Epstein-Barr Virus and Monoclonal Gammopathy of Clinical Significance in Autologous Stem Cell Transplantation for Multiple Sclerosis

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Introduction. Autologous hematopoietic stem cell transplantation (AHSCT) with anti-thymocyte globulin (ATG) conditioning as treatment of active multiple sclerosis (MS) is rapidly increasing across Europe (EBMT registry data 2017). Clinically significant Epstein-Barr virus reactivation (EBV-R) following AHSCT with ATG for severe autoimmune conditions is an underrecognized complication relative to T-cell deplete transplants performed for hematological diseases. This retrospective study reports EBV-R associated significant clinical sequelae in MS patients undergoing AHSCT with rabbit ATG.

Methods. Retrospective data were analyzed for 36 consecutive MS-AHSCT patients at Kings College Hospital, London. All patients routinely underwent weekly EBV DNA polymerase chain reaction monitoring and serum electrophoresis for monoclonal gammopathy (MG or M-protein). EBV-R with rising Epstein-Barr viral load, M-protein, and associated clinical sequelae were captured from clinical records.

Results. All patients had evidence of rising EBV DNA-emia, including 7 who were lost to long-term follow-up, with a number of them developing high EBV viral load and associated lymphoproliferative disorder (LPD). Nearly 72% (n = 18/29) developed de novo MG, some with significant neurological consequences with high M-protein and EBV-R. Six patients required anti-CD20 therapy (rituximab) with complete resolution of EBV related symptoms. Receiver operating characteristics estimated a peak EBV viremia of >500 000 DNA copies/mL correlated with high sensitivity (85.5%) and specificity (82.5%) (area under the curve: 0.87; *P* = .004) in predicting EBV-R related significant clinical events.

Conclusion. Symptomatic EBV reactivation increases risk of neurological sequelae and LPD in MS-AHSCT. We recommend regular monitoring for EBV and serum electrophoresis for MG in MS patients in the first 3 months post-AHSCT.

Keywords. multiple sclerosis; autologous hematopoietic stem cell transplantation; Epstein-Barr virus infection; monoclonal gammopathy; post-transplant lymphoproliferative disorder.

Multiple sclerosis (MS) is a chronic autoimmune, inflammatory, demyelinating disease of the central nervous system [1, 2], with a relapsing-remitting MS (RRMS) presentation in the majority of patients at diagnosis. Recovery from relapses may be complete or partial [3, 4]. After a variable period of time, people with RRMS may develop a more progressive disability accumulation with or without superimposed relapses, termed secondary progressive MS (SPMS). A minority experience progressive disability from the onset of disease, termed primary progressive MS (PPMS) [4]. A number of immunomodulatory disease modifying therapies (DMTs) are currently licensed for treatment of RRMS with an aim of reducing number of relapses and accrual of disability, although with variable efficacy [5]. Since 1996, autologous hematopoietic stem cell transplantation (AHSCT) has been a novel approach for MS management, using immunoablation followed by immunomodulation mechanisms, with evidence of significant suppression of inflammatory activity and qualitative changes in the reconstituted immune system (immune reset theory) [6–8]. AHSCT appears most effective for MS patients with evidence of inflammatory activity on magnetic resonance imaging, younger age, a shorter disease duration, low to moderate disability levels (expanded disability status scale <6 or up to 6.5 if recent progression), and failure of at least 1 highly active DMT (natalizumab or alemtuzumab) with no significant comorbidities [9–11]. Recently reported preliminary results of randomized MIST study [12] found AHSCT to be superior to standard DMT for RRMS with respect to both treatment failure and disability progression.

However, risk of subsequent rise in opportunistic infections following such immunosuppressive therapies remain a potential concern [13]. MS patients undergoing AHSCT have often been exposed to a number of immunomodulating DMTs; the addition of immunosuppressive rabbit anti-thymocyte globulin (rATG) to their conditioning regimen may confer a higher risk of viral reactivation in these patients. The number of AHSCTs performed for MS is rising significantly in Europe [14] and as more centers perform AHSCT for this indication, it is increasingly important to recognize the unique problems faced by these patients post-AHSCT. This retrospective study reports for the first time, Epstein-Barr virus reactivation (EBV-R) associated neurological sequelae and lymphoproliferative disorder (LPD) in MS patients undergoing rATG conditioned AHSCT in our center.

