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Effect of Early Epstein-Barr Virus and/or Cytomegalovirus Viraemia on Graft Function and Acute Cellular Rejection in Paediatric Liver Transplantation

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Introduction

Cytomegalovirus (CMV) and Epstein-Barr virus (EBV) infections following paediatric liver transplantation have been associated with a variety of clinical syndromes (1). The spectrum of EBV infection ranges from asymptomatic primary infection, glandular fever, hepatitis and post-transplant lymphoproliferative disease which encompasses a range of disorders from mononucleosis-like syndrome to lymphoma (2). CMV infection may manifest as fever, bone marrow suppression and organ-invasive disease, which most often involves the gastrointestinal tract such as CMV gastritis, oesophagitis, enteritis and colitis, although the virus can disseminate widely (3). Clinically important EBV and CMV infections occur more frequently in patients who are susceptible pre-transplantation, especially babies or infants (1).

In the early post-transplant period, EBV and CMV infections are most likely transmitted via the graft or blood products (4,5). In paediatric transplantation settings, the increased use of living donors and split grafts has increased the age disparity between the donor and recipient making the possibility of an EBV and CMV antibody status mismatch between recipient and donor more common. Eighty percent of EBV and CMV IgG negative children who undergo liver transplantation develop virological evidence of EBV and CMV infection within three months post-transplant (6-12). This is a period where high levels of immunosuppression are maintained and as symptoms of EBV infection are related to the immune response, many children infected with EBV or CMV, especially those of young age, may be asymptomatic (9,13). Symptoms, when present, may be non-specific and difficult to discriminate during the early post-transplant period. Infections can be associated with an isolated flare in the aspartate aminotransaminase (13,14) and indistinguishable from acute allograft rejection (13,14). In these patients specific

virological surveillance for EBV and CMV infections is mandatory in order to make the diagnosis.

Few data are available on EBV and CMV infections in the early post-transplant period. The aims of the present study were to investigate the prevalence and timing of EBV and CMV infections during the first 21 days after transplantation in relation to graft function and acute cellular rejection in a large cohort of paediatric liver transplantation recipients treated in a single centre.

Materials and Methods

We reviewed retrospectively the clinical notes of 63 consecutive children who received 69 liver transplants at King's College Hospital, London, UK, a supraregional referral centre, from April 2007 to February 2008. Gender, age, clinical history, liver function tests, virological and liver histopathological data from the first 21 days after liver transplantation were recorded. Two children died in the immediate post-transplant period: one was excluded from the analysis, while the other, who had received four transplants, was excluded from the analysis only in regard of his final transplant.

Pre-transplantation EBV viral capsid antigen (VCA) IgG (Liaison EBV VCA IgG, Diasorin) and CMV IgG (ETI-CYTOK-G Plus, Diasorin) results for the recipients were recorded. Donor EBV serology data were incomplete and therefore not recorded, while donor CMV IgG data were available for all patients. Post-transplant quantitative EBV DNA and CMV DNA using in-house real time-polymerase chain reaction tests (RT-PCRs) were carried out weekly on whole blood in EDTA samples and whenever a graft dysfunction episode occurred according to the local transplant protocol.

The primary immunosuppressive agent was tacrolimus at a dose aimed at maintaining a trough level of 10 - 15 µg/litre. All patients received antibacterial and antifungal prophylaxis for a minimum of 5 days post-transplant, which was modified according to the culture results. CMV IgG negative or indeterminate recipients of a CMV IgG positive graft were given 2 weeks of intravenous ganciclovir (10 mg/kg/day) (15), starting from the 7^{th} day post-transplant.

Recipients were considered CMV and/or EBV uninfected if CMV IgG and CMV DNA and VCA IgG and EBV DNA results were negative (Table 1). Recipients were considered to have had previous CMV and/or EBV exposure when there was a positive CMV IgG result and CMV DNA was negative and/or a positive VCA IgG result and EBV DNA was

negative. Primary CMV or EBV infection was diagnosed when CMV DNA or EBV DNA became positive in patients previously negative for CMV IgG, or VCA IgG in peripheral blood samples. CMV and/or EBV reactivation or reinfection was diagnosed when CMV DNA or EBV DNA became positive in patients with previously positive CMV IgG or positive VCA IgG. VCA IgG positive and/or CMV IgG positive results in recipients of less than 18 months of age were considered of maternal origin and the EBV or CMV status was defined as indeterminate.

