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ORIGINAL ARTICLE

Characteristics of Epstein–Barr viraemia in adult liver transplant patients: A retrospective cohort study

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Summary

Therapeutic immunosuppression following solid organ transplantation increases the risk of Epstein–Barr (EBV) viraemia, which is implicated in post-transplant lymphoproliferative disease (PTLD). We retrospectively analysed the incidence of EBV viraemia and clinical outcomes in 98 liver transplant recipients. Patients underwent EBV DNA monitoring by whole-blood PCR: EBV levels were correlated with clinical parameters and outcomes for a median of 249 days. 67% patients developed EBV viraemia (EBV DNA ≥100 copies/ml) and 30% had sustained viraemia. There was a trend towards higher hazard ratios for viraemia with exposure to aciclovir (HR 1.57, $P = 0.12$) or in recipients of a poorly HLA-matched graft (HR 1.62, $P = 0.10$). These associations became significant in the subgroup with >90 days surveillance; HR 2.54 ($P = 0.0015$) for aciclovir and HR 1.99 ($P = 0.03$) for poorly matched grafts. The converse was true with ganciclovir (HR 0.56 $P = 0.13$). Viraemia was more prolonged in men (median duration 7 days vs 1; $P = 0.01$) and in those with lower UKELD scores (11 days vs 1 day; $P = 0.001$) but shortened with ganciclovir exposure ($P = 0.06$). Younger patients were more likely to have high peak viral loads ($P = 0.07$). No clinical signs or symptoms or adverse outcomes were associated with EBV reactivation.

Introduction

With the availability of immunosuppressive (IS) drugs, the overall survival rates following solid organ transplantation have improved, resulting in an increasing number of immunocompromised patients. A resultant problem is infection with, or reactivation of, persistent viruses. EBV is an oncogenic herpesvirus that infects and activates B lymphocytes leading to their proliferation. Following primary infection, usually in childhood, EBV establishes a life-long latent persistence in B cells [1]. The virus intermittently reactivates in seropositive individuals, but the infection is kept at a subclinical level by EBV-specific memory T cells [2]. Immunosuppression following transplantation suppresses memory T-cell function, allowing EBV to drive B-cell proliferation, and in up to 10% of transplant patients, this may lead to PTLD [3]. PTLD in liver transplant recipients occurs in up to 2.4% of adult and 15% of paediatric patients [4,5]. The incidence of PTLD is highest during the first year post-transplant, although can develop at any stage. High cumulative doses of immunosuppressive drugs and primary EBV infection or reactivation [6,7] are risk factors for PTLD. The first line of treatment is reduction in immunosuppression, which carries the risk of graft rejection and loss. In addition, chemotherapy, radiotherapy, rituximab and EBV-specific CTL immunotherapy have been used [8–11]. Despite treatment, relapses are common and the mortality remains high [12]. It is important to identify patients at risk of developing EBV-associated PTLD and intervene early.

Quantitative polymerase chain reaction (qPCR) assays have been used to retrospectively quantify EBV DNA in the

blood of patients with PTLD. EBV DNA levels are high in the majority of patients with PTLD; however, there are PTLD patients with low or undetectable EBV DNA [11,13]. Conversely, transplant recipients without PTLD can have EBV DNA reaching levels seen in PTLD [14]. High EBV DNA levels are not predictive of PTLD development in children receiving cardio-thoracic transplants [15], whereas another study demonstrated that stem cell transplant recipients with PTLD had higher EBV levels compared with the non-PTLD group [16]. A single PCR test is not helpful, and several centres include PCR on sequential blood samples in their surveillance protocols. Persistence of high levels of EBV DNA over 6 months is associated with an increased risk of developing PTLD in paediatric heart transplant patients [13]. In patients with rising or high viral loads, pre-emptive reduction in immunosuppression reduces PTLD incidence compared with historic controls [17]. There is a theme that EBV surveillance with pre-emptive intervention is beneficial in the prevention of PTLD. We investigated the incidence of, and risk factors for, development of EBV viraemia in adult liver transplant (LT) patients at the Royal Free Hospital, London, where EBV DNA is routinely tested in serial blood samples post-transplantation.