METHODS

Patients and Procedures

Data were collected retrospectively on 36 consecutive MS patients undergoing AHSCT between February 2012 and February 2017 at Kings College Hospital, London. Peripheral blood stem cells were collected following standard mobilization strategy consisting of cyclophosphamide 4 g/m^2 over 2 days and granulocyte colony-stimulating factor for 7 days. Thirty-five (97%) patients were conditioned using standard protocol of cyclophosphamide (50 mg/kg for 4 days) and rATG (2.5 mg/kg/day for 3 days) for in vivo lymphodepletion followed by stem cell infusion. One patient was conditioned with carmustine/etoposide/cytarabine/melphalan regimen along with an equivalent dose of rATG (BEAM-ATG) prior to stem cell infusion. The median CD34 stem cell dose returned was 7.17 × 10^6/kg (range 4.0–17.1 × 10^6/kg).

Prior exposure to EBV was assessed by serological evidence (EBV viral capsid antigen [VCA] immunoglobulin G [IgG]). EBV DNA load monitoring was performed on whole blood samples by standardized quantitative real-time polymerase chain reaction (RT-PCR) using Rotor-Gene^{TM} (Qiagen) assay of EBV BZLF1 DNA. This assay was adapted from our published assay using LightCycler (Roche) [15] and since has been validated against the recently published World Health Organization (WHO) standard, with our lab's EBV DNA quantification of 10 copies/mL considered equivalent to 1 IU/mL DNA reported with the WHO reference method [16]. EBV-R was defined as rising EBV DNA load of >10 copies/mL detected on 2 consecutive tests based on our assay sensitivity. Symptomatic EBV-R was captured by peak blood EBV DNA load, presence of B symptoms (defined by presence of either unexplained weight loss, recurrent fever, night sweats); which was, in turn, defined by clinical, radiological, and/or histological evidence based on recent ECIL-6 guidelines [17]. In addition, significant "clinical events" were also defined as new and persistent organ dysfunction (eg, neurological events) temporally associated with rising EBV viremia in MS patients. Serum protein electrophoresis was routinely tested around 3 months post HSCT as part of our institutional practice, with immunoglobulin subclasses identified by immunofixation electrophoresis. Patient outcomes were assessed at last follow-up as of April 2017.

Statistics

The database of transplants and outcomes was built in Microsoft Excel 2016, and statistical analyses were performed using IBM SPSS Statistics version 24.0. Patient characteristics are presented as medians (with interquartile ranges [IQRs]) for data with nonnormal distribution. Comparisons of baseline characteristics used Mann-Whitney U test, Fisher exact test, or χ^2 test for trend as appropriate. Receiver operating characteristics (ROC) curve was obtained correlating LPD and clonal gammopathy associated clinical events with rising EBV viremia (copies/mL).

RESULTS

Baseline characteristics are presented in Table 1. Most MS patients (88.9%) had RRMS phenotype with median of 2 (range 0–3) DMTs prior to AHSCT. Twenty-two MS patients had prior exposure to natalizumab, and 7 were treated with alemtuzumab (6 patients received both). All 36 (100%) MS patients were serologically positive for EBV VCA IgG pretreatment, indicating prior EBV exposure and had detectable EBV DNA post-AHSCT. Seven MS patients were lost to long-term follow-up for EBV monitoring. The median time to first EBV DNA detection post-transplant was 30 days (IQR 23–46 days). EBV DNA levels peaked at a median of 32 days post-transplant (IQR 31–53 days). All MS patients had normal baseline lymphocyte counts (median) pre-HSCT with a median time of 46 days (range 14–404 days) to lymphocyte recovery (defined by total lymphocyte count >1.0 × 10^6/mL) following AHSCT (see Figure 1). A high proportion (86%; n = 25/29) of the MS patients in active follow-up recovered lymphocyte counts around D56 with a median lymphocyte count of 1.56 (10^6 cells/mL). Four patients remained lymphopenic at last follow-up.

All patients were stratified into following 3 groups according to peak rise/burden of EBV DNA-emia (copies/mL): <100 000 (<100 k) copies/mL, 100 001–50 000 (100 k–500 k) copies/mL, and >500 000 (>500 k) copies/mL to identify any specific thresholds for clinically significant events related to rising EBV-R (Table 1). The majority of patients (76%) with rising Epstein-Barr viral load >100 k copies/mL were routinely screened by computed tomographic (CT) scans to assess for evidence of LPD, in line with our institutional policy. One third (34.5%) of patients developed peak EBV viremia of >500 k copies/mL. Eight patients (27.6%) developed symptomatic EBV-R; defined as persistent fever, lymphadenopathy, and/or B symptoms. Of these 8 patients, only 1 (12.5%) had a peak EBV viremia <100 k