A diagnosis of acute rejection was based on graft dysfunction [elevation or flare of aspartate aminotransaminase (AST) and γ-glutamyl transferase (γ-GT), or alkaline phosphatase levels (ALP)] in association with characteristic histological features on liver biopsy, or, when a liver biopsy was contraindicated (abnormal coagulation parameters, platelet count less than 50,000/μL, ascites, sepsis) with a rapid response to high dose steroid therapy (pulsed methyl-prednisone 10 mg/kg), after exclusion of other possible causes of graft dysfunction, including vascular, biliary and infectious complications.

Liver function tests were monitored daily until normalisation according to the local post-transplant protocol. AST, γ-GT, ALP, and total bilirubin values were entered in the database. For statistical purposes, four different patterns were identified: "normal", "gradual reduction to normal value", "flare episode(s)", and "always abnormal, no flare episode(s)".

Data were processed with the SPSSX (SPSS 11.0) statistical package (SPSS Inc, Chicago, IL). Two tailed *p* values were used and *p* values < 0.05 were considered statistically significant. Differences in frequencies were evaluated by χ^2 test or Fisher's exact probabilities. Odds ratio (OR) and 95% confidence intervals (95% CI) were calculated. For continuous variables, the t-test, and the ANOVA tests were used with natural logarithmic

transformation of non-normal distributed variables. Results were expressed as mean levels and standard deviations (SD) or median and interquartile ranges as appropriate.

Results

Sixty-two patients (33 males, 53.2%, median age 25.7 months, interquartile range 211.5) underwent 67 transplants. All transplants were orthotopic, of which 2 (3%) were auxiliary cadaveric and 14 (20.9%) from living donors. The indications for liver transplantation were biliary atresia [32 (51.6%)], acute liver failure [11 (17.7%)], progressive intrahepatic cholestasis [6 (9.7%)], Crigler-Najjar syndrome type 1, cystic fibrosis, hyperoxaluria [2 (3.2%) each], Alagille syndrome, type IV glycogenosis, hepatoblastoma, carbamyl phosphate synthetase deficiency, sclerosing cholangitis, propionic acidemia, and Wilson disease [1 (1.6%) each].

EBV infection

Data regarding pre- and post-transplant EBV infection are summarised in table 2 and figures 1 and 2. As 18 months of age was the cut off taken at which passively acquired maternal antibody would not be detected, the pre-transplantation VCA IgG status was determinable in 48 transplant recipients.

Twenty-four (50%) patients VCA IgG negative pre-transplantation, and therefore susceptible to EBV infection, were significantly younger than those with evidence of previous infection (mean age at transplantation 48.2 months, SD 29.8 and 100.6 months, SD 63.9, respectively; $p = 0.01$). Twenty of sixty seven (29.9%) recipients developed an EBV viraemia within 21 days from transplantation. This was due to a primary infection in 3, reactivation or reinfection in 16, while one had indeterminate VCA IgG results pre-transplantation. EBV viraemia was more common in recipients who were VCA IgG positive (16/24, 66.7%) pre-transplant when compared to those who were VCA IgG negative (3/24, 35.8%; $p < 0.000$; OR 14; 95%CI 3.3-57.4) or indeterminate (1/19, 5.3%; $p < 0.000$; OR 35; 95%CI 5-242).

Transplantation recipients with a primary EBV infection were significantly younger than recipients who developed a reinfection or reactivation (mean age 16.5 months, SD 8.3; 90.5 months, SD 64.3; 121.4 months, SD 62.1 respectively; p = 0.008). Recipients with EBV viraemia were significantly younger than those without viraemia (mean age 50.8 months, SD 59.2 and 75.3 months, SD 65.1, respectively; p = 0.04). No significant difference was found in the interval between transplantation and the first time EBV DNA was detected between recipients with reinfection or reactivation and those with primary infection (mean 7.3 days, SD 3.8 and 7.7 days, SD 4, respectively). Recipients with primary EBV infection tended to have higher EBV DNA loads compared to recipients with reinfection or reactivation (mean 560,886 copies/ml, SD 629,159 and 176,664 copies/ml, SD 357,713, respectively; p = 0.15).

CMV infection

Data regarding pre- and post-transplant CMV infection are summarised in table 2 and figures 1 and 2. Having taken 18 months as the age at which passively acquired maternal antibody ceased to be detected, the pre-transplantation CMV IgG status was determinable in 47 transplant recipients, of whom 31 (65.9%) were CMV IgG negative pre-transplantation.

