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Balance between herpes viruses and immunosuppression after lung transplantation

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Chapter 11

Low rate of Bronchiolitis Obliterans Syndrome following Pre-emptive Treatment of Post-Transplant Lymphoproliferative Disease after Lung Transplantation

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Submitted

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Abstract

Results of lung transplantation are hampered by both chronic rejection, accounting for 30% of mortality after the first year, and development of malignancies such as post transplant lymphoproliferative disease (PTLD). Development of PTLD has been shown to be related to Epstein-Barr virus (EBV) - DNA load, and pre-emptive strategies to prevent PTLD have been suggested. In June 2001, we started a new immunosuppressive protocol for lung transplant patients including pre-emptive reduction of immunosuppression based on EBV-DNA load. In this report we describe the results of this new protocol with a follow-up of 4.5 years.

Of the 94 patients included, 12 developed EBV reactivation which was approached by reduction of immunosuppression. A significant ($p < 0.001$) decrease in EBV-DNA load was observed after reduction of immunosuppression. Only one case of PTLD was seen in 94 patients treated according to this new protocol. This contrasted to the 16 cases of PTLD out of 152 patients treated according to the previous protocol ($p = 0.10$). Most importantly, pre-emptive strategy for PTLD proved safe, as none of the patients in whom immunosuppression was reduced developed bronchiolitis obliterans syndrome (BOS), and only one treatable episode of acute rejection was seen during the phase of reduced immunosuppression. Overall, a remarkably low rate of BOS ($< 10\%$ after 4.5 years) was seen in patients treated according to this new protocol ($P < 0.0003$ versus the occurrence of BOS during the previous protocol). Survival, from four months after transplantation, also significantly improved ($p < 0.05$). We conclude that EBV-DNA guided pre-emptive reduction of immunosuppression is a safe procedure after lung transplantation and may not only reduce the incidence of PTLD but also improve the BOS free survival.

Introduction

Lung transplantation has become a generally accepted and frequently applied treatment modality for end stage lung diseases. Survival, although improving, is still limited due to acute as well as chronic complications, and is much lower than that of other solid organ transplantations (1-4).

This lower survival rate is partly due to higher peri-operative mortality. However, acute rejection and chronic allograft dysfunction (bronchiolitis obliterans syndrome (BOS)), also occurs much more frequently when compared with other solid organ transplantations, with BOS accounting for over 30% of mortality after the third postoperative year(5;6). This, and the fact that there is no alternative therapy for lung transplantation, is the reason that levels of immunosuppression after lung transplantation are higher than used in other solid organ transplantations.

Related to this high level of immunosuppression, results of lung transplantation are also hampered by the development of posttransplant lymphoproliferative disease (PTLD) (7-9). Reported incidence of PTLD after lung transplantation varies from 5 to 15 percent, and PTLD is associated with high morbidity and mortality (7-9).

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 lymphomas such as diffuse large B-cell lymphoma (10). Generally, PTLD is considered to be an iatrogenic complication of immunosuppression-driven dysfunction of EBV specific T-cells, which in turn may lead to uncontrolled proliferation of EBV infected B-cells (11).

Previously, we and others have shown a relation between the development of PTLD and EBV-DNA load in the peripheral blood (12-15). We also observed that peripheral blood EBV-DNA can often be detected long before the development of PTLD (15). This offers the possibility of early identification of patients at risk and a subsequent pre-emptive strategy to prevent PTLD.

It has been postulated that pre-emptive reduction of immunosuppression may lead to a lower incidence of PTLD, by reconstitution of T-cell control. Recent data, indeed, suggest that this approach is effective: in paediatric liver transplant recipients the incidence of PTLD decreased after pre-emptive reduction of immunosuppression (16;17). This strategy, however, bears the obvious risk of allograft rejection, which is highly relevant in “rejection prone” lung transplant recipients (5;6).

Based on these considerations and on our experience with EBV-infection, we embedded EBV-DNA guided pre-emptive reduction of immunosuppression in our lung transplant protocol revision of 2001. In this report efficacy (i.e. prevention of PTLD) and safety (i.e. rate of acute rejection and BOS) in a series of 94 consecutive lung transplant recipients is described.

Patients and Methods

Patients and baseline characteristics

From the introduction of routine measurement of EBV-DNA load at our centre (June 1st, 2001) until January 1st, 2006, 94 consecutive adult lung transplant recipients were included (“new protocol patients”) (Table 1).

