WE&ME Projects - Stage 1 Short Proposal (Draft v2)

Neuroimmune-Autonomic Signature of ME/CFS: A Multimodal Case-Control Study with Recovered-Infection and Immune-Disease Comparators, Deep Immune Phenotyping, and Naturalistic Immunomodulation Follow-up

Working draft prepared ahead of the WE&ME Foundation "WE&ME Projects" call (Stage 1 deadline: 25 August 2026, 14:00 CET). Revision of the AXIS-ME draft of 17 July 2026: absorbs the Th17/autoantibody, cellular-immunophenotyping and discovery-proteomics layers previously drafted as PARITY-ME; adds recovered-post-infection, young healthy and adolescent-IBD comparator arms; adds a neurocognitive work package quantifying cognitive post-exertional malaise; rebalances the budget to the EUR 180,000 ceiling. Bracketed items and character counts are notes for Andrea, not part of the submitted text.

Basic Information

Project title: Neuroimmune-Autonomic Signature of ME/CFS: A Multimodal Case-Control Study with Recovered-Infection and Immune-Disease Comparators, Deep Immune Phenotyping, and Naturalistic Immunomodulation Follow-up

Acronym: AXIS-ME

Keywords: ME/CFS; post-COVID ME/CFS; dysautonomia; neuroaxonal injury (NfL/GFAP/S100B); cellular immunophenotyping; cognitive post-exertional malaise

Project duration: 24 months

Lay Summary

Max. 1,000 characters (incl. spaces) - current length: 926 characters.

ME/CFS, including cases triggered by COVID-19, causes overwhelming fatigue that worsens after activity (post-exertional malaise), an unstable autonomic nervous system, and brain fog. We compare patients with three groups: healthy volunteers, people who caught a similar infection but fully recovered, and healthy young adults. In all of them we measure blood markers of nerve-cell injury and immune activation, look directly at immune cells, record heart rate, blood pressure and brain blood flow on standing, and test thinking speed while standing and again in the days after a standardised mental effort - making brain fog measurable. In patients already receiving immunoglobulin in our clinic as routine care, we repeat these measures to see whether biological change tracks clinical improvement. Patients help design and interpret the study; those too unwell to attend hospital can still contribute through questionnaires.

Scientific Abstract

Max. 2,000 characters (incl. spaces) - current length: 1991 characters.

Rationale: ME/CFS, including post-COVID-onset cases, combines post-exertional malaise (PEM), dysautonomia and neurocognitive impairment. Biomarker studies conflict, plausibly because unstratified cohorts mix patients with and without a genuine neuroimmune phenotype, and because healthy controls alone cannot separate what is specific to ME/CFS from the residue of having been infected.

Design: prospective case-control study at Fondazione Policlinico Universitario A. Gemelli IRCCS, extended onto existing institutional biobanks. Arms: ME/CFS (CCC/ICC, PEM mandatory; pre- and post-pandemic onset), matched healthy controls, fully recovered post-infection controls, a young healthy age-anchor, and an immune-mediated-disease reference (rheumatoid arthritis/other autoimmune disease; adolescent-onset IBD) from biobank material.

Measures. Layer 1 (all arms): serum NfL, GFAP, S100B; IL-6, TNF-alpha, CRP, IL-17, IL-23; functional beta2-adrenergic and M3/M4-muscarinic autoantibodies. Layer 2 (nested): flow cytometry of Th17, Treg, B-cell and NK/monocyte subsets. Layer 3 (nested, exploratory): untargeted nLC-MS/MS proteomics. Autonomic: HRV, active stand/NASA lean, near-infrared cerebral oximetry. Neurocognitive: cognition at rest and during orthostasis, then a standardised cognitive load with remote retesting at 24 and 48 h and paired blood sampling - an objective, exertion-linked measure of cognitive PEM.

In the subgroup already receiving IVIg as standard care, all layers are repeated longitudinally, comparing responders and non-responders. Pre-registered analyses: comparison against healthy and recovered controls; equivalence/non-inferiority testing of a composite immune-activation score against the autoimmune reference; model-based clustering for subtypes.

Innovation: first study combining deep immune phenotyping, simultaneous autonomic and cerebral-perfusion measurement and quantified cognitive PEM across ME/CFS, recovered-infection and immune-disease comparators.

