Preoperative introduction and maintenance immunosuppression therapy of oral-only tacrolimus, mycophenolate mofetil and steroids reduce acute rejection episodes after lung transplantation

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Abstract

Objective: Immunosuppression therapy in lung transplantation (LTX) remains unsatisfactory due to a high incidence of infection and frequent acute rejection (AR), leading to early onset of the bronchiolitis obliterans syndrome (BOS). The long-term success of LTX is limited by BOS, associated with marked morbidity and mortality. The strongest risk factor for BOS is frequent AR. Decreasing frequent AR episodes might lead to improved long-term survival following LTX. Methods: Despite the introduction of many novel agents, the basis of currently applied protocols remains a calcineurin inhibitor, that is, cyclosporine/tacrolimus (TAC). Eighty-two lung recipients received oral-only administered immunosuppression with oral TAC, mycophenolate mofetil (MMF) and intravenous (IV) methylprednisolone as introduction 2 h prior to skin incision. Intra-operatively, patients received additional methylprednisolone prior to unclamping the pulmonary arteries. Postoperatively oral TAC/MMF and prednisolone were continued and trough levels closely monitored (target 8—12 ng ml⁻¹). Pulmonary function tests were performed frequently and daily after discharge by means of a self-measuring device (daily forced expiratory volume in 1 s (FEV₁)) as the major part of a close follow-up and monitoring programme. Trans-bronchial biopsies were rarely performed. Patient data were collected prospectively and stored in transplantation registries. LTX survival was analysed according to the Kaplan—Meier method. Results: The follow-up of the LTX patients through frequent ambulatory care unit visits and close monitoring of the immunosuppressive regimen and the medication response was 100% complete. The mean duration of observation per patient was 1.8 ± 1.7 years (median 1.4, range: 0.0—6.4 years) and this study included 176.5 patient-years of follow-up. The 1-, 3- and 5-year survival following LTX was 70%, 60% and 55%, respectively. Eight patients (10%) underwent high-dose intravenous (IV) bolus methylprednisolone treatment and taper for AR. Two additional patients developed BOS more than 4 years following LTX. The AR- and BOS-related mortality was 0% within the 7-year interval of LTX. Alterations in FEV₁ were associated with significant anastomotic airway and infectious complications, requiring frequent bronchoscopy interventions, stenting and laser therapy as well as frequent IV antibiotic treatment. The 30-day and in-hospital mortality of 19.5% was markedly related to primary graft failure and viral infection. Long-term survival was limited predominantly by cytomegalovirus (CMV) infection and sepsis. Conclusions: Our results suggest that a standard immunosuppressive regimen of TAC and MMF orally administered and introduced prior to skin incision for LTX surgery and maintained long-term might reduce the incidence of acute and chronic rejection. Viral infections and not BOS seemed to be the limiting factor of long-term survival.

Keywords: Lung transplantation; Immunosuppression regimen; Oral tacrolimus

1. Introduction

During the past two decades, lung transplantation (LTX) has become a life-saving intervention for patients presenting with end-stage pulmonary disease. A successful LTX may result in complete restoration of lung function and normal quality of life in these very ill patients. The enhanced long-term risk of LTX recipients compared with other solid organ transplant recipients likely relates to several factors. The lung is the only allograft in continuous contact with the external environment, and local defences may be altered by the intense immunological activity taking place within the graft. The majority of LTX recipients experience at least one episode of acute rejection (AR), regardless of the immunosuppressive regimen. AR has been observed as early as a few days and as late as several years after transplantation. The...
highest risk is during the first year [1], but a lower hazard persists for years. It has been shown that frequent episodes of AR can lead to chronic rejection or bronchiolitis obliterans syndrome (BOS). The two leading causes of death after LTX are BOS and infections [2]. Recent data suggest that also alloantigen-independent mechanisms such as viral infections and chronic gastro-oesophageal reflux are probably involved in the pathogenesis of BOS. Both, chronic rejection and infections, are consequences of inadequate immunosuppression and observed frequently, independent of various immunosuppressive strategies. This clinical study focusses on the efficacy, simplicity of application and surveillance of a conventional oral-only immunosuppressive regimen (calcineurin inhibitor tacrolimus (TAC), cell-cycle inhibitor mycophenolate mofetil and steroids), which was initiated 4 h before skin incision and maintained following LTX surgery.

2. Material and methods

We performed a single institutional transplantation data bank analysis of the prospectively collected data of our LTX recipients to determine the incidence of rejection, changes in lung function and BOS (chronic rejection) and their impact on outcomes. The Institutional Data Use Committee at the Heart and Lung Transplantation Center of the University of Leipzig approved this study, and patients provided written and informed consent for data registration and analysis at institutional and national and international data registries (German Transplant Procurement Agency, Eurotransplant and the Registry of the International Society for Heart and Lung Transplantation). To identify the early onset of potential complications, follow-up data including laboratory values, pulmonary function test data, bronchoscopy results, immunosuppressive protocol, survival status and cause of death were collected. The ability of the patients to understand the procedure and the risks involved with it, and to comply with follow-up care and surveillance is achieved through intensive teaching and adherence to guidelines and to the transplant centre’s specific information booklet of ‘Care of the lung transplant patient’. Monitoring and follow-up remains 100% complete through the evaluation of the frequently assessed complications, follow-up data including laboratory values, immunosuppressive therapy and medication levels of all transplanted patients by our central laboratory and lung transplant unit.

