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Balance between herpes viruses and immunosuppression after lung transplantation

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Chapter 12

Epstein-Barr virus-DNA load Monitoring Late after Lung Transplantation: A surrogate Marker of the Degree of Immunosuppression and a Safe Guide to Reduce Immunosuppression

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Abstract

Background Posttransplant lymphoproliferative disease (PTLD) is a serious complication after lung transplantation and its relation with Epstein-Barr virus (EBV) is well recognized. It has been postulated that pre-emptive reduction of immunosuppression guided by EBV-DNA load may lead to a significantly lower incidence of PTLD, because of the reconstitution of T-cell control.

In this report, we describe the efficacy of this approach in terms of PTLD prevention and safety with regard to the risk of acute as well as chronic allograft rejection in 75 lung transplant recipients transplanted between 1990 and 2001 and followed for this study from June 1, 2001 until January 1, 2006.

Methods From all patients visiting our outpatient clinic, EBV-DNA load was measured at least twice a year during the study period. In patients with positive results, measurements were repeated every 2 to 4 weeks. EBV reactivation was defined as two consecutive EBV-DNA load measurements with a rising trend; with the last measurement exceeding 10.000 copies/ml under *stable* immunosuppression. In such case, immunosuppression was reduced.

Results EBV reactivation was observed in 26/75 patients (35%). One (1.5%) of these patients developed PTLD during the study period. No acute rejection was observed after reduction of immunosuppression. EBV-reactivation and subsequent reduction of immunosuppression was not associated with a worse survival or acceleration of bronchiolitis obliterans syndrome.

Conclusions Pre-emptive reduction of immunosuppression after lung transplantation guided by EBV-DNA load appears to be a safe approach for the prevention of PTLD in lung transplant recipients late after transplantation.

Introduction

Over the last decade, lung transplantation has become a generally accepted and frequently applied treatment modality for end stage lung diseases. Although survival rates have significantly improved since the start of lung transplantation, especially in the early posttransplant period (1), long term survival is still hampered by acute as well as chronic complications.

The major acute complications are infections and acute rejection, whereas chronic allograft dysfunction (bronchiolitis obliterans syndrome (BOS)) accounts for the majority of late transplant related morbidity and mortality (1). Also, the development of lymphoma's associated with Epstein-Barr virus (EBV) in the posttransplant host (posttransplant lymphoproliferative disease, PTLD) is frequently observed in lung transplant recipients (2).

PTLD encompasses a heterogeneous group of lymphoproliferative diseases, ranging from Epstein-Barr virus (EBV) driven polyclonal proliferation resembling infectious mononucleosis to highly aggressive monomorphic proliferations which may be indistinguishable from aggressive types of lymphoma such as diffuse large B-cell lymphoma (3). Generally, PTLD is considered an iatrogenic complication of immunosuppression after transplantation, leading to decreased function of EBV specific T-cells, which in turn may lead to uncontrolled proliferation of EBV infected B-cells (4).

Reported incidence of PTLD after lung transplantation varies from 5 up to 15 percent (2,5,6). Major risk factors for development of PTLD include the amount and intensity of immunosuppression after transplantation, especially induction and rejection therapy (7-9), and EBV seronegativity of the recipient before transplantation (leading to primary EBV infection) (8).

With the increasing emphasis on the role of EBV in PTLD, much effort has been put on developing methods that can identify patients at risk for developing PTLD by measuring the amount of circulating EBV-DNA in the peripheral blood of transplant recipients (10,11). Elevation of EBV-DNA load in the circulation is considered to reflect aberrant EBV induced B-cell proliferation due to impaired EBV T-cell control. It has already been shown that transplant recipients with PTLD have a significantly higher EBV-DNA load when compared with transplant recipients without PTLD or the non-transplant population, and that a high EBV-DNA load is associated with PTLD development (12,13).

