Study Protocol

Official Title: Poor Sleep and Inflammation in HIV-Infected Adults (SASH)

ClinicalTrials.gov ID (NCT number): NCT03848325

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Scientific Background

Background:

Although antiretroviral therapy (ART) has significantly increased the life expectancy of HIV-infected individuals, HIV+ individuals are still at an increased risk of non-AIDS associated chronic diseases such as cardiovascular and pulmonary disease. This increased risk has been associated with persistent immune activation and increased levels of systemic inflammation. Indeed, people living with HIV (PLWH) with higher levels of inflammatory biomarkers have higher hazards of long-term mortality. Furthermore, this chronic inflammation and immune activation has been associated with premature cardiovascular disease and obstructive pulmonary disease. Even with virologic suppression and complete immune reconstitution on ART, PLWH have greater levels of immune activation and persistent inflammation compared to the HIV- population. Poor sleep, a common comorbidity observed among PLWH, may worsen the burden of long-term cardiovascular and pulmonary morbidity since it is also associated with both inflammation and inflammation-related long-term morbidity among HIV- individuals.

We propose a novel mechanistic framework by which acute, sub-acute and chronic sleep disruption may synergistically interact with HIV infection to increase systemic inflammation leading to an increased risk of cardiopulmonary dysfunction. Both, poor sleep acting via reduced expression of adenosine receptors and HIV infection with reduced frequencies of T cells expressing the rate-limiting ectonucleotidase CD73 can lead to reduced adenosine signaling. This loss of the normal immunosuppressive actions of adenosine results in an unregulated inflammatory output predisposing to the long-term cardiovascular and pulmonary complications associated with HIV. The disrupted adenosine signaling may also feedback to perpetuate chronic sleep disruption.

Significance:

Given the high prevalence of poor sleep among PLWH and the growing understanding of the adverse consequences of poor sleep, obtaining a better biological understanding of how poor sleep may interact with HIV infection not only will provide the rationale to develop public health interventions to improve sleep in this high-risk population, but also will inform the identification of novel interventions that might minimize the adverse effects of poor sleep and improve non-infectious outcomes in PLWH. Drugs that modulate adenosine signaling (such as dipyridamole which increases extracellular adenosine levels) are already clinically available and could be readily utilized to improve outcomes if our hypotheses are proven accurate.

This proposal is highly innovative in being the first to test the impact of poor sleep on adenosine signaling in lymphocytes and monocytes, a pathway already established to be impaired in the setting of HIV infection and hypothesized to contribute to the persistent inflammation and long-term cardiopulmonary morbidity observed in PLWH. Further, this proposal will be the first to directly test the hypothesis that poor sleep and HIV infection have a synergistic impact on inflammation through the adenosine pathway.

From a methodologic standpoint, this will be one of the first large-scale assessments of sleep in an HIV+ population to use objective assessments of sleep. In addition, we will use a multi-dimensional framework to comprehensively assess sleep. Finally, we will separately assess both long-term effects of chronically poor sleep as well as short-term effects of acute and sub-acute

sleep loss, providing a comprehensive understanding of the interaction between sleep, inflammation, and adenosine signaling that can lead to the development of intervention strategies to decrease the levels of inflammation and subsequently the risk for cardiopulmonary comorbidities in PLWH.

Study Objectives

In this proposal we seek to test the hypothesis that one mechanism by which poor sleep may impact inflammation and cardiopulmonary risk in people living with HIV is via effects on peripheral adenosine signaling. We will do this by performing a cross-sectional evaluation to test Hypotheses 1-2 and an interventional study to perform a within-subject analysis to test Hypothesis 3.

Hypothesis 1: Objectively measured poor sleep is associated with greater levels of inflammation and markers of cardiopulmonary disease among antiretroviral therapy (ART)-treated people living with HIV (PLWH) compared to those without HIV.

Hypothesis 2: Among ART-treated PLWH, downregulation of the adenosine pathway is associated with objectively measured markers of poor sleep.

Hypothesis 3: Acute sleep deprivation in ART-treated PLWH does not produce a compensatory increase in extracellular adenosine generation and signaling, leading to increased inflammation and endothelial dysfunction.

