Cytokine Profiles and Sleep Health in Relapse and Remission Phases of Relapsing-Remitting Multiple Sclerosis: A Push to Improve Quality of Life for Multiple Sclerosis Patients in Relapse

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Project Summary

Multiple sclerosis, or MS, is an autoimmune disease of the central nervous system that causes fatigue, numbness, and muscle weakness. There are many forms of MS, but in this study, we will focus on the most common form: relapsing-remitting. There are two distinct phases of relapsing-remitting multiple sclerosis. When a patient starts experiencing more severe symptoms and their MS is appearing to progress, their state is defined as a relapse. Otherwise, their state is defined as remission. For a few decades, research has shown a bidirectional relationship between sleep and MS: as one worsens, so does the other. Nonetheless, the distinctive impacts of a relapse on sleep quality are still not well understood. In light of this knowledge gap, we plan to address two major research questions. First, we want to study the directional relationship between sleep health, inflammatory molecules, and MS relapses. Moreover, we aim to examine differences in the levels of the inflammatory molecules during daytime and nighttime hours. We will recruit a sample of patients diagnosed with relapsing-remitting multiple sclerosis and healthy controls. All participants will fill out a self-report questionnaire to measure six dimensions of their sleep, and researchers will collect blood samples to assess inflammation levels during the day and at night. Following these procedures, we expect to see increased levels of inflammatory molecules and decreased sleep health in patients in a relapse compared to patients in remission and other control individuals. The relationship between sleep health and inflammation is anticipated to be stronger during a relapse than during remission. Additionally, we foresee increased inflammatory molecules in the day and night and decreased anti-inflammatory molecules during the day in relapses compared to remission and controls. These anticipated results suggest that considerable, defining characteristics may differ in relapse and remission states of relapsing-remitting MS. In particular, the levels and temporal patterns of inflammation may affect sleep differently in relapses and remission. With more research, the specific definitions of relapse and remission can be refined, and treatments can be adapted to improve sleep quality and quality of life for patients in a relapse.

Project Narrative

Relapsing-remitting multiple sclerosis, or RRMS, affects hundreds of thousands of individuals worldwide, decreasing their societal productivity and quality of life with each relapse. A gap in our scientific knowledge of the underlying biological bases for inflammatory relapses has prevented progression in our treatment of the lowered sleep quality and quality of life for individuals in relapse. Our research intends to develop a more coherent understanding of the relationship between sleep health, relapses, and inflammation so as to form a foundation for future research to explore the temporal effects of inflammation and develop better treatments for relapses.

Specific Aims

As of 2019, MS affected 913,925 people in the United States, and even more worldwide (Wallin et al., 2019). The main symptom of MS is motor decline, but in recent years, MS has been connected to myriads of other symptoms, including diminished sleep quality (Caminero & Bartolomé, 2011).

Aim One: Identify a relationship among serum cytokine levels, sleep health, and relapse phase in RRMS.

Previous research demonstrates a relationship between increased pro-inflammatory cytokine levels and poor sleep quality (Heesen et al., 2006). Moreover, sleep disturbances have also been proposed as triggers for relapses (Sahraian et al., 2017). To assess the relationship between these three variables, we will collect cytokine samples and assess sleep health in three groups: MS patients in relapse, MS patients in remission, and age and sex-matched controls. Participants will give venous daytime blood samples, and ELISA chemiluminescent assay will assess cytokine levels. Sleep health will be assessed with a brief survey. We hypothesize that MS patients in relapse will have lower sleep health scores, higher levels of pro-inflammatory cytokines, and lower levels of anti-inflammatory cytokines. In addition, relapse state will act as a moderator for the relationship between pro-inflammatory cytokines and sleep health.

Sleep health has been shown to influence physical quality of life for MS patients (Lobentanz et al., 2004). This research will aid in our understanding of the biological bases of poor sleep in MS, establish empirical levels for future MS cytokine analysis, and kickstart our long-term goal to develop new, better-targeted treatments for patients in relapse.

Aim Two: Define differences in the daytime and nighttime cytokine profiles in RRMS.

In individuals without autoimmune diseases, pro-inflammatory cytokines including IL-6, IL-1, IL-8, IL-17, INF-γ, and TNF-α tend to have higher levels at night (van Leeuwen et al., 2009; Hohagen et al., 1993; Huang et al., 2017). Contrastingly, anti-inflammatory cytokines such as IL-10 and IL-4 are higher during the day and inhibit sleep (Krueger, Majde, & Rector, 2017). The exact variations in pro- and anti-inflammatory cytokines at different times of the day are not well understood in RRMS. To investigate this question, we will use the same sample and methodological procedures as with aim one, but blood collection will now occur at two points for all participants: before bed and after awakening. We hypothesize increased levels of pro-inflammatory cytokines at both time points and decreased anti-inflammatory cytokine levels during the day in relapse compared to remission and controls.

Thus far, little research has been conducted with regard to the temporal release of cytokines in relapse and remission phases of RRMS. Differences could prove important in the clinical distinction between the two phases and the types and timings of treatment in each phase. This research builds the foundation for future in-depth analysis of temporal cytokine release and inflammation in RRMS.

Research Strategy

Multiple sclerosis is an autoimmune disease of the central nervous system defined by increased blood brain barrier permeability, influxes of oligoclonal immune cells, and eventual degradation of oligodendrocytes and impaired neural conduction (van Waesberghe et al., 1999; Wucherpfennig, Newcombe, Keddy, Cuzner, & Halfer, 1992). The precise progression of the disease varies by individual and depends on the diagnosis of one of four sub-types: relapsing-remitting, primary progressive, secondary progressive, or progressive relapsing. RRMS encompasses 85 percent of all cases and is the main focus of our research (Lublin & Reingold, 1996). RRMS receives its name from its unique and cyclical progression: increased inflammation, a relapse, alternating with decreased inflammation, remission (Lublin & Reingold, 1996).

Generalized fatigue and daytime sleepiness are long-observed symptoms of MS, and sleep has even been proposed as a primary focus point of interdisciplinary treatment plans for MS (Stanton, Barnes, & Silber, 2006). Within the last decade, myriads of studies have drawn connections between decreased sleep quality and MS. For example, sleep disorders and disturbances such as restless leg syndrome and obstructive sleep apnea are more common in MS patients than in the general population (Caminero & Bartolomé, 2011). Nonetheless, the underlying biology and causes of the unwanted fatigue and sleepiness in MS are still poorly understood.

The goal of this study is to generate a more comprehensive understanding of the biological bases for sleep disturbances in the two distinct phases of relapsing-remitting multiple sclerosis: relapse and remission. Long-term, this research aims to uncover the driving force behind sleep disturbances and apply this knowledge to the modification of therapeutic treatments for relapses. Overall, our goal is to build the foundations on which future research can help patients improve their sleep quality and, by proxy, their quality of life.

Significance

As of 2019, in the United States alone, over 900,000 individuals had been diagnosed with MS, a vast majority of whom had RRMS (Wallin et al., 2019). Relapses result in a major increase in inflammation and an escalation of symptoms including numbness, cognitive difficulties, and loss of mobility (Cavallo, 2017). Beyond the timeframe of the relapse, symptoms can linger long-term. A relapse takes a massive mental and physical toll on a patient and their support system. Matt Cavallo, an author who documents his experiences with MS explains his relapse symptoms: “Memory problems are the worst. People can see my physical limitations right now, but not the brain fog that is taking a toll on me cognitively […] I [sleep] on my back because it’s the only place I can find momentary comfort” (Cavallo, 2017). From Cavallo’s narrative, one can see the negative physical, mental, and psychological impact of a

relapse on quality of life. This research will help build a foundation on which we can search for methods to improve the quality of life and sleep quality for patients in the midst of a relapse.

Present clinical definitions of relapse and remission require improvement. Clinical diagnosis of MS relapses is routine in modern medicine. To limit costs and time, the diagnosis of a relapse is often completed without an MRI scan. Once a relapse is established, treatment with relapse-management therapies, such as corticosteroids, begins. Without the use of an MRI scan, however, there are frequent misdiagnoses of relapses (Jagnnadha, 2017). These improper diagnoses inflate the effectiveness of acute relapse treatments, because many of the individuals with prescriptions for the treatments are not actually in a relapse. Dr. Avasarala Jagnnadha (2017) suggests an increase in routine MRIs for proper diagnoses of relapses. Although this would improve diagnostic accuracy, this proposed solution is still unappealing in terms of the expense. With further analysis, our research on daytime and nighttime cytokine profiles and sleep health in relapses and remission could be implemented as an accurate diagnostic tool for identifying an acute relapse. For example, a sudden decrease in sleep health in combination with an increased inflammatory cytokine profile, as measured from questionnaire and blood sample respectively, could indicate a relapse and be used as both a predictive and diagnostic tool. Sleep health measures and blood samples can be recorded frequently throughout the disease's progression and are far less time consuming than an MRI scan. Additionally, the use of these relapse markers is far less expensive than routine MRI scans and could provide economic relief to MS patients and their families, who face an average of over $15,000 in medical expenses annually, almost $13,000 more than individuals without MS (Jennum, Wanscher, Frederiksen, & Kjellberg, 2012).

Even according to current diagnostic criteria, relapses and remission define drastically different stages of RRMS on a physiological level. Treatment of each phase, therefore, should be individualized according to its distinct biological bases. The relative daytime levels of pro-inflammatory and anti-inflammatory cytokines in RRMS have been analyzed in depth. Nevertheless, there remains a major gap in our knowledge regarding potential changes to the rhythmic release of cytokines in RRMS. In order to fully understand the inflammation, we need to determine if the temporality of cytokine release changes. Changes to the temporal pattern of cytokine release could generate further knowledge about the biological bases for poor sleep in a MS. If the temporality has been adjusted, a treatment that changes the general cytokine levels may not prove effective for improving sleep health in MS patients. Instead, treatments need to be temporally targeted to readjust the rhythmic release of pro-inflammatory and anti-inflammatory cytokines. To date, rhythmic release of cytokines has never been compared between relapse and remission phases of RRMS. Our study begins this temporal analysis by analyzing differences at two time points, day and night. With time, our foundational data and future data could change how the field defines relapses and remission in RRMS.

Innovation

Research on MS and sleep has grown over the last two decades, and we have an improved understanding of the types of sleep disruptions that affect MS patients. Nonetheless, there are aspects of the relationship between sleep and MS that we have yet to explore. This study builds on our previous knowledge of the relationship between cytokine profiles and sleep in four main ways.

Primarily, by focusing specifically on differences between relapse and remission phases in MS, this study goes beyond the previous analyses of MS, which do not tend to compare the two phases. The relapse phase of RRMS is the most devastating to the patients' physical and mental well-being. Improving our understanding of the difference between these phases will be critical to providing better treatment options for patients, especially those experiencing symptoms of an acute relapse.

Our first aim uses a novel measure for sleep quality: sleep health. A majority of previous research on sleep in MS focuses on sleep disorders (Caminero & Bartolomé, 2011). In contrast, sleep health (Buysse, 2014) positively frames our analyses of sleep, and rather than focusing on solely on disorders and disease in sleep, it highlights the positive impacts that healthy sleep can have in MS patients' lives. Patients diagnosed with MS face the distressing realities of their diagnosis on a daily basis. In fact, MS patients have a higher frequency of mental health disorders, including major depressive disorder and generalized anxiety disorder, when compared to the general population (Chwastiak & Ehde, 2007). Rather than bombarding these patients with continued negative feedback, the use of sleep health measures attempts to positively frame their sleep quality. Furthermore, sleep health measures have high ecological validity and serve to help make the results in this study more understandable to both patients and physicians in clinical environments. Sleep is a multi-dimensional concept, so it requires a multi-dimensional measure. In the past, sleep quality has mainly been assessed in terms of duration and deficiencies, yet we now understand that sleep is affected by many other factors (Buysse, 2014). Sleep health addresses the multi-dimensionality of sleep quality by addressing multiple metrics. By analyzing efficiency, timing, satisfaction, alertness, and regularity in addition to duration, sleep health helps generate a more wholistic picture of someone's sleep (Buysse, 2014). Sleep health measures, which are comprehensive, positively framed, and easily understood, are a much-needed change of pace for MS patients, who normally face discouraging and confusing evaluations.

The combination of immunoassay cytokine quantification and sleep health measures also gives our study a novel quality. Cytokine profile and sleep quality have each been studied extensively in separate investigations, but previous literature illustrates that their combined measurement is relatively unstudied in the MS population. Through a combined study of these two variables, we will be able to establish a more straightforward relationship between the induced inflammation and decreased sleep

quality in MS and eventually develop treatment options directly aimed at improving sleep quality in MS patients.

Finally, and most importantly, our second aim uses an innovative methodological approach to study the relationship between sleep health and cytokine release. No study to date has made a connection between the temporal release of cytokines and the relapse and remission phases of RRMS. Cytokine profiles are regularly examined and used in analyses of RRMS progression, but serum levels collected during the day are normally studied. Under normal circumstances, sleep is influenced by cyclical changes in cytokine profile, so if the rhythmic patterns of cytokine release are altered in MS, they could drive the unwanted changes in sleep quality. Therefore, the comparison of daytime and nighttime cytokine levels could provide information about the underlying mechanisms for the decreased sleep health scores in relapses, as studied in aim one.

Approach

Participants

We will recruit participants according to the following inclusion criteria: extended disability status scale, or EDSS, scores less than or equal to six (Kurtzke 1983), an official diagnosis of the relapsing-remitting form of MS from a physician, and a diurnal activity schedule (no shift workers). The EDSS quantifies disability in MS, and scores greater than six indicate that the disease has progressed considerably. Patients with higher scores have been excluded because their symptoms are not comparable to patients with lower EDSS scores (Kurtzke 1983).

Participants will receive referrals from their neurologists for participation. All MS participants will be allowed to continue their current course of disease-modifying therapies (interferon-beta, glatiramer acetate, etc.), but the use of relapse management therapies, such as corticosteroids, will not be permitted during relapse data collection. Immediately after data collection, however, corticosteroid treatment can begin again. Corticosteroids and comparable relapse management therapies have been shown to significantly impact pro-inflammatory cytokine levels in RRMS relapses, making the cytokine profile appear more like that of the remission phase (Matsushita et al., 2013). Relapse management therapies are intended to reduce unpleasant symptoms, so without corticosteroids, the participants will likely begin to develop symptoms, such as numbness, tingling, and cognitive fogginess. The well-being of our participants is a concern during this time period. To decrease the distress of the participants, the measurements will all occur with an 18-hour period, a standard amount of time a patient may need to wait for home healthcare treatment anyway (Cavallo, 2017). Moreover, the course of their disease will not be impacted by the lack of corticosteroids, so there should be no persisting symptoms once corticosteroid treatment begins again.

MS patients in the relapse and remission groups are the same individuals at different time points. Control participants will be age- and sex-matched to the MS participants. Both age and sex have been shown to impact many aspects of sleep including sleep latency, efficiency, and architecture, so it is important that there are no major differences in these features for the controls and MS patients (Mallampalli & Carter, 2014; Ohayon, Carskadon, Guilleminault, & Vitiello, 2004).

Methods

Before any measurements are taken, the MS participant is diagnosed in one of two phases, relapse or remission. They are diagnosed by their neurologist with an MRI scan, in accordance with the McDonald diagnostic criteria and are then referred to the study by the neurologist (Polman et al. 2011). After diagnosis, the participant begins the first period of data collection within 2 days. This short duration of only two days attempts to ensure that the participant's disease status does not change between the diagnosis and data collection period.

To start the study, each participant signs informed consent documents. Each participant is reminded of their voluntary participation and ability to withdraw from the study at any time without penalty. On the first day of data collection, participants are asked to arrive at the lab approximately 60 minutes before their bedtime. Each participants' arrival to the lab varies in accordance with their normal bedtime, meaning that each individual may not arrive at the lab at the same time. Researchers will then take a three-milliliter blood sample. Participants then return to their homes and proceed with their normal routines for the evening. After awakening, participants will fill out the sleep health questionnaire, consisting of six questions (See Appendix A). Again, participants will proceed with their normal daily routines, but now they must return to the lab before 12PM for another blood collection. Researchers take a three-milliliter blood sample within 30 minutes of 12PM. This blood collection marks the completion of the data collection period. For MS participants, the study is not complete, however. Participants with MS wait until they are diagnosed in the opposite stage of RRMS from their previous data collection period. For instance, if the participant was in remission during the first period of data collection, then they would wait for a diagnosis of a relapse phase from their neurologist before completing their second period of data collection. The neurologist who refers the participant to the study must be actively monitoring the MS patient's status between data collection periods. The second period of data collection follows identical protocol to the first, with the only difference being the relapse state of the participant. Likewise, controls follow identical protocol, except they do not need to complete data collection more than one time, nor do they require a referral from a neurologist. Note that this protocol fulfills all the necessary data collection for aims one and two, but different data will be used for each aim's hypothesis testing.

Sleep Health Measures

To evaluate sleep health, participants fill out a questionnaire designed to assess the six metrics of sleep health (See Appendix A). The questionnaire consists of six questions that are each answered on a five-point Likert scale. All scores for each question are added together to generate a comprehensive score ranging from 0 to 30. Scores at the low end of the range (0) indicate poor sleep health, while scores at the high end of the range (30) indicate good sleep health (Buysse, 2014).

Each participant will evaluate their sleep health only one time per data collection period. Despite the statistical benefits, we do not include more days of data collection because the longer duration could pose significant mental and physical distress for MS patients facing a relapse without their relapse-management therapies.

Blood Collection and ELISA

With blood collection, proper sterilization precautions are taken with an alcohol swab to prevent contamination from skin flora. Likewise, precautions are taken to properly dispose of the needles in biohazard waste, and clean gloves are worn during each collection. Cytokine profiles are assessed from the venous blood samples. The three milliliter blood samples are put into centrifuge tubes and spun down at 3,000 rotations per minute at $4^{\circ C}$ for 10 minutes. The supernatant, containing the liquid serum is carefully removed with Pasteur pipettes for further analysis. All serum samples are stored at $-80^{\circ C}$ until inspection, once all data collection is complete (Periavian et al. 2016). Sequential ELISA chemiluminescent assays are performed on the serum samples to assess levels of each cytokine: IL-1, IL-6, IL-17, IFN-γ, TNF-α, IL-8, IL-4, and IL-10 (See Table 1). We will follow the protocol for sequential ELISAs as outlined by Chiswick, Duffy, Japp, and Remick (2012) to conduct our tests.

Statistical Analyses

For our first aim, we hypothesized that MS patients in relapse would have lower sleep health scores, higher levels of pro-inflammatory cytokines, and lower levels of anti-inflammatory cytokines compared to both controls and MS patients in remission. For our second aim, we hypothesized increased levels of pro-inflammatory cytokines at night and during the day in a relapse compared to remission and controls. Additionally, we hypothesized decreased anti-inflammatory cytokine levels during the day in a relapse compared to remission and controls (See Table 1).

Table 1

Cytokine Predicted Levels

Cytokine	Type	Night Relapse	Day Relapse
IL-1	Pro	+	+
IL-6	Pro	+	+
IL-17	Pro	+	+
IFN- γ	Pro	+	+
TNF- α	Pro	+	+
IL-8	Pro	+	+
IL-4	Anti	x	-
IL-10	Anti	x	-

Note. An x indicates no prediction. Pro- and anti- refer to the inflammatory categorization of the cytokines. Predicted levels in Night Relapse and Day Relapse refer to an increase (+) or decrease (-) relative to remission and control levels at the same time of day.

To test our hypotheses, we will calculate mean levels of each cytokine and mean sleep health scores for all three groups: control, remission, and relapse. Then, to address our first aim, we will conduct one ANOVA to look at differences in sleep health between relapse and remission conditions. Likewise, another ANOVA will be conducted to look at differences in sleep health between relapse and control conditions. These two analyses of variance will determine if there are any significant differences in sleep health for the relapse condition. A p-value of 0.05 is used as the threshold for significance in all testing, and any values below will indicate significant differences. Moreover, a Cohen's d is calculated for each ANOVA to determine effect sizes (Cohen's d = $\frac{\mu_1-\mu_2}{s_{pooled}}$) (Rosnow & Rosenthal, 2003).

The analysis of cytokines requires a different statistical test, a MANOVA, or a multivariate ANOVA. For our first aim, we will conduct a MANOVA to look for differences in each cytokines' levels for relapse, remission, and control groups. For our second aim, we will conduct two separate MANOVAs, one for daytime and one for nighttime, to assess the differences in the levels of each cytokine in the three groups. Note that we are looking to see differences between groups at nighttime and between groups during the day, not differences between daytime and nighttime cytokine profiles themselves. Our use of a MANOVA to simultaneously compare levels of cytokines helps to reduce the type I error associated with repeated ANOVAs (Genser, Cooper, Yazdanbakhsh, Barreto, & Rodrigues, 2007). An effect size, η^2, is also calculated for each MANOVA ($\eta^2 = \frac{d^2}{d^2+4}$) (Steyn & Ellis, 2009).

For our moderator analysis in aim one, we hypothesize that relapse phase will act as a moderator for the relationship between sleep health and pro-inflammatory cytokine profile. Specifically, we hypothesize that the relationship between sleep health and inflammation is stronger during a relapse than

during remission. To study this relationship, we first need to find the mean levels of pro-inflammatory cytokines (pg/mL) in each participant. We will then calculate two Spearman's r correlation coefficients for the relationship between sleep health scores and pro-inflammatory cytokine levels, one in relapse and one in remission. Then, we will complete a Fisher's r to z transformation on the pair of Spearman's r coefficients to determine if there was any difference between the correlations for relapse and remission. A value for Cohen's q is calculated to determine the effect size (Cohen's q = $Z_{r1} - Z_{r2}$) (Rosnow & Rosenthal, 2003).

Potential Problems and Challenges

The sleep health metrics developed by Buysse (2014) have shown high validity for predicting and identifying many health problems, including mental health issues like clinical depression (Furihata et al., 2017). Nonetheless, sleep health measures have not been enabled in MS research to date. As a result, there are no analogous values to which we can compare our outcomes for each sleep health metric. Without comparable values, it may be difficult to ascertain whether the reported sleep metrics from our study correctly represent the actual sleep quality our MS participants experience, or if the awareness of relapse state is inflating the subjective evaluation of poor sleep quality.

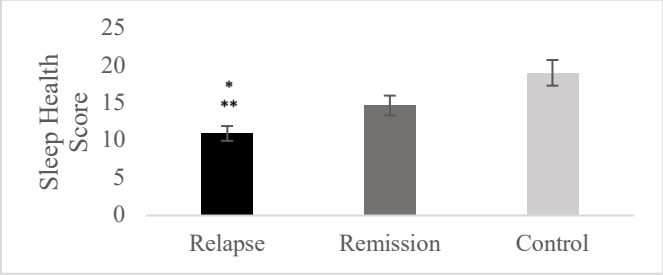
Additionally, due to the detailed protocol and small population of study, it may be difficult to obtain a large sample size. Previous research with cytokine profiling in RRMS patients tend to have relatively small sample sizes. Hollifield et al. (2003) only had 15 patients, while Periavian et al. (2016), one of the studies with the most participants, still had less than 50. Our within-participant design attempts to increase statistical power and decrease the required sample size. Nevertheless, if our study follows trends similar to those of previous research, it will still be difficult to secure a large sample.

Expected Results

For our first aim, in accordance with our hypothesis, we expect to see a significant relationship between sleep health, pro-inflammatory and anti-inflammatory cytokine profile, and relapse state in our RRMS patients. Specifically, patients in a relapse have lower sleep health scores (See Figure 1), higher levels of pro-inflammatory cytokines, and lower levels of anti-inflammatory cytokines (See Figure 2) compared to patients in remission and controls. Furthermore, relapse condition acts as a moderator for the relationship between sleep health and levels of pro-inflammatory cytokines. In other words, we expect that the relationship between pro-inflammatory cytokine levels and sleep health will be stronger in relapse than in remission, and that the Spearman's r values calculated for the relationship will be significantly higher in relapse than in remission.

Figure 1

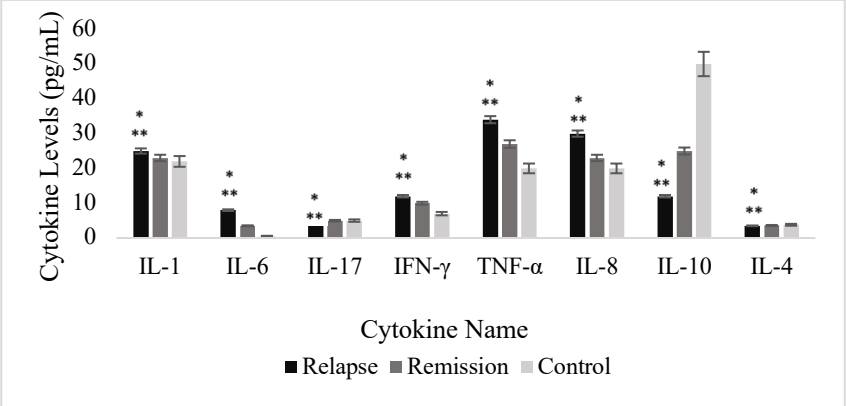
Mean Sleep Health Scores in Relapse, Remission, and Controls



Note. * indicates significant difference from remission. ** indicates significant difference from control. Error bars represent standard deviations.

Figure 2

Cytokine Profile in Relapse, Remission, and Controls



Note. * indicates significant difference from remission for the same cytokine. ** indicates significant difference from control for the same cytokine. Error bars represent standard deviations.

These results suggest that relapse state of an individual impacts both sleep health and biological inflammation. More specifically, we see that relapse state correlates with higher inflammation levels and lower sleep quality compared to remission and controls. This data gives a framework of empirical cytokine levels for relapse and remission to which future studies can compare. Although more research should be conducted to reinforce these cytokine levels, our initial data outlines cytokine levels for relapse and remission classification in RRMS (See Table 2). It's important to note, however, that these cytokine levels alone should not be the only indicator of relapse state. Using biomarkers, such as cytokines, should always have the eventual goal of improving clinical outcomes and should be assessed alongside the patients' reported symptoms (Strimbu & Tavel, 2010). From our data, it seems further assessment of sleep health, in addition to cytokine profile, could help improve the accuracy of relapse identification.

Table 2

Cytokine Levels for Multiple Sclerosis Relapses and Remission

Cytokine	Range for Relapse (pg/mL)	Range for Remission (pg/mL)
IL-1	21.16 – 24.84	24.25 – 25.75
IL-6	7.76 – 8.24	3.22 – 3.78
IL-17	6.79 – 7.21	4.60 – 5.40
IFN- γ	11.64 – 12.36	9.20 – 10.80
TNF- α	32.98 – 35.02	24.84 – 29.16
IL-8	29.1 – 30.9	27.6 – 32.4
IL-4	11.64 – 12.36	23.0 – 27.0
IL-10	3.61 – 3.40	3.42 – 3.78

Note. The range of cytokine levels for remission and relapse span one standard deviation in either direction of the mean for that cytokine.

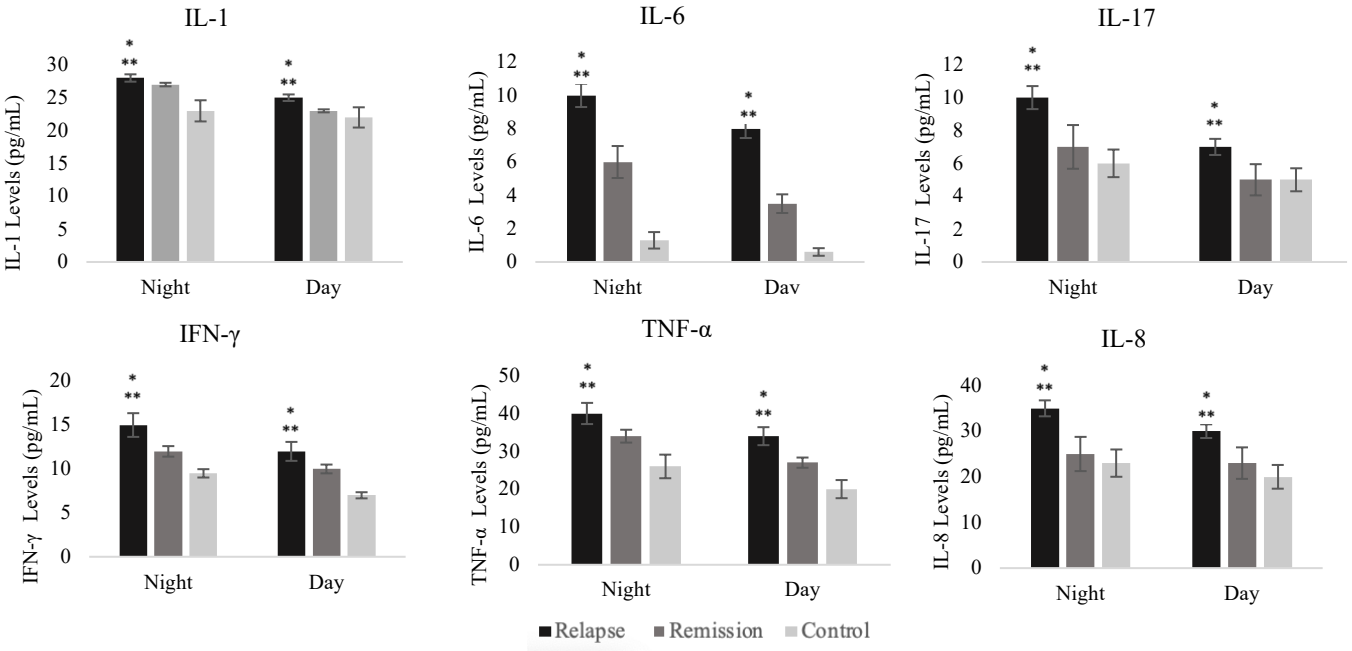
The distinct differences in sleep health and cytokine profiles in relapses and remissions indicates that sleep health measures, cytokine levels, or a combination of the two may be defining features of the two stages of RRMS. Sleep health scores or cytokine profiles could be used as indicators for relapse state and, with further research, could even be utilized as potential diagnostic tools for relapse state identification. Instead of using MRI scans to assess disease state and progression, the levels of cytokines from blood samples and sleep health scores could be used to identify relapse and remission with high degrees of accuracy, at lower cost, and with less participant burden.

In our second aim, we anticipate differences in the nighttime and daytime cytokine profiles for patients in relapse compared to patients in remission and controls. In specific, daytime cytokine profiles will have higher levels of pro-inflammatory cytokines (See Figure 3) and lower levels of anti-inflammatory cytokines (See Figure 4) in relapse compared to remission and controls. Likewise,

nighttime cytokine profiles will also have higher levels of pro-inflammatory cytokines compared to remission and controls (See Figure 3).

Figure 3

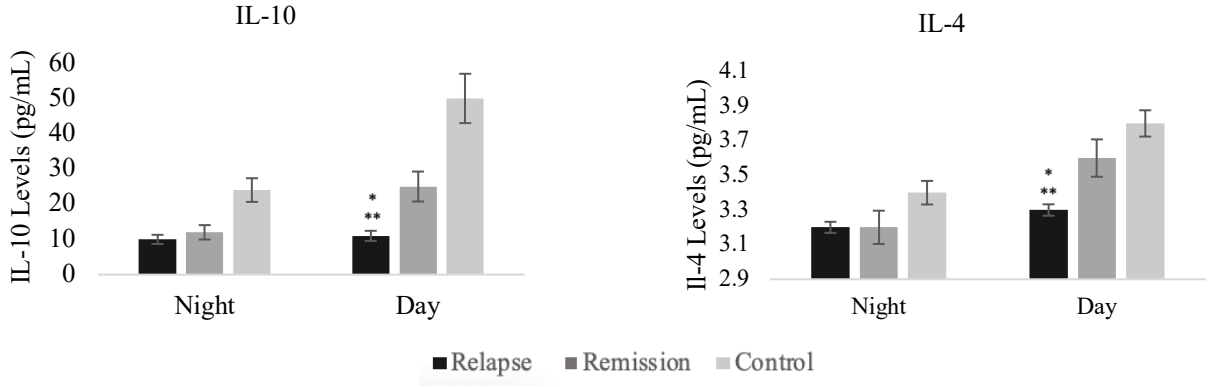
Pro-inflammatory Cytokine Profiles During the Day and Night



Note. * indicates significant difference from remission for the same time of day. ** indicates significant difference from control for the same time of day. Error bars represent standard deviations.

Figure 4

Anti-inflammatory Cytokine Profiles During the Day and Night



Note. * indicates significant difference from remission. ** indicates significant difference from control. Error bars represent standard deviations.

From these results, we see that the relapse state of MS impacts the daytime and nighttime cytokine profiles differently. During the day, there is a decrease in the normal anti-inflammatory state alongside an increase in the pro-inflammatory state that is normally not present. Similarly, at night there is also an increase in the normal pro-inflammatory state. Clearly, the rudimentary statement that "multiple sclerosis increases inflammation" does not encompass all of the complexities of the relationship between relapse state and cytokine profile. The varying levels of pro- and anti-inflammatory cytokines at different time points necessitate further research. Temporal dissection of cytokine secretion is required in order to understand the underlying biological nature of the inflammatory immune responses in RRMS, specifically with regard to how it affects sleep, a process profoundly impacted by changes in inflammation.

Appendix A: 5-Point Likert Scale for Assessment of Sleep Health

Questions developed from Buysse (2014).

Question 1: Are you satisfied with your sleep?

1	2	3	4	5
Almost Never	Seldom	Sometimes	Frequently	Almost Always

Question 2: Do you stay awake all day without dozing?

1	2	3	4	5
Almost Never	Seldom	Sometimes	Frequently	Almost Always

Question 3: Are you asleep or trying to sleep between 2:00am and 4:00am?

1	2	3	4	5
Almost Never	Seldom	Sometimes	Frequently	Almost Always

Question 4: Do you spend less than 30 minutes awake at night? (This includes the time to fall asleep and awakenings in the middle of the night)

1	2	3	4	5
Almost Never	Seldom	Sometimes	Frequently	Almost Always

Question 5: Do you sleep between 6 and 8 hours per day?

1	2	3	4	5
Almost Never	Seldom	Sometimes	Frequently	Almost Always

Question 6: Do you tend to go to bed within 60 minutes of the same time every night?

1	2	3	4	5
Almost Never	Seldom	Sometimes	Frequently	Almost Always

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