Table 1: *Baseline characteristics of all patients treated according to the new and old protocol.*

Alpha-1 AT def = alpha-1-antitrypsin deficiency, BE = Bronchiectasies, R/D = Recipient/Donor

Patients	New protocol		Old Protocol		P-value
	n	(%)	n	(%)	
Patients	94	100	152	100	
Age at transplantation (year, median, range)	50	(18-68)	45	(19-64)	0,005
Indication for transplantation					0.28
Emphysema incl. Alpha-1-AT def.	40	43	72	47	
Cystic Fibrosis/BE	26	28	43	28	
Pulmonary Fibrosis/ Sarcoidosis	20	21	20	14	
Primary Pulmonary hypertension	7	7	17	11	
other	1	1			
Gender					0.90
male	51	54	85	44	
female	43	46	67	56	
Bilateral/unilateral lung transplant					0.051
unilateral	26	28	25	16	
bilateral	68	72	127	84	
EBV status pre-transplant					0.03
Positive	93	99	141	93	
Negative	1	1	11	7	
Matching for CMV pre-transplant					0.20
R/D negative/negative	24	26	52	34	
R/D negative/positive	25	27	28	18	
recipient positive	45	48	72	47	

All adult lung transplant recipients transplanted before June 1st 2001 (n=152) served as a control population (“old protocol patients”). None of these patients was lost to follow-up during the study period.

The following parameters were available from all patients: recipient age at transplantation, gender, unilateral vs. bilateral lung transplantation, indication for transplantation, BOS stage at endpoint of the study (according to ISHLT criteria)(18), conversion of immunosuppressive maintenance regimen, rejection treatment, CMV serostatus of donor and recipient, active primary or secondary CMV infection after transplantation (defined as a positive CMV antigenemia (19)), recipient pre-transplant EBV status, and follow-up time after transplantation. In addition, prospective EBV-DNA measurements were available for the patients treated according to the new protocol starting June 1st, 2001. Baseline patient characteristics are shown in table 1.

Immunosuppressive protocols

Old protocol, patients transplanted between November 1990 and June 1st, 2001:

Immunosuppressive induction therapy consisted of 3-5 gifts of rabbit-antithymocyte-globulins, 3 mg/kg, (Merieux, France) during the first 10 days after transplantation. Maintenance immunosuppressive regimen consisted of cyclosporine-A, azathioprine (2 mg/kg/d), and prednisolone (0.2 mg tapering to 0.1 mg/kg/d after 3 months). Cyclosporine A administration was aimed at a trough level of 400 ng/ml at the start, as determined by high-performance liquid chromatography, which was tapered in 3 weeks to 150 ng/ml.

Acute rejection, defined as transplant dysfunction not explained by other causes (e.g. infection), was treated with pulse methylprednisolone intravenously, 500 to 1000 mg for 3 days. This was done with or without histological confirmation. In patients with recurrent rejection or development of BOS stage 1 immunosuppression was converted. Initially, cyclosporine-A was changed to tacrolimus, and in case of progressive transplant deterioration azathioprine was converted to mycophenolate mofetil (MMF). In case of steroid resistant rejection horse-ATG was administered.

Additionally, all patients received, during the first 3 months after transplantation, acyclovir 200 mg qd orally for herpes prophylaxis, and co-trimoxazole, 960 mg orally on alternate days for pneumocystis jirovecii prophylaxis (previously pneumocystis carinii). A pre-emptive strategy was used for treatment of CMV infection with routine measurements of CMV antigenemia.

New Protocol, patients transplanted between June 1st, 2001 and January 1st, 2006:

Induction treatment consisted of 2 gifts of Basiliximab 20mg on day 0 and 4 after transplantation. Maintenance immunosuppressive regimen consisted of

tacrolimus aiming at trough levels of 18 to 20 ng/ml during the first 3 weeks, then 13 to 15 ng/ml until 3 months, and 10 to 12 ng/ml thereafter. Azathioprine and prednisolone remained unchanged as to the old protocol.

Acute rejection was treated with pulse methylprednisolone intravenously, 1000 mg for 3 days. In patients with recurrent rejection or development of BOS stage 1 azathioprine was converted to mycophenolate mofetil (MMF). In case of steroid resistant rejection and failure to respond to conversion of azathioprine to MMF, OKT3 was administered.

CMV prophylaxis was given. Patients at risk for CMV infection (that is in case a recipient and/or donor was seropositive) received 3 months treatment of valganciclovir (900 mg od). Patients not at risk (seronegative match) received 3 months of acyclovir, 200 mg qd. Co-trimoxazole 960 mg orally on alternate days for pneumocystis jirovecii prophylaxis (previously pneumocystis carinii) remained unchanged.

