Cancer incidence, mortality, and immunotherapy outcomes in relation to sleep problems: Results from Cardiovascular Health Study and a cancer immunotherapy cohort

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Abstract

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Background: Sleep problems (SP) affect a large proportion of adults: an estimated 50-70 million U.S. adults suffer from sleep problems. Among numerous potential health consequences, sleep problems may be adversely associated with cancer risk and cancer mortality. There has been evidence from human studies and numerous animal studies linking sleep problems to cancer development and proliferation in recent years. The mechanisms underlying such cancer-related effects likely reflect the adverse downstream consequences of disruptions to the 24-hour circadian rhythm or physiologic insults of sleep disorders such as sleep apnea. In light of the high prevalence of sleep problems in the population, the potential connection of these problems to cancer occurrence and mortality is a pressing public health concern.

Beyond its impact on cancer risk, sleep disturbances are a prominent concern of cancer patients, with up to 80% reporting disturbed sleep. One particular concern in this patient population is the fact that disturbed and insufficient sleep adversely affects immune health. The presence of a strong T-cell response in cancer, indicating activation of the adaptive immune system, has been consistently associated with better patient outcomes. Existing and emerging immunotherapies are attempting to harness the T-cell response to treat several forms of cancer by targeting immune-suppressive proteins, such as programmed cell death-1 receptor (PD-1), its

ligand PD-L, and cytotoxic T lymphocyte antigen 4 (CTLA-4) using immune checkpoint inhibitors (ICIs). Accordingly, disruptions to the circadian rhythm could plausibly have implications for the effect of these therapies.

Therefore, we sought to quantify the associations of SP with cancer incidence, mortality, aggressiveness, and ICI outcomes in the context of the longitudinal Cardiovascular Health Study (CHS) and a pilot study of cancer patients initiating ICIs (The Lifestyle Attributes and Sleep in Immunotherapy Response (LASIR) Study).

Methods: We assessed the association of self-reported SP with incident cancer (N=3930, excluding prevalent cancers) and cancer mortality (N=4580) among Cardiovascular Health Study (CHS) participants, a population-based study of adults aged >=65 years recruited from four US communities. Participants reported sleep apnea symptoms (SAS) and insomnia symptoms at each visit between 1989–1994. Cancer incidence was ascertained through linkage with state cancer registries through 2005; cancer-specific death was adjudicated through 2015. We used Cox proportional hazards regression to calculate hazard ratios (HR) and 95% confidence intervals (CI) for associations of baseline and longitudinal SP with subsequent cancer incidence and cancer mortality, adjusting for a priori selected confounders, including gender, study phase, age, smoking, body mass index, diabetes, physical activity levels, and alcohol consumption.

For the LASIR cohort, 33 participants consented to the study, of whom 32 initiated an ICI treatment. We collected questionnaire data on primary SP (sleep apnea risk) and secondary SP, including insomnia and general sleep patterns. We extracted patient attributes on the date corresponding to the patient's last visit at SCCA prior to or at ICI initiation from the Electronic health records (EHR) six months post-ICI initiation. We also extracted information on their cancer attributes at diagnosis, prior cancer treatments information, vital status, the type, dates,

number of ICI infusions, and response assessment indicators. Our primary analyses included the associations of SP with tumor aggressiveness at diagnosis and ICI tolerance, indicating a likely favorable response to treatment. Tumor aggressiveness was defined by M-stage (M0 vs. M1). Six or more infusions was considered ICI tolerability. We ran a Poisson regression with robust standard errors to assess the association between SP and tumor aggressiveness, adjusting for age, gender, body mass index, and reported prevalence ratio (PR) and associated 95% CI for the associations. We used logistic regression models to assess the association between sleep apnea and insomnia risk with the number of ICI infusions received, adjusting for male gender, age at ICI, and prior cancer treatment. We calculated the odds ratio (OR) and 95% confidence intervals (CI) for associations with this outcome.

Results: The mean age of the CHS study population was 73 years, 57% were female, and 83% were white. Overall, 885 first incident cancers and 804 cancer deaths were identified over a median follow-up of 12 and 14 years, respectively. Briefly, compared to participants who reported no SAS, the risk of incident cancer was inversely associated [(HR(95%CI)] with snoring [baseline: 0.84 (0.71, 0.99), time-dependent: 0.76 (0.65, 0.89)]. We noted an elevated cancer incidence for prostate cancer for time-dependent analyses of apnea [2.34 (1.32, 4.15)], baseline snoring [1.69 (1.11, 2.57)] and cumulative average snoring [2.17(1.22, 3.86)]. We found a significantly elevated HR for lymphatic or hematopoietic cancers [baseline snoring: 1.81 (1.06, 3.08)]. We also noted an inverse relationship for cancer mortality with respect to snoring [time-dependent: 0.73 (0.62, 0.86); cumulative average: (0.67 (0.50, 0.90)) and baseline apnea (0.69 (0.51, 0.94)]. We found a significant inverse relationship between difficulty falling asleep and colorectal cancer death [baseline: 0.32 (0.15, 0.69), time dependent: 0.41 (0.17, 0.98) and

cumulative average: 0.28 (0.09, 0.84)] and baseline snoring with lung cancer death [0.56 (0.35, 0.89)].

The mean age of the LASIR cohort was 61 years, 61% were male, 85% were white, 64% were partnered, 70% had a college degree, 79% had a BMI of at least 25 kg/m^2, 3% were current smokers, 18% reported sufficient weekly physical activity (150 min/week at moderate equivalent) and 18% high stress. The prevalence of low, intermediate, and high-risk OSA risk was 36%, 42%, and 21%, respectively. Of the secondary SP considered, 58% of participants reported clinically significant insomnia, 72% experienced an average or restless night sleep, 30% reported taking longer than 15 min to fall asleep, 46% had ideal night sleep, and 36% reported an evening chronotype. We did not find a significant association [(PR(95%CI)] between intermediate or high-risk OSA and metastatic cancer compared to low-risk OSA [1.01 (0.28, 3.67)] Of the secondary sleep attributes considered, patients who reported taking more than 15 minutes to fall asleep were 3.6 times more likely to be diagnosed with metastatic cancer compared to those reporting shorter sleep latency [95% CI (1.74, 7.35)]. Additionally, patients reporting a morning chronotype were less likely to be diagnosed with metastatic cancer compared to those reporting evening chronotypes [0.23 (0.09, 0.58)]. Similarly, we did not find any significant association [(OR (95%CI)] between intermediate or high-risk OSA and six or more infusions compared to low-risk OSA [0.27 (0.02, 3.41)]. Similarly, we found no significant association between insomnia and six or more infusions [0.23 (0.03, 1.60)].

Conclusion: Despite the mostly null results in the CHS study, there were a few notable elevated and inverse relationships between SP and cancer-site specific incidence and cancer mortality. The results add to the growing evidence suggesting the physiologic effect of sleep problems on cancer is heterogeneous across cancer sites. Therefore, future larger community based

prospective studies addressing more cancer site and molecular type-specific associations and improved SP self-report documentation over time are needed.

Additionally, the immunotherapy cohort gives insights into the potential burden and impact of SP on tumor aggressiveness and ICI treatment response. The results could inform larger-scale observational studies with an ultimate goal of informing clinical trials focused on finding effective sleep quality improvement interventions in ICI cancer treatment populations.

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Dedication

To my parents Marie Pamela Sillah and George Oldman Sillah

Chapter 1: Introduction

Sleep problems (SP) affect a large proportion of adults: an estimated 50-70 million U.S. adults suffer from chronic sleep and wakefulness disorders[1], and about 35% of adults average less than the recommended 7 hours of daily sleep.[1] Among numerous potential health consequences, sleep problems may be adversely associated with cancer risk and cancer outcomes. There has been evidence from both human studies and numerous animal studies linking SP to cancer development and aggressiveness in recent years.[2-6]

The mechanism for the effect of SP on cancer development and progression could be through disruptions in the 24-hour circadian rhythm.[5,7] This rhythm is generated endogenously in coherence with external cues, thus enabling alignment of physiological process changes in the day–night cycle.[7] Disruptions to the circadian rhythm result in increased inflammation, and reduced production of hormone melatonin which help prevent cellular damage.[5] The effects of these physiologic insults have been seen in numerous cancer cell lines, which likely contribute to aggressive tumor proliferation.[8] Taken together, there are biologically plausible reasons to expect an effect of SP induced circadian rhythm disruption on cancer pathways. In light of the high prevalence of sleep problems in the population, the potential connection of these problems to the occurrence of cancer is a pressing public health concern.

Despite strong biological plausibility and suggestive epidemiologic evidence, methodologic challenges in evaluating the relationship of sleep problems with cancer have limited epidemiologic studies on this topic. For instance, because historic sleep patterns may be difficult for study participants to accurately recall, study questionnaires typically inquire about recent sleep patterns (i.e., in the past month). Sleep data collected at a single baseline

interview may then be related to subsequent cancer incidence and mortality. However, sleep patterns are likely to change over time and with age. In failing to capture such changes, important information regarding the relationship of sleep with cancer may be lost. Likewise, wide domains of SP indicative of OSA have not been studied together with respect to cancer incidence and mortality in a cohort of older individuals. As such, it is important to consider other study designs that focus on several dimensions of SP and their impact on cancer incidence and mortality. Using longitudinally collected sleep data from the Cardiovascular Health Study (CHS), we attempted to mitigate this limitation in relating sleep problems with subsequent cancer incidence and mortality (**Chapter 2**).

The focus of **Chapter 3** on sleep in cancer patients addresses an important gap in knowledge. Sleep problems in cancer patients are common and may result as a side-effect of cancer therapy[9,10] or as a consequence of cancer-related anxiety, but may also reflect the previously established carcinogenic role of SP, [11-23] with increasing evidence demonstrating that the overall level of T cells within a tumor is favorably associated with prognosis.[24-32] As such, given the role of circadian rhythms in regulating healthy immune responses, sleep disruptions could have implications for the effectiveness of therapeutic strategies targeting the immune system in cancer patients, such as immune checkpoint inhibitors (ICIs). Therefore, regardless of their etiology, the consequences of such sleep problems in cancer patients have the potential to be far-reaching. Thus, as ICIs become increasingly common options for many cancer patients, it is critical to understand how sleep problems may relate to cancer aggressiveness and impact treatment outcomes in cancer immunotherapy patients.

Thus, our study provides a further understanding of the role of sleep problems in cancer incidence, prognosis, and immunotherapy outcomes.

Chapter 2: Cardiovascular Health Study

Title: Sleep problems and risk of cancer incidence and mortality in an older cohort: The Cardiovascular Health Study (CHS)

Abstract

Introduction: Even in the absence of a formal diagnosis, sleep problems (SP) are frequently indicative of an underlying sleep disorder, such as obstructive sleep apnea, which may be adversely associated with cancer risk and cancer mortality.

Methods: We assessed the association of self-reported SP with incident cancer (N=3930, excluding prevalent cancers) and cancer mortality (N=4580) among the participants of Cardiovascular Health Study (CHS), a population-based study of adults aged >=65 years recruited from 4 US communities. Participants reported sleep apnea symptoms (SAS) and insomnia symptoms at each visit between 1989–1994. Cancer incidence was ascertained through linkage with state cancer registries through 2005; cancer specific death was adjudicated through 2015. We used Cox proportional hazards regression to calculate hazard ratios (HR) and 95% confidence intervals (CI) for associations of baseline and longitudinal SP with subsequent cancer incidence and cancer mortality, adjusting for gender, study phase, age, smoking, body mass index, diabetes, physical activity levels, and alcohol consumption.

Results: The mean age (SD) of the study population was 73 (6) years, 57% were female, and 83% were white. Overall, 885 first incident cancers and 804 cancer deaths were identified over a median follow-up of 12 and 14 years, respectively. Briefly, compared to participants who reported no SAS, the risk of incident cancer was inversely associated [(HR(95%CI)] with snoring [baseline: 0.84 (0.71, 0.99), time-dependent: 0.76 (0.65, 0.89)]. We noted an elevated cancer incidence for prostate cancer for time-dependent analyses of apnea [2.34 (1.32, 4.15)] and baseline snoring [1.69 (1.11, 2.57)] and cumulative average snoring [2.17(1.22, 3.86)]. We also noted a significant elevated HR for lymphatic or hematopoietic cancers [baseline snoring: 1.81 (1.06, 3.08)]. We found an inverse relationship for cancer mortality with respect to snoring [time-dependent: 0.73

(0.62, 0.86); cumulative average: (0.67 (0.58, 0.97)) and baseline apnea (0.69 (0.51, 0.94))]. We noted a significant inverse relationship between difficulty falling asleep and colorectal cancer death [baseline: 0.32 (0.15, 0.69), time dependent: 0.41 (0.17, 0.98) and cumulative average: 0.28 (0.09, 0.84)] and baseline snoring with lung cancer death [0.56 (0.35, 0.89)].

Conclusions: Our study showed mixed results for the association between SP and cancer incidence and mortality. Future larger community based prospective studies addressing more cancer site and molecular type-specific associations and improved SP self-report documentation over time are needed.