Patients and methods

Patients and clinical features

Ninety-eight LT patients were included. Seven patients were regrafted within 35 days of the first transplant and were each analysed as a single case from the second transplant. All patients were enrolled in a surveillance programme with fortnightly EBV DNA testing during the first 90 days. The duration of testing was extended in viraemic patients or at clinicians' request, which occurred for various reasons including individual clinician's routine practice, infections with other opportunistic or immunosuppression-related agents, unexplained graft dysfunction and established high levels of EBV viraemia. Demographic and clinical parameters were recorded from the transplant registry database, clinic letters and inpatient flow charts. IS medication was recorded for the first 7 days from transplant and then at days 7, 30, 60 and 90. Graft rejection was identified by the presence of a liver biopsy showing acute cellular rejection or by administration of antirejection therapy. Rejection episodes were considered separate if they occurred 5 days apart. Comparative statistical analysis was performed for number of treated rejection episodes only. All patients with cytomegalovirus (CMV) DNA of ≥3000 copies/ml received ganciclovir or valganciclovir until 2 negative CMV PCR results were obtained. There was no policy for routine changes in IS medications following detection of EBV viraemia of any level.

Human Leucocyte Antigen (HLA) matching

Data on HLA A, B and DR loci for both donor and recipient were collated, and the HLA mismatch was scored as grade 1: most favourable, no HLA mismatch at HLA A/B/DR (000), grade 2: 0–2 mismatches at A, 0 or 1 mismatch at B and no DR mismatch (100, 010, 110, 200, 210), grade 3: no mismatch at DR with up to 2 mismatches at A and B or 1 mismatch at DR with up to 2 mismatches at A and 1 at B (020, 120, 220, 001, 101, 201, 011, 111, 211) or grade 4: least favourable, 2 DR mismatches with any level of mismatch at A and B, or 1 DR mismatch with two mismatches at B (021, 121, 221, 002, 102, 202, 012, 112, 212, 022, 122, 222) [18].

Viral serology

The presence of anti-EBV viral capsid antigen IgG was determined using a commercially available enzyme immunoassay (DiaSorin, Wokingham, Berkshire, UK) on all recipients' sera as pretransplantation work-up and on donor sera available at transplantation.

Quantification of EBV DNA

EBV DNA levels were assayed by real-time quantitative PCR amplification of the EBNA 1 gene from whole blood using an ABI Prism 7000 system (Taqman®, Applied Biosystem, Worrington, UK) using a published method [19]. The lower limit of detection of the assay was 100 copies/ml of blood.

Classification of EBV viraemia group

Based on PCR results, patients were classified as having no viraemia or viraemia (EBV DNA ≥100 copies/ml in any sample). Sustained viraemia was defined as the presence of two consecutive samples positive for EBV DNA. For analysis of peak viral load, patients were divided into groups with low peak viral load (≤10 000 copies/ml) and high peak viral load (≥10 000 copies/ml). Duration of EBV viraemia was taken as the number of days from the first positive EBV PCR result to the last positive result with no intervening negative results.

Statistical analyses

Univariate analysis of the associations between donor, recipient and graft variables and the development of EBV viraemia, the peak viral load, duration of viraemia and duration of sustained viraemia was performed including all patients, irrespective of the duration of surveillance using standard survival methods where necessary to account for different follow-up times. Duration of viraemia, grouped

per variable, is expressed as a median with interquartile ranges. Time to development of EBV viraemia was assessed using standard survival methods. Individuals were followed from the date of transplantation until the time of first detection of EBV viraemia ≥100 copies/ml, or the date of the last EBV viral load measurement. Kaplan–Meier curves were plotted describing both the time to first viraemia for all patients and when subdivided on the basis of HLA matching group (group 4 compared with all others), and hazard ratios were calculated using a Cox proportional hazards regression model. Only univariate hazard ratios were calculated, due to the small number of EBV viraemia events. Subgroup analysis including only patients who had EBV PCR testing for more than 90 days following LT to determine associations between clinical parameters and risk of EBV viraemia in this subgroup was also performed. Analyses were performed using SAS version 9.2 (SAS Institute Inc, Cary, NC, USA).