2.1. Patient populations

From November 2002 (first LTX at our cardiothoracic unit) through April 2009, 93 patients underwent LTX surgery. The mean age of the recipients was 52.5 ± 8 years, and 63% were male. Of these, 51 patients (55%) underwent single-LTX and 42 patients had sequential bilateral LTX for various end-stage lung diseases. In addition to their LTX procedure, eight patients underwent orthotopic heart replacement surgery and various heart operations such as aortic, tricuspid and mitral valve interventions as well as congenital heart defect repair and coronary artery bypass grafting. The majority of these patients had restrictive pulmonary disorders (53%) and 34% had chronic obstructive lung disease. Cystic fibrosis was the indication for LTX in 7% and primary pulmonary hypertension was documented in 6% of the recipients. Invasive pulmonary artery pressure monitoring revealed systolic pulmonary artery pressures of greater than 60 mmHg in 41% of the recipients.

2.2. Surgical technique

We used well-established criteria for accepting donor lungs including objective evidence of adequate gas exchange and bronchoscopic evaluation to exclude aspiration or purulent secretions [3]. Standardised organ procurement and recipient implantation techniques were used for lung transplantation. A cold crystalloid preservation solution (low potassium dextrose (LPD), Vitrolife, Gottenberg, Sweden) was infused via the donor pulmonary artery at low pressure in an antegrade fashion, immediately following prostaglandin intrapulmonary artery injection. During the procurement, the vascular structures were divided in situ and the trachea dissected well, proximal to the carina. With the lungs partially inflated, the trachea was divided between staple lines and the organ transported to the centre immersed in LPD. The most common recipient operation performed was a single lung transplant accessed through a lateral thoracotomy. Double LTX was performed using a sequential single-lung implantation technique through bilateral anterolateral thoracotomies without transverse incision of the sternum. The need for cardiopulmonary bypass was determined based on trial occlusion of the pulmonary artery with one lung ventilation as indicated by pulmonary pressures changes, intolerance of single-lung ventilation or increasing haemodynamic instability. Once the donor lung was present in the operating room, the recipient pneumonectomy was completed. The bronchial anastomosis was accomplished first, usually followed by the vascular pulmonary artery and left atrial cuff anastomoses. De-airing was done thoroughly through the atrial cuff anastomosis.

2.3. Immunosuppressive therapy and medication protocol

The standard recipient immunosuppressive protocol (Table 1) is a per os (PO) or orally applied triple medication regimen consisting of glucocorticoids, TAC and mycophenolate mofetil (MMF). The immunosuppressive medications are

<table>
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<th>Cause of death</th>
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<tr>
<td>Primary graft failure</td>
<td>6</td>
</tr>
<tr>
<td>Intracranial bleeding, infarct</td>
<td>2</td>
</tr>
<tr>
<td>Abdominal, haemorrhagic shock</td>
<td>1</td>
</tr>
<tr>
<td>Pulmonary embolism</td>
<td>1</td>
</tr>
<tr>
<td>Infection, sepsis</td>
<td>6</td>
</tr>
<tr>
<td>Viral (CMV)</td>
<td>3</td>
</tr>
<tr>
<td>Viral (varicella, HSV)</td>
<td>2</td>
</tr>
<tr>
<td>Bacterial</td>
<td>1</td>
</tr>
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</table>

Primary graft failure (severe reperfusion injury) and cytomegalovirus (CMV) infection were the main causes of in-hospital death. One patient died of CMV sepsis 9 months post-transplant following successful acute rejection treatment. The varicella zoster infection and sepsis led to therapy-refractory meningitis and intracranial oedema. Herpes simplex virus (HSV) was diagnosed by chest X-ray, new onset of massive haemoptysis and PCR determined HSV-1 DNA in the BAL and blood while treated for lung transplant pneumonia.
started preoperatively (usually on call to the operating room following donor-lung evaluation) in all recipients and maintained dose-adjusted as a long-term oral regimen following LTX. Only the preoperative and intra-operative steroids are given IV in the form of methylprednisolone, continued IV for 24 h postoperatively and then converted to a PO regimen. Induction therapy is not employed in LTX surgery.

