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Natural history of EBV replication and viral load dynamics after alemtuzumab based allogeneic stem cell transplantation

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Highlights:

- EBV detection post-transplant may trigger pre-emptive therapies.
- Performance of EBV PCR assays influences their utility for directing treatment.
- We report a combined assessment of EBV load and clinical signs of EBV-disease.
- This strategy may reduce overtreatment whilst not adversely affecting outcomes.

Abstract

Background:

Epstein-Barr virus (EBV) load monitoring post-allogeneic haematopoietic stem cell transplantation (HSCT) enables earlier detection of EBV replication and is often used as a trigger for pre-emptive therapies aiming to reduce EBV-related diseases. Our institutional strategy is to treat patients with clinical signs of EBV-related disease accompanied by a rising viral load, rather than to intervene solely based on viral load. This affords an opportunity to study the natural history of EBV replication and to assess if our strategy reduces over-treatment without compromising outcomes.

Objective:

Our objective was to assess the natural history of untreated EBV replication in patients who have received an alemtuzumab-based allogeneic haematopoietic stem cell transplant and to examine whether our clinical strategy reduced over-treatment without compromising patient outcomes.

Study Design:

We present a retrospective, single-centre, observational study of 515 consecutive patients (≥18 years) undergoing T-cell depleted allogeneic haematopoietic stem cell transplantation incorporating alemtuzumab. Patients underwent surveillance monitoring for EBV by qPCR in the peripheral blood at least weekly up to 100 days post-transplant and longer if they remained on immunosuppressive therapy. Cumulative incidence of EBV detection and EBV-related disease were assessed.

Results:

192 patients had EBV DNA detectable on ≥1 occasion, with a cumulative incidence of 35.8% (31.8-40.4%), although this remained below the limit of quantification in 93 patients. Median time to first detection was 89.5 days (0-2254 days). The incidence was higher in sibling donor transplants (45.4% vs 30%, P= 0.00021) when compared to unrelated donor transplants. 20 patients developed EBV-related disease (cumulative incidence 3.9%). Two had immunosuppression reduction alone, 18 received rituximab, and 5 required additional therapies. Five patients died due to PTLD and all five had received rituximab. The positive predictive value of EBV load for disease was higher in the unrelated donor cohort but remained <75% regardless of EBV threshold (57.1-72.7%).

Conclusions:

The cumulative incidence of EBV-related disease in our study (3.9%) was comparable to other studies incorporating alemtuzumab and our clinical strategy reduced over-treatment in this patient population. There are limitations of PCR-based surveillance strategies as reflected in the relatively low sensitivity of the assay coupled with the low positive predictive value which may influence the potential choice of threshold for pre-emptive intervention. We conclude that it remains unclear whether treatment based on rising EBV viral load alone gives superior overall results to treatment based on the development of clinical signs of EBV-related disease in the context of a rising viral load.

Natural history of EBV replication and viral load dynamics after alemtuzumab based allogeneic stem cell transplantation

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Introduction

Epstein-Barr virus (EBV) is a highly prevalent member of the human gamma-herpesvirus family, infecting up to 90% of individuals by adulthood. The primary infection may manifest clinically as infectious mononucleosis but may also be asymptomatic. Following infection the virus enters a phase of latency, kept quiescent by the host immune system[1] with the major viral reservoir residing within B lymphocytes and the oropharyngeal lymphoid tissues. The immunocompromised state following allogeneic haematopoietic stem cell transplantation (HSCT) predisposes to 'reactivation' from this latent phase[2], which can cause a rapid EBV-driven B cell proliferation resulting in post-transplant lymphoproliferative disorders (PTLD) or EBV-related disease such as encephalitis, pneumonia and hepatitis.