It has been postulated that pre-emptive reduction of immunosuppression may lead to a significantly lower incidence of PTLD, because of the reconstitution of T-cell control. Recent results, indeed, suggest that this

approach is effective, at least in paediatric liver transplant recipients where incidence of PTLD decreased significantly after pre-emptive reduction of immunosuppression (14,15). However, a possible complication of reduction of immunosuppression is allograft rejection, which may be especially relevant in lung transplant recipients because of the lack of alternative organ replacement therapy.

At our centre, routine EBV-DNA measurements and subsequent pre-emptive reduction of immunosuppression has been applied to all lung transplant recipients transplanted since June 2001.

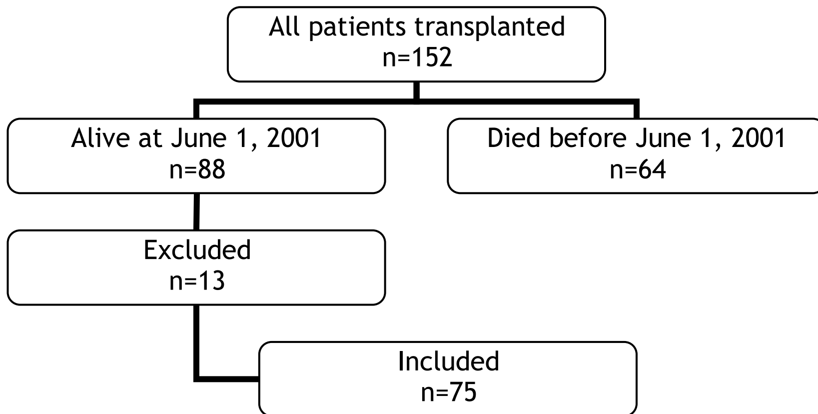
The same protocol was also applied to all patients transplanted since the start of the program in 1990 until June, 2001 and still alive at that date. In this report, we describe the efficacy of this approach in terms of PTLD prevention and safety with regard to development of acute as well as progressive chronic allograft rejection in this group of patients. Along with these issues, we describe the frequency and dynamics of EBV reactivations occurring late after lung transplantation.

Patients and Methods

Patients

Routine EBV-DNA load measurements and subsequent reduction of immunosuppression based on EBV-DNA load were introduced at our centre from June 1, 2001 onwards. Since then, all adult lung transplant recipients, transplanted between November 1990 (start of the transplant program) and June 2001, and alive at June 1, 2001, were also treated according to this protocol. Of the 152 patients transplanted in this period, 88 patients were alive at June 1, 2001 (Fig. 1). Of these 88 patients, 13 patients were excluded from analysis. Reasons for exclusion were: i. follow-up elsewhere (n=4), ii. dying within 2 months after inclusion date (no EBV-DNA measurements available, n=6), iii. Grade of BOS could not be evaluated because of concomitant disease (n=3). So, ultimately 75 patients were included who all had a complete follow-up until January 1, 2006 or until death. None of the patients was lost to follow-up during the study period.

***Figure 1**: Flow-chart illustrating patient selection.*



Baseline patient characteristics

The following parameters were recorded from all patients at June 1, 2001: recipient age at transplantation, sex, type of lung transplant (unilateral/bilateral), BOS grade (according to ISHLT criteria, (16)), initial immunosuppressive regimen, conversion to another immunosuppressive regimen before June 1, 2001, treatment of rejection, pre-transplant serostatus of CMV of donor and recipient, active primary or secondary CMV infection after transplantation (defined as positive CMV antigenemia (17)), recipient pre-transplant EBV status, and time after transplantation. Baseline patient characteristics are shown in table 1.

Table 1: Baseline patient characteristics at June 1, 2001

	N	%
Patients	75	100
Sex (male)	35	47
Age at inclusion date* (year)	48	(19-68)
Time after transplantation* (year)	4.25	(0.10-10.13)
Bilateral transplant	64	85
EBV negative pre transplantation	2	3
CMV match**		
neg/neg	15	20
neg/pos	14	19
pos/neg, pos/pos	46	61
Active CMV infection after transplantation		
no	22	29
primary infection	13	17
secondary (reactivation)	40	54
Rejection treatment***		
no	18	24
steroids	53	71
ATG/OKT3	4	5
PTLD before June 1, 2001	3	4
BOS grade at June 1, 2001		
BOS 0	57	76
BOS 1	10	13
BOS 2	2	3
BOS 3	6	8
BOS 1,2,3	18	24
Initial Immunosuppression		
Cyclo, Aza, Pred	70	93
Cyclo, MMF, Pred	5	7
Conversion of immunosuppression		
no	50	67
cyclosporine > tacrolimus	15	20
and Azathioprine > MMF	10	13