Results

Study population

Ninety-eight adult patients who consecutively underwent LT at the Royal Free Hospital, London, between August 2007 and January 2009 were included. Sixty-six (67%) were men and the median age at transplant was 52 years (range 23–69). The demographic details are shown in Table 1. Ninety-seven (99%) of the 98 patients were EBV-seropositive (R+) pretransplantation and one (1%) was seronegative (R−). EBV serostatus was known for 76/98 (78%) donors; 70 (92%) were EBV-seropositive (D+) and six (8%) seronegative (D−). Of the 76 cases with known EBV serostatus for both donor and recipient, 69 (91%) were D+R+, six (8%) were D−R+, and one (1%) was D+R−. EBV surveillance testing was scheduled every 2 weeks post-transplantation for the first 90 days, with adjustment made at clinicians' request. The median number of EBV samples tested per patient within 90 days of transplant was seven (range 2–14) with 69% having at least six samples within 90 days of transplantation. The median total number of samples per patient was nine (mean 10, range 2–25), and the median duration of testing was 249 days (range 13–930) from LT. 83 patients (85%) had EBV PCR performed for more than 90 days following LT.

Transplant details and clinical course

Eighty-eight (90%) patients were alive at the end of data collection (July 2010) and 10 (10%) patients died (median time to death 329 days; range 32–958). Causes of death were varied, but no patients died of EBV-related disease.

All patients received IS medication in the first week. During days 1–7 post-transplantation, 92 (94%) patients were treated with corticosteroids, 97 (99%) with tacrolimus, 29 (30%) with mycophenolate mofetil (MMF), 44 (45%) with azathioprine and 20 (20%) with basiliximab

Table 1. Demographics and baseline clinical features of all patients and transplanted organs.

Characteristic	*n* (%)	Range
Total	98 (100)	
Age (median)		
Years	52	23–69
Gender		
Male	66 (67)	
Female	32 (33)	
Aetiology of liver disease		
Viral*	28 (29)	
Autoimmune group†	23 (23)	
Alcoholic liver disease	21 (21)	
Other‡	26 (27)	
Presence of HCC	20 (20)	
UKELD§§ score pretransplant (median)	53	0–71
MELD score pretransplant (median)§	15	6–55
HLA matching¶		
Level 1	3 (3)	
Level 2	1 (1)	
Level 3	21 (24)	
Level 4	62 (71)	
Donor cardiac Status‖		
Prior to death	87 (90)	
After death	10 (10)	
Cold ischaemic time** (median)		
Minutes	503	61–999
Recipient EBV serostatus		
Positive	97 (99)	
Negative	1 (1)	
Donor EBV status††		
Positive	70 (92)	
Negative	6 (8)	
Recipient CMV serostatus		
Positive	71 (72)	
Negative	27 (28)	
Donor CMV status‡‡		
Positive	39 (49)	
Negative	40 (51)	

*****Chronic hepatitis B and hepatitis C virus infections.
†Autoimmune hepatitis, primary sclerosing cholangitis, primary biliary sclerosis, overlap conditions.
‡Cryptogenic, (sub)acute seronegative liver failure, familial amyloid polyneuropathy, epithelioid haemangioendothelioma, paracetamol toxicity, nonalcoholic steatohepatitis/nonalcoholic fatty liver disease, Budd-Chiari.
§n = 95.
¶n = 87.
‖n = 97.
******n = 93.
††n = 76.
‡‡n = 79.
§§UKELD = [(5.395Xln(INR)) + (1.485Xln(creatinine)) + (3.13Xln(bilirubin)) − (81.565Xln(Na))] + 435 (21).

Table 2. Immunosuppressant treatment of recipients.

Medication	*n* (%)
Immunosuppressant drugs administered in D0-7	
Corticosteroid	92 (94)
Tacrolimus	97 (99)
Mycophenolate mofetil	29 (30)
Azathioprine	44 (45)
Basiliximab	20 (20)
Immunosuppressant drugs administered during D7-90	
Corticosteroid	89 (91)
Tacrolimus	98 (100)
Mycophenolate mofetil	46 (47)
Azathioprine	43 (44)
Ciclosporin	7 (7)

(usually on day 1 and 4). One additional patient received basiliximab more than 7 days after transplantation. In total, 45 (46%) patients received MMF, 16 of whom did not receive it in the first week post-transplant. Table 2 summarizes the immunosuppressive therapy of transplant recipients. Twelve (12%) patients received hepatitis B immunoglobulin in the first week.