Table 1.   Baseline Patient Characteristics and Epstein-Barr Virus (EBV) Related Clinical Events According to Peak EBV DNA-emia Burden

| Baseline Characteristics (n = 36)			Patient Groups According to Peak EBV DNA in copies/mL		
			0–100 000 (n=29)	100 001–500 000	>500 000
Median age at time of AHSCT in years (range)	43.5 (36–47)	No. of patients (%)	16 (55.2)	3 (10.3)	10 (34.5)
Sex					
Male	19 (52.8%)	M-Protein (n)	11	0	7
Female	17 (47.2%)				
Disease type (n; %)					
Relapsing remitting MS	22 (61.1%)	Median EBV DNA log value at peak (IQR)	4.8	5.5	6.25
Secondary progressive MS	10 (27.8%)		(3.5–4.8)	(N/A)	(6.1–6.9)
Primary progressive MS	4 (11.1%)				
Median number of previous DMT (range)	2 (0–6)	Median number of prior DMTs	2	3	2
Previous use of high efficacy DMT (n)		Symptomatic EBV (n)	1	0	7
Natalizumab	22	LPD diagnosis (CT/Biopsy) (n)	0	0	3 by CT alone
Alemtuzumab	8	Neuro/autoimmune complications (n)	0	0	3
Both	6	Treated with Rituximab (n, %)	0	0	6
Median EDSS (range)	6.0 (2.5–8.0)	Confirmed EBV resolution at last follow up (n)	7	2	7
Median follow-up post-AHSCT in days (range)	436 (188–785)	Detectable EBV DNA at last follow up (n, %)	9	1	3
No. of patients with prior EBV exposure (n, %)	36 (100%)				
No. of patients with detectable EBV post-AHSCT (n, %)	29 (80.5%)				
No. of patients lost to long-term follow-up (n, %)	7 (19.5%)				
Median time to EBV detection post-AHSCT in days (IQR)	30 (23–46)	Median time for EBV resolution (IQR in days)	67 days (44–155)	47 days (N/A)	63 days (45–170)
Median Time to peak EBV DNA levels in days (IQR)	32 (31–53)	Median Time to peak EBV DNA levels in days (IQR)	40 days (25–85)	30 days (N/A)	39 days (32–43)

Abbreviations: AHSCT, autologous hematopoietic stem cell transplant; CT, computed tomography; DMT, disease modifying therapy; EBV, Epstein-Barr virus; EDSS, Kurtzke expanded disability status scale; IQR, interquartile range; LPD, lymphoproliferative disease; M-Protein, monoclonal paraprotein or gammopathy; MS, multiple sclerosis.
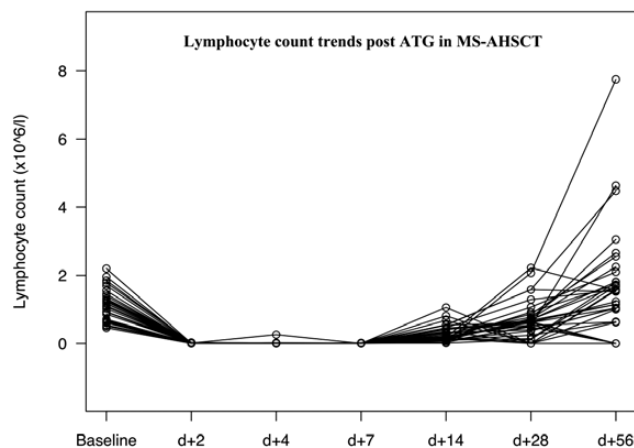
Figure 1. Trend of lymphocyte count recovery following ATG in MS patients. Figure shows trends of lymphocyte count from baseline to recovery post-AHSCT for MS patients. Majority of patients had normal baseline lymphocyte counts pre-HSCT and became lymphopenic post-ATG with counts recovering towards d+28 and >85% of patients recovered counts by d+56, with some overshooting from their baseline, possibly reflective of EBV related lymphoproliferation in some of these patients. Abbreviations: AHSCT, autologous hematopoietic stem cell transplantation; ATG, anti-thymocyte globulin; d+, days post-AHSCT; MS, multiple sclerosis.

DNA copies/mL with the remaining 7 (87.5%) patients having a peak EBV viremia of >500 k copies/mL. Three patients with rising EBV viremia >500 k copies/mL had findings consistent with probable LPD on CT imaging; however, none had definitive histological diagnosis. Three MS patients had worsening neurological symptoms concurrent with rising EBV viremia >500 k copies/mL and clonal gammopathy, as described below.