Core Team Members

Short CVs to be uploaded separately per person. Competencies and role fields below, each max. 500 characters.

PI&C - Andrea De Angelis

Scientific competencies (500 char max): Neuropsychiatrist, Fondazione Policlinico Universitario A. Gemelli IRCCS. Extensive clinical experience assessing and following patients with Long Covid and ME/CFS (neuropsychiatric evaluation, cognitive and affective phenotyping, risk assessment). Established use of intravenous immunoglobulin in clinical practice for a subset of these patients, with a documented good clinical response rate, and routine ordering of serum neurofilament light chain in privately admitted patients.

Role (500 char max): Principal Investigator and Coordinator. Leads clinical and neuropsychiatric phenotyping, designs and oversees the neurocognitive work package (orthostatic cognition and cognitive-PEM challenge), oversees the IVIg sub-study, interprets biomarker results in clinical context, and coordinates the project. Point of contact for WE&ME Foundation.

Co-PI - Danilo Buonsenso

Scientific competencies (500 char max): Physician-researcher, Fondazione Policlinico Universitario A. Gemelli IRCCS, with expertise in post-acute infection syndromes, including Long Covid natural history, cohort characterisation, and follow-up study design; access to adolescent and young-adult cohorts.

Role (500 char max): Co-Principal Investigator. Contributes diagnostic rigour, pre/post-pandemic onset stratification, recruitment of the recovered-post-infection and young healthy arms, and dissemination.

[Andrea: please expand with specific publications/grants for the portal CV and competency field - kept generic pending your input.]

Collaborating core facilities (non-PI, to be formalised)

Scientific competencies (500 char max): GSTeP Immunology & Cytometry Core Facility (multiparametric flow cytometry, cell sorting) and GSTeP Proteomics Core Facility (untargeted nLC-MS/MS, PTM analysis).

Role (500 char max): Named facility scientists to be confirmed and added to the team list before submission; costed quotes required from both.

Project Description

Max. 10,000 characters (incl. spaces) - current length: 10029 characters. Must cover: research questions/hypotheses; mechanistic relevance to PAIS and ME/CFS; proposed methods/innovation; team composition; patient involvement.

RESEARCH QUESTIONS AND HYPOTHESES

ME/CFS, including post-COVID onset, combines PEM, autonomic dysfunction and neurocognitive impairment. Two weaknesses recur in the literature: cohorts are unstratified, averaging patients with and without a genuine neuroimmune phenotype - the likeliest source of the contradictory NfL/GFAP findings; and healthy controls are the only comparator, which cannot separate what is specific to ME/CFS from what follows any infection. AXIS-ME addresses both.

Primary hypothesis: a definable subset of ME/CFS patients shows a coherent neuroimmune-autonomic signature - elevated NfL, GFAP and S100B, Th17-skewed cytokines, functional adrenergic/muscarinic autoantibodies, autonomic dysfunction and reduced orthostatic cerebral oxygenation - relative both to healthy controls and to people who had a comparable acute infection and fully recovered.

Secondary hypotheses. (1) Cellular: the signature is cellular, not a soluble epiphenomenon - Th17, Treg and memory B-cell/plasmablast frequencies differ from healthy and recovered controls and approach the immune-disease reference. (2) Parity: a composite immune-activation score in ME/CFS is at parity with rheumatoid arthritis and other autoimmune disease, tested by formal equivalence rather than by failure to reject a null. (3) Cognitive PEM: cognition degrades disproportionately under orthostatic challenge and fails to recover normally 24-48 h after a standardised cognitive load. (4) Reversibility: the signature shifts alongside clinical change in the IVIg subgroup, comparing responders with non-responders.

Exploratory: clustering for subtypes predicting IVIg response; proteomics in an extreme-phenotype subset; ADHD and autism-spectrum trait prevalence (~24% reported in ME/CFS and Long Covid).

MECHANISTIC RELEVANCE TO POST-ACUTE INFECTION SYNDROMES AND ME/CFS

Autonomic dysfunction - reduced orthostatic cerebral blood flow, small-fibre neuropathy, POTS - is among the most consistent objective findings in ME/CFS and Long Covid, and neuroaxonal/glial markers are a candidate peripheral readout of the process behind it. The mixed literature is itself informative: it suggests a signal confined to a definable subgroup rather than present in the average patient. Cellular immunophenotyping on the same platform as the autoimmune comparators tests whether that soluble signature reflects real shifts in the Th17, Treg and B-cell compartments - the level at which 'is this immune-mediated?' is decided.

The comparator structure is the second lever. Recovered post-infection controls hold the trigger constant and vary only the outcome; the immune-disease reference converts an unanchored 'abnormal versus healthy' result into a statement about how abnormal, against a disease nobody disputes. Naturalistic immunomodulation tests state-dependence.

PROPOSED METHODS AND INNOVATIVE ASPECTS

Design

Prospective case-control study, single-centre (Gemelli IRCCS), 24 months, with the immune layers extended onto existing institutional biobanks. The hybrid is deliberate: the autonomic, cognitive and neuroaxonal core must be collected prospectively under one harmonised protocol, whereas the immune-disease reference groups already exist as consented biobank material assayable on the same pipeline far more cheaply than de novo recruitment.

Comparator structure

Healthy controls, age- and sex-matched, screened to exclude PEM. Recovered post-infection controls: documented acute SARS-CoV-2 or other infection, full return to pre-illness function, recovered at least six months - the decisive specificity comparator. Young healthy age-anchor (16-25 years): NfL and GFAP rise steeply and non-linearly with age and young-onset ME/CFS is over-represented post-COVID, so an age-appropriate reference distribution is needed to interpret young patients.

Immune-disease reference (biobank; Layer 1, and Layer 2 where PBMCs permit): rheumatoid arthritis and other autoimmune disease, plus adolescent- and young-adult-onset IBD. IBD is included because fatigue persists in

40-50% of patients in objective remission, making it the closest available model of fatigue after inflammation is controlled. Treatment status is a confound rather than a detail - anti-TNF and other biologics suppress analytes on our panel - so these arms are stratified a priori into treatment-naive and treated strata, with treatment-naive samples carrying the primary comparison.

Layer 1 - serum immune and neuroaxonal panel (all arms)

Serum NfL, GFAP and S100B (neuroaxonal, astroglial, blood-brain-barrier); IL-6, TNF-alpha and CRP (inflammatory context); IL-17 and IL-23 (Th17 axis); functional ELISA autoantibodies against beta2-adrenergic and M3/M4-muscarinic receptors. Approximately 215 timepoints including IVIg follow-up. A pilot on banked aliquots confirms analyte stability given freeze-thaw history before the full set is committed.

Layer 2 - cellular immunophenotyping (nested)

Multiparametric flow cytometry on PBMCs at the GSTeP Cytometry Core Facility, ~20 per arm across ME/CFS, healthy, recovered and autoimmune reference (~80 samples): T-cell subsets (CD3/CD4/CD8), Th17 (CCR6+CXCR3-CCR4+), Treg (CD25high/FoxP3+), B cells (CD19/CD27/CD38), NK (CD56) and monocytes (CD14/CD16). PBMCs are isolated fresh in the prospective arms; in the biobank arms this layer depends on viable cryopreserved PBMCs - a stated dependency, not an assumption.

Layer 3 - exploratory discovery proteomics (nested)

Untargeted nLC-MS/MS serum profiling with post-translational modification analysis at the GSTeP Proteomics Core Facility in an extreme-phenotype subset (~12 per group, ~48 samples), selected high- versus low-signature by a pre-specified rule. Hypothesis-generating, outside the confirmatory framework.

Autonomic and cerebral perfusion

Resting and paced heart-rate variability; active stand and NASA lean test with continuous beat-to-beat monitoring; near-infrared spectroscopy cerebral oximetry throughout. Oximetry is a low-cost addition that turns orthostatic intolerance into a measured perfusion variable and bridges the autonomic and cognitive arms.

Neurocognitive work package - quantifying cognitive PEM

Brain fog is the symptom patients rank as most disabling and the one least often measured objectively. We operationalise it in three steps. (i) Seated baseline: Symbol Digit Modalities Test, digit span backward, a brief n-back and simple/choice reaction time (under 20 minutes). (ii) Orthostatic cognition: n-back and reaction time repeated during the active stand/lean test with simultaneous cerebral oximetry and beat-to-beat haemodynamics; the index is the position-dependent decrement in speed and accuracy - a within-person measure robust to education, effort and baseline ability. (iii) Cognitive-load challenge with delayed retest: a standardised sustained load of about 30 minutes, then brief remote retesting at 24 and 48 hours with daily DSQ-PEM ratings and a repeat blood draw at 24 hours. The primary endpoint is the group difference in recovery trajectory; the mechanistic endpoint is whether cognitive exertion alone moves NfL/GFAP and autonomic indices.

Delayed, effort-triggered deterioration is the signature separating ME/CFS from depression, deconditioning and ordinary fatigue, and single-timepoint testing cannot capture it. The battery runs across two visits with pacing built in; the challenge is separately consented, carries a stopping rule and is reviewed by patient advisors. Instruments: DSQ-PEM, Chalder, Bell, COMPASS-31, HADS, ASRS-5, AQ-10, optional Beighton score, and a structured psychiatric interview establishing that fatigue is not better explained by a primary mood disorder.

IVIg naturalistic sub-study

Patients already receiving IVIg as routine care are followed longitudinally (baseline, ~6 months) with all layers repeated; trajectories are compared between responders and non-responders on pre-specified criteria. Observational, nested in usual care, making no efficacy claim.

Statistical approach

Pre-registered. Primary comparisons by mixed-effects models with multiple-comparison correction, contrasting ME/CFS against healthy and recovered controls. Parity is tested by equivalence/non-inferiority on the composite

immune-activation score with margins fixed in advance - a positive claim of similarity, not an argument from a non-significant p value. Cognitive PEM is a group-by-time interaction across the 0/24/48-hour retests; IVIg responder-by-time is the key longitudinal analysis.

Innovation

To our knowledge the first ME/CFS study to combine, in one pipeline, recovered-infection and immune-disease comparators, three-layer immune phenotyping, cerebral-perfusion measurement and objective quantification of cognitive PEM.

TEAM COMPOSITION

PI&C Andrea De Angelis (neuropsychiatry): clinical phenotyping, neurocognitive work package, IVIg oversight, coordination. Co-PI Danilo Buonsenso (post-acute infection syndromes): onset stratification and recruitment of the recovered-infection and young arms. Co-PI Enrico De Lorenzis (rheumatology): autoimmune comparator arms and Th17 layer. A fourth co-PI in autonomic physiology, to be finalised, leads the autonomic and cerebral-oximetry protocol. Collaborating: GSTeP Cytometry and Proteomics Core Facilities.

PATIENT INVOLVEMENT

Please leave your signature if you feel this project support your views or comment on how to improve the study protocol

Study Sample and Recruitment

Diagnostic criteria, comparator rationale, pre/post-pandemic composition, and recruitment plan, as required by the call.

Diagnostic criteria. ME/CFS cases are defined by the Canadian Consensus Criteria (or revised CCC) or the International Consensus Criteria, with mandatory post-exertional malaise; PEM alone is not sufficient. Participants whose post-COVID exercise intolerance is attributable to documented cardiac or pulmonary organ damage are excluded to avoid conflating distinct mechanisms. Autoimmune comparators are classified by standard rheumatological criteria (e.g. ACR/EULAR for RA); IBD by ECCO criteria with documented disease activity and treatment status.

Comparator rationale. Healthy controls answer 'is this abnormal?'. Recovered post-infection controls answer 'is this specific to persistence rather than to having been infected?'. The young healthy arm answers 'is this abnormal for this age?', which matters because NfL and GFAP reference ranges are strongly age-dependent and a substantial share of post-COVID ME/CFS onset is in adolescence and young adulthood. The autoimmune and IBD arms answer 'how abnormal, relative to a disease no one disputes?'. Each comparator is tied to a specific inferential job; none is decorative.

Pre/post-pandemic composition. Our active outpatient population is predominantly post-pandemic-onset. To meet the call's requirement for pre-pandemic comparison we recruit pre-pandemic-onset ME/CFS patients able to attend in person from our own non-COVID CFS/ME caseload and, where insufficient, via collaboration with an Italian ME/CFS patient association. Onset date and trigger are documented for every participant.

In-person eligibility and severely affected patients. Autonomic testing, cognitive testing and venepuncture require attendance, so the core sample is restricted to patients able to attend hospital. This under-represents the most severely affected. We mitigate this with a parallel low-burden remote symptom-questionnaire pathway (DSQ-PEM, Chalder, Bell, COMPASS-31, HADS, ASRS-5, AQ-10), analysed as a separate supplementary cohort rather than pooled with the objectively tested sample, and we state the limitation explicitly rather than obscuring it.

Ethics. A single ethics submission covers the prospective arms, the secondary use of banked samples with new assay panels, the cognitive-load challenge (separately consented, with a stopping rule), and the participation of minors in the adolescent arms (assent plus parental consent). Planned as an early Stage 2 activity, with

acknowledgement of receipt submitted alongside the full proposal and final approval obtained before project start.

Target numbers by arm:

Arm	Target n	Source	Notes
ME/CFS, post-pandemic onset	45	Prospective	Post-COVID-triggered; CCC/ICC with mandatory PEM. All layers eligible.
ME/CFS, pre-pandemic onset	15	Prospective	Required by the call. Recruited from our non-COVID CFS/ME caseload and, if insufficient, via an Italian patient association.
Healthy controls	35	Prospective	Age- and sex-matched; screened to exclude PEM/CFS-consistent symptoms.
Recovered post-infection controls	30	Prospective	Documented acute infection, full return to pre-illness function, recovered >=6 months. The key specificity comparator.
Young healthy age-anchor	20	Prospective	16-25 years. Age-appropriate reference distribution for NfL/GFAP, which are strongly age-dependent.
Autoimmune reference (RA and other)	25	Biobank	Layer 1, plus Layer 2 where viable PBMCs exist. Stratified by treatment status.
Adolescent/young-adult IBD	25	Biobank + clinic	Layer 1. Chosen as a 'fatigue despite controlled inflammation' model; stratified treatment-naive versus on immunomodulation.
IVIg longitudinal subgroup	~20	Nested, prospective	Drawn from the ME/CFS arms; baseline and ~6 months, all layers repeated.
Remote questionnaire-only cohort	open	Prospective	Severely affected/housebound patients; symptom instruments only, analysed as a separate supplementary cohort.

Nested layers: Layer 2 cellular immunophenotyping in ~20 per arm across ME/CFS, healthy, recovered and autoimmune reference (~80 samples); Layer 3 discovery proteomics in ~12 per group as phenotypic extremes (~48 samples). Final sample sizes confirmed by formal power calculation at Stage 2, including the equivalence margin for the parity test.

Indicative Budget

Bulk sums only, per Stage 1 requirements - no detailed cost-line justification needed until Stage 2.

Cost category	Indicative amount (EUR)	% of total	Notes
Personnel	90,000	50%	Research fellow/data analyst (24 months: cross-cohort coordination, statistics, pre-registration); research-nurse time for testing sessions, venepuncture, PBMC isolation and IVIg follow-up visits; sessional neuropsychology technician time for the cognitive work package including the 24/48-hour remote retests.
Non-personnel (cap 40%)	72,000	40%	Layer 1 serum panel across ~215 timepoints - NfL/GFAP/S100B, IL-6/TNF-alpha/CRP, IL-17/IL-23, beta2-adrenergic and M3/M4-muscarinic autoantibody ELISAs, vendor quote pending (~38,000); Layer 2 multiparametric flow cytometry, GSTeP Immunology & Cytometry Core, ~80 samples (~15,000); Layer 3 exploratory discovery proteomics, GSTeP Proteomics Core, ~48 samples (~7,000); autonomic and NIRS cerebral-oximetry consumables, cognitive test licences and remote-retest platform, each item <=EUR 1,500 (~4,000); biobank access and aliquot

Cost category	Indicative amount (EUR)	% of total	Notes
			retrieval (~2,000); patient involvement compensation (~3,000); data management/repository (~2,000); open-access publication (~1,000).
Overhead (cap 10%)	18,000	10%	Institutional overhead, Fondazione Policlinico Universitario A. Gemelli IRCCS.
Total	**180,000**	**100%**	**At the ceiling of the EUR 120,000-180,000 range. The added comparator arms and the cellular/proteomic layers are affordable only because the reference groups are drawn from existing biobank material rather than recruited de novo.**

Signatures

- *please sign it if you support this protocol as a patient representative*