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Case Report

HERV Dysregulation in a Case of Myalgic Encephalomyelitis and Multiple Sclerosis Responsive to Rituximab

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Abstract: This article summarizes the case of 30-year-old male diagnosed with Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) and its longitudinal follow up that provided a secondary diagnosis of Multiple Sclerosis (MS) eight years after. The most impactful result being his response to rituximab treatment after the systematic failure of prior treatments. Although the expression of endogenous retroviral proteins has been associated with autoimmunity, the patient did not show increased expression of the toxic protein HERV (human endogenous retrovirus)-W ENV, a target of the ongoing clinical trials with temelimab in MS and long COVID-19 cases. However, genome-wide HERV transcriptome analysis by high density microarrays clearly revealed a distinct profile in patient's blood supportive of an altered immune system. Limitations of the study include MRI imaging follow up to monitor lesion progression and reassessment of HERV profiles after rituximab. As an overall, the coincidence of HERV alterations and the impactful response to rituximab presents the possibility of additional, more specific, therapeutic targets encoded by other HERV elements yet to be discovered.

Keywords: Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS); Multiple Sclerosis (MS); Human Endogenous Retrovirus (HERV); rituximab; autoimmunity; Epstein-Barr Virus (EBV)

1. Introduction

Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) is defined as a chronic disorder classified by the WHO as postviral fatigue syndrome (ICD-11 8E49 code), while Multiple Sclerosis (MS) (ICD-11 8A40 codes), is known as a chronic, inflammatory demyelinating disease of the central nervous system that includes three categories (relapsing/remitting, secondary progressive and primary progressive) [1].

Diagnosis criteria for ME/CFS relies on clinical symptoms [2–4], as no validated biomarker exists, with post-exertional malaise or PEM playing a significant role including fatigue, pain, cognitive, intestinal and sleep disturbances among others, limiting patient's daily performance in the mildly affected and driving bed confinement in the severe. By contrast, MS is a complex neurological disorder characterized by inflammation in the central nervous system (CNS), leading primarily to

demyelinating tissue damage. It affects approximately 2.8 million people worldwide [5]. MS represents a primary chronic, non-traumatic neurological disorder affecting young individuals with a high impact on patients' quality of life [6]. The predominant presentation of MS manifests as relapsing episodes – relapsing MS (RMS) [7]. Clinical relapses lead to acute neurological damage, characterized by varied clinical manifestations and subsequent recovery. Natural history studies indicate that close to 40% of these patients undergo steady neurological deterioration without evident relapses, transitioning into a progressive phase of the disease – progressive MS (PMS) [8–10].

While no directional treatment exists for ME/CFS, being often multimedicated to palliate their symptoms [11], there is a wide range of disease-modifying therapies available for MS, spanning from low- to high-efficacy options. These therapies enable neurologists to tailor treatment strategies based on individual prognostic factors [12,13]. In recent years, there has been a shift toward initiating treatment with high-efficacy DMTs early in the disease course, as accumulating evidence suggests superior long-term outcomes [14–20]. Among these therapies, anti-CD20 monoclonal antibodies have emerged as a key approach, specifically targeting B-cell populations to modulate disease activity [21].

This case report describes the longitudinal follow-up of a patient diagnosed with ME/CFS who developed central nervous system lesions compatible with a diagnosis of MS, the medication received and patient response to treatments. In an effort to understand the potential mechanisms behind patient's diagnosis and response to rituximab, we evaluated the potential association of his diagnosis with derangement of PBMC human endogenous retrovirus (HERV) expression, using as reference previously reported healthy and ME/CFS HERV profiles [22].

2. Case description

We present here the case of a male in his 30s diagnosed with ME/CFS and MS, who experienced a significant improvement in his fatigue following initiation of treatment with rituximab.

The patient's symptoms began in 2008, characterized by episodic dizziness, malaise, blurred vision, weakness, and mental fatigue, with no identifiable trigger. Additionally, he had sustained fractures in his right/left 5th metacarpal and his clavicle in 2008, followed by a bimalleolar ankle fracture in 2010. Throughout this period, cognitive fatigue was his predominant symptom, significantly impacting his daily life. He experienced difficulties in concentrating, understanding, or solving problems, which forced him to change his academic pursuits towards less demanding studies. While physical fatigue occasionally led to episodes of being bedridden, he managed to maintain a relatively normal level of physical activity in the periods between extreme fatigue episodes.