Interestingly, we also observed frequent de novo monoclonal gammopathy (MG or M-protein) in 18 MS patients (62.4%) following rising EBV viremia, the majority (n = 16) of whom developed IgG subtype, and the remaining 2 developed immunoglobulin A (IgA) and immunoglobulin M (IgM) M-protein. Concerningly, 2 of these patients developed clinically significant M-Protein burden; one patient with IgG Kappa M-protein of 45.6 g/L developed hyper-viscosity and neurological symptoms mimicking MS relapse, requiring plasma exchange. Another patient developed significant lumbosacral radiculopathy with rising EBV-R and high IgM lambda M-protein (IgM 48.5 g/L) (see Supplementary Case Vignettes). Figure 2 highlights the association of neurological symptom onset following rising EBV viremia (log copies), falling lymphocyte counts (× 10^6/mL) with
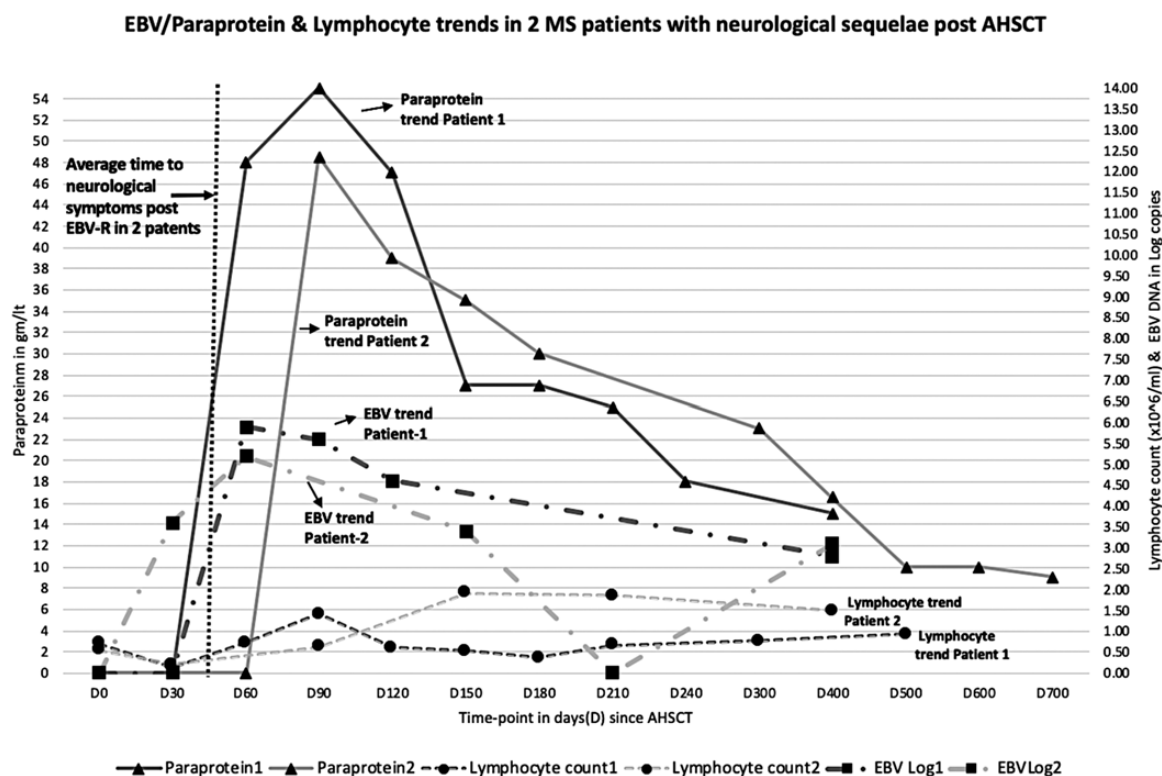
Figure 2. Paraprotein, EBV, and lymphocyte trends in 2 MS patients with significant neurological sequelae post-AHSCT. Figure demonstrates trends of EBV copies (log), paraprotein levels (g/lt), and lymphocyte levels (counts ×10^6/mL) in 2 MS patients with significant neurological symptoms following EBV reactivation. Both patients had significant EBV viremia (log>5.2 or >500 000 copy number) and developed significant paraproteinemia, which was only noted after persistent unexplained neurological symptoms. The trend reversed following anti-CD20 (rituximab) therapy, with limited recovery in neurological symptoms. Abbreviations: AHSCT, autologous hematopoietic stem cell transplants; D, days post -HSCT; EBV-R, Epstein-Barr virus reactivation; MS, multiple sclerosis.

significant rise in M-protein (gm/lt) levels post-AHSCT. A third patient developed painful lower limb paresthesia following rising EBV viremia >500 k copies/mL, although there was not any M-protein detected. Their symptoms persisted at last follow-up despite no evidence of MS related new disease activity.