PTLDs are defined as lymphoid or plasmacytic proliferations developing as a consequence of immunosuppression in allogeneic transplant recipients, and are usually EBV-driven[3]. They are subcategorised into probable or biopsy proven[4]. Their incidence post-allogeneic HSCT is 0.5-17%[5], the frequency varying according to diagnostic criteria and presence of risk factors, including HLA-mismatch, anti-thymocyte globulin (ATG) usage, graft-versus-host disease (GvHD), age of ≥50 years and T-cell depletion of the donor graft[6,7]. Presentations include pyrexia, lymphadenopathy, or extranodal involvement. Current treatment strategies are directed at achieving B cell depletion and restoring the EBV-specific T-cell responses, often by a combination of rituximab and reduced immune suppression. A poor response to rituximab has been associated with age, involvement of extranodal tissue, acute GvHD

and lack of reduction of immunosuppression therapy upon PTLD diagnosis[8]. In rituximab-refractory patients, therapeutic options include systemic chemotherapy, EBV-specific cytotoxic T-cell therapy (CTL) or, in allogeneic HSCT recipients, donor lymphocyte infusions (DLI)[9,10].

The introduction of EBV DNA load monitoring by quantitative real-time polymerase chain reaction (qPCR) has enabled earlier detection of EBV reactivation, although the enhanced sensitivity of new assays compared to precedents impacts both on reported incidence and potentially also the apparent efficacy of clinical interventions. Intervention at low viral loads may result in treatment of patients whose immune systems would have responded sufficiently to constrain viral replication without treatment. Although the toxicity profile associated with rituximab use is generally modest, it can cause late-onset, prolonged immune-mediated neutropenia, acute infusion reactions, B-cell depletion and an increased risk of infections[11]. A number of studies have investigated pre-emptive treatment strategies based on qPCR, though optimal thresholds for intervention remain unclear[12-17] and evaluation is confounded by differences in PCR measurements between institutions[4]. Recently, the World Health Organization (WHO) International Standard for EBV was developed based on the results of a worldwide collaborative study group, and was released for the standardization of qPCR[18]. With this standard, comparisons across institutions will become easier.

Our institutional strategy is to treat patients with clinical signs of PTLD/EBV-related disease accompanied by a rising EBV viral load, rather than to intervene solely based on a specific pre-determined viral load. This affords an opportunity to assess the natural history of asymptomatic EBV replication, to evaluate the performance characteristics of the assay, and to examine if this approach reduces over-treatment without compromising patient outcomes. We report the characteristics of 515 consecutive recipients of allogeneic HSCT incorporating alemtuzumab.

Methods

This was a retrospective, single centre, observational study of consecutive adult patients (≥18 years) treated with T-cell depleted allogeneic HSCT at University College London Hospital (UCLH) between January 2006 and February 2017. Patients received either one of two myeloablative regimens or one of two reduced intensity regimens, incorporating differing dose schedules of alemtuzumab (Table 1). Choice of myeloablative regimen depended on donor source (Cy/TBI for sibling donor and Flu/Cy/TBI for unrelated donor transplants). Choice of reduced intensity regimen depended on underlying disease diagnosis and history of prior autologous transplantation (BEAM-alemtuzumab was used for a subset of transplant-naïve patients with Hodgkin Lymphoma, Diffuse Large B Cell Lymphoma or Mantle Cell Lymphoma). Ciclosporin (CSA) was used as GvHD prophylaxis in all regimens from Day -1, tapered from 2 months post-transplant if there was no evidence of GvHD. All patients received anti-viral prophylaxis with aciclovir (200mg orally bd post-engraftment).

Patients underwent surveillance monitoring for EBV in peripheral blood by qPCR at least weekly up to 100 days post-transplant and longer if they remained on immunosuppressive therapy. EBV surveillance was restarted if they recommenced immunosuppressant therapy. PCR was performed on plasma using the Artus® EBV RG PCR Kit (Qiagen) which targets the *EBNA1* gene. The viral load was quantified if it was equal to or over 200 copies/ml, or was referred to as "below the limit of quantification" (BLQ) if it was detectable but <200 copies/ml. The conversion factor to the WHO standard is 1 copy = 0.17 IU or 1 IU = 5.88 copies ie 200 copies/ml = 34 IU/ml. Patients with detectable EBV DNA were not treated pre-emptively, and only received treatment if they had clinical features consistent with EBV disease. In these cases, a biopsy was performed wherever feasible and imaging (either CT or PET/CT) was performed for staging and treatment response assessment. In the first instance, immunosuppression was reduced and rituximab (375mg/m^2) given once per week for 4 weeks. Other therapies were considered in unresponsive cases.