Immunosuppression therapy is initiated through methylprednisolone 500 mg, IV upon arrival in the preoperative holding area. On call to the operating room, TAC 3 mg PO and MMF 1.5 g PO are introduced (approximately 2 h prior to skin incision). Intra-operatively, prior to the release of each pulmonary artery cross-clamp, additional methylprednisolone 250 mg IV are administered. Immunoglobulin G preparation with anti-viral properties (50 mg ml⁻¹) are infused (1 ml kg⁻¹) following skin incision slowly over the duration of 4 h. Postoperatively, methylprednisolone 125 mg IV is given thrice in 8-h intervals and converted to a PO prednisone taper of 0.4 mg kg⁻¹ divided in two doses per day. Prednisone is further reduced to 0.25 mg kg⁻¹ single morning dose following the first week after transplantation. Over the course of the following 6 months post-transplant, prednisone is slowly tapered to a single-day maintenance dose of 10–15 mg (Fig. 1).

Immediately postoperatively, oral TAC administration is continued trans-enterically (naso-gastric tube or NGT) in 1 mg doses exactly every 12 h. The trough level-associated dose is adjusted after the morning of the initial 24-h post-transplant. Daily trough levels are assessed and the dose-adjusted to target trough levels between 10 and 12 ng ml⁻¹ during the first year and reduced to 8–10 ng ml⁻¹ in the following years. MMF 2–3 g day⁻¹ is administered per NGT or PO in two divided doses. PO, TAC and MMF are maintained postoperatively. TAC trough-level determinations are continued biweekly in the outpatient setting. If deviations from the target levels are observed, the daily dose is adjusted accordingly, followed by more frequent trough-level determinations. In cases of overdosing, leading to very high TAC levels, discontinuation of the daily dose is avoided; however, the dosage is markedly reduced. MMF levels are rarely assessed. In general, IV administration of TAC or MMF is not performed.

In case of recurrent AR, medication toxicity, severe adverse events, gastrointestinal disorders, leucopenia or BOS, alternate immunosuppressive medications are discussed based on a case-by-case decision.

2.4. Infection prophylaxis

A single dose of 1 g IV vancomycin is started 1 h prior to skin incision. A routine cephalosporine-based antibacterial regimen is administered immediately preoperatively and continued postoperatively and changed to PO broad-spectrum quinilone following the discontinuation of the chest tubes. IV antibiotic therapy is continued or expanded in case of infection based on culture and sensitivity results. Cytomegalovirus (CMV) prophylaxis and treatment are performed in case of donor–recipient mismatch and donor–recipient positivity for CMV-IgG by means of ganciclovir IV and PO for 6 months. Since 2006, CMV prophylaxis has been changed to valganciclovir administered for 12 months post-transplant. Further anti-viral medication prophylaxis consists of oral acyclovir for 6 months in all lung transplant recipients. Additional oral prophylactic medications include double-strength co-trimoxacol (twice weekly) and fluconazole applied daily for at least 12 months.

Fig. 1. Immunosuppressive therapy and medication protocol in lung transplantation (LTX). Despite a very limited perioperatively intravenous (IV) administration of methylprednisolone (MP) the standard recipient immunosuppressive protocol consist of a strictly per os (PO) or orally applied triple medication regimen. Tacrolimus (TAC) and mycophenolate mofetil (MMF) are started PO preoperatively, continued PO postoperatively and maintained long-term as a twice a day or bis per diem (BD) administration. Following the initial IV doses of MP, which are converted to a PO formulation on day 2 after lung transplantation (LTX) the dose is rapidly tapered to a once a day or quis per diem (QD) administration. The immunosuppressive medications are introduced 2 h preoperatively in all recipients after acceptance of the organ and maintained dose-adjusted as a long-term oral regimen following lung transplantation. PA stands for pulmonary artery. IgG relates to immunoglobulin G anti-CMV prophylaxis.

2.5. Rejection monitoring and routine post-transplant care

Patients remain under the care of the cardiovascular and thoracic surgery service until discharge from the hospital after LTX to a specific rehabilitation centre for an exercise-testing rehabilitation program. Thereafter, they are followed primarily by the respiratory medicine service with cardiovascular and thoracic surgery service consultations as needed. Every patient receives a hand-held home spirometer and thorough instructions and teaching.

While on mechanical ventilatory support, daily routine bronchoscopic evaluations and bronchoalveolar lavage (BAL) are performed. Following discharge, clinic visits and bronchoscopic examinations are individualised depending on patient needs. Trans-bronchial biopsies (TBBs) are not routinely performed. Surveillance tests and bronchoscopic procedures are scheduled for a patient without complications as follows:

- Two visits a week for 1–2 weeks.
- One visit every week for 1 month.
- One visit every 2 weeks for 1 month.
- One visit every 2 months for the remainder of the first year.
- One visit every 3 months for the second year.
- One visit every 4–6 months for the following years.
Home monitoring of pulmonary function and clinical status assessment occurs daily by means of the home spirometer AM1 (145 g including batteries, 112 mm x 82 mm x 34 mm; Jaeger, Viassys Healthcare, Hoechberg, Germany). Patients record data daily and download them to our monitoring centre. This system has the capability to detect decreasing pulmonary function (deviation from the new target forced expiratory volume in 1 s (FEV₁) baseline value) before routine measurements are performed. AR and/or infection are diagnosed through decreasing values on the pulmonary function tests with or without clinical signs, bronchoscopic examinations (cell differentiation and cell count, culture and sensitivity, viral DNA-polymerase chain reaction (PCR)) and procalcitonin for differential diagnosis of graft rejection and infection according to the Lung Rejection Study Group criteria [4]. AR is treated with methylprednisolone at a dose of 1000 mg day⁻¹ for 3 consecutive days. TBBs are not routinely performed for the confirmation of the diagnosis; they are, however, discussed as a case-to-case decision if lung function does not show improvement. The diagnosis of chronic lung rejection/BOS is established using the International Society for Heart and Lung Transplant (ISHLT) definition [5] and graded 0–3 defined by current FEV₁ decline from the baseline FEV₁ [6]. The management of BOS is individualised for each patient. This includes optimising the dose/levels of the maintenance immunosuppressive agents, considering a change of the immunosuppressive medications and the administration of macrolide antibiotics.

2.6. Statistical analysis

Statistics are calculated using SPSS software version 15.0.2 for Windows (SPSS Inc., Chicago, IL, USA). Actuarial survival is calculated using the Kaplan–Meier method. A Cox regression model is then used to examine synergistic effects of potential predictors (AR, BOS and survival). Results were considered statistically significant at P < 0.05.

3. Results

Nine patients were excluded from the analysis due to major deviation from the standard oral immunosuppression medication protocol. Three patients underwent combined heart–LTX and received induction therapy with antithymocyte globulin (ATG). Four patients, who were under sedation and had extracorporeal membrane oxygenation (ECMO) support preoperatively, received induction therapy. Further two patients received ATG induction therapy due to unknown and uncertain preoperative adherence to the immunosuppression protocol. Therefore, 82 LTX patients were analysed experiencing the same medication protocol of conventional oral immunosuppressive agents. The oral immunosuppressive combinations of calcineurin inhibitors (cyclosporine or CsA, administered as an oil-based solution or TAC) and MMF were employed. In all of these patients, the immunosuppressive therapy was introduced preoperatively and maintained long-term following LTX. During the early lung transplant phase of the lung transplant unit, eight patients (9.8%) were initially treated with oral CsA and later converted to oral TAC (five patients). In more than 90% of patients, oral TAC was introduced preoperatively (3 mg) and maintained as combination immunosuppressive therapy with MMF and prednisolone following LTX. In all of these patients and independent of the underlying end-stage lung disease (including cystic fibrosis patients with associated gastrointestinal disorders), a rapid and effective gastrointestinal absorption was observed and target trough levels of greater than 10 ng ml⁻¹ achieved within 48 h of oral TAC therapy initiation. Early seizures (between the postoperative days 5 and 16), presumably induced by TAC, were observed in five patients independent of the trough TAC level (range 8.4–26 ng ml⁻¹). Cranial computed tomography (CT) did not reveal significant findings. Anti-seizure medications were started and administered for 14 days; however, TAC immunosuppressive therapy continued and the doses reduced to target levels between 8 and 12 ng ml⁻¹. Two patients developed severe tremor in the midterm follow-up (3 and 5 months post-lung transplant). These patients were converted to CsA and the TAC treatment was discontinued. Two patients were treated with everolimus and reduced doses of TAC (target trough levels between 4 and 6 ng ml⁻¹) after the discontinuation of MMF due to intractable gastrointestinal adverse effects. Eight patients required dose reduction or pausing of MMF secondary to severe leucopaenia. Following long-term immunosuppressive therapy, new renal insufficiency was observed in 12 patients and treated through dose reduction or conversion from CsA to TAC (three patients or 37% of the CsA-treated patients and 17% of the TAC-treated patients). None of the patients required dialysis. Three patients underwent combined lung and renal transplantation for preoperatively existing terminal renal insufficiency or failure. New onset of immunosuppressive therapy-induced diabetes mellitus was observed in six patients requiring insulin treatment in three patients. A total of six patients received IV methylprednisolone prematurely, although LTX surgery was not performed because the donor lung was not accepted. The majority of these donor organs were rejected due to on-site assessment of poor organ quality or unexpected donor organ function deterioration. However, major hazards or adverse effects were not observed, and the occasionally steroid-induced hyperglycaemia subsided without major interventions.