Six patients were treated with anti-CD20 antibody, rituximab (375 mg/m2 weekly up to 4 weeks), due to clinical severity of EBV reactivations and leading to reduction in EBV viral load and concurrent improvement/resolution of EBV related symptoms. ROC curve analysis (Figure 3) confirmed EBV viremia of >500 k copies/mL correlated with high sensitivity (85.5%) and specificity (82.5%) (area under the curve: 0.87; 95% confidence interval: 0.73–1.0; P = .004) in predicting significant EBV related clinical events (evidence of LPD and/or neurological symptoms) that may require treatment with rituximab. The sensitivity dropped significantly on lower estimates for events below 500 k copies/mL.

The median time to resolution of EBV viremia post-rituximab was 21 days (IQR 19–124 days) in 5 patients with >500 k copies/mL (1 patient was treated for late onset persistent symptomatic clonal gammopathy, despite fall in EBV levels). No significant adverse events were noted in the treated group. Nine patients had a persistent low-level EBV viremia detectable at last follow-up. All patients who underwent AHSCT were alive as of April 2017.

DISCUSSION

MS as an autoimmune disorder (AD) is theorized to have generally similar underlying pathophysiological immune dysregulation mechanisms [18–22] relative to other chronic autoimmune conditions. EBV is increasingly implicated in the pathogenesis of MS by virtue of epidemiological and serological evidence, impaired CD8+ T cell immune responses to EBV, and possible underlying genetic susceptibility for autoimmunity (with EBV encoded protein interactions), as recently described by Harley et al and others [1, 23–25].

Generally, EBV-R related LPD has been reported in both allogeneic (allo-HSCT) and solid organ transplants treated with immunosuppressive therapy, often with a significant impact on organ function and overall survival [26–30]. It is observed that reduced intensity allo-HSCT for malignant hematological conditions using alemtuzumab have a relatively lower overall risk of LPD compared to ATG based treatments, possibly mediated by more effective pan-B and T-cell lymphodepletion. In contrast, ATG primarily affects the T-cell repertoire with delayed EBV specific CD8+ T-cell recovery [31]. Clinically significant endogenous viral infections including EBV following ATG conditioned AHSCT for severe ADs such as Crohn's disease and systemic sclerosis is increasingly recognized, but the development of LPDs remains rare in these ADs [13, 32, 33]. Nash et al [32] concerningly reported 2 deaths (1 MS and 1 systemic sclerosis patient) from EBV-related LPD in 56 ATG conditioned AHSCT for autoimmune diseases. Additionally, EBV associated hemophagocytosis in ATG-AHSCT for ADs have also been reported [34], with one resulting in death of the patient [35].

Our report of suspected EBV-related LPDs (~10%) in MS-AHSCT group is relatively higher than published reports with allo-HSCTs (4.5–7%) [36, 37] and our own center's unpublished T-cell depleted allo-HSCT experience (6.5%), possibly a reflection of underlying immunopathological state of MS itself [38]. This is further corroborated by the fact that similar LPD risk has not been observed in other ADs, for example, Crohn's disease, treated with ATG -AHSCT in this center. Another example from our center's experience of severe aplastic anemia (n = 40) treated with ATG/ciclosporin, only 52% (n = 21/40) developed EBV-R (unpublished data), and none had LPD or required any treatment, suggesting that the problem may not be ATG specific.