Statistical analyses were performed using the NCSS statistical software programme (version 12). Cumulative incidence was calculated by time-to-event analysis. Competing risks were time-to-relapse without EBV reactivation (defined as the time from the date of transplant to the date of relapse without EBV DNA viraemia detected) and time-to-death without EBV reactivation (defined as the time from the date of transplant to the date of death without EBV DNA viraemia detected). Differences in cumulative incidences were assessed using Gray's test. A P value <0.05 was considered statistically significant. Multivariate analysis was performed using Cox regression. EBV PCR assay performance was expressed as sensitivity, specificity, positive and negative predictive value, positive and negative likelihood value and diagnostic odds ratio using the calculator at http://statpages.org/ctab2x2.html. The predictive values related to the development of EBV-related disease.

Results

Patient Characteristics

Patient characteristics are shown in Table 2. Median age was 48 years (18-70 years). The majority were treated with reduced intensity conditioning (414/515; 80.4%), FM-Alemtuzumab being the commonest (336/515; 65.2%). The median follow-up was 820 days (range: 21-4249 days).

EBV DNA detection

EBV DNA was detected in 192/515 patients on at least one occasion, with a cumulative incidence of 35.8% (31.8-40.4%) (Figure 1A). In 93 of these the viral load remained BLQ (200 copies/ml = 34 IU/ml). The median time to first detection was 89.5 days post-transplant (range 0-2254 days) and the median viral load at first detection was BLQ (BLQ – 260000 copies/ml). The median time to the maximum viral load in individual patients was 128.5 days (range 0-2254 days), and the median maximum viral load was 240 copies/ml (range: BLQ-4300000 copies/ml).

The cumulative incidence of a quantifiable PCR was 18.6% (15.5-22.3%) (Figure 1B). In this cohort, the median first positive viral load was also BLQ (BLQ-260000 copies/ml), detected at a median of 81 days (range: 0-1048 days) post-transplant. The median maximum viral load was 3100 copies/ml (range: 200-430000