*median (range); ** serum CMV IgG antibodies recipient/donor pair *** rejection treatment > 1 month after transplantation

Immunosuppressive protocols

All patients received immunosuppressive induction treatment with 3 mg/kg rabbit-antithymocyte globulin (rATG, Thymoglobulin; Pasteur-Merieux, Lyon,

France), 2-5 times after transplantation. Immunosuppressive maintenance therapy consisted of cyclosporine A (dose adjusted to whole blood trough levels of 400 µg/L, tapering to levels of 150 µg/L after 3 weeks), azathioprine (1.5-3 mg/kg/d) and prednisolone (3 times 125 mg the first day, 0.2 mg/kg/d from day 2 to the third month, and 0.1 mg/kg/d thereafter). Furthermore, all transplant recipients received 960 mg Co-trimaxozole on alternate days for Pneumocystis jerovici prophylaxis (previously Pneumocystis carinii), and 200 mg acyclovir four times a day for 6 months.

Acute allograft rejection was diagnosed clinically in case of deteriorating pulmonary function without infection or airway complications and with a positive response on high-dose methylprednisolone. Histological diagnosis of rejection was defined according to Yousem et al. (18). Episodes of acute rejection were treated with pulse therapy of methylprednisolone (500-1000 mg/d IV for 3 days). Recurrent rejection was treated by replacement of cyclosporine A by tacrolimus (Prograf; Astellas) and subsequently, replacement of azathioprine by mycophenolate mofetil (cellcept; Roche). In case of an insufficient response (further decline in lung function), Muromonab-CD3 (Orthoclone OKT3, Ortho Pharmaceutical Corporation, Biotech Division, Raritan, NJ) (20 mg for 10 subsequent days) or horse antithymocyte globulin (hATG, Lymfoglobuline, Pasteur-Merieux, Lyon, France) was administered (5 infusions, 100-300 mg/day, in ten days).

Active CMV infection was diagnosed by positive pp65-antigenemia (tested weekly after transplantation during admission and at all outpatient visits) and treated with IV gancyclovir (Cymevene, Roche) or foscarnet (Foscavir, Astra Pharmaceuticals, Wilmington, DE) until two weeks after pp65-antigenemia levels dropped below the limit of detection.

EBV-DNA measurements

From all patients visiting our outpatient clinic, EBV-DNA load was determined twice a year during routine follow-up and during admission because of complications. In all patients with positive EBV-DNA load measurements (>2.000 copies/ml, lower limit of detection), EBV-DNA load was subsequently determined every two to four weeks thereafter until it was not detectable anymore.

Before June 2003, a semi quantitative EBV-DNA PCR was used, which has been described previously (13). After June 2003, a real time TaqMan quantitative PCR was introduced. The BioRobot EZ1 Robotic workstation (Qiagen) was used for automated DNA purification (EZ1 DNA Blood Card). DNA was extracted from 200-µl portions of whole blood and eluted in 200µl of buffer AE (Qiagen).

For the TaqMan PCR assay a primer-probe set for EBNA-1 was used; the nucleotide sequences (5′→3′) were as follows: upstream primer, CCGGTGTGTTCGTATATGG; downstream primer, AAAGGGGAGACGACTCAATG; and minor groove binding (MGB) probe, CTATTCCACAATGTCGTCTTA, designed with Primer express software version 1.5.

