TRANSPLANT INTERNATIONAL

Transplant International ISSN 0934-0874

ORIGINAL ARTICLE

(Val-)Ganciclovir prophylaxis reduces Epstein-Barr virus primary infection in pediatric renal transplantation

Britta Höcker,[1] Stephan Böhm,[2,3] Helmut Fickenscher,[2,4] Uta Küsters,[2] Paul Schnitzler,[2] Martin Pohl,[5] Ulrike John,[6] Markus J. Kemper,[7] Henry Fehrenbach,[8] Marianne Wigger,[9] Martin Holder,[10] Monika Schröder,[11] Reinhard Feneberg,[1] Sabine Köpf-Shakib[1] and Burkhard Tönshoff[1]

1 University Children's Hospital, Heidelberg, Germany
2 Department of Infectious Diseases, Virology, University of Heidelberg, Heidelberg, Germany
3 Department of Gastroenterology, University Hospital Leipzig, Leipzig, Germany
4 Institute for Infection Medicine, University of Kiel, Kiel, Germany
5 University Children's Hospital, Freiburg, Germany
6 University Children's Hospital, Jena, Germany
7 University Children's Hospital, Hamburg, Germany
8 Children's Hospital, Memmingen, Germany
9 University Children's Hospital, Rostock, Germany
10 Olga Children's Hospital, Stuttgart, Germany
11 Clementine Children's Hospital, Frankfurt, Germany

Keywords
Epstein-Barr virus, ganciclovir, pediatric renal transplantation, post-transplant lymphoproliferative disease, prophylaxis, valgancivlovir.

Correspondence
Burkhard Tönshoff MD, PhD, Department of Pediatrics I, University Children's Hospital, Im Neuenheimer Feld 430, D-69120 Heidelberg, Germany. Tel.: ++49 (0)6221 568401; fax: ++49 (0)6221 564203; e-mail: burkhard.toenshoff@med.uni-heidelberg.de

Conflicts of Interest
Britta Höcker, Stephan Böhm, Uta Küsters, Paul Schnitzler, Martin Pohl, Ulrike John, Markus J Kemper, Henry Fehrenbach, Marianne Wigger, Martin Holder, Monika Schröder and Reinhard Feneberg have no conflicts of interest.

Received: 6 December 2011
Revision requested: 12 January 2012
Accepted: 19 March 2012
Published online: 25 April 2012

doi:10.1111/j.1432-2277.2012.01485.x

Summary

Epstein-Barr virus (EBV) primary infection constitutes a serious risk for pediatric transplant recipients, particularly as regards the development of EBV-related post-transplant lymphoproliferative disease (PTLD). Currently, there is no established prophylactic regimen. We investigated the association between chemoprophylaxis with valganciclovir (VGCV) or ganciclovir (GCV) and the incidence of EBV viremia in EBV-naïve pediatric renal transplant recipients (R−) who had received a graft from an EBV-positive donor (D+) and are therefore at high risk of EBV primary infection. In a prospective, multicenter trial ($n = 114$), we compared a cohort on chemoprophylaxis ($n = 20$) with a similar control cohort without chemoprophylaxis ($n = 8$). Over the 1-year study period, antiviral prophylaxis with VGCV/GCV was associated with a significantly decreased incidence of EBV primary infection: 9/20 patients (45%) in the prophylaxis group experienced an EBV primary infection compared to 8/8 controls (100%) ($P < 0.0001$). Chemoprophylaxis was associated with a significantly lower EBV viral load ($P < 0.001$). Type or intensity of immunosuppressive therapy did not influence the occurrence of EBV primary infection or the level/persistence of EBV viral load. Chemoprophylaxis with VGCV/GCV is associated with a reduced incidence of EBV viremia in high-risk pediatric kidney allograft recipients in the first year post-transplant.
(ClinicalTrials.gov number: NCT00963248).

Introduction

Epstein-Barr virus (EBV) is a ubiquitous herpesvirus, which infects most of the population. In transplant recipients, whose cellular and humoral immune response is suppressed by immunosuppressive therapy, EBV infection may constitute a serious risk. Its clinical presentation varies and ranges widely between asymptomatic EBV viremia,

unspecific flu-like symptoms, infectious mononucleosis syndrome, and malignant lymphoproliferation [1]. Furthermore, subclinical EBV infection may be associated with chronic allograft injury and enhanced loss of transplant function in pediatric kidney transplant recipients [2].

One of the most serious complications of EBV infection in transplant patients is post-transplant lymphoproliferative disorder (PTLD), which occurs in approximately 0.5–10% of recipients [3–5]. The physiological control of EBV-infected B-cells by T-lymphocytes is disturbed due to drug-induced immunosuppression, which can result in uncontrolled B-cell proliferation [3]. Recipient EBV seronegativity at the time of transplantation plays a crucial role in the emergence of PTLD [4–6]. As most of adult organ donors are EBV-seropositive, pediatric renal allograft recipients being often EBV-naïve at the time of transplantation are exposed to an especially increased risk of developing EBV primary infection and EBV-associated PTLD [5–7]. As an EBV vaccine is currently not available [8], antiviral agents, which have been shown to sufficiently prevent cytomegalovirus (CMV) infections in transplant patients [9–11], could also act as prophylaxis against EBV infections, thereby potentially lowering the risk of EBV-associated PTLD. Funch and co-workers conducted a retrospective case–control study among 100 patients with biopsy-confirmed benign or malignant PTLD and 375 controls [12]. Chemoprophylaxis with ganciclovir (GCV) or aciclovir was associated with an up to 83% reduction in PTLD risk, particularly during the first 12 months after transplantation; the prophylactic effect of GCV was more pronounced than that of aciclovir [12].

