Mechanisms of Persistent Fatigue (MAP-FAT): A prospective observational cohort study of persistent fatigue following Epstein-Barr virus infection in adolescents and young adults

Study protocol

Summary
Persistent fatigue (PF) is a highly prevalent transdiagnostic symptom across countries and cultures, and an important cause of disability and reduced quality of life. Acute infection is a common trigger of PF, as exemplified by the 'Long COVID' phenomenon. Despite substantial burden for the suffering individuals as well as their next-of-kins, the healthcare systems and the economy, PF is an under-researched field, with scarce knowledge of disease mechanisms as well as treatment and preventive measures.
Existing knowledge on PF pathophysiology suggests complex interactions between functional brain alterations, immunological aberrations and disturbances of autonomic nervous system activity. Previous findings have been interpreted in light of two alternative models: A body-to-brain mechanism highlighting immunological aberrations as the primary mechanism, and a brain-to-body mechanism where functional brain alteration is seen as the central element whereas immunological alterations are regarded secondary phenomena mediated by autonomic disturbances.
The multinational and collaborative Mechanism of Persistent Fatigue (MAP-FAT) project is determined to scrutinize these potential brain-body interactions in PF. The main objectives are to determine a) the relationship between PF, brain network dynamics, sympathetic nervous activity and immunological alterations and b) the relative importance of strong priors and current interoceptive input within a central inferential model of PF. To achieve these objectives, MAP-FAT will conduct a *de novo* post-infective cohort study of n=150 individuals with acute Epstein-Barr virus (EBV) infection and n=150 healthy controls followed for six months. Investigations include a) Clinical and demographic assessment; b) Questionnaire charting; c) Multimodal brain MRI; d) Autonomic cardiovascular assessment; e) Deep immunological profiling; and f) Behavioural experiments (The Affect & Symptom Paradigm (ASP), The Virtual Hill Bicycling Paradigm (VHBP), and The Belief Updating Task Paradigm (BUTP).

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Versions/revisions
Version 01 (28.11.2025), original

Version 02 (19.05.2026), following pilot experiments

- Adjusted details regarding the VHBP (fewer trials (a total of 12), lower actual slopes, no adjustment to previous fitness level).
- Adjusted details regarding the BUTP (a total of three categories of events, a total of 60 events altogether).
- Adjusted minor details regarding the isometric exercise experiment (handgrip).
- Adjusted minor details regarding autonomic cardiovascular monitoring
- Adjusted minor details regarding the ASP experimental protocol
- Ajusted minor details regarding serological criteria for diagnosing acute EBV-infection

1. Background/rationale

Introduction. *Persistent fatigue (PF)* is a highly prevalent transdiagnostic symptom across countries and cultures, and an important cause of disability and reduced quality of life.[38,75] Adolescents and young adults (AYAs) are particularly vulnerable,[4] and PF is a common cause of chronic disabilities, reduced working capacity and related insurance reimbursements among young people. Despite substantial burden for the patients and their next-of-kins, the healthcare systems and the economy, PF is an under-researched field,[98] with scarce knowledge of disease mechanisms as well as treatment and preventive measures. Hence, more research is needed. The multinational and collaborative Mechanism of Persistent Fatigue (MAP-FAT) project is determined to address critical knowledge gaps related to the role of bidirectional brain-body interactions in the etiology, pathophysiology and treatment of PF.

Fatigue is common in the general population and a feature of several clinical conditions, such as infections, cancers, autoimmune disorders, various neurological conditions, and mental disorders, and may become persistent after resolution of the initial triggering event, such as in the post-infective fatigue syndrome (PIFS)[43] and the post-cancer fatigue syndrome.[33] Also, PF is a hallmark of the common but poorly understood conditions often referred to as functional somatic syndromes; which include chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME), fibromyalgia, and irritable bowel syndrome.[78] Among these conditions, PIFS is the best characterised. Certain endemic infections, such as infectious mononucleosis caused by the Epstein-Barr Virus (EBV), are common triggers,[43,67,100] and many cases of the post-COVID-19 condition ("Long COVID") are likely variants of PIFS.[83,85] Phenotypically, PIFS manifests as an abnormal prolongation of the acute sickness response, which is a stereotyped collection of physiological, behavioural, and psychological manifestations of most acute infections.[99] Across multiple prospective cohort studies of certain systemic infections, *up to 40-45 % fulfil commonly accepted case definitions of PF and 10–15 % suffer from full-blown PIFS with significant disability at six months follow-up*.[43,67,85] Thus, acute infections may be regarded a natural experimental model of the mechanisms of PF risk and resilience. Compared to other fatigue states, PIFS is regarded as a relatively homogeneous clinical condition and therefore a relevant target for scientific inquiry with implications for the understanding of PF in general.

Models of PF. While PIFS is triggered by acute infection, the pathophysiology of prolonged symptoms is incompletely understood. Notable facets include autonomic nervous system alterations characterised by sympathetic over parasympathetic predominance,[46] and immunological aberrations characterised by low-grade inflammation and altered NK-cell functions.[74,76] Previous findings have been interpreted in light of two alternative models (Fig. 1). Model A highlights immunological mechanisms[8] possibly linked to autoimmune processes due to associations with auto-antibodies[104] and human leukocyte antigen (HLA) alleles.[59] This resonates with experimental research showing that fatigue may be induced by peripheral immunological signalling.[39] Hence, *Model A suggests a primary body-to-brain mechanism*. Model B considers functional brain alterations as the key driver and immune responses as secondary phenomena mediated by autonomic disturbances, i.e. *a primary brain-to-body mechanism*. This model is supported by clinical trials,[57] studies showing that inflammatory proteins are associated with sympathetic nervous activity predominance in fatigued patients,[54] and that a sympathetic inhibitor lowers inflammation but not symptoms.[93] Model B aligns with evidence that fatigue arises from central inferential processes[55] and

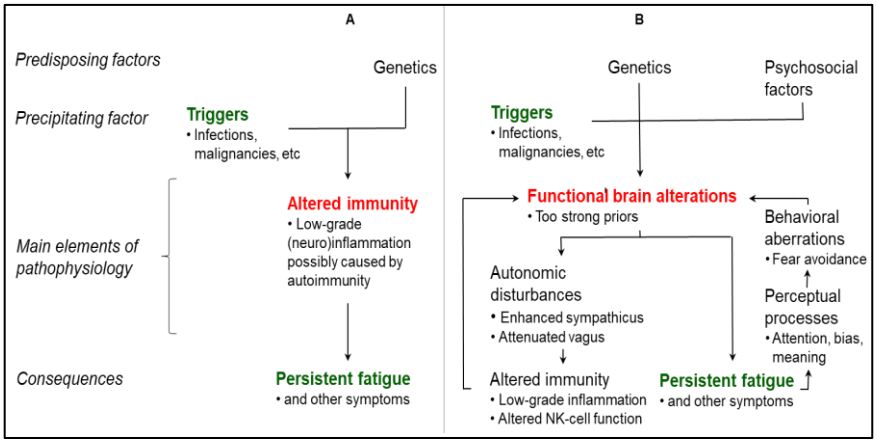

Figure 1. *Mechanistic models of PF. In A, altered immunity is the main determinant of PF; i.e., a body-to-brain model. In B, functional brain alterations are the main determinant of PF as well as altered immunity, i.e., a brain-to-body model. The models are not mutually exclusive and bidirectional brain-body interaction may be important for the perpetuation of symptoms, cf. the two 'vicious circles' indicated in panel B.*

that PF can result from 'erroneous' predictions (too strong priors) associated with trait negative affectivity.[9,96] These simplified models are not mutually exclusive, and bidirectional brain-body interactions may be important for symptom persistence and dynamics (Fig. 1, part B). Key facets of the models will be elaborated below.