Despite evaluations by various specialists in the public health system, including internal medicine, neurology, rheumatology, psychiatry, and digestive medicine, no abnormalities were detected in clinical tests, leading to a referral to mental health services, where depression or anxiety was ruled out. In 2014, his symptoms worsened when he began working in a physically demanding job, leading to new symptoms such as headache, fainting, tinnitus, dyspnea, difficulty speaking, abdominal pain, pain in the hands, neurovegetative crises during sleep, intolerance to temperature changes, and orthostatic intolerance. This resulted in prolonged sick leaves, with the patient living mostly confined to bed.

Further evaluations ruled out autoimmune, thyroid, myasthenia, endocrine, and infectious pathologies, except for Epstein-Barr Virus (EBV) IgG positivity in 2015. Magnetic resonance imaging (MRI) in 2016 revealed a small demyelinating juxtacortical lesion in the left temporal pole, but other investigations were unremarkable, leading to a diagnosis of ME/CFS. Patient met the 2011 International Consensus Criteria [2], as well as Canadian [3] and IOM (Institute of Medicine) 2015 criteria [4]. Prescribed supplements, including magnesium, NADH, D-ribose, L-carnitine, ubiquinol, melatonin, vitamin B1, alpha-lipoic acid, and LDN (Low Dose Naltrexone), failed to improve symptoms.

In June 2017, he decided to stop all treatments, and in July 2017, he experienced sensory loss in his legs with a sensory spinal cord level from C6 to D2, prompting a referral to the Neurology

Department. Electromyoneurography (EMG) was normal, and a lumbar puncture revealed intrathecal production of antibodies (oligoclonal bands) not present in serum (IgG and IgM). Evoked sensory potentials were altered on both sides. During the diagnostic panel, anti-Acetylcholine receptor (AChR) antibodies were positive, although this finding was of unknown significance with the normal EMG. Brain and spinal cord MRIs revealed a new demyelinating lesion at the spinal cord level, fulfilling spatial and temporal dissemination criteria for MS diagnosis according to McDonald criteria 2017 [23], with two foci in the medulla and parietal lobe (Figure 1).

He was initially prescribed oral teriflunomide, a low-efficacy disease-modifying therapy (DMT) [12], which was discontinued due to adverse effects, including tachycardia and tiredness. Subsequently, a switch to subcutaneous glatiramer acetate (GA) proved effective for 3 years. To address fatigue symptoms, amantadine, modafinil, and fampidrine [24] were trialed but proved ineffective. Amitriptyline was added for paresthesic symptoms at night, along with lormetazepam to improve sleep disturbances. Although well-tolerated in general, several episodes of immediate post-injection reaction were experienced by the patient, characterized by shortness of breath and palpitations for 15 minutes, which became uncomfortable due to their increased frequency.

Following a new relapse of disease activity for MS with evidence of radiological inflammation in the MRI (Figure 1), rituximab, an anti-CD20 agent aimed at eliminating the B cell population of lymphocytes, was prescribed in December 2021.

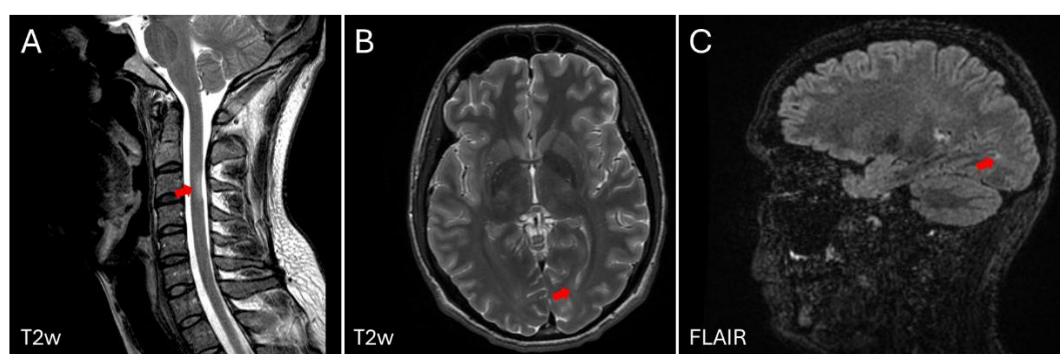


Figure 1. Magnetic resonance imaging (MRI) of the brain and spinal cord of the patient described in the clinical case. Two subtle lesions can be observed in T2 sequences of the cervical spinal cord at the level of C3 (A) and in the white matter (WM) of the brain at the level of the temporal horn of the left lateral ventricle (B). Image obtained in FLAIR sequence, which enhances the lesions in WM (C). The images were obtained in a 3-Tesla MRI (General Electric, Signa HDx, Boston, MA, USA).

The treatment regime consisted of an initial dose of 1 gram on days 1 and 15 of the first month, followed by 9-monthly doses of 1 gram, adjusted based on the repopulation of B cells. This treatment resulted in a significant improvement in fatigue after the third dose, enabling increased mobility and participation in daily activities such as walking and dining out. The patient remains stable of his neurological condition, with fatigue stabilized.

EBV, as well as other viral infections unleashes the otherwise silenced expression of human endogenous retrovirus (HERV) [25], potentially leading to sterile inflammation and additional symptoms that resemble those of acute exogenous infections. In particular, overexpression of the toxic HERV-W ENV protein has been detected in MS patients [26], as well as in ME/CFS and post-COVID condition [27] indicating a relationship of this protein with post-viral disease chronicity.

To determine whether HERV-W ENV is overexpressed in the plasma of this patient, positive for EBV IgGs in 2015, we assessed HERV-W ENV protein levels following the protocol previously described in [27] and compared it to those in other ME/CFS cases and control samples (Figure 2) (Supplementary Table S1) (patient's archived sample National Registry in Biobanking collection C0006024 obtained in 2016). The results showed that mean HERV-W ENV level of this patient's was

1.36E+05 (AUC), which was below the positivity cutoff value (mean AUC: 2.30E+06), defined as the mean + 2 standard deviations of control samples.

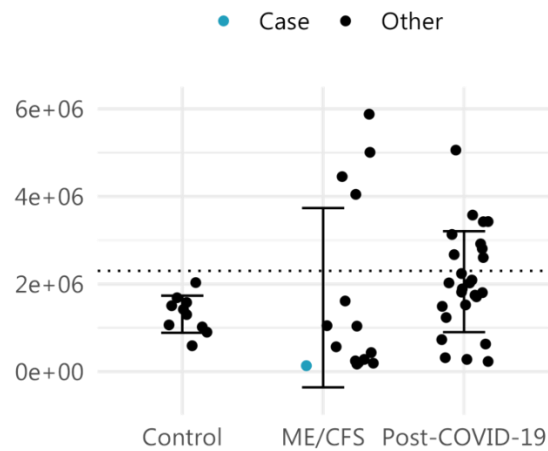


Figure 2. HERV-W envelope (ENV) antigenemia in control cases (n=10), ME/CFS (n=15) and post-COVID-19 condition subjects (n=26). Levels of HERV-W ENV protein in plasma are shown as mean area under the curve (AUC). HERV-W ENV protein level in plasma is highlighted in blue for the study case. Cutoff line shows the minimum value to be considered positive.

To further explore potential HERV alterations in this patient, we performed a genome-wide analysis using high-density microarrays, as described in [22]. Results of this study case were compared to those of other age- and BMI-matched ME/CFS cases and healthy volunteers. Bioinformatic analysis, conducted with the R "limma" package [22,28], identified 502 differentially expressed HERV probesets between ME/CFS patients and healthy controls (p-value < 0.1 and absolute log2 fold-change > 1) (Supplementary Table S2). Hierarchical clustering of HERV expression profiles revealed that the patient's sample clustered with other ME/CFS cases, despite being from a male subject (Figure 3A). To further investigate the patient's clustering pattern, we conducted Principal Component Analysis (PCA). This analysis revealed that while the patient's sample did not perfectly align with female ME/CFS cases, his HERV profile was closer to ME/CFS patients' than to control's (Figure 3B), indicating potential epigenetic alterations in his immune cells that despite differing from both comparison groups aligns with that of ME/CFS females.

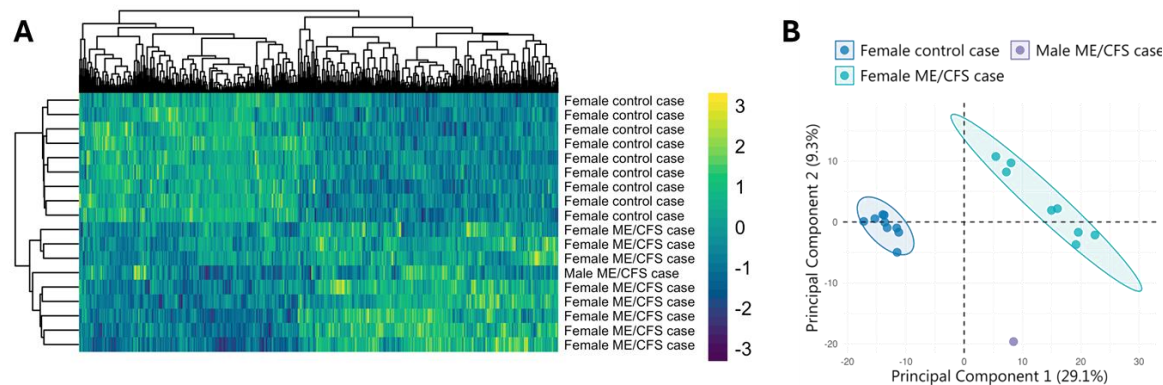


Figure 3. HERV clustering (A) and principal component analysis (B) of the ME/CFS male case reported in this study along with 8 female ME/CFS cases and 9 healthy volunteer samples. Analysis includes all HERV probes of custom HERV-V3 arrays [29], displaying significant differential expression between ME/CFS and healthy

control groups ($FDR < 0.1$ and $|\log_2 FC| > 1$). Clustering heatmap was built with pheatmap [30] and PCA with FactoMineR package [31].

To understand the potential cell role of top overexpressed HERVs in the study case, we first selected those with highest expression levels in the study case sample with respect to the rest (control or ME/CFS case), which turned out to be 29 out of the 502 differentially expressed (about 6% of them) (Supplementary Table S3), and then searched for their closest annotated genes. Gene names, genomic distances to HERVs with highest expression in the study case and their position within the matched gene were determined using the Goldmine package in RStudio [32] (Supplementary Table S4). The data shows that of the 29 HERV allocated, 10 (34.5%) corresponded with intergenic regions less than 100,000bp away while the remaining 19 (65.5%) seemed to be encoded within genes. From the latter, most (thirteen) were encoded within introns (68.4%), four lied within promoter regions (21%), one within the 3'UTR region and one within a coding exon (5.3% each). In addition to regulatory lncRNAs, novel transcripts, hit genes associated with development, infection, metabolism and neural homeostasis, as for example QKI involved in myelinization and oligodendrocyte differentiation or LINGO2 with a role in the glutamatergic synapse.

3. Discussion

This case report informs of a patient having received an initial diagnosis of ME/CFS (2015) and a later diagnosis of MS (2017), with a lack of response to several treatments for the following three years and striking response to rituximab in 2020, having kept the patient in remission until present. Although MS relapse frequency is highly variable, as it is the case for factors affecting MS-associated fatigue [33], recent meta-analysis reports a reduction in MS relapses with rituximab treatment [34]. Clinical trials assessing the potential benefit of ME/CFS with rituximab are more limited, with an initial partial success in the reduction of symptoms [35] and failure to show benefits by a continuation trial [36]. Discrepancies across trials may be associated with patient heterogeneity, medical regime applied or other. In this case report the patient not only showed clear improvement after only three doses of treatment with rituximab, but also remains stable of his neurological condition, with fatigue stabilized now for some years after the 9-month treatment regime applied. Whether the successful evolution of the patient is due to additional factors is unknown.

The patient displayed auto-antibodies against AchR (AchR-aAbs), a typical finding in myasthenia gravis (MG), an autoimmune disease displaying impaired neuromuscular junction transmission with about 85% of patients presenting AchR-aAbs [37]. Thus, a mechanism mediated by AchR-aAbs contributing to patient improvement by rituximab therapy cannot be discarded [38].

The lack of overexpression of the toxic HERV-W ENV protein, also known as MSR/V, frequently reported in MS patients [39], but also reported in some ME/CFS patients [40] was unexpected. Targeting MSR/V with the monoclonal antibody temelimab is being studied [41]. On another hand, our mechanistic findings on differential HERV profiles are limited by the lack of comparison of HERV profiles of this case to male ME/CFS and MS cases. In fact, the literature reports molecular differences in the immune systems of male and female ME/CFS patients [42–44]. Whether molecular sex-differences in ME/CFS include HERV profiles remains unknown at present. Nevertheless, the results of this patient's HERV profile seem to align better with other ME/CFS cases, despite the absence of sex-matching, than with profiles of the healthy participants, in support of his ME/CFS diagnosis. In addition, the findings fit with those of previous reports of EBV infections leading to important endogenous retroviral repression in CD19 + B cells [45]. Despite an important downregulation of HERV expression in the PBMCs of our patient, we observed prominent overexpression (overexpressed above all other reference samples; $n=17$) (Supplementary Table S3), on about 6% of the differentially expressed HERVs. Interestingly associated gene functions match with potential alterations of the patient's physiology (Supplementary Table S4). Although it is not possible to attribute our findings to CD19+ B cell epigenetic alterations or to any other PBMC subpopulations,

the observed response of the patient to rituximab therapy suggests this may be a mechanism for EBV disease triggering worth exploring.

EBV infections seem to constitute a causal factor for developing MS [46] as found for EBNA1-peptide epitope mimicry mechanisms with the central nervous system protein glial cell adhesion molecule (GlialCAM) leading to nervous tissue lesions [47]. Similarly, mechanisms leading to autoimmune encephalitis after herpes simplex infections [48] or to other types of autoimmunity by still unknown mediators (unknown foreign antigens) [49], have been described. Additional auto-antigen-related viral sequences will possibly be discovered in the years to come. In this respect, WHO's definition of ME/CFS as a post-viral chronic disease, the rich bibliography relating ME/CFS with EBV, herpes or other infectious agents [50–52], in addition to the presentation of symptom overlaps between ME/CFS, MS and MG, including muscle weakness, sleep and sensory disturbances, or pain [53–55], poses the pertinence of co-exploring the mechanisms underlying these diseases. The case reported here appears singular from the angle that the patient presents with features, to a certain extent, for a triplet disease. Further work towards defining differential markers across these and other symptom related diseases, thus promoting the development of targeted therapeutics, is urgently needed.

4. Conclusions

In summary, we present a male case with a co-diagnosis of ME/CFS and MS, with a positive EBV serology, also expressing AchR-aAbs, and showing multiple symptomatology and lesions in the spine and brain, who positively responded to a 9-month treatment regime of rituximab doses of 1 gram and stayed in remission for at least 3 years after treatment. Despite no evidence of HERV-W ENV overexpression, as expected, patient's PBMC HERV profile was clearly in closer proximity of other ME/CFS profiles than with those of healthy participants, with potential sex-discrepancies and/or connections to MS and MG, confirmation pending. Derangement of HERV expression in B-cells contained within the explored PBMCs appear as a feasible mechanistic explanation for the response of this patient to rituximab, seemingly worth exploring in future cases. In addition, the possibility of individual responses to viral infections triggering the development of complex chronic states deserves further assessment.

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Informed Consent Statement: Informed consent was obtained from the subject involved in the study.

Data Availability Statement: The original contributions presented in this study are included within the article. Further inquiries can be directed to the corresponding author/s.

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Abbreviations

The following abbreviations are used in this manuscript:

| | |
|--------|---|
| ME/CFS | Myalgic Encephalomyelitis/ Chronic Fatigue Syndrome |
|--------|---|

| | |
|------------|---|
| MS | Multiple Sclerosis |
| MG | Myasthenia Gravis |
| HERV | Human Endogenous Retrovirus |
| HERV-W ENV | Human Endogenous Retrovirus type W (tryptophan), Envelope protein |
| MSRV | Multiple Sclerosis Retrovirus |
| PBMC | Peripheral Blood Mononuclear Cells |
| EBV | Epstein Bar Virus |
| AchR | Acetylcholine Receptor |
| aAb | Autoantibody |

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