It is likely that the potential beneficial effect of GCV chemoprophylaxis on the development of PTLD is partially due to EBV viremia prevention in an EBV-naïve patient receiving a transplant from an EBV-positive donor; however, this has never been proven formally. In the framework of a prospective, multicenter study, we therefore performed the present subanalysis. Its aim was to investigate the potential beneficial association between chemoprophylaxis with valganciclovir (VGCV) or GCV, which was administered initially for prophylaxis of CMV infection, and the incidence of EBV infection in pediatric renal transplant patients at high risk of EBV primary infection.

Materials and methods

Study design and patient population

The present controlled cohort study was carried out in the context of a prospective, multicenter trial investigating the epidemiology and morbidity of EBV infection in pediatric renal transplant recipients during the first post-transplant year. The trial was registered at clinicaltrials.gov (NCT00963248). All patients and their parents or guardians provided written informed consent. The study was conducted in full accordance with the principles of the Declaration of Helsinki and Good Clinical Practice guidelines and approved by the ethics committee of each contributing center.

For this subgroup analysis, eligible subjects were EBV-seronegative, nonviremic (R−) patients receiving a kidney allograft from an EBV-seropositive donor (D+), thus bearing a high risk (D+/R−) of developing EBV primary infection. The aim of the analysis was to investigate the potential association of antiviral chemoprophylaxis with GCV or VGCV and the incidence of EBV viremia in pediatric renal transplant recipients at high risk of EBV primary infection.

Between July 2003 and June 2009, 114 patients were recruited for a prospective, multicenter study on the epidemiology and morbidity of EBV infection in pediatric renal transplant recipients. In this substudy, we compared two cohorts of patients at high risk (D+/R−) of developing post-transplant EBV primary infection, one cohort on chemoprophylaxis ($n = 20$) (''prophylaxis group'') and a similar control cohort, which received no antiviral prophylaxis ($n = 8$) (''control group''). All EBV-seronegative patients who received an organ from a positive donor (D+/R−; $n = 28$) were included in the analysis, corresponding to 24.6% (28/114) of the entire study population, and followed up for 1 year post-transplant. The cohort of all high-risk (EBV D+/R−) patients was assigned retrospectively to two groups, one receiving an antiviral prophylaxis (prophylaxis group) and the other undergoing no prophylaxis (control group). No patient was excluded from this allocation. In the prophylaxis group ($n = 20$), 13 were administered VGCV and seven GCV; seven (six VGCV, one GCV) of 20 patients were given, in addition, one dose of anti-cytomegalovirus hyperimmunoglobulin (Cytotect®) at a dosage of 100 mg/kg resp. 200 units/kg i.v. immediately prior to engraftment. The control group neither received chemoprophylaxis with VGCV or GCV nor anti-CMV hyperimmunoglobulin. Patient characteristics are shown in Table 1. The prophylaxis group and the control group were well comparable in terms of age, gender, ethnicity, type of donor, cold ischemia time, HLA mismatch, CMV status, immunosuppressive regimen, baseline estimated glomerular filtration rate (eGFR), and number of acute rejection episodes during the first year post-transplant. In addition, immunosuppressive maintenance therapy did not differ significantly between the prophylaxis and the control groups (Table 1): Overall immunosuppressive score (modified Vasudev score), mean dose of the respective immunosuppressive agents and the respective predose concentrations were comparable. All patients had received

Table 1. Patient characteristics.

Characteristic	Prophylaxis group (n = 20)	Control group (n = 8)	Statistical significance
Age at transplantation (mean ± SD), years	8.5 ± 5.8	6.1 ± 3.8	P = 0.37
Male gender, n (%)	15 (75.0)	4 (50.0)	P = 0.37
Caucasian, n (%)	19 (95.0)	7 (87.5)	P = 0.50
Living-related donation, n (%)	10 (50.0)	2 (25.0)	P = 0.40
Cold ischemia time (mean ± SEM), h	9.3 ± 1.7	8.7 ± 2.5	P = 0.86
HLA mismatch (mean ± SEM), n	2.4 ± 0.9	2.5 ± 1.2	P = 0.90
CMV status, n (%)			
D+/R−	6 (30.0)	1 (12.5)	P = 0.63
D+/R+	5 (25.0)	1 (12.5)	P = 0.64
D−/R+	1 (5.0)	1 (12.5)	P = 0.50
D−/R−	8 (40.0)	5 (62.5)	P = 0.41
Initial immunosuppressive regimen, n (%)			
IL-2 receptor antagonist	1 (5.0)	1 (12.5)	P = 0.50
TAC	14 (70.0)	5 (62.5)	P = 1.00
CSA	6 (30.0)	3 (37.5)	P = 1.00
MMF	20 (100.0)	8 (100.0)	P = 1.00
Corticosteroids	20 (100.0)	8 (100.0)	P = 1.00
Maintenance immunosuppressive regimen* (mean ± SEM)			
TAC dose, mg/m^2/day	6.7 ± 0.6	6.3 ± 0.7	P = 0.71
TAC predose level, ng/ml	7.9 ± 0.4	7.8 ± 0.7	P = 0.88
CSA dose, mg/m^2/day	256 ± 62	227 ± 40	P = 0.72
CSA predose level, ng/ml	145 ± 13	107 ± 10	P = 0.06
MMF dose, mg/m^2/day	791 ± 50	956 ± 81	P = 0.09
MPA predose level, ng/ml	2.3 ± 0.3	2.0 ± 0.3	P = 0.65
Equivalent PRED dose, mg/m^2/day	3.7 ± 0.3	3.4 ± 0.7	P = 0.65
Modified Vasudev score†	9.5 ± 0.5	8.8 ± 0.8	P = 0.47
Baseline eGFR‡ (mean ± SEM), ml/min/1.73 m^2	71.3 ± 5.6	83.5 ± 5.9	P = 0.37
BPAR§, n (number and percentage of patients)	14 (9; 45.0)	3 (3; 37.5)	P = 1.00

