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

Statistical Analysis Plan

Clinical Trials ID
NCT07394855

Versions/revisions
Version 01 (28.11.2025), original

Version 02 (19.05.2026), revised following pilot experiments
- Specification on managing multiplicity testing issues
- Minor adjustments of wording throughout

1. Aim and study design

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.

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 Mechanism of Persistent Fatigue (MAP-FAT) project is designed to scrutinize these potential brain-body interactions in PF. The main objectives are to determine:

- The relationship between PF, brain network dynamics, sympathetic nervous activity and immunological alterations and
- 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 observational cohort study of n=150 individuals with acute Epstein-Barr virus (EBV) infection and n=150 healthy controls followed for six months (Fig. 1). 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).

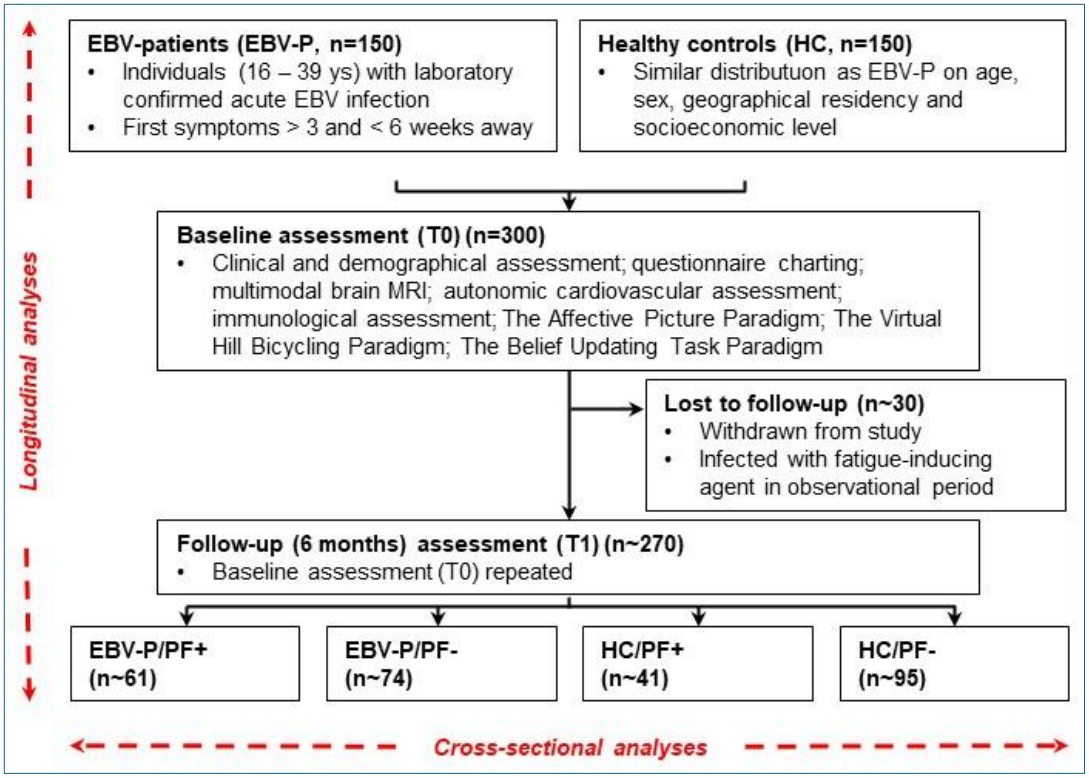

Figure 1. *Study outline. Lost to follow-up and group members at T1 is estimated based on a previous study.*

Fatigue caseness at T1 is the primary outcome, defined as a total sum score ≥4 on the Chalder Fatigue Questionnaire, applying bimodal scoring (0-0-1-1) of single items. The behavioural experiments (ASP, VHBP, BUTP) are seen as the most important part of the assessment program and therefore used for sample size considerations.

EBV-patients (EBV-P) will be recruited from the South-Eastern region of Norway (total population ~3.1M) based upon laboratory analyses indicating acute EBV infection. Healthy controls (HC) will be recruited by asking each EBV patient to invite a friend of the same sex and approximate age. Inclusion and exclusion criteria are listed below (Tab. 1).

Table 1. 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

An overview of the assessment program is given in Table 2.

Table 2. 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

2. Sample size considerations

A previous observational study of young people with acute EBV-infection had less than 5 % loss to follow-up. Hence, we consider 10 % as the upper rate of protocol deviations (including loss to follow-up), which would result in n=270 participants for per-protocol analyses at T1 (cf. paragraph 4).