For the PCR, the ABI prism 7900 HT-RealTime-PCR system (384 wells) was used. Samples of 10 µl whole blood were used as input in the PCR, while 10 µl of exogenous internal positive control mix (Applied Biosystems) was used as a template to identify possible inhibition of the PCR. The concentration of EBV-DNA was determined from a reference standard quantified by electron microscopy (Advanced Biotechnologies Incorporated, Columbia, US). The viral load was expressed as the number of copies per millilitre. Each sample was tested in fourfold and EBV-DNA load was expressed as the mean of these four samples.

Before this new PCR was introduced, both tests were compared with each other for reproducibility of results which showed no difference for both methods (data not shown).

EBV reactivation and intervention

EBV reactivation was defined as two consecutive EBV-DNA load measurements with a rising trend; with the last measurement exceeding 10.000 copies/ml under *stable* immunosuppression. In such case, immunosuppression was lowered according to our protocol. The first step was reduction of the proliferation inhibitor (azathioprine or mycophenolate mofetil was reduced with 50%) and instigation of antiviral therapy (valacyclovir, 1000 mg, 3x daily). In case of an insufficient response (no decrease in EBV-DNA load), azathioprine or mycophenolate mofetil was stopped.

Statistics

Endpoints in this study were overall survival (OS) and freedom from BOS progression (FFBP). Overall survival and FFBP were measured from inclusion date (June 1, 2001) until date of event; patients without event were censored at date of last follow-up. For OS, an event was defined as death due to any cause. For FFBP, an event was defined as progression to a higher BOS grade compared with BOS grade at inclusion date.

Continuous variables were expressed as mean with standard deviation or median with range, and categorical variables as counts and percentages. Shapiro-Wilk test, together with normality plots, were used to assess normal distribution of the continuous variables. Differences between groups were evaluated by

Student's t-test or Mann-Whitney U test for continuous data and by Fisher's exact test or Chi-Square test for categorical data. Survival curves were depicted by the method of Kaplan–Meier. Hazard ratios (HR) for survival and freedom from BOS progression were obtained using Cox proportional hazard survival analysis. In a multivariable analysis all clinically relevant covariates significant at a p-level of 0.10 were included in the initial model. A backward elimination strategy was used to achieve the most suitable model to estimate the hazard ratios. In addition, possible risk factors for EBV reactivation were evaluated by multivariable logistic regression.

The response to reduction of immunosuppression (indicated as EBV-DNA load at 1, 3, 6 and 12 months after intervention) was graphically presented. The extent of changes over time was evaluated by repeated measures analysis.

A two-tailed p-value of less than 0.05 was considered to indicate statistical significance. All analyses were performed using SAS software, version 9.1 (SAS-Institute inc., Cary, North Carolina, USA).

Results

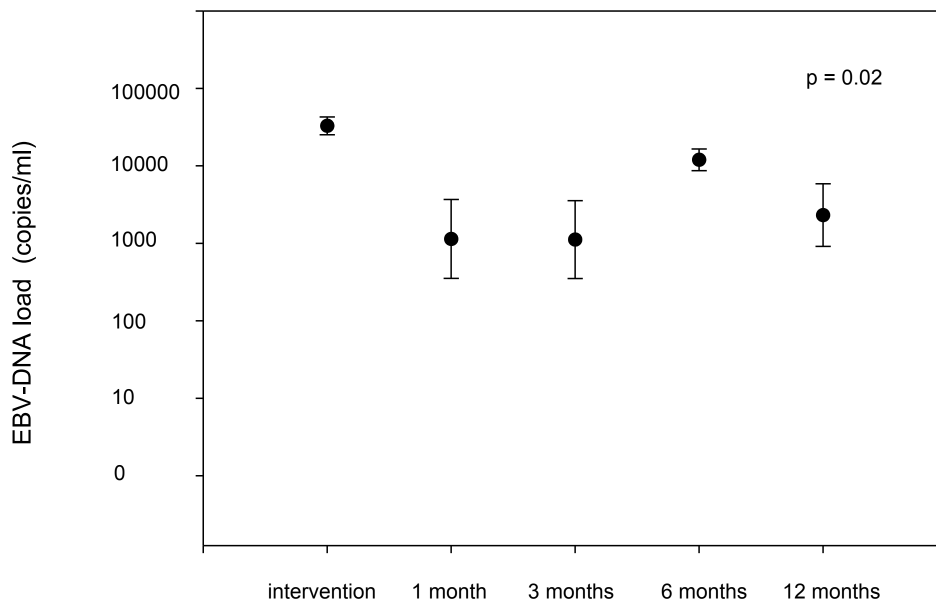
EBV reactivation

EBV reactivation was observed in 26 (35%) of all patients during the study period. Of these, 19 were treated with reduction of immunosuppression according to our treatment protocol (Fig. 2). In seven patients lowering immunosuppression was not regarded safe as immunosuppression was already lowered before for other reasons (n=5), or severe concomitant disease or suspicion of rejection was present (n=2).

PTLD development and acute rejection

Of the 75 patients, one (1.5 %) with an EBV reactivation developed PTLD 9 years after transplantation (25 months after date of study inclusion). Despite reduction of immunosuppression according to the protocol, the patient deteriorated and died. Diagnosis of PTLD was based on post mortem examination, which showed a massive tumour mass in the patients' abdomen. Immunohistochemistry showed an EBV *negative* PTLD, without expression of CD-20, which was classified as diffuse large B-cell lymphoma type according to the WHO classification (19). None of the patients with EBV reactivation developed acute rejection following reduction of immunosuppression.

***Figure 2**: Dynamics of EBV-DNA load measured from date of intervention onwards at 1, 3, 6 and 12 months after intervention (mean +standard error). Overall, a significant reduction (p=0.02) of EBV-DNA load was observed.`*



Response to reduction of immunosuppression

To assess whether reduction of immunosuppression was associated with a concomitant decrease in EBV-DNA load, a repeated measures analysis was performed.

After a sharp decrease in EBV-DNA load 1 month after intervention, patients tended to stabilize, after which a rising trend was observed 6 months after intervention (Fig. 2). Overall, there was a significant reduction of EBV-DNA load (p=0.02) after reduction of immunosuppression.

Survival

To assess whether EBV reactivation (and subsequent reduction of immunosuppression) affected overall survival, a Cox regression analysis was performed (Table 2). In univariate analysis, EBV reactivation was associated with a worse outcome (p=0.04). Based on univariate analysis, also the presence of BOS (shown as BOS 0 versus BOS grade 1, 2 or 3) at inclusion date (p<0.001), conversion of immunosuppression (p=0.03) and unilateral transplant (p=0.08) were included in the multivariable analysis (Table 2). In multivariable analysis, only the presence of BOS at inclusion date remained as negative predictive factor for overall survival in our patients. When comparing patients with and without

reduction of immunosuppression at date of EBV reactivation, no significant differences in survival were observed (data not shown).

BOS progression

We also analyzed which factors were predictive for BOS progression during the study time. Based on univariate analysis, the presence of BOS at inclusion date (p=0.001), active CMV infection (p=0.01) and a history of rejection treatment (p=0.02) were included in the multivariable analysis (Table 3). In multivariable analysis, only the presence of BOS at inclusion date remained as predictive factor for BOS progression in our patients (p=0.007). There was a trend for active CMV infection as predictive factor for BOS progression, but this was not significant (p=0.06). EBV reactivation (and subsequent reduction of immunosuppression) was not associated with BOS progression (p=0.15).

Risk factors for EBV reactivation

Based on univariate analysis, the time after transplantation (p=0.003), unilateral transplantation (p=0.04), active CMV infection (p=0.03) as well as conversion of immunosuppression (p=0.06) were included in the multivariable analysis (Table 4).

In multivariable analysis, only the time after transplantation was independently associated with EBV reactivation (p=0.003). The median time between transplantation and study inclusion date was 5.94 years (range 0.56-10.13 year) in patients with EBV reactivation versus 2.83 years (range 0.10-9.35 year) in patients without EBV reactivation (data not shown). In the three patients with a previous history of PTLD, no EBV reactivation was observed during the study time frame. In these three patients, immunosuppression was already reduced as part of PTLD treatment.

Table 2: Risk factors for mortality

Risk Factor	RR*	95% CI	*P*
Univariate Cox regression			
Male	0.92	0.36-2.34	0.87
Age at inclusion date	1.00	0.96-1.04	0.93
Time after transplantation (years after inclusion date)	1.09	0.92-1.29	0.31
Unilateral transplant (versus bilateral)	2.56	0.90-7.14	*0.08*
recipient EBV status pre-transplantation (negative)	NE**	NE	NE
CMV match			0.27
neg/neg	1.00		
neg/pos	5.87	0.69-50.31	0.11
pos/neg, pos/pos	4.24	0.55-32.61	0.17
Active CMV infection after transplantation			0.71
no	1.00		
primary infection	1.78	0.44-7.11	0.42
secondary (reactivation)	1.46	0.46-4.64	0.53
Rejection treatment***			0.20
no	1.00		
steroids	6.35	0.84-47.91	0.07
ATG/OKT3	5.77	0.36-92.32	0.22
PTLD before June 1, 2001	NE**	NE	NE
BOS 1,2,3	5.05	1.98-12.82	<0.001
Initial Immunosuppression			
Cyclo, Aza, Pred (standard)	1.00		
Cyclo, MMF, Pred	2.01	0.46-8.77	0.35
Conversion of immunosuppression			0.03
no	1.00		
Cyclo > tacrolimus	2.12	0.69-6.49	0.19
and Aza > MMF	4.69	1.53-14.40	0.007
EBV reactivation	2.63	1.04-6.67	0.04
Multivariable Cox regression			
Unilateral transplant	2.26	0.79-6.43	0.13
BOS 1,2,3	4.67	1.82-11.90	<0.001
EBV reactivation	1.98	0.77-5.13	0.15

*RR= 1.00 indicates reference group **Not evaluable (too few events to calculate RR)
*** rejection treatment > 1 months after transplantation

***Table 3**: Risk factors for BOS progression*

Risk Factor	RR*	95% CI	*P*
Univariate Cox regression			
Sex (male)	1.20	0.55-2.50	0.68
Age at inclusion date (June 1, 2001)	1.01	0.98-1.05	0.53
Time after transplantation (years after inclusion date)	1.05	0.91-1.20	0.51
Unilateral transplant (vs bilateral)	1.77	0.71-4.41	0.22
Recipient EBV status pre-transplantation (negative)	NE**	NE	NE
Active CMV infection after transplantation			*0.01*
no	1.00		
primary infection	2.65	1.02-6.90	0.05
secondary (reactivation)	0.68	0.27-1.73	0.42
Rejection treatment***			*0.02*
no	1.00		
steroids	2.03	0.69-5.96	0.20
ATG/OKT3	7.19	1.79-28.81	0.005
PTLD before June 1, 2001	NE**	NE	NE
BOS 1,2,3	3.62	1.66-7.92	*0.001*
Initial Immunosuppression			0.68
Cyclo, Aza, Pred (standard)	1.00		
Cyclo, MMF, Pred	0.65	0.09-4.83	
Conversion of immunosuppression			0.19
no	1.00		
Cyclo > tacrolimus	1.98	0.84-4.67	0.12
and Aza > MMF	2.15	0.71-6.52	0.18
EBV reactivation	1.49	0.69-3.22	0.31
Multivariable Cox regression			
Active CMV infection after transplantation			0.06
no	1.00		
primary infection	2.09	0.79-5.55	0.14
secondary infection	0.68	0.27-1.73	0.42
BOS 1,2,3	3.03	1.34-6.71	0.007

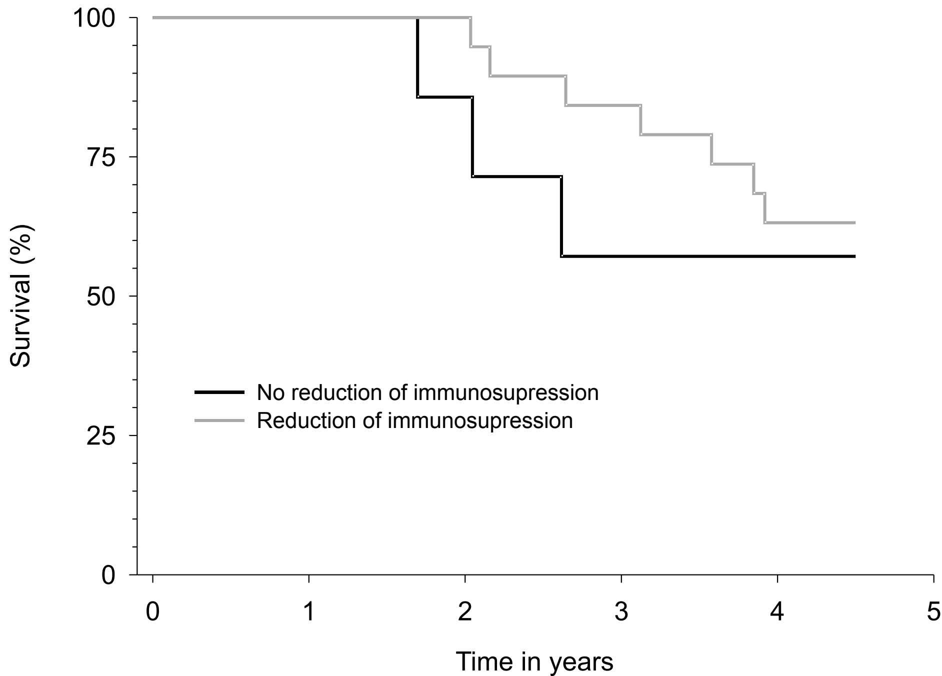
*Relative risk 1.00 indicates reference group **Not evaluable (too few events to calculate RR)*** rejection treatment > 1 month after transplantation

***Table 4**: Risk factors for EBV reactivation*

Risk Factor	RR*	95% CI	*P*
Univariate logistic regression			
Sex (male)	0.81	0.31-2.11	0.67
Age at inclusion date (June 1, 2001)	1.12	0.43-2.95	0.82
Time after transplantation (years after inclusion date)	1.35	1.08-1.66	*0.003*
Unilateral transplant (vs bilateral)	0.24	0.06-0.92	*0.04*
Recipient EBV status pre-transplantation (negative)	NE**	NE	NE
Active CMV infection after transplantation			*0.03*
no	1.00		
primary infection	5.43	1.12-32.26	0.04
secondary (reactivation)	4.67	1.32-22.22	0.02
Rejection treatment***	1.88	0.69-8.53	0.21
PTLD before June 1, 2001	NE**	NE	NE
BOS 1,2,3	0.58	0.20-1.71	0.32
Initial Immunosuppression			0.49
Cyclo, Aza, Pred (standard)	1.00		
Cyclo, MMF, Pred	2.22	0.24-20.98	
Conversion of immunosuppression			*0.06*
no	1.00		
Cyclo > tacrolimus	3.50	1.08-12.20	0.04
and Aza > MMF	0.58	0.08-2.68	0.52
Multivariable logistic regression			
Time after transplantation	1.35	1.08-1.66	*0.003*

*Relative risk 1.00 indicates reference group **Not evaluable (too few events to calculate RR)*** rejection treatment > 1 month after transplantation

***Figure 3**: Survival of patients with EBV reactivation; a comparison between patients with (n=19) and without (n=7) reduction of immunosuppression.*



Discussion

The present report aimed to describe the efficacy reduction of immunosuppression guided by EBV-DNA load in terms of PTLD prevention and safety with regard to development of acute as well as progressive chronic allograft rejection in a group of patients late after lung transplantation.

The rationale behind reduction of immunosuppression in patients with EBV reactivation was based on prevention of PTLD development in these patients. Because of the heterogeneous composition of our cohort and the lack of a comparable control group, it cannot be firmly concluded from our data that the institution of this new protocol has led to a lower incidence of PTLD. However, the observed incidence of 1.5% during the study period in our group is lower than that reported in the literature, also taken in account the interval between transplantation and the start of the study (1). It seems, therefore, that this approach is, at least, helpful for the prevention of PTLD developing late after transplantation.