After transplantation, 13/67 (19.4%) recipients developed CMV viraemia. This was due to primary CMV infection in 3 of 31 (9.7%) recipients who were CMV IgG negative pre-transplantation, and to reinfection or reactivation in 5 of 16 (31.3%) who were CMV IgG positive pre-transplantation. The remaining 5 belonged to a group of 20 (25%) children who had indeterminate CMV IgG results before transplant. There was no association between CMV viraemia post-transplant and CMV IgG status pre-transplant. Age at transplantation was not correlated with pre-transplant CMV IgG status and with post-transplant CMV viraemia: the mean age of CMV IgG negative recipients was 75.6 months,

SD 62.4, and for CMV IgG positive recipients was 83.8 months, SD 66.9. The mean age of those with evidence of primary infection was 92.2 months, SD 46.5; of those with CMV reinfection/reactivation 48 months, SD 27.3; and of those with evidence of previous CMV exposure, but negative for CMV DNA, 100.1 months, SD 74.2. No significant difference in age was found between patients with or without CMV viraemia post-transplant (viraemic: mean age 44 months, SD 40.6; non viraemic: 61.6 months, SD 64.6). Timing of appearance of CMV viraemia post-transplant was similar in recipients with primary infection or reinfection/reactivation (mean 11.7 days, SD 8.1 and 13.8 days, SD 8.3, respectively). Transplantation recipients with a primary CMV infection had a higher CMV DNA load than those with reinfection or reactivation, but this was not statistically significant (mean 22,842 copies/ml, SD 38,572 and 7,473 copies/ml, SD 9,562, respectively; $p = 0.5$).

Liver Function Tests

Aspartate aminotransferase (AST), gamma glutamyl transpeptidse (γ-GT), alkaline phosphatase (ALP) and bilirubin results from the first 21 days after transplant are summarised in table 3. Abnormal AST, γ-GT, ALP, and bilirubin levels were significantly more frequent in recipients who underwent liver biopsies (31/67, 46.3%) than in those who did not (table 4). Recipients with an EBV viraemia had abnormal bilirubin levels more often than those who were EBV DNA negative [8/20 (4%) and 4/43 (9.3%), respectively; $p = 0.004$; OR 7.2: 95%CI 1.9-26.4]. No difference was found in the AST, γ-GT and ALP levels in relation to EBV viraemia and bilirubin, AST, γ-GT, and ALP levels in relation to CMV viraemia.

Rejection

In 22 of the 67 transplantation episodes (32.8%) the recipient experienced acute rejection, diagnosed histologically in 17 (77.3%) and on the basis of anti-rejection treatment

response in the other 5 (22.7%), in whom liver biopsy could not be performed. No significant relationship was found between rejection and gender [11/29 (37.9%) females compared with 11/38 (28.9%) males], age at transplantation (rejection: median age 20,2 months, interquartile range 61.7; no rejection: 36.1 months, interquartile range 86.7), VCA and CMV IgG before transplantation, and EBV and CMV viraemia after transplantation (table 5).

Patients with Combined EBV DNA and CMV DNA Detection

Five of 62 first-time liver transplant recipients (8.1%) developed both EBV and CMV viraemia in the first 21 days post-transplant. No significant difference was found between age at transplantation (median 64.7 months, SD 98.2 and 24.3 months, SD 75, respectively), or AST, γ-GT, ALP, and bilirubin levels between recipients with combined EBV and CMV viraemia and the other recipients. EBV and CMV viraemic recipients did not have a higher incidence of rejection when compared with the rest of the cohort [2/5 (40%) and 20/62 (32.3%), respectively].

Ganciclovir

Ganciclovir prophylaxis was given to all CMV IgG negative and indeterminate recipients whose donor was CMV IgG positive and ganciclovir treatment was given to one patient who developed CMV viraemia with abnormal liver function tests soon after transplant [total number of patients treated with ganciclovir 26 (40%)]. There was no difference between recipients who had (5/26) or had not (8/41) received ganciclovir in respect to the time and detection of CMV viraemia (given ganciclovir: mean 9.2 days, SD 6.7; not given: mean 9.7 days, SD 8.4; $p = 0.9$). No significant difference in viral load at the time of first CMV DNA detection was observed between recipients who were, or were not given ganciclovir (median 456 copies/mL, interquartile range 33,989 and 1,545 copies/mL, interquartile range 21,940, respectively; $p = 0.5$).

Six of 26 recipients who had received ganciclovir developed EBV viraemia compared with 14 of 41 recipients who had not received ganciclovir, but the difference was not statistically significant (p = 0.4). Among recipients who were EBV VCA IgG positive pre-transplantation, no significant difference in EBV viraemia was seen between those receiving (5/7) and those not receiving (11/17) ganciclovir (p = 1). The time interval between first EBV DNA detection and transplantation was similar between children treated or not treated with ganciclovir (mean 7 days, SD 3.6 in ganciclovir treated; mean 7.1 days, SD 4.1 in untreated). However, the first EBV DNA value was higher in recipients not given ganciclovir when compared to those who were treated (median 42,041.5 copies/ml, interquartile range 490,164 and 655.5 copies/ml, interquartile range 256,656, respectively; p = 0.2). No significant difference was found between patients given and not given ganciclovir in regard to the time interval for CMV DNA and EBV DNA values to become undetectable (lower than 10 copies/mL) (p = 0.9 and 0.3, respectively, table 6).

Follow up

The median follow up for all transplant recipients was 28.1 months (interquartile range 7). Overall, 53 (79.1%) recipients developed EBV viraemia, of whom 20 (37.7%) developed it during the first 21 days post surgery, and 28 (41.8%) recipients developed CMV viraemia, of whom 13 (46.4%) within the first 21 days. EBV DNA was detected in the entire cohort at a median of 1.3 months, interquartile range 2.15 and CMV DNA at a median of 1 month, interquartile range 1.9. Among the 57 patients who were alive at the end of the follow up, EBV DNA and CMV DNA were persistently detected in 8/14 and 3/11 who developed viraemia in the first 21 days after transplantation and in 20/31 and 5/14 who developed viraemia later, respectively (EBV p = 0.6; CMV p = 0.7). Among patients with EBV viraemia, EBV DNA was detected persistently during the follow up in 14 of the 18 VCA

IgG negative and in 5 of the 15 VCA IgG positive patients ($p < 10^{-3}$; OR 52.5; 95%CI 6.2-393.1).

Five patients died, One was a 17-year-old girl who had received a first transplant 12 years previously for progressive intrahepatic cholestasis and required a second transplant for graft failure due to chronic rejection associated with poor adherence to immunosuppressive treatment. She had severe renal impairment before re-transplant, was EBV viraemic and died a few hours after surgery with multiorgan failure (patient excluded from the analysis, Figure 1). A second death was that of a 2-year-old boy who was first transplanted for cryptogenic fulminant hepatic failure. Forty-nine days after transplant he developed EBV viraemia (maximum load 91,142 copies/mL, day 58^{th}) and EBV-related lymphoprolipherative disease associated with progressive graft failure. Sixty-four days after transplant, he was retransplanted. EBV DNA load from explanted liver was 1,087,665 copies/ml. The patient, who was persistently EBV viraemic, lost the second graft after 62 days for severe rejection, the third graft after 64 days for rejection associated with vascular and biliary complications and died few hours after the fourth transplant (data from this last transplant were excluded from the analysis, Figure 1). A 13-year-old boy with progressive intrahepatic cholestasis died having been re-transplanted 14 days after the first transplant because of severe rejection. He developed EBV viraemia 7 days after the first transplant and CMV viraemia 8 days after the second. He experienced severe Steven-Johnsons syndrome and died in his local hospital with bacterial sepsis 15 months after the second transplant. The fourth death was a 15-year-old girl transplanted for sclerosing cholangitis who was not EBV or CMV viraemic and died 19 months after the transplant for sudden unexplained cardiac death. Finally, a 14-year-old-boy with human immunodeficiency virus (HIV) infection who had received a first transplant for acute liver failure probably related to HIV treatment, required a second transplant for primary graft failure 19 days later and

died 26 months after the second transplant for complications related to his underlying disease. He developed CMV viraemia 1 month and EBV viraemia 2 months after the second transplant. Overall, EBV viraemia was in 4 of the 5 children who died and CMV viraemia in 2.

During the follow up, 1 patient who had a primary EBV-infection 30 days after the transplant, developed post-transplant lymphoprolipherative disease 15 months after the transplant (maximum load 2,595,750 copies/mL). The immunosuppression was completely withdrawn till, 9 months later, signs of graft dysfunction appeared and the liver biopsy showed severe rejection. The immunosuppression was then restarted at low levels (tacrolimus of 3-5 µg/L) and EBV DNA values were monitored closely (load persistently < 400 copies/mL).

Of the 4 patients who received more than one liver transplant, 3 received 2 and 1 received 4 transplants; 2 recipients developed EBV viraemia and 1 CMV viraemia, all after the first graft. Overall, 6 recipients lost their graft, of whom 4 (66.7%) developed EBV viraemia. EBV viraemia, however, was also detected in 16/61 (26.2%) recipients with successful engraftement ($p = 0.06$). CMV viraemia was detected in 1/6 (16.7%) patients who lost their graft and in 12/61 (19.6%) who had a successful transplant ($p = 0.8$).

Discussion

This paper provides novel information on CMV and EBV infections within the first 21 days after paediatric liver transplantation and their relationship to early graft function and acute cellular rejection.

The clinical impact of EBV and CMV infections in patients receiving a transplant is dependent on the serological status of the recipient and on the level of immunosuppression. Since severe liver disease presents commonly in infancy, many children undergoing liver transplantation are susceptible to primary EBV and CMV infections (9,16-19). In the present paediatric cohort, 50% and 65% of the recipients older than 18 months were VCA IgG negative and CMV IgG negative at the time of transplantation, respectively. The number of children susceptible to EBV and CMV infections reported in previous studies is highly variable ranging from 50 to 90% for EBV and from 20 to 40% for CMV (9,16-19). No comparison can be made between the present and the previous results as patients of different ages were enrolled and different laboratory methods and samples used. However, in the present as in previous studies, children who were VCA IgG negative and CMV IgG negative before transplantation were younger than the rest of the cohort confirming that younger patients are at higher risk of primary infection (9,10,16-23).

One of the first aims of the present study was to determine the prevalence and timing of EBV and CMV infections in the first three weeks after liver transplantation. Overall, EBV viraemia was detected in 30% and CMV viraemia in 20% of the cohort at a mean interval from transplantation of seven days for EBV and nine days for CMV. It is difficult to compare these findings with those of other studies that were not designed to obtain information on the early post-transplantation period, had less intensive virological monitoring and longer follow up and were focused more on the relationship between EBV infection and post-transplant lymphoproliferative disease (6-12). The high prevalence and

the early detection of EBV and CMV viraemia found in the present study suggest that the intense virologic testing in the first 21 days after transplantation is an effective and advisable approach.

The primary EBV and CMV infections were demonstrated in 3 of the 67 recipients for both EBV and CMV (4.5% each). The value raises to 6% for EBV and to 12% for CMV if the viraemic children younger than 18 months with positive VCA IgG and CMV IgG are considered of having acquired passively the antibodies from the mother and are included in the group of EBV and CMV naïve children. In the patients with early primary infection transmission via the graft or blood products is the most likely route of infection (4-12). EBV reinfection or reactivation was seen in 66% of the VCA IgG positive transplantation recipients while CMV reinfection or reactivation in 30% of the CMV IgG positive transplantation recipients. Interestingly, the rates of reinfection/reactivation were higher than those reported in previous studies with longer follow up (9,16-19,24,25). It may be speculated that during the early post-transplant period, the risk of reactivation of latent viruses is increased by the immunosuppressive treatment as well as by stimuli activating the infections, characteristic of the early post-transplant period, such as inflammatory cytokines released because of ischemia/reperfusion injury (26).

Few data are available on EBV and CMV viraemia and liver function tests (9,14). No significant relationship was demonstrated in the present study between CMV viraemia and abnormalities of liver function tests and between EBV viraemia and γ-GT, ALP and, according to previous data (9), transaminase flare. A significant association between EBV viraemia and abnormal bilirubin was demonstrated but due to the small sample size of this study this result needs to be confirmed in larger cohorts.

CMV infection is known to increase the risk of acute rejection (27). On this basis it was of interest to evaluate whether CMV and/or EBV were potentially related to early rejection.

Overall, rejection's rate was similar in transplantation recipients with and without EBV and CMV viraemia suggesting the absence of any relationship between viraemia and acute rejection in the first 21 days after transplantation. Eight percent of the transplantation recipients in the cohort developed a simultaneous EBV and CMV viraemia but no association was found with either abnormalities of the liver function tests or graft rejection. Ganciclovir prophylaxis due to a CMV IgG mismatch between donor and recipient has been demonstrated to be highly effective in preventing CMV disease after liver transplantation (4,20). In the present study, all the recipients with negative or indeterminate CMV IgG who received a graft from CMV IgG positive donors were given ganciclovir prophylaxis. The prophylaxis had no effect on the incidence of EBV and CMV viraemia in all settings (primary infection, reinfection/reactivation). There was no difference in the timing of the detection of CMV and EBV viraemia in transplantation recipients who were given, compared with those not given ganciclovir. The CMV and EBV DNA load were higher in transplantation recipients who did not receive ganciclovir, suggesting the efficacy of ganciclovir in controlling viral replication, but this was not statistically significant.

Within the first 21 days post-transplant, 5 of the 26 CMV mismatched transplant recipients given ganciclovir developed CMV viraemia 9 days post-transplantation while 6 of the 26 CMV mismatched transplant recipients given ganciclovir developed EBV viraemia 7 days post-transplant. Although, the ganciclovir prophylaxis did not have a significant effect on the EBV DNA load at first detection, the median EBV DNA level in these recipients was 655 copies/ml whereas it was 42,041 copies/ml in 14 of the 41 who did not receive ganciclovir. The number of recipients who developed an EBV viraemia was small and the effect of antiviral therapy on EBV replication is uncertain (28). It would therefore be interesting to investigate this further in a larger post-transplant cohort.

The present study was not designed to collect information on the long term follow up of liver transplantation in paediatric recipients but the data available demonstrate that more around 40% of the EBV and 50% of the CMV viraemic patients developed the viraemia in the first three weeks post-transplant. The presence of detectable EBV or CMV DNA in the first 21 days after transplantation was not predictive of a persistent viraemia. Confirming the result of a previous study, EBV infection in VCA IgG negative patients was associated with a sustained EBV DNA detection (17).

In conclusion, the present study reported results of early surveillance for EBV and CMV viraemia in paediatric liver transplantation recipients. Based on our findings, EBV and CMV infection can be detected at very early stages post-transplantation but do not appear to affect the early outcome of the transplant. The significance of early post-transplant infection in terms of risk of complications of EBV and CMV infection with particular regard to post-transplant lymphoproliferative disease is still unknown. Further prospective studies are needed to evaluate the long term impact of early infection on graft function, rejection and other complications associated in particular with EBV infection.

Table 1. Epstein-Barr virus and Cytomegalovirus pre- and post-transplant status according to IgG and DNA detection.

Recipient status	***CMV IgG***	***CMV DNA***	***VCA IgG***	***EBV DNA***
CMV and EBV uninfected	Negative	Negative	Negative	Negative
CMV previous exposure	Positive	Negative	NA	NA
EBV previous exposure	NA	NA	Positive	Negative
Primary CMV infection	Negative	Positive	NA	NA
Primary EBV infection	NA	NA	Negative	Positive
CMV reinfection or reactivation	Positive	Positive	NA	NA
EBV reinfection or reactivation	NA	NA	Positive	Positive

Note: CMV, Cytomegalovirus; VCA, viral capsid antigen; EBV, Epstein-Barr virus; Ig, immunoglobulin; NA, not applicable.

Table 2. Epstein-Barr virus and Cytomegalovirus status and infection in 67 paediatric transplant recipients.

	EBV ***(67 transplant recipients)***	***CMV*** ***(67 transplant recipients)***
Infection assessment before transplantation (based on VCA IgG for EBV and on CMV IgG for CMV)		
Negative	24 (36%)	31 (46%)
Positive	24 (36%)	16 (24%)
Indeterminate (less than 18-month-old, IgG positive)	19 (28%)	20 (30%)
Infection assessment in the first 21 days after transplantation (based on VCA / CMV IgG pre-transplantation and CMV/EBV DNA testing post-transplant)		
Not infected (CMV/ EBV IgG and DNA negative)	21 (31%)	28 (42%)
Indeterminate (less than 18-month-old, CMV / EBV DNA negative)	18 (27%)	15 (22%)
Infected (CMV/EBV IgG and/or DNA positive)	28 (42%)	24 (36%)
Previous exposure (CMV/EBV IgG positive, DNA negative)	8/28 (29%)	11/24 (46%)
Primary infection (CMV/EBV IgG negative, DNA positive)	3/28 (11%)	3/24 (13%)
Reactivation, reinfection (CMV/EBV IgG positive, DNA positive)	16/28 (57%)	5/24 (21%)
Infected/indeterminate (less than 18-month-old, CMV/EBV DNA positive)	1/28 (4%)	5/24 (21%)
Viraemic in the first 21 days after transplantation	20/67 (30%)	13/67 (19%)
Interval from transplantation to first CMV and EBV DNA detection, days mean (standard deviation) (n)	7.05 (3.85) (20)	9.54 (7.58) (13)
First CMV and EBV DNA value copies/mL, median (interquartile range) (n)	5,391.5 (440,878) (20)	483 (16,868.5) (13)

Note: EBV, Epstein-Barr virus; CMV, Cytomegalovirus; VCA, viral capsid antigen; Ig, immunoglobulin.

Table 3. Patterns of aspartate aminotransaminase, γ-glutamyl transferase, alkaline phosphatase, and total bilirubin in 67 transplant recipients in the first 21 days after transplant.

	AST	***γ-GT***	***ALP***	***bilirubin***
Always abnormal, no flare episode(s)	3 (5%)	10 (15%)	3 (5%)	4 (6%)
Flare episode(s)	23 (34%)	31 (46%)	10 (5%)	8 (12%)
Gradual reduction to normal value	41 (61%)	25 (37%)	6 (9%)	49 (73%)
Normal	-	1 (2%)	48 (72%)	6 (9%)

Note: AST, aspartate aminotransaminase; γ-GT, γ-glutamyl transferase; ALP, alkaline phosphatase.

Table 4. Patterns of aspartate aminotransaminase, γ-glutamyl transferase, alkaline phosphatase, and total bilirubin in transplantation recipients who underwent liver biopsy in the first 21 days after transplantation.

	aspartate aminotransaminase		***γ-glutamyl transferase***		***alkaline phosphatase***		***total bilirubin***	
	abnormal	*normal/gradual reduction*	*abnormal*	*normal/gradual reduction*	*abnormal*	*normal/gradual reduction*	*abnormal*	*normal/gradual reduction*
Liver biopsy not performed n (%)	6/26 (23)	30/41 (73)	17/41 (42)	19/26 (73)	3/13 (23)	33/54 (61)	1/12 (8)	35/55 (64)
Liver biopsy performed n (%)	20/26 (77)	11/41 (27)	24/41 (49)	7/26 (27)	10/13 (77)	21/54 (39)	11/12 (92)	20/55 (36)
p; OR; 95%CI	$<10^{-3}$; 9.09; 2.95-27.92		0.014; 3.83; 1.84-10.9		0.027; 5.29; 1.37-19.66		$<10^{-3}$; 22.75; 3.46-143.48	

Note: abnormal is "always abnormal, no flare episode(s)" plus "flare episode(s)". See text for details. OR, odds ratio; 95%CI, 95% confidence intervals.

Table 5. Pre-transplantation EBV VCA IgG and CMV IgG status, and post-transplant EBV and CMV infection in relation to rejection episodes.

	Epstein-Barr virus		***Cytomegalovirus***	
	Rejection (n/ %)	*No rejection(n/ %)*	*Rejection(n/ %)*	*No rejection(n/ %)*
Infection assessment pre-transplantation (based on VCA IgG for EBV and CMV IgG for CMV)				
Negative	5 (21%)	19 (79%)	11 (36%)	20 (65%)
Positive	10 (42%)	14 (58%)	4 (25%)	12 (75%)
Indeterminate (less than 18-month-old)	7 (37%)	12 (63%)	7 (35%)	13 (65%)
Infection assessment in the first 21 days post-transplantation (based on VCA/CMV IgG pre-transplantation and CMV and EBV DNA post-transplant) n (%)				
Not infected (CMV/EBV IgG and DNA negative)	4 (19%)	17 (81%)	10 (36%)	18 (64%)
Indeterminate (less than 18 month-old, CMV / EBV DNA negative)	7 (39%)	11 (61%)	6 (40%)	9 (60%)
Infected (CMV/EBV IgG and/or DNA positive)	11 (39%)	17 (61%)	6 (25%)	18 (75%)
Previous exposure (CMV/EBV IgG positive, DNA negative)	3 (38%)	5 (63%)	3 (27%)	8(73%)
Primary infection (CMV/EBV IgG negative, DNA positive)	1 (33%)	2 (67%)	1 (33%)	2 (67%)
Reactivation, reinfection (CMV/EBV IgG positive, DNA positive)	7 (44%)	9 (56%)	1 (20%)	4 (80%)
Infected/indeterminate (less than 18-month-old, CMV/EBV DNA positive)	0	1 (100%)	1 (20%)	4 (80%)
Viraemic in the first 21 days after transplantation	8 (42%)	11 (58%)	3 (23%)	10 (77%)

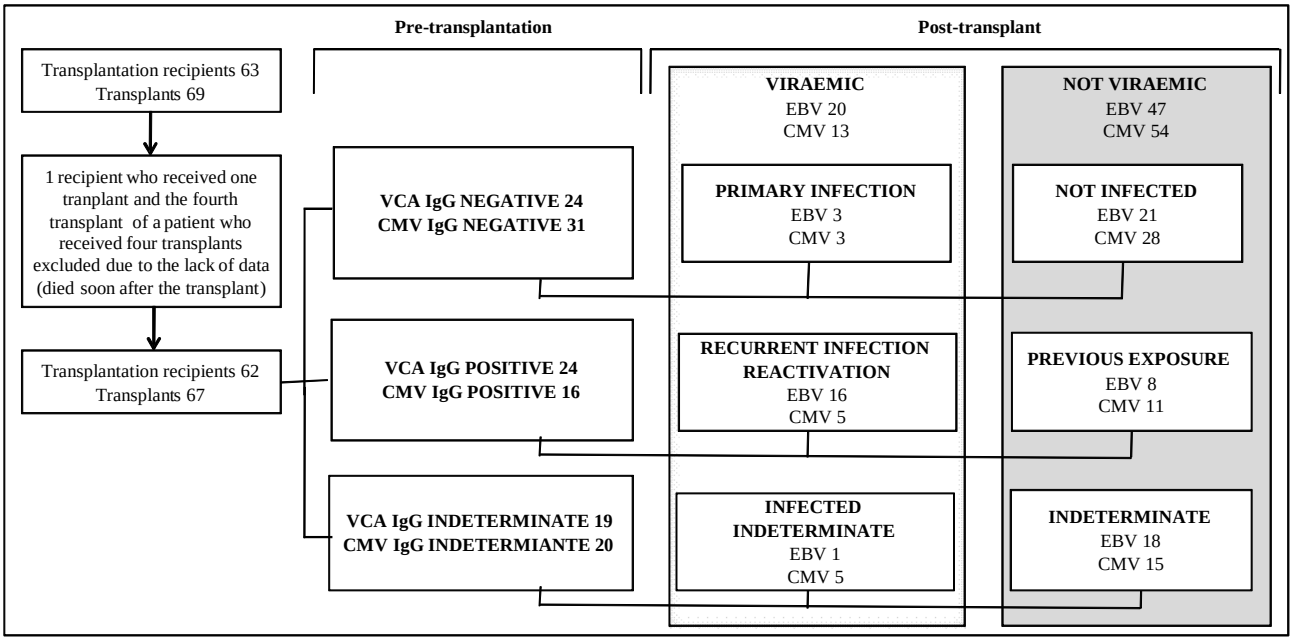
Note: EBV, Epstein-Barr virus; CMV, Cytomegalovirus; VCA, viral capsid antigen; Ig, immunoglobulin.