Introduction

Sleep problems affect a large proportion of adults: an estimated 50-70 million U.S. adults suffer from chronic sleep and wakefulness problems [1] and about 35% of adults average less than the recommended 7-8 hours of daily sleep.[1] Even in the absence of a formal diagnosis, sleep problems (SP) are frequently indicative of an underlying sleep disorder, such as obstructive sleep apnea (OSA)[33] and insomnia.[34] Sleep problems have been linked to numerous adverse health outcomes including type 2 diabetes and hypertension,[35] cardiovascular disease,[36] mortality[37], a substantial increase in healthcare utilization[38], and motor vehicle accidents.[39] Together, these effects have substantial consequences for older adults.[40]

Evidence has emerged suggesting SP and circadian rhythm disruptions are risk factors for cancer development and progression. Animal and in vitro studies provide evidence for the role of the circadian system as a tumor suppressor through cell cycle freeze, inhibition of cellular proliferation, promotion of apoptosis, and anti-angiogenesis.[41-43]

Most epidemiologic studies of SP in relation to cancer in humans have focused on the impact of shift work, as well as sleep duration measures. Specifically, numerous empirical

findings of the adverse impact of shift work on cancer development and progression have led the International Agency for Research on Cancer (IARC) to classify shift work involving significant circadian disruptions as a probable carcinogen.[22,23] Evidence of this carcinogenic effect is strongest for breast cancer,[44] but also suggests an increased risk of incident prostate and colorectal cancers.[44] Furthermore, short sleep duration (typically <7 hours) has been linked with increased risks of cancer overall,[45,46] gastric cancer[47], and breast cancer[48] and poor overall cancer and breast cancer survival particularly in combination with snoring.[49,50] With respect to other aspects of SP, some recent studies have provided suggestive evidence for an association between OSA and cancer incidence[3,51-53] and mortality.[54,55] A recent meta-analysis of eight studies also found an overall increased risk of cancer for individuals with insomnia compared to those without insomnia and reported that risk was mostly driven by studies conducted in women.[56]

Beyond this prior work, several methodologic challenges in evaluating the relationship of sleep disturbances with cancer have limited epidemiologic studies on this topic. For instance, sleep patterns are likely to change over time and with age and wide domains of sleep disordered symptoms indicative of OSA and insomnia have not been simultaneously assessed with respect to cancer incidence and mortality in a cohort of older individuals. To advance our understanding of various components of sleep quality and sleep disorder symptoms, we assessed several dimensions of SP and their impact on cancer incidence and cancer mortality using longitudinally collected sleep data from the Cardiovascular Health Study (CHS).

Methods

Study Participants and settings

The CHS is an observational cohort study of men and women aged >=65 years recruited from a random sample of the Health Care Financing Administration Medicare eligibility list of 4 US communities: Forsyth Country, North Carolina: Sacramento County, California; Washington County, Maryland; and Pittsburgh, Pennsylvania.[57] Participants forming part of the original cohort were enrolled between 1989-1990 (94% White, 5% African Americans, 1% other ethnic group; N=5201), and an additional supplemental cohort was enrolled between 1992–1993 (predominantly African American (AA), N=687).[57] We excluded participants missing SP and covariate data at baseline (n=1308); for analyses of cancer incidence, we also excluded participants who had history of cancer at the time of recruitment (n=650). The excluded participants did not differ from the analytic sample with respect to age, sex, and racial distribution.

Sleep problems ascertainment

Longitudinal sleep data was ascertained through self-reported sleep symptoms collected at baseline (1989-90) and study exams in 1991-92, 1993-94, and 1994-95 for the original CHS cohort; for the supplemental cohort, these data were collected at baseline (1992-93) and the study exam in 1994-95. The same set of sleep questions were asked at each of the visits and have been previously validated with polysomnography (PSG) data from a subset of participants enrolled in the Sleep Heart Health Study (n= 1240)**.** [58,59] Participants reported the following sleep apnea symptoms (SAS):

i) Are you usually sleepy in the daytime? (Daytime sleepiness)

ii) Has your spouse/roommate complained about your loud snoring? (Snoring)

iii) Has anyone observed you while sleeping to have episodes where you stop breathing for a while & then snore? (Observed apnea)

In addition to daytime sleepiness, participants also reported the following insomnia symptoms (IS):

i) Do you usually have trouble falling asleep? (Problem falling asleep)

ii) Do you usually wake up several times at night? (Problem maintaining sleep)

iii) Do you usually wake up far too early? (Early morning wakefulness)

Other variables

We included demographic attributes [i.e., sex, age, race (white, black other), ethnicity (Hispanic, non-Hispanic) marital status (partnered, unpartnered), and the number of years of education completed)] and behavioral factors (i.e., smoking (current, former and never), weekly alcohol consumption, and physical activity), collected by questionnaires at baseline and follow-up visits. Alcohol consumption per week was computed from the reported usual frequency of consumption of beer, wine, and liquor, and the usual number of drinks consumed on each occasion [57] and categorized into <1, 1-6, 7-13, and 14+ alcohol beverages per week. Physical activity (in kilocalories per week) was collected using a modified Minnesota Leisure-Time Activities questionnaire[57,60] and categorized into quartiles. Body mass index (BMI) was calculated by dividing study staff-measured weight in kilograms by height in meters squared and grouped into standard categories (< 25 kg/m^2, 25-29 kg/m^2, ≥ 30 kg/m^2). Participants were also asked about their disease history (diabetes, hypertension, and coronary heart disease) and verified from hospital and physician records, as previously described.[59,61]

Cancer incidence ascertainment

Cancer incidence (overall and site-specific) was ascertained through 5 population-based cancer registries serving the 4 CHS regions; linkage to these data is complete through 2005.[62]

Cancer-specific mortality

Standardized protocols for the identification of cardiovascular events and deaths were implemented during follow-up.[57] Deaths among CHS participants have been adjudicated through 2015 by a study-wide Event Review Committee made up of physicians representing the 4 study sites.[63,64] The committee reviewed medical records, death certificates, and other information to adjudicate the underlying cause of death.

Statistical analysis

All analytical procedures were conducted using Stata 14.0 (College Station, Texas)[65], with statistical significance considered at a 2-sided alpha value of 0.05. In descriptive analyses, we examined the distribution of participants' baseline characteristics overall and stratified by the number of SAS. We calculated means (standard deviation) for normally distributed continuous variables and median (interquartile range) for non-normally distributed continuous variables and with percentages for categorical measures. We tabulated the overall and site-specific counts for incident cancers and subsequent cancer mortality along with their tumor attributes according to tumor summary stage.

We used Cox proportional hazards regression to evaluate the association between SP and cancer incidence and cancer mortality overall and for cancer sites with at least 50 incident cases and deaths. We calculated the hazard ratios (HR) and 95% confidence intervals (CI) for the associations. For the cancer incidence outcome analysis, 3930 participants free of cancer at baseline were included in the analysis. Participants contributed person-time to the analyses until

cancer incidence or at the time of the last attended visit, all-cause death, or end of follow up (31 December 2005) whichever came first. With respect to the cancer mortality outcome, 4580 participants were included regardless of their baseline cancer status. Participants contributed person-time to the analyses until cancer mortality or at the time of the last attended visit, other cause-specific death, or end of follow up (31 December 2015) whichever came first.

For both analyses, we modeled SP in several ways: 1) using baseline values only, 2) allowing SP to be time-varying by incorporating follow-up data as time-dependent, and 3) allowing SP variables to be time-varying by modeling a cumulative average of previous values of the SP variable (coded as 0,1) each year to examine chronic effects of SP. We also allowed BMI to vary for the time-varying analysis given its strong correlation with both SP and cancer outcomes.[66] Each missing value of the time-varying SP and BMI was replaced by the last observed value of that variable.

In addition to running a separate model for the SAS, we also assessed the joint effects of the baseline values coded as 0 for no sleep apnea symptoms, 1 for having any symptom, and 2 for two or three symptoms. We treated the IS variables similarly by combining symptoms based on the number of the symptoms reported.

Finally, we ran several sensitivity analyses. We censored the first 2-years and 5-years of follow-up for cancer incidence and cancer mortality analyses, respectively, to reduce the possible influence of reverse causation (i.e. undiagnosed cancer or worsening cancer contributing to SP). In addition, we calculated a modified version of the 8-item validated STOP-BANG for assessment of OSA risk with scores ranging from 0-8 and categorized into low risk (0-2), intermediate risk (3-4), and high risk (5-8).[67] The STOP-BANG uses information on whether a patient snores (S), experiences daytime tiredness (daytime sleepiness, T), breathing cessation

during sleep (observed apnea, O), has high blood pressure (P), body mass index (B) >35 kg/m^2, older than 50 years of age (A), > 40cm neck circumference (N), and male gender (G). Since neck circumference was only assessed for years 1994-95, we upweighted baseline BMI by assigning 0 for <25 kg/m^2, 1 for 25-34 and 2 for ≥ 35 kg/m^2. We also computed a STOP-BANG score for the supplementary cohort which was enrolled 1-year prior to neck circumference ascertainment. We opted to use the modified STOP-BANG (which had excellent agreement with the original STOP-BANG, kappa=0.76) to make use of most of the data in our sensitivity analysis. Furthermore, because of the strong relationship between BMI and SP and cancer outcomes, we also assessed the possibility of BMI as an effect modifier of the relationship of SP with cancer incidence and cancer mortality by presenting HR estimates stratified by BMI categories. In addition, because late stage cancer generally has poorer treatment response it is unlikely that treated late stage cancer will mitigate the effects of SP, and also since SP provides a more favorable environment for tumor growth, angiogenesis, and metastasis, it is likely that it may play more important roles in cancer progression than initiation (thus more strongly associated with the incidence of advanced cancers), we also presented cancer stage-specific estimates. Lastly, because most prior studies of SP and cancer have primarily focused on breast and prostate cancers, we presented a sex-stratified analysis.

Models were adjusted for a priori selected confounders, including gender, study phase, baseline age (continuous), smoking, BMI (continuous), diabetes, physical activity levels (continuous), and alcohol consumption (continuous). A formal test for proportional hazards assumptions was performed by evaluating the nonzero slope of the scaled Schoenfeld residuals on ranked failure times.[68]

Results

Patient characteristics

The mean age of the study population was 73 years, 57% were female, 83% were white, 70% were partnered, 20% were at least a college graduate, 60% were overweight or obese, and 12% were current smokers. The distribution was similar across the SAS groups except that, among those with 2+ SAS symptoms, 35% were female, 81% were partnered, 70% were overweight or obese. (**Table 2.1**)

Prevalence of sleep problems

The prevalence of SAS was 17% for daytime sleepiness, 9% for observed apnea, and 24% for snoring; 63% reported none of the three SAS. The prevalence of IS was 21.9% for difficulty falling asleep, 63% for problems staying asleep, and 32% for early morning awakenings; 38% reported no IS (including daytime sleepiness).

Cancer characteristics

Overall, 885 first incident cancers and 804 cancer deaths were identified over a median follow-up of 12 and 14 years, respectively. The median interval between SP data collection and cancer incidence ranged from 1.6 years for cervical cancer to 9 years for lymphatic or hematopoietic cancer; for cancer mortality, follow-up ranged from 2.9 years for cervical to almost 13 years for uterine cancer death. The most common cancer sites were prostate and lung (13%), colorectal cancer (12%), lymphatic or hematopoietic cancers (9%), and breast cancer (8%). The most common cancer sites for cancer death were lung (17%), lymph and colorectal cancer (11%), prostate (8%), pancreas (6%), urinary bladder, stomach, and breast cancer (4%). Among those diagnosed with cancer during follow-up, 41% were diagnosed with early-stage disease. Conversely, among participants for whom cancer stage information was available

(~50%) and who died during follow-up, the largest proportion were diagnosed at a late stage (18%). (**Table 2.2**)

Association between SP and cancer incidence

Table 2.3 presents the adjusted HRs and 95% CIs [reported as HR (95% CIs)] for cancer incidence in relation to baseline, time-dependent, cumulative averages, and baseline cumulative number of SAS and insomnia symptoms respectively. Compared to participants who reported no SAS, the risk of incident cancer was inversely associated with snoring [baseline: 0.84 (0.71, 0.99), time-dependent: 0.76 (0.65, 0.89)]. There were no observed significant associations with cancer incidence for daytime sleepiness and apneas. The total number of baseline SAS showed an inverse association for increasing number of symptoms compared to those reporting no SAS [any symptom: 0.81 (0.69, 0.95); 2+ symptoms 0.77 (0.61, 0.97)]. We found no evidence of association for symptoms of insomnia, whether considered individually or cumulatively.
Of the specific cancer sites evaluated, significantly elevated cancer incidence was noted for prostate cancer for time-dependent analyses of apnea [2.34 (1.32, 4.15)] and baseline snoring [1.69 (1.11, 2.57)]; the observed association with snoring was increased when modeling this exposure as cumulative average [2.17 (1.22, 3.86)]. For prostate cancer, we also found a dose-response relationship for baseline cumulative SAS symptoms compared to those reporting no symptoms [any symptom: HR=1.30 (0.84, 2.01); 2+ symptoms HR=2.22 (1.30, 3.79)]. We also noted a significantly elevated HR for lymphatic or hematopoietic cancers [baseline daytime sleepiness: 1.81 (1.06, 3.08)]; with respect to the IS symptoms, a significant inverse relationship was noted for problem staying asleep (cumulative average 0.54 (0.31, 0.92)).

Association between SP and cancer mortality

Table 2.4 presents the adjusted HRs and 95% CIs [reported as HR (95% CIs)] for cancer mortality in relation to baseline, time-dependent, cumulative averages, and baseline cumulative number of SAS and insomnia symptoms respectively. We found significant inverse relationship for cancer mortality with respect to snoring [time-dependent: 0.73 (0.62, 0.86); cumulative average: 0.67 (0.58, 0.97)) and baseline apnea (0.69 (0.51, 0.94)]. The total number of baseline SAS showed an inverse association for increasing number of symptoms compared to those reporting no SAS [any symptom: 0.90 (0.76, 1.06); 2+ symptoms 0.75 (0.58, 0.97)]. None of the IS was significantly associated with cancer mortality. We noted a significant inverse relationship between difficulty falling asleep and colorectal cancer death [baseline: 0.32 (0.15, 0.69), time dependent: 0.41 (0.17, 0.98) and cumulative average: 0.28 (0.09, 0.84)] and baseline snoring with lung cancer death (0.56 (0.35, 0.89)). We also found an inverse dose response relationship between lung cancer mortality and baseline cumulative SAS compared to those reporting no symptoms [any symptom: HR= 0.84 (0.56, 1.24); 2+ symptoms HR= 0.35 (0.16, 0.77)].

Sensitivity analysis

The overall patterns observed in the main results did not differ for cancer incidence overall and cancer mortality when we imposed 2- and 5-years outcome censoring and stratified by sex (**Sup Table 2.1**), when we stratified by BMI and cancer stage (**Sup. Table 2.2**) or when we used modified STOP-BANG measure instead of combined SAS (**Sup Table 2.3**).

Discussion

Despite the mostly null results in the present study, there were a few notable elevated and inverse relationships between SP and cancer-site specific incidence and cancer-specific mortality. We found an inverse association between cancer incidence overall and snoring, and a

null association with daytime sleepiness and apneas. In addition, we found an inverse dose response with combined SAS and no evidence of association for symptoms of insomnia. Of the site-specific cancer incidence considered, we noted an association of snoring, apnea, and a dose-response for cumulative SAS with elevated prostate cancer incidence. We also found an association between daytime sleepiness and elevated lymphatic or hematopoietic cancers incidence and an inverse relationship with problem staying asleep for these cancers. To the best of our knowledge, no other studies have noted a relationship between a SAS and increased incidence of lymphatic or hematopoietic cancers. With respect to cancer mortality, we found an inverse relationship with snoring, apnea, and combined SAS. Of the cancer sites considered for site-specific mortality, colorectal cancer and lung cancer were inversely associated difficulty falling asleep and snoring, respectively. We also found an inverse dose-response relationship between lung cancer mortality and baseline cumulative SAS compared to those reporting no symptoms.

The noted inverse and null relationships are inconsistent with the adverse effects of SAS on cancer development and progression expected based on prior evidence. Notably, numerous mechanisms for the effect of SP on cancer development and progression are possible, given the many downstream implications of disruptions in the 24-hour circadian rhythm.[5,7] Animal and *in vitro* studies provide evidence for the potential role of the circadian system in tumor suppression through cell cycle freeze, inhibition of cellular proliferation, promotion of apoptosis, and anti-angiogenesis.[41-43,69] Furthermore, misalignment in the circadian clock could result in reduced secretion of melatonin.[70,71] This could be significant given that melatonin, at sufficient physiologic levels, has been shown to impede the initiation of tumorigenesis by preventing the accumulation of DNA adducts that contribute to DNA damage and neoplastic transformation.[72,73]

Another specific aspect of SP, apnea (i.e., recurrent cessations of breathing during sleep and a symptom of OSA), has been associated with accelerated tumor progression and metastatic potential through enhanced oncogenic pathways in the presence of intermittent hypoxia.[51,74-77] Suggested mechanisms by which intermittent hypoxia could contribute to cancer include the generation of excessive reactive oxygen species (leading to oxidative stress and induced DNA damage)[78,79], overexpression of the transcriptional regulator hypoxia-inducible factor-1alpha (HIF-1α), and suppressed immune function.[80-83] Additionally, human studies have provided suggestive evidence for an association between OSA and cancer incidence[3,6,51-53,84] and cancer mortality in adults.[54,55] With respect to insomnia symptoms, a recent meta-analysis of eight studies suggested an overall increased risk of cancer incidence for individuals with insomnia symptoms in comparison to those without insomnia symptoms.[56] Consistent with our study findings, another meta-analysis consisting of 6 studies found a null association between insomnia symptoms and cancer mortality.[85]

In considering the differences between the a priori anticipated effects of SP and cancer incidence and cancer mortality and our results, we note that the population under study is ≥65 years whereas some studies have suggested an elevated association could be mostly restricted to younger people.[86,87] For instance, Christensen et al. found no evidence of the relationship between SAS and incidence of cancer overall but observed an elevated risk in persons younger than 50 years.[86] Moreover, a murine model of SP and lung cancer, old age was protective rather than deleterious, suggesting that type of cancer and age may be interactively implicated along with SP to modify their epidemiological characteristics,[88] which is consistent with the inverse association between SP and lung cancer and colorectal cancer mortality, respectively, observed

in our study. Therefore, the mostly null result in this older cohort does not necessarily rule out elevated associations in other age groups.

Furthermore, not all cancers are equally susceptible to the physiologic insults of SP as noted in the heterogeneity across the cancer sites in our present study and several other studies.[53,84,89,90] Of note is the elevated prostate cancer incidence with respect to snoring, apnea, and cumulative SAS. Given the age range of the study population and the fact that about 75% of prostate cancer are diagnosed in men who are 65 or older[91] it may be suggestive of SP impacting increased incidence of prostate cancer in this population. This finding is inconsistent with previous observational studies of SP and prostate cancers.[92-94] For instance, Markt et al. found no association between SP and prostate cancer incidence in 12,976 men with 785 cases of incident prostate cancer over 13 years.[93] A more recent study by Tan et al. in 2322 Swedish men aged 50 years or older followed for 40 years also found no significant association between SP and 263 prostate cancer incidence.[95] Furthermore, a recent meta-analysis consisting of 18 large studies found no association between rotating or night-shift work and prostate cancer.[96] Conversely, Chung, et al. study revealed a 35% higher risk of prostate cancer in patients aged ≥65 years with sleep disorders compared to their non-sleep disorder counterparts.[97] These studies illustrate some inconsistency in the findings from of the association of sleep problems and cancer to date.

Our study examining the association between SP and cancer incidence and cancer mortality in the CHS cohort should be interpreted in the context of some key study limitations. First, the physiologic insults of sleep apnea could not only vary across cancer sites, but also molecular subtypes of a given cancer site. Marhuenda et. al noted tumor cell growth varied according to the presence of a representative oncogenic mutation on different cell lines of the most prevalent histological subtypes of non–small cell lung cancer (adenocarcinoma and

squamous cell carcinoma) in response to intermittent hypoxia (IH) mimicking OSA.[98] In particular, they found significant differences in HIF-1α activation in two of the four histologic lung cancer cell lines that were exposed to IH compared with normoxia.[98] As such, while colorectal cancer and lung cancer-specific mortality were inversely associated with difficulty falling asleep and combined SAS respectively in this present study, our findings could have been impacted by the distribution of histologic cancer cell types that are less susceptible to the physiologic insults of SP. However, the lack of histologically defined cancer sites precludes us from teasing out the potential heterogeneous relationship across molecular subtypes of a given cancer site. Second, different cancers have different treatments, and given that data on treatment is unavailable, we could not account for this in our analysis of cancer mortality. However, analysis of cancer mortality stratified by cancer stage did not differ from the overall results which suggests that treatment is unlikely to fully explain the results since we would expect late-stage cancers to be less impacted by treatment. Moreover, the lack of this treatment information could also have biased our estimates to the null. Third, sleep patterns change over time and with age, which remains a challenging exposure to capture. For instance, some participants had resolved sleep problems but with no documented reason and the SP is self-report which is subject to misclassification. However, given that the measures were validated in a subset of the participants, misclassification is unlikely to significantly impact the results. Additionally, given that 70% of the cohort is partnered, SAS such as snoring, apnea could be very reliable since it relies on other people observing them sleep. Moreover, when we restricted the analysis to the 70% of the partnered cohort, the estimates did not change by much. Furthermore, we did not have information on sleep duration which is key sleep quality metrics that could vary substantially across participants with SAS or insomnia symptoms and an individual with short

sleep duration might not have a SAS or insomnia symptoms.[99,100] Individuals with both a SAS and short sleep duration might be experiencing a more severe underlying SP and therefore subsequent worse cancer outcomes.[49] Despite these limitations, the reported SP were pre-diagnostic (which reduces the likelihood that our results were impacted by reverse causality), and we were able to assess the impact of baseline and longitudinal SP on cancer incidence and mortality which is lacking in the current literature.

A key strength of our study is the relatively large sample size which allowed us to evaluate cancer incidence and cancer mortality overall and site-specific. Furthermore, the study design ensured temporality between the SP and the study endpoints with several covariates (including demographic and lifestyle factors) which allowed us to adjust for confounders selected a priori. Furthermore, the cancer incidence data was ascertained through a cancer registry (the gold standard in cancer research) while the cancer mortality data was also thoroughly assessed through half-yearly adjudication leveraging hospital diagnostic and procedure codes with associated text fields, death certificate diagnoses, and other information on the study criteria for underlying cause-of-death categories.

Conclusions

In conclusion, our study showed mixed results for the association between SP modeled at baseline and longitudinally and cancer incidence and mortality. We noted an inverse association between SP and lung and colorectal cancer mortality. Our results also showed an elevated risk of prostate cancer with respect to SAS. Given the high prevalence in men 65 years and older, and the ease with which SAS can be ascertained and managed, this finding may suggest an opportunity for reducing the burden of prostate cancer in older men if the association was causal.

Future larger community based prospective studies addressing more cancer site and molecular type-specific associations and improved SP self-report documentation over time are needed.

Tables

Table 2.1: Selected baseline characteristics overall and according to composite sleep apnea symptoms (SAS) in CHS

		SAS composite group		
Characteristics	**Total (n=4580)***	**0 symptoms (n=2864)**	**Any 1 symptom (n=1235)**	**2+ symptoms (n=481)**
Age at baseline, mean (SD)	72.6 (5.5)	72.5 (5.4)	72.8 (5.8)	72.6 (5.4)
age group				
64-69	35.6	36. 1	34.9	34.7
70-74	32.1	32.2	31.7	32.6
75-79	19.8	19.5	20.6	19.8
80-84	9.1	9.1	8.4	11.0
85+	3.4	3.2	4.45	1.9
Partnered living	70.2	67.9	71.5	80.5
Female	56.6	62.4	55.7	34.9
Education				
<HS	29.9	27.1	34.5	34.1
HS or GED	27.7	29.2	25.8	25.2
Vocational	8.3	8.7	7.9	7.5
Some college	14.0	14	14	13.5
College degree	10.2	10.4	9.8	10.2
Grad or professional deg	9.8	10.7	7.9	9.6
Race				
White	83.4	85.1	81.4	78.6
African American/Black	16.2	14.6	18.4	20.6
other	0.4	0.4	0.24	0.8
Hispanic	1.2	1.0	1.3	1.5
BMI, kg/m^2				
Mean (SD)	26.7 (4.7)	26.2 (4.5))	27.8 (5.0)	27.7 (4.8)
<25	39.3	43.4	33.4	29.9
25-29	41.1	39.2	45.0	42.8
30+	19.6	17.5	21.6	27.2
Smoking				
Never	46.6	49.5	43.7	36.2
former	42.0	39.2	44.8	51.4
current	11.5	11.3	11.5	12.5
Alcohol beverages per week				
median (IQR)	0.0 (0, 1.25)	0.0 (0, 1.25)	0.0 (0, 1.02)	0.019 (0, 1.5)
none	51.3	50.4	54.7	48.4
<1	18.3	18.8	16.7	20.0
1-6	16.9	17.6	15	17.7
7-13	5.8	6.11	5.6	4.6

14+	7.6	7.16	8.1	9.4
Physical Activity (kcal/week)				
median (IQR)	1080 (373.1, 2305.6)	1132.5 (405.0, 2376.9)	916.3 (307.5, 2160)	990 (292.5, 2265)
quartiles				
<405	24.3	22.5	27.6	27.0
408.8-1110	25.0	24.8	25.6	24.5
1113.8-2415	25.3	26.5	23.1	23.9
2420-14805	25.4	26.2	23.7	24.5
Baseline Coronary Heart Disease	19.0	17.7	20.4	23.5
Baseline Hypertension	66.0	64.4	69.3	67.4
Baseline Diabetes	16.1	13.5	19.9	21.8

*Includes prevalent cancer

Categorical variables are in percentages and continuous variables in mean (SD) or Median (IQR)

SAS: Sleep Apnea Symptoms

Table 2.2: Selected characteristics of incident cancers and cancer deaths in CHS

	Cancer group					
	Incidence (n=885)			Death (n=804)		
	Median time at risk (yrs.)	n	%	Median time at risk (yrs.)	n	%
Overall	12.0			13.5		
Common cancer sites						
breast	4.9	73	8.3	11.2	29	3.6
buccal	7.0	12	1.4	11.1	11	1.4
cervix	1.6	1	0.1	2.9	1	0.1
colorectal	7.0	102	11.5	9.2	84	10.5
digest	5.6	8	0.9	9.5	10	1.2
esophagus	6.8	9	1.0	7.7	11	1.4
kidney	6.4	27	3.1	11.8	17	2.1
liver	5.6	7	0.8	5.9	8	1.0
lung	6.0	113	12.8	8.8	138	17.2
lymphatic or hematopoietic	9.1	78	8.8	11.2	87	10.8
melanoma	5.0	2	0.2	8.1	2	0.3
ovary	8.4	11	1.2	12.1	15	1.9
pancreas	4.9	28	3.2	7.9	47	5.9
prostate	3.4	114	12.9	8.8	66	8.2
stomach	7.7	20	2.3	8.9	22	2.7
bladder	4.8	25	2.8	12.2	30	3.7
uterus	8.6	25	2.8	12.8	16	2.0
Cancer stage						
localized	5.9	360	40.7	11.1	106	13.2
regional	6.3	188	21.2	9.0	115	14.3
distant	7.6	174	19.7	8.6	144	17.9
unknown	6.1	76	8.6	7.2	48	6.0

*Cancer stage does not sum up to 100% due to missing stage information

Table 2.3: Association of SP with cancer incidence overall and with specific cancer sites in CHS, n=3930

	Cancer overall (n=885)	Breast (n=73)	Colorectal (102)	Lung (113)	Lymphatic or hematopoietic (78)	Prostate (114)
Sleep apnea symptoms (SAS): (Primary exposure)	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**
Daytime Sleepiness						
Baseline	0.93 (0.77, 1.12)	0.99 (0.44, 1.88)	0.98 (0.58, 1.67)	1.38 (0.86, 2.19)	1.81 (1.06, 3.08)*	0.95 (0.55, 1.62)
Time dependent	0.84 (0.69, 1.01)	0.81 (0.44, 1.49)	0.85 (0.52, 1.38)	1.05 (0.67, 1.62)	1.21 (0.77, 1.92)	1.20 (0.78, 1.86)
Cumulative average	0.78 (0.59, 1.04)	0.77 (0.31, 1.90)	0.75 (0.36, 1.57)	1.33 (0.68, 2.58)	1.57 (0.80, 3.08)	0.99 (0.51, 1.93)
Stopped breathing (Apnea)						
Baseline	0.80 (0.61, 1.05)	0.63 (0.15, 2.72)	0.64 (0.26, 1.61)	0.65 (0.28, 1.52)	0.74 (0.31, 1.73)	1.28 (0.71, 2.29)
Time dependent	0.98 (0.72, 1.36)	0.42 (0.12, 1.45)	0.25 (0.058, 1.06)	0.54 (0.20, 1.44)	0.71 (0.26, 1.94)	2.34 (1.32, 4.15)**
Cumulative average	0.92 (0.60, 1.42)	0.25 (0.027, 2.41)	0.37 (0.09, 1.46)	0.39 (0.08, 1.78)	0.49 (0.12, 1.93)	2.25 (1.03, 4.88)
Snoring						
Baseline	0.84 (0.71, 0.99)*	1.06 (0.56, 2.01)	0.78 (0.45, 1.35)	0.50 (0.30, 0.83)*	1.06 (0.62, 1.80)	1.69 (1.11, 2.57)*
Time dependent	0.76 (0.65, 0.89)**	0.78 (0.48, 1.27)	1.03 (0.70, 1.52)	0.80 (0.55, 1.15)	0.75 (0.49, 1.13)	1.28 (0.83, 199)
Cumulative average	0.80 (0.62, 1.04)	0.57 (0.22, 1.44)	1.01 (0.052, 1.94)	0.56 (0.28, 1.11)	0.98 (0.49, 1.98)	2.17 (1.22,3.86)**
Combined baseline SAS (Reference: 0 symptoms)						
1	0.81 (0.69, 0.95)**	0.99 (0.57, 1.71)	1.01 (0.66, 1.56)	0.81 (0.53, 1.25)	1.13 (0.69, 1.85)	1.30 (0.84, 2.01)
2+	0.77 (0.61, 0.97)*	0.80 (0.23, 2.71)	0.46 (0.20, 1.09)	0.46 (0.22, 0.96)*	1.27 (0.63, 2.57)	2.22 (1.30, 3.79)**
Insomnia Symptoms (IS)						
Difficulty falling asleep						
Baseline	0.92 (0.77, 1.10)	0.87 (0.49, 1.53)	0.81 (0.49, 1.33)	0.74 (0.44, 1.24)	1.06 (0.56, 2.02)	0.49 (0.27, 0.92)
Time dependent	0.90 (0.74, 1.10)	0.83 (0.27, 1.46)	0.74 (0.44, 1.26)	0.64 (0.37, 1.11)	1.40 (0.86, 2.25)	0.20 (0.093, 0.43)**
Cumulative average	0.92 (0.72, 1.19)	0.94 (0.45, 1.95)	0.78 (0.40, 1.50)	0.54 (0.27, 1.06)	1.21 (0.62, 2.37)	0.15 (0.064, 0.37)**
Problems staying asleep						
Baseline	1.07 (0.92, 1.10)	1.00 (0.61, 1.64)	1.00 (0.65, 1.53)	1.35(0.90, 2.02)	0.81 (0.49, 1.33)	1.08 (0.72, 1.62)
Time dependent	1.07 (0.92, 1.24)	0.77 (0.48, 1.24)	0.85 (0.58, 1.25)	1.04 (0.73, 1.48)	0.77 (0.52, 1.13)	1.42 (0.97, 2.11)*
Cumulative average	0.98 (0.79, 1.20)	0.86 (0.46, 1.59)	0.85 (0.50, 1.44)	1.20 (0.71, 2.00)	0.54 (0.31, 0.92)*	1.31 (0.79, 2.17)

Early morning Awakenings						
Baseline	1.11 (0.96, 1.29)	0.82 (0.48, 1.38)	1.42 (0.93, 2.16)	1.44 (0.96, 2.18)	1.17 (0.69, 1.97)	1.01 (0.65, 1.55)
Time dependent	0.92 (0.78, 1.08)	1.08 (0.67, 1.71)	1.25 (0.84, 1.86)	0.87 (0.57, 1.31)	0.84 (0.55, 1.28)	1.03 (0.70, 1.52)
Cumulative average	1.12 (0.89, 1.45)	0.94 (0.43, 2.04)	1.49 (0.78. 2.84)	1.19 (0.61, 2.32)	1.19 (0.58, 2.43)	1.40 (0.76, 2.56)
Combined baseline IS (Reference: 0 symptoms)						
1	1.06 (0.89, 1.26)	0.85 (0.49, 1.50)	0.96 (0.58, 1.61)	1.28 (0.78, 2.09)	1.03 (0.56, 1.88)	1.36 (0.86, 2.16)
2	0.96 (0.78, 1.17)	0.51 (0.24, 1.06)	0.92 (0.52, 1.65)	1.12 (0.74, 1.96)	1.44 (0.77, 2.68)	0.80 (0.45, 1.44)
3+	1.19 (0.89,1.26)	0.82 (0.42, 1.62)	1.33 (0.74, 1.36)	1.72 (0.99, 3.00)	1.19 (0.57, 2.50)	0.85 (0.44, 1.61)

* *p-value* < 0.05 ** *p-value* < 0.01

Adjusted for: gender, study phase, baseline age, smoking, body mass index, diabetes, physical activity levels, and alcohol consumption

Table 2.4: Association of SP with cancer death overall and site-specific in CHS, n=4580

	Cancer Overall (n=804)	Colorectal (n=84)	Lung (138)	Lymphatic or hematopoietic (n=87)	Prostate (n=66)
Sleep apnea symptoms (SAS): (Primary exposure)	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**
Daytime Sleepiness					
Baseline	1.08 (0.89, 1.31)	1.37 (0.80, 2.35)	1.11 (0.70, 1.77)	1.69 (0.99, 2.85)	1.42 (0.77, 2.61)
Time dependent	1.01 (0.83, 1.23)	1.03 (0.57, 1.86)	1.40 (0.90, 2.18)	1.02 (0.57, 1.83	0.93 (0.45, 1.94)
Cumulative average	1.11 (0.82, 1.49)	1.29 (0.53, 3.13)	1.45 (0.71, 2.97)	2.08 (0.97, 4.49)	1.91 (0.71, 5.13)
Stopped breathing (Apnea)					
Baseline	0.69 (0.51, 0.94)*	0.87 (0.34, 2.20)	0.58 (0.25, 1.36)	0.61 (0.24, 1.52)	0.71 (0.27, 1.86)
Time dependent	0.99 (0.70, 1.41)	1.05 (0.37, 2.96)	0.64 (0.23, 1.78)	0.74 (0.23, 2.39)	1.93 (0.67, 5.58)
Cumulative average	0.94 (0.58, 1.55)	1.22 (0.29, 5.18)	0.36 (0.07, 2.02)	0.56 (0.11, 2.79)	1.68 (0.35, 8.10)
Snoring					
Baseline	0.86 (0.72, 1.03)	0.71 (0.34, 1.20)	0.56 (0.35, 0.89)*	0.88 (0.51, 1.49)	1.50 (0.84, 2.69)
Time dependent	0.73 (0.62, 0.86)**	0.88 (0.53, 1.46)	0.76 (0.51, 1.12)	0.66 (0.41, 1.09)	0.83 (0.45, 1.53)
Cumulative average	0.67 (0.50, 0.90)**	0.72 (0.29, 1.83)	0.48 (0.22, 1.02)	0.57 (0.24, 1.38)	1.43 (0.51, 4.09)
Combined SAS (Reference: 0 symptoms)					
1	0.90 (0.76, 1.06)	0.82 (0.49, 1.37)	0.84 (0.56, 1.24)	1.04 (0.64, 1.70)	1.56 (0.90, 2.69)
2+	0.75 (0.58, 0.97)*	0.89 (0.43, 1.83)	0.35 (0.16, 0.77)**	0.93 (0.46, 1.89)	1.46 (0.64, 3.30)
Insomnia Symptoms					

Difficulty falling asleep					
Baseline	0.90 (0.74, 1.08)	0.32 (0.15, 0.69)*	0.80 (0.49, 1.30)	0.91 (0.50, 1.65)	0.63 (0.32, 1.23)
Time dependent	0.89 (0.72, 1.10)	0.41 (0.17, 0.98)*	0.69 (0.40, 1.21)	1.04 (0.56, 1.94)	0.44 (0.17, 1.12)
Cumulative average	0.97 (0.74, 1.28)	0.28 (0.09, 0.84)*	0.70 (0.35, 1.39)	0.90 (0.39, 2.09)	0.46 (0.15, 1.41)
Problems staying asleep					
Baseline	1.02 (0.88, 1.20)	1.13 (0.69, 1.83)	1.18 (0.82, 1.69)	1.05 (0.65, 1.68)	0.97 (0.58, 1.62)
Time dependent	1.01 (0.86, 1.20)	1.01 (0.61, 1.67)	0.97 (0.65, 1.42)	1.06 (0.64, 1.74)	1.45 (0.76, 2.79)
Cumulative average	0.89 (0.71, 1.11)	1.03 (0.51, 2.07)	1.16 (0.66, 2.03)	0.73 (0.38, 1.39)	1.25 (0.52, 2.99)
Early morning Awakenings					
Baseline	1.13 (0.96, 1.33)	1.47 (0.69, 1.82)	1.08 (0.72, 1.62)	0.88 (0.54, 1.42)	1.09 (0.65, 1.83)
Time dependent	1.00 (0.85, 1.19)	0.93 (0.54, 1.59)	1.03 (0.68, 1.56)	1.26 (0.77, 2.06)	0.95 (0.50, 1.80)
Cumulative average	1.23 (0.17, 1.64)	1.32 (0.53, 3.30)	1.25 (0.61, 2.61)	1.60 (0.70, 3.68)	1.20 (0.40, 3.67)
Combined IS (Reference: 0 symptoms)					
1	1.05 (0.88, 1.26)	1.25 (0.72, 2.19)	1.08 (0.70, 1.66)	1.39 (0.80, 2.42)	0.64 (0.34, 1.19)
2	0.97 (0.79, 1.20)	1.34 (0.73, 2.48)	0.89 (0.54, 1.46)	1.12 (0.59, 2.14)	0.66 (0.33, 1.32)
3+	1.14 (0.91, 1.42)	0.75 (0.34, 1.70)	1.29 (0.77, 2.13)	1.26 (0.61, 2.59)	0.76 (0.36, 1.59)

* *p-value* < 0.05 ** *p-value* < 0.01

Adjusted for: gender, study phase, baseline age, smoking, body mass index, diabetes, physical activity levels, and alcohol consumption

Supplementary tables:

Table 2.1: Association of SP with cancer incidence and cancer death overall in CHS for 2- and 5-years censored outcomes, and sex specific estimates

	Cancer incidence (n=3980)		Cancer mortality (4580)	
	2 yrs. censorship	5 yrs. censorship	2 yrs. censorship	5 yrs. Censorship
Sleep apnea symptoms (SAS): (Primary exposure)	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**
Combined SAS (Reference: 0 symptoms)				
1	0.80 (0.68, 0.96)*	0.87 (0.71, 1.07)	0.85 (0.72, 1.01)	0.87 (0.72, 1.05)
2+	0.78 (0.61, 1.01)	0.71 (0.51, 0.97)*	0.74 (0.57, 0.97)*	0.67 (0.49, 0.91)**
Insomnia Symptoms				
Combined IS (Reference: 0 symptoms)				
1	1.11 (0.92, 1.34)	1.08 (0.86, 1.35)	1.04 (0.87, 1.25)	1.02 (0.83, 1.25)
2	1.05 (0.86, 1.30)	1.02 (0.79, 1.32)	0.97 (0.78, 1.19)	1.01 (0.80, 1.27)
3+	1.28 (1.03, 1.61))	1.33 (1.02, 1.73)*	1.07 (0.85, 1.34)	1.17 (0.91, 1.50)
	Females (n=2240)	**Males (n=1690)**	**Females (n=2594)**	**Males (n=1986)**
Sleep apnea symptoms (SAS): (Primary exposure)	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**
Combined SAS (Reference: 0 symptoms)				
1	0.86 (0.64, 1.17)	0.90 (0.68, 1.20	0.83 (0.65, 1.06)	0.98 (0.78, 1.23)
2+	0.52 (0.26, 1.04)	0.79 (0.55, 1.13)	0.85 (0.54, 1.33)	0.73 (0.53, 1.00)
Insomnia Symptoms				
Combined IS (Reference: 0 symptoms)				
1	0.89 (0.65, 1.24)	1.26 (0.92, 1.74)	1.11 (0.85, 1.45)	0.99 ().78, 1.27)
2	1.02 (0.71, 1.44)	1.02 (0.70, 1.49)	1.05 (0.78, 1.41)	0.90 (0.68, 1.20)
3+	1.09 (0.76, 1.57)	1.63 (1.11, 2.40)*	1.10 (0.81, 1.50)	1.17 (0.86, 1.61)

* *p-value*< 0.05, ** *p-value* < 0.01

Adjusted for: gender, study phase, baseline age, smoking, body mass index, diabetes, physical activity levels, and alcohol consumption

Table 2.2: Tumor stage- and BMI-specific associations of SP with cancer incidence and cancer death overall in CHS

	Cancer incidence (n=3980)			Cancer Mortality (n=4580)		
	localized (n=360)	**Regional (n=188)**	**Distant (n=174)**	**Localized (n=106)**	**Regional (n=115)**	**Distant (144)**
Sleep apnea symptoms (SAS): (Primary exposure)	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**
Combined SAS (Reference: 0 symptoms)						
1	0.87 (0.67, 1.12)	0.56 (0.39, 0.81)**	0.94 (0.68, 1.32)	0.80 (0.51, 1.27)	0.66 (0.42, 1.04)	1.06 (0.74, 1.54)
2+	0.98 (0.70, 1.38)	0.41 (0.22, 0.76)**	0.62 (0.35, 1.12)	0.60 (0.29, 1.22)	0.45 (0.21, 0.98)*	0.71 (0.39, 1.32)
Insomnia Symptoms						
Combined IS (Reference: 0 symptoms)						
1	1.03 (0.79, 1.35)	0.99 (0.68, 1.44)	0.88 (0.61, 1.28)	1.21 (0.73, 2.02)	1.11 (0.67, 1.84)	0.85 (0.56, 1.28)
2	1.06 (0.78, 1.43)	0.89 (0.58, 1.38)	0.76 (0.49, 1.17)	1.12 (0.63, 1.99)	1.33 (0.78, 2.27)	0.68 (0.42, 1.11)
3+	1.18 (0.85, 1.64)	1.24 (0.80, 1.91)	0.72 (0.43, 1.18)	1.68 (0.91, 3.08)	1.27 (0.70, 2.29)	0.74 (0.43, 1.28)
	BMI <25kg/m^2 (n=1542)	**BMI 25-29 kg/m^2 (n=1619)**	**BMI 30+kg/m^2 (n=769)**	**BMI <25kg/m^2 (n=1798)**	**BMI 25-29 kg/m^2 (n=1884)**	**BMI 30+kg/m^2 (n=898)**
Sleep apnea symptoms (SAS): (Primary exposure)	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**
1	0.80 (0.61, 1.05)	0.80 (0.63, 1.01)	0.85 (0.60, 1.20)	0.99 (0.74, 1.32)	0.96 (0.75, 1.22)	0.89 (0.62, 1.28)
2+	1.00 (0.70, 1.44)	0.65 (0.44, 0.95)*	0.68 (0.41, 1.12)	1.30 (0.88, 1.90)	0.56 (0.35, 0.89)*	0.89 (0.55, 1.44)
Combined IS (Reference: 0 symptoms)						
1	1.06 (0.80, 1.40)	1.12 (0.86, 1.46)	0.94 (0.65, 1.37)	1.10 (0.82, 1.48)	1.07 (0.81, 1.42)	0.89 (0.60, 1.32)
2	1.14 (0.84, 1.55)	0.84 (0.61, 1.16)	0.87 (0.56, 1.34)	1.17 (0.84, 1.64)	0.84 (0.61, 1.17)	0.86 (0.55, 1.34)
3+	0.93 (0.65, 1.33)	1.52 (1.12, 2.07)**	1.07 (0.68, 1.68)	0.99 (0.79, 1.43)	1.24 (0.89, 1.73)	0.92 (0.56, 1.52)

* *p-value*<0.05, ** *p-value* <0.01

Adjusted for: gender, study phase, baseline age, smoking, body mass index, diabetes, physical activity levels, and alcohol consumption

BMI: Body mass index

Table 2.3: Associations of SAS symptoms vs Modified STOP-BANG with cancer incidence and cancer death overall in CHS

	Cancer overall (n=3930) **HR (95%CI)**	**Cancer mortality (n=4580)** **HR (95%CI)**
SAS symptoms (Ref: 0 symptoms)		
Any symptoms	0.81 (0.69, 0.95)*	0.90 (0.77, 1.06)
2+ symptoms	0.77 (0.61, 0.97)*	0.75 (0.58, 0.97)*
Modified STOP-BANG (Ref: low)		
Intermediate OSA risk 3-4	0.95 (0.79, 1.14)	1.04 (0.86, 1.26)
High OSA risk 5+	0.74 (0.56, 0.97)*	0.91 (0.68, 1.22)

Sleep Apnea Symptoms (SAS): snore, apnea and daytime sleepiness
Modified STOP-BANG: Snoring, daytime Tiredness, observed apnea, Blood Pressure, Body Mass Index, Age, Neck circumference and Gender: upweighted body mass index (0: <25 kg/m^2, 1: 25-34 kg/m^2 and 2: 35+ kg/m^2) to replace Neck circumference
OSA: Obstructive Sleep Apnea
* *p-value*< 0.05, ** *p-value* < 0.01
Adjusted for: gender, study phase, baseline age, smoking, body mass index, diabetes, physical activity levels, and alcohol consumption

Chapter 3: Immunotherapy cohort

Title: Sleep problems, tumor aggressiveness, and immune checkpoint treatment outcomes: A pilot study

Abstract

Background: Sleep problems (SP) are a prominent concern of cancer *patients* and are frequently indicative of an underlying sleep disorder, such as obstructive sleep apnea. SP may be adversely associated with tumor aggressiveness and poor treatment response via upregulation of immune-suppressive proteins.

Methods: Thirty-three participants consented to an immunotherapy cohort study, of whom 32 initiated an immune checkpoint inhibitor (ICI) treatment. We collected questionnaire data on primary SP (sleep apnea risk) and secondary SP, including insomnia and general sleep patterns. We extracted patient, tumor, and ICI attributes on the date corresponding to the patient's last clinic visit prior to or at ICI initiation from the *Electronic health records (EHR)* six months post ICI initiation. Tumor aggressiveness was defined by M-stage (M0 vs. M1). Six or more ICI infusions was considered ICI tolerability. We ran an adjusted multivariate Poisson regression with robust standard errors to assess the association between sleep problems and tumor aggressiveness and reported prevalence ratio (PR) and associated 95% CI for the associations. We used adjusted logistic regression models to assess the association between sleep apnea and insomnia risk with the number of ICI infusions (6+ vs. <6). We calculated the odds ratio (OR) and 95% CI for associations with this outcome.

Results: Among those with sleep symptoms in the immunotherapy cohort, the prevalence of low, intermediate, and high-risk OSA risk was 36%, 42%, and 21%, respectively. Of the secondary sleep problems considered, 58% of participants reported clinically significant insomnia, 72% experienced an average or restless night sleep, 30% reported taking longer than 15 min to fall asleep, 46% had ideal night sleep, and 36% had an evening chronotype. We did not find significant association [PR (95%CI)] between intermediate or high risk OSA and

metastatic cancer compared to low risk OSA [1.01 (0.28, 3.67)] or with continuous OSA risk scores [1.15 (0.74, 1.77)]. Of the secondary sleep attributes considered, patients who reported taking more than 15 minutes to fall asleep were 3.6 times more likely to be diagnosed with metastatic cancer compared to those reporting shorter sleep latency [95% CI (1.74, 7.35)]. Additionally, patients reporting a morning chronotype were less likely to be diagnosed with metastatic cancer compared to those reporting evening chronotypes [0.23 (0.09, 0.58)]. We did not find any significant association [OR (95%CI)] between intermediate or high risk OSA and six or more infusions compared to low risk OSA [0.27 (0.02, 3.41)] and continuous OSA risk score [0.72 (0.37, 1.39)].

Conclusion: This study gives insights into the potential burden and impact of sleep problems on tumor aggressiveness and ICI treatment tolerability. This could potentially inform clinical trials focused on finding effective sleep quality improvement interventions in ICI cancer treatment populations.

Keywords: OSA risk, immune checkpoint inhibitors, tumor aggressiveness, circadian rhythm

Introduction

Sleep problems are a prominent concern of cancer patients. Disturbed sleep is reported by 45-80% of cancer patients, compared to 29-32% of the general population.[101] Sleep problems result from sleep disorders [e.g., sleep-disordered breathing (obstructive sleep apnea (OSA), central sleep apnea, upper airway resistance syndrome), insomnia, narcolepsy], poor sleep quality (e.g., non-restorative sleep, sleep fragmentation), improper timing (e.g., not occurring at night), irregularity (e.g., constant variation in bedtimes and wake times, frequent random nap episodes), or a non-ideal sleep duration (<7 hrs/night or >8 hrs/night).[2] Cancer therapy and cancer-related anxiety can cause sleep problems[9,10] and/or reflect previously established carcinogenic roles of sleep problems themselves.[11,12,18,20,21]

Sleep problems may have implications for cancer progression and treatment response, as illustrated in figure 3.1. Multiple studies show a consistent positive association between OSA (a common sleep afflicting an estimated 25 million US adults[102]) and the incidence and severity of melanoma and kidney cancers in humans[6,76,84,103,104]. The mechanism underlying this cancer-related effect is likely through induced intermittent hypoxia (IH, a hallmark of OSA)[76,105,106] and adverse downstream consequences of sleep fragmentation and disruption to the 24-hour circadian rhythm.[5,107-110] With respect to IH, mouse models of melanoma and kidney cancers shows enhanced tumor growth, invasiveness, and metastasis.[76,105,106] Circadian rhythms are generated endogenously in coherence with external cues (e.g., zeitgebers) enabling alignment of physiological processes to the day-night cycle.[8] Disruption to these rhythms increases inflammation and reduces melatonin hormone production which in both instances promotes cellular damage. [5,107-110] The effects of these physiologic insults are observed in numerous

cancer cell lines contributing to aggressive tumor proliferation due to corresponding reductions in T-cell activation and overall cytotoxicity.[5,107,109]

Increasing evidence shows higher tumor related T cell levels improve prognosis.[25,29,31,32,111,112] Existing and emerging immunotherapies are harnessing this T-cell response to successfully treat several forms of cancer [e.g., melanoma, renal cell carcinoma (RCC)] by inhibiting immune-suppressive proteins such as programmed cell death-1 receptor (PD-1), its ligand PD-L1 (PD-1/PD-L1), and cytotoxic T lymphocyte antigen 4 (CTLA-4).[113-118] As a result, the US Food and Drug Administration (FDA) has approved seven immune checkpoint inhibitors (ICI) that target CTLA-4 (ipilimumab), PD-1 (nivolumab, pembrolizumab, and cemiplimab), or PD-L1 (atezolizumab, avelumab, and durvalumab).[119,120] Despite the promise of ICI patient response is not uniformly favorable. Thus a better understanding of factors predictive of ICI response is needed.[121-124]

Immune checkpoint inhibitors (ICIs) are increasingly prescribed for late stage cancer patients. In this context, the field must better understand the impact of circadian rhythms (and/or "circadian rhythm disruption") and sleep problems such as OSA on immune response and cancer aggressiveness in this patient population. Despite the numerous studies of sleep problems in the context of other cancer treatments (e.g., chemotherapy),[125-128] to date, there are no studies correlating sleep problems with tumor aggressiveness and outcomes in cancer patients receiving ICI therapy.[129] We, therefore, describe the burden of sleep problems, its relation to tumor aggressiveness at diagnosis, and the impact on ICI tolerability in cancer patients previously unexposed to ICI therapy (The Lifestyle Attributes and Sleep in Immunotherapy Response (LASIR) Study).

Methods

Setting

The LASIR Study was conducted at the Seattle Cancer Care Alliance (SCCA). The SCCA is the clinical care arm of the Cancer Consortium formed in partnership with Fred Hutchinson Cancer Research Center, the University of Washington, and Seattle Children's Hospital. The SCCA is the only National Cancer Institute-designated comprehensive cancer center serving the five state Washington, Wyoming, Alaska, Montana, and Idaho region.[130] Based on current clinical practice, which stipulates ICI use as second-line therapy for advanced kidney cancer [131] and first-line therapy for some with metastatic melanoma and lung cancers,[132,133] the vast majority of study participants had advanced-stage disease (stage III/IV) at ICI initiation.

Participant recruitment

Patients were study eligible if they were: 1) An adult initiating outpatient treatment of a commercial ICI agent for the first time at the Renal cell carcinoma /Melanoma (Ren/Mel) and throat head and neck (THN) clinics, 2) Aged between 18-84 years, 3) Able to provide informed consent and 4) Able to complete the questionnaire in English. Patient recruitment began in April 2019 and was interrupted by the COVID-19 global pandemic in mid-March 2020. Of the 370 patients screened, 94 were eligible, 63 (67%) were approached for consenting, and 31 (33%) could not complete a consent conference due to time constraints or COVID-19 restrictions (Figure 3.2). Of those approached, 33 (52%) enrolled in the study, and one participant died before initiating ICI.

Data collection and definitions

Data was collected from study participants via three sources: a self-administered patient questionnaire, a sleep monitoring device, and electronic medical records.

The questionnaire was self-administered at the time of study enrollment and included an assessment of OSA risk, insomnia, and general sleep patterns. In particular, the ***primary*** exposure sleep data was a self-reported 8-item validated ***STOP-BANG*** questionnaire for the assessment of OSA risk with scores ranging from 0-8; scores were categorized into low risk (0-2), intermediate risk (3-4), and high risk (5-8) of OSA. Details of the ***STOP-BANG*** questionnaire content is described elsewhere.[67] Secondary sleep data included the 5-item Women's Health Initiative Insomnia scale (***WHIIS***).[134] The ***WHIIS*** requires individuals to rate the quality of their sleep and the frequency with which they experience certain sleep problems in the last month with scores ranging from 0-20 in increasing order of insomnia symptoms; a score >9 was considered clinically significant. Details of the WHIIS are described elsewhere.[135] Other self-reported secondary sleep data included: history of OSA diagnosis, chronotype (e.g., preferred sleep timing over a 24 hour period), sleep latency, and typical sleep duration categorized as shown in Table 3.2.

Additional relevant lifestyle and patient attribute data collected in the questionnaire included: physical activity, smoking status, alcohol consumption, perceived stress, educational level, and marital status. These variables were all categorized as in Table 3.1. Participants also reported previous diagnoses of hypertension, diabetes, and high cholesterol and treatment status for these conditions.

The SleepScoreMax[136] was used to measure nighttime sleep pattern for a maximum 30 days post ICI initiation in a subset of consented participants' (n=12). The SleepScoreMax is a non-contact sleep sensor which uses smartphone app and web-based app to record sleep patterns. Specifically, it uses radiofrequency waves to assess body movement including chest and abdominal respiratory movement to measure key sleep attributes. The device recorded data on

total night sleep time (TST), sleep onset latency (SOL), wake after sleep onset (WASO), number of awakenings (A) , sleep architecture (non-rapid eye-movement (NREM) and rapid eye-movement (REM)) and overall sleep quality (Sleep score, SC).

Electronic health records (EHR) data was collected six months post ICI initiation. Extracted (EHR) data included age, gender, weight, and height on the date corresponding to the patient's last visit at SCCA prior to or at ICI initiation. Body mass index (BMI) was calculated by dividing weight in kilograms by height in meters squared and grouped into three categories: <25 kg/m^2, 25-29 $kg/m^{2,}$ and ≥30 kg/m^2. We extracted information pertaining to cancer diagnosis date, tumor attributes (as a measure of tumor aggressiveness) at diagnosis and ICI initiation, and prior cancer treatments (chemotherapy, radiotherapy, surgery, gene therapy, and other treatment regimens). Tumor attributes included T-stage, N-stage, M-stage, number and sites of metastases, and summary stage (I-IV). We extracted dates of the first six and last ICI infusions along with the type of each ICI initiated, physician-noted impressions based on tumor pathology of treatment response from Computed tomography (CT) scans (favorable response, stable disease, and disease progression), and ICI adverse events incidence within the first six months of ICI initiation. We extracted participant vital status (death status, cause of death, and date of death) within the first six months of ICI initiation.

Main outcomes definitions

Tumor aggressiveness was defined by M-stage (M0 vs. M1). Immune checkpoint inhibitor tolerance was defined in close consultation with experienced SCCA oncologists. Six or more infusions were considered to represent both ICI tolerability and treatment benefit within the six-month assessment period.

Statistical analysis

All analytical procedures were conducted using Stata 14.0 (College Station, Texas)[65], with statistical significance considered at a 2-sided alpha value of 0.05. In descriptive analyses, we examined the distribution of participants' baseline characteristics overall and stratified by OSA risk (low vs. intermediate/high). Our main analyses included the associations of sleep problems with tumor aggressiveness at diagnosis and ICI tolerance.

We ran a multivariate Poisson regression with robust standard errors (SEs) to assess the association between sleep problems and tumor aggressiveness [M-stage (M0 vs. M1)], adjusting for age, gender, and BMI. We opted to use a Poisson model instead of a logistic model due to sleep problems being common in cancer populations and the high prevalence of advanced disease in cancer patients initiating ICI. We used robust SEs in the Poisson model to account for any violation of the distribution assumption that the variance equals the mean. We reported the prevalence ratio (PR) and associated 95% CI for the associations.

We used logistic regression models to assess the associations of OSA and insomnia risk with the number of ICI infusions, adjusting for the following selected attributes: male gender, age at ICI treatment, and prior cancer treatment. We calculated the odds ratio (OR) and 95% confidence intervals (CI) for associations with these outcomes.

We ran several sensitivity analyses. First, we focused on treatment tolerability, defined according to first CT scan indicating favorable tolerance (e.g., favorable response) or non-tolerance (defined by disease progression or stable disease). Second, we assessed change in ICI regimen within six months of initiation. For both these outcomes, we used logistic regression as described for the number of ICI infusions. In addition, we used Cox proportional hazards regression [hazard ratios (HR) and 95% confidence intervals (CI)] to evaluate the association

between OSA, insomnia risk and time to treatment failure (TTF, changed ICI due to disease progression).

Results

Patient characteristics

The mean age of the cohort was 61 years, 61% were male, 85% were white, 64% were partnered, 70% had a college degree, 49 % had BMI of 30 kg/m^2, 3% were current smokers, 18% reported sufficient weekly physical activity and 18% high stress. Patients with intermediate or high risk OSA were on average older and had: 1) Fewer years of education, 2) Lower weekly physical activity, 3) High stress levels, and 4) Higher prevalence of diabetes, high cholesterol, and high blood pressure. (**Table 3.1**)

Prevalence of sleep problems

The prevalence of OSA symptoms was 21% for daytime sleepiness, 33% for observed apnea, and 21% for snoring; 51% reported none of these three symptoms. Among those with sleep apnea symptoms, the prevalence of low, intermediate, and high OSA risk was 36%, 42%, and 21% respectively. The prevalence of a self-reported OSA diagnosis at enrollment was 18%.

Of the secondary sleep problems considered, 58% of participants reported clinically significant insomnia, 72% experienced average or restless sleep, 30% reported taking longer than 15 min to fall asleep, 56% had ideal night sleep and 36% had an evening chronotype. (**Table 3.2)**

With respect to the SleepScoreMax data, overall, the mean nighttime total sleep duration was 6 hours, mean latency was 19 min, mean number of wake times was five. Participants had an average of four hours of light, one hour of deep and REM sleep. The sleep quality overall measured by the Sleep Score was 80%. (**Sup. Table 3.1)**

Tumor attributes

The most common cancer site was melanoma (52%). Seventy three percent were diagnosed with a late-stage disease (III/IV), 42% with a metastatic disease, 12% with numerous nearby nodal involvement (N2/N3) and 33% with a large tumor size or disease progression (T3/T4). The majority of patients had prior cancer treatment (64%), of which 66.7% had surgery, 23.8% were treated with chemotherapy, and 19% with radiotherapy. (**Table 3.3**)

Immune checkpoint inhibitor treatment attributes

The majority of patients initiated a PD-1 blockade (94%), 16% initiated ICI with chemotherapy, and 72% had six or more ICI infusions within a six-month period. About 44% of patients changed ICI on average, three months post ICI initiation. Participants reported on average, three adverse events post ICI initiation. The most common incident adverse events were rash and vitiligo (47%), general body pain (39%), hypothyroidism (25%), severe diarrhea (22%), dry mouth (19%), rheumatologic symptoms (19%), hypophysitis (13%), and adrenal insufficiency (13%). According to CT scans in relation to their ICI response assessment, 68% showed a stable or favorable response. Thirteen percent of enrolled participants died within the six-month follow-up period. The average time from cancer diagnosis or ICI initiation to death was 13 months and two months, respectively. (**Table 3.4)**

Association between sleep problems and tumor aggressiveness

Table 3.5 presents PRs and 95% CIs [reported as PR (95% CIs)] for associations between sleep problems and metastatic cancer at diagnosis. We did not find a significant association between intermediate or high risk OSA and metastatic cancer compared to low risk OSA [1.01 (0.28, 3.67)] and continuous OSA risk scores and metastatic cancer [1.15(0.74, 1.77)].

Of the secondary sleep attributes considered, patients reporting more than 15 minutes to fall asleep were 3.6 times more likely to have been diagnosed with metastatic cancer compared to those reporting shorter sleep latency [95% CI (1.74, 7.35)]. Additionally, patients reporting a morning chronotype were less likely to have been diagnosed with metastatic cancer compared to those reporting evening chronotypes [0.23 (0.09, 0.58)].

Association between sleep problems and ICI treatment tolerance

Table 3.6 presents HRs and 95% CIs [reported as HR (95% CIs)] for associations between sleep problems and the number of ICI infusions as a measurement of ICI treatment tolerance. We did not find any significant association between intermediate or high risk OSA and six or more infusions compared to low risk OSA [0.27 (0.02, 3.41)] and between continuous OSA risk scores and six or more infusions [0.72 (0.37, 1.40)]. Similarly, we found no significant association between insomnia and six or more infusions [0.23 (0.03, 1.60)] and between insomnia total scores and six or more infusions [0.77 (0.59, 1.02)].

Sensitivity analysis

We found a similar non-significant association and mostly similar directionality of the association between OSA risk (intermediate/high vs. low risk) and insomnia (clinically significant insomnia vs. non) and ICI tolerance when we used CT impressions (favorable vs. stable or progressive disease), changed ICI status (participants who did not change vs. those who did) or when we used TTF. (**Sup. Table 3.2**)

Discussion

We found a high burden of sleep problems in this ICI cohort. For instance, two-thirds of enrolled participants had intermediate to high sleep apnea risk and an average / restless night sleep, more than half experienced clinically significant insomnia, and about a third reported

taking longer than 15 min to fall asleep and evening chronotype. In a multivariable-adjusted regression analysis, we did not find a statistically significant association between intermediate or high risk OSA and metastatic cancer compared to low risk OSA. However, of the secondary sleep problems assessed, patients who reported taking longer to fall asleep were more likely to have been diagnosed with metastatic tumors compared to those reporting shorter sleep latency. Additionally, patients reporting an evening chronotype were more likely to have been diagnosed with metastatic cancer compared to those reporting a morning chronotype. Our second goal was to determine the association between sleep problems and ICI treatment tolerance. While we did not find any significant association between OSA risk, insomnia, and six or more infusions, the direction of the estimates showed higher odds for poor ICI treatment tolerance in patients with certain sleep problems.

Numerous mechanisms could explain our suggestive associations of sleep problems and higher likelihood of aggressive tumors at diagnosis and increased odds of poor ICI tolerance. Sleep exerts a strong regulatory influence on the adaptive immune system. In particular, sleep problems causing circadian rhythm dysregulation mediate cytotoxic cell activities via up-regulation of adaptive T-cell immune responses.[5,71,107,109,137] Specifically, misalignment in the circadian clock could reduce the secretion of melatonin[71,137], which has been shown to downregulate the expression of immune suppressive protein PD-1/PD-L1 via a membrane and nuclear receptors.[71,107,137] Thus, reduced melatonin allows increased activity of immune suppression proteins. Additionally, PD-1/PD-L1 binding cascade impedes T cell function and activation of autologous T-lymphocytes to inhibit cytotoxic activity of $CD8^+$ T-cells.[83,138,139] The effects of these physiologic insults are seen in numerous cancer cell lines and thought to contribute to tumor proliferation.[8]

Another specific aspect of sleep problems, apnea (i.e., recurrent cessations of breathing during sleep and a symptom of OSA), is associated with accelerated tumor progression and metastatic potential through enhanced oncogenic pathways in the presence of intermittent hypoxia.[51,74-77] Suggested mechanisms by which intermittent hypoxia contributes to tumor aggressiveness include the generation of excessive reactive oxygen species (leading to oxidative stress and induced DNA damage)[78,79], overexpression of the transcriptional regulator hypoxia-inducible factor-1alpha (HIF-1α), and suppressed immune function.[80-83] Specific to sleep problems and ICI response, intermittent hypoxia enhances the interaction of PD-1/PD-L1 on the T-cell surface.[83,138] The mechanism by which (HIF)-1 upregulates PD-L1 is through hypoxia response factors of the PD-L1 promoter, which activate a series of steps resulting in PD-L1 expression.[140] Nomad et al. showed hypoxia significantly increased HIF-1α and the mRNA expression levels of PD-L1 and its receptor PD-1 along with CTLA-4. In particular, they found that HIF-1α increased PD-L1 mRNA and subsequent PD-L1 protein. They reported that PD-L1 is a direct HIF-1α target gene that binds to the proximal promoter of the PD-L1 gene with a greater affinity at hypoxia-response element (HRE-4) promoter sites. Their work conclusively revealed hypoxia increased expression of HIF-1α, which then binds to a transcriptionally active HRE in the PD-L1 proximal promoter resulting in increased mRNA expression levels of PD-L1 and subsequent PD-L1 proteins.[140] Furthermore, Cubillos-Zapata and colleagues demonstrated the impact of intermittent hypoxia resulting in over-expression of HIF-1α in OSA patients and conditioned mice.[83,141] Collectively, their study found upregulation of PD-1/PD-L1 cascade in patients with OSA due to IH leads to suppression of autologous T-cell proliferation and decreased CD8+ T-cell cytotoxicity. Thus, the level of the PD-1/PD-L1 activation, and therefore impairment of the cytotoxic activity of $CD8^+$ T-cells, is most pronounced in individuals with

severe sleep problems, as demonstrated in severe OSA.[83] These physiologic effects could explain the suggestive reduced ICI tolerance in patients with intermediate to high risk OSA and clinically significant insomnia.

Given our study is the first to assess sleep problems in a cancer ICI treatment cohort, there is nothing for comparison besides the aforementioned biological data. However, in a cross-sectional study, Martinez et al.[6] found 35% of melanoma patients had intermediate to severe OSA with an increased likelihood of more aggressive melanoma as measured by melanoma-specific tumor attributes, including Breslow index, mitotic index, and presence of ulceration. Furthermore, our study corroborates other studies of sleep problems in more traditional cancer treatment cohorts such as radiotherapy and chemotherapy.[125-128] In particular, several of these studies noted a high burden of sleep problems, including insufficient sleep duration, insomnia symptoms, and poor overall sleep quality[125-128] are linked with poor cancer prognosis. [4,49,142] For instance, cancer patients with insufficient sleep duration (≤ 6 h sleep/night) and who snore might be experiencing more severe underlying sleep problems and, therefore, subsequent worse cancer outcomes. [4,49]

Our study, examining the burden of sleep problems, tumor aggressiveness, and ICI response, has some key limitations. Chief amongst them is the limited small sample size, which could explain the mostly non-statistically significant results. Due to the small sample size, we could not do a more sensitive analysis of the association between sleep problems and specific cancer sites (Table 3). The physiologic insults of sleep problems on cancer prognosis are heterogeneous across cancer sites and possibly molecular types. [53,84,143] Marhuenda et al. noted tumor aggressiveness varied according to a representative oncogenic mutation on different cell lines of the most prevalent histological subtypes of non–small cell lung cancer (adenocarcinoma

and squamous cell carcinoma) in response to OSA.[98] In particular, they found significant differences in HIF-1α activation in two of the four histologic lung cancer cell lines that were exposed to OSA compared with normoxia.[98] However, the results observed could be mostly driven by melanoma and RCC since they constitute 73% of cancer sites in the cohort and have been consistently associated with severe sleep problems.[6,53,84,90,144,145].

Secondly, study participants self-reported their sleep problems. Given the focus of this study on the patient population initiating ICI for the treatment of their late-stage cancer, several factors might be impacting participant sleep patterns (e.g., stress, side effects from previous lines of therapy); thus, observed sleep patterns may not be reflective of pre-diagnostic sleep patterns. In addition, it is possible that poor cancer prognosis could cause sleep problems instead of the reverse. However, this is less concerning for the ICI response outcome analysis since it is downstream of reported sleep problems at enrollment. Additionally, our study did not measure immune response biomarkers (e.g., inflammatory markers cytokines, including IL-1, IL-6, and TNF-α) that may be more sensitive to underlying sleep problems.[146,147] However, our sleep data is based on validated questionnaires and, unlike most studies assessing sleep problems in cancer which have focused on a single sleep dimension (e.g., sleep duration)[148], we evaluated multiple sleep dimensions, including ***STOP-BANG*** OSA risk levels.[67,149,150] As such, if the direction of our estimates (suggesting more aggressive cancer at diagnosis and potentially poor ICI response) can be replicated in larger studies, the ***STOP-BANG*** score can be used to 1) Non-invasively screen ICI patients for underlying sleep disorders, 2) Stratify patients who may respond more favorably to treatment, and 3) Motivate integration of assessment and treatment for sleep problems with ICI treatment to enhance response in patients with moderate to severe sleep problems.

Another important study limitation is in the assessment of ICI response. The primary response measure in ICI studies and the clinic settings is based on RECIST 1.1 guidelines.[151] The guideline is based on tumor imaging data incorporating information on changes in lesion size and new lesions to distinguish ICI "responders" from "non-responders".[151] We were unable to incorporate the guidelines into our study due to lack of this data at the end of 6 months follow-up. However, we were able to obtain mostly consistent result across ICI tolerance outcomes defined primarily by the number of ICI infusions and additional sensitivity analysis utilizing CT impressions (favorable vs. stable or progressive disease), changed ICI status (participants who did not change vs. those who did) or time to treatment failure (TTF) as response indicators (**Sup. Table 3.1**).

Finally, there is also an issue of representativeness of the SCCA cancer population to the general ICI cancer treatment population. Specifically, our study population is racially homogenous (mainly of European descent, 85% white) and likely have higher socio-economic status.

Despite these study limitations, this study is the first, to our knowledge, to examine the biologically plausible and potentially impact of sleep problems in cancer patients receiving ICI therapy. In result, given the burden and potential impact of sleep problems on ICI treatment response, we believe the study limitations are outweighed by the importance of this study in setting the stage for larger studies with comprehensive sleep and gold standard (such as RECIST 1.1 guidelines) ICI response assessment.

Conclusions

This study provides new insights into the potential burden and impact of sleep problems on tumor aggressiveness and ICI treatment response. We hope these results will motivate larger

studies of predictors of ICI response to include sleep problems in their assessment[129] and could potentially inform clinical trials focused on finding effective sleep quality improvement interventions in ICI cancer treatment populations.

Figures

Figure 3.1: Conceptual model linking sleep problems to tumor aggressiveness and Immune Checkpoint Inhibitor (ICI) response

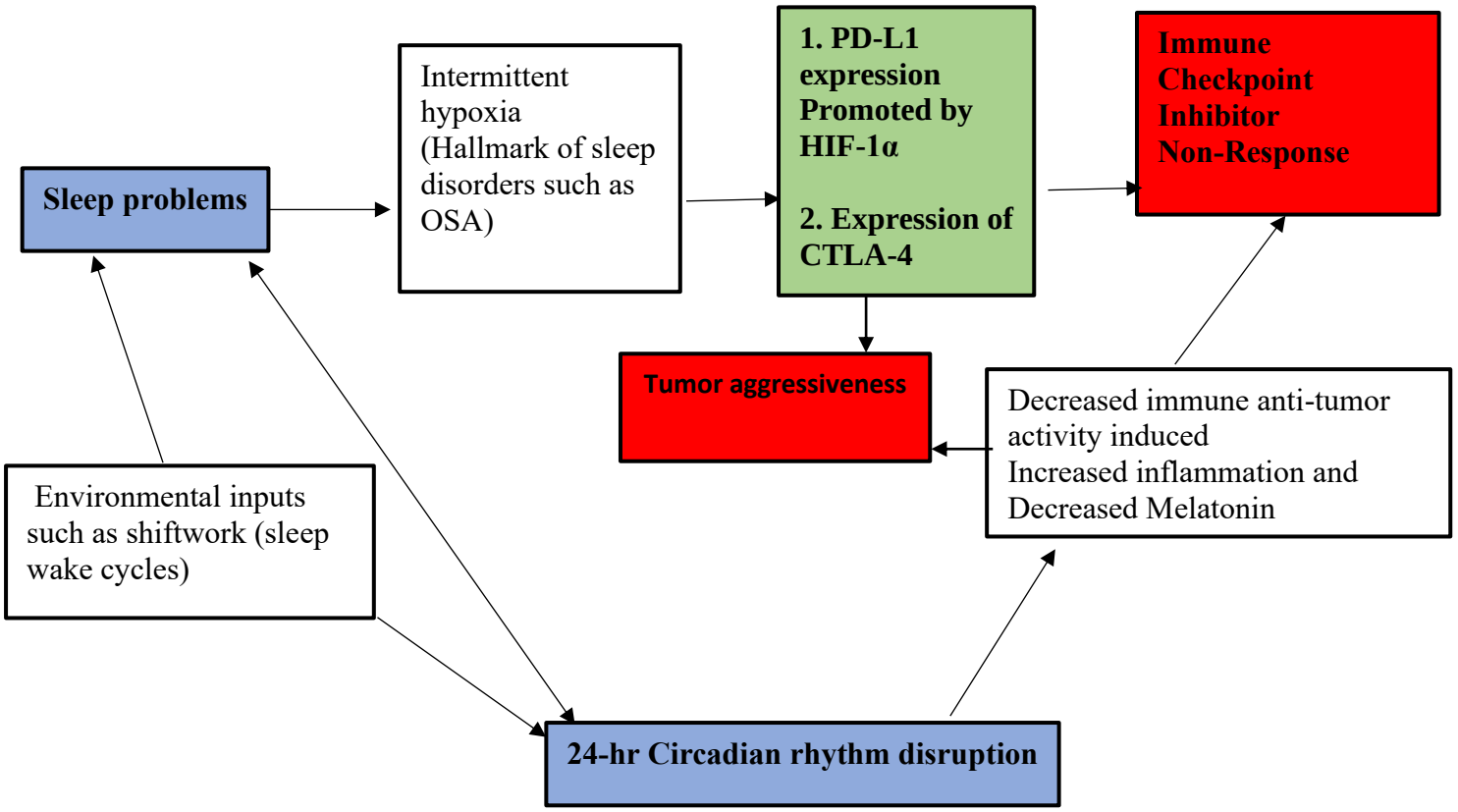
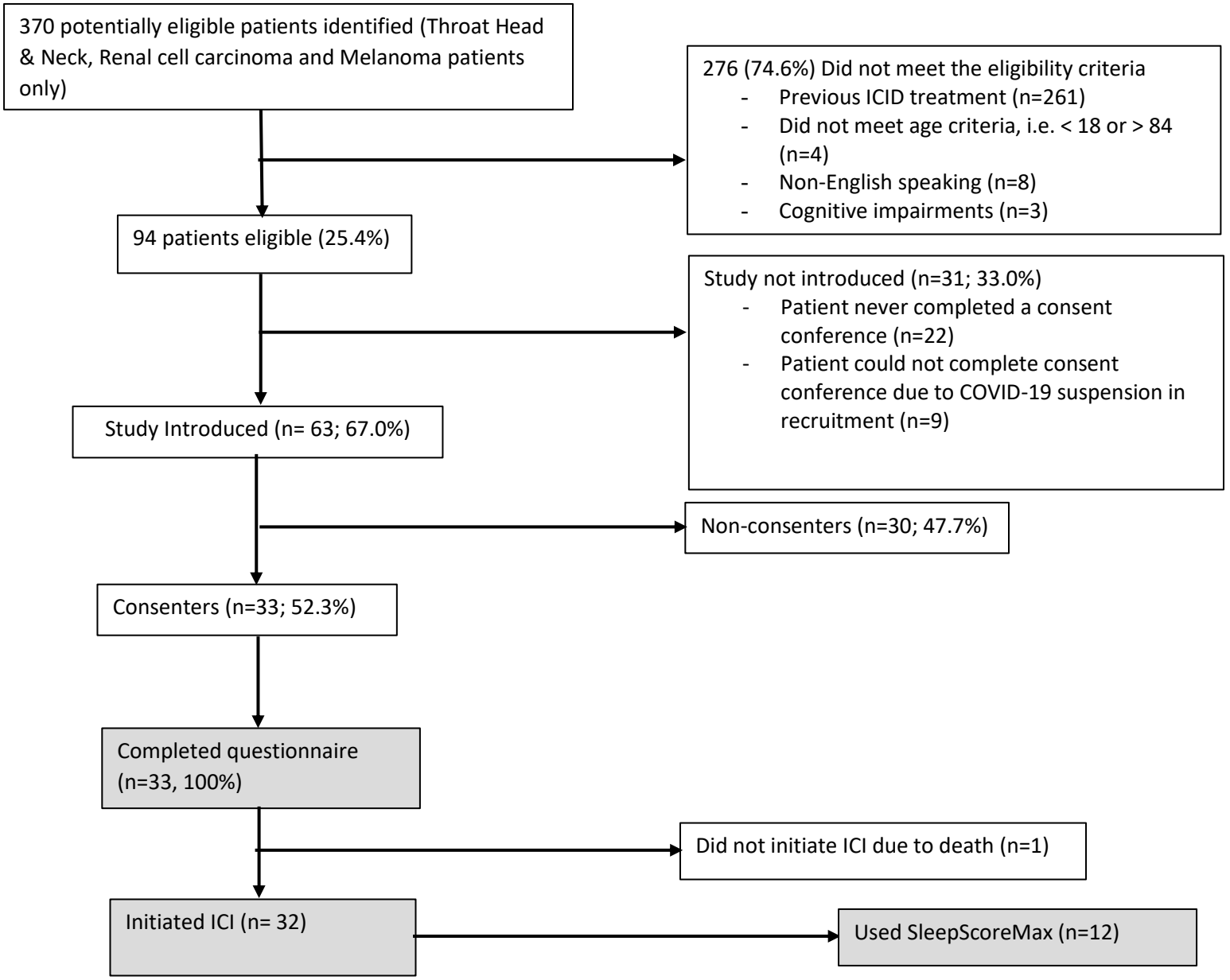


Figure 3.2: LASIR Study recruitment and retention



Tables

Total 3.1: Selected baseline characteristics overall and according to OSA risk in the LASIR cohort, n=33

		Sleep apnea risk	
	Total (n=33)	**Low (n=12)**	**Intermediate/High (n=21)**
Mean age at enrollment (SD)	61.1 (13.4)	58.4 (13.9)	62.5 (13.1)
Males	60.6	16.7	85.7
married, domestic partnered	63.6	50.0	71.4
Education			
< college	30.3	25.0	33.3
College degree	45.5	66.7	33.3
Grad or professional deg	24.2	8.3	33.3
White	84.8	83.3	84.7
Hispanic	3.3	0	4.8
smoking at cancer diagnosis			
Current	3.3	0	4.8
Former	45.5	33.3	52.4
Never	51.5	66.7	42.9
BMI at cancer dx kg/m2 mean (SD)	30.9 (8.1)	29.8 (11.4)	31.4 (5.7)
<25	21.2	41.7	9.5
25-29	30.3	16.7	38.1
30+	48.5	41.7	52.4
Alcohol beverages per week			
median (IQR)	1.1 (0.25, 5)	1.0 (0.25, 6)	1.1 (0.25, 5)
none	18.2	16.7	19.1
>0-6	57.6	58.3	57.1
7+	24.4	25	23.8
Physical Activity (mins/week)			
median (IQR)	405.0 (1.59.3, 819)	400.8 (78.8, 591.5)	474.0 (159.3, 856.5)
Sufficient 150+	18.2	25.0	14.3
In-sufficient 0-149 min	69.7	66.7	71.4
Perceived stress			
median (IQR)	5.0 (3.0, 6.0)	5.0 (2.5, 6.0)	6.0 (3.0, 7.0)
Low stress (<8)	81.8	83.3	81
High stress (8-16)	18.2	16.7	19.1
Self-reported disease history/ medication			
High blood pressure/ hypertension medications	48.5	25.0	61.9
high cholesterol/ Cholesterol medications	39.4	25.0	47.6
Diabetes/ treated diabetes	9.1	0.0	14.3

Categorical variables are in percentages, continuous measures in mean (SD: Standard Deviation) or median (IQR: Inter quartile range), *% may not sum to 100% due to missing data or rounding

Total 3.2: Selected baseline sleep problems in the LASIR cohort, n=33

	Total (n=33)
Primary Sleep Problems	
Sleep Apnea Symptoms	
Daytime sleepiness	21.2
Snore	33.3
Apnea	21.2
Sleep apnea risk (STOP-BANG)	
mean (SD)	3.2 (1.7)
High risk (5-8)	21.2
Intermediate risk (3-4)	42.4
low risk (0-2)	36.4
Self-report sleep apnea diagnosis	18.2
Secondary Sleep Problems	
Insomnia Risk (0-20)	
mean (SD)	9.5 (4.3)
Clinically significant insomnia (9+)	57.6
Overall sleep quality	
Sound	27.3
Average	39.4
Restless	33.3
Sleep latency, min	
0-14	69.7
15-30	12.1
31+	18.2
Total sleep duration, h	
<=6 or 9+	54.6
7-8	45.5
Chronotype	
Morning	63.6
Evening	36.4

*% may not sum up to 100% due to missing data or rounding
SD: Standard Deviation

Total 3.3: Selected baseline cancer attributes in the LASIR cohort, n=33

		Total (n=33)
Cancer site		
Melanoma		51.5
Squamous Cell Carcinoma		6.1
Renal Cell Carcinoma		21.2
Lung		21.2
Cancer attributes at diagnosis		
T-stage		
	T-0	6.1
	T-1	12.1
	T-2	21.2
	T-3	27.3
	T-4	6.1
	Unknown (TX)	6.1
N-stage		
	N0	15.2
	N1	27.3
	N2	9.1
	N3	3
	Unknown (TX)	18.1
M-stage		
	M0	57.6
	M1	42.4
number of metastatic sites, mean (SD)		1.1 (0.8)
summary stage		
	I	9.1
	II	9.1
	III	33.3
	IV	39.4
Cancer treatment prior to ICI		63.6
Chemotherapy		23.8
Radiotherapy		19.1
Surgery		66.7
Gene therapy		9.5
Adjuvant		4.8
Other		
	high-dose interleukin-2	4.8

*% may not sum to 100% due to missing data or rounding
SD: Standard Deviation

Table 3.4: Distribution of ICI attributes in the LASIR Cohort, N=32

	Total (n=32)
Months from cancer diagnosis to ICI, Mean (SD)	
ICI initiated	
Cemiplimab (PD-1)	6.3
Ipilimumab (CTLA-4)	6.3
Nivolumab (PD-1)	43.8
Pembrolizumab (PD-1)	43.8
ICI initiated with chemo	16.1
Years from cancer dx to ICI, median (IQR)	0.4 (0.2, 2.3)
Total ICI initiation mean (SD)	6.8 (3.6)
% <4 infusions	18.8
% 4-5 infusions	9.4
% 6+ infusions	71.9
Changed ICI	43.8
months between 1st and changed ICI (time to ICI failure)	2.9 (1.31)
Time between 1st and last Infusion, months (SD)	4.00 (2.08)
% Incidence Adverse Events	
Dry mouth	18.8
Hypothyroidism	25.0
Hypophysitis	12.9
Severe Diarrhea	21.9
adrenal insufficiency	12.5
Pneumonitis	9.0
immune-related hepatitis	9.0
Rash & vitilgo	46.9
Rheumatologic symptoms	19.4
General body pain	39.3
Allergic reactions	6.3
Hospitalization	9.4
Total events, mean (SD)	3.3 (2.5)
% CT overall CT impressions	
Favorable response	25.8
Disease progression	32.3
Stable disease	41.9
Vital Status	
% Death *	12.5
Time from cancer dx to death, months mean (SD)	13.2 (7.7, 39.8)
Time from ICI to death, months mean (SD)	2.1 (1.32, 5.2)

SD: Standard Deviation

Table 3.5: Prevalence ratios (PRs) for tumor aggressiveness comparing across sleep problem groups in the LASIR cohort, N=33

	Diagnosis M-stage (M0 vs M1)	
	PR (95%CI)	***p-values***
Primary Sleep problems		
Sleep apnea Risk (0-8)		
Total score (1-unit increment)	1.15 (0.74, 1.77)	0.533
Intermediate risk (3-4)/High risk (5-8)	1.01 (0.28, 3.67)	0.990
low risk (0-2) (ref)	1	
Secondary sleep problems		
Insomnia Risk (0-20)		
Total score (1-unit increment)	1.08 (0.97, 1.20)	0.147
Clinically significant insomnia (9+)	1.24 (0.49, 3.14)	0.649
Not clinically significant (<9) (ref)	1	
Sleep latency, min		
15+	3.58 (1.74, 7.35)	0.001
0-14 (ref)	1	
Total sleep duration, h		
<=6 or 9+	0.70 (0.25, 1.93)	0.489
7-8 (ref)	1	
Chronotype		
Morning	0.23 (0.09, 0.58)	0.002
Evening (ref)	1	
Overall sleep quality		
Restless	2.25 (0.51, 9.91)	0.285
Average	1.31 (0.40, 4.28)	0.659
Sound (ref)	1	

M-stage - Metastatic cancer stage
Adjusted for age, male, BMI

Table 3.6: Total Association between Sleep problems and number of ICI infusions in the LASIR Cohort, N=32

	Number of infusions (6+ vs <6)	
Tumor attributes	**OR (95%CI)**	***p-values***
Sleep apnea risk (STOP-BANG, 0-8)		
Total score (1-unit increment)	0.72 (0.37, 1.39)	0.335
Intermediate risk (3-4)/High risk (5-8)	0.27 (0.02, 3.41)	0.308
low risk (0-2) (reference)	1	
insomnia Risk (WHIIS, 0-20)		
Total score (1-unit increment)	0.77 (0.59, 1.02)	0.071
Clinically significant insomnia (9+)	0.23 (0.03, 1.60)	0.138
Not clinically significant <9 (reference)	1	

WHIIS: Health Initiative Insomnia scale
STOP-BANG: Snoring, daytime Tiredness, observed apnea, Blood Pressure, Body Mass Index, Age, Neck circumference and Gender
Treatment tolerability: Number of infusions >6
Adjusted: age, sex, prior cancer treatment

Supplementary tables:

Table 3.1: SleepScoreMax data summary in the Lifestyle Attributes and Sleep in LASIR study, N=12

SleepScoreMax Data	N =12
Total sleep time, mean (SD) hrs.	6.4 (1.7)
Sleep latency, mean (SD) mins	18.6 (21.5)
Wake time, mean (SD) hrs.	1.2 (1.0)
Light, mean (SD) hrs.	4.1 (1.2)
Deep, mean (SD) hrs.	1.3 (0.6)
REM, mean (SD) hrs.	1.0 (0.5)
Sleep Score in %, mean (SD)	80.2 (17.8)
# of wake times, mean (SD)	5.1 (3.0)

REM: rapid eye movement, SD: Standard Deviation

Table 3.2: Total Association between Sleep problems and CT impressions, unchanged ICI status and TTF in the LASIR Cohort, N=32

	CT impressions (favorable vs stable or progression)		unchanged ICI		Survival Analysis (TTF)	
Tumor attributes	**OR (95%CI)**	***p-values***	**OR (95%CI)**	***p-values***	**HR (95%CI)**	***p-values***
Sleep apnea risk (STOP-BANG, 0-8)						
Intermediate risk (3-4)/High risk (5-8)	5.25 (0.45, 61)	0.186	0.27 (0.03, 2.74)	0.269	6.16 (0.70, 54.20)	0.101
low risk (0-2) (reference)	1		1		1	
Insomnia Risk (WHIIS, 0-20)						
Clinically significant insomnia (9+)	0.64 (0.13, 3.19)	0.586	0.71 (0.14, 3.47)	0.669	2.00 (0.30, 13.8)	0.483
Not clinically significant <9 (reference)	1		1		1	

WHIIS: Health Initiative Insomnia scale

STOP-BANG: Snoring, daytime Tiredness, observed apnea, Blood Pressure, Body Mass Index, Age, Neck circumference and Gender

Adjusted: male, sex, age at, prior cancer treatment

TTF: time to treatment failure (changed ICI due to disease progression)

Treatment tolerability: Ct impressions, changed immune checkpoint inhibitor, TTF

Chapter 4: Conclusions

Despite the mostly null results in the CHS study (**Chapter 2**), there were a few notable elevated and inverse relationships between sleep problems (SP) and cancer-site specific incidence and cancer-specific mortality. Our results also showed regardless of how SP is modeled (baseline or longitudinally), the associations remain consistent. Furthermore, our results have also added to the growing body of evidence for the heterogenous physiologic effect of sleep problems across cancer sites. Therefore, future larger community based prospective studies should address more cancer site and molecular type-specific associations, and improved SP self-report documentation over time are needed.

Additionally, the LASIR study (**Chapter 3**) gives insights into the potential burden and impact of SP on tumor aggressiveness and immune checkpoint inhibitor (ICI) treatment response. This is the first study that has considered the potential role of SP in relation to ICIs outcomes. As such, the results could inform larger-scale observational studies with an ultimate goal of informing clinical trials focused on finding effective sleep quality improvement interventions in ICI cancer treatment populations. Finally, if our findings are confirmed in future larger studies, we hope it could lead to a shift in cancer ICIs paradigms, motivating greater emphasis on patient sleep patterns and promoting the recognition and management of sleep disorders in ICI cancer population.

Finally, our project is methodologically innovative. We incorporated multiple dimensions of sleep problems in cancer patients with multiple outcomes in recognition that sleep problems may simultaneously promote cancer development and progression. Furthermore, our project is conceptually innovative. We recognized that ICIs are becoming an increasingly available option for a growing population of cancer patients and has resulted in a growing interest in identifying factors important to ICI response. However, most of this effort has focused on tumor biomarkers

with little to no consideration for lifestyle factors. As a result, the LASIR Study is the first to quantify the burden of SP in a cancer ICI cohort and the potential role in relation to ICI outcomes.

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