The close follow-up of the LTX patients through frequent ambulatory care unit visits and close monitoring of the immunosuppressive regimen and the medication response was 100% complete. The mean duration of observation per patient was 1.8 ± 1.7 years (median 1.4, range: 0.0–6.4 years) and this study included 176.5 patient-related years of follow-up. Using the Kaplan–Meier survival analysis (Fig. 2), the 1-, 3- and 5-year survival rates following LTX were 70%, 60% and 55%, respectively. The 30-day and in-hospital mortality was 20%. The causes of death are depicted in Table 1. One of these patients developed signs and symptoms of hyperacute rejection [7], immediately following transfer to the intensive care unit after single LTX, which was confirmed histopathologically. Serious cardiopulmonary instability occurred that was unresponsive to inotropic–vasoactive medications and aggressive mechanical ventilation requiring early ECMO support and re-transplantation evaluation. This 55-year-old female patient with obstructive end-stage lung disease suffered grade IV AR and severe
respiratory insufficiency, diagnosed by TBB 3 weeks following successful single lung re-transplantation. She responded to high-dose IV steroid treatment. She could be weaned from mechanical ventilation; however, renal replacement treatment and haemodialysis were required. This patient developed a massive spontaneous gastrointestinal and retroperitoneal bleeding and died of the complications of haemorrhagic shock 6 weeks following re-transplantation. The outcome analysis and the causes of deaths of all the patients, who were discharged from the hospital, are summarised in Table 2. Very frequently, negative outcome was related to viral infection and therapy-resistant virus-related sepsis and multi-organ failure. Fatal CMV infection was the major cause of death in the median and long term. CMV-positive (+) or negative (–) and donor (D) and recipient (R + or –) were matched as follows: D(–) and R(–) 44%, D(+) and R(–) 40%, D(–) and R(+) 7% and D(+) and R(+) 9%. All CMV-related deaths occurred in D/R mismatched patients. Following an unusually high rate of early virus-infection-related deaths in 2005 (five patients of 19 LTXs) the anti-viral infection prophylaxis medication regimen was expanded from 3 months to 6 months (acyclovir) and 12 months (valganciclovir).

3.1. Lung function test changes, AR and BOS

Following discharge from the transplantation unit and after accomplishment of the rehabilitation programme, nearly all patients (95%) reported on regular self-assessment lung function tests (LFTs) through the hand-held home monitoring spirometer devices. Significant changes and decreases of greater than 10% from newly established baseline FEV₁ values were reported. These 20 patients (24%) were frequently admitted to the hospital to undergo multiple bronchoscopies with BAL, LFT in the pulmonary laboratory, CT, IV antibiotic treatment to assess for rejection and to define the causes of LFT decline. The majority of patients with marked FEV₁ decline had developed bronchial anastomotic narrowing and strictures requiring frequent bronchoscopic dilatations, laser interventions and main bronchus stenting (16%). Despite stent placement and immediate improvement in LFTs, recurrent and frequent laser therapy was performed for in-stent stenosis due to uncontrolled growth of granulation tissue. Four patients had positive cultures for methicillin-resistant Staphylococcus aureus (MRSA) infections and two patients had additional Pseudomonas-positive BAL. One patient showed positive culture results for Stenotrophomonas. Five other patients needed IV anti-viral treatment for recurrent CMV and herpes simplex virus infections.

In the majority of treated patients, a slow but steady improvement of LFTs was assessed. One patient with MRSA and recurrent Pseudomonas infection had previously multiple treatments for CMV and herpes simplex virus infections. Later in the course and 6 years following bilateral LTX, this female patient showed significant deteriorations in LFTs; aspergillosis was diagnosed due to culture and chest CT results. Following wedge resection, which did not confirm the diagnosis (instead, a consolidated area of bleeding was described resulting in a dense round shape with some air inclusion), the patient was stable. However, no significant improvement in LFTs was observed. Further histopathological analysis revealed BOS. Presumably, the chronic rejection was triggered by the multiple infectious states. Eight patients (10%) underwent high-dose IV bolus methylprednisolone treatment and taper for AR. The majority of these patients responded to treatment showing a slow steady FEV₁ incline, including two patients with CsA conversion to TAC. With the exception of one very early AR episode in one patient (severe, grade A4), TBBs were not routinely performed. Six of the patients with AR were more than 18 months post-LTX. Two patients with limited response to steroid bolus treatment were started on additional oral everolimus therapy (steroids, MMF, TAC and everolimus) after trials of customised aerosolised CsA were discontinued due to severe intolerance and toxic reactions. Over the course of 6 weeks, they improved from FEV₁ 50% of baseline to greater than 80% of baseline FEV₁. In general, AR was observed in two of the eight CsA-treated patients and in four of the 72 TAC-treated patients (see Table 3). Further, two of the CsA-treated patients developed stage II (FEV₁ 51–65% of baseline) and stage III (FEV₁ 50% or less of baseline) BOS, 4 and 6 years

Table 2

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<th>Cause of death</th>
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<td>Unknown cause</td>
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<tr>
<td>Malignancy</td>
<td>2</td>
</tr>
<tr>
<td>Bleeding (intestinal, pulmonary)</td>
<td>3</td>
</tr>
<tr>
<td>Infection, sepsis</td>
<td>4</td>
</tr>
<tr>
<td>Viral (CMV)</td>
<td>3</td>
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<tr>
<td>Bacterial lung abscess</td>
<td>1</td>
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</tbody>
</table>

Three patients died at home of unknown cause. Major adverse cardiac and cerebral event (heart failure, stroke) were documented in two patients. Malignant diseases relate to the development of metastatic bronchial cancer in two patients and implanted metastatic thyroid cancer of the transplanted lung. Cytomegalovirus (CMV) infection remains a dominant cause of infectious death in the long-term following lung transplantation. MACCE: major adverse cardio-cerebral event.