SD, standard deviation; SEM, standard error of the mean; HLA, human leukocyte antigen; CMV, cytomegalovirus; IL-2, interleukin-2; TAC, tacrolimus; CSA, cyclosporine microemulsion; MMF, mycophenolate mofetil; MPA, mycophenolic acid; PRED, prednisone; eGFR, estimated glomerular filtration rate; BPAR, biopsy-proven acute rejection.

*Immunosuppressant dosage, predose levels and immunosuppressive scores were documented at the time of transplantation, 6 weeks and 3, 6, 9 and 12 months after engraftment. Table 1 shows the average values of all measurements.

†Defined as shown in Table 2.

‡Defined as eGFR at time of discharge after renal transplantation.

§Including borderline changes (Banff 97 '09 update).

their first kidney allograft. None of them experienced delayed graft function. Patient and transplant survival amounted to 100% at 1 year post-transplant.

Antiviral chemoprophylaxis

Antiviral prophylaxis was administered based on international guidelines for CMV prophylaxis in renal transplant recipients [9,13–17]. Patients at high risk of CMV infection (D+/R−, D+/R+) received GCV or, since the year 2006, VGCV. However, nine patients in the prophylaxis group were given antiviral prophylaxis despite a moderate or low risk of CMV infection (D−/R+, D−/R−). This is because, based on the findings by Funch *et al.*, some transplant centers have administered antiviral prophylaxis with GCV or VGCV since the year 2005, not only to patients at high risk of CMV infection (D+/R−, D+/R+) but also to those at high risk (D+/R) of EBV infection [12]. Two patients did not receive antiviral prophylaxis although bearing a high risk of CMV infection (Table 1). Oral GCV (Cymeven®) or VGCV (Valcyte®) was administered for the first 100 post-transplant days in conformity with the previously published dosing recommendations [16–19]. GCV dose was calculated according to the following scheme: eGFR > 100 ml/min/1.73 m^2: 100 mg/kg/day; eGFR ≤ 100 ml/min/1.73 m^2: dose = eGFR in mg/kg/day [16]. VGCV dose was calculated using the formula: 7 × body surface area (m^2) × eGFR (ml/min/1.73 m^2) = dose (mg/day) (published as abstract in 2006 [17] and as full article in 2009 [19]). ''Responders'' to antiviral prophylaxis were defined as patients with 1-year EBV viremia-free survival, ''nonresponders'' to antiviral

prophylaxis were defined as patients with EBV viremia during the first year post-transplant.

In terms of hematological safety parameters, anemia was defined as hemoglobin below 10.0 g/dl. Leukocytopenia was defined as leukocyte count below 4000/μl; neutropenia as neutrophil count below 1000/μl; and agranulocytosis as granulocyte count below 200/μl. Thrombocytopenia was defined as thrombocyte count below 100 000/μl.

Diagnosis of EBV primary infection

EBV primary infection was defined as positive EBV viremia and/or EBV seroconversion. The clinical pattern of EBV infection was categorized into asymptomatic infection, unspecific flu-like symptoms (fever, malaise, chills), infectious mononucleosis (fever, pharyngitis, lymphadenopathy with or without hepatosplenomegaly), and PTLD [[20, 21]]. Although physicians were not blinded to antiviral prophylaxis administration, and EBV viral load and antibody measurements were taken prospectively, they did not know whether a patient had an EBV infection at the time of documenting clinical symptoms, as laboratory results were forwarded with a 1- to 3-day delay.

A central laboratory (Department of Infectious Diseases, Virology, University of Heidelberg) performed EBV viral load and EBV-specific antibody measurements in recipients prospectively, based on fresh blood samples and the same assays. According to the study protocol, they took place immediately prior to transplantation, 6 weeks and 3, 6, 9 and 12 months post-transplant. In case of EBV primary infection, EBV viral load and EBV-specific antibodies were determined once every month. If EBV disease or PTLD was suspected, measurements were made at weekly intervals for at least 6 weeks and at monthly intervals thereafter. In 18/20 (90%) patients of the prophylaxis group and 7/8 (87.5%) controls, measurements were performed at scheduled times. Only one patient in each cohort missed one measurement. Both the mean number and range of EBV viral load and of EBV-specific antibody measurements were comparable between the prophylaxis and the control groups, and amounted to 7.4 ± 0.4 (5–11) vs. 7.3 ± 0.6 (5–10) and to 6.7 ± 0.3 (5–9) vs. 6.8 ± 0.5 (5–9). An EBV viral load $\geq 10^4$ genomes/ml was considered as high viral load. A detectable EBV viral load in >50% of blood samples was defined as persistent EBV viral load. Although positive EBV viremia was defined as detection of one single positive EBV viral load, all patients who developed EBV primary infection had at least two positive results. Seroconversion was defined as detection of at least one of the below-mentioned EBV-specific antibodies. Patients receiving EBV antibody-containing CMV hyperimmunoglobulin or transfusions, who showed a single marginal or positive antibody result only once at 6 weeks after administration but negative results thereafter, were regarded as seronegative.

Quantitative real-time polymerase chain reaction (PCR) was performed for detection of EBV-DNA in whole-blood; nucleic acids were extracted from 200 μl EDTA-blood using the QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions [22]. PCR was carried out in a total reaction volume of 20 μl with the LightCycler 1.5 or 480 Probes Master ready-to-use master mix (Roche Diagnostics, Mannheim, Germany). Amplification and detection were performed in a LightCycler 1.5 or 480 instrument (Roche Diagnostics, Mannheim, Germany) with a thermocycling profile at 95 °C for 5 min, followed by 50 cycles of 95 °C for 5 s and 60 °C for 20 s. The lower limit for quantification with a 95% confidence interval was set at 1000 copies/ml whole-blood.

Recipients' EBV-specific antibodies were determined in serum samples using the Enzygnost Anti-EBV IgG and Enzygnost Anti-EBV IgM enzyme linked immunosorbent assays (Siemens, Eschborn, Germany) according to the manufacturer's instructions. Donor EBV IgG seropositivity (D+) was analyzed by certified laboratories of country-specific organ procurement organizations such as the ''Deutsche Stiftung Organtransplantation'' or by other affiliated laboratories of the Eurotransplant International Foundation.

Immunosuppressive regimen

Immunosuppressive medication was administered according to center practice (Table 1). Exposure to the respective immunosuppressive drugs (TAC, CSA and mycophenolic acid [MPA]) was monitored by predose concentrations. In addition, a score modified according to Vasudev *et al.* for assessment of the overall immunosuppressive load was calculated by adjusting the adult score to a body surface area (BSA) of 1.0 m^2, assuming an adult BSA of 1.73 m^2, and by including interleukin 2-receptor antibody induction and steroid pulse therapy corresponding to two immunosuppressive units each (Table 2) [23]. Immunosuppressant dosage, predose concentrations and immunosuppressive score were documented at the time of transplantation, 6 weeks and 3, 6, 9 and 12 months after engraftment.

Statistical analysis

Data were analyzed using PASW (SPSS) Statistics 18.0 (IBM Corporation, Armonk, New York, NY, USA). Unless stated otherwise, results for continuous variables are given as mean ± standard error of the mean (SEM), while categorical parameters are expressed as number and percentage

Table 2. Immunosuppressive score.

Immunosuppressant	''Vasudev score'': dose per unit (mg/day)	Pediatric score: dose per unit (mg/m^2/day)	Immunosuppressive unit
TAC	2	1.2	1
CSA	100	58	1
SRL	2	1.2	1
MMF	500	290	1
AZA	100	58	1
Prednisone equivalent	5	2.9	1

Interleukin-2 receptor antagonist induction and steroid pulse therapy (as anti-rejection treatment) corresponded to two immunosuppressive units each.
TAC, tacrolimus; CSA, cyclosporine microemulsion; SRL, sirolimus; MMF, mycophenolate mofetil; AZA, azathioprine.

of patients showing the respective outcomes. Normal distribution of the data was evaluated using Shapiro-Wilks test. Any divergences between two groups were analyzed by means of the Student's *t*-test or, if normality failed, the Mann–Whitney rank-sum test. Time to EBV primary infection was summarized using Kaplan–Meier curves and tested for significance based on the two-sided log-rank test. Rates in both groups were compared using Fisher's exact test. Differences of means or proportions with a two-tailed $P < 0.05$ were considered statistically significant.

Results

Antiviral prophylaxis with VGCV or GCV was associated with a significantly ($P < 0.0001$) lower incidence of EBV primary infection (Fig. 1a) in the first year post-transplant (95% confidence intervals for EBV primary infection-free survival: 6.7–10.2 vs. 1.6–3.4 months). Patients received a mean VGCV dose of 473 ± 49 mg/m^2/day for 102 ± 3 days, a mean oral GCV dose of 42.2 ± 6.9 mg/kg/day for 101 ± 2 days, and a mean single anti-cytomegalovirus hyperimmunoglobulin dose of 96.7 ± 3.9 mg/kg equivalent to 193 ± 7.9 units/kg. In the prophylaxis group, the majority of patients who developed EBV primary infection, did so only after termination of antiviral prophylaxis; during prophylaxis, 80% experienced no EBV primary infection (Fig. 1a). Whereas at 5 months post-transplant, no patient in the control group remained EBV primary infection-free, 75% of the prophylaxis group did not develop EBV primary infection; the rate of EBV primary infection-free patients at 12 months post-transplant was 55%. When analysis was restricted to patients who only received VGCV or GCV and no anti-CMV hyperimmunoglobulin, a comparable difference was observed between the prophylaxis

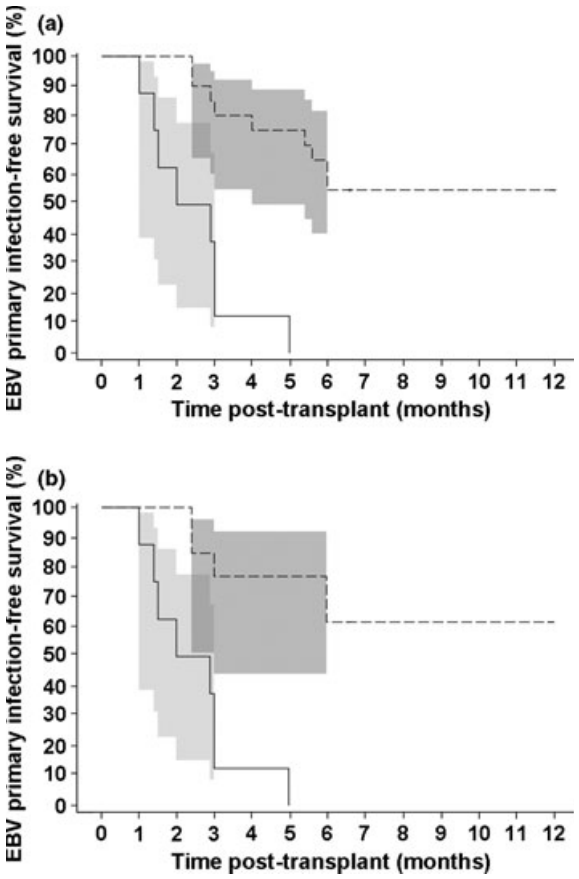

Figure 1 Epstein-Barr virus (EBV) primary infection-free survival in high-risk (D+/R–) patients according to Kaplan–Meier. (a) EBV primary infection-free survival rates were 55.0% and 0% for the prophylaxis group (dotted line, $n = 20$) and the control group (solid line, $n = 8$), respectively ($P < 0.0001$). (b) EBV primary infection-free survival rates were 61.5% and 0% for the prophylaxis group excluding recipients of anti-CMV hyperimmunoglobulin (dotted line, $n = 13$), and the control group (solid line, $n = 8$), respectively ($P < 0.0001$). Shaded areas depict 95% confidence intervals.

group and controls (Fig. 1b). Additionally, the prophylaxis group had significantly less often a high and/or persistent EBV viral load over the 1-year study period than controls (Fig. 2a); also, the peak EBV viral load was ten times and the EBV viral load AUC 100 times lower in the prophylaxis group than in controls (Fig. 2b). Within the prophylaxis group, no significant difference in EBV viral load was observed between patients receiving VGCV or GCV (peak EBV viral load 1.4 ± 2.8 vs. 1.1 ± 1.0 · 10^4 genomes/ml, $P = 0.86$; EBV viral load AUC 3.8 ± 6.8 vs. 2.1 ± 1.8 · 10^4 genomes/ml, $P = 0.58$) [12].

All patients with EBV primary infection developed EBV viremia. Two in the prophylaxis and one in the control group exhibited no EBV seroconversion (3/17, 17.6%) during the first post-transplant year, despite EBV primary

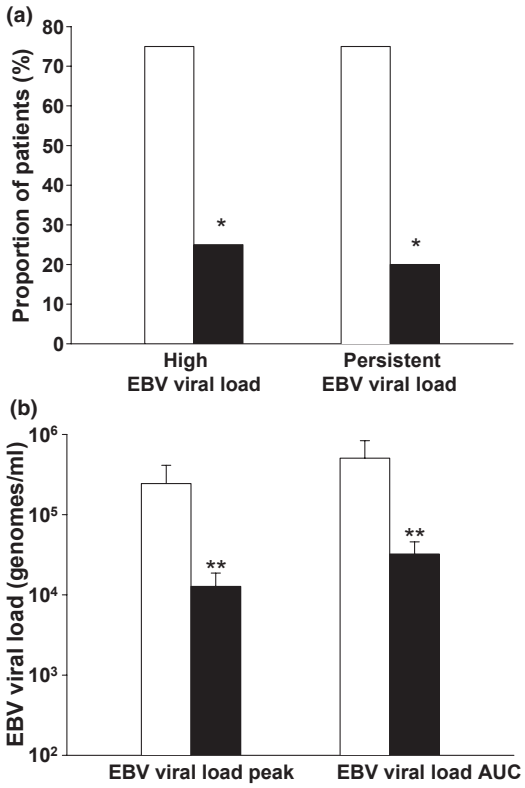

Figure 2 Epstein-Barr virus (EBV) viremia in high-risk (D+/R–) patients. (a) Proportions of patients with high and/or persistent EBV viral load in the prophylaxis group (black bars) and the control group (white bars) (*$P < 0.05$). High EBV viral load is defined as viral load $\geq 10^4$ genomes/ml whole-blood; persistent EBV viral load is defined as a detectable viral load in more than 50% of blood samples. (b) Comparison of mean EBV viral load peak and EBV viral load area-under-the-curve (AUC) in the prophylaxis group (black bars) and the control group (white bars) (**$P < 0.001$). Data are given as mean ± SEM.

infection; but all patients with EBV seroconversion also had already developed EBV viremia beforehand. On average, seroconversion occurred at 8.1 ± 0.5 months post-transplant and at 5.6 ± 0.7 months after detection of EBV viremia.

Next we sought to analyze whether the degree of immunosuppressive therapy affected EBV viremia-free survival during the first year post-transplant. The overall immunosuppressive load quantified by the modified Vasudev score as well as mean doses and predose concentrations of single specific immunosuppressants were comparable in responders and nonresponders to antiviral prophylaxis (results not shown). In addition, maintenance immunosuppression was comparable between the prophylaxis and the control group (Tables 1 and 3).

We also investigated whether CMV viremia affected EBV viremia-free survival during the first post-transplant year. There was no significant difference in the rate of CMV infection between responders and nonresponders to antiviral prophylaxis: 1/11 (9.1%) responders vs. 2/9 (22.2%) nonresponders developed CMV viremia during the first post-transplant year ($P = 0.57$). In the control group, 2/8 (25%) patients developed CMV viremia. Beyond that, we found no significant difference in the overall immunosuppressive load between patients with and without CMV infection (modified Vasudev score, 9.7 ± 0.6 vs. 8.7 ± 0.4, $P = 0.25$). No patient required antiviral treatment in the first post-transplant year.

Table 3. Immunosuppressive maintenance therapy.

Modified Vasudev score*	Prophylaxis group ($n = 20$)	Control group ($n = 8$)	Statistical significance
Baseline†	11.5 ± 0.9	10.9 ± 1.2	$P = 0.71$
Month 3	9.8 ± 0.5	9.1 ± 0.9	$P = 0.48$
Month 6	9.4 ± 0.6	8.8 ± 1.1	$P = 0.56$
Month 9	7.5 ± 0.4	7.4 ± 0.6	$P = 0.95$
Month 12	7.0 ± 0.5	6.7 ± 0.6	$P = 0.71$

Data are given as mean ± SEM.
*Defined as shown in Table 2.
†Defined as modified Vasudev score at time of discharge after renal transplantation.

During the 1-year study period, high-risk patients of the prophylaxis group (5/20 [25%]) tended to develop EBV-related clinical symptoms less often than controls (5/8 [62.5%], $P = 0.09$). Patients receiving antiviral chemoprophylaxis showed unspecific EBV-associated flu-like symptoms significantly less frequently than controls (2/20 [10%] vs. 4/8 [50%], $P = 0.04$). However, because further causes of flu-like symptoms like other infections were not investigated systematically according to the study protocol, these data should be interpreted with caution. The infectious mononucleosis (2/20 [10%] vs. 0/8 [0%], $P = 1.00$) or PTLD rate (1/20 [5%] vs. 1/8 [12.5%], $P = 0.50$) was too low to make a clear statement. Interestingly, patients displaying clinical symptoms had a significantly ($P < 0.01$) lower peak EBV viral load (6.5 ± 2.7 vs. 8.7 ± 7.7 · 10^4 genomes/ml) and EBV viral load AUC (15.5 ± 5.0 vs. 17.5 ± 15.2 · 10^4 genomes/ml) than asymptomatic recipients.

Mean eGFR at baseline was comparable in both groups (Table 1) and remained stable over the 1-year study period: the mean loss of eGFR did not differ statistically between prophylaxis (−3.9 ± 1.7 ml/min/1.73 m^2) and control groups (−6.0 ± 4.9 ml/min/1.73 m^2; $P = 0.60$).

Overall, antiviral prophylaxis was well tolerated. The incidence of anemia was comparable in the prophylaxis group (9/20 [45.0%]) and controls (5/8 [62.5%]; $P = 1.00$), likewise the rate of leukocytopenia (prophylaxis group, 4/20 [20%]; controls 2/8 [25.0%]; $P = 1.00$) and neutropenia (prophylaxis group, 3/20 [15.0%]; controls 1/8 [12.5%]; $P = 1.00$). None of the patients

developed agranulocytosis or thrombocytopenia. In addition, none withdrew or interrupted antiviral prophylaxis due to adverse events.

Discussion

This is the first study investigating the association between antiviral chemoprophylaxis with VGCV or GCV and EBV primary infection in high-risk pediatric renal transplant recipients. Our data demonstrate that this prophylaxis is associated with a significantly reduced incidence of EBV viremia in the first 12 months after renal transplantation. GCV and its prodrug VGCV are known to be active against EBV in the initial, so-called lytic, phase of EBV infection [24]. A possible explanation for a reduced EBV viremia rate in high-risk patients could be that GCV, when given at the time of transplantation, eliminates the virus as soon as it is transmitted with the donor kidney, thus preventing significant viremia and infection. This potential mechanism could also explain our finding that patients receiving antiviral prophylaxis experienced not only less frequently an EBV primary infection, but also, in case of infection, a significantly lower EBV viral load than controls. As a consequence of GCV erasing EBV in the lytic phase, the virus might be further inhibited from entering the second (latent) phase of infection, which is accompanied by B-cell immortalization with the potential of malignant transformation [25].

Besides a reduced EBV primary infection rate, we also found that patients under prophylaxis with GCV or VGCV, who contracted EBV infection, exhibited less frequently EBV-associated flu-like symptoms. One of the most severe EBV-related complications known is post-transplant lymphoproliferation. Funch *et al.* found in their case–control study among pediatric and adult renal transplant recipients that chemoprophylaxis using one of the antiviral agents GCV or aciclovir in the first months post-transplant was associated with a significantly reduced risk of EBV-associated PTLD [12]. Our observation that GCV or VGCV is associated with a significantly decreased incidence of EBV primary infection in high-risk patients is a likely explanation for the reduced PTLD rate noticed by Funch and coworkers [12]. In the adult solid-organ transplant population, two retrospective studies have also shown a lower incidence of EBV-related PTLD in EBV-naïve patients receiving an antiviral agent such as GCV or VGCV [26, 27].

While Funch *et al.* included both benign and malignant PTLD cases in their study, Opelz *et al.* retrospectively analyzed the effect of CMV prophylaxis with anti-CMV hyperimmunoglobulin or antiviral drugs on malignant post-transplant non-Hodgkin lymphoma (NHL) [28]. They did not observe a relevant influence of GCV or aciclovir on NHL incidence [28]. However, as donor EBV serology was often unknown, patients were not stratified by risk of EBV infection. It therefore remains unclarified whether an effect of antiviral agents on NHL incidence would have occurred in EBV high-risk patients.

In our study, patients with acute EBV-associated clinical symptoms had a lower EBV viral load than asymptomatic recipients. This observation is consistent with the finding that acute clinical symptoms like flu-like signs or infectious mononucleosis are caused by the host's immune response rather than by the virus itself [20, 29, 30]. For example, Silins *et al.* observed massive T-cell expansions in immunocompetent patients with infectious mononucleosis in contrast to homeostatic T-cell control in asymptomatic patients, while high levels of EBV DNA were found in both symptomatic and asymptomatic persons [31].

A limitation of our study consists in its small sample size and lack of randomization; instead, we analyzed two similar cohorts at high risk of primary EBV infection, one group on antiviral prophylaxis and one control group, in the framework of a large prospective, multicenter trial. Both groups were well comparable in terms of their baseline characteristics, immunosuppressive regimen, and graft function during the first year post-transplant. Clearly, a prospective, randomized trial investigating the effect of chemoprophylaxis with GCV or VGCV on EBV primary infection would have been preferable; however, such a study would be virtually unfeasible because antiviral prophylaxis with VGCV or GCV is currently recommended for patients at high risk of CMV infection [9,15], and nowadays, there are only few patients at high risk of EBV primary infection who do not require VGCV for prevention of CMV infection, given the strong association between recipient and donor CMV and EBV serostatus [4]. For example, the number of patients in this multicenter study carrying a high risk of primary EBV infection (D+/R−) and a low risk of CMV (i.e., D−/R− or D−/R+), who therefore would need no VGCV for CMV infection prevention, was very low (15/114 [13.2%]) [32]. In the light of this low number of potentially eligible patients, a randomized, controlled study on the effect of VGCV on EBV viremia incidence in high-risk pediatric renal transplant recipients would therefore be practically impossible to conduct. Thus, we feel that the evidence provided by this prospective cohort study is the maximum realistically achievable.

In conclusion, our data demonstrate that VGCV- or GCV-based chemoprophylaxis in high-risk pediatric renal transplant recipients is associated with a significant reduction of the incidence of EBV primary infection during the first year post-transplant. Chemoprophylaxis was well tolerated. The observed beneficial association between chemoprophylaxis and EBV viremia is expected to translate into a reduced incidence of EBV-related PTLD in this vulnerable patient population.

Authorship

BH and BT: participated in literature search, study design, data collection, analysis and interpretation, and preparation of the manuscript. SB and UK: participated in literature search, sample and data collection, data analysis, and interpretation of results. HF: participated in study design, grant application, data collection and analysis, and setup of methods. PS: participated in data collection and analysis. MP, UJ, MJK, HF, MW, MH and MS: participated in data collection, data analysis and interpretation of results. RF: participated in data analysis and interpretation of results. SK-S: participated in literature search, study design, data collection, analysis, and interpretation of results.

Funding

This study was performed by the German Society for Pediatric Nephrology (GPN) and supported by a grant from the Else Kröner-Fresenius-Stiftung, Bad Homburg, Germany (to Burkhard Tönshoff and Helmut Fickenscher) and the Medical Faculty of the University of Heidelberg (Young Investigator Grant to Sabine Köpf-Shakib). Britta Höcker has been awarded an Olympia Morata Grant by the Medical Faculty of the University of Heidelberg.

Acknowledgements

We thank Axel Rahmel and Jan de Boer, Eurotransplant International Foundation, and Günter Kirste, Deutsche Stiftung Organtransplantation, for their assistance in providing information on the EBV serostatus of organ donors.

References

1. Ho M, Jaffe R, Miller G, *et al.* The frequency of Epstein–Barr virus infection and associated lymphoproliferative syndrome after transplantation and its manifestations in children. *Transplantation* 1988; **45**: 719.
2. Smith JM, Corey L, Bittner R, *et al.* Subclinical viremia increases risk for chronic allograft injury in pediatric renal transplantation. *J Am Soc Nephrol* 2010; **21**: 1579.
3. Hopwood P, Crawford DH. The role of EBV in post-transplant malignancies: a review. *J Clin Pathol* 2000; **53**: 248.
4. Opelz G, Daniel V, Naujokat C, Dohler B. Epidemiology of pretransplant EBV and CMV serostatus in relation to posttransplant non-Hodgkin lymphoma. *Transplantation* 2009; **88**: 962.
5. Quinlan SC, Pfeiffer RM, Morton LM, Engels EA. Risk factors for early-onset and lateonset post-transplant lymphoproliferative disorder in kidney recipients in the United States. *Am J Hematol* 2011; **86**: 206.
6. McDonald RA, Smith JM, Ho M, *et al.* Incidence of PTLD in pediatric renal transplant recipients receiving basiliximab, calcineurin inhibitor, sirolimus and steroids. *Am J Transplant* 2008; **8**: 984.
7. Dharnidharka VR, Sullivan EK, Stablein DM, Tejani AH, Harmon WE. Risk factors for posttransplant lymphoproliferative disorder (PTLD) in pediatric kidney transplantation: a report of the North American Pediatric Renal Transplant Cooperative Study (NAPRTCS). *Transplantation* 2001; **71**: 1065.
8. Rees L, Tizard EJ, Morgan AJ, *et al.* A phase I trial of Epstein–Barr virus gp350 vaccine for children with chronic kidney disease awaiting transplantation. *Transplantation* 2009; **88**: 1025.
9. Kotton CN, Kumar D, Caliendo AM, *et al.* International consensus guidelines on the management of cytomegalovirus in solid organ transplantation. *Transplantation* 2010; **89**: 779.
10. Humar A, Lebranchu Y, Vincenti F, *et al.* The efficacy and safety of 200 days valganciclovir cytomegalovirus prophylaxis in highrisk kidney transplant recipients. *Am J Transplant* 2010; **10**: 1228.
11. Rostaing L, Weclawiak H, Mengelle C, Kamar N. Viral infections after kidney transplantation. *Minerva Urol Nefrol* 2011; **63**: 59.
12. Funch DP, Walker AM, Schneider G, Ziyadeh NJ, Pescovitz MD. Ganciclovir and acyclovir reduce the risk of posttransplant lymphoproliferative disorder in renal transplant recipients. *Am J Transplant* 2005; **5**: 2894.
13. European Best Practice Guidelines for Renal Transplantation (part 1). *Nephrol Dial Transplant* 2000; **15**(Suppl. 7): 1.
14. Bueno J, Ramil C, Green M. Current management strategies for the prevention and treatment of cytomegalovirus infection in pediatric transplant recipients. *Paediatr Drugs* 2002; **4**: 279.
15. Humar A, Michaels M. American Society of Transplantation recommendations for screening, monitoring and reporting of infectious complications in immunosuppression trials in recipients of organ transplantation. *Am J Transplant* 2006; **6**: 262.
16. Filler G, Lampe D, von Bredow MA, *et al.* Prophylactic oral ganciclovir after renal transplantation-dosing and pharmacokinetics. *Pediatr Nephrol* 1998; **12**: 6.
17. Bouw R, Vaudry W, Walker R, Ettenger RB, Jara P, Varela-Fascinetto G. Ganciclovir pharmacokinetics in pediatric solid organ transplant recipients after administration of valganciclovir oral solution. *Transplantation* 2006; **82**: 215 [Abstract].
18. Zhang D, Lapeyraque AL, Popon M, Loirat C, Jacqz-Aigrain E. Pharmacokinetics of ganciclovir in pediatric renal transplant recipients. *Pediatr Nephrol* 2003; **18**: 943.
19. Vaudry W, Ettenger R, Jara P, *et al.* Valganciclovir dosing according to body surface area and renal function in pediatric solid organ transplant recipients. *Am J Transplant.* 2009; **9**: 636.

20. Straus SE, Cohen JI, Tosato G, Meier J. NIH conference. Epstein–Barr virus infections: biology, pathogenesis, and management. *Ann Intern Med* 1993; **118**: 45.
21. Cohen JI. Epstein–Barr virus infection. *N Engl J Med* 2000; **343**: 481.
22. Plentz A, Jilg W, Kochanowski B, Ibach B, Knoll A. Detection of herpesvirus DNA in cerebrospinal fluid and correlation with clinical symptoms. *Infection* 2008; **36**: 158.
23. Vasudev B, Hariharan S, Hussain SA, Zhu YR, Bresnahan BA, Cohen EP. BK virus nephritis: risk factors, timing, and outcome in renal transplant recipients. *Kidney Int* 2005; **68**: 1834.
24. McGavin JK, Goa KL. Ganciclovir: an update of its use in the prevention of cytomegalovirus infection and disease in transplant recipients. *Drugs* 2001; **61**: 1153.
25. Faller DV, Mentzer SJ, Perrine SP. Induction of the Epstein–Barr virus thymidine kinase gene with concomitant nucleoside antivirals as a therapeutic strategy for Epstein–Barr virus-associated malignancies. *Curr Opin Oncol* 2001; **13**: 360.
26. Davis CL, Harrison KL, McVicar JP, Forg PJ, Bronner MP, Marsh CL. Antiviral prophylaxis and the Epstein Barr virus-related post-transplant lymphoproliferative disorder. *Clin Transplant*. 1995; **9**: 53.
27. Malouf MA, Chhajed PN, Hopkins P, Plit M, Turner J, Glanville AR. Anti-viral prophylaxis reduces the incidence of lymphoproliferative disease in lung transplant recipients. *J Heart Lung Transplant*. 2002; **21**: 547.
28. Opelz G, Daniel V, Naujokat C, Fickenscher H, Dohler B. Effect of cytomegalovirus prophylaxis with immunoglobulin or with antiviral drugs on post-transplant non-Hodgkin lymphoma: a multicentre retrospective analysis. *Lancet Oncol* 2007; **8**: 212.
29. Rickinson AB, Kieff E. Epstein–Barr virus. In: Knipe DM, Howley PM, Griffin DE, Martin MA, Lamb RA, Roizman B, eds. *Fields Virology*, 4th edn. Philadelphia, PA: Lippincott Williams & Wilkins, 2001: 2575–627.
30. Feigin RD, Cherry JD, Demmler GJ, Kaplan SL. *Textbook of Pediatric Infectious Diseases*, 5th edn. Houston: Saunders, 2004: 10934 pp.
31. Silins SL, Sherritt MA, Silleri JM, *et al.* Asymptomatic primary Epstein–Barr virus infection occurs in the absence of blood Tcell repertoire perturbations despite high levels of systemic viral load. *Blood* 2001; **98**: 3739.
32. Höcker B, Köpf S, Küsters U, *et al.* Surveillance of EBV infection as a risk factor for PTLD in pediatric renal transplant recipients – interim report from a multicenter prospective study. *Am J Transplant* 2007; **7**(Suppl. 2): 366.