Presentation and early detection of posttransplant lymphoproliferative disorder after solid organ transplantation
Bakker, Nicolaas Arjen

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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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ME Erasmus³
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NJGM Veeger⁵
CGM Kallenberg⁶
W van der Bij²

Department of Haematology¹, Pulmonary Diseases², Cardiothoracic Surgery³, Pathology and Laboratory Medicine⁴, Clinical Epidemiology⁵, Clinical Immunology⁶, University Medical Centre Groningen, University of Groningen, The Netherlands

Transplantation, in press
ABSTRACT

Background: Posttransplant lymphoproliferative disease (PTLD) is a serious complication after lung transplantation and its relation with Epstein-Barr virus (EBV) is well recognised. 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 feasibility of this approach in terms of 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, acceleration of chronic allograft rejection or worse survival were observed after reduction of immunosuppression.

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\(^1\), 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 rejection (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\(^3\), and is generally 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\(^4\). Major risk factors for development of PTLD include the amount and intensity of immunosuppression after transplantation, especially induction and rejection therapy\(^5\)-\(^7\), and EBV seronegativity of the recipient before transplantation (leading to primary EBV infection)\(^6\).

Over the last years, much effort has been put on developing methods that can identify patients at risk for development of PTLD by measuring the amount of circulating EBV-DNA in the peripheral blood of transplant recipients\(^8\)-\(^9\). 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\(^10\)-\(^11\). Recent results in paediatric liver transplant recipients suggest that pre-emptive reduction of immunosuppression may lead to a significantly lower incidence of PTLD, probably because of the reconstitution of T-cell control\(^12\)-\(^13\). However, a possible complication of reduction of immunosuppression is allograft rejection.

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 our experience with this approach in terms of PTLD prevention and safety with regard to development of acute as well as progressive chronic allograft rejection in these patients.

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 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 (see figure 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.

Baseline patient characteristics

The following parameters were recorded from all patients at June 1, 2001 (table 1): recipient age at transplantation, sex, type of lung transplant (unilateral/bilateral), BOS grade (according to ISHLT criteria14), 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 transplantation15), recipient pre-transplant EBV status, and time 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, and 200 mg acyclovir four
Reduction of immunosuppression late after lung transplantation

Table 1. Baseline patient characteristics at June 1, 2001

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patients</strong></td>
<td>75</td>
<td>100</td>
</tr>
<tr>
<td><strong>Sex (male)</strong></td>
<td>35</td>
<td>47</td>
</tr>
<tr>
<td><strong>Age at inclusion date</strong> (year)</td>
<td>48</td>
<td>(19-68)</td>
</tr>
<tr>
<td><strong>Time after transplantation</strong> (year)</td>
<td>4.25</td>
<td>(0.10-10.13)</td>
</tr>
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<td><strong>Bilateral transplant</strong></td>
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<td>85</td>
</tr>
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<td>3</td>
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<tr>
<td>neg/neg</td>
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<td>20</td>
</tr>
<tr>
<td>neg/pos</td>
<td>14</td>
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</tr>
<tr>
<td>pos/neg, pos/pos</td>
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<td>61</td>
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<td><strong>Active CMV infection after transplantation</strong></td>
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<td></td>
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<tr>
<td>no</td>
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<td>no</td>
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<tr>
<td>steroids</td>
<td>53</td>
<td>71</td>
</tr>
<tr>
<td>ATG/OKT3</td>
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<td>5</td>
</tr>
<tr>
<td><strong>PTLD before June 1, 2001</strong></td>
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<td>4</td>
</tr>
<tr>
<td><strong>BOS grade at June 1, 2001</strong></td>
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<td></td>
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<tr>
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<td>76</td>
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<td>BOS 1</td>
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<td>BOS 3</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>BOS 1,2,3</td>
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<td>24</td>
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<td><strong>Initial Immunosuppression</strong></td>
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<td></td>
</tr>
<tr>
<td>Cyclo, Aza, Pred</td>
<td>70</td>
<td>93</td>
</tr>
<tr>
<td>Cyclo, MMF, Pred</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td><strong>Conversion of immunosuppression</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>no</td>
<td>50</td>
<td>67</td>
</tr>
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<td>Cyclosporin &gt; Tacrolimus</td>
<td>15</td>
<td>20</td>
</tr>
<tr>
<td>and Azathioprin &gt; MMF</td>
<td>10</td>
<td>13</td>
</tr>
</tbody>
</table>

*median [range]; *serum CMV IgG antibodies recipient/donor pair *rejection treatment > 1 month after transplantation.

