroke and OSA ($r=0.32$) ⁸. A small, non-controlled study involving patients with OSA reported higher morning values of fibrinogen after sleep than the evening prior, suggesting an overnight increase in fibrinogen associated with untreated OSA ⁹. In contrast, another study reported no effect of OSA on thrombin/antithrombin III complex (TAT), fibrin D-dimer, and von Willebrand factor antigen (vWF) ¹⁰. However, the non-apneic group in this study included patients with AHI 5-15 (mild OSA). Rangemark et al found higher levels of plasminogen activator inhibitor (PAI-1), suggesting impaired fibrinolytic activity, in patients with OSA ¹¹. However, the number of subjects in this study was small, controls did not have any sleep investigation, and no relation between the values and OSA severity was reported. Presence or absence of blood glucose abnormalities and hypertriglyceridemia, factors which may affect PAI-1 levels were also not reported.

Patients with OSA have higher systemic levels of the potent vasoconstrictor endothelin-1 than their healthy counterparts ¹². Moderate to severe OSA is associated with lower circulating levels of nitric oxide derivatives and less endothelium-dependent flow-mediated vascular dilatation ¹³⁻¹⁵. Renin-angiotensin system causes vasoconstriction, endothelial damage and cell growth, especially *via* AT1 angiotensin receptor. Angiotensin-converting enzyme (ACE) inhibitors and Angiotensin II antagonists improve endothelial function in patients with hypertension ¹⁶. Activation of renin-angiotensin by recurrent hypoxia may lead to hypertension in OSA patients. Indeed, Fletcher et al demonstrated an increase in mean arterial pressure in rats exposed to intermittent hypoxia akin to that seen in OSA, and attenuation of this response by using an AT1 receptor inhibitor ¹⁷. Higher plasma levels of aldosterone and angiotensin II also have been reported in patients with OSA ¹⁸.

The Sleep Heart Health Study database constitutes a valuable, ethnically diverse and comprehensive resource to study the above parameters so as to elucidate any abnormalities in endothelial function in patients with OSA. Offspring and Omni Cohorts of Framingham Heart Study (FHS) cohort provide indices of sleep disordered breathing as well as levels of diverse coagulation factors and vasoactive mediators in the subjects. The Cardiovascular Health Study (CHS) and Strong Heart Study (SHS) provide data regarding sleep disordered breathing

and levels of coagulation factors in their SHHS enrolled subjects. The analysis of this data can yield important information about the association between presence and severity of OSA and endothelial dysfunction (indicated by a prothrombotic and vasoconstrictive state). Many of the currently available studies lack one or more of the following: adequate size, controls, or the assessment of effect of OSA *severity* on hemostatic or vasoactive mediators ¹⁹. Our proposal aims to address all these issues so as to reach a more reliable conclusion regarding the association of OSA with endothelial dysfunction.

6. Hypothesis

We hypothesize that the levels of vasoconstrictive mediators and procoagulant factors are increased and anticoagulant/fibrinolytic and vasodilator activity decreased in patients with OSA proportionate to the disease severity

7. Data [variables, time window, source, inclusions/exclusions]:

Design: The proposed research will be a retrospective cross-sectional analysis of available data.

Subjects: We will utilize data from 1000 members of the Sleep Heart Health Study (SHHS) who participated in Framingham Offspring exam 7 and Omni exam 2 as well as 1950 subjects derived from Cardiovascular Health Study (CHS, 1,350 participants) and Strong Heart Study (SHS, 600 participants) cohorts for study of the levels of coagulant mediators in relation to severity of sleep disordered breathing. However, only the 1000 subjects from FHS will be studied to assess the levels of vasoactive mediators.

Inclusion Criteria: All subjects on whom complete and technically adequate data are available for aforementioned sleep as well as blood variables will be factored in the analysis.

Exclusion criteria: Use of warfarin, heparin products, decompensated heart failure, significant mitral or aortic valvular heart disease, pulmonary hypertension, recurrent thromboembolic disease or hepatic, renal or hematologic disease.

The **dependent variables** will be a) Levels of *vasoactive mediators* (renin, aldosterone, endothelin-1 and brain natriuretic peptide levels) and b) *thrombotic/fibrinolytic mediators* (von Willebrand factor, plasminogen activator inhibitor-1, P-selectin, soluble-intercellular adhesion molecule-1 (sICAM-1), Plasmin/Antiplasmin Complex, Factor VIIc, factor VIIIc, fibrinogen and D-dimer).

Analysis

We will compare demographic characteristics, cardiovascular risk factors, and cardiovascular disease prevalence among subjects with and without OSA using the *t* test for means and χ^2 test for proportions.

MANCOVA will be used for between-groups (4 groups of OSA severity: AHI≤5, AHI 6-15, AHI 16-30 and AHI>30) comparisons for dependent variables that are

vasoactive mediators ((renin, aldosterone, endothelin-1 and brain natriuretic peptide levels) or thrombotic/fibrinolytic mediators (von Willebrand factor, plasminogen activator inhibitor-1, P-selectin, soluble-intercellular adhesion molecule-1 (sICAM-1), Plasmin/Antiplasmin Complex, Factor VIIc, factor VIIIc, fibrinogen and D-dimer). Age, BP, blood sugar and triglycerides will be used as covariates (since these factors have been shown to affect the levels of variables being studied in earlier trials). We will also determine the relative strength of association of the biomarkers with OSA using correlation.

The relationship between the SDB variables and fibrinogen and endothelin-1 will also be analyzed using multiple linear regression. Due to the expected skewed distribution of SDB measures and to prevent undue influence of observations with extreme values, these variables will be log-transformed (natural log [x+0.1]). Significance will be assessed at $P < 0.05$.

Logistic regression will be used to calculate the odds ratio (OR) of elevated levels of fibrinogen, elevated levels of endothelin-1, and decreased levels of brain natriuretic peptide comparing SDB categories, while adjusting for possible confounders.

Since the presence of Coronary artery disease (CAD) may influence various dependent variables, we will also perform analyses excluding subjects with prevalent CVD to see if the relations are driven by subjects with prevalent disease.

All statistical analyses will be conducted using SPSS software v.10 (SPSS Inc., Chicago, IL).

8. References

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