Generally, the planned cross-sectional analyses at T1 across two groups (e.g., fatigue vs non-fatigue cases) have adequate power (>80%) to detect small-to-medium effect sizes (Cohen's *d* ~0.35 to 0.50), which we find satisfactory. Based on previous studies of ASP and BUTP, a sample size of n=270 is sufficient to detect medium-sized group differences (Cohen's $d \geq 0.40$, alpha=0.05; power=90%; independent samples *t*-test). For a 2×2 mixed factorial ANOVA (alpha=0.05; power=90%), a sample size of n=270 is sufficient to detect medium-sized (f=0.25) between-group effects. The VHBP is a novel paradigm, which has not previously been used to examine ratings of fatigue as an outcome. Given this, it is not possible to generate precise power estimates without first obtaining preliminary data. However, a minimum total sample size of n=118 is assumed sufficient to provide 90% power (at alpha=0.05) to detect small within-between interaction effects (f=0.10) in a mixed repeated measures design.

3. Variables

An overview of variables is given in Table 3.

Table 3. Variables	
Main category	*Variables and explanations (not exhaustive list)*
Background/demographics	Sex
	Age
	Height, weight, Body Mass Index (BMI)
	Ethnicity
	Diseases/comorbidities (previous and present)
	Medicines/other treatments (previous and present)
	Vaccines
Social and behavioural markers	Household members
	Socioeconomic level/Level of education
	Chronic disease, family member
	Smoking
	Alcoholic beverages, illicit drugs
	Average level of physical activity
	UCLA loneliness questionnaire, total sum score
Psychological traits	NEO-FFI-30, subscore neuroticism
	Penn State Worry Questionnaire, total score
	Toronto Alexithymia Scale (TAS-20), emotional awareness subscore
	Highly Sensitive Person Scale (HSP-12), average score
	Hierarchial Taxonomy of Psychopathology, self-report (HiTOP-SR), somatoform items. Total sum score, as well as the five somatoform subscales (bodily distress, somatic preoccupation, conversion symptoms, health anxiety, and disease conviction)
Symptoms, function and quality of life	Chalder Fatigue Questionnaire (CFQ), total sum scores, both Likert-based (0-1-2-3) and bimodal (0-0-1-1) – the latter is used for fatigue caseness (≥ 4).
	Accompanying symptoms (post-exertional malaise; sleep disturbances; pain, general infectious symptoms; cognitive, digestive, cardiac, autonomic symptoms).
	Hospital Anxiety and Depression Symptoms (HADS), subscores.
	Positive and Negative Affect Schedule, short-form (PANAS-SF), total sum score for negative affect
	Brief Illness Perception Questionnaire (BPIQ), total sum score
	Patient Health Questionnaire (PHQ-15) on pre-existing symptoms, total sum score
	SF-36 subscores on functional abilities/quality of life
Clinical findings	General clinical examination, pathological findings
	Neurological examination, pathological findings
	Brief psychiatric assessment, pathological findings
Blood analyses	Standard whole blood haematological variables (Haemoglobin; Leucocyte total and differential count; Platelet count)
	Standard plasma biochemistry variables (C-reactive protein (CRP); Sodium; Potassium; Calcium; Creatinine; ALT; GT; Albumin; Bilirubin; CK, Glucose; HbA1c; Vitamin B12; Folic Acid; Ferritin; NT-proBNP; INR; Thyroxine; TSH; Cortisol)
	Plasma catecholamines (norepinephrine; epinephrine)