0

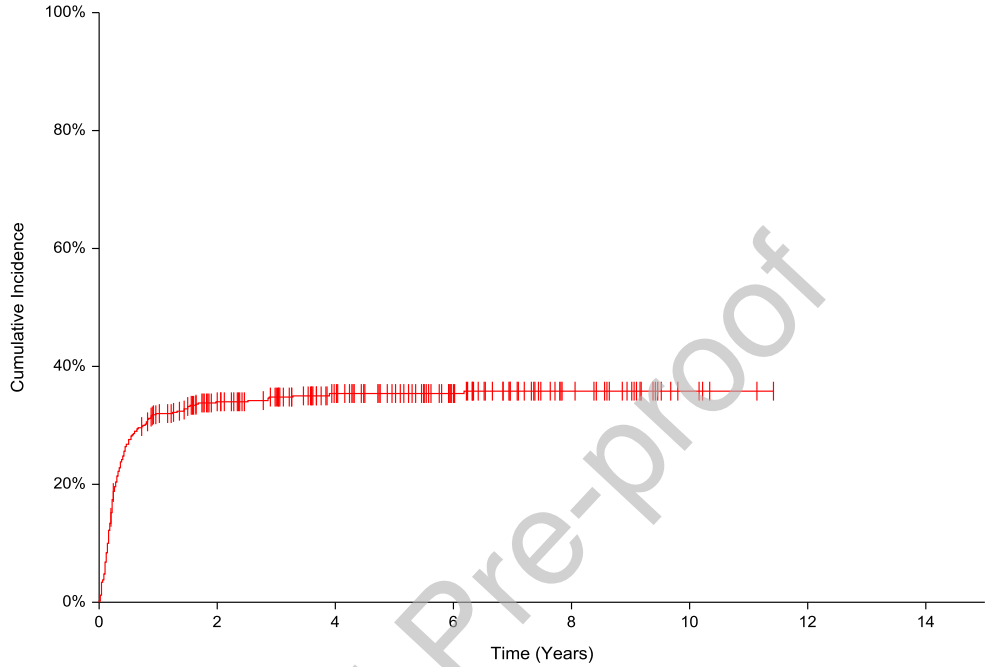
1A

copies/

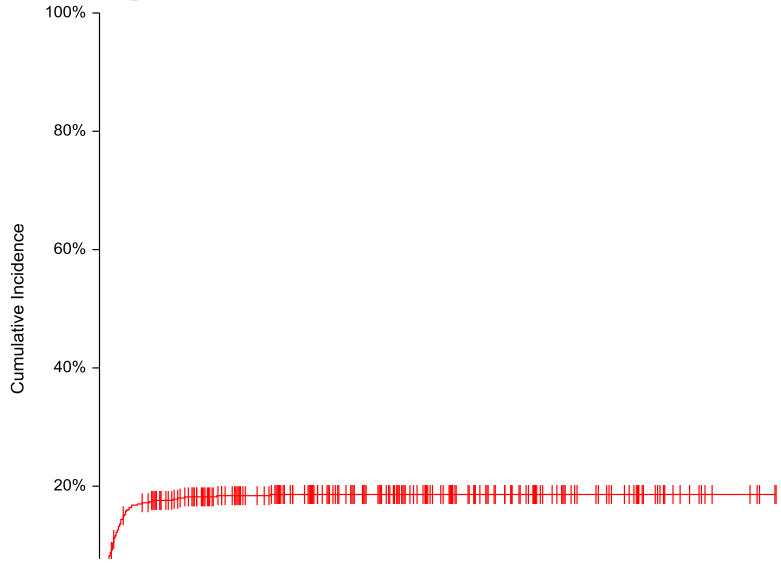
ml),

which

was



detected at a median of 123 days (0 -1930 days) post-transplant.



1B

8

Figure 1: Cumulative incidence of EBV detection in blood for the 515 patients. *Figure 1A*: Cumulative incidence of EBV detection of any EBV level: 35.8% (31.8-40.4%). *Figure 1B*: Cumulative incidence of quantifiable EBV detection: 18.6% (15.5-22.3%).

Factors associated with EBV DNA detection

There was no significant difference in the cumulative incidence of EBV detection in patients who received a reduced intensity versus a myeloablative conditioning regimen (data not shown). The cumulative incidence of EBV detection either of any level (Figure 2A) or of a quantifiable level (Figure 2B) was significantly higher in patients who received a sibling- compared to an unrelated-donor allograft (45.4% vs 30.0% respectively, P = 0.00021 for any positive; 27.9% vs 13.0%, P = 0.00002 for EBV>200 copies/ml), with similar values when analyses were restricted to reduced intensity transplants (Figure 2C and 2D).

One potential difference between sibling and unrelated donor cohorts is the dose of alemtuzumab. The optimal dose schedule remains unclear, and we have performed both dose de-escalation studies and inter-institute comparisons to address this issue[19,20] resulting in an overall reduction in dose delivered in the reduced intensity transplant cohort over the study period. Figure 3 shows the cumulative incidence of EBV DNA detection of any positive viral load (Figure 3A) and of a quantifiable viral load (Figure 3B) within the cohort receiving reduced intensity conditioning, analysed according to alemtuzumab dose. Because some dosing cohorts were small, we performed these analyses using 3 groupings: 20-40mg, 50-60mg and 100mg. Alemtuzumab dose conveyed significant differences both for any level of EBV DNA detection (P = 0.0023) and for quantifiable viral loads (P = 0.0013) in univariate analyses. Interestingly, higher doses were associated with lower cumulative incidences. Thus, for quantifiable viral loads, the cumulative incidences for 20-40mg, 50-60mg and 100mg doses were 29.9% (95% CI: 22.6-39.5%), 19.7% (95% CI: 12.3-31.5%) and 13.5% (95% CI: 9.7-18.9%) respectively (Figure 3B). Alemtuzumab dose and donor type are, however, correlated variables, since the lower 20-40mg doses were used only in the sibling setting and the majority of the unrelated donor transplant recipients received 100mg (n=213). When the impact alemtuzumab dose on the cumulative incidence of quantifiable EBV load was analysed in the sibling and unrelated donor cohorts independently it failed to reach statistical significance (P = 0.8025 and P = 0.78717 respectively). Likewise, when donor source was used as the grouping variable and analyses performed in the 50-60mg dose and 100mg dose cohorts independently the donor type was not