Neurocognitive mechanisms of PF. The experience of fatigue is likely mediated by various brain networks, in line with other complex traits.[11,55,94] The specific networks involved and their dynamical properties remain to be characterized; however, *insular cortex* (IC) and *anterior cingulate cortex* (ACC) are reported as key nodes,[105] and there appears to be substantial overlap with networks involved in pain sensation, interoceptive integration and salience signaling, reward and motivation, autonomic control and brain-immune-interactions.[26] A "fatigue network" in the brain can conceivably be activated by afferent sensory signaling (cf. below). However, any subjective experience is a product of *central inferential processes* where the brain's predictions (commonly labelled 'priors') shapes the experience of sensory information[66] and may even "overrule" the sensory input, leading to biased or erroneous experiences as exemplified by visual illusions (e.g., the "hollow mask" illusion). The importance of central inferential processes for the experience of fatigue has been documented,[55] and erroneous predictions (too strong priors) has been put forward as a mechanism for the persistence of symptoms.[56,96] There are substantial individual differences in the tendency to prioritize prior experiences over sensory input, and those scoring higher on negative affectivity tend to rely more on these prior experiences.[10,45,97]

Immunological mechanisms of PF. Accumulating evidence suggests a link between mental states (e.g., fatigue and depression) and neuroinflammation, supporting a close connection between immunological processes and brain function.[2] There is a well-established causal relationship between peripheral immunological activation and the feeling of fatigue as observed in the acute sickness response, i.e., a body-to-brain mechanism.[26] For instance, experimental studies have revealed strong correlations between dosages of the inflammation inducer lipopolysaccharide (LPS), host cytokine responses and fatigue report.[60] Likewise, inflammation induced by typhoid vaccine caused fatigue as well as functional alterations in brain "fatigue network nodes".[39] Enhanced innate immune responses have been reported across different PF conditions,[30,63,71] but the findings are inconclusive and *the associations between immunological aberration, fatigue and its brain network underpinnings remain obscure*. For instance, a study of PIFS following EBV-infection reported predictive value of baseline C-reactive protein (CRP) levels for later PIFS development,[67] but only a very subtle CRP increase in PIFS sufferers and no correlation with fatigue severity,[54] in line with another EBV study.[18] Likewise, our recent *Long-term effects of COVID-19 in adolescents* (LoTECA) study revealed no evidence of peripheral inflammation[90] nor neuroinflammation[40] associated with PF. As for other facets of immune function, there are several previous reports of altered NK cell count and functional properties in PF.[14,24,76,77] This is supported by recent evidence from LoTECA suggesting that NK-cell function is fundamentally altered in PF conditions independently of the trigger; that is, whether the fatigue could be related to COVID-19 ("Long COVID") or not.[91] This supports a brain-to-body mechanism; i.e., that altered NK cell functionality may be a consequence rather than a cause of fatigue.

Autonomic alterations and sympathetic-immunological interactions in PF. *A sympathetic over parasympathetic predominance has been consistently reported in PIFS*[47,54,100] as well as in the more heterogeneous CFS/ME.[61,106,108,109] This autonomic activity pattern seems to be of central origin,[106,107] which supports the notion of fatigue as a "salience signal" indicating a potential threat to internal homeostasis.[65] Furthermore, studies have shown that autonomic nervous activity exerts important immunomodulatory effects. Sympathetic activity during stressful situations promotes proinflammatory gene transcription and cytokine production via beta-adrenergic receptor actions[27,53] and alters the functionality of NK-cells[20] as well as CD8+ T-cells.[36] Hence, the *immunological aberrations reported in PF may be a consequence of central alterations of autonomic activity*; i.e., they are caused by a brain-to-body mechanism. Some preliminary evidence favors this hypothesis: In PIFS following EBV, sympathetic activity was associated with CRP levels,[54] and in CFS/ME, the centrally acting sympathetic inhibitor clonidine lowered CRP without affecting fatigue nor functional capabilities.[93]

2. Aims, objectives and hypotheses

The overarching aim of MAP-FAT is to assess the dynamic relationship between fatigue and brain, autonomic and immunological activity, exploiting a *de novo* prospective cohort of EBV-infected patients (EBV-P) and healthy controls (HC). Through two specific objectives (O), MAP-FAT will:

- **O1:** Determine the relationships between PF, brain network dynamics, sympathetic nervous activity and NK-cell functions. **Hypothesis(H)1:** A "brain fatigue network" underpins the experience of fatigue and modulates efferent sympathetic activity and subsequent NK-cell functions.
- **O2:** Determine the relative importance of strong priors and current interoceptive input for PF within a central inferential model. **H2:** PF is dependent on strong priors.

3. Relevance

PF is a common and debilitating yet poorly understood. It commonly affects AYAs, with profound negative implications.[4] The female-to-male ratio is about 3:1; hence, *PF is a major threat to women's health* and gender equality.[43,67,85] Further, *PF is a transdiagnostic challenge* pertaining not only to sequels of acute infections but also to e.g., cancer survivors,[33] patients with various neurological conditions,[51] and patients with autoimmune disorder despite otherwise successful treatment.[21] Still, *PF is an under-researched field*, and the resource allocation is disproportionally low,[98] despite the likely prospects of future pandemics with PIFS development in the aftermath. Hence, MAP-FAT is relevant for several stakeholders and will generate clinical and scientific as well as societal and economic impact.
For *individual patients*, PF results in disability and poor quality of life (QoL), and they are often unable to attend work/school and normal social life. For AYAs, lower education and reduced work participation due to PF may have detrimental consequences. MAP-FAT will provide potentially amendable disease mechanisms and pave the way for new therapies, rehabilitation measures and preventive strategies.
Healthcare providers for PF patients may feel helpless when exposed to suffering which seem difficult to explain and alleviate and are devoid of clinical tools. Increased knowledge of disease mechanisms will inform the development of tools for risk assessment and primary care-based interventions.
For the *scientific community*, MAP-FAT will provide new knowledge and enable its diffusion through open science practice. This will facilitate future clinical trials. Our approach focusing on bidirectional brain-body-interaction will help overcoming dualistic models and resolve the ongoing scientific controversies between proponents of a purely biomedical and purely psychosocial understanding of PF.
At the *societal level*, PF causes significant strain. Caregivers suffer emotional distress from having a disabled family member and a loss of income and increase of expenditures. Healthcare services on all levels of the value chain are affected with one report estimating a $528 billion increased expenditure in the USA from long COVID alone.[25] PF is a major cause of sick leave and disability, hampers academic and career development and reduces the total work force.[37] Hence, PF is costly, in both human and financial terms. MAP-FAT will generate economic impact by helping patients to continue work/education and reduce the burden on insurance and welfare systems.