Fig. 2. Survival of patients following lung transplantation surgery. Eighty-two lung transplantation recipients were prospectively followed over time (years). The mean duration of observation per patient was 1.8 ± 1.7 years (median 1.4, range 0.0—6.4) and the study included 176.5 patient-related years of follow-up. The 1-, 3-, and 5-year survival rate following lung transplantation were 70%, 60%, and 55%, respectively. The in-hospital mortality was 20%.

Table 2

<table>
<thead>
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<th>Causes of late mortality post-lung transplantation (15 patients or 18%).</th>
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<tbody>
<tr>
<td>Cause of death</td>
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</tr>
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<td>Unknown cause</td>
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Three patients died at home of unknown cause. Major adverse cardiac and cerebral event (heart failure, stroke) were documented in two patients. Malignant diseases relate to the development of metastatic bronchial cancer in two patients and implanted metastatic thyroid cancer of the transplanted lung. Cytomegalovirus (CMV) infection remains a dominant cause of infectious death in the long-term following lung transplantation. MACCE: major adverse cardio-cerebral event.
following single and bilateral LTX. Both patients were on CsA-based immunosuppressive therapy. Conversion to TAC was not tolerated. The disease progressed gradually in a stepwise fashion in one patient who was relisted for single LTX in 2009 after bilateral LTX six years ago. Macrolide antibiotics were started and moderate improvement observed. The course of both patients was remarkable for multiple bacterial, viral and Aspergillus infections requiring multiple long-lasting IV antibiotic treatments. Both patients were ruled out for gastro-oesophageal reflux disease (GERD). Two more patients improved on chronic macrolide antibiotic treatment following a slow gradual decline of unknown cause in FEV\textsubscript{1} from baseline. Chest CT and several TBB assessments were inconsistent with BOS. The BOS-related mortality was 0% within the 7-year interval of LTX. Therefore, the Cox regression analysis was not performed because a BOS-related mortality was not observed, and this model is unable to evaluate for a possible association between AR, BOS and death.

4. Discussion

During the past two decades, LTX has become an effective and life-saving operation for many patients suffering from a variety of end-stage pulmonary diseases. Lung transplant may result in complete restoration of lung function and near-normal quality of life in this very ill patient population. Improvements in surgical technique, lung preservation, immunosuppression and the management of acute allograft failure and ischaemia—reperfusion syndrome have increased the 1-year survival of lung transplant recipients to over 80% [8]. Despite these improvements, the survival of allograft recipients is significantly lower than other solid organ transplants. This limited long-term survival is due to the development of BOS, which affects over 50% of the patients [11—13]. A recent consensus statement from the ISHLT classified AR as a probable risk factor for BOS [5]. Subsequently, to decrease long-term mortality, it seems to be of utmost importance to keep the rate of ARs at a minimum.

Korom et al. [14] summarised the favourable limited single- and multicentre trial results of TAC in comparison to CsA in his recent review ‘Immunosuppressive therapy in lung transplantation: state of the art’. Emerging from these investigations, there seems to be a trend of fewer AR episodes in the presence of TAC compared to CsA. Likewise, ISHLT registry data between 2000 and 2005 indicated a slightly lower average number of AR per year in patients under TAC and MMF versus CsA and MMF immunosuppression therapy [15]. At the time of transplantation, many centres are now using induction chemotherapeutic agents to deplete the recipient immune system in the immediate post-transplant period to decrease early interaction between the recipient immune cells and donor allograft antigens. In 2005, approximately 45% of lung transplant recipients received an induction agent with transplantation. We share the concern that early alloreactivity leads not only to increased AR but also to chronic low-level inflammation associated with injury and remodelling, subsequently leading to pathophysiological responses and BOS [10]. Currently, there is significant debate regarding the clinical efficacy of induction therapy in the setting of LTX. Although induction therapy has proven to decrease incidence and severity of acute and chronic rejection in other solid organ transplantation, the beneficial effects of induction therapy on AR and BOS in LTX have not been consistently demonstrated in clinical trials.

Based on the beneficial TAC trial results and the study data of Keenan et al. [16], showing a clear advantage of TAC over CsA for the development of BOS at 2 years, TAC became the primary immunosuppressant for LTX in our transplant unit. Further favourable aspects, clinical in nature, using primarily TAC as part of a standard immunosuppressive regimen relate to the improved bioavailability following oral application, the ease of drug-concentration assays (unlike needless time-consuming C\textsubscript{2} drug-level assays) and the rapid achievement of target trough levels, even if only orally administered as described in this study.