We can, however, conclude from our data that reduction of immunosuppression was safe in this heterogeneous group of patients late in their clinical course, as none of the patients developed acute rejection following reduction of immunosuppression. This is in concordance with previous reports in paediatric liver transplant recipients (14,15).

Furthermore, EBV reactivation and subsequent reduction of immunosuppression were not associated with a worse survival or with progression of BOS in our group of patients.

In a recent paper, Savoldo et al. postulate that, because of the potentially harmful effects of reduction of immunosuppression to the graft, infusion of EBV-specific cytotoxic T-lymphocytes (CTL) for the prevention of PTLD development may be a safer approach as it could spare both the patient and the graft (20). Our observations, however, suggest that a careful stepwise reduction of immunosuppression guided by EBV-DNA load is a safe and effective initial approach in lung transplant recipients and that there is only a minimal chance of graft loss.

We hypothesize that a rising EBV-DNA load in the transplant recipient can be considered to reflect a general state of decreased T-cell surveillance as a result of, probably, overimmunosuppression. If this is he case, only a minimal chance of allograft rejection is present when reduction of immunosuppression is applied. Some observations support this hypothesis. EBV negative PTLD cases, that is negative staining for EBV in the tumour as observed for the only case of PTLD in this study, may develop simultaneously with a sharp increase in peripheral blood EBV-DNA load (21). This observation suggest that, although increased EBV-DNA load is generally considered to represent an increase in circulating EBV-positive tumour cells, rising EBV-DNA load may here result from a population of proliferating B-cells, apart from PTLD development. Rising EBV-DNA load may, thus, be considered as a surrogate marker for depressed T-cell function and, possibly, overimmunosuppression. The observation that a rise in EBV-DNA load is associated with impaired T-cell control of EBV (22,23) further supports this hypothesis. If this is correct, reduction of immunosuppression is, probably, the safest and most effective preventive therapy in patients considered to be at high risk for PTLD development.

To assess the efficacy of reduction of immunosuppression in terms of EBV-DNA load, we conducted a repeated measures analysis (Fig. 2). Overall, we observed a significant decrease in EBV-DNA load ($p=0.02$), which suggests that reduction of immunosuppression indeed leads to a decrease in EBV-DNA load, probably by reconstitution of EBV specific T-cell surveillance, consequently lowering the risk for PTLD development. Although we observed a moderate

increase in EBV-DNA load 6 months after reduction of immunosuppression, EBV-DNA load remained low, even 12 months after reduction of immunosuppression.

In this study we furthermore showed that EBV reactivation is a frequent event after lung transplantation, and occurs also *late* after transplantation. These results indicate that EBV reactivation constitutes a life long risk in the posttransplant host, and that EBV-DNA load measurements should be consequently performed, also late after transplantation. Furthermore, it is interesting that in none of the three patients with a history of PTLD, EBV reactivation was observed. One could, however, hypothesise that this was the result of the already lowered immunosuppression in these patients because of PTLD in the past.

A limitation of this study may be the fact that there obviously is a selection bias as we have excluded patients not alive at the study inclusion date. We cannot exclude that these patients, who had been transplanted and had been died between 1990 and 2001, had been at an increased risk for EBV reactivation and, consequently, PTLD development. As shown in figure 1, 64 out of the 152 patients who were transplanted during that period, died and 13 out of these 64 patients had developed PTLD. Together with the three surviving cases with PTLD, this leads to an incidence of 16 cases of PTLD in this cohort of 152 patients during the period from 1990 to 2001, in which levels of immunosuppression were not guided by EBV-DNA load. The present data should be evaluated in view of this selection bias.

In conclusion, our findings demonstrate that i. EBV reactivation after lung transplantation is a frequent event, also late after transplantation. ii. Reduction of immunosuppression in patients with an EBV reactivation appears to be safe with respect to acute as well as progression of chronic allograft rejection and survival. iii. Reduction of immunosuppression in patients with an EBV reactivation may prevent PTLD development.

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