4. Study design

MAP-FAT is based on a *de novo* prospective cohort of AYAs (16–39 yrs) with acute EBV-infection (EBV-P) and matched healthy controls (HC) (Fig. 2). By combining longitudinal and experimental methods we can disentangle key body-to-brain and brain-to-body mechanisms and model individual trajectories and intra- and inter-individual variability that may otherwise obscure group differences.
Participants will be assessed at two time points: The first (T0) at early convalescent stage (3-6 weeks after symptom onset), and the second (T1) six months later. At each visit, a standardised protocol will be completed (detailed below), including: a) Clinical and demographic assessment; b) Questionnaires; c) Brain MRI; d) Autonomic cardiovascular assessment; e) Deep immunological profiling; and f) Behavioural experiments (The Affect & Symptom Paradigm (ASP, two versions), The Virtual Hill Bicycling Paradigm (VHBP) and The Belief Updating Task Paradigm (BUTP)).
Electroencephalography (EEG) during rest and cognitive tasks is an optional element of the protocol, and will provide electrophysiological correlates of decision making, belief updating and other cognitive processes.
Participants will be recruited from the catchment population of Akershus University Hospital (AHUS), cf. details on recruitment below. Based on previous experience,[67] we assume to complete the inclusion within 1.5 years. Two consecutive days will be used for completion of the investigational program at

each time point (T0 and T1): One visit at AHUS, Norway (point a, b, d, e, f, g and h), and one visit at Oslo University Hospital (OUH), Norway (point c). All procedures will be carried out in a quiet room in a fixed sequence. EEG, if included, will be performed at the Dept. of Psychology, UiO. Participants will receive a gift card (NOK 500) after completion of the assessment program, and their expenses will be reimbursed.

Fatigue caseness at T1 is the primary endpoint, defined as a score ≥4 on the Chalder Fatigue Questionnaire[19] (cf. below) applying bimodal scoring (0-0-1-1) of single items, as in previous studies.[85,101] We will use linear mixed effects models to test for main effects of group and group by time interactions on the various outcomes, considering the following groups: a) EBV-P with fatigue; b) EBV-P without fatigue; c) HC with fatigue; d) HC without fatigue, avoiding potential flaws related to high prevalence of PF in the population (Fig. 2).[75]

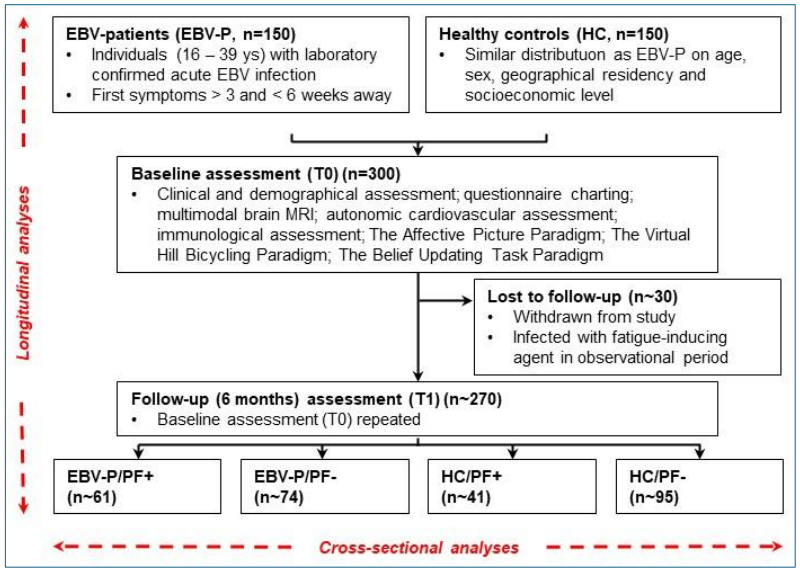

Figure 2. *Study outline. Lost to follow-up and group members at T1 is estimated based on a previous study of AYAs having a PF prevalence of 45% and 30% at six months' follow-up in the EBV and HC group, respectively.[67] PF=Persistent Fatigue, as defined by the Chalder Fatigue Questionnaire bimodal scoring.[19,101]*

5. Participants

The yearly incidence of symptomatic EBV-infection in AYAs is ~0.7%.[29] The population served by the South-Eastern Norway Regional Health Authority (the feasible catchment area of AHUS) is ~3.1M, of which ~35% are within the age range of the present study; hence, the expected number of new symptomatic EBV cases is about 7500/year. In a majority, diagnosis is confirmed by laboratory assays of EBV-serology (based on blood samples obtained by patients' GPs) (Table 1). As successfully done in previous studies,[67,85] recruitment will be based on access to laboratory results followed by invitation (short text message) to potentially eligible participants. This procedure, which implies a limited confidentially waiver, has been approved by the Norwegian Regional Committee for Medical Research Ethics in previous studies. Those who respond with interest will be screened according to exclusion and inclusion criteria (Table 2). Based on experience we assume that ~20% of screened patients will be included[67,85]. HC will be recruited by

Table 1. Serological pattern confirming acute EBV-infection

	EBV-VCA IgM	*EBV-VCA IgG*	*EBNA IgG*	*Heterophilic antibodies**
#1	Positive (≥40 U/ml)	Negative (<22 U/ml)	Negative (<20 U/ml)	Positive
# 2	Positive (≥40 U/ml)	Positive (≥22 U/ml)	Negative (<20 U/ml)	-

**The test for heterophilic antibodies will only be executed when the specific tests alone are inconclusive*

Table 2. Inclusion and exclusion criteria

Inclusion criteria	*Exclusion criteria*
EBV PATIENTS (EBV-P)	
• Acute EBV infection, laboratory confirmed. • First symptoms > 3 and < 6 weeks away • Age 16-39 years	• Co-morbidity, including mental disorder (seasonal allergy/asthma is accepted if no signs/symptoms) • Usage of pharmaceuticals (hormonal contraception and paracetamol/ibuprofen is accepted) • Concurrent demanding life event causing fatigue. • Disability impacting on daily living. • Regular smoking • Usage of illicit drugs/alcoholism • Pregnancy
HEALTHY CONTROLS (HC)	
• Age 16-39 years	• All criteria pertaining to EBV-P • Acute/recent infection with onset < 3 weeks away • Persistent post-infective symptoms

asking each EBV patient to invite a friend of the same sex and approximate age. This procedure, applied in previous projects,[67] lowers the risk of volunteer bias and ensures similar geographic residency and socioeconomic level. If needed, other recruitment strategies will be considered, such as advertising in social media. Family members of EBV-Ps (first cousins or closer) are not eligible as HCs.

6. Assessments

Details of the assessment protocol is provided below and summarized in Table 3.

Table 3. Assessments and main outcomes	
Assessment	*Main outcome*
Clinical examination, blood sampling, registry linkages, questionnaires	PF caseness, defined by the Chalder Fatigue Questionnaire bimodal scoring
	Persistence of other symptoms (sleep disturbances, cognitive difficulties, etc.)
	Trait negative affectivity
Multimodal brain MRI	fMRI responses in regions of interest (ROI) during rest and ASP-task. ROI include nodes of the salience network, such as the insula and the anterior cingulate cortex (ACC).
	Connectivity patterns in ROI at rest and during ASP-task, cf. above.
Autonomic cardiovascular assessment	Changes in HRV indices during controlled breathing; provides information on autonomic cardiovascular modulation of the sinus node of the heart
	Responses in standard cardiovascular variables and fatigue report during isometric exercise (handgrip) with and without nocebo instructions
	Changes in HRV indices during ASP-task
Immunological profiling	NK-cell functionality at rest and during catecholaminergic stimulation
Behavioural experiments	
Affect & Symptom Paradigm	Difference of general symptom reports between the negative and neutral picture condition (ASP-general)
	Difference of fatigue reports between the negative and neutral picture condition (ASP-fatigue)
Virtual Hill Bicycling Paradigm	The proportion of the regression coefficients for virtual hill gradient association with fatigue and workload association with fatigue
Belief Updating Task Paradigm	The degree of updating expectations regarding risk for adverse events while provided with official statistics.
Electroencephalography (optional)	Neural activity at rest and during task performance

