Complete Epstein-Barr virus seropositivity in a cohort of Pediatric Onset Multiple Sclerosis: a comparison to other autoimmune diseases

Gabriele Monte[1], Giuseppe Tiralongo[2], Gianmarco Bellucci[3,4], Laura Papetti[1], Michela Ada Noris Ferilli[1], Marco Salvetti[3,6], Massimiliano Valeriani[1,5]

[1]Developmental Neurology Unit, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy; [2]Academy of Pediatrics, Tor Vergata University of Rome, Rome, Italy; [3]Centre for Experimental Neurological Therapies, Department of Neurosciences, Mental Health and Sensory Organs, Sapienza University, Rome, Italy; [4]Department of Neurology, University of California, San Francisco, USA; [5]Systems Medicine Department, Tor Vergata University of Rome, Rome, Italy; [6]Istituto Ricovero e Cura a Carattere Scientifico Istituto Neurologico Mediterraneo Neuromed, Pozzilli, Italy

Introduction: The role of Epstein-Barr virus (EBV) in MS pathogenesis is supported by the increased MS risk after infectious mononucleosis. This study aimed to evaluate the rate of previous EBV infection in our large pediatric-onset MS (POMS) cohort and we also wanted to test the disease-specificity of this association.

Materials and Methods: We retrospectively included patients with POMS followed up at Bambino Gesù Children's Hospital. Demographics, clinical data and blood samples were collected at MS onset. "Previous EBV infection" was diagnosed when anti-EBV nuclear antigen (EBNA) Immunoglobulin G (IgG) and/or anti-viral capsid antigen (VCA) IgG resulted positive. The control group consisted of two subgroups matched for both age and sex: non-autoimmune control (primary headaches) and non-neurological pediatric-onset autoimmune control cohort (juvenile idiopathic arthritis (JIA), ulcerative colitis (UC) and Crohn's disease (CD)). Fisher's exact test was used to compare frequencies, Kruskal-Wallis and Mann-Whitney tests were applied for continuous variables. Correlation between continuous variables was assessed through Spearman's rho.

	POMS (n = 57)	Total control group (n = 162)	IBD (n = 80)	JIA (n = 32)	Headaches (n = 50)
Median age at EBV serostatus, years (range)	14 (5-17)	12 (6-17)	13 (6-17)	12 (6-17)	12 (6-17)
F/M	1.85	1.79	1.67	1.9	1.94
Anti-EBNA IgG positive, n (%)	57 (100%)	88 (54%)	45 (56.2%)	13 (40.6%)	30 (60%)
Anti-VCA IgG positive, n (%)	57 (100%)	96 (59%)	47 (58.7%)	14 (43.7%)	35 (70%)
Anti-VCA IgM positive, n (%)	1 (2%)	11 (7%)	5 (6.2%)	0	6 (12%)
EBV seropositive, n (%)	57 (100%)	96 (59%)	47 (58.7%)	14 (43.7%)	35 (70%)

Results: 57 patients with POMS were included and all had a previous EBV infection. 162 patients were included in the control group and EBV seropositivity was 59%: 50 patients had primary headaches and 35 (70%) had a previous EBV infection; 112 patients had pediatric-onset autoimmune disease and EBV seropositivity was 54%. EBV seropositivity was significantly higher in POMS ($p=3.9\times10^{-11}$) than in the whole controls cohort, corresponding to an Odds Ratio (OR) of 79.2 (95% C.I. 4.8-1305). This difference was also significant when we separately compared POMS patients to headache patients ($p=2.98\times10^{-6}$) and non-neurological autoimmune diseases patients ($p=4.66\times10^{-12}$), as well as POMS to inflammatory bowel diseases (IBD) and JIA separately ($p=1.01\times10^{-9}$ and $p=1.56\times10^{-10}$, respectively). Looking at absolute levels (titres) of EBV antibodies across the groups, EBNA IgG and VCA IgG were significantly higher in POMS compared to both autoimmune and non-autoimmune controls ($p<0.0001$ in all settings). Age and biological sex did not affect EBV serostatus or absolute antibody titres.