Affect and Symptom Paradigm (ASP), general	Valence and arousal, 1-9 scales
	Checklist of Symptoms in Daily life (CSD), general version, total sum score
	CSD-gen-dif: CSD-gen score negative pictures minus CSD-gen score neutral pictures
	HR-gen-dif: Heart rate (HR) negative pictures minus HR neutral pictures
	HRV-gen-dif: Heart rate variability (HRV) indices (LF-power, HF-power, LF/HF, RMSSD, pNN50) during negative pictures minus HRV indices neutral pictures
Affect and Symptom Paradigm (ASP), fatigue	Valence and arousal, 1-9 scales
	Checklist of Symptoms in Daily life (CSD), fatigue version, total sum score
	CSD-fat-dif: CSD-fat score negative pictures minus CSD-fat score neutral pictures
	HR-fat-dif: Heart rate (HR) negative pictures minus HR neutral pictures
	HRV-fat-dif: Heart rate variability (HRV) indices (LF-power, HF-power, LF/HF, RMSSD, pNN50) during negative pictures minus HRV indices neutral pictures
	Nocebo-fat-effect: CSD-fat-dif during nocebo instruction minus CSD-fat-dif during non-nocebo instruction.
Belief Updating Task Paradigm (BUTP)	E1: Probability estimate (instructed to be in the range 5 – 75 %) of experiencing a particular event oneself.
	eBR: Probability estimate (5 – 75 %) for a similar other of experiencing the same event.
	E2: Updated probability estimate (5 – 75 %) of experiencing a particular event oneself having been informed about the Base Rate (BR).
	Estimation error: | E1 – BR |
	Valence: E1 > BR vs. E1 < BR (“good” vs “bad” news)
	Update: E1-E2 / EE (if “good news”); E2-E1 / EE (if “bad news”).
	Update bias: Mean update “good news” minus mean update “bad news”.
	Distance: E1 minus eBR.
Virtual Hill Bicycling Paradigm (VHBP)	Perceived fatigue (scale from 0 to 10, 15 times)
	Distance travelled
	Power (Watt)
	Physical slope
	Virtual slope
	Conventional cardiovascular variables (HR, blood pressures, cardiac output, total peripheral resistance), sampled beat-to-beat
	Virtual slope vs. fatigue ratings association (regression coeff. β_{vs})
	Power vs. fatigue ratings association (regression coeff β_{p})
Brain MRI	fMRI neuronal activity (BOLD) in regions of interest (ROI), in particular insula and anterior cingulate cortex (ACC),.at rest
	fMRI responses in regions of interest (ROI) during ASP-fatigue
	Connectivity patterns in ROI at rest
	Connectivity patterns in ROI during ASP-fatigue
Autonomic cardiovascular assessment	Supine resting value for conventional (HR, BP, CO, TPR) cardiovascular variables and HRV indices (LF-power, HF-power, LF/HF, RMSSD, pNN50)
	Changes in conventional variables and HRV indices during controlled breathing at 0.2 Hz
	Mean changes in conventional cardiovascular variables and fatigue ratings from rest to max exercise during 3 consecutive bouts of handgrip (each bout 30 % of max force, 60 seconds duration).
	Mean changes in standard cardiovascular variables and fatigue ratings from rest to max exercise during 3 consecutive bouts of handgrip following a standardized nocebo instruction [Variable obtained at 6 months follow-up only]
Immunological profiling	Plasma concentration of selected immukines, cytokines, and growth factors, assayed by multiplex technology
	Proportion of NK-cells, B-cells and T-cells at different stages of differentiation, unstimulated.
	Proportion of NK-cells, B-cells and T-cells following unspecific mitotic stimulation (PMA) and co-cultured with epinephrine
Study design characteristics	Screening results: Number of screened, number/characteristics of excluded/declined, reasons for exclusion
	Lost to follow-up: Total number, reasons for being lost to follow-up, number of incomplete cases
	Time span from T0 – T1
	Protocol deviations: Lost to follow-up, primary endpoint missing, infected with fatigue-inducing pathogen during observational period

4. Analysis sets

The per-protocol analysis set (PPAS) will be used for the main analyses, and is defined as all enrolled participants who attended and completed the assessment program 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 pathogen (e.g., SARS-CoV-2) during the observational period. Missing data will not be imputed in the PPAS, and outliers will not be removed.

The full analysis set (FAS) is defined as all enrolled participants (n=300) and will be used for sensitivity analyses (cf. below). Missing data will be replaced by multiple imputation techniques. The number of data sets will be guided by the proportion of cases that are incomplete. All available data from background, study design and efficacy variables will be used to generate imputed data sets. Rubin's rule will be used to combine estimates and standard errors.

5. Statistical methods

The main results will be presented according to the STROBE recommendations for reporting on prospective observational cohort studies. 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).

Descriptive statistics
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. Transformation of variables will only be done if necessary for complying with formal requirements of planned statistical analyses, and information on variable transformation will be reported.

Population characteristics and attrition
The two groups (EBV-P and HC) will be compared using descriptive statistics and statistical tests as appropriate. For participants who fulfilled inclusion criteria, an attritional analysis featuring logistic regression will be carried out assessing the associations between characteristics of the invited individuals and their decision to decline/accept the invitation to participate in the study.

Loss to follow-up
Number and proportions of losses to follow-up will be reported. Descriptive statistics for key background and outcome variables will be reported across the group lost to follow-up and the group remaining in the study, respectively. Also, for all these variables, associations to being lost to follow-up will be formally assessed by logistic regression.

Statistical tests related to outcomes
Generally, we will apply generalized linear models on the PPAS, with the distributional family and link functions determined by the nature of the outcome variables. For example, analysis of risk factors for 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. For cross-sectional analyses at T1, 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). 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.

All statistical tests carried out will be two-sided. 95 % confidence intervals and effect sizes will be reported as applicable. A p-value <0.05 is considered statistically significant. "Raw" p-values will be

reported throughout; i.e., not adjusted for multiple testing. However, for the main analyses related to the behavioural experiments (ASP, VHBP, BUTP), we will also report adjusted p-values that adhere to a family wise error rate (FWER) at an overall 5% level.

Sensitivity analyses
Sensitivity analyses using the FAS instead of the PPAS will be applied for key outcome variables. Additional sensitivity analyses will be considered in the presence of substantial outliers within key outcome variables (i.e., analyses will be carried out after removal of outliers).

Subgroup analyses
Each participant within the EBV-P group will be assessed according to the modified Fukuda-criteria of post-infective fatigue syndrome (PIFS). Classification in three groups (certain PIFS case; uncertain PIFS case; no-PIFS case) will be performed by two researchers independently; if disagreement, the classification will be discussed with a third researcher until consensus is reached. Subgroup analyses will be carried out for the main outcomes if the number of certain PIFS cases > 25 at T1.

Interim analysis
No interim analysis will be performed.