EBV measurements

From all patients EBV-DNA load was determined weekly after transplantation during admission and at every outpatient visit during the first post transplant year. Thereafter, EBV-DNA load measurements were performed at routine follow-up twice a year. In all patients with positive EBV-DNA load measurements (>2.000 copies/ml, lower limit of detection), EBV-DNA load was consistently determined at every outpatient visit until EBV-DNA load became undetectable.

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- μ l portions of whole blood and eluted in 200 μ l 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, CCGGTGTGTTTCGTATATGG; 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 μ l whole blood were used as input in the PCR, while 10 μ l 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).

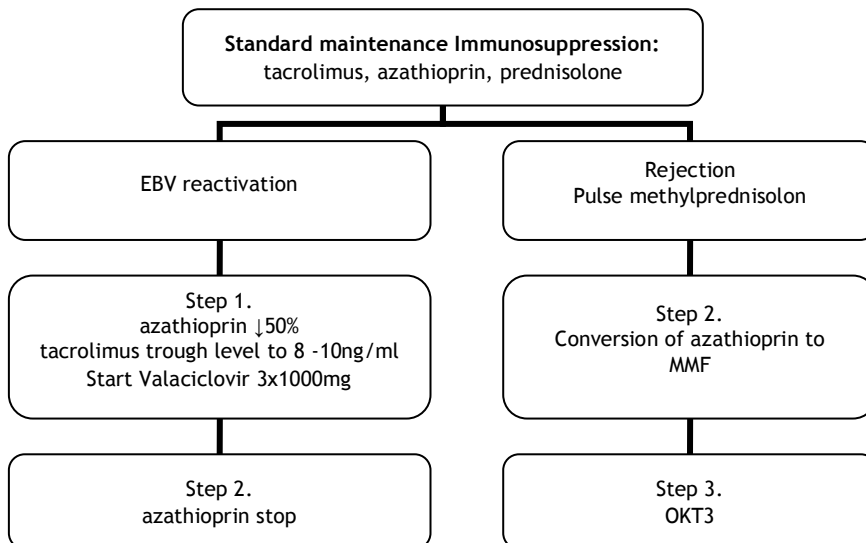
EBV re-activation and immunosuppressive intervention protocol

EBV re-activation was defined as two consecutive EBV-DNA load measurements with a rising trend compared to the previous sample, with the last sample exceeding 10.000 copies/ml (temporary rises after rejection treatment with OKT3 were excluded). If this occurred, immunosuppression was lowered according to the protocol (Fig. 1). The first step was reduction of the dose of azathioprine with 50% and instigation of antiviral therapy (Valaciclovir, 1000 mg td). In case of a further rise in EBV-DNA load, azathioprine was stopped.

On the other side, immunosuppression was augmented if a patient developed a late rejection. Acute rejection during the first 3-4 weeks after transplantation was treated with pulse methylprednisolone while maintenance immunosuppression was not changed. A histologically proven acute rejection thereafter, or a clinically defined rejection, after exclusion of other causes of symptoms (e.g. infection), led to conversion of azathioprine to MMF. In case of steroid resistant rejection OKT3 was used (Fig. 1).

BOS was defined and graded I to III according to ISHLT criteria (18). For comparison of frequency of rejection between the patients with and without EBV reactivation treated according to the “new protocol” (Table 2), only rejections that occurred later than three weeks after transplantation were evaluated. This approach was chosen since we aimed to evaluate late rejections as a marker of failure of maintenance immunosuppression and since EBV reactivations occur usually later than 3 weeks after transplantation.

Figure 1: Intervention scheme of immunosuppression as used in the “new protocol”. In case of EBV reactivation the left arm was used and in case of rejection, patients were treated according to the right arm.



Statistics

Endpoints in this study were overall survival (OS), freedom from PTLD development and freedom from BOS. Overall survival, freedom from PTLD and freedom from BOS were measured from transplantation date until date of event; patients without event were censored at January 1st, 2006. For OS, an event was defined as death due to any cause. For freedom from BOS, an event was defined as progression to BOS stage I.

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. 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 8.0 (SAS-Institute inc., Cary, North Carolina, USA).