EBV viral load (copies/ml)		Sibling Donor						
		PPV (%)	NPV (%)	Sensitivity (%)	Specificity (%)	Positive Likelihood Value (+LR)	Negative Likelihood Value (- LR)	Diagnostic Odds Ratio (DOR)
>60000		28.6 (5.3 ±63.5)	97.3 (96.5 ±98.6)	28.6 (5.3 ±63.5)	97.3 (96.5-98.6)	10.7 (1.5 ±46.8)	0.7 (0.4 ±1.0)	14.6 (1.5 ±126.5)
>50000		37.5 (10.9 ±66.5)	97.9 (96.7 ±99.1)	42.9 (12.5 ±76.0)	97.3 (96.2 ±98.6)	16.1 (3.3 ±53.3)	0.6 (0.2 ±0.9)	27.4 (3.6 ±218.7)
>40000		37.5 (10.9 ±66.5)	97.9 (96.7 ±99.1)	42.9 (12.5 ±76.0)	97.3 (96.2 ±98.6)	16.1 (3.3 ±53.3)	0.6 (0.2 ±0.9)	27.4 (3.6 ±218.7)
>30000		30.0 (8.6 ±54.2)	97.8 (96.7 ±99.1)	42.9 (12.3 ±77.4)	96.3 (95.1 ±97.6)	11.5 (2.5 ±31.8)	0.6 (0.2 ±0.9)	19.4 (2.8 ±137.5)
>20000		25.0 (9.2 ±38.4)	98.3 (96.9 ±99.5)	57.1 (21.0 ±87.7)	93.6 (92.3 ±94.8)	9.0 (2.7 ±16.7)	0.5 (0.1 ±0.9)	19.6 (3.2 ±128.7)
>10000		31.6 (16.1 ±36.6)	99.4 (97.8 ±100)	85.7 (43.8 ±99.2)	93.1 (91.5 ±93.6)	12.4 (5.2 ±15.5)	0.2 (0.0 ±0.6)	80.8 (8.4 ±1923.8)

EBV viral load (copies/ml)		Unrelated Donor						
		PPV (%)	NPV (%)	Sensitivity (%)	Specificity (%)	Positive Likelihood Value (+LR)	Negative Likelihood Value (- LR)	Diagnostic Odds Ratio (DOR)
>60000		57.1 (21.2 ±87.5)	97.1 (96.3 ±97.8)	30.8 (11.4 ±47.1)	99.0 (98.2 ±99.7)	31.5 (6.4 ±165.3)	0.7 (0.5 ±0.9)	45.0 (7.0 ±311.7)
>50000		66.7 (33.1 ±90.2)	97.7 (96.8 ±98.4)	46.2 (22.9 ±62.5)	99.0 (98.0 ±99.7)	47.2 (11.7 ±217.6)	0.5 (0.4 ±0.8)	86.9 (14.9 ±577.8)
>40000		66.7 (33.1 ±90.2)	97.7 (96.8 ±98.4)	46.2 (22.9 ±62.5)	99.0 (98.0 ±99.7)	47.2 (11.7 ±217.6)	0.5 (0.4 ±0.8)	86.9 (14.9 ±577.8)
>30000		72.7 (42.9 ±91.7)	98.4 (97.3 ±99.1)	61.5 (36.3 ±77.6)	99.0 (98.0 ±99.7)	63.0 (17.7 ±259.4)	0.4 (0.2 ±0.7)	162.1 (27.2 ±1152.2)
>20000		71.4 (46.9 ±86.0)	99.0 (97.9 ±99.7)	76.9 (50.5 ±92.6)	98.7 (97.6 ±99.4)	59.0 (20.8 ±144.6)	0.2 (0.1 ±0.5)	252.5 (41.1 ±1934.9)
>10000		66.7 (43.4 ±80.6)	99.0 (97.9 ±99.7)	76.9 (50.1 ±93.0)	98.4 (97.2 ±99.1)	47.2 (18.1 ±98.0)	0.2 (0.1 ±0.5)	201.3 (35.2 ±1382. 4)

Table 4. The positive predictive value (PPV), negative predictive value (NPV), sensitivity, specificity, positive (+LR) and negative (-LR) likelihood value and diagnostic odds ratio (DOR) of EBV viral load values of predicting EBV-related disease in sibling and unrelated donors. 95% Confidence Intervals are shown in parentheses.