Clinical examination, blood sampling, registry linkages, questionnaires

A comprehensive and structured clinical interview will encompass exposures, medical history, and current symptoms and disabilities combined with standard clinical assessment (including psychiatric and neurological screening) (Appendix 1). Blood samples will be obtained from antecubital venous puncture following application of a local anesthetic (lidocaine) and used for routine in-house analyses and biobanking; total blood draw is 49 mL (Appendix 1). In addition, urine dipstick analysis, urine pregnancy test (women) and spleen ultrasound will be carried out. The specimen handling procedures will be based on best practice from the Collaborative On Fatigue and other related symptoms Following Infection (COFFI).[23]

As a quality control of clinical and demographic data and to ensure that inclusion and exclusion criteria are met, the assessment will be supplemented by linkage with four nationwide health registries: The Norwegian Patient Registry (NPR, diagnoses and treatment in secondary care); Norwegian Registry for Primary Health Care (NRPHC, diagnoses and treatment in primary care); The Norwegian Prescribed Drug Registry; Norwegian Immunization Registry; and the Norwegian Surveillance System for Communicable Diseases.[41] Registry variables include current and previous infectious diseases one year prior to inclusion; immunizations one year prior to inclusion; co-occurring disorders; current medications; current and previous (one year prior to inclusion) usage of health care services.

In the same session as the clinical examination, all participants will complete an electronic questionnaire composed of validated inventories charting fatigue[19] and associated symptoms (post-exertional malaise,[7] pain,[50] and sleep disturbances[1]), as well as trait negative affectivity,[58] anxiety/depression,[111] illness beliefs,[15] health anxiety,[84] and functional capabilities/QoL (Appendix 2).[44]

Multimodal brain MRI

Clinical conditions and mental states which are prototypical products of bidirectional brain-body interactions (e.g., fatigue) and their corresponding brain network dynamics can be manipulated and measured using experimental paradigms and functional MRI (fMRI), which is sensitive to dynamic changes in blood oxygenation. The blood-oxygenation-level-dependency (BOLD) signal serves as a proxy for alterations in metabolic demands in response to neural activation and can be probed with spatial resolution at the millimeter scale) at a temporal resolution corresponding to seconds.

The MRI protocol includes fMRI during task (the ASP-fatigue experiment, cf. below) and rest. In addition, the protocol includes MRI sequences assessing macro- and microstructural properties of the brain (see below). fMRI data will be analyzed using different approaches, including activation-based and combinations of multivariate analysis and network modeling. The former reveals brain regions where the BOLD signal shows co-variation with the task conditions, e.g., increased or decreased activation as a function of task load. In contrast, functional connectivity targets the *coordination* between brain regions, and aids the understanding of the dynamic processing systems in the brain.[22] Our main analysis include conventional task-based analysis to reveal brain regions involved in task performance, brain network modeling[5] and graph-theoretical analysis providing clinically informative brain network properties.[16,17,79] In addition, we will assess sensitive measures of brain morphology (e.g., apparent cortical thickness, global and regional brain volumes) based on T1-weighted MRI and microstructural characteristics based on diffusion MRI (dMRI), which is sensitive to inflammation, microglial alterations and provides markers for myelin and other white matter microstructural characteristics.[64]

MRI (3T) will be performed at the Core Facility for Translational Neuroimaging, OUH, which has the infrastructure and expertise required for clinical imaging studies. All scans will be assessed by a neuroradiologist. Incidental findings will be followed up routinely, in line with established procedures.

Autonomic cardiovascular assessment

As successfully done in previous studies,[67] autonomic cardiovascular assessment will be undertaken using the Task Force Cardio™ (CNSystems Medizintechnic, Graz, Austria), which is a combined hardware and software device providing continuous, non-invasive recording of cardiovascular variables. Instantaneous RR intervals (RRIs) and heart rate (HR) will be obtained from the electrocardiogram. Continuous arterial blood pressure and stroke volumes will be measured noninvasively beat-to-beat by finger plethysmography. The RRIs will be further processed to obtain Heart Rate Variability (HRV) indices in the time and frequency domains according to international standards where the low-frequency (LF) band is defined at 0.04-0.15 Hz and the high-frequency (HF) band at 0.15-0.5 Hz.[42] Briefly, parasympathetic activity is considered the main contributor to HF-variability of heart rate (respiratory sinus arrhythmia), whereas both parasympathetic and sympathetic activity contributes to LF-variability. Task Force Cardio recordings will be performed during the following experimental conditions:

- During 5 minutes of supine rest with spontaneous breathing.
- During 5 minutes of metronome-controlled breathing at 0.2 Hz (i.e., a fixed rate of 12 breaths/min). This fixation rate increases respiratory sinus arrhythmia in healthy individuals and is a well-validated technique to assess the ability to enhance parasympathetic modulation of the sinus node; in addition, this technique standardizes the respiratory rate across study visits thereby reducing intra-individual variability of HRV indices.[82]
- During isometric exercise (handgrip). The participants are instructed to apply 30 % of max. voluntary force (self-adjusted using feedback from a force monitor) for 60 seconds three times with 60 secs. rest in-between. In addition, at T1, and second bout of isometric exercise will instruct the participants to perform the same repeated handgrip 10 times; however, the exercise is terminated after three rounds of handgrip, making the total physical challenge equal across the two bouts. Fatigue sensation is scored at the end of both bouts.
- During the ASP-fatigue experiment, cf. below.

In addition, sympathetic autonomic activity at supine rest will be assessed by measuring the plasma levels of norepinephrine and epinephrine exploiting a validated and sensitive method of high-performance liquid chromatography (HPLC) combined with tandem mass spectrometry (MS/MS).

Immunological profiling

Blood samples will be drawn from venous puncture and viable Peripheral Blood Mononuclear Cells (PBMCs) will be isolated and stored applying standard laboratory procedures. Following the sample acquisition phase, a batch of PBMCs from the entire cohort will be co-cultured with sympathetic transmitter molecules (epinephrine, norepinephrine) and stimulated with phorbol 12-myristate 13-acetate (PMA); unstimulated cultures will serve as controls (Fig. 3). This will enable us to address sympathetic-immunological interactions *in vitro*. For staining we will use a cocktail of antibodies targeted against surface receptors to provide coverage for major cell types (including antibody panel for deep NK cell profiling) and against intracellular antigens to assess stimulation-induced cytokine response within the immune cells. The assay will be optimized to minimize experimental and technical variability by acquiring multiplexed/barcoded samples on a spectral flow cytometer, which allows simultaneous recording of 40-50 parameters. A bioinformatic pipeline designed for high-dimensional single-cell cytometry data will be applied to characterize cellular phenotypes and global cytokine response profiles to stimulations. This experimental set-up will enable us to address sympathetic-immunological inter-actions *in vitro*. For staining we will use a cocktail of antibodies targeted against surface receptors to provide coverage for major cell types (including a well-designed antibody panel for deep NK cell profiling) and against intracellular antigens to assess stimulation-induced cytokine response within the immune cells. The assay will be optimized to minimize experimental and technical variability by acquiring multiplexed/barcoded samples on a spectral flow cytometer, which allows simultaneous recording of 40-50 parameters. A bioinformatic pipeline designed for high-dimensional single-cell cytometry data will be applied to characterize cellular phenotypes and global cytokine response profiles to stimulations. The immune cell population frequencies and response patterns will be compared across groups and related to clinical and pathophysiological markers.