Table 2: Baseline characteristics of all patients treated according to the new protocol comparing patients with and without EBV reactivation. CMV pre-transplant match describes serostatus of donor and recipient at the time of transplantation, CMV active infection indicates patients with positive CMV-antigenemia after transplantation, R/D = recipient/Donor

Patients	EBV reactivation		No EBV reactivation		P-value
	n	(%)	n	(%)	
Patients	12	100	82	100	
Age at transplantation (years, median, range)	50 (21-61)		45 (18-68)		0.76
Gender					1.00
male	7	42	44	54	
female	5	58	38	46	
Bilateral/unilateral lung tx					0.30
unilateral	5	58	21	26	
bilateral	7	42	61	74	
EBV status pre-transplant					0.13
positive	11	92	82	100	
negative	1	8	0	0	
CMV pre-transplant match					0.41
R/D negative/negative	3	25	21	26	
R/D negative/positive	5	42	20	24	
recipient positive	4	33	41	50	
CMV active infection					0.51
no active infection	9	75	66	80	
active infection: primary	3	25	12	15	
active infection: secondary	0	0	4	5	
Rejection					0.29
no rejection	8	67	41	50	
Methylprednisolon	4	33	21	26	
conversion to MMF	0	0	18	22	
OKT-3	0	0	2	2	

Results

Patients and baseline characteristics

Baseline patient characteristics are shown in Table 1.

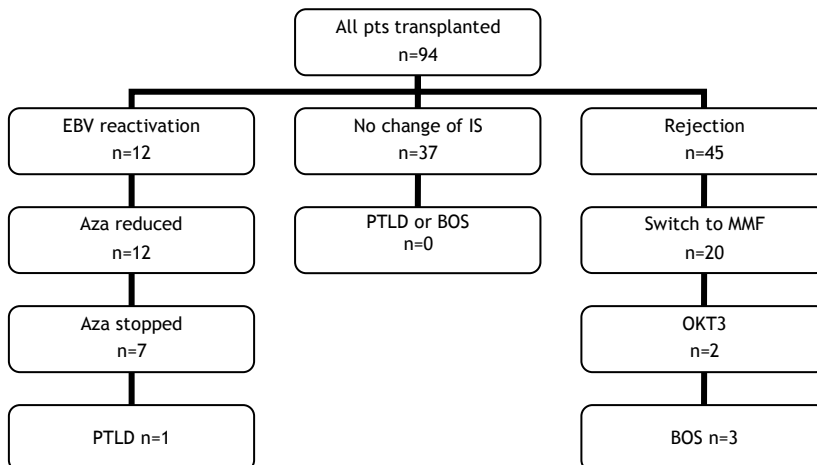
Patients treated according to the new protocol were older (median age of 50 versus 45 years, $p=0.005$) and tended to have undergone more frequently unilateral lung transplantation ($p=0.051$) (Table 1). In the cohort of “old protocol patients” more EBV seronegative recipients were present. No difference in CMV recipient/donor match was found between the patients treated according to the “new protocol” and the “old protocol”.

Analysis of the baseline characteristics of the patients treated according to the “new protocol” showed no differences between the patients with or without EBV reactivation in age, gender, uni- versus bilateral transplantation, CMV sero-match or rejection episodes (Table 2).

EBV-reactivation and immunosuppression

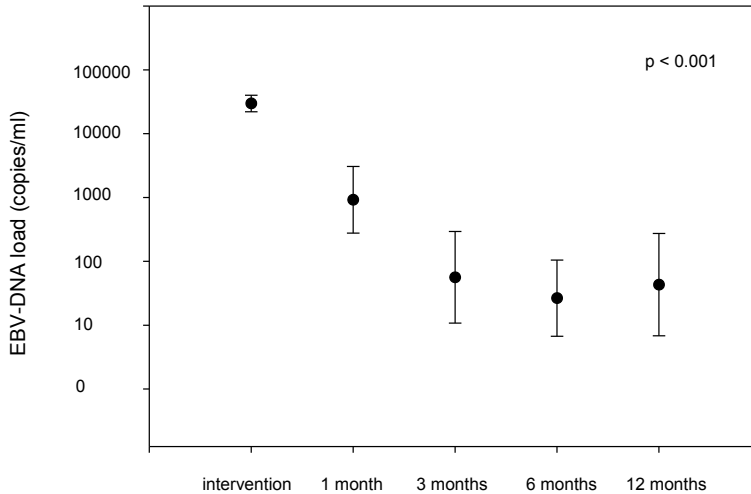
In 12 of the 94 patients (13%) transplanted since June 1, 2001, EBV-reactivation was observed. In all 12 patients with an EBV reactivation immunosuppression was decreased according to the protocol (Fig. 2) and in 7 patients a further rise in EBV-DNA load led to complete withdrawal of azathioprine.

Figure 2: Patients treated according to the new protocol Pathways of treatment. Pts = patients, IS = immunosuppression. Aza = azathioprine, MMF = mycophenolate mofetil, PTLD = posttransplant Lymphoproliferative disease, BOS = bronchiolitis obliterans syndrome.