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.¹⁶. 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, Muromonab-CD3 (Orthoclone OKT3, Ortho Pharmaceutical Corporation, Biotech Division, Raritan, NJ) (20 mg for 10 subsequent days) or horse antithymocyte globulin (hATG, Lymfoglobulaire, 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. 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). A primer-probe set for EBNA-1 was used; the nucleotide sequences (5′→3′) were: upstream primer, CCGGTGTTCTCGTATATGG; 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, 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). 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 for reproducibility of results showing no difference between both methods (data not shown).

**EBV reactivation and intervention**

Based on previous experiences, EBV reactivation was defined as two consecutive EBV-DNA load measurements with a rising trend; the last measurement exceeding 10,000 copies/ml under stable immunosuppression. In such case, immunosuppression was lowered according to a protocol. First, the proliferation inhibitor (azathioprine or mycophenolate mofetil) was reduced with 50%, and, because of the reported
beneficial effect on PTLD development\textsuperscript{17}, antiviral therapy was added (valacyclovir, 1000 mg td). 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 the 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 that at inclusion.

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 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 the protocol. In seven patients, lowering immunosuppression was not regarded safe as immunosuppression was already lowered before for other reasons (n=5), or because severe concomitant disease or suspicion of rejection was present (n=2).

PTLD development and acute rejection
One out of 75 patients (1.5 \%) developed PTLD 9 years after transplantation (25 months after date of study inclusion) following EBV reactivation. Despite reduction of immunosuppression, the patient deteriorated and died. Diagnosis of PTLD was based on post mortem examination, which showed a massive abdominal tumour mass. Immunohistochemistry and EBV-encoded RNA (EBER) in situ hybridisation showed an EBV negative PTLD, classified as diffuse large B-cell lymphoma type according to the WHO classification\textsuperscript{18}. 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 analysed by repeated measures analysis (mean ±standard error). Overall, a significant reduction (p=0.02) of EBV-DNA load was observed.

Response to reduction of immunosuppression

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

Survival

In univariate analysis (Table 2a), EBV reactivation was associated with a worse outcome (p=0.04). Based on univariate analysis, also the presence of BOS 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.

BOS progression

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 2b). Only the presence of BOS at inclusion date remained as predictive factor for BOS progression (p=0.007).

Risk factors for EBV reactivation

Based on univariate analysis, the time after transplantation (p=0.003), unilateral
Table 2a. Risk factors for mortality

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>RR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Univariate Cox regression</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Unilateral transplant (versus bilateral)</td>
<td>2.56</td>
<td>0.90-7.14</td>
<td>0.08</td>
</tr>
<tr>
<td>BOS 1,2,3</td>
<td>5.05</td>
<td>1.98-12.82</td>
<td>&lt;0.001</td>
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<tr>
<td>Conversion of immunosuppression</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>no</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyclo &gt; Tacrolimus</td>
<td>2.12</td>
<td>0.69-6.49</td>
<td>0.19</td>
</tr>
<tr>
<td>and Aza &gt; MMF</td>
<td>4.69</td>
<td>1.53-14.40</td>
<td>0.007</td>
</tr>
<tr>
<td>EBV reactivation</td>
<td>2.63</td>
<td>1.04-6.67</td>
<td>0.04</td>
</tr>
<tr>
<td>Multivariable Cox regression</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unilateral transplant</td>
<td>2.26</td>
<td>0.79-6.43</td>
<td>0.13</td>
</tr>
<tr>
<td>BOS 1,2,3</td>
<td>4.67</td>
<td>1.82-11.90</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>EBV reactivation</td>
<td>1.98</td>
<td>0.77-5.13</td>
<td>0.15</td>
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</table>

Table 2b. Risk factors for BOS progression

<table>
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<tr>
<th>Risk Factor</th>
<th>RR</th>
<th>95% CI</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td>Univariate Cox regression</td>
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<tr>
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<tr>
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<td>1.00</td>
<td></td>
<td></td>
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<tr>
<td>primary infection</td>
<td>2.65</td>
<td>1.02-6.90</td>
<td>0.05</td>
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<td>secondary (reactivation)</td>
<td>0.68</td>
<td>0.27-1.73</td>
<td>0.42</td>
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<tr>
<td>Rejection treatment</td>
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<td>no</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>steroids</td>
<td>2.03</td>
<td>0.69-5.96</td>
<td>0.20</td>
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<td>ATG/OKT3</td>
<td>7.19</td>
<td>1.79-28.81</td>
<td>0.005</td>
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<td>BOS 1,2,3</td>
<td>3.62</td>
<td>1.66-7.92</td>
<td>0.001</td>
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<tr>
<td>Multivariable Cox regression</td>
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<tr>
<td>Active CMV infection after transplantation</td>
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<td></td>
<td>0.06</td>
</tr>
<tr>
<td>no</td>
<td>1.00</td>
<td></td>
<td></td>
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<tr>
<td>primary infection</td>
<td>2.09</td>
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<td>0.27-1.73</td>
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<tr>
<td>BOS 1,2,3</td>
<td>3.03</td>
<td>1.34-6.71</td>
<td>0.007</td>
</tr>
</tbody>
</table>