Thirty-four patients (35%) had detectable CMV DNA in blood with 16 (47%) receiving pre-emptive treatment with ganciclovir or valganciclovir as per Royal Free Hospital protocol (described elsewhere [20]). Twenty-three (23%) patients received aciclovir or valaciclovir therapy, 15 for treatment of active herpes simplex disease and one for prophylaxis, and the reason for treatment was unclear in the remaining patients.

Forty-one patients (42%) had biopsy-proven acute cellular rejection of the transplanted liver, with 17, 12, 5, 5 and 2 patients having one, two, three, four and five rejection episodes, respectively. Twenty-six patients (27%) received treatment for acute cellular rejection, typically with 1 gram intravenous methylprednisolone daily for 3 days. Eighteen patients received treatment for a single episode, 4 patients for two episodes and 4 patients for three episodes.

EBV DNA in blood

Sixty-six (67%) patients had detectable EBV DNA (≥100 copies/ml blood) on at least one occasion and 29 (30%) had viraemia in two or more consecutive samples (sustained viraemia). Five patients had peak EBV DNA ≥10 000 copies/ml. The median peak viral load was 2111 copies/ml (range 142–906 518) in all cases and 3990 copies/ml (range 463–906 518) in sustained viraemia. Amongst those who developed viraemia the median time to first detection of EBV DNA was 14 days post-transplant (range 2–658). Forty-three (65%) of the 66 patients with viraemia developed viraemia within 4 weeks of transplant (Fig. 1a). The

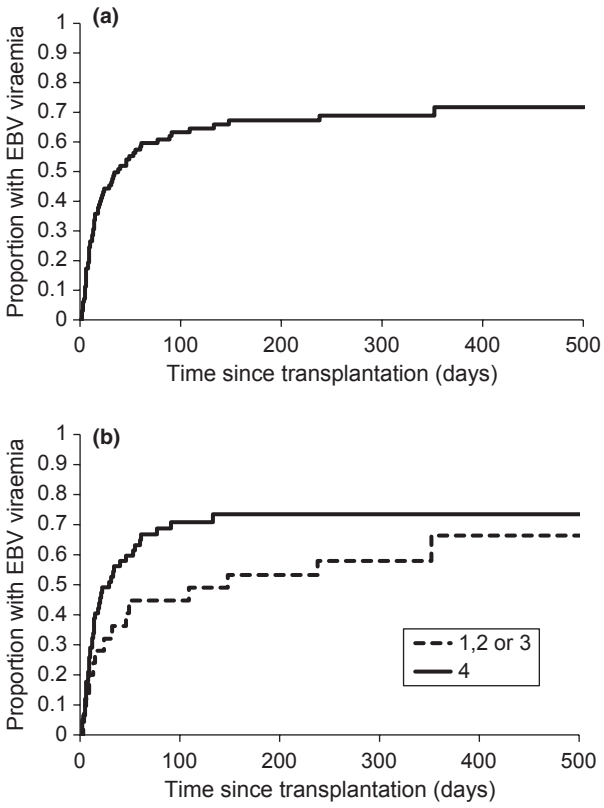

Figure 1 Kaplan–Meier curves demonstrating the development of first detectable EBV viraemia with time. (a) Time to developing first EBV viraemia in all patients. (b) Time to developing first EBV viraemia in patients with Grades 1, 2 and 3 HLA matching compared with the most poorly HLA-matched patients in Grade 4 ($P = 0.10$ log rank test).

median duration of EBV viraemia was 42 days (range 6–911) for patients with sustained viraemia. Of those who developed viraemia 56% had two or more episodes (Table 3). No differences were noted when only those patients with EBV PCR testing for >90 days following LT were included (data not shown).