Study Design & Methods

Research Activities:

Recruitment

Individuals with and without HIV will be recruited through advertising in local longitudinal research cohorts studying PLWH, by advertising through clinics that care for PLWH and patients at high risk for developing HIV, and through platforms developed for recruiting people in the community interested in participating in research. Telephone screening will be performed to assess eligibility and review the study protocol. Individuals who meet eligibility criteria and are interested in participating will be scheduled for an in-person study visit.

Cross-Sectional Protocol

Visit 1

A point-of-care urine pregnancy test will be given to women of child-bearing potential. If the pregnancy test is positive, the participant will be ineligible for the study and procedures will be discontinued. The participant will then be asked to complete a battery of interviewer-administered questionnaires. This battery consists of questions on demographics, education, work schedule, caffeine use, nicotine use, and the reduced Morningness Eveningness Questionnaire (rMEQ) to assess circadian preference. Participants will have a wrist actigraph (Respironics Spectrum) placed on the non-dominant wrist and instructed to wear for two weeks. They will be given instructions

on pressing the event monitor whenever getting into or out of bed and also asked to complete a sleep diary each morning on awakening. Participants will also be given a wrist device for home sleep apnea testing (Itamar Medical WatchPAT) to place on their dominant wrist to wear for one night while they are sleeping. They will be given instructions for activating and wearing the device as well as proper placement of the fingertip sensor cap. Participants will be asked to return the WatchPAT device at Visit 2 where the data will be downloaded and reviewed. One week after the visit, a staff member will call participants to remind them to continue wearing the actigraph and completing the sleep diary.

Visit 2

Visit 2 will take place as soon as possible at least two weeks after Visit 1. Participants will be instructed to continue to wear the actigraph until this visit and to bring the completed sleep diaries with them to this visit. Actigraphy data will be downloaded and reviewed as will the sleep diaries. If data are incomplete (< one week of complete data), participants will be asked to repeat sleep diary and actigraphy testing. They will be issued a new actigraph and diaries to wear for an additional two weeks and be rescheduled to complete Visit 2 activities described below. Urine will be collected and frozen for adenosine analysis. Phlebotomy will be performed by research staff to obtain 100cc of blood. One tube will be prepared with a solution that specifically blocks adenosine metabolism and the rest will be standard Na-EDTA tubes. A battery of interviewer-administered questionnaires will be administered including: the Pittsburgh Sleep Quality Index, Epworth Sleepiness Scale, Insomnia Severity Index, Munich Chronotype Questionnaire, PROMIS 8a sleep disturbance scale, PROMIS 8b sleep-related impairment scale, eight-item Patient Health Questionnaire, seven-item Generalized Anxiety Disorder screener, Alcohol Use Disorders Identification Test, and the ten-item Drug Abuse Screen Test. At the end of Visit 2, staff will ask permission from HIV+ participants to be contacted at a later date to present the interventional study and discuss possible enrollment.

Interventional Protocol

Upon detailed analysis of actigraphy data from the cross-sectional study, HIV+ participants who may be eligible for the interventional study will be contacted by telephone. Staff will review the protocol with the participant, and if they are interested, screen them to confirm that they still meet inclusion criteria. They will also be screened for any changes in their medical history or medications since they participated in the cross-sectional study as these changes might make them ineligible based on exclusion criteria for Aim 3.

Visit 3

At Visit 3, eligibility for the interventional protocol will be evaluated by administering a pregnancy test to women of childbearing potential followed by administration of number of questionnaires. Participants will be asked about caffeine and nicotine use as well as the Mood Disorder Questionnaire and Ask Suicide-Screening Questions to identify those at elevated risk for adverse effects from sleep deprivation (bipolar disorder episode and suicide risk, respectively). The PSQI (used in Visit 2) will be repeated to help confirm that the participant has had a regular sleep schedule in the month prior to the study. A medical history and physical examination (including an evaluation of drug/alcohol use) will be completed by a study physician. The protocol will be reviewed and informed consent obtained. The participant will then be given a wrist

