

ME/CFS: Phenotypic Overlap with Infection-Associated Syndromes and Craniofacial Sampling for Neuroimmune Investigation

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Abstract

Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) affects up to 3.3 million Americans, yet its pathogenesis remains poorly understood. To support ME/CFS research, we developed two targeted evidence syntheses: (i) phenotypic overlap between ME/CFS and syndromes associated with SARS-CoV-2, Epstein-Barr virus (EBV), and other pathogens; and (ii) needle-free, incision-free craniofacial sampling sites anatomically linked to intracranial sites with pathogen detection. To illustrate pathogen detection constraints, we examined EBV studies in ME/CFS conducted by U.S.-affiliated investigators. Only 1 of 21 studies (4.8%) measured EBV nucleic acids or viral proteins, and sampling was restricted to capillary blood. These design limitations may reduce sensitivity to intracranial infection or inflammation, including processes involving EBV, a virus implicated in multiple sclerosis and an encephalitis variant with overlapping clinical features. Observed challenges motivated the proposal of two reporting frameworks, J.O.A.N. and M.I.K.E.: J.O.A.N. aims to reduce false-negative risk in pathogen detection studies while M.I.K.E. seeks to advance neuroimmune research with needle-free, incision-free craniofacial sampling. Overall, the current understanding of ME/CFS may be incomplete due to methodological gaps. If ME/CFS, like Long COVID, encompasses biologically distinct subgroups, these syntheses and frameworks could support more rigorous investigation and interpretation.

1 Objectives

1. To evaluate the methods used in studies of the association between Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) and Epstein-Barr virus (EBV).
2. To inform craniofacial sampling strategies for ME/CFS research by synthesizing evidence on (i) phenotypic overlap between ME/CFS and post-infectious syndromes

and (ii) needle-free, incision-free craniofacial sampling sites anatomically linked to intracranial sites with evidence of pathogen detection.

3. To introduce the J.O.A.N. and M.I.K.E. frameworks: J.O.A.N. aims to reduce false-negative risk in pathogen detection studies and improve cross-study comparability, while M.I.K.E. aims to advance neuroimmune research using needle-free, incision-free craniofacial sampling.

2 Background

Pathogens may invade the brain parenchyma or intracranial cerebrospinal fluid (CSF) through the blood-brain barrier (BBB), the blood-CSF barrier, or neural pathways.[1] For instance, HIV-1 hijacks infected monocytes to translocate across the BBB and establish central nervous system (CNS) infection.[2] In contrast, herpes simplex virus 1 (HSV-1) can access the CNS via the trigeminal and olfactory cranial nerve pathways and undergo asymptomatic shedding on mucosal and cutaneous surfaces.[3, 4] Certain microbial agents, including HSV-1 and varicella-zoster virus (VZV), achieve bidirectional movement by exploiting both retrograde and anterograde axonal transport.[5, 6] Both viruses can cause clinically consequential CNS disease while also causing common syndromes – herpes labialis (cold sores) for HSV-1, and varicella (chickenpox) and herpes zoster (shingles) for VZV.[7–9]

Once intracranial, pathogens can drive neurological dysfunction via compartmentalized persistence or reactivation, as demonstrated by measles virus persistence in subacute sclerosing panencephalitis, John Cunningham virus reactivation in progressive multifocal leukoencephalopathy (PML), and VZV reactivation in CNS vasculopathy.[10–12]

2.1 Myalgic Encephalomyelitis/Chronic Fatigue Syndrome

ME/CFS is a chronic illness defined by post-exertional malaise (PEM), unrefreshing sleep, a substantial reduction or impairment in the ability to engage in pre-illness levels of activity, and either cognitive impairment or orthostatic intolerance per the 2015 Institute of Medicine (IOM), now the National Academy of Medicine (NAM).[13] In the United States, up to an estimated 3.3 million people are affected, with many cases unreported due to heterogeneous presentation and subjective diagnostic methods governed by symptom-based exclusion criteria.[14] Encephalomyelitis refers to inflammation of the brain and spinal cord, whereas encephalitis refers to inflammation of the brain.

Microbial infection has long been suspected to initiate or promote ME/CFS. Hypothesized risk factors include various bacteria, EBV, VZV, human herpesvirus 6, human cytomegalovirus, parasites, and other pathogens.[15–20] Despite numerous studies, the evidence remains inconclusive.

Regardless of etiology, ME/CFS-defining symptoms have been associated with differences in the brain and broader CNS. Orthostatic intolerance has been associated with reduced cerebral blood flow during head-up tilt testing, including in patients without classic heart rate or blood pressure abnormalities.[21–23] In translocator protein (TSPO) PET studies, cognitive impairment scores correlate positively with higher ligand binding in multiple

brain regions.[24] In unrefreshing sleep investigations, ME/CFS cohorts show differences in hypothalamic volume and connectivity in sleep-wake regulatory networks.[25–27] Studies on PEM also report differential post-exercise brain activation and resting-state measures in ME/CFS cohorts.[28–31]

Brainstem findings have been reported across multiple ME/CFS symptoms. The brainstem ascending reticular activating system, with projections to the hypothalamus and other targets, mediates wakefulness and can influence sleep-wake regulation.[32, 33] Orthostatic intolerance and blood-pressure control are mediated by brainstem baroreflex networks, while PEM studies report exertion-associated differences in the brainstem.[28, 34] Two brainstem regions, the midbrain and pons, show higher TSPO ligand binding in ME/CFS, with midbrain binding correlating with cognitive impairment scores.[24]

2.2 Epstein-Barr Virus

Multiple lines of evidence have motivated investigation of EBV in ME/CFS. First, EBV causes a form of encephalitis with clinical features overlapping those of ME/CFS, including post-exertional symptom exacerbation, cognitive dysfunction, myalgia, and sleep disturbances.[35–38] Studies further demonstrate EBV association in multiple sclerosis (MS), a demyelinating disease of the brain and spinal cord, with Lanz et al. reporting a molecular mimicry mechanism and implicating the virus as a potential causal factor in some patients.[39–41] Moreover, infectious mononucleosis – the classic presentation of primary EBV infection – precedes ME/CFS-like illness in 9-13% of patients within 6 months; EBV is a recognized precipitant of post-infectious fatigue; and ME/CFS subgroups show EBV-specific immune dysregulation.[42–46]

EBV, or human herpesvirus 4, is a double-stranded DNA virus from the Herpesviridae family. Over 90% of individuals worldwide carry the virus, usually asymptotically, and infection typically occurs before adulthood. Approximately 50% of people are infected by age 10 and 80% by age 18, with asymptomatic transmission persisting for weeks primarily via saliva but also potentially via blood and other body fluids. EBV achieves lifelong persistence by tethering its episomal genome to host chromosomes and limiting immune recognition via various methods. Although B lymphocytes constitute the primary reservoir, the virus also infects epithelial cells, T lymphocytes, monocytes, and macrophages.[47–50]

Classified as a Group 1 carcinogen, EBV is an established etiological agent of Burkitt lymphoma, nasopharyngeal carcinoma (NPC), gastric carcinoma subtypes (EBVaGC), NK/T-cell lymphomas, Hodgkin lymphoma, and pulmonary lymphoepithelioma-like carcinoma.[51, 52]

Although findings of EBV infecting brain parenchyma remain controversial, entry receptors for the virus – including CD21, CD35, and Ephrin receptor A2 (EphA2) – can be expressed on astrocytes, microglia, and brain endothelial cells.[53–57]

The EBV genome consists of 170-180 kb of DNA encoding over 80 proteins and 40 non-coding RNAs. Strains are designated type 1 or type 2, with the former predominating globally and the latter concentrated in tropical regions. Similar to other herpesviruses, EBV maintains a latent-lytic life cycle. During the lytic stage, the virus is generally immunogenic while producing the broad array of gene products essential for viral replication and infection.