Sixty-five of the 97 (67%) EBV-seropositive patients (R+) developed viraemia (EBV reactivation). There were no cases of PTLD or other EBV-mediated disease. The single EBV-seronegative patient received a liver from an EBV-seropositive donor and became viraemic 32 days later. They had three episodes of viraemia, lasting up to 467 days with a peak viral load of 906 518 copies/ml and a sustained viral load over 50 000 copies/ml for more than 431 days. The patient developed hepatitis, histologically consistent with EBV hepatitis on day 447.

Factors associated with EBV viraemia

Univariate analysis of factors associated with the development of EBV viraemia is shown in Table 4. Age, gender,

Table 3. Features of EBV viraemia occurring post-transplantation in all patients.

EBV outcome	*n* (%)	Range
EBV viraemia at any point	66 (67)	
Sustained viraemia		
≥2 consecutive positive measurements	29 (30)	
Time to first EBV		
Median (days)	14	2–658
Time to peak EBV titre		
Median (days)	45	3–930
Peak EBV titre (all cases)		
Median (copies/ml)	2111	142–906 518
Peak EBV titre (sustained viraemia)		
Median (copies/ml)	3990	463–906 518
Duration of longest EBV viraemia		
Median (days)	1	1–911
Duration of longest sustained EBV viraemia		
Median (days)	42	6–911
Number of viraemia episodes		
1	29 (44)	
2	20 (30)	
3	12 (18)	
4	3 (5)	
5	1 (1.5)	
6	1 (1.5)	

aetiology of liver disease, the presence of hepatocellular carcinoma (HCC), United Kingdom model for end-stage liver disease (UKELD) [21] score (divided around the mean), cardiac status of the donor, total number of IS agents, use of individual IS drugs or hepatitis B immunoglobulin in the first week did not significantly alter the hazard ratio (HR) for EBV viraemia. There was a trend towards increased HR of EBV viraemia in poorly HLA-matched cases (grade 4) compared with others (HR 1.62, $P = 0.10$) and with use of aciclovir (HR 1.57, $P = 0.12$). The inverse was seen with ganciclovir use (HR 0.56, $P = 0.13$). There was no correlation between CMV and EBV viraemia ($P = 0.66$). Poorly HLA-matched cases (grade 4 vs. grades 1–3) developed EBV viraemia earlier (Fig. 1b) ($P = 0.10$).

Univariate analysis including patients with >90 days of EBV PCR monitoring following LT did not reveal any novel associations. However, a significant increase in the HR of EBV viraemia was seen with aciclovir exposure (HR 2.54, 95% CI 1.43–4.52, $P = 0.0015$) and with poor HLA matching (HR 1.99, 95% CI 1.04–3.81, $P = 0.03$) in this subgroup.

Duration of viraemia and peak viral load

The median duration of viraemia was longer in men compared with women (7 days (IQR1,49) vs. 1 day (IQR1,1), $P = 0.01$) and in those with low compared with high pretransplant UKELD (11 days (IQR1,56) vs. 1 day (IQR1,7), $P = 0.001$) when analysing all episodes of viraemia. With sustained viraemia, the gender difference remained [49 days (IQR 14,84) vs. 11 (8, 239)], but the difference with UKELD did not [41 days (12, 86) vs. 42 days (9, 84)]. No association was demonstrated with age, gender, cause of liver disease, cardiac status of the donor, HLA matching, IS drug use (either individual drugs or any combination of 3 or more agents in the first week vs. 2 or fewer) or aciclovir use with duration of viraemia. Ganciclovir exposure was associated with a shortened median duration of viraemia vs. nonexposure [1 day (IQR1,1) vs. 1 day (IQR1,42) $P = 0.06$)].

Univariate analysis of factors associated with a peak EBV viral load ≥10 000 copies/ml demonstrated that younger patients were more likely to develop higher peak viral loads compared with older patients ($P = 0.07$). No other factors were associated with high peak viral load.

Discussion

Our data describe the natural history of EBV viraemia in an adult LT population. Sixty-seven per cent of our patients developed EBV viraemia post-transplantation, which is higher than other studies in adult LT (0–48% [22–24]) although similar to a recent study at 72% [25]. The frequency of EBV sampling in other cohorts was similar or more intense; but in some studies lower limits of EBV detection were higher than in ours. Our study and Schaffer *et al.* [25] used whole blood for EBV PCR, whereas others used plasma or serum. There is debate about whether to measure EBV DNA in whole blood, plasma or lymphocytes [26–28], but testing whole blood has greater sensitivity compared with plasma [25,26].

EBV viraemia is common following LT, usually due to reactivation of latent infection, occurs early post-transplantation and most often during the period of maximal immunosuppression, as shown previously [23–25]. We found no evidence of adverse clinical outcomes associated with EBV reactivation. Primary EBV infection was associated with a profound, sustained EBV viraemia and subsequent EBV hepatitis, suggesting that EBV-seronegative recipients are at risk of EBV-associated disease following transplantation.

Fewer than half of episodes of viraemia were sustained, although it may persist for years. Men were more likely to have more prolonged viraemia. Schaffer *et al.* [25] demonstrated higher peak EBV loads in men, although we did not replicate this finding. Patients with lower UKELD scores had more prolonged episodes of viraemia. Whether these differences reflect confounders such as age or cause of liver disease which were not independently identified due to small numbers is unclear. Schaffer *et al.* [25] demonstrated lower peak viral loads in younger patients, but our data show the converse. No cases of PTLD occurred in either of

Table 4. Univariate analysis of factors associated with the development of any EBV viraemia in all patients.

Variable (*n*)	EBV viraemia *n* (%)	No EBV viraemia *n* (%)	Hazard ratio	95% CI	*P* value
Age group					
≤40 years	9 (60)	6 (40)	0.50	0.21, 1.21	0.34
41–50 years	13 (59)	9 (41)	0.49	0.22, 1.09	
51–60 years	33 (72)	13 (28)	0.62	0.31, 1.23	
≥61 years	11 (73)	4 (27)	1.00	–	
Gender					
Male	47 (71)	19 (29)	1.27	0.74, 2.16	0.39
Female	19 (59)	13 (41)	1.00		
Aetiology of liver disease					
Autoimmune	17 (74)	6 (26)	1.44	0.83, 2.51	0.20
All others	49 (65)	26 (35)	1.00	–	
HCC					
Present	14 (70)	6 (30)	1.32	0.73, 2.39	0.36
Absent	52 (67)	26 (33)	1.00	–	
UKELD* score pretransplant					
>53	37 (71)	15 (29)	1.10	0.67, 1.79	0.71
≤53	29 (63)	17 (37)	1.00	–	
Donor cardiac status					
Prior to death	60 (69)	27 (31)	1.52	0.61, 3.80	0.37
After death	5 (50)	5 (50)	1.00	–	
Cold ischaemic time					
≥503 min	33 (70)	14 (30)	1.09	0.66, 1.79	0.74
<503 min	29 (63)	17 (37)	1.00	–	
Matching category					
4	44 (71)	18 (29)	1.62	0.90, 2.94	0.10
1, 2 or 3	15 (60)	10 (40)	1.00	–	
Number of IS drugs (days 1–7)					
3–5	44 (67)	22 (33)	0.94	0.56, 1.58	0.82
1–2	22 (69)	10 (31)	1.00	–	
Steroids (days 1–7)					
Yes	63 (68)	29 (32)	1.27	0.40, 4.06	0.68
No	3 (50)	3 (50)	1.00	–	
MMF (days 1–7)					
Yes	20 (69)	9 (31)	1.04	0.62, 1.77	0.88
No	46 (67)	23 (33)	1.00	–	
Azathioprine (days 1–7)					
Yes	30 (68)	14 (32)	1.07	0.66, 1.74	0.78
No	36 (67)	18 (33)	1.00	–	
Basiliximab (days 1–7)					
Yes	16 (80)	4 (20)	1.51	0.86, 2.65	0.16
No	50 (64)	28 (36)	1.00	–	
MMF ever used					
Yes	34 (76)	11 (24)	1.38	0.85, 2.23	0.20
No	32 (60)	21 (40)	1.00	–	
Basiliximab ever used					
Yes	16 (76)	5 (24)	1.33	0.76, 2.34	0.32
No	50 (65)	27 (35)	1.00	–	
Acyclovir ever used					
Yes	17 (74)	6 (26)	1.57	0.90, 2.74	0.12
No	49 (65)	26 (35)	1.00	–	
Ganciclovir ever used					
Yes	8 (50)	8 (50)	0.56	0.27, 1.19	0.13
No	58 (70)	24 (30)	1.00	–	

Table 4. continued

Variable (*n*)	EBV viraemia *n* (%)	No EBV viraemia *n* (%)	Hazard ratio	95% CI	*P* value
Use of HBIg (days 1–7)					
Yes	8 (67)	4 (33)	0.99	0.47, 2.08	0.98
No	58 (67)	28 (33)	1.00	–	

*UKELD = [(5.395Xln(INR)) + (1.485Xln(creatinine)) + (3.13Xln(bilirubin)) − (81.565Xln(Na))] + 435 (21).

these studies, but a previous large study of adult liver transplant recipients demonstrated increasing age, hepatitis C infection, alcohol-related cirrhosis and use of antithymocyte globulin or OKT3, but not gender as risk factors for PTLD [29]. The importance EBV viral load in predicting the evolution PTLD in adult LT recipients will require larger long-term studies.

We did not find an association with the number of IS agents administered, the use of mycophenolate mofetil (as has been shown previously in a renal transplant cohort [30]) nor basiliximab with EBV viraemia. However, HLA matching appears to be important and poorly HLA-matched organs were associated with a higher risk of viraemia which was more likely to occur early post-transplantation. Median trough tacrolimus levels were not different between these groups (data not shown). Without a measure of functional immunosuppression, we cannot demonstrate that this difference in viraemia is due to increased immunosuppression in the poorly matched cases. The source of EBV in secondary infection may be from recipient-derived or donor-derived B cells. If recipient B cell derived, donor HLA matching would be less likely to influence the development of EBV viraemia. Conversely poorly matched donor B cells would be more likely to escape recipient immune control, which may explain the increased viraemia, although evidence to confirm this is lacking. In primary EBV infection in lung transplant patients, closer HLA matching was associated with PTLD [31] although in mixed primary and secondary EBV infection no association has been shown [32].

EBV viraemia is of interest as a surrogate marker of the degree of immunosuppression [33] and if so should associate with CMV and HSV viraemia and reduced rejection, but we found no evidence of this. We demonstrated a possible increase in EBV viraemia in those exposed to aciclovir, and increasing EBV titres on aciclovir therapy have been demonstrated in liver transplant patients previously [24]. Aciclovir is a known inhibitor of EBV replication *in vitro* although it has limited effect clinically. Whether the effect seen was due to the action of the drug or confounded by greater immunosuppression and poor cellular immune responses allowing simultaneous HSV disease and EBV replication, or another mechanism remains unclear. There was a reduced risk and duration of EBV viraemia with ganciclovir exposure, again if this was due to the drug, the presence of CMV activation or changes in immune control is unclear. Some authors have suggested a role for ganciclovir in treating EBV disease [24,34,35] and EBV-encoded protein kinase is essential in phosphorylating the drug and the expression of this enzyme induces viral susceptibility to the drug [36].

Whilst this study captures a large volume of EBV monitoring data, the conclusions drawn are based upon retrospective post hoc analysis. EBV sampling was not even across all patients, which could introduce bias. There is a potential for late onset PTLD, the evolution of which may have been missed due to the relatively short follow-up period.

EBV reactivation was not associated with adverse clinical outcomes in this study. The EBV-seropositive status of the majority of recipients may confer protection against EBV-mediated diseases and PTLD even in the presence of viraemia. Longer-term evidence will be required to reassure clinicians that EBV viraemia is a benign process, particularly in those with high EBV titres. Therefore, it would be prudent to continue close monitoring, especially of those at high risk such as EBV-seronegative recipients, men and those with poorly HLA-matched grafts.

Authorship

NH: study concept. NH, DT and TH: study design. CA, JO'B, DP, AKB, DT and TH: provided data. NH and TH: data collection. NH, CS and TH: data analysis.

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