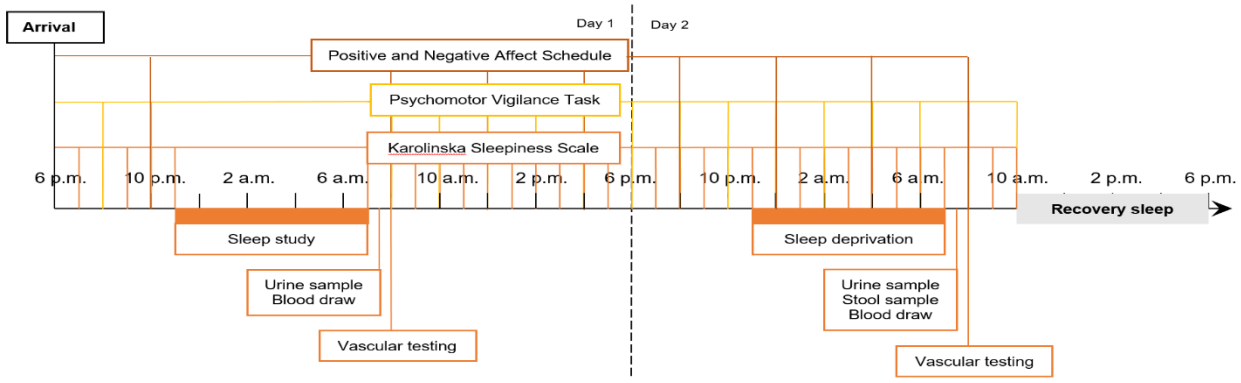
actigraph, sleep diaries, and asked to maintain a regular sleep schedule (both regular bedtime and regular duration of at least seven hours of sleep per night) until Visit 4.

Visit 4

Participants will be asked to maintain their normal sleep patterns during the week prior to this visit. They will arrive in the sleep lab at 6 p.m., and the actigraph will be removed and data reviewed to confirm the participant maintained a regular sleep schedule. In addition, the sleep midpoint will be calculated from the actigraphy data to define the participant's normal 8-hour period of sleep (lights-off and lights-on) and determine the timing of all study procedures in relation to it. In other words, those with a sleep midpoint of 3 a.m. lights-out for the sleep study will be 11 p.m. and lights-on will be 7 a.m. For those with a sleep midpoint of 2 a.m., lights-out would be 10 p.m. with lights-on at 6 a.m., and for those with a sleep midpoint of 4 a.m., it would be 12 a.m. and 8 a.m. The single-item Karolinska Sleepiness Scale (KSS) and the 10-minute Psychomotor Vigilance Task (PVT) will be administered prior to sleep. The participant will then be prepared for full polysomnography with application of EEG, EOG, ECG, chin EMG, and pretibial EMG electrodes. A nasal cannula will measure nasal airflow, a thermistor will measure body temperature and a pulse oximeter will be placed on the finger to measure oxygen. Respiratory effort belts will be placed around the chest and the abdomen. Four hours after the participant's calculated sleep midpoint, the lights will be turned on and the participant awoken. The KSS and the PVT will again be administered. After this, the participant will be sent home. Individuals found to have sleep-disordered breathing (AHI>15 events/hr) on overnight polysomnography will be excluded from further testing.

Visit 5

The timing of all procedures of Visit 5 is relative to the participant's sleep midpoint. The descriptions in the Figure below are based on a hypothetical midpoint of 3 a.m. and will be adjusted accordingly to maintain the same amount of time between each procedure and the participant's actual sleep midpoint. For those participants who are eligible to complete this protocol, they will need to abstain use of caffeine, alcohol, nicotine or any illicit drugs for the entirety of their two-day visit.



Day 1

Participants will arrive to the sleep lab by 6 p.m. The KSS is administered upon arrival and then every hour until lights-out, which will occur four hours before the participant's sleep midpoint. The PVT is administered upon arrival and then every two hours until lights-out. The Positive and Negative Affect Schedule (PANAS) is administered upon arrival and then every four hours until lights-out. Overnight polysomnography with a limited montage using only EEG, EOG, and chin EMG will be performed in order to assess sleep/wake state and sleep staging. Lights-on will be four hours after sleep midpoint. Upon awakening in the morning, participants will provide a urine sample and have a 100cc blood sample drawn. The KSS, PVT, and PANAS will again be administered every hour, every two hours, and every four hours respectively, beginning one hour after lights-on. Vascular ultrasound for brachial artery reactivity will be performed as described below. In addition to abstaining from caffeine, alcohol, nicotine, and illicit substances for the entire visit, participants will need to fast (except water) from the time of lights out through the end of vascular testing. Morning antihypertensive doses will be delayed until after vascular testing is completed. Breakfast will be served after this vascular testing. Participants will stay onsite all day, and staff will remain with them at all times (except bathroom breaks) to ensure their safety and to make sure that they stay awake with no daytime naps.