After reduction of immunosuppression, EBV-DNA load decreased significantly ($p < 0,001$, Fig. 3). None of the patients being EBV seropositive before transplantation developed acute rejection after reduction of immunosuppression. Interestingly, the patient with a primary EBV infection developed acute rejection at the time EBV-DNA became undetectable. This patient was treated with pulse methylprednisolone followed by a complete recovery of lung function.

Figure 3. EBV-DNA load after intervention. Decrease in EBV-DNA load following reduction in immunosuppression according to the new protocol. Time dependent analysis showed a significant decrease in EBV-DNA load after reduction of immunosuppression ($p < 0.001$). (Mean and standard error)



Of the 82 patients without EBV reactivation, 41 patients had a rejection episode and 20 patients were converted to MMF. Two patients developed steroid resistant rejection and were subsequently treated with OKT3. Three patients from this cohort developed BOS, including both patients treated with OKT3 and one patient in whom immunosuppression was reduced early after transplantation due to tacrolimus toxicity. None of the patients without EBV reactivation developed PTLD

Development of PTLD

There was a trend to a decreased incidence of PTLD in the 94 patients treated according to the new protocol as compared to the “old protocol” patients. One patient (1%) developed PTLD, compared to 16 of the 152 (11%) patients in the “old protocol” group (Hazard ratio 5.5, confidence interval 0.7-42.5, $p=0.10$) (Table 3, Fig. 4). The only PTLD encountered in the “new protocol” group occurred in a patient very early (4 weeks) after transplantation. It was of donor origin (as demonstrated by single cell PCR and microsatellite analysis), and was easily managed by reduction of immunosuppression (azathioprine was stopped, tacrolimus trough level was reduced) and one course of Rituximab ($375\text{mg}/\text{m}^2$). The patient presented with progressive dyspnoea and diffuse infiltrative abnormalities on chest X-ray. Before diagnosis, a strong rise in

Pre-emptive treatment for PTLD

EBV-DNA load (6.000 to 54.000 copies/ml) was observed starting 2 weeks after transplantation.

This led, in absence of other infectious causes, to transbronchial biopsies in which large EBER positive, CD20 positive B-cells were found. It was classified as a diffuse large B-cell lymphoma according to the WHO classification. Using this new protocol, no mortality occurred due to PTLD.

In summary, this new protocol of immunosuppression and pre-emptive therapy after lung transplantation resulted in a trend to a reduced incidence of PTLD and, so far, no mortality due to PTLD.

Figure 4: PTLD free survival in a Kaplan Meyer plot in the patients treated according to the new protocol versus the historical cohort. (Hazard ratio 5.5, confidence interval 0.7-42.5, $p=0.10$).