Conversely, the latent stage expresses a sparse set of gene products and typically evades immune surveillance. EBV is believed to exist primarily in the latent stage, which comprises four sub-stages, or types, marked by disparate protein and RNA expression: 0, I, II, and III.

The only EBV gene products with consensus evidence of expression across all latency sub-stages are two non-coding RNAs, EBV-encoded RNA 1 (EBER1) and EBV-encoded RNA 2 (EBER2). These non-polyadenylated transcripts, roughly 160-180 nucleotides long and abundantly expressed during latency, are the targets of the gold standard for EBV detection, EBV-encoded RNA in situ hybridization (EBER-ISH).

2.3 Limitations of EBV Detection Protocols

EBV detection is influenced by protocol design and data source characteristics.

The Cancer Genome Atlas (TCGA) molecularly characterized over 20,000 primary cancer and matched normal specimens spanning 33 cancer types, and is widely used in oncology studies. However, TCGA's mRNA-seq libraries employed poly(A) enrichment, which captures polyadenylated coding transcripts but depletes non-polyadenylated transcripts such as EBER1/2 and other viral non-coding RNAs.[58–60] In addition, microRNA (miRNA) sequencing libraries were enriched for mature miRNA species and not constructed to capture the longer EBER1/2 transcripts.[59]

Separately from library preparation constraints, EBV biology can affect detectability across protocols. EBV gene expression is shaped by both the infected cell type and latency program, so the abundance of any given transcript can differ across tissue compartments. For example, assays keyed to lytic-only transcripts may miss latent infection. Strain polymorphism across coding and non-coding regions may create primer-template mismatches, making single reference primer sets vulnerable to allelic dropout across EBV type 1/2 and regional variants. A review of EBV studies in American breast cancer patients documented potential methodological gaps related to EBV biological variability. 5/7 (71%) studies chose primer sequences with binding sites mismatched to over 50% of EBV genomes in NCBI GenBank. 0/7 (0%) studies used adenocarcinoma-derived reference sequences and instead relied on sequences derived from infectious mononucleosis or lymphoma samples.[61] Finally, low viral burden below one copy/cell with spatial heterogeneity in subclones or infiltrating lymphocytes may dilute signals in bulk tissue and fall below the detection threshold of conventional qPCR.[62–66]

EBV operates through diverse mechanisms. In classic EBV-driven tumors such as NPC, EBVaGC, and Burkitt lymphoma, viral genomes and latent gene products are retained in virtually all malignant cells while oncoproteins such as LMP1 and EBNA1 reprogram signaling, epigenetic, and immune pathways to sustain transformation. Experimental work also indicates that EBV can promote chemoresistance in epithelial and hematologic cancer cell lines by upregulating the multidrug-resistance transporter MDR1 and related efflux pumps.[66, 67]

Beyond canonical pathways where tumor cells contain EBV, emerging evidence supports speculative models where the virus shapes carcinogenesis and the immune response without widespread persistence in tumor cells. Under a "hit-and-run" model, transient EBV infection initiates oncogenesis but episomal genomes are subsequently lost, yielding

EBER-ISH/EBNA1- negative tumors as clonal evolution favors EBV-negative progeny. Mechanistically, EBV drives epigenetic reprogramming via CpG-island hypermethylation and broader chromatin remodeling, silencing tumor suppressor genes and dysregulating additional oncogenic and immune pathways. These epigenetic changes can sustain malignant phenotypes even after loss of the viral genome.[52, 68, 69] In contrast to these "hit-and-run" scenarios, the virus may promote carcinogenesis without direct infection via a "remote hit" mechanism: infected cells secrete exosomes harboring viral transcripts and oncoproteins such as LMP1, which can promote angiogenesis, proliferation, and immune evasion in remote cells. In NPC, Meckes Jr et al. found that exosomes from malignant cells activated the ERK and AKT signaling pathways in recipient cells.[70–72]

2.4 Neurodiagnostic Sampling and Craniofacial Readouts

Because sampling brain parenchyma typically requires surgical biopsy, clinical neurologic evaluation often relies on neuroimaging and surrogate biofluids, primarily CSF and blood. Conventional neuroimaging modalities, including MRI and FDG-PET, detect surrogate markers of inflammation rather than directly measuring pathogens or inflammatory mediators.

Due to distal sampling sites, CSF and blood assays may lack sensitivity to infection or inflammation localized to intracranial compartments. CSF is typically obtained via lumbar puncture, yet its composition differs substantially between ventricular and lumbar compartments.[73] Blood is routinely collected via antecubital venipuncture and similarly may not reflect localized CNS pathology because of BBB exclusion and peripheral dilution. Moreover, serology reflects systemic antibody responses rather than pathogen presence and does not establish active or localized infection.

Clinical data underscore these constraints. In one VZV vasculopathy cohort, CSF PCR for VZV DNA was negative in 21/30 (70%) of cases.[74] Similarly, in a study of 597 patients with all-cause encephalitis, 25% lacked CSF pleocytosis on initial lumbar puncture, including 23.7% of HSV-1 cases. Beyond acute infection, CSF assay limitations extend to chronic neurologic disease. In a 46-analyte CSF screen in AD, only 15 analytes were robustly detectable, and none achieved more than 70% discriminative power in distinguishing AD from controls.[75]

Craniofacial sampling has demonstrated measurable diagnostic performance and correlative associations in select neurologic contexts, though most indications remain investigational. In human prion disease, olfactory-mucosa nasal brushings assayed by real-time quaking-induced conversion (RT-QuIC) achieved 97% sensitivity and 100% specificity, exceeding matched CSF sensitivity (77%) in the same cohort.[76] In Parkinson's disease, α -synuclein RT-QuIC positivity in olfactory nasal swabs increased from 45% to 84% when targeting a region of higher neuronal density, demonstrating substantial yield variation within the same compartment.[77] Ocular-surface studies likewise report elevated tear total tau in individuals with neurodegeneration, inversely correlated with CSF A β 42.[78] In the oral cavity, salivary CGRP varies across migraine phases and typically rises during attacks, consistent with peripheral tracking of trigeminal-system activation.[79, 80]

Beyond biomarker data, anatomical and functional studies support connectivity between select craniofacial sites and intracranial compartments. In humans, following lumbar

intrathecal administration of an MRI contrast agent, serial imaging demonstrated the presence of Gadovist along the trigeminal nerve, including Meckel's cave, the foramen ovale, and the inferior alveolar nerve.[81] In animal tracer studies, CSF and interstitial solutes can drain along peri-olfactory pathways across the cribriform plate into nasal lymphatic drainage, although human peri-olfactory CSF efflux remains inconclusively characterized.[81–86] Separately, transcutaneous auricular vagus nerve stimulation at the cymba conchae elicits fMRI activation of the nucleus tractus solitarius (NTS) versus earlobe stimulation, consistent with functional afferent connectivity from the external ear to brainstem vagal nuclei.[87]

However, CNS attribution from peripheral biofluids can be confounded by non-CNS sources. For example, proteins from systemic circulation may enter saliva via glandular epithelial transport or may enter gingival crevicular fluid via microvascular transudation or inflammatory exudation.[88]

3 Materials and Methods

3.1 Literature Search Methodology: EBV and ME/CFS Association

To find prior studies investigating the association of EBV in U.S. ME/CFS patients, our literature screening employed a two-tier approach.