Vascular Testing

After 10 minutes of lying quietly, brachial artery diameter and flow velocities will be measured with high-resolution ultrasound equipped with an 11-L linear transducer. Image acquisition will be ECG-gated, so all measurements are done at end diastole. The brachial artery will be identified in the longitudinal plane above the antecubital fossa and a segment with clear anterior and posterior intimal interfaces between the lumen and vessel wall will be selected for continuous 2D-grayscale imaging. After obtaining baseline images, blood pressure cuff on the arm will be inflated to 50 mm Hg above resting systolic blood pressure (up to 300 mm Hg) for five minutes. After deflation, images will be obtained continuously and the maximal diameter between 30 seconds and two minutes used for calculating change. Endothelial function will be quantified as the ratio of the maximum diameter identified following cuff release to baseline diameter.

Day 2

Participants will stay awake for the remainder of procedures. Study staff will remain with the participants at all times (except for during bathroom trips) during sleep deprivation to monitor mood and to keep them awake through conversations, playing board games, and watching television. The KSS, PVT, and PANAS will be administered every hour, every two hours, and every four hours, respectively. The participants will again be prepared with limited montage polysomnography equipment described above and will need to remain fasting (except water) starting four hours prior to sleep midpoint until vascular testing is completed (the same time frame as the prior day). In the morning, the participant will provide a urine sample and have 100cc of blood drawn at the same time as the previous day. Vascular testing will be performed as described above, after which breakfast will be served. The KSS, PVT, and PANAS will continue to be administered until seven hours after the participant's normal sleep midpoint time. At this point the participant will be provided the opportunity to have eight hours of recovery sleep, and staff will encourage them to do so for their safety before returning home. They will be allowed to leave without recovery sleep if another adult is able to drive them home.

Eligibility Criteria

Aims 1-2
Inclusion- HIV+
- 18-75 years old
- On contiguous anti-retroviral therapy (ART) regimen for at least 48 weeks
- CD4+ cell count > 200 cells/mm3
Inclusion- HIV- (Aim 1 only)
- 18-75 years old
*Note: HIV- participants are only eligible for participation in Aim 1.
Exclusion- all
- Presence of a significant sleep disorder (restless legs syndrome, narcolepsy, sleep disordered breathing on active treatment)
- Presence of an active chronic inflammatory or autoimmune disease (e.g., scleroderma, Crohn's disease)
- Regular use of medications which would impact assessment of inflammatory markers (systemic glucocorticoids, interleukins, anti-TNF drugs)
- Use of medications impacting adenosine function (theophylline or dipyridamole)
- Any initiation of an investigational/experimental therapy in the prior 30 days
- Current pregnancy

Aim 3
Inclusion
- HIV+
- Completion of Aim 1 with at least 5 days of valid actigraphy data
- On continuous anti-retroviral therapy (ART) regimen for at least 48 weeks
- CD4+ cell count > 200 cells/mm3
Exclusion
- Atypical sleep patterns (mean sleep duration < 6 hours, sleep efficiency < 85%, standard deviation of sleep duration > 60 minutes, sleep midpoint < 2 a.m., sleep midpoint > 5 a.m.)
- Elevated risk of adverse health effects from sleep deprivation (bipolar disorder, epilepsy, history of suicidal ideation in the past 6 months)
- Heavy caffeine use (>3 caffeinated beverages per day)
- Alcohol abuse
- Drug abuse
- Daily use of medications for treatment of insomnia
- Sleep disordered breathing (AHI >15 on in-lab sleep study)
- Current pregnancy

Statistical Analysis Plan

Hypothesis 1:

The primary exposures will be four domains of sleep health: sleep duration (mean hrs/night during the main rest period), sleep fragmentation index (mean across main rest periods), sleep regularity (interdaily stability over a 14-consecutive day period), and sleep apnea severity (pAHI). Secondary measures will be mean sleep midpoint, mean diary-reported sleep quality, and

proportion of days with naps. The primary measures of inflammation will be serum IL-6 and sCD14 levels. Secondary measures will include the other 12 cytokines both inflammatory and anti-inflammatory on the Luminex platform including TNFα, CRP, IL-10, and sCD163. All inflammatory biomarkers will be transformed as needed to approximate a normal distribution. Given that some sleep exposures (particularly sleep duration) have U-shaped associations with cardiovascular outcomes, we will initially model sleep exposures in quintiles to assess for non-linear relationships with inflammation measures. If a linear association is confirmed, we will then model sleep exposures continuously to maximize power and use multivariable linear regression to regress IL-6 and sCD14 levels on each of the three sleep exposures among HIV+ subjects. Initial analyses will adjust for age and sex. Alternatively, we will use fractional polynomial regression to test the nonlinear relationship between sleep measures and inflammatory markers. In all models, we will further adjust for CD4+ T cell count and viral load to determine if sleep exposures are associated with inflammation in HIV+ subjects independent of severity of infection. Subsequently, we will generate models including all four sleep exposures simultaneously to understand the independent effects of each sleep domain. If needed, we will apply dimension reduction methods (e.g., principal components analysis) to overcome collinearity in sleep domains. Secondary analyses will utilize structural equation modelling to evaluate the extent to which identified associations represent effects mediated by sleep directly versus effects specific to depression. Furthermore, we will stratify by sex to separately evaluate the association between sleep and inflammation in men and women. Formal testing of effect modification will be performed by including an interaction term in the multivariable models. We will repeat models in HIV- subjects and then test whether HIV infection moderates the association between sleep and inflammation by modeling data from those with and without HIV infection in one model with a sleep by HIV interaction term, using a two-sided $p<0.05$ as the criterion for rejecting the null hypothesis of no effect modification.

Hypothesis 2:

We will compare the frequencies of immune cells expressing ectonucleotiinterdases (CD39+ T cells, CD73+ T cells, and CD39+ CD73+ T cells), monocyte adenosine receptor expression (A1R and A2AR), ADO and inosine levels, and measures of T cell activation between those HIV+ subjects with healthy and poor sleep. Ectonucleotidase expression on B cells and monocytes, additional purine pathway metabolites, and frequencies of CD14+ monocytes expressing IL-6 and/or TNFα will also be compared in secondary analyses. Analyses will initially use t-tests for normally distributed variables or Mann Whitney tests for non-normal distributions. Subsequent analyses will consider potential confounders (age, sex, CD4+ T cell count, viral load, habitual caffeine use, chronotype, sleep midpoint) and adjust in multivariable linear regression for the set of variables that differ between the healthy and poor sleep groups at $p<0.10$. Sex-related differences will be tested by evaluating the statistical significance of a sex * sleep interaction term.

Hypothesis 3:

The primary outcome measures are levels of soluble CD14, soluble CD163, and circulating IL-6 levels. Secondary outcome measures are flow mediated dilation (percent change in brachial artery diameter to reactive hyperemia), monocyte expression of IL-6, monocyte expression of TNFα, frequency of CD4+ T cells expressing HLA-DR and CD38, and frequency of CD8+ T cells expressing HLA-DR and CD38. Additional pre-specified outcomes include plasma adenosine and inosine levels, urinary 3'5'-cAMP, and frequencies of CD4+ and CD8+ T cells expressing CD39

and CD73. The impact of sleep deprivation will be assessed by performing paired t-tests of each of these outcomes comparing baseline to post-sleep deprivation values. For variables with non-normal distributions, we will either transform variables or use Wilcoxon signed-rank tests. The correlation between the changes in each domain (expressed as percent change from baseline) will be calculated to assess the extent to which change in endothelial function may be predicted by change in inflammation or ADO signaling and change in inflammation by change in ADO signaling. Secondary analyses will consider the extent to which sex, CD4+ T cell count, and viral load impact sensitivity to sleep deprivation by regressing each of these potential effect modifiers on the percent change in each of the primary outcome measures for inflammation, endothelial function, and ADO signaling.