Neurohr et al. [17] reported in 2009 for the Munich Lung Transplant Group (single-centre analysis, enrolment of patients in the TAC trials) unique long-term results of the combination of TAC, MMF and prednisolone maintenance immunosuppression therapy in 155 LTX recipients, which was started intravenously immediately after transplantation and switched to oral administration after extubation [17]. The mean duration of follow-up per patient was 3.54 years and the study included a total of 549 patient-years of follow-up. The overall survival rates for the entire cohort of patients was 86.4% at 1 year, 74.9% at 3 years, 60.3% at 5 years, 52.0% at 7.5 years and 32.4% at 10 years. These are excellent survival data despite a relatively high proportion of high-risk patients with idiopathic pulmonary fibrosis. Early survival (<12 months post-transplant) was limited predominantly by non-CMV infection (27.3%), cardiovascular events (18.2%) and graft failure (27.3%). The main cause of limited long-term survival was BOS in 48.5% and non-CMV infection in 18.2%, respectively. AR episodes were detected in 35.4% of
lung graft recipients, which occurred predominantly within the first year of LTX. This was an overall cause of death in 5.5% of the recipients.

Comparable outcome data were observed in this study; however, the volume of patients studied and the accumulated patient-related years of follow-up were smaller. The 1-, 3- and 5-year survival rates following LTX were 70%, 60% and 55%, respectively. The relatively high 30-day and in-hospital mortality of 20% influenced the reduced 1-year and long-term survival rates. The observed early mortality rate might be related to the unusually large subset (55%) of high-risk LTX patients with restrictive end-stage lung disease and pulmonary hypertension. Still, these data compare well with the ISHLT registry outcome analysis of idiopathic pulmonary fibrosis patients [15]. It is remarkable that in more than 80 patients, the early mortality as well as the late mortality is absolutely unrelated to AR or BOS within our close follow-up of 176 patient-years. These are very favourable results in transplantation medicine in general and in LTX in particular, where acute and chronic rejection is more prevalent compared to all other solid organ allografting. However, these findings have still to be discussed cautiously and considered as a preliminary analysis since the median follow-up of 1.4 years was relatively short. The low incidence of AR in eight patients and BOS in two further patients cannot be explained only through the tac and MMF maintenance immunosuppression regimen. The surveillance programme and the close monitoring of drug levels of a single central unit in the long-term and the high degree of patient compliance may mainly account for the positive results. The immunosuppression medication protocol for itself and the mode and time point of application might be contributing factors to the very low incidence of acute and chronic rejection. In comparison to the existing literature and published data, the conventional immunosuppression medications were already started hours prior to LTX surgery and only orally administered (with the exception of the initial perioperative IV steroid administration). This is the main difference to many other immunosuppression medication protocols and LTX units where the immunosuppression therapy is initiated a few minutes or seconds prior to the release of the pulmonary artery cross-clamp through the administration of a high dose of IV methylprednisolone. We have to presume that the broad-ranging immunosuppressive action and effectiveness of the steroids cannot be fully elucidated because it is a time-consuming process. The early immunologic response to the implanted allograft might have an important impact on its fate. It is of highest importance to control early alloreactivity. We are allowed to speculate that the time-dependent activation of the immune response following cross-clamp release through anti-donor effector T-cells is already effectively suppressed by the very early administration of steroids and TAC as described in our immunosuppressive approach. The already preoperatively started cell deactivation leads to decreased early interaction between the recipient immune cells and donor allograft antigens. Is the low incidence of rejection related to over-immunosuppres-}