The first tier utilized a PubMed query to include studies meeting all these criteria:

- Investigated EBV in patients with ME/CFS
- Published between 2010-2025
- MEDLINE-indexed journals
- Primary research studies (excluded reviews, meta-analyses, case reports, editorials, letters, preprints)
- Included at least one U.S.-affiliated author
- English language

Qualifying papers were manually screened and included if they met all these conditions:

- Direct detection of EBV nucleic acids or proteins: included if papers investigated EBV association in human specimens by measuring EBV nucleic acids or viral proteins and reporting (1) sampled specimen compartments; (2) detection targets; (3) assay methodologies; and (4) detection criteria. Studies using EBV assays solely for participant exclusion screening were excluded. Serological assays measuring antibodies against EBV were excluded.
- Primary ME/CFS cohort: included if ME/CFS was the primary condition under investigation. Papers were excluded if ME/CFS was studied as a secondary symptom of another primary disease (e.g., chronic fatigue in multiple sclerosis or systemic lupus erythematosus patients).

- Sample size: included if the ME/CFS sample size was ≥ 30 patients.
- U.S. patient cohorts: included if study cohorts recruited patients from U.S. clinical sites or populations. Papers with U.S.-affiliated authors but non-U.S. cohorts were excluded.

We designated 2010 as the publication cutoff to prioritize studies using contemporary EBV detection methods and reporting practices.

The search identified one paper from U.S.-affiliated investigators that attempted direct detection of EBV viral components in primary ME/CFS patient specimens and provided sufficiently detailed assay methodology in cohorts of at least 30 patients.

Our minimum sample size of 30 was grounded in statistical reliability standards. The National Center for Health Statistics (NCHS) Data Presentation Standards commonly treat estimates based on fewer than 30 observations as potentially statistically unreliable. We applied this NCHS reliability threshold, acknowledging the risk of missing rare EBV-positive subtypes. With 0/30 patients testing EBV-positive, the one-sided exact binomial 95% upper confidence limit is 9.5%, which cannot exclude EBV-associated subtypes with true prevalence up to 9.5%.

	Excluded	Included
Website: https://pubmed.ncbi.nlm.nih.gov		
Search query: (("Epstein-Barr Virus Infections"[Mesh] OR "Herpesvirus 4, Human"[Mesh] OR "Epstein-Barr"[tiab] OR "Epstein Barr"[tiab] OR EBV[tiab] OR HHV4[tiab] OR "human herpesvirus 4"[tiab]) AND ("Fatigue Syndrome, Chronic"[Mesh] OR "myalgic encephalomyelitis"[tiab] OR "chronic fatigue syndrome"[tiab] OR ME/CFS[tiab] OR "systemic exertion intolerance disease"[tiab] OR SEID[tiab]) AND 2010:2025[dp] AND ("United States"[ad] OR USA[ad] OR "U.S."[ad] OR "U.S.A."[ad] OR "United States of America"[ad]) AND medline[sb]) NOT (review[pt] OR meta-analysis[pt] OR case reports[pt] OR comment[pt] OR editorial[pt] OR letter[pt] OR preprint[pt])		21
Excluding non-human studies and human studies without direct detection of EBV nucleic acids or proteins	20	1
After screening for EBV association studies with direct EBV detection in human specimens		1
Excluding studies below the NCHS reliability threshold	0	1
After screening for EBV association studies with direct EBV detection in human specimens and minimum sample size		1

	Excluded	Included
Excluding studies where ME/CFS was not the primary condition investigated	0	1
After screening for EBV association studies with direct EBV detection in human specimens, minimum sample size, and ME/CFS as the primary cohort		1
Excluding studies without U.S. patient cohorts	0	1
After screening for EBV association studies with direct EBV detection in human specimens, minimum sample size, and ME/CFS patients from the U.S. as the primary cohort		1
Final list		1

Table 1. Literature Search and Study Selection for EBV Detection Studies in U.S. ME/CFS Patient Cohorts (2010–2025). A PubMed search and manual screening identified primary studies utilizing direct detection of EBV nucleic acids or proteins in ME/CFS cohorts with at least one U.S.-affiliated author, applying prespecified eligibility filters and a minimum sample size of ≥ 30 .

3.2 Evidence Synthesis: Phenotypic Overlap Between ME/CFS and Infection-Associated Syndromes

We conducted a targeted evidence synthesis to characterize phenotypic overlap between infection-associated syndromes and the 2015 diagnostic criteria for ME/CFS from IOM/NAM. These criteria include substantial reduction or impairment in pre-illness activity accompanied by fatigue, PEM, unrefreshing sleep, and either cognitive impairment or orthostatic intolerance. We selected representative pathogens with documented post-acute or post-infectious syndromes to illustrate phenotypic overlap across diverse infectious etiologies. Because this synthesis focuses on symptom-feature overlap, we mapped infection-associated evidence to PEM, unrefreshing sleep, cognitive impairment, and orthostatic intolerance. We did not operationalize the required activity-impairment with fatigue criterion. Feature mapping described overlap in reported manifestations and did not infer diagnostic equivalence.

Sources were eligible if they reported human clinical manifestations or objective measurements attributable to a specified pathogen-associated syndrome consistent with one or more prespecified ME/CFS features. Eligible source types included primary clinical studies, secondary syntheses, and consensus or public health guidance. We excluded non-human studies, reports lacking sufficient clinical detail to support feature mapping, and studies lacking a defined syndrome context.

Publications were identified via targeted PubMed searches and supplemented by backward citation searching of included reviews and key primary studies. Search strings combined post-acute and post-infectious terms with feature-aligned terms, including: PEM and post-exertional symptom exacerbation; cognitive impairment and neurocognitive dysfunction;

orthostatic intolerance, postural orthostatic tachycardia syndrome, and dysautonomia; and unrefreshing sleep, nonrestorative sleep, and sleep disturbance. Titles and abstracts were screened for relevance. Full texts were reviewed to confirm syndrome context and extract feature-aligned evidence. Findings were synthesized descriptively; no pooled effect estimates were generated.

3.3 Evidence Synthesis: Needle-Free, Incision-Free Craniofacial Sampling Sites Anatomically Linked to Intracranial Sites With Pathogen Detection

We conducted a targeted evidence synthesis to map craniofacial sampling sites to intracranial sites with reported pathogen detection and linked by cranial nerve pathways or CSF-contiguous routes. Eligibility criteria for craniofacial sites, intracranial sites, anatomical pathways, and intracranial pathogen detection were prespecified as follows.