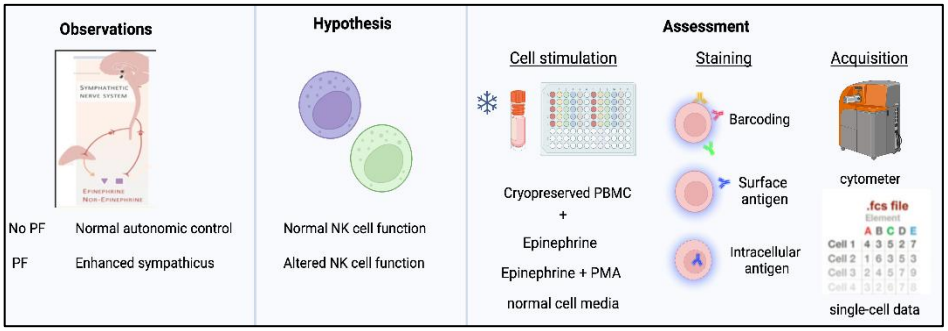

Figure 3. In-vitro *assessment of sympathetic-immunological interactions. Previous studies suggest enhanced sympathetic activity and altered NK-cell function in PF. We hypothesize that these findings are causally related and will test this assumption by co-culturing PBMCs from cases and controls with sympathetic transmitters (right lower part of the figure), followed by spectral flow cytometry assessment.*

Behavioural experiments

The Affect & Symptom Paradigm (ASP) investigates the causal influence of affective stimuli on somatic symptoms.[10] The presentation of negative pictures (compared to neutral pictures) reliably causes increased somatic symptom reports in the ASP, and this effect is moderated by chronic somatic symptom distress, i.e., higher levels of somatic symptom reports are correlated with stronger ASP effects.[69] Recent studies have demonstrated the stability of the ASP effect over time within and between experimental sessions.[68,70] As further evidence for validity, patients diagnosed with a functional disorder have demonstrated significantly larger ASP effects both on a behavioral level and regarding somatosensory neural activity.[9] Moreover, the ASP effect was mediated by stronger activation patterns in somatosensory and nociceptive brain networks in patients compared to controls. In MAP-FAT, two versions of the ASP will be used: a previously validated version targeting general somatic symptoms (ASP-General),[69,70] and a novel version of the ASP targeting fatigue (ASP-Fatigue).

In ASP-General, 48 pictures from the International Affective Picture System (IAPS) serve as visual stimuli. The pictures are presented in two blocks with 24 pictures each; one negative-valence block and one neutral-valence blocks. Each picture is displayed for 10 seconds. Block order is randomised between participants. To ensure the comparability of the two blocks, 8 pictures including animals, 8 pictures

including humans, and 8 pictures including objects will be used. Each block is followed by an assessment of valence and arousal (Self-Assessment Manikin, SAM)[13] as well as somatic symptoms assessed with a short version (12 items) of the checklist for symptoms in daily life (CSD)[102] state version (Appendix 2). The primary dependent variable is the difference of symptom reports in the CSD between the negative and neutral picture condition.

In ASP-Fatigue, the analogue methodology as in the ASP-General will be used (including negative and neutral picture sets from the IAPS). In contrast to the ASP-General, participants will not receive the CSD but a list of fatigue related symptoms (mental vs. physical aspects of fatigue after each set of pictures. A further modification of the ASP-Fatigue concerns the active manipulation (within subjects) of the task instruction (control vs. nocebo): the nocebo instruction will provide the information that viewing the following set of pictures might cause an increase in subjective levels of fatigue, whereas the control condition will not contain this information. This manipulation of task instruction allows for directly testing the role of prior expectations according to the predictive processing theory.[96]

The ASP-Fatigue will be carried out three times in each participant at each time point. The two first bouts (with no-nocebo and nocebo instructions, respectively) during supine rest with the participant attached to the Task Force Cardio, enabling simultaneous recording of autonomic cardiovascular control, cf. above. The third bout in the MRI scanner (no-nocebo instruction), enabling assessment of corresponding brain activity, cf. above.

The Virtual Hill Bicycling Paradigm (VHBP) will be used to measure the extent to which participants rely on interoceptive input versus prior expectation in the perception of exercise-induced fatigue. We will use an adaptation of a recently developed task that has produced reliable results in the context of breathlessness perception.[32] In this study, healthy participants cycled repeatedly 100m distances on a cycle ergometer while wearing a VR headset depicting a hill. Across the trials, two variables were manipulated independently: (1) pedal resistance affecting the actual power (W) that participants needed to exert to cycle; (2) virtual hill slope, impacting the expected power to cycle. It was shown that 18% of the variability in breathlessness after the cycling trials was explained by differences in actual power, whereas 19% was explained by differences in virtual hill slopes. This result shows that breathlessness perception relies on the integration of input from peripheral body and (implicit) expectations. Similar conclusions were reached in another study with this paradigm.[81] We will adapt this task in several ways by (1) measuring exercise induced fatigue rather than breathlessness; (2) adapt the trial structure to assess inter- and intra-individual differences beyond group differences; (3) assess autonomic cardiovascular responses (heart rate, blood pressures, cardiac output, total peripheral resistance); and (4) avoid maximal and sub-maximal physical efforts.

During the virtual hill cycling task, participants will complete a course consisting of three practice trials followed by 12 trials of 150m each with physical slope to take on values of 0.5%, 2% or 4%, and the virtual slope to take on values of 3%, 5%, 7% and 9%. The trials will be interspersed with 50m intervals of 0% virtual and actual slopes. To rule out order/time effects as far as possible, a balanced latin square will be used to distribute the trials. Cardiovascular variables will recorded beat-by-beat using the Task Force Cardio. After each trial, participants will retrospectively rate fatigue induced by that particular hill ride on a Likert scale ranging from 0 to 10. From this task, we will determine the relative contributions of interoceptive input and prior expectations to the perception of fatigue as follows. “Interoceptive input” will be quantified as power (Watt) exerted by the participant during the trial, while “prior expectations” will be quantified as the virtual hill slope. A random intercept random slope linear mixed model on the entire study sample will be fit with fatigue ratings as the dependent variable, and exerted power and virtual hill slope as independent variables, controlling for age, sex, BMI, estimated fitness level, and the effects of heart rate and breathing behavior. For inferential purposes, the interoceptive input x prior expectations x group interaction will be examined. From this model, parameter estimates of the participant specific slope (βi) for exerted power and virtual hill slope will be extracted (with i = the individual). For each participant, the ratio between the two - β(i) exerted power/β(i) virtual hill slope - will serve as an indicator of the relative contribution of interoceptive input versus prior expectations.