Supplemental data: abbreviated clinical history details of the 5 patients with PTLD-related deaths

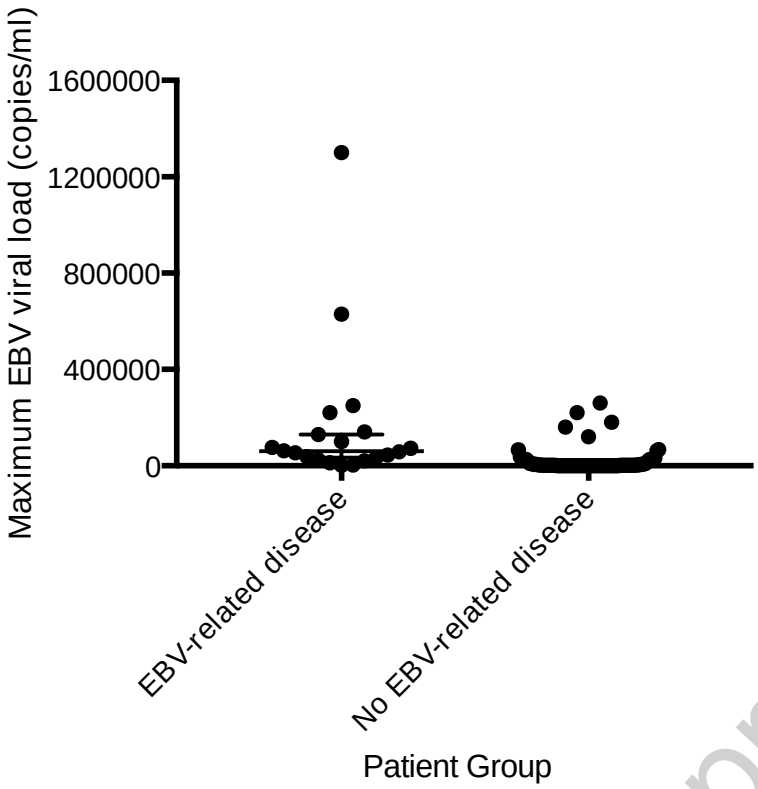
Case 2 presented with diarrhoea, rash, lymphadenopathy, confusion, limb weakness, fever and jaundice. He was treated for GvHD with steroids. He subsequently developed multi-organ failure. A post-mortem confirmed PTLD as a cause of death. He received rituximab 2 days following a load of 77,000 copies/ml (9 days following a viral load of 15,000 copies/ml) but died the following day.

Case 3 presented with fever and diarrhoea with a rising EBV load. His immunosuppression was reduced, and he received rituximab 2 days following a viral load of 40000 copies/ml. He subsequently developed multi-organ failure and died. A post-mortem confirmed PTLD as a cause of death.

Case 4 presented with fevers and a respiratory tract infection with rising EBV load. Immunosuppression was reduced, and he received rituximab 5 days following a viral load of 68,000 copies/ml. His clinical course was complicated by a large gastrointestinal bleed, infective complications and hypoadrenalism. A post-mortem confirmed PTLD as a contributory cause of death.

Case 5 presented with abdominal pain and diarrhoea. A PET scan showed bowel disease; a biopsy confirmed PTLD. He was given rituximab and steroids but developed a bowel perforation and multiple gastrointestinal bleeds. His EBV load did not rise higher than 2300 copies/ml and he received rituximab 27 days before his death.

Case 13 presented with a dry cough and right-sided cervical lymphadenopathy in the context of a rising EBV viral load. She had recently been diagnosed with pulmonary graft-versus-host disease and was taking a weaning dose of prednisolone at the time of her PTLD diagnosis. A PET/CT scan confirmed lymphadenopathy above and below the diaphragm. She had refractory PTLD and received multiple lines of therapy. Notably, the illness was particularly protracted with the time from first rituximab to death being 438 days.



Supplemental Figure S1: The maximum EBV viral load. The median maximum EBV viral load measured for the patients with EBV-related disease (60000 copies/ml [2300 – 1300000]) was significantly higher than for the patients who did not develop EBV-related disease (1700 copies/ml [200 – 260000]) (P<0.0001).