Craniofacial Sites. We included craniofacial sites satisfying all of the following: (i) tissue type consistent with mucosal epithelium, glandular tissue, or cutaneous epithelium; (ii) specimen type limited to biofluids or exfoliated cellular material obtainable via needle-free, incision-free collection; and (iii) anatomical linkage to an eligible intracranial site via a cranial nerve pathway or CSF-contiguous route.

Intracranial Sites. We included intracranial neural structures or CSF compartments with reported pathogen detection in human subjects and an anatomical linkage to at least one eligible craniofacial site via a cranial nerve pathway or CSF-contiguous route.

Anatomical Pathways. We included only cranial nerve pathways and CSF-contiguous drainage routes that link eligible craniofacial and intracranial sites. Cranial nerve pathways were included regardless of physiological direction, given the ability for neurotropic viruses to exploit axonal transport bidirectionally.

Intracranial Pathogen Detection. We included pathogens detected at eligible intracranial sites in human subjects, documented in case series or larger studies.

Publications were identified via PubMed searches using combinations of craniofacial site terms, cranial nerve and CSF pathway terms, intracranial site terms, and pathogen detection terms, supplemented by backward citation searching of included reviews and primary studies. Titles and abstracts were screened for relevance. Full texts were reviewed as needed to confirm eligibility and extract the minimal anatomical and pathogen-detection information specified a priori.

Evidence was extracted and tabulated to populate the synthesis tables, capturing craniofacial site, anatomical pathway, intracranial site, and pathogen evidence. Pathogen evidence was recorded at the intracranial-site level, capturing pathogen identity and evidence type.

3.4 J.O.A.N. Framework

The Joint Omics Adaptive Nosological (J.O.A.N.) framework proposes a reporting standard for pathogen detection studies. J.O.A.N. is motivated by the risk of false-negative results arising from pathogen genetic diversity, tissue tropism, latency patterns, and pre-analytical inconsistencies. This approach is designed to accommodate disorders with suspected microbial contributions, including ME/CFS, oncology, and autoimmune disease. J.O.A.N. requires investigators to justify assay selection relative to target pathogen biology, specimen type, and hypothesized disease compartment. By standardizing detection rationale and assay reporting, J.O.A.N. seeks to reduce false-negative risk, enhance reproducibility, and facilitate the identification of pathogen-associated disease subtypes.

Certain J.O.A.N. items are study-dependent. Authors should label unavailable or inapplicable fields as "N/A" and briefly note the reason when omission could affect interpretation. J.O.A.N. complements, but does not replace, assay-specific reporting guidelines such as MIQE, dMIQE2020, STARD, and STORMS.

Section and Topic	Item No.	Item
TITLE		
Title	1	Identify the report as a J.O.A.N. study.
ABSTRACT		
Abstract	2a	State the primary objectives of the study, including the target conditions and microbial agents investigated.
	2b	State whether the investigation is Primary, Secondary, or Hybrid. If Secondary or Hybrid, identify all specimen-derived datasets analyzed, including the repository or consortium name, version, accession identifiers when applicable, and the access date.
	2c	Summarize the study population, including cohort size, age, race and/or ethnicity, and sex and/or gender.
	2d	Summarize the assay specimen types and targets.
	2e	Summarize the applicability analysis.
	2f	Summarize the key results.
	2g	Summarize the key conclusions and implications.
	2h	Summarize the key limitations.
	2i	Specify funding sources for the study.
INTRODUCTION		
Introduction	3	State the primary objectives of the study, including the target conditions and microbial agents investigated.
METHODS		
Target condition	4	State the disease or condition investigated.
Study design	5	State whether the investigation is Primary, Secondary, or Hybrid. If Secondary or Hybrid, identify all specimen-derived datasets analyzed, including the repository or consortium name, version, accession identifiers when applicable, and the access date.
Study population	6	For each analytic cohort, report the total number analyzed and, if applicable, the number analyzed per group.

Section and Topic	Item No.	Item
	7	For each analytic cohort, summarize age, race and/or ethnicity, sex and/or gender, and other clinically relevant characteristics.
Microbial agents	8	For each agent, report its NCBI Taxonomy scientific name and TaxId.
	9	For each microbial agent, list the reference genome(s) with accession.version, assembly name and level, submission date, and persistent identifier or stable URL.
Sequence record sources	10	State all sources queried for sequence records.
Sequence record search strategy	11	For each search query, state the data source, microbial agent queried, search tool and version, query parameters and filters applied, taxonomic limits, deduplication approach, search timestamp in ISO 8601 format, and provide either: accession.version list, runnable retrieval script, or stable URL to the result set.
Complete genome records	12	For each microbial agent, report the number of complete genomes retrieved and the definition of "complete genome" used by the source.
Assay specimens	13a	Anatomical sites: Identify each site by UBERON ID and label.
	13b	Specimen types: State the specimen matrix and collection method for each specimen type.
	13c	Cell types: State the Cell Ontology (CL) ID and label for each cell type.
	13d	Pre-analytical coding: Report the SPREC version, code, and labels for each specimen type.
	13e	Pre-analytical standards: Cite the standards followed for specimen collection, stabilization, transport, processing, and storage, including version/date and a persistent identifier or stable URL.
	13f	Storage history: Report storage temperature, duration, freeze-thaw handling, and any deviations likely to alter nucleic acid or antigen integrity.
Detection reference standards	14	For each assay, state the reference standard used to evaluate detection calls, including the source, version and date, and a persistent identifier or stable URL. If no consensus standard exists, justify a surrogate or composite standard.
Target reference sequences	15	For each assay target, state the reference sequence records from which the target was derived, including accession.versions, coordinate system and coordinate convention, region coordinates, submission date, and stable URL or DOI.
Target lineages	16	State the lineage scheme and version, the target lineages covered by the assay, and sufficient documentation for reproducing in silico inclusivity analysis.
	17	For each target lineage, cite evidence of association with the investigated condition.
	18	For each target lineage, cite evidence of tropism to the assay specimen types.

Section and Topic	Item No.	Item
Assay targets	19a	For each target, state the strategy for addressing the minimum abundance reported in the literature.
	19b	For each target, state the strategy for addressing the spatial heterogeneity reported in the literature.
	19c	For each target, cite evidence of stage-invariant expression. If no evidence exists, summarize the rationale for selecting a target not expressed throughout the microbial agent's life cycle.
	19d	For each target region (e.g., probe, primer, epitope), report the minimum percent identity observed against all complete genome records for the relevant microbial agent.
	19e	For each transcript target, state the polyadenylation status.
Assay sensitivity	20	For each assay, report the limit of detection with the validation reference.
Assay sequencing	21	State the order in which assays were applied and the criteria for specimen advancement between stages.
Applicability analysis	22a	Target-condition applicability: For each target lineage, indicate if the evidence supports applicability to the target condition (YES/NO).
	22b	Specimen applicability: For each target lineage, indicate if evidence supports detectability in the assay specimen types (YES/NO).
	22c	Detection reference standard applicability: For each microbial agent, indicate if reference standards were followed for detection (YES/NO).
	22d	Target region applicability: For each target region, indicate if 100% sequence identity is observed across all complete genome records (YES/NO).
	22e	Stage-invariant applicability: For each transcript or protein target, indicate if the target is expressed in all stages of the microbial agent's life cycle (YES/NO).
	22f	Sensitivity applicability: For each assay target, indicate if methods support detection at the reported minimum abundance (YES/NO).
	22g	Spatial heterogeneity applicability: For each assay target, indicate if methods support detection given the reported spatial heterogeneity (YES/NO).
	22h	Polyadenylation applicability: For each transcript target, indicate if the methods account for polyadenylation status (YES/NO).
	22i	Pre-selection applicability: For each microbial agent, indicate if testing proceeded from most sensitive to least sensitive assays to reduce false-negative specimen exclusion (YES/NO).
Deviations from standards	23	Summarize deviations from governing standards or consensus guidelines cited in the methods, justifying omissions or modifications.
RESULTS		