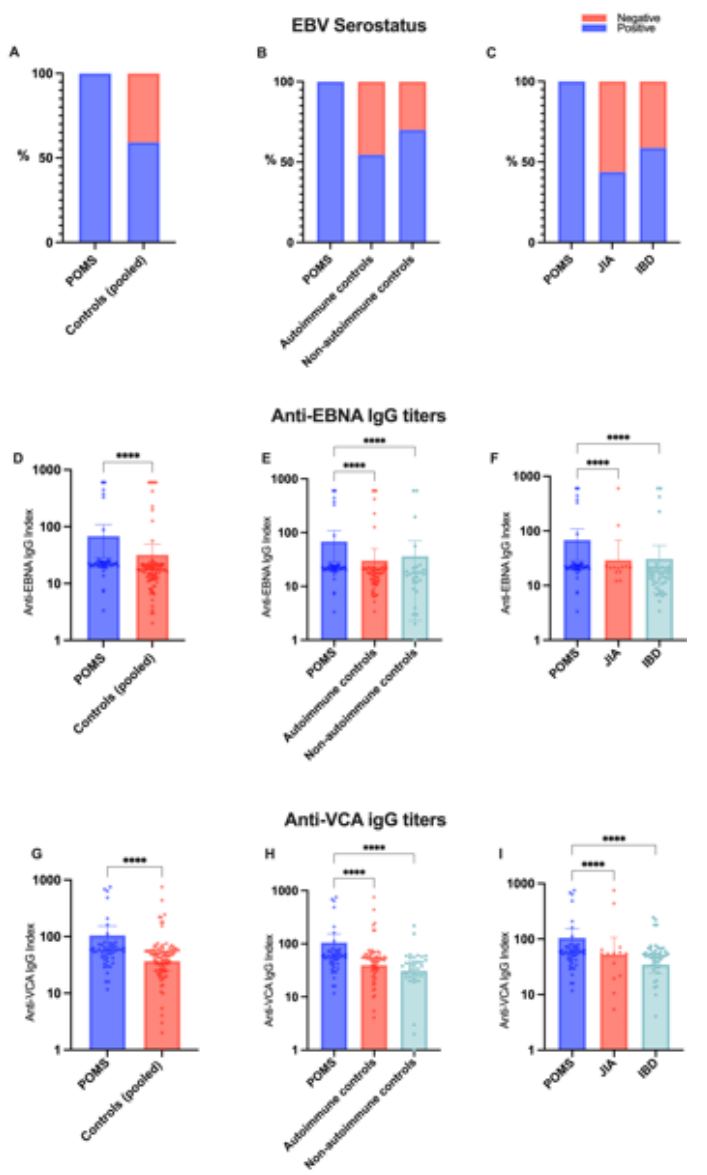

Figure legend: (A–C) Percentage of EBV seropositive individuals in POMS versus (A) pooled controls, (B) autoimmune and non-autoimmune controls, and (C) disease-specific controls: JIA and IBD. (D–F) Anti-EBNA IgG titers (log scale) in the same groups. (G–I) Anti-VCA IgG titers (log scale) across the same comparisons. (D-F) Bars represent the mean and 95% confidence interval. Statistical significance was assessed using non-parametric tests. ****$p < 0.0001$.

Conclusions:

- EBV seropositivity of our cohort was 100%, as observed in adult-onset MS. This rate is higher than previously reported for children, further strengthening the role of EBV in the pathogenesis of POMS.
- EBV infection has also been suggested to play a key role in other non-neurological autoimmune diseases in both adults and children. EBV seroprevalence and antibody titres in our POMS population were significantly higher than in patients with non-neurological autoimmune diseases, possibly pointing to a stronger link between EBV and POMS onset and supporting a disease specificity.
- Larger and prospective studies are needed and it will be important to understand how EBV may initiate disease in this population. These evidence could support the development of preventive strategies, as EBV vaccines.

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