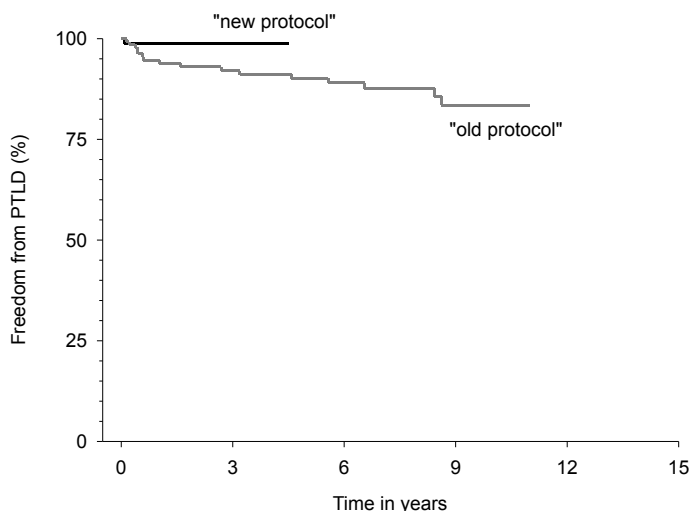


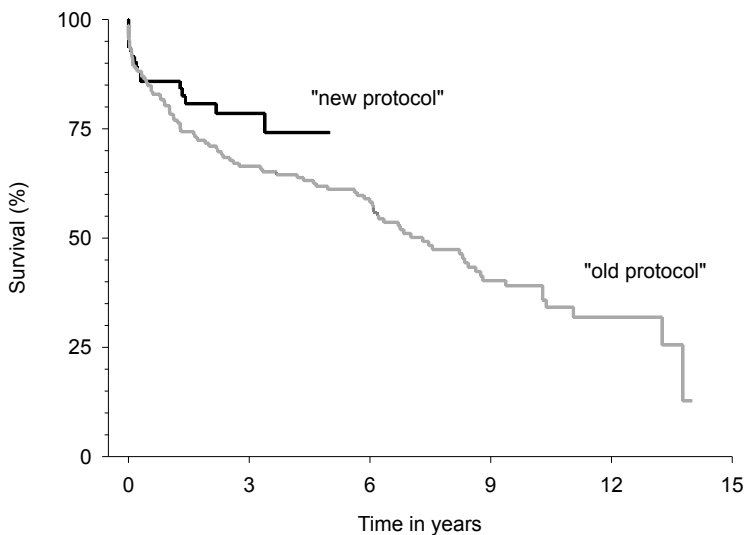
Table 3: Results of patients with and without EBV reactivation treated according to the new protocol. PTLD=post transplant lymphoproliferative disease, BOS= bronchiolitis obliterans syndrome,

Patients	EBV reactivation		no EBV reactivation		P-value
	n	(%)	n	(%)	
Patients	12	100	82	100	
PTLD					
yes	1	8	0	0	0.13
no	11	92	82	100	
Development of BOS					
yes	0	0	3	4	1.00
no	12	100	79	96	
Mortality					
died	2	17	16	20	1.00
alive	10	83	66	80	

Survival

A significant improvement in long term survival was observed with the new protocol. A time-dependent analysis showed no difference in survival during the first 4 months after transplantation ($p=0.74$), but, thereafter, survival in the “new protocol” group significantly improved when compared with the “old protocol” group ($p<0.03$). Mortality in the first 4 months was 14%, one year survival was 86%, three year survival 76%, and amounted 74% after 4.5 year in the “new protocol group”. Survival rates were 80% at 1 year, 66% at 3 years, and 62% after 4.5 years in the “old protocol group”(Fig 5).

Figure 5: Overall survival of patients treated according to the new protocol versus survival of patients from the “old protocol” group. The survival was comparable in the first 4 months after transplantation but was significantly better in the patients treated according to the new protocol after 4 months compared to the “old protocol” group. ($p<0.03$)



In the patient group treated according to the new protocol no difference was observed in survival later than 4 months after transplantation, between groups with and without EBV reactivation (Fig 6). Two out of 12 patients with EBV reactivation died, due to sudden death and haemolytic uremic syndrome, respectively.

Figure 6. There was no difference in survival in the patients treated according to the new protocol between patients with EBV reactivation, who got reduction of immunosuppression, and patients without EBV reactivation ($p=0.33$, analysis of survival later than 4 months after transplantation).

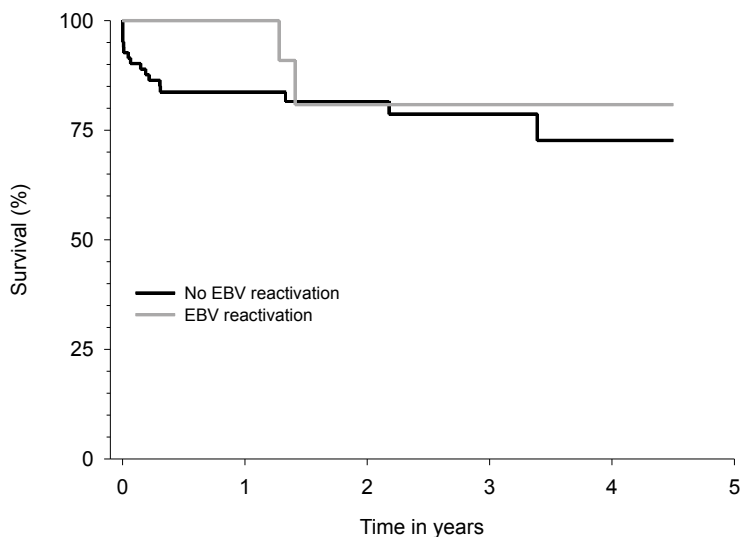
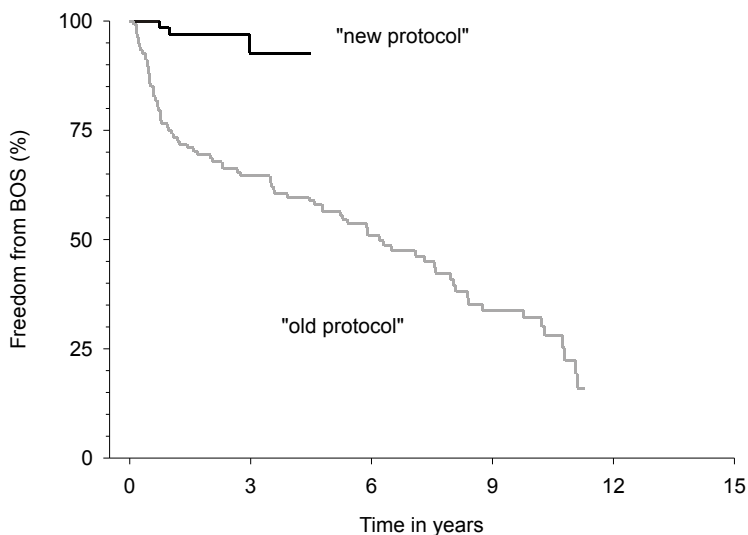