Since target drug levels were closely monitored and kept within target range. Furthermore, there was a relatively low rate of observed TAC side effects such as new onset diabetes mellitus, nephrotoxicity, and post-transplantation malignan- cies or MMF therapy-induced leucopaenia. Despite the constantly measured appropriate TAC target levels and the low incidence of side effects of the regimen, late CMV infection remained a dominant cause of morbidity and mortality, thus reiterating the potential of over-immunosuppression in the long term. Subsequently, we will adjust the TAC target levels to a reduced range of 6—8 ng ml⁻¹ after the first-year post-LTX. TBBs for rejection diagnosis were rarely employed, although frequent bronchoscopies were performed. Therefore, occult and clinically silent AR episodes could have been missed or remained undiagnosed. Surveillance bronchoscopy with TBB and pre-emptive treatment of clinically silent or occult AR are controversial topics in LTX. Transplant centres performing surveillance broncho- scopy have reported yields between 15% and 40% with the greatest yield during the first 6 months [18]. The chance of developing AR does not reach a 0 value after 6 months; nevertheless, most centres are dissuaded from performing surveillance TBB beyond the first 2 years because of low yields [19]. The majority of ARs were diagnosed following more than a year of LTX and treated by pre-emptive high-dose steroid therapy in this study. Proponents of surveillance bronchoscopy cite the high yield for rejection and infection and the likelihood for untreated AR to progress to clinically evident rejection as grounds for using pre-emptive therapy [20]. These centres view the strategy as a routine and low-risk procedure with high yield for detecting a treatable entity linked to chronic rejection. Meanwhile, some centres have reported satisfactory long-term outcomes without perform- ing regular surveillance bronchoscopy with TBB [21]. Centres report of occult ARs that resolve spontaneously [22], suggesting a variable natural history for occult AR that is not fully understood. Most importantly, opponents criticise its ongoing use despite a lack of convincing data that pre- emptive therapy minimises the subsequent burden of chronic rejection. In addition, many programmes as well as ours do not perform surveillance TBB but rely on low thresholds for clinically necessary investigations. There has been less attention focussed on the findings and how best to use the information. The majority of abnormal surveillance findings have been minimal or mild AR (A1) and the natural history of A1 rejection is not fully understood and the findings are often controversially interpreted. It is still unclear as to which rate and grade of AR derived from longitudinally collected biopsies might serve as a better predictor of chronic rejection by including all levels of allograft injury. Based on this discussion, it seems more likely that a certain rate of low-grade AR was not detected in this study. Low-grade rejection might have particularly prevailed in these patients where alterations in FEV₁ were explained by infectious complications. It is well documented in LTX medicine that infection and rejection trigger each other and frequently occur combined. Those were not fully segregated since no bronchoscopic biopsies were performed. In the majority of these presentations, the patients improved following bronchoscopic evaluation and alveolar lavage, thorough infectious work-up, thoracic high-resolution radiographic
imaging and specific antibiotic treatment. In the rare event of further deterioration or a very delayed improvement, high-dose IV bolus methylprednisolone treatment and taper for AR was administered. Two patients in this study improved on chronic macrolide antibiotic treatment following a slow gradual decline in FEV1 from baseline of unknown cause (presumed low-grade chronic rejection). The results of our study show that there is no relation between frequent AR episodes and the conversion to chronic rejection and BOS. The two patients in this study with BOS had frequent episodes of bacterial, viral and fungal infections, but not a single diagnosed event of AR. Other markers were investigated to decipher rejection and/or airway-centred inflammation such as procalcitonin [4], high-resolution chest CT and changes in exhaled breath condensate nitrite, which we previously could pass on to severe mechanical lung stress increases and lung injury [23]. Unfortunately, these markers and tests were unreliable to discriminate between prevailing acute or chronic rejection and infection in relatively stable patients. Usually, high-resolution CT has been shown to be of clinical value in diagnosing infectious pathology and small focal consolidations.

Spirometry remained the most widely used tool for monitoring the allograft in LTX recipients. When a patient’s course stabilises after the first few months, lung function remains stable or continues to improve based on frequent forced vital capacity measurements. Generally, a greater than 10% decline from baseline warrants further evaluation. This algorithm, including frequent home FEV1 monitoring, identified 20 patients or 24% of recipients within the first year after LTX with a gradual decline in lung function. The majority of these patients were further assessed in the pulmonary lung function laboratory. As diminution of spirometry cannot discriminate between infection and rejection, frequent bronchoscopy revealed definite explanations. Following frequent bronchoscopic interventions, airway dilatation, laser therapy and stenting, a marked improvement in lung function was immediately measured. Proximal airway problems and anastomotic narrowing and strictures were often diagnosed. With the exception of a single right bronchial narrowing, the majority of strictures requiring stent placement and frequent laser interventions for overgrowth of granulation tissue were related to the left bronchial anastomosis. This was interpreted as a technical surgical problem when the donor left bronchus was not trimmed appropriately up to two bronchial cartilage rings proximally to the origin of the left upper-lobe bronchus. The left donor-lung preparation protocol was revised and this challenging problem disappeared. Notably, the incidence of bronchopleural fistula or bronchial anastomotic breakdown was 0%.

A subset of the patients still required frequent hospital admissions for IV antibiotic treatment of bacterial, viral and fungal infections, leading to further improvements in lung function. Despite aggressive and frequent in-hospital antimicrobial treatment, infection with predominantly therapy-resistant CMV sepsis remained the main cause of late mortality years after LTX. BOS is not the limiting factor of long-term success in this just-described LT programme.

The main limitations of this study are the retrospective analysis of prospectively collected data, single-centre design and the absence of randomisation and control group, which might have led to biased patient selection. The strength of our study lies in close monitoring and 100% follow-up and prospective data registration of a patient population consecutively undergoing LTX surgery and the same centre-specific perioperative immunosuppressive management for all patients. Several aspects of this study are not only innovative, but also controversial. A standard immunosuppressive regimen of TAC, MMF and steroids was strictly applied orally and initiated and introduced prior to skin incision for LTX surgery. The maintenance immunosuppression therapy of all lung transplant patients was closely monitored by the same group of specialists. Frequent bronchoscopies without associated TBBs were a major part of a rigid surveillance programme for alterations in chronically monitored lung function.

The long-term results of this programme compare favourably with international data and support the conclusion of Neurohr et al. [17] that, in particular, the use of TAC as the initial calcineurin inhibitor may delay the development of BOS. However, our programme did not achieve long-term survival rates superior to many other LTX centres because of the high incidence of early and late mortality caused by serious viral infections. Eventually, new trends in long-term immunosