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Circadian rhythmicity of symptomatic phenotypes in multiple sclerosis: the CircaMS study protocol and feasibility of biomarker collection

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ABSTRACT

Introduction Multiple sclerosis (MS) is a chronic autoimmune neurological disease with a variable prognosis and unpredictable course. Fatigue, pain and low mood are common symptoms that tend to fluctuate in people with MS (pwMS). Disrupted circadian rhythms may have a role in the symptoms' variability. Distinguishing interindividual differences and temporal daily fluctuations in MS symptoms may help to define specific symptomatic phenotypes. Understanding how these phenotypes are associated with quality of life and their immunological underpinnings—immune profiles—could shape new MS management strategies. Our primary aim is to document ongoing fluctuations in fatigue, pain and mood in a cohort of pwMS to determine whether symptom variability is associated with differential quality of life. Our secondary aim is to evaluate the feasibility of our study design to identify immune profiles of circadian rhythmicity in MS.

Methods and analysis This observational cohort study examines individual temporal fluctuations in MS symptomatology via ecological momentary assessment in a cohort of pwMS. All participants complete (1) a baseline battery of questionnaires and (2) electronic symptom-tracking diaries to rate fatigue, pain intensity and mood on a 0–10 scale at three time points (08:00, 14:00 and 20:00) for 10 days. Participants will be grouped into symptomatic phenotypes based on longitudinal data from e-diaries. We will assess whether exhibiting a specific phenotype is associated with certain baseline measures. A subgroup of 20 participants—feasibility study—will also complete blood sample collection two times within 24 hours to study immune profiles and molecular markers of circadian rhythmicity in MS. Flow cytometry, whole blood RNA sequencing and plasma analyses will be applied to determine changes in immune profiles indicative of circadian rhythmicity.

This work has the potential to reduce the burden of this complex disease on a global scale. Future studies will build on our work to understand individual variability in MS symptomatology, including disease severity; identification of biomarkers underlying the association between rhythmic symptomatology profiles and symptomatic phenotypes in MS; and designing personalised interventions focused on interindividual differences in symptomatology and circadian rhythmicity.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ We use a battery of baseline questionnaires validated in the multiple sclerosis (MS) populations and electronic symptom diaries to measure temporal variations in fatigue, pain and mood in people with MS (pwMS).
⇒ Data from a national study will be collected using self-report questionnaires and ecological momentary assessment to provide a broader view of changes in MS symptoms.
⇒ The use of online/smartphone data collection may affect the diversity in our sample (eg, the representation of rural and/or underprivileged communities).
⇒ A feasibility study assessing changes in immune cell phenotype by flow cytometry will determine whether these methodologies can be used to characterise circadian rhythmicity in pwMS.

Ethics and dissemination The CircaMS project and its associated procedures have been reviewed and approved by the Queen's University Health Sciences and Affiliated Teaching Hospitals research ethics board (File number: 6039383). Participants provide informed consent to participate, and their data will not be identifiable in any publication or report. All documents are stored securely and only accessible by study staff and authorised personnel. The results will be presented to academic and lay audiences via national/international conferences, publications in peer-reviewed journals, social media and through an official website created to engage pwMS, caregivers, clinicians and researchers.

INTRODUCTION

Multiple sclerosis (MS) is a chronic immune-mediated disease of the central nervous system characterised by demyelination and neurodegeneration. This heterogeneous disease has a variable prognosis and unpredictable disease course.[1] The prevalence of MS has seen a global increase since 1990, with the highest age-standardised prevalence in high-income regions such as North America, Western Europe and Australasia.[2]

Fatigue,[3] pain (eg, headache, neuropathic pain and lower back pain)[4] and low mood[5] are among the most common symptoms reported by people with MS (pwMS). Emerging evidence suggests substantial variability in the occurrence and intensity of MS symptoms such as fatigue (physical and cognitive),[6] pain and low mood[7] and how they are perceived on a moment-by-moment and day-to-day basis. The ecological momentary assessment (EMA) is a valid methodology to characterise temporal variations in self-reported symptoms and identify daily fluctuations in pain, fatigue and low mood within the MS population.[8 9]

It is necessary to consider the influence of circadian rhythms (or, 24-hour cycles) when investigating variations in processes that have both a psychological and physiological component, such as fatigue, pain and low mood. Disrupted circadian rhythms can impact MS symptoms and may play a role in their daily fluctuations.[10] Circadian rhythms align physiological functions with the environment[11] and are directed by an endogenous clock system, located in the suprachiasmatic nucleus, which controls the release of hormones and other secreted factors. Circadian clocks work in a tightly regulated feedback process lasting ~24 hours that influences biomarkers (eg, gene expression and neuroinflammation) and psychosocial experiences (eg, fatigue, mood).[10]

Normal fluctuations of clock genes across 24 hours are significantly reduced in the mouse model of MS (experimental autoimmune encephalomyelitis), suggesting clock-dependent circadian rhythm disturbances.[12] Genetic polymorphisms in key circadian genes are associated with the risk of developing MS in the clinical population.[11] Additionally, higher levels of some proinflammatory cytokines (eg, TNF-alpha) and lower levels of some anti-inflammatory cytokines and secreted mediators (eg, melatonin) are observed in both pwMS and preclinical models.[13–15] Furthermore, the association between fatigue and levels of inflammatory markers overall has been equivocal.[16] Thus, understanding whether rhythmic expression of immune markers (ie, at the RNA or protein level) and serum proteins involved in MS impact symptoms such as fatigue, pain and low mood may help identify new therapeutic avenues.

Approved biomarkers for MS (eg, MRI, spinal fluid analysis, evoked potentials, etc) are mostly used for disease diagnosis and detection of neuroinflammation related to disease activity.[17] The integration of biomarkers has revolutionised the management of other chronic conditions such as cancer, where oncologists have been able to tailor treatment based on patients' molecular profiles.[18] Similarly, we believe it is crucial to identify prognostic biomarkers in MS to improve the management of various MS symptoms and to apply individualised treatments based on molecular profiles and symptomatic phenotypes. Currently, the burden of MS symptoms such as fatigue, pain and low mood is substantial, and there is an urgent need for more targeted biomarkers that can guide multidisciplinary symptom management and monitoring.

Our CircaMS study uses a multidisciplinary approach to investigate the role of circadian rhythmicity in MS. Our focus is on characterising key symptomatic phenotypes (daily fluctuations in fatigue, pain and mood) and understanding how they are associated with baseline quality-of-life measures. Furthermore, we aim to identify immune biomarkers of circadian rhythmicity in whole blood immune cells (including both peripheral blood mononuclear cells (PBMCs) and polymorphonuclear leucocytes (PMNs)) of pwMS. This work may help bring us closer to developing innovative personalised treatment strategies, targeting both interindividual differences in symptomatology and circadian dysfunction at a molecular level. These are important steps towards the primary missions and research priorities set by the Wellness Research Working Group (National MS Society) and the International Progressive MS Alliance[19 20] that will lead to ultimately reducing the burden that this complex disease has on Canadian and global populations.

We expect that people with rhythmic EMA symptomatic phenotypes (based on fatigue and pain daily fluctuations) will report better quality-of-life measures and healthier molecular profiles through examination of gene expression patterns as well as PBMC and PMN activation states, compared with people with constant EMA symptomatic phenotypes.

METHODS AND ANALYSIS

This study adheres to the principles of the Declaration of Helsinki,[21] and the results will be reported in accordance with Strengthening the Reporting of Observational Studies in Epidemiology guidelines.[22] The start date for both national and local feasibility studies is October 2023, and the anticipated end date is October 2027 or when the recruitment target is reached for both studies.

Study design and aims

The CircaMS study explores how circadian rhythms and daily fluctuations in fatigue, pain and mood in pwMS might be associated with quality of life assessed at baseline. Our primary aim (*national/international cohort*) is to document ongoing intradaily fluctuations in fatigue, pain and mood (symptomatic phenotypes) in a cohort of pwMS to determine whether symptom variability is associated with specific measures of quality of life. Our secondary aim (*local feasibility study*: subcohort of 20 people) is to evaluate the feasibility of our study design to identify immune profiles of circadian rhythmicity in pwMS (figure 1). To address these outcomes, the CircaMS study will recruit pwMS for approximately 4 years—based on our previous chronic pain study recruitment timeline[23]—or until the recruitment target is reached. We collect data using established methods from epidemiology (surveys and e-diaries) and neuroimmunology (blood samples).

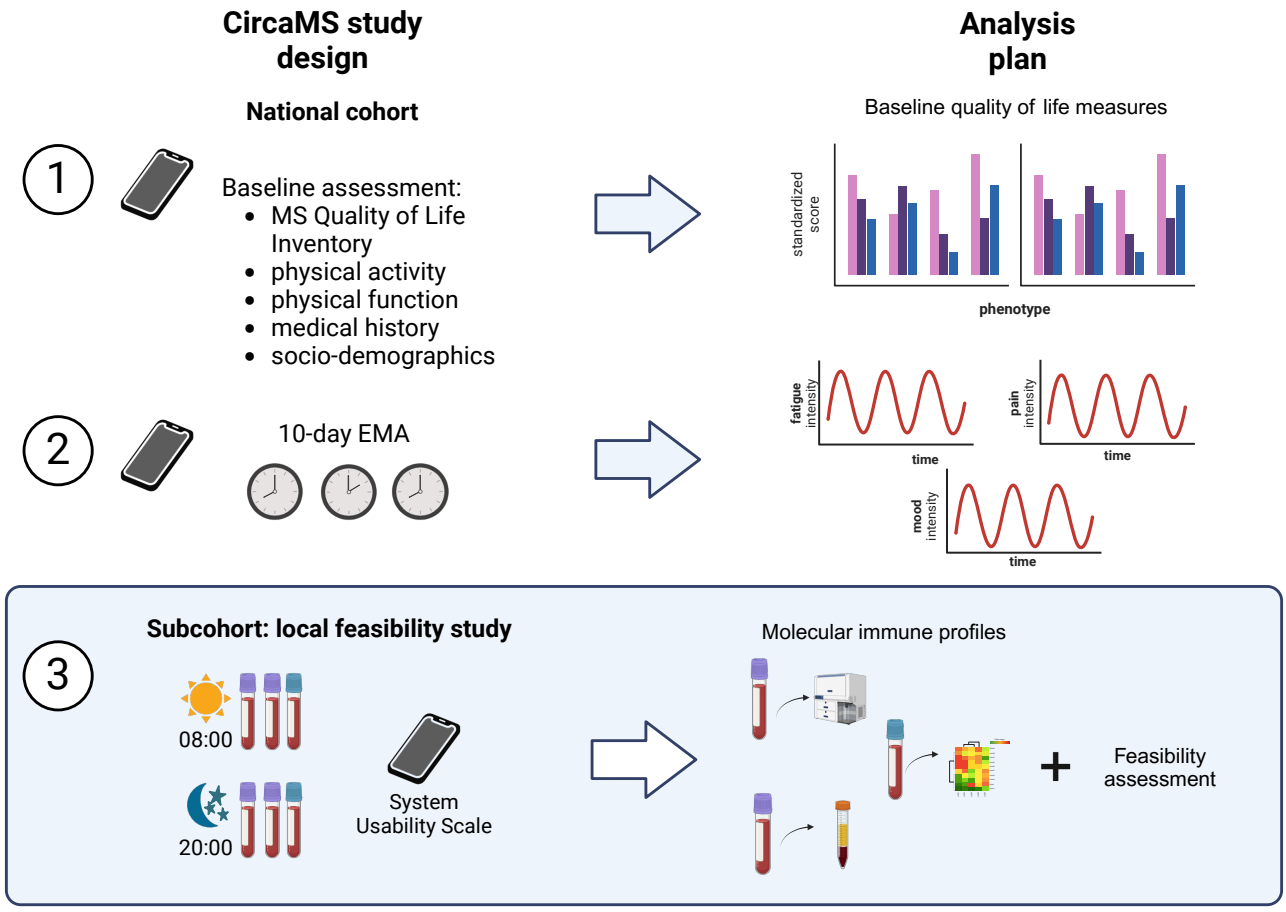

Figure 1 CircaMS study design: The study is divided into two parts: National cohort consisting of a baseline assessment that includes a battery of questionnaires and a 10-day e-diary. In addition, a subcohort of 20 people will also complete the collection of biological samples two times per day for flow cytometry, plasma and RNA sequencing. The feasibility component of the protocol will be assessed in this subcohort, recruited in Kingston through the local multiple sclerosis (MS) clinic and research centre.

Secondary aim—local feasibility study

Secondary aim is to evaluate the feasibility of our study design in pwMS, including survey completion and blood sample collection to study the circadian expression of inflammatory markers/immune profiles of circadian rhythmicity.

Eligibility criteria—national/international cohort and local feasibility study

Participants who are at least 18 years of age, have internet access (any device is accepted), are fluent in English or French (or additional languages, as the study is translated) and have subjective complaints of fatigue or pain are eligible to participate in CircaMS; participants with mood complaints alone (eg, no fatigue or pain) will not be eligible. Participants are excluded if they have any other neurological condition (eg, stroke, dementia), severe and untreated psychotic disorder (eg, schizophrenia), any active infections, diagnosis of an untreated and severe primary sleep disorder such as narcolepsy (sleep apnoea is accepted) and/or history of travel across time zones in the past 3 weeks or are planning to travel to a different time zone during the period of data collection. We include both untreated and DMT-treated pwMS. For participants in the local feasibility study, we access medical records to verify the type of medications and dosage.

Recruitment and participants—national/international cohort

The study is open nationally and internationally for collection of epidemiological data (surveys) to reach >200 participants with complete data in approximately 2 years. These numbers are expected to be sufficient to group participants in meaningful symptomatic phenotypes, based on our previous unpublished results on a population of people with chronic low back pain where we found four major phenotypes for pain, fatigue and mood symptoms (constant low, constant high, rhythmic and mixed patterns) at an approximate ratio of ~1:1:1:1. The sample size calculation was based on ANOVA assuming a type 1 error α=0.05 and type 2 error β=0.2 (ie, 80% power). To detect a difference in pain, fatigue and mood across phenotypes, we will require around ~50 participants per phenotype.

The study is advertised via the research team's official social media pages (Facebook, X, etc), a dedicated study website CircaMS and paper forms (eg, posters, handouts). We also leverage MS networks, people with lived experience (PWLEs) and collaborators to help with recruitment.

Recruitment and participants—local feasibility study

The feasibility study will recruit 20 adults from Kingston Health Science Centre (KHSC) MS clinics within 2 years of study recruitment initiation, with a clinician's diagnosis. Based on our previously described unpublished study, detecting differences in circadian genes will require 20 participants per phenotype. We will start collecting blood from 20 pwMS to assess the feasibility of these procedures before expanding to a larger sample sufficiently powered for this aim. Participants for the local study are recruited primarily through the KHSC MS clinic by clinicians and researchers. Additionally, recruitment material will be sent to other clinics and local services accessed by pwMS. Individuals have the freedom to follow the link to initiate study enrolment (eligibility screening questions) through the REDCap platform.[24 25] Participants can opt in or out of the blood collection using the REDCap link.

Data collection—national/international cohort

Eligible pwMS complete an online form with a link to a REDCap survey for informed consent, and baseline questionnaires, followed by our e-diary using the EMA approach.[8]

The baseline questionnaire battery includes self-report questionnaires covering different domains: fatigue, pain, sleep, mood and other quality-of-life variables, physical activity, physical function and medical history and sociodemographic characteristics (table 1).

Table 1 Validated and non-standardised measures completed by participants in the CircaMS baseline battery and EMA symptom diaries

Questionnaire	Focus	Description
MSQLI:(MFIS)[47]	Fatigue	MSQLI:(MFIS) assesses the effects of fatigue in terms of physical, cognitive and psychosocial functioning.
PROMIS Fatigue MS-8a[48]		PROMIS Fatigue MS-8a assesses the intensity of fatigue and how these factors impact quality of life.
MSQLI:(PDQ)[47]	Perceived cognitive deficit	MSQLI:(PDQ) provides an assessment of several domains of cognitive functioning that are frequently affected in MS: attention, retrospective memory, prospective memory, and planning and organisation.
BPI[49]	Pain	BPI characterises the nature of a participant's chronic pain and the degree to which it interferes in their daily activities.
MSQLI:(PES)[47]		MSQLI:(PES) to assess the ways in which pain and unpleasant sensations interfere with mood, ability to walk or move, sleep, work, recreation and enjoyment of life.
NRS (0–10)	Pain, fatigue and mood	NRS ia an 11-point scale that depicts the severity of pain, fatigue and sadness a participant is feeling at that moment, used in EMA diary.
rMEQ[50]	Sleep	rMEQ assesses alertness of participants and when they feel they are at their 'peak' functioning level. Timing intervals in the questionnaire are consistent with the original questionnaire.
ISI[51]		ISI assesses severity of insomnia components experienced by a participant.
MSQLI:(MHI)[47 52]	Mood and other psychological characteristics	MSQLI:(MHI) assesses participants' mental health including anxiety, depression, behavioural control, positive affect and general distress.
PHQ-4[53]		PHQ-4 briefly assesses for the presence of anxious and depressive symptoms among participants along with psychological distress.
POMS[54]		POMS assesses the mood states of participants. Negative affect items were isolated and used in the EMA diary.
GLTEQ[55]	Physical activity	GLTEQ assesses a participant's typical time expenditure on physical activity at different intensity levels.
PROMISnq PFMS-15a[56]	Physical function	PROMISnq PFMS-15a assesses changes in functional states in people with MS.
Non-standardised medical history questionnaire	Medical history and sociodemographic characteristics	Records participants' past and present medical treatments, comorbidities and sociodemographic information.
SUS[26]	Usability	SUS offers information about usability to define user requirements in a way that can help analyse, design and evaluate the interface.

The total time required to complete the baseline survey is around 15–20 min. EMA e-diaries require around 1 min per completion (3 min per day).
ANOVA, Analysis of Variance; BPI, Brief Pain Inventory; DMT, Disease Modifying Therapy; EMA, ecological momentary assessment; GLTEQ, Godin Leisure-Time Exercise Questionnaire; ISI, Insomnia Severity Index; MFIS, Modified Fatigue Impact Scale; MS, multiple sclerosis; MSQLI:(MFIS), Multiple Sclerosis Quality of Life Inventory Modified Fatigue Impact Scale; MSQLI:(MHI), Multiple Sclerosis Quality of Life Inventory Mental Health Inventory; MSQLI:(PDQ), Multiple Sclerosis Quality of Life Inventory Perceived Deficit Questionnaire; MSQLI:(PES), Multiple Sclerosis Quality of Life Inventory MOS Pain Effects Scale ; NRS, Numerical Rating Scale; PHQ-4, Patient Health Questionnaire-4; POMS, Profile of Mood States; PROMIS Fatigue MS-8a, Patient-Reported Outcomes Measurement Information System Short Form—Fatigue—Multiple Sclerosis 8a; PROMISnq PFMS-15a, Patient-Reported Outcome Measurement Information System Physical Function Measure for Multiple Sclerosis; rMEQ, Reduced Morningness-Eveningness Questionnaire; SUS, System Usability Scale; TNF-alpha, Tumour Necrosis Factor-alpha.

See online supplemental material 1–3 for a complete list of questions for both national and local study.

10-day e-diary (EMA)

Following baseline assessment, all participants are asked to rate their fatigue, pain, current mood and activity levels using a Numerical Rating Scale three times a day for 10 days using a 10-day e-diary (see online supplemental material 4 for details). Participants complete the e-diary three times a day (8:00, 14:00 and 20:00) for 10 days, providing an email address or phone number to receive a personalised link tracking their daily reports via REDCap/Twilio Integration Module. An additional question about hours of sleep and perceived sleep quality is included in the 8:00 survey. Invites are sent according to the local time where the participant is based. Surveys completed past 10:00, 16:00 and 22:00 are excluded from the analysis as timed out (no longer ecologically valid). Any symptom diary containing ≥5 days with submitted diaries at all time points over the 10 days will be used in the analyses; all other symptom diaries are excluded from main analyses and may be used for secondary analyses.

Data collection—local feasibility study

PwMS participating in the local feasibility study complete all the measures mentioned in the previous section. In addition, MS clinicians/researchers will record information relevant to the study (such as vitamin D levels or medication use) using electronic medical records.

Blood collection: blood draws are collected after the baseline assessment and around the time the last day of EMA is completed. Participants will be recruited through the MS clinic. They will be given the choice of either attending the Research facility in Kingston two times per day or receiving a home visit by a certified phlebotomist in the research team if they live in the Kingston area. They will provide two blood samples (≤20 mL/draw) within a 12-hour period (morning (7:00–10:00) and evening (19:00–22:00) in one single 24-hour cycle. Blood is collected in Tempus or PAXgene tubes for whole blood RNA sequencing and K2EDTA tubes for flow cytometry and serum proteins. Samples are processed and/or stored as described in the sample processing and storage section of this paper.

20 participants who completed both blood samples and surveys are sent an additional survey following the last diary completion (10 days) for their assessment of our local study procedures (feasibility subaim 3) and the usability of our EMA diary based on the System Usability Scale.[26] See online supplemental material 5 for details.

Analyses—national/international cohort and local feasibility study

We assess the variability of fatigue, pain and mood symptoms as previously done in the MS population using EMA data.[7 9] Furthermore, to accomplish our primary aim, participants are grouped by the mean and SD of their reported EMA pain scores across all time points available using the SD 50th percentile to determine low/high variability groups.[27] When the full sample size is reached, participants with distinct rhythmicity trajectories (ie, fatigue, pain, mood phenotypes) are identified by EMA scores using a latent class mixed effect model (LCMM, based on the *lcmm* R package,[28] a probabilistic modelling algorithm approach that clusters longitudinal data accounting for correlation between repeated measures[29 30] and has been used to characterise pain chronicity over months.[31] To ensure biologically meaningful phenotypes are identified, functional data analysis with high-dimensional data clustering (based on the *funHDDC R* package[32]) will be considered in addition to LCMM as an alternative approach; this allows for participants with similar fatigue, pain and mood fluctuation phenotypes to be clustered together. Graphical tools (eg, Sankey plots) will be used to visualise changes in phenotype. Furthermore, we will use linear mixed-effect models[33 34] to test for differences in average fatigue, pain and mood scores in the morning versus evening across time points. We will use descriptive statistics to characterise the sample and inferential statistics (eg, Fisher's Exact tests, χ^2, Kruskal-Wallis, multinomial logistic regression and other regression models) to determine the association between symptomatic phenotypes and baseline well-being/quality-of-life measures, including possible confounders. All statistical analyses will be performed halfway through and at the end of the study recruitment using R[35] or SPSS[36] where appropriate (figure 1).

Analyses—local feasibility study

Our secondary aim—feasibility of the procedures[37]—both self-report measures and blood collection and specific subaims are assessed as follows.

Recruitment capability

- *Can we recruit 20 pwMS within 18 months for both surveys and two blood samples collected within 12 hours?*

This pilot study will help us to answer the question of being able to recruit this number of people for two complete blood samples within 12 hours (yes or no). The recruitment rate is also measured by the number of people enrolled over the number of participants approached plus screened plus inquired (these numbers are recorded using enrolment and recruitment logs).

- *Are the eligibility criteria suitable or too inclusive or restrictive?*

We assess the suitability of eligibility criteria by counting the number of people who entered the study but were deemed ineligible following initial screening as recorded in the REDCap eligibility form. Eligibility criteria will be considered suitable if more than 50% of people who entered the study are deemed eligible to take part in the study. If one specific criterion results in the non-eligibility of 50% of participants, that criterion will be considered restrictive and therefore might be adapted.

- *What are the reasons for ineligibility or refusal?*

Recurrent themes/reasons for declining are recorded whenever participants are willing to share them. These will be considered to guide changes in recruitment procedures.

Data collection procedures

- *Are data collection procedures (ie, repeated venipuncture: blood sample collection) manageable for the MS population?*

The feasibility of repeated blood sample collection in the MS population is evaluated by counting the number of people who were eligible for the local study but declined the blood sample collection.

- *Are the planned EMA e-diary times manageable for participants?*

The appropriateness of EMA times is assessed by considering the number of missed time points for morning, afternoon and evening diary completions. Less than 50% completion per time point in more than 50% of the enrolled participants will be considered unmanageable.

- *Are the planned times for blood collection convenient for participants or do they need to be changed?*

Appropriateness of blood collection times is assessed by considering the number of missed time points for morning and evening blood collection sessions. Furthermore, the location of blood collection is evaluated based on the number of appointments scheduled at this location. Alternative locations (eg, home collection) may be proposed to facilitate recruitment.

Compliance is assessed through examination of the percentage of people who completed ≥5 days with submitted diaries at all time points over the 10-day period and who completed two blood draws. The completion rate is evaluated by dividing the number of people who completed the survey by the total number who started the survey without completing it.

Acceptability of the procedures

- *Can fidelity to the protocol be achieved?*

Adherence to the protocol during data collection is evaluated. The number of deviations from the protocol will be recorded by members of the research team and Prinicipal Investigator. No more than five deviations from the protocol will be considered acceptable.

- *Are there any unexpected adverse events involving both participants and the research team?*

The safety of procedures for researchers and participants is estimated by recording the number of adverse events related to blood collection procedures.

- *Are the procedures/measures used acceptable and usable?*

Scores and responses to the System Usability Scale[26] are used to determine the acceptability and usability of our study procedures.

Proof of concept

- *Are there any circadian changes in the immune profiles of participants as expected?*

Changes in immune cell surface markers, global gene expression among immune cells and plasma protein within and between participants will be studied to determine whether circadian rhythms can be detected using the proposed methods.

- *Are the preliminary findings congruent with the hypothesis?*

We will measure changes in mRNA expression of circadian genes (eg, *BMAL1, CLOCK, PER1/2* and *CRY1/2*) between the two collection time points to assess rhythmicity within and between participants. This may also serve as a proxy for potential circadian fluctuations in global gene expression patterns. The bioinformatics analysis pipeline has been previously established by our group.[30 38] Although changes in gene expression do not always translate to protein expression, we will use results from flow cytometry (ie, immune cell population size, median fluorescence intensity, immune cell population ratios) and multiomics analyses to determine whether the proposed protocol can capture circadian changes in neuroinflammation among pwMS.

This will allow us to explore whether the results are congruent with the hypothesis or whether the procedures need to be changed.

- *If there are no changes, are the collection procedures appropriate?*

Statistical analyses will be performed halfway through the study recruitment to explore whether the results are congruent with the hypothesis or whether the procedures need to be changed.

Our national study is modelled after our previous chronic low back pain study[30] and our larger CircaPain study.[23] The results from this CircaMS local feasibility study will inform the conceptualisation and planning of future studies that include blood draws and the possible extension of the national CircaMS study, which currently only includes surveys and EMA data. This will inform the potential expansion of large-scale studies that include both EMA and blood collection in the MS population.

Sample Processing and Storage—local feasibility study

Sample collection: blood collection is conducted two times on the same day for each participant at 7:00–10:00 and 19:00–22:00, and the time of collection is recorded for each sample. Samples arrive at the laboratory and sample processing starts at 10:00–11:00 and 22:00–23:00, respectively.

Flow cytometry: up to 6 mL of blood is used to characterise circadian fluctuations in the immune cell population using cell surface markers. On arrival, human PBMCs and PMNs are isolated using a Ficoll gradient following published protocols.[39] Briefly, the Ficoll gradient is performed following the manufacturer's instruction; the buffy coat is collected and PMNs are isolated by treating the granulocyte–erythrocyte layer using a red blood cell lysis buffer. PMBCs and PMNs are checked for viability and phenotyped as follows: CD4+ T cells (CD45+CD3+CD4+CD8−), CD8+ T cells (CD45+CD3+CD4 CD8+), B cells (CD45+CD3 CD19+), neutrophils (CD45+CD11b+CD16+CD14low/int), monocytes (CD45+CD11b+CD16±CD14+) as well as intermediate monocytes

and macrophages (CD45+CD11b+CD16+CD14hi). The markers HLA-DR and HLA-ABC are used to identify specific activation states.

Whole blood RNA sequencing and bioinformatic analysis: up to 3 mL of blood is used for whole blood RNA sequencing. Immediately on arrival, Tempus tubes (whole blood) are stored at −80°C and/or in liquid and sent to a genome facility for simultaneous processing and sequencing to avoid batch-to-batch variability. Purified RNA samples from whole blood will be used to assess circadian oscillations in global gene expression patterns using differential gene expression, network analysis and other bioinformatic tools.

Plasma: up to 6 mL of blood is used to collect plasma through centrifugation on arrival (×300*g* for 10 min at room temperature). Then the plasma is aliquoted into 500 µL aliquots and stored at −80°C and/or in liquid nitrogen. Plasma samples will be processed using multiomics technologies to profile circadian changes in mediator expression (eg, proteins, lipids). While serum analysis remains outside the scope of this study, it will be banked for future analysis (figure 1).

Patient and public involvement

All research questions and outcome measures were developed in collaboration with PWLEs of MS, taking into account their priorities and experiences with the disease. This was done through consultations with the research team. They completed the survey and returned feedback about the questions. This guided the final version of our CircaMS measures. CircaMS also has a patient partner as part of the study team, who contributes to the knowledge translation and dissemination efforts by helping the research team summarise the research objectives and background in clear user-friendly language and ensure that the information is accessible to a public audience.

ETHICS AND DISSEMINATION

The CircaMS project and its associated procedures have been reviewed and approved by the Queen's University Health Sciences and Affiliated Teaching Hospitals Research Ethics Board (File number: 6039383), with the latest approval on 9 May 2024. The research team will ensure that the participants' confidentiality is maintained. The participants are identified only by a study participant ID on any electronic database. All documents are stored securely and only accessible by study staff and authorised personnel. The study complies with The Personal Information Protection and Electronic Documents Act and the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans.

All information is stored in the REDCap[24 25] online data collection software or in a secure, password-protected file. Medical records are accessed to gather information relevant to the study and only by an authorised member of the research team with a valid research appointment. Since some of the in person testing sessions occur outside of normal working hours, a second member of the research team may be present on-site to maintain both participant safety and that of the researchers.

Participants will not be identified in any publication or reports, nor will information such as email addresses appear in any study documents. REDCap has specialized functionality which allows only select users to view and input identifying information, with all other users seeing exclusively deidentified data and prohibiting the downloading of identifying information when exporting the dataset. The study team will present results from this study to research, clinical and lay audiences via national and international conferences, publications in peer-reviewed journals and ongoing knowledge translation efforts (eg, blog, optional return of results to participants) spearheaded by investigators at the lead site. The authors will acknowledge eventual funding and other contributors. The official website CircaMS is used to post updated resources (blogs, videos, research updates, etc) to engage people who live with MS and their families or friends, clinicians or anyone interested in the topic.

DISCUSSION AND EXPECTED RESULTS

Theoretical framework and considerations for the analyses

The National Institutes of Health established a symptom science model to help guide precision medicine and develop innovative interventions[40]. According to this model, it is important to consider the presentation of different symptoms (eg, psychological and physiological comorbidities) to determine their phenotypic characteristics and to test biomarkers (eg, genes, protein).[40 41] This allows clinicians/researchers to consider several domains that interact with symptom experiences, such as personal characteristics, functional outcomes, health/illness factors, environment and management strategies.[42] When patient-reported outcomes in electronic form[43] are employed to capture data, using validated and standardised measures (eg, Patient-Reported Outcomes Measurement Information System (PROMIS)),[44] it becomes possible to include mental, physical and social well-being components in the model.[45] This is particularly important when studying chronic and multifactorial diseases like MS; where the combination of clinical profiling, real-world data collection and biomarker identification is key for a comprehensive understanding of individual differences within the disease.[46]

The CircaMS baseline measures include several standardised questionnaires to account for quality of life in MS (eg, Multiple Sclerosis Quality of Life Inventory,[47] PROMIS Fatigue MS-8a[48] (table 1). We will include those variables as covariates in our regression model, and our final modelling will be based on all these considerations. Ultimately, this, together with phenotyping based on the EMA e-diary measures and biomarker identification via blood sample, will establish a solid model to study MS, with the vision of fostering precision medicine and personalised treatment.

Expected results, goals and limitations

We expect that people with rhythmic EMA symptomatic phenotypes (based on fatigue and pain daily fluctuations) will report better quality-of-life measures and healthier molecular profiles through examination of gene expression patterns as well as PBMC and PMN activation states, compared with people with constant EMA symptomatic phenotypes. Our short-term goal is to examine the feasibility of this study design in the MS population and identify circadian rhythmicity as a biomarker for fatigue, pain and mood phenotypes. This will respond to the immediate need (1) to find biomarkers to monitor and prognosticate common MS symptoms, (2) to develop effective symptom management strategies by targeting circadian rhythms; important steps toward the primary missions and research priorities set by the Wellness Research Working Group[19 20] to reduce the burden that this complex disease has on the global population.

Our long-term goal is to build the foundation to develop new treatment strategies targeting circadian dysfunction. Non-pharmacological means can include light therapy to reset circadian clocks or chronotherapy to time medication dosing according to symptom need; pharmacological strategies can be used to target clock gene expression or PBMC and PMN activation states at a molecular level. This work will help reduce the burden this complex disease has on the global population. Future studies will build on our work to understand individual variability in MS symptomatology, including disease severity; identification of biomarkers underlying the association between rhythmic symptomatology profiles and symptomatic phenotypes in MS; and designing personalised interventions focused on interindividual differences in symptomatology and circadian rhythmicity.

Our study examines interindividual and temporal variations in fatigue, pain and mood in pwMS, using electronic symptom diaries to ensure the ecological validity of self-reported symptoms and baseline questionnaires validated in the MS population.[7 9] Collecting blood two times per day in our local feasibility study will allow us to understand whether immune profiles and molecular markers of circadian rhythmicity exist in pwMS. Some limitations of our study design include the fact that no formal medical validation of participants' MS diagnosis is included in the epidemiological portion of the study as all measures are self-reported. For our local feasibility study, a medical validation is instead required. Due to the difficult nature of this methodology with repeated blood collection, we did not include a healthy control group for this feasibility study; however, we plan to add a healthy control group and a larger cohort of pwMS once feasibility for this design is established. Furthermore, participation may be difficult for individuals with limited computer skills as the study is available online through a web-based platform. However, for our local feasibility study, researchers can offer alternatives to complete the intake survey and support participants in setting up reminders to complete e-diaries. The feasibility study will be based in Kingston, Ontario, and this might not allow results to be reflective of other settings. This study will inform methodology and procedures for future recruitment with additional sites across Canada.

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