The Belief Updating Task Paradigm (BUTP) assesses abnormal fixity of conscious beliefs that can play the role of priors. The BUTP has been increasingly used in clinical depression studies, showing that negative beliefs about oneself are less updated in individuals with depression as compared with controls.[34,52,87,92] Thus, for example, individuals’ conviction of self-unworthiness remains despite cumulating counter evidence, while evidence in favor reinforces unworthiness. According to the

predictive processing model, these "stubborn" priors explain why patients are trapped into a vicious circle of symptoms perpetuation.[110] Interestingly, compared to HC, patients with treatment resistant depression updated their beliefs more after good than bad news following a single ketamine infusion.[12] This suggests that abnormal belief updating is a core cognitive psychopathological feature closely related to symptom evolution. BUTP quantifies the flexibility versus fixity of specific priors, enabling targeted, personalized CBT interventions. The procedure can be delivered online.
BUTP has not been used to study PF, despite related models of disease mechanisms.[56,96] Because it focuses on the cognitive content of priors, i.e., conscious beliefs, the BUTP is a good complement to the ASP (that focuses on emotions rather than cognitive content) and the VHBP (that focuses on the integration of sensory cues and priors to shape perceptions). The three complementary tasks enable a comprehensive description of the behavioral phenotype for all participants, which is a truly unique opportunity to better understand PF and identify subgroups related to different facets of predictive processing. Additionally, BUTP offers the chance to separate between priors that have distinct cognitive content and that can be targeted by distinct therapeutic tools, in particular priors related to negative affectivity in general (e.g., "I am at risk for self-unworthiness") and priors related specifically to health anxiety and fatigue (e.g., "I am at risk for chronic fatigue after acute infection").
The BUTP setup will be provided as an online version to all participants at T0 and T1. Participants will be presented on a trial-by-trial basis with a total of 60 different negative life events targeting general negative affectivity (e.g., the risk for not living in a happy relationship) as well as negative events related specifically to health issues (e.g., the risk for developing hypertension) and post-infective issues (e.g., the risk for experience long-term sick leave after an acute infection), and information about the events' likelihood in the general population (base rates, BR) (20 issues in each of the three categories). BR will range between 5% and 75% and be normally distributed to avoid confounds of optimism biases. Participants rate their own likelihood of experiencing this specific negative event in the future (E1), and the likelihood for someone else (eBR). Then, they are provided with the actual BR of occurrence of this specific negative life event in form of global population estimates adapted to the Norwegian context. At the end of each trial, participants re-estimate (E2) (update) their likelihood of experiencing the given negative life event after being informed about the true average incidence rate.

Electroencephalography (EEG) (optional)
Pending additional funding, EEG will be used as a non-invasive method to measure brain activity during computerized test paradigms. The recordings provide high-temporal-resolution electrophysiological measures of neural activity related to cognitive processes relevant to persistent fatigue, including attention, decision-making, and the integration of expectations with sensory information. Standard scalp electrodes will be applied according to established safety procedures, and the session will take approximately 1-2 hours. The method involves no discomfort beyond routine electrode placement. The gel applied between EEG electrodes and the scalp may, in rare cases, cause a slight rash. Monitoring and standard cleaning procedures will be used to minimize this risk.

7. Statistical methods

Generally, continuous variables will be reported with parametric (mean/standard deviation) or non-parametric (median, quartiles) descriptive statistics, depending on the distribution which will be assessed through visual inspection of plots as well as formal tests of normality. Ordinal/nominal variables will be reported as frequencies and relative frequencies.
Statistical tests will primarily be performed on per protocol data sets, which is defined as all participants who completed the observation period (attended and completed consultation at baseline (T0) and at 6 months (T1)) without any of the following protocol deviations: a) Lost to follow-up; b) Primary endpoint missing at T1; c) Infected with a fatigue-inducing agent during the observational period. Missing data will not be routinely imputed but considered on a case-by-case basis determined by rates of missingness, and eventually applied for sensitivity analyses using multiple imputation techniques.
All statistical tests carried out will be two-sided. We will apply generalized linear models, with the distributional family and link functions determined by the nature of the outcome variables. For example, the primary endpoint of fatigue caseness at T1 will utilize a modified Poisson approach (log-link and robust error variances) to allow estimation of relative risks with corresponding confidence intervals. A p-value <0.05 is considered statistically significant. Generally, the planned cross-sectional analyses at

T1 will have adequate power (>80%) to detect small-to-medium effect sizes (Cohen's *d* ~0.35 to 0.50), which we find satisfactory. These models will include group as a fixed factor, and control for demographic differences between groups and corresponding values obtained at T0 (the functional form for which will be determined based on visual inspection of fit and AIC metrics). As far as possible, researchers performing statistical analyses will be blinded for data that may be a source of potential bias (such as group adherence). Using Bayesian modeling we will also be able to estimate the evidence of the null hypothesis (i.e., the lack of effect), which may be particularly relevant in studies of complex conditions with heterogeneous etiology and small group-level effect sizes.[48]
Further details on statistical methods will be provided in a separate Statistical Analysis Plan made publicly available prior to participant inclusion.

8. Dissemination and exploitation

The target audiences for dissemination are the international medical and public health communities, PF-sufferers/their relatives, as well as politicians, health care administrators, and the general audience. MAP-FAT will adhere to the EU's Open Science policy, maximising impact. We will a) Follow an *open methodology* approach to research by sharing results, methods and data (pending relevant approvals) openly; b) Ensure *open research data*, applying the Findable, Accessible, Interoperable, Reusable (FAIR) principles; c) Promote *open access and review* guaranteeing free access to all peer-reviewed publications. Also, results will be disseminated broadly by a) Actively publicizing research results in traditional media and b) social media; and c) Distribution of results through patients' organizations.
A roadmap for exploitation will be developed during the last part of the project period. Of particular importance is a strategic grant plan intended to fund clinical trials that specifically target disease mechanisms identified through MAP-FAT. Intervention may be therapeutic (e.g., pharmaceuticals interacting with autonomic and/or immune functions) or prophylactic (e.g., behavioural interventions in the aftermath of acute infections to modify strong priors). Notably, the AHUS consortium partner has a strong track record of conducting complex clinical trials and has developed a similar translational strategy within the Long COVID field.[62,88]

9. Partners, advisors, consumer involvement

MAP-FAT is led by a consortium of four partners: *Prof. Vegard Wyller*, MD, PhD (AHUS, Norway) chairs the consortium. He is adjunct professor of medicine (pediatrics), head of research at a clinical department, and chairs the international COFFI consortium.[23] The other partners are *Prof. Lars T. Westlye*, PhD (University of Oslo, Norway) who directs the Multimodal Imaging Group at the Centre for Precision Psychiatry, UiO/OUH; *Prof. Michael Witthöft,* PhD (Ruhr University Bochum, Germany), who is a full professor of clinical psychology, psychotherapy, and experimental psychopathology; and *Ass. Prof. Victor Pitron,* MD, PhD (Assistance Public-Hôpitaux de Paris, Université Paris Cité, France), who is a trained psychiatrist. *Em. Prof. Omer Van den Bergh* (University of Leuven, Belgium) will be affiliated with the project as an expert advisor. *Ass. Prof. Erin Cvejic* (University of Sydney, Australia) is a biostatistician and will advise on statistical methods during the course of MAP-FAT. The consortium partners will meet regularly (monthly during the first six months, thereafter quarterly) to oversee progress in the specific aims, milestones and performance indicators, and the associated timelines. The collaboration will be promoted by internal workshops and seminars to ensure real integration.
The *Consumer Advisory Committee (CAC)* of the COFFI collaborative will serve as a consumer advisory board for MAP-FAT. The COFFI CAC consists of eight members from five countries, all of them previously or currently suffering from PIFS.[23] The CAC will be involved in all stages of the project, such as priority settings among research questions, plans for recruitment, data interpretation and dissemination. The CAC will meet with the consortium partners at least twice a year.

10. Ethics, data management, gender perspectives

Participation in the project will be based upon informed consent, and thorough information will be provided orally as well as in writing. Approval will be obtained from the Regional Committees for Medical and Health Research Ethics as well as the Data Protection Officer at Ahus and Oslo University Hospital. All participants' travel expenses will be reimbursed. In addition, each participant will receive a NOK 500 gift card once all study procedures are completed. The project will be pre-registered with

ClinicalTrials.gov, the study protocol and statistical analysis plan will be made publicly available prior to inclusion of any participant, and results will be reported following the STROBE guideline for observational cohort studies. Efforts will be focused on minimizing burden on individual participants, including the provision of routine local anaesthetic ointment prior to blood sampling. The procedures carry a very low risk of harm.

EBV-patients will be included based upon laboratory tests indicating acute EBV infection. This requires a limited confidentiality waiver, providing a study secretary with information that identifies potentially eligible participants so that they may be sent a short text message inviting them to receive further study information. We regard this violation of the confidentiality principle to be rather limited with only minor potentially negative consequences for the participants, and that it is outweighed by the potentially large individual and societal benefits of the project.

MRI data will be stored and analysed together with data from the ongoing BRAINMINT project (REK 2019/943), led by Prof. Lars T. Westlye. A separate informed consent will be obtained from the participants regarding BRAINMINT participation.

All data generated from MAP-FAT will be considered for inclusion in the COFFI database (under establishment), containing totally anonymized data from 15 post-infective cohort studies across 7 countries (cf. www.coffi-collaborative.com). The COFFI database is managed by Ahus and approved by the Data Protection Officer.

Data management will comply with obligations and requirements outlined in ethics and data protection regulations such as the GDPR (2016/679) and the ePrivacy Directive (2002/58/EC). The Guidelines for Data Management in Horizon Europe and the European Clinical Research Infrastructure Network (ECRIN) certification standards will be applied. A detailed Data Management Plan will be set up before the project starts. A data repository as well as a secure portal for the data access among consortium members will be established using the infrastructure provided by the Services for Sensitive Data (TSD) at the University of Oslo.[86] Totally anonymized data will also be shared at a trusted, public repository such as ZENODO at project end. We will adhere to the FAIR (findable, accessible, interoperable, reusable) principles:

- A unique and persistent Digital Object Identifier (DOI) will be assigned to each item in the public repository, thus making them easily findable.
- MAP-FAT (meta)data will be retrievable by its identifier; all beneficiaries will ensure open access to the deposited data, following the principle ‘as open as possible as closed as necessary’.
- MAP-FAT (meta)data will use a formal, accessible, shared, and broadly applicable language for knowledge representation. Interoperability resources such as FAIRsharing will be applied.
- The conditions under which the data can be used will be made transparent through easy-to-use copyright licenses, such as Creative Commons (Creative Commons Attribution International Public License, CC BY) or Creative Commons Public Domain Dedication (CC 0).

11. Risks and mitigations considerations

The MAP-FAT consortium is uniquely positioned to contribute to the field, with low risk of failure, and with ample experiences in all methodological facets from previous projects. The following risks and corresponding mitigation efforts are identified:

- *Recruitment failure of the EBV cohort:* The study may increase the age span of eligible participants.
- *Failure of data acquisition:* The design of the EBV cohort is similar to previous cohort studies conducted by the consortium coordinator.[67,85] Should there be more losses to follow-up than expected the number of included patients may be increased.

12. Funding

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13. Potential extensions

The MAP-FAT project group is currently drafting two possible extensions of the present project:

- An experiment conducted during the second study visit involving intravenous infusion of one dosage *dexmedetomidine* to inhibit sympathetic nervous activity, followed by assessment of symptoms and immunological functions
- A mechanistic clinical trial recruiting participants with persistent fatigue at the second study visit; the trial encompasses two psychological-behavioral treatment techniques that may influence brain networks hypothesized to underpin the experience of fatigue.

If these extensions are implemented, separate study protocols and formal approvals will apply, and inclusion will be based upon separate informed consent.

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Appendix 1. Clinical examination and blood sampling

CLINICAL EXAMINATION	
History taking	Sex/gender, ethnicity, symptoms of severe depression/anxiety, concurrent adverse life events
General examination	Blood pressures, heart rate, respiratory rate, height, length, body temperature, rashes, enlarged lymphatic nodes
Organ specific examination	Heart/lung auscultation, abdominal palpitation (liver/spleen enlargement), throat (redness, tonsillar enlargement), otoscopy, neurological signs, psychiatric signs
Additional	Urine dipstick, urine pregnancy test (women).
BLOOD ANALYSES	
Routine in-house analyses	Hb, WBC with differentials, platelets, Na, K, Creatinine, ALT, GT, bilirubin (fractioned), CRP, albumin, INR, glucose, HbA1c, vit B12, folate, ferritin, proBNP, TSH, FT4, CK, IgG, IgA, IgM, VCA-IgM, VCA-IgG, EBNA-IgG, EBV-PCR, CMV-IgM, CMV-IgG. *Total blood draw 11.5 mL*
Biobanking (cf. below).	Serum, plasma, whole blood (DNA), PAXgene (RNA), PBMC. *Total blood draw 33.5 mL*

MAP-FAT BIOBANKING OVERVIEW

Specimen	Volume	Processing	Time from collection to storage	Aliquoting	Storage
Serum	7 ml	2000 g at RT for 10 min	Within 2 h	5 x 0.5ml	-80°C
Li-Hep plasma For catecholamine analysis	4 ml	Just after collection the tubes should be placed on ice and centrifuged for 10 min at 2500 g at 4°C within 30 minutes	Within 1 h	2 x 1.0ml	-80°C
EDTA whole blood for DNA extraction	8 ml	Direct storage after inversion before centrifugation for plasma:	Within 2 h	2 x 0.5ml	-80°C
EDTA plasma		2000 g at RT or 4°C for 10 min	Within 2 h	4 x 0.5ml	-80°C
PAX tubes for RNA extraction	2,5 ml	Store tube upright for 2 h at RT. Freeze at -20°C for 24 h before transfer to -80°C for long-term storage		1	-80°C
Viable PBMCs using CPT tubes	16 ml	Turn CPT tubes 8-10 times right before centrifugation at 1700 g for 20 min	Within 2 h	2 x 1ml	-150 °C
	37,5 ml				

EDTA- Ethylene diamine tetra acetic acid; PBMC-peripheral blood mononuclear cells; RT- room temperature; h-hour; min-minutes; sec-seconds

Appendix 2. Questionnaire overview

MAP-FAT: Composite questionnaire: Constructs, inventories and scoring procedures

Construct(s)	Name of inventory	Description and scoring procedures
BACKGROUND AND DEMOGRAPHICS		
Household, socioeconomic level	Not applicable	Household members; education; occupation; the international socio-economic index (ISEI) of occupational status were used to score socio-economic level. (Ganzeboom 1992; Ganzeboom 2008)
Smoking, alcohol, illicit substances	Not applicable	Answered on a 5-point Likert scale, where 1 is "never" and 5 is "every day/almost every day".
Physical activity	Not applicable	Answered on a 5-point Likert scale, where 1 is "a lot less active than peers" and 5 is "a lot more active than peers".
Diseases/medicines	Not applicable	Comorbidity; chronic disease affecting parents or siblings; chronic medicines
SYMPTOMS AND DISABILITY		
Fatigue	Chalder Fatigue Questionnaire (CFQ)	A total of 11 items scored on 4-point Likert scales. In order to obtain a continuous variable, each item is scored 0-3 where 0 is "less than usual" and 3 is "much more than usual"; then, a total sum score across all items is obtained ranging from 0 to 33, where higher scores indicate more fatigue. (Chalder 1993) In addition, bimodal scoring (0-0-1-1) of each item will be performed; a total sum score across all items of 4 or higher is defined as fatigue caseness.
Clinical symptoms of PIFS	CDC symptom inventory for Chronic Fatigue Syndrome	A total of 30 items addressing frequency of specific symptoms since falling ill from acute EBV-infection on 5-point Likert scales, where 1 is "never" and 5 is "all the time". (Wagner 2005). At follow-up, the questions will be slightly rephrased in order to address symptom frequency during the last months. Follow-up answers will be used to define caseness of PIFS according to the Fukuda criteria. The average score across four items addressing cognitive symptoms (memory, decision making, concentration, and confusion/disorientation) is taken as an index of subjective cognitive difficulties.
Post-exertional malaise (PEM)	PEM items from the DePaul Symptom Questionnaire	A total of five items addressing frequency of PEM symptoms on 5-point Likert scales, where 0 is "never" and 4 is "all the time"; answers are to be averaged across all items and multiplied with 25 to get a 100 point scoring scale where higher scores indicate more PEM.(Jason 2018; Bedree 2019).
Sleep disturbances	Karolinska Sleep Questionnaire (KSQ)	A total of 12 items addressing frequency of sleep disturbances on 6-point Likert scales, where 1 is "never" and 6 is "all the time"; then, the scoring is reversed, and total sum score will be computed across all items ranging from 12 to 72, where *lower* scores indicate more sleep disturbances. (Åkersted 2008). Accordingly, indexes for insomnia, awakening problems, and sleepiness will be computed as sum scores across relevant items.
Depression and anxiety symptoms	Hospital Anxiety and Depression Symptoms (HADS)	A total of 14 items addressing different symptoms of depression and anxiety on 4-point Likert scales scored 0 – 3; for eight of the items, scoring is reversed, after which total sum score is computed ranging from 0 to 42, where higher scores indicate more symptoms of depression and anxiety. (Zigmond 1983) Accordingly, separate indexes for depression and anxiety will be computed as sum scores across relevant items (seven each, total range 0 to 21). A score of 15 or higher on HADS-D is taken to suggest possible severe depression.
Negative affect	Positive and Negative Affect Schedule, short-form (PANAS-SF)	A total of five items addressing negative affects (shameful, anxious, nervous, hostile, offended) on 5-point Likert scales, where 1 is "disagree completely" and 5 is "agree completely"; total sum score will be computed ranging from 5-25, where higher scores indicate more negative affects.(Thompson 2007)
Illness perception	Brief Illness Perception Questionnaire (BPIQ)	A total of eight items addressing perceived impact of acute infection are scored on 11-point Likert scales 0 – 10; total sum score will be computed ranging from 0 to 80, where higher scores indicate more perceived impact. (Broadbent 2006). At follow-up, the questions will be slightly rephrased in order to address symptoms following acute infection.
Functional disability and Quality of life	Short Form Health Survey 36 (SF-36)	A total of 36 items, scored on Likert scales and recoded to achieve 100 point scales (higher score means better functional capabilities/quality of life); average scores will be reported. Eight subdomains: Physical functioning; role limitations due to physical problems; role limitations due to emotional problems; vitality; mental health; social functioning; bodily pain; general health. (Ware 1992).
Pre-existing symptoms	Patient Health Questionnaire (PHQ-15)	A total of 15 symptoms addressing pre-existing symptom burden in the past 4 weeks; each item scored on a 3-point Likert scale where 0 means "not bothered at all" and 2 means "bothered a lot"; total sum score ranges from 0 to 30 (Kroenke et al 2002)
Miscellaneous	Not applicable	• One item addressing avoidance behavior on a 11-point Likert scale, where higher scores indicate more avoidance tendency. • One item addressing school/work absenteeism as number of totally absent days during the last month.
PSYCHOLOGICAL TRAITS AND SOCIAL FACTORS (ONLY ASKED AT FIRST VISIT (T0, BASELINE))		
Neuroticism	NEO Five-Factor Inventory-30 (NEO-FFI-30)	A total of six items making up the neuroticism axis is included and scored on 5-point Likert scales where 0 is "disagree completely" and 4 is "agree completely"; total sum score across all items will be computed ranging from 0 to 24, where higher scores indicate stronger neuroticism tendencies.(Körner 2008).

Worrying tendencies	Penn State Worry Questionnaire (PSWQ)	A total of 16 items addressing worrying tendencies are scored on 5-point Likert scales where 1 is "disagree completely" and 5 is "agree completely"; scoring is to be reversed on five items, after which the total sum score across all items will be computed ranging from 16 to 80, where higher scores indicate stronger worrying tendencies. (Pallesen 2006)
Emotional awareness	Toronto Alexithymia Scale (TAS-20)	A total of seven items making up the index of Difficult identifying feelings are included and scored on 5-point Likert scales where 1 is "disagree completely" and 5 is "agree completely"; total sum score will be computed across all items ranging from 7 to 49, where higher scores indicate poorer emotional awareness (ie. more difficulties identifying feelings). (Bagby 1994)
Loneliness	UCLA Loneliness Scale	A total of 20 items addressing loneliness are scored on 4-point Likert scales where 1 is "never" and 4 is "always"; scorings will be reversed on nine items, after which the total sum score will be computed ranging from 20 to 80, where higher scores indicate more loneliness. (Russel 1980).
Sensory processing sensitivity	Highly Sensitive Person Scale, short version (HSP-12)	A total of 12 items addressing sensory processing sensitivity on 7-point Likert scales where 1 is "not at all" and 7 is "extremely"; the average across all items provides a general sensitivity score where higher values indicate higher sensitivity (Pluess et al, 2023). Accordingly, subscores for three different facets of sensory processing sensitivity (ease of excitation, aesthetic sensitivity, low sensory threshold) are computed across relevant items.
Previous psychological distress	Hierarchial Taxonomy of Psychopathology, self-report (HiTOP-SR), somatoform items	A total of 26 items rated on a 4-point Likert scale (1=not at all; 2=a little; 3=moderately; 4=a lot) (Measurement of the Hierarchical Taxonomy of Psychopathology (HiTOP). Dimensions include bodily distress, body focus, conversion symptoms, disease conviction, and health anxiety
Miscellaneous	Not applicable	A total of four self-invented items addressing interoceptive awareness and positive expectancies, scored on 5-point Likert scales where 1 is "disagree completely" and 5 is "agree completely.

STATE QUESTIONNAIRES DURING ASP EXPERIMENT		
Emotion	Self-assessment manekin (SAM)	Valence and arousal rated on 9-point Likert scales corresponding with simple drawings (ie., language-free) (Bradley 1994).
Symptoms	Checklist Symptoms Daily life – short version (CSD-12)	A total of 12 items on specific symptoms rated on a 5-point Likert scale where 1 is "not at all" and 5 is "extremely". (Wientjes et al 1994).
	Checklist Symptoms Daily life – fatigue version (CSD-FV)	A total of 12 items on specific symptoms rated on a 5-point Likert scale where 1 is "not at all" and 5 is "extremely".

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