Section and Topic	Item No.	Item
	24a	Assay outcomes: Report the number of detected, not detected, and indeterminate results for each assay and target, stratified by study group and specimen type, according to the standards cited in Methods.
	24b	Applicability outcomes: Report unmet applicability criteria by microbial agent and assay target, and summarize the frequency of unmet criteria.
	24c	Assay concordance: If multiple assays were applied to the same specimens, report concordance and the final classification of discordant specimens. Classify unresolved specimens as indeterminate.
	24d	Genomic search outcomes: Report the number of complete genome records retrieved, stratified by microbial agent and genomic sequence source, and state each source's definition of "complete genome."
DISCUSSION		
	25a	Key results: Summarize key results with reference to the study objectives.
	25b	Interpretation: Interpret results in the context of study objectives and limitations, highlighting key implications.
	25c	Detection risk assessment: For each assay target, assess false-negative risk based on analytical sensitivity, target inclusivity, specimen applicability, and unmet applicability criteria. Evaluate false-positive risk based on cross-reactivity, environmental contamination, and reagent contamination.
	25d	Limitations: Discuss study limitations, including specimen and population constraints, genomic variability not captured by reference sequences, and unmet applicability criteria.
	25e	Future work: Propose future research to validate or expand upon the findings.
OTHER		
Support	26	Describe sources of financial or non-financial support for the study, and the role of the funders or sponsors in the study.
Competing interests	27	Declare any competing interests of the authors.
Availability of data, code, and other materials	28	Report where and how to access source data, analytic code, and other materials, including persistent identifiers or accession codes and any access restrictions.

Table 2. Reporting Items for the Joint Omics Adaptive Nosological (J.O.A.N.) Framework. J.O.A.N. defines minimum reporting elements for pathogen detection studies.

3.5 M.I.K.E. Framework

Multimodal Inflammatory Kinetics Evaluation (M.I.K.E.) proposes a reporting framework for investigating neuroimmune conditions via needle-free, incision-free craniofacial sampling. M.I.K.E. is motivated by the invasiveness of intracranial sampling and the risk of

CSF and venipuncture blood lacking sensitivity to compartmentalized intracranial infection and inflammation. Recognizing the anatomical pathways between craniofacial and intracranial sites, M.I.K.E. pairs craniofacial data with optional intracranial correlates such as neuroimaging, CSF, or neural tissue. This modular architecture allows investigators to conduct standalone craniofacial studies or paired compartmental analyses. By standardizing craniofacial pre-analytics, compartment linkage justification, and temporal sampling, M.I.K.E. aims to reduce patient burden, enhance reproducibility, and facilitate cross-study comparisons.

Certain M.I.K.E. items are study-dependent, particularly those addressing intracranial correlates. Authors should label unavailable or inapplicable fields as "N/A" and briefly note the reason when omission could affect interpretation. M.I.K.E. complements, but does not replace, assay-specific reporting guidelines such as MIQE, dMIQE2020, STARD, and STORMS.

Section and Topic	Item No.	Item
TITLE		
Title	1	Identify the report as a M.I.K.E. study.
ABSTRACT		
Abstract	2a	State the primary objectives of the study, including the target conditions investigated.
	2b	State whether the investigation is Primary, Secondary, or Hybrid. If Secondary or Hybrid, identify all specimen-derived datasets analyzed, including the repository or consortium name, release or version information, accession identifiers when applicable, and the access date.
	2c	Summarize the study population, including cohort size, age, race and/or ethnicity, and sex and/or gender.
	2d	Define the study as craniofacial-only or craniofacial-intracranial paired. Specify sampled craniofacial sites and specimen matrices. If paired, specify any intracranial correlate modalities and intracranial regions of interest (ROI).
	2e	Summarize the key results.
	2f	Summarize the key conclusions and implications.
	2g	Summarize key limitations, addressing local craniofacial confounders and the inferential gap between peripheral and central compartments.
	2h	Specify funding sources for the study.
INTRODUCTION		
Introduction	3	State the primary objectives of the study, including the target conditions investigated.
METHODS		
Target condition	4	State the disease or condition investigated.
Study design	5a	State whether the investigation is Primary, Secondary, or Hybrid. If Secondary or Hybrid, identify all specimen-derived datasets analyzed, including repository or consortium, release or version, accession identifiers when applicable, and access date.

Section and Topic	Item No.	Item
	5b	State design and setting, including whether craniofacial and correlate data were paired prospectively or linked retrospectively.
	5c	Define inclusion and exclusion criteria, case definitions, and phenotyping instruments used.
	5d	State key confounders relevant to craniofacial sampling and neuroimmune biomarkers, indicating whether they were pre-specified or post hoc, and describe measurement methods.
Study population	6a	For each analytic cohort, report the total number analyzed and, if applicable, the number analyzed per group.
	6b	For each analytic cohort, summarize age, race and /or ethnicity, sex and /or gender, and other clinically relevant characteristics.
Craniofacial sites	7a	Anatomical sites: Identify each site by UBERON ID and label.
	7b	Specimen types: Define each specimen matrix using the SNOMED CT code and label.
	7c	Pre-analytics: Report the SPREC version, code, and labels for each specimen type.
	7d	Assay targets: State each molecular target, including taxonomic level for pathogens.
	7e	Assays: For each assay, state the method class, platform, and kit or protocol identifier when applicable. Report standard-mandated sensitivity metrics and the limit of detection when applicable.
	7f	Reference standards: Specify the reference standard for each assay, including source, version, date, and persistent identifier. If no consensus standard exists, justify the surrogate or composite standard used.
	7g	Kinetics: State sampling timing, frequency, time-zero reference, and schedule type.
Intracranial correlates	8a	Correlate provenance: Specify correlate modality and whether clinical-care derived or prospective research-acquired.
	8b	Region of interest: Define each intracranial region of interest, including coordinate system or anatomical atlas for imaging, or pathology-defined boundaries for tissue.
	8c	Modality methods: For imaging, report acquisition parameters, preprocessing pipeline, and analysis software. For CSF or tissue, report acquisition context and SPREC-coded pre-analytics. Report sufficient detail for replication.
Anatomical linkage	9	Specify the anatomical linkage for each craniofacial-intracranial pairing. Characterize the evidence basis as human anatomical, tracer and transport, clinical-pathology, or preclinical.
Association analysis	10	Define the primary model or metric linking craniofacial read-outs to intracranial correlates. Specify handling of repeated measures, compartmental lags, and prespecified confounding control.
Deviations from standards	11	Summarize deviations from governing standards or consensus guidelines cited in the methods, justifying omissions or modifications.

Section and Topic	Item No.	Item
RESULTS		
	12a	Study population: Report the final analytic cohort size and summary demographics, including age, race or ethnicity, and sex or gender. Report participant counts at each study stage and exclusions with reasons.
	12b	Craniofacial results: Report results for each site per the governing standards cited in Methods, including detection frequency and summary statistics. Document missing data and quality control failures.
	12c	Intracranial results: For paired studies, report results for each intracranial region of interest as defined in Methods. Report summary statistics, missing data, and quality control failures per the governing modality standards cited in Methods.
	12d	Compartmental concordance: Report the pre-specified metric used to measure the relationship between craniofacial and intracranial data, including pre-specified time-lagged analyses.
	12e	Statistical outcomes: Report effect estimates with measures of precision. Document analysis exclusions and changes to the prespecified analysis plan.
DISCUSSION		
	13a	Key results: Summarize key results with reference to the study objectives.
	13b	Interpretation: Interpret results and highlight key implications, considering multiplicity of analyses, study limitations and objectives, and prior evidence. For paired studies, discuss the strength and direction of craniofacial-intracranial associations and whether the observed pattern is consistent with evidence of the anatomical linkage.
	13c	Craniofacial limitations: Address site-specific sources of error, including local pathology that may not reflect intracranial status, and plausible alternative explanations.
	13d	Limitations: Discuss broader limitations, including correlate sensitivity, the inferential gap between compartments, and generalizability across diverse populations.
	13e	Future work: Propose future research to validate or expand upon the findings.
OTHER		
Support	14	Describe sources of financial or non-financial support for the study, and the role of the funders or sponsors in the study.
Competing interests	15	Declare any competing interests of the authors.
Availability of data, code, and other materials	16	Report where and how to access source data, analytic code, and other materials, including persistent identifiers or accession codes and any access restrictions.

Table 3. Reporting Items for the Multimodal Inflammatory Kinetics Evaluation (M.I.K.E.) Framework. M.I.K.E. defines minimum reporting elements for neuroimmune studies using needle-free, incision-free craniofacial sampling, with optional pairing to intracranial correlates.

4 Results

4.1 Epstein-Barr Virus Detection Studies

4.1.1 Literature Search Results

Our PubMed query returned 21 candidate publications referencing EBV in the context of ME/CFS. Because the query was intentionally broad, we conducted manual screening to eliminate studies not investigating EBV association in U.S.-based ME/CFS patient specimens using direct viral detection methods.

After applying pre-specified inclusion criteria, 1/21 (4.8%) studies remained eligible.

4.1.2 Study Results

A single study performed by Wang et al. met the inclusion criteria for investigating EBV association in a U.S. ME/CFS cohort.[89] The parent cohort comprised 166 ME/CFS participants and 83 healthy controls. Blood was collected as fingerstick capillary whole blood using micro-collection devices designed for ambient stabilization and shipment. Viral assays were completed for a subset of 240 participants.

Methodological parameters of the included study:

- Specimen compartment: Fingerstick capillary whole blood.
- Specimen processing: DNA extraction from fingerstick whole blood stabilized in micro-collection devices at ambient temperature.
- Assay methodology: Real-time PCR performed by an external clinical laboratory.
- Detection target: EBV DNA; assay target region and primer/probe details were not reported.
- Positivity criteria: Not reported.
- Analytical limit of detection: Not reported.
- Sample timing: Primary analyses used first collection; 33 ME/CFS and 15 controls provided a second sample ~4 weeks later.
- Detection outcome: EBV DNA was reported as detected in 1/240 participants (Ct 37.67; 6.7 copies/mL); the detected sample was from the ME/CFS cohort.
- CNS-associated sampling: No sampling of CSF or other CNS-associated compartments reported.
- Disease characteristics: Symptom duration spanned 1-63 years; 17% reported 1-5 years; 81% reported ≥ 6 years; 49% of ME/CFS participants housebound or bedridden.

4.2 Evidence Synthesis: Phenotypic Overlap Between ME/CFS and Infection-Associated Syndromes

We conducted a targeted evidence synthesis mapping symptom domains in the 2015 IOM/NAM ME/CFS criteria against post-acute manifestations reported in selected infection-associated syndromes.

Across the selected examples, each symptom domain is represented among viral (SARS-CoV-2, EBV, West Nile virus) and bacterial (*Borrelia burgdorferi*, *Coxiella burnetii*) triggers. Table 4 links each domain to assessment modalities reported in the cited sources, spanning validated patient-reported instruments and objective physiological or neurobehavioral testing. These modalities include cardiopulmonary exercise testing measures like peak VO_2 , autonomic testing with head-up tilt protocols, standardized neuropsychological batteries, and sleep measurement tools including actigraphy and polysomnography.

ME/CFS Core Feature	Infection-Associated Syndromes	Evidence Type
PEM	SARS-CoV-2: PEM and post-exertional symptom exacerbation reported in post-acute cohorts.[90, 91] EBV: Prolonged exertion intolerance persisting ≥6 months following acute mononucleosis reported in prospective cohorts.[92, 93] Coxiella burnetii: PEM reported in Q-fever fatigue syndrome cohorts at long-term follow-up.[94]	Two-day cardiopulmonary exercise testing (CPET) (e.g., peak VO ₂); submaximal exercise testing; prospective/-longitudinal cohorts; systematic reviews.
Cognitive impairment	SARS-CoV-2: Executive function, memory, and attention deficits reported in post-acute cohorts and reviews.[90, 95] EBV: Slowed information processing and reaction time deficits reported following acute mononucleosis; prospective cohorts identify post-infectious ME/CFS risk.[92, 96] West Nile virus: Persistent neurocognitive deficits reported in long-term survivors.[97, 98]	Structural MRI (e.g., cortical thinning) where available; standardized neuropsychological test batteries (including attention/processing-speed paradigms); prospective/longitudinal cohorts.
Orthostatic intolerance	SARS-CoV-2: POTS and orthostatic intolerance reported in post-acute cohorts.[99] EBV: POTS reported following infectious mononucleosis.[100] Borrelia burgdorferi (PTLDS): Dysautonomia and POTS reported in post-treatment Lyme disease syndrome cohorts and reviews.[101]	Tilt-table testing and autonomic function testing; supportive mechanistic assays reported in subsets; cohorts and case series.
Unrefreshing sleep	SARS-CoV-2: Sleep disturbance and non-restorative sleep reported in post-acute cohorts and systematic reviews/meta-analyses.[102] EBV: Prolonged hypersomnolence reported following mononucleosis.[103] Borrelia burgdorferi (PTLDS): Sleep disturbance reported in post-treatment Lyme disease syndrome cohorts.[104]	Polysomnography where available; actigraphy; validated sleep questionnaires; longitudinal cohorts and systematic reviews.

Table 4. Phenotypic Overlap Between ME/CFS and Infection-Associated Syndromes. Rows correspond to the ME/CFS Core Feature domains. Infection-Associated Syndromes summarizes post-acute manifestations reported in the cited sources. Evidence Type represents primary assessment modalities and study designs, including patient-reported instruments and objective physiological or neurobehavioral testing.

Abbreviations: CPET, cardiopulmonary exercise testing; IOM, Institute of Medicine; NAM, National Academy of Medicine; PEM, post-exertional malaise; POTS, postural orthostatic tachycardia syndrome; PTLDS, post-treatment Lyme disease syndrome; VO₂, oxygen consumption.

4.3 Evidence Synthesis: Needle-Free, Incision-Free Craniofacial Sampling Sites Anatomically Linked to Intracranial Sites With Pathogen Detection

We conducted a targeted evidence synthesis mapping needle-free, incision-free craniofacial sampling sites with pathways to intracranial sites with pathogen detection. Table 5 characterizes craniofacial sites with pathways to intracranial sites while Table 6 summarizes

reported pathogen detection at these intracranial sites, categorized by evidence type.

Table 5 catalogs 15 craniofacial sites, six anatomical pathways, and six intracranial targets. CN V is the predominant pathway, linking 10 craniofacial sites to trigeminal sensory nuclei. 10 craniofacial sites map to a single pathway while five map to multiple pathways. Distinct mappings include a CN I route from olfactory-region nasal mucosa to the olfactory bulb and olfactory tract, and a perineural route from the same site to subarachnoid CSF at the cribriform plate. A cutaneous CN X route via the auricular branch of the vagus nerve (ABVN), historically termed Arnold’s nerve, links the cymba conchae epithelium to the NTS in the brainstem. The subarachnoid space represents the sole intracranial fluid compartment included.

Craniofacial Site	Anatomical Pathway	Intracranial Site
Anterior nasal mucosa	CN V	TSN
Conjunctival mucosa	CN V	TSN
Cymba conchae epithelium	CN X	NTS
Gingival sulcus	CN V	TSN
Lacrimal gland	CN VII	SSN
	CN V	TSN
Nasolacrimal duct mucosa	CN V	TSN
Nasopharyngeal mucosa	CN V	TSN
	CN IX	NTS
	CN X	NTS
Olfactory region nasal mucosa	CN I	OB and olfactory tract
	CN V	TSN
	Perineural CSF pathway	SAS CSF
Oral cavity mucosa	CN V	TSN
Oropharyngeal mucosa	CN IX	NTS
	CN X	NTS
Parotid gland	CN IX	ISN
Submandibular gland	CN VII	SSN
Sublingual gland	CN VII	SSN
Tongue mucosa	CN V	TSN
	CN VII	NTS
	CN IX	NTS
Trigeminal cutaneous epithelium	CN V	TSN

Table 5. Craniofacial Sites and Anatomical Pathways to Intracranial Targets. Craniofacial mucosal, glandular, and cutaneous sampling sites accessible via needle-free, incision-free collection are listed. For each site, mapped cranial nerve and perineural CSF pathways and the corresponding intracranial targets are reported. Anatomical pathways are listed regardless of physiological direction, given bidirectional axonal transport by neurotropic viruses. These pathways denote anatomical connectivity and may reflect mixed afferent or efferent components.

Abbreviations: CN, cranial nerve; CSF, cerebrospinal fluid; ISN, inferior salivatory nucleus;

NTS, nucleus tractus solitarius; OB, olfactory bulb; SAS, subarachnoid space; SSN, superior salivatory nucleus; TSN, trigeminal sensory nuclei.

Table 6 summarizes six intracranial sites with 10 pathogen-site evidence entries across six viral pathogens. Pathogen evidence is site-attributed and does not constitute route-resolved evidence from craniofacial sites. Evidence types include clinical guidelines, human intracranial tissue and CSF studies, and pre-clinical models. Human autopsy studies report SARS-CoV-2 and influenza A virus detection in the olfactory bulb and olfactory tract, and SARS-CoV-2 detection in brainstem regions including trigeminal sensory nuclei and the NTS. CSF testing guidance supports NAAT-based evaluation for HSV-1 and VZV in suspected encephalitis, while EBV CSF NAAT is reported in clinical cohorts and case series and requires cautious attribution given frequent co-etiologicals. Pre-clinical pseudorabies virus studies support retrograde and transneuronal labeling of trigeminal and salivatory nuclei, including TSN, SSN, and ISN.

Intracranial Site	Pathogen Examples	Evidence Type	Evidence Detail
OB and olfactory tract	SARS-CoV-2	Human tissue	Autopsy studies reporting SARS-CoV-2 detection in olfactory bulb tissue using molecular and histopathologic assays.[105]
	Influenza A virus	Human tissue	Case-based human evidence reporting influenza A virus detection along olfactory structures.[106]
SAS CSF	HSV-1	Guideline	CSF NAAT is guideline-supported for suspected viral encephalitis, with HSV-1 as a primary target.[107]
	VZV	Guideline	CSF NAAT and CSF antibody-based testing are included in encephalitis diagnostic guidance for VZV neuroinvasive disease.[107]
	EBV	Human CSF NAAT	EBV DNA detection in CSF by PCR is reported across clinical cohorts and case series, with interpretive cautions due to coetiologies in many positives.[108]
TSN	SARS-CoV-2	Human tissue	Autopsy evidence reporting SARS-CoV-2 detection in brainstem regions including TSN.[109]
	PRV	Pre-clinical	Animal infection studies describe trigeminal-route propagation with transfer to spinal trigeminal nucleus.[110]
NTS	SARS-CoV-2	Human tissue	Autopsy evidence reporting SARS-CoV-2 detection in brainstem regions including NTS.[109]
SSN	PRV	Pre-clinical	Retrograde transneuronal labeling localizes parasympathetic preganglionic neurons within SSN after peripheral inoculation in animal models.[111]
ISN	PRV	Pre-clinical	Retrograde transneuronal labeling after parotid-gland inoculation identifies parotid preganglionic neurons within ISN in animal models.[112]

Table 6. Pathogen Detection Evidence at Intracranial Targets. Evidence for pathogen detection at the intracranial targets mapped in Table 5 is summarized by site, representative pathogen, evidence type, and detection detail from clinical guidelines, human intracranial tissue studies, or pre-clinical models. Evidence is site-attributed and does not imply route-resolved spread from any specific craniofacial sampling site.

Abbreviations: CN, cranial nerve; CSF, cerebrospinal fluid; HSV-1, herpes simplex virus 1; ISN, inferior salivatory nucleus; NAAT, nucleic acid amplification test; NTS, nucleus tractus solitarius; OB, olfactory bulb; PRV, pseudorabies virus; SAS, subarachnoid space; SSN, superior salivatory nucleus; TSN, trigeminal sensory nuclei; VZV, varicella-zoster virus.

5 Discussion

Three central points emerge from this work. First, clinico-immunological features of ME/CFS recur across infection-associated syndromes and implicate shared organ systems and neural circuits. Second, our EBV findings illustrate how methodological constraints in pathogen detection and localized intracranial pathology can limit interpretation of negative findings. Finally, these challenges motivate the proposal of two reporting frameworks: J.O.A.N. seeks to reduce false-negative risk in pathogen detection studies while M.I.K.E. aims to advance neuroimmune research with minimally invasive craniofacial sampling.

Core ME/CFS features recur across post-infectious syndromes, supporting investigation of potential infectious contributions in a subset of cases. Prospective cohorts following EBV-associated infectious mononucleosis reinforce this observation: a measurable minority meet ME/CFS criteria months after infection, despite resolution of the acute illness. These core features jointly implicate distributed networks, including brainstem circuits, engaged in autonomic regulation, sleep-wake control, and cognitive function.

In our review of U.S.-affiliated studies, 95.2% of EBV investigations relied on indirect serology, with only one study attempting direct viral detection – via capillary blood alone. These approaches may lack the resolution to detect compartmentalized pathology and do not account for neurological precedents such as PML, where viral pathology may dominate intracranially despite uninformative peripheral results. Furthermore, negative lumbar CSF results may not exclude intracranial processes due to site-dependent variability of CSF analytes. These gaps suggest negative findings may reflect constraints in sampling location and sensitivity rather than the absence of pathogen association.

Beyond sampling location, complex microbial biology may further confound pathogen detection. EBV illustrates these challenges. Reference sequences derived from lymphoma or infectious mononucleosis isolates may inadequately represent strains adapted to non-lymphoid tissue or responsible for neurological disorders, increasing the risk of false-negative results from divergent strains. The latent-lytic EBV life cycle further complicates assay design due to stage-dependent expression signatures, although even widely used targets may pose problems. TCGA and other genomic projects often prioritize human coding transcripts and systematically deplete non-polyadenylated RNAs such as the canonical EBV detection targets EBER1/2. Finally, low per-cell viral burden and infection confined to minority cell populations may each dilute bulk-sample signals below conventional detection thresholds.

Despite inconclusive evidence, EBV remains a pathogen of interest because of its cellular tropism and neuroimmunological associations. The virus establishes lifelong persistence in over 90% of adults, yet only a minority develop associated malignancies or demyelinating disease. EBV causes a form of encephalitis whose clinical features overlap with those of ME/CFS, is implicated as a causal factor in some cases of MS, and 9-13% of patients in prospective cohorts report ME/CFS-like illness following infectious mononucleosis. Mechanistically, EBV persists in B cells and has been reported in other circulating leukocytes, which could facilitate BBB transit via leukocyte trafficking. Neuroinvasive potential is also underscored by reported expression of EBV-interacting receptors, including CD21, CD35, and EphA2, in select brain contexts, although receptor-mediated entry into CNS cells remains uncharacterized.

These challenges motivated the proposal of two reporting frameworks. J.O.A.N. aims to reduce false-negative risk and facilitate cross-study comparisons in pathogen detection investigations by requiring justification of assay design relative to pathogen biology, specimen type, and disease compartment. The framework addresses potential detection errors related to genetic diversity, tissue tropism, life-cycle stage, and pre-analytical variability, extending beyond ME/CFS to cancer and other disorders with suspected microbial contributions.

M.I.K.E. proposes a framework for needle-free, incision-free craniofacial biospecimen collection, mapping craniofacial sites to intracranial targets to enable anatomically-driven sampling. Evidence supports the plausibility of craniofacial readouts correlating with CNS pathology or reflecting CNS-relevant biomarkers, as demonstrated by olfactory-mucosa brushings in prion disease and in vivo tracer imaging along human trigeminal pathways. Neurotropic pathogens such as HSV-1 reinforce this strategy because viral shedding at oral mucosa can occur without overt local pathology, supporting craniofacial sampling in the absence of clinical signs. Informative readouts across afferent and efferent pathways, together with bidirectional axonal transport exploited by neurotropic pathogens, motivate site inclusion regardless of physiological direction. Beyond site selection, variation in α -synuclein RT-QuIC positivity in Parkinson's disease suggests sub-site selection may materially influence detection yield.

For ME/CFS, Long COVID, and other syndromes involving autonomic dysfunction, the cymba conchae may merit investigation despite uncharacterized biomarker accessibility, mixed auricular innervation, and afferent vagal connectivity. Tape-strip proteomics recover cytokines and inflammatory mediators from keratinized skin, and predominantly afferent sensory fibers release neuropeptides including substance P and CGRP at peripheral terminals via antidromic axon reflexes, establishing precedent for molecular sampling at keratinized sites.[113, 114] Brainstem circuits, including the NTS and broader medulla, contribute to autonomic regulation and influence sleep-wake and neuroimmune pathways. Innervated by the only cutaneous branch of the vagus nerve, the cymba conchae offers a distinctive site with validated functional connectivity to the NTS, a key brainstem hub for autonomic reflex integration.

Even if craniofacial readouts are partially mediated by systemic circulation rather than direct CNS origin, needle-free sampling could complement blood draws and potentially enable low-burden, low-cost monitoring and longitudinal intra-subject tracking.

Future research could prioritize three domains. First, pilot studies and prospective validation of J.O.A.N. and M.I.K.E. are needed to assess reliability and demonstrate impact. Second, mechanistic studies must correlate craniofacial biomarkers with CNS pathophysiology while M.I.K.E. could expand to incorporate directional and functional annotation of craniofacial pathways – categorizing each site innervation as afferent, efferent, or mixed, and characterizing known or hypothesized neuroimmune functions (e.g., sensory surveillance, parasympathetic secretion, immunomodulation). These details would strengthen mechanistic inferences when interpreting correlations between peripheral biomarkers and intracranial pathology. Third, future studies should account for pathogen biology and sampling location when testing whether pathogen-associated and non-pathogen-associated ME/CFS subtypes can be identified within immunogenetically predisposed subpopulations.

Collectively, the evidence suggests the need to robustly investigate whether microbial factors contribute to ME/CFS, given the gap between neurotropic pathogenesis and current

investigative practices. In particular, studies optimized for pathogen biology and intracranial detection may clarify whether prior approaches were adequate to assess microbial contributions across heterogeneous populations. By aligning assay designs to pathogen biology and disease compartments, J.O.A.N. and M.I.K.E. aim to support rigorous investigations of ME/CFS, Long COVID, and other applicable disorders. Whether studies employing these frameworks support pathogen involvement or reveal non-infectious drivers, the resulting data could deepen mechanistic understanding of ME/CFS pathophysiology and inform therapeutic strategies.

6 Limitations

Many inferences about ME/CFS pathogenesis and neurotropic pathogen association are extrapolated from heterogeneous literature on microbial infection, encephalitis, and cancer. The clinico-immunological parallels between ME/CFS and infection-associated syndromes are correlative, may be confounded by comorbidities or treatment effects, and cannot exclude non-microbial mechanisms or secondary neuroimmune dysregulation.

Our emphasis on EBV and other pathogens reflects current evidence and neuroinvasive potential but does not preclude contributions from unprofiled microbes, polymicrobial interactions, non-infectious triggers, or local tissue confounders not captured by existing detection strategies. In particular, since EBV establishes lifelong persistence in memory B cells, peripheral EBV signals may reflect baseline latent carriage rather than mechanistic promotion of ME/CFS pathogenesis. Viruses with demonstrated capacity to infect nerve terminals and undergo retrograde axonal transport, such as HSV-1 or VZV, may represent more mechanistically aligned exemplars for entry into the brain.

Importantly, microbial detection does not establish causality. Microbes identified in affected tissues or biofluids may represent opportunistic passengers, reactivated latent infections secondary to immune dysregulation, or incidental bystanders rather than pathogenic drivers – a distinction that demands functional validation beyond detection alone.

Finally, J.O.A.N. and M.I.K.E. have not been prospectively validated, may be constrained by real-world resource limitations, and rely on surrogate tissues and neuroimaging readouts not formally qualified as quantitative reporters of pathogenic or neuroimmune activity.

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