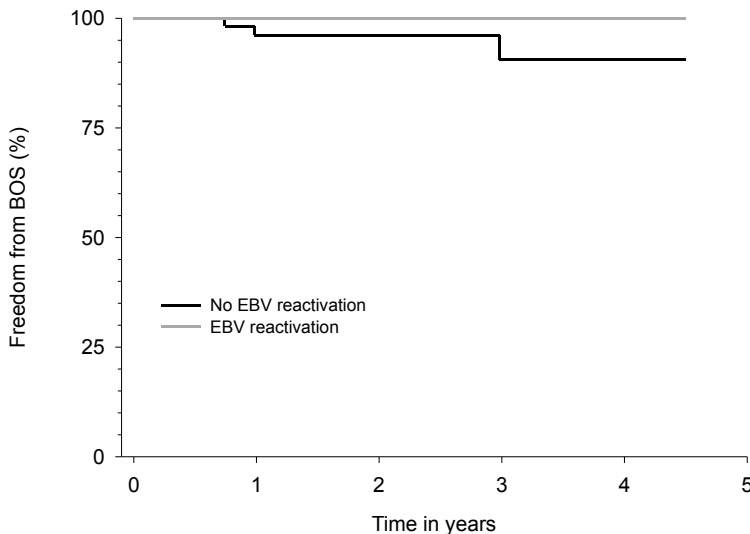
Figure 7: Development of BOS in patients treated according to the new protocol versus the patients from the "old protocol" group. There was a marked decrease in the development of BOS in the new protocol group. ($p<0,001$)



Development of Bronchiolitis Obliterans Syndrome

A significant decrease in the development of BOS was observed in patients treated according to the “new protocol” ($p < 0.0005$). Of the 94 patients, only 3 patients (3 %) developed BOS, resulting in a BOS free survival of over 90% after 4.5 year. This contrasted with the group of patients treated according to the old protocol and with data from the literature (Fig. 7)(2-5;21). None of the patients with EBV reactivation in whom immunosuppression was reduced developed BOS during the follow-up, thus all three patients who developed BOS remained EBV-DNA negative (Fig. 8).

Figure 8: Development of Bronchiolitis Obliterans Syndrome (BOS) in patients treated according to the new protocol, Comparison of patients with EBV reactivation versus those without EBV reactivation.



Discussion

Introduction of the new protocol in our lung transplant program in June 2001 resulted in a major change in outcome. The protocol had two major changes: first, the immunosuppressive approach, and, second, the pre-emptive therapy for PTLD. It was introduced for two reasons. In the historical control group, BOS presented early with about 20% of patients having developed BOS at one year. This suggests that maintenance immunosuppression should be increased. On the other hand, however, the percentage of patients with PTLD amounted 10%, which is substantial. To address both of these problems, we

designed a new immunosuppressive protocol in which 1) tacrolimus was introduced, aimed at trough levels we considered more intense than the cyclosporine A levels in the previous protocol, and, 2) EBV-DNA guided, pre-emptive reduction of immunosuppression was introduced to prevent PTLD.

Our primary goal was to reduce the occurrence of PTLD. Indeed, the reduction in PTLD incidence was considerable (1 out of 94 patients in the new protocol versus 16 out of 152 patients in the previous protocol). Given the relatively small number of patients, this resulted in a trend to reduction of PTLD. Furthermore, no mortality from PTLD was seen in our cohort during 4.5 years of follow-up.

Probably even more important is the observation that reduction of immunosuppression, in case of rising EBV DNA load, can be considered safe as only one of the patients developed acute rejection after reduction of immunosuppression and none developed BOS. Strikingly, the only patient who developed acute rejection was the one with primary EBV infection, and the rejection occurred at the time EBV-DNA became undetectable. This contrasts with the observation that 41 of the 82 patients who were persistently EBV-DNA negative did present a rejection episode, necessitating conversion to MMF in 20 patients. This suggests that patients presenting with an EBV reactivation during standard maintenance immunosuppression may require lower levels of immunosuppression, thereby maintaining EBV directed T-cell response at a level that controls EBV without increased risk for rejection. Indeed, in all patients a significant reduction in EBV-DNA load was seen following reduction of immunosuppression, suggesting that we succeeded in improving the immune response against EBV.

Another striking observation was the sofar unpublished low incidence of BOS that we encountered using the new protocol. Only three patients developed BOS resulting in less than 10% BOS during 4.5 years of follow-up. This is in sharp contrast to its prevalence in our historical group and that reported in the literature (2-5;21).

Several changes in our protocol could be responsible for this sharp decrease in BOS.

The first change relates to the immunosuppressive regimen. Although the introduction of tacrolimus at the level described, indeed, may have contributed to the improved outcome, the immunosuppressive protocol we used was not unique and has not been associated with a low rate of BOS as found in this study. We also introduced CMV prophylaxis. The role of CMV in the development of BOS is controversial (22-24). In both our historic control group and in the group treated according to the new protocol, CMV was not a risk factor for BOS. Also,

many centres have introduced CMV prophylaxis without reporting a decrease in BOS to the level we here report. This suggests that the introduction of CMV prophylaxis is not a major factor in the decrease in incidence of BOS in our centre.

The pathogenesis of BOS is regarded to be induced both by rejection, caused by alloreactivity, and non-alloimmune events, such as recurrent (subclinical) infections in which (over-) immunosuppression plays a role (25;26). Clinically, it can be very difficult to distinguish between these two entities.

According to this concept, we hypothesize that our extremely low incidence of BOS is due to the fact that patients at risk for BOS, as a result of over-immunosuppression, were recognized at an early stage by routine measurements of EBV-DNA load. Timely reduction of immunosuppression, triggered by a rise in EBV-DNA load, may have prevented the development of BOS due to over-immunosuppression-associated infections. Transplant dysfunction in the remaining EBV-DNA negative patients could be considered to be due to alloreactivity based, true rejection.

Also some theoretical arguments support the hypothesis that measuring EBV-DNA load may be a useful marker for assessing over-immunosuppression. EBV infection afflicts over 90% of the western population, so EBV is present in almost all patients. As EBV persists lifelong, it can always reactivate, and become detectable, after transplantation. Besides, EBV infects B-cells, also the circulating pool of B-cells. So EBV reactivation can be measured in peripheral blood (27;28). Being an intracellular virus that induces B-cell proliferation, antiviral drugs have little or no effect (29), which makes reduction of immunosuppression a logical treatment. As EBV is controlled by EBV specific cytotoxic T-lymphocytes (11), and, T-lymphocytes are the main target of immunosuppression because they are fundamental for rejection, EBV-DNA load may therefore be a useful surrogate marker for the level of T-cell immune responsiveness. Thus, EBV-DNA load may represent, in almost all patients, a reflection of the balance between EBV and cell-mediated immunity. Following this hypothesis, a positive EBV DNA load can be seen as a surrogate marker for T-cell failure and used to recognize those patients that are over-immunosuppressed. Reduction of immunosuppression in the presence of increasing EBV-DNA load may restore the balance in such a way that T-cell control of EBV returns without the concurrent occurrence of rejection. This way EBV DNA load can be used as a surrogate marker for T-cell immunity and clinically used to bring the immunosuppression to the level appropriate for the individual patient.

Pre-emptive treatment for PTLD

This study, however, despite its promising results, cannot prove that the pre-emptive reduction of immunosuppression based on EBV-DNA load was responsible for the low incidence of BOS, due to some confounding factors. The simultaneous changes of immunosuppressive strategy and introduction of pre-emptive reduction of immunosuppression makes it impossible to clarify which factor is most important for improvement of the outcome. Also, this study does not explore the most optimal level of immunosuppression. Even if EBV-DNA load is a marker of over-immunosuppression (as suggested here), should we aim to reduce immunosuppression until all patients become EBV-DNA negative?

Nevertheless, careful pre-emptive reduction of immunosuppression seems to reduce the number of cases of PTLD. This approach is safe with respect to BOS, even in the high risk lung transplant population. Additional, prospective controlled studies are needed to clarify the usefulness of EBV-DNA monitoring in preventing PTLD, and possibly, BOS. Ideally, these studies should include assessment of over-immunosuppression by measuring both allo-reactivity and EBV-specific T-cell responsiveness.

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