Table 6. Cytomegalovirus and Epstein-Barr virus in relation to Ganciclovir prophylaxis.

	Cytomegalovirus		**Epstein-Barr Virus**	
	Given ganciclovir (primary infections)	*Not given ganciclovir (primary infections or reactivations)*	*Given ganciclovir (primary infections)*	*Not given ganciclovir (primary infections or reactivations)*
Viraemic in the first 21 days post- transplantation	5 (19.2%)	8 (19.5%)	6 (23.1%)	14 (34.1%)
Time interval from transplantation to first DNA detection, days mean (standard deviation)	9.2 (6.7)	9.7 (8.4)	7 (3.6)	7.1 (4.1)
First DNA value copies/mL, median (interquartile range) (n)	456 (33,989)	1,545 (21,940)	655.5 (256,656)	42,041 (490.164)
Time interval from transplantation to first DNA value <10 copies/ml , days mean (standard deviation)	30 (18)	31.3 (17.2)*	38.8 (27.9)	95.9 (129.8)**

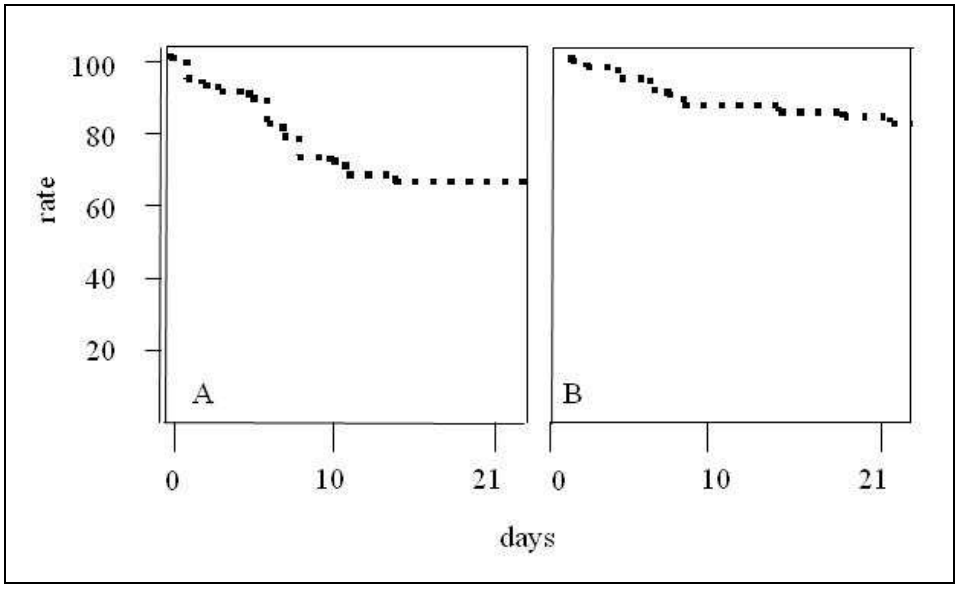
Note: * 2 and ** 6 patients with ongoing viraemia at the end of the follow up.

Figure 1. Flow chart describing the features of EBV and CMV infections before and after transplantation.



Note: EBV, Epstein-Barr virus; CMV, Cytomegalovirus. For definitions see text (Materials and Methods; Definitions).

Figure 2. Kaplan-Meier curves describing the percentage of patients viraemic for Epstein-Barr virus (A) and viraemic for Cytomegalovirus (B) during the first 21 days after transplantation.



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