*aOnly factors with a p-value < 0.10 in univariate analysis are shown  
*bRelative risk 1.00 indicates reference group  
cRejection treatment > 1 month after transplantation

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 3). Only the time after transplantation was independently associated with EBV reactivation
### Table 3. Risk factors for EBV reactivation

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>RR(^a)</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
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<tr>
<td><strong>Univariate logistic regression</strong></td>
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<td>Sex (male)</td>
<td>0.81</td>
<td>0.31-2.11</td>
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<tr>
<td>Age at inclusion date (June 1, 2001)</td>
<td>1.12</td>
<td>0.43-2.95</td>
<td>0.82</td>
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<td>Time after transplantation (years after inclusion date)</td>
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<td>Unilateral transplant (vs bilateral)</td>
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<td>0.06-0.92</td>
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<tr>
<td>Recipiënt EBV status pre-transplantation (negative)</td>
<td>NE(^b)</td>
<td>NE</td>
<td>NE</td>
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<tr>
<td>Active CMV infection after transplantation</td>
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<td></td>
<td>0.03</td>
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<tr>
<td>no</td>
<td>1.00</td>
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<tr>
<td>primary infection</td>
<td>5.43</td>
<td>1.12-32.26</td>
<td>0.04</td>
</tr>
<tr>
<td>secondary (reactivation)</td>
<td>4.67</td>
<td>1.32-22.22</td>
<td>0.02</td>
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<tr>
<td>Rejection treatment(^c)</td>
<td>1.88</td>
<td>0.69-8.53</td>
<td>0.21</td>
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<td>PTLD before June 1, 2001</td>
<td>NE(^b)</td>
<td>NE</td>
<td>NE</td>
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<td>0.58</td>
<td>0.20-1.71</td>
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<td>0.49</td>
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<td>Cyclo, Aza, Pred (standard)</td>
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<tr>
<td>Cyclo, MMF, Pred</td>
<td>2.22</td>
<td>0.24-20.98</td>
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<tr>
<td>Conversion of immunosuppression</td>
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<td>0.06</td>
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<td>no</td>
<td>1.00</td>
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<tr>
<td>Cyclo &gt; Tacrolimus</td>
<td>3.50</td>
<td>1.08-12.20</td>
<td>0.04</td>
</tr>
<tr>
<td>and Aza &gt; MMF</td>
<td>0.58</td>
<td>0.08-2.68</td>
<td>0.52</td>
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<tr>
<td><strong>Multivariable logistic regression</strong></td>
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<tr>
<td>Time after transplantation</td>
<td>1.35</td>
<td>1.08-1.66</td>
<td>0.003</td>
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</tbody>
</table>

\(^a\)Relative risk 1.00 indicates reference group \(^b\)Not evaluable (too few events to calculate RR)\(^c\)rejection treatment > 1 month after transplantation

(p=0.003). Median time between transplantation and study inclusion 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 period. In these three patients, immunosuppression had already been reduced as part of PTLD treatment.
DISCUSSION

The present report aimed to describe the feasibility of reduction of immunosuppression guided by EBV-DNA load late after lung transplantation. We can firmly conclude from our data that reduction of immunosuppression was safe, as none of the patients developed acute rejection following reduction of immunosuppression. Also, EBV reactivation (and subsequent reduction of immunosuppression) was not associated with acceleration of BOS or worse survival.

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 into 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.

In a recent paper, Savoldo et al. postulate that 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\(^19\). 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 with only a minimal chance of graft loss. We hypothesise that a rising EBV-DNA load can be considered to reflect a general state of decreased T-cell surveillance as a result of, probably, overimmunosuppression. If this is the case, only a minimal chance of allograft rejection is present following reduction of immunosuppression. The observation that a rise in EBV-DNA load is associated with impaired T-cell control of EBV\(^20,21\) supports this hypothesis. Also, EBV negative PTLD cases, that is negative staining for EBV in the tumour as observed for the only case of PTLD in our group, may develop simultaneously with a sharp increase in peripheral blood EBV-DNA load\(^22\). 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 possibly due to overimmunosuppression.

A limitation of this study may be a selection bias as we have excluded patients not alive at study inclusion date. We cannot exclude that these patients, who had been transplanted and 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 EBV reactivation appears safe with respect to acute as well as progression of chronic allograft rejection and survival. iii. Reduction of immunosuppression in patients with EBV reactivation might prevent PTLD development.
References


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Reduction of immunosuppression late after lung transplantation