Our study's observation of significant persistent neurological events (with no evidence of new MS disease activity) associated with clonal gammopathy suggest a potentially new clinical syndrome, described for the first time in ATG conditioned AHSCTs in MS and possibly induced by clonal B-cell dysregulation following EBV-R. It could be hypothesized that any remaining EBV infected latent B cells, surviving despite high doses of cyclophosphamide (given with mobilization and conditioning in MS ASHCT) [8] and compounded by depletion of CD8+ T cells by ATG, may serve as potential source for EBV escape while interacting abnormally within the host immune micro-environment [39] and leading to rise in M-protein, LPD, and neuro-inflammatory insults in some of the MS patients
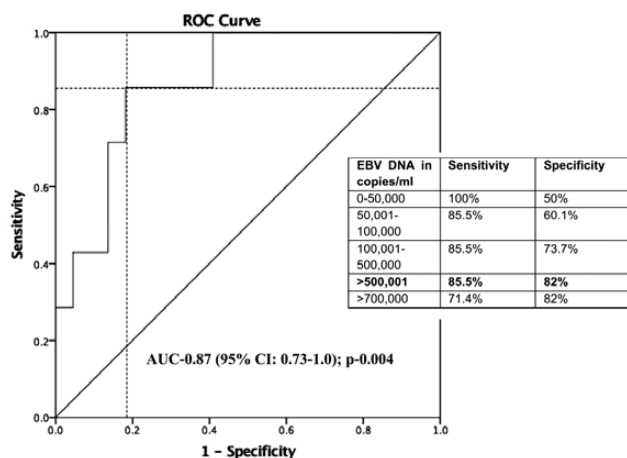
Figure 3. ROC curve estimates for peak EBV viremia levels and significant clinical events in MS post-AHSCT. ROC curve demonstrating significant correlation between high EBV levels and clinical events (LPD and neurological events) in MS-AHSCT patients, with highest sensitivity and specificity noted with peak EBV viremia of >500 000 copies/mL (P = .0004). Abbreviations: EBV, Epstein-Barr virus; LPD, lymphoproliferative disorder; MS-AHSCT, multiple sclerosis patients treated with autologous haematopoietic stem cell transplants; ROC, receiver operating characteristics.

EBV DNA in copies/ml	Sensitivity	Specificity
0-50,000	100%	50%
50,001-100,000	85.5%	60.1%
100,001-500,000	85.5%	73.7%
>500,001	85.5%	82%
>700,000	71.4%	82%

AUC-0.87 (95% CI: 0.73-1.0); p-0.004

post-AHSCT. Reports of lower incidence of EBV-R and LPD in another commonly used protocol for MS-AHSCT using BEAM-ATG (Ricardo Saccardi, Carregi University Hospital, Florence; personal communication) may reflect the greater myeloablative effect of BEAM chemotherapy, which could further deplete the residual B-cell pool and thus lower potential for EBV proliferation. It is plausible that dose of rATG is critical, given we have not seen similar reports from other centers where less rATG doses were given for MS-AHSCT (range between 5.0 and 6.5 mg/Kg; personal communication), but there seems to be some variability in prospective serial EBV monitoring in these patients.

The clinical threshold for Epstein-Barr viral load as a significant risk factor for post-transplant LPD is widely debated. This study reports peak Epstein-Barr viral load >500 k copies/mL post-AHSCT is significantly associated with probable LPD and neurological events in MS patients with high sensitivity (85.5%) and specificity (82.5%) (P = .004) (Figure 3, ROC curve). Our ROC curve estimates are potentially limited by the relatively small number of events analyzed, but this has consistently been useful in our MS-AHSCT experience for predicting clinical events with high EBV load. Our EBV PCR assay has been validated against the recently defined standard WHO reference method (ie, 10 copies/mL = 1 IU/mL EBV DNA) [16], and thus this EBV threshold for preemptive treatment with Rituximab can potentially be applied in relevant clinical context in other centers using similar validated essays. Rituximab treatment delivered good overall response in our symptomatic patients, with resolution of EBV related clinical symptoms and no subsequent viral or bacterial infections at last follow-up. The role of prophylactic or pretransplant rituximab in MS-AHSCT is also a potential area of interest in reducing risk of EBV-LPD in stem cell transplants as observed by Burns et al [36], and future randomized studies are required to investigate its potential benefit.

Our study limitations include its retrospective nature and that no suspected LPD patients had histological confirmation, mainly related to patient refusal or technical difficulties. Seven MS patients were lost to follow-up for EBV monitoring following discharge, which limits the findings of this study. Additionally, our numbers were too small to identify any association of EBV-related clinical events with previous DMT exposure in MS patients.

In conclusion, symptomatic EBV reactivation increases risk of neurological sequelae and LPD in MS-AHSCT. Regular monitoring for rising EBV viremia, as recommended by Snowden et al [10] and Muraro et al [11], and serum electrophoresis for M-protein should be considered in the first 3 months post-AHSCT for MS. We recommend persistent high EBV viremia > 500 k DNA copies/mL as potential trigger for consideration of preemptive anti-CD20 therapy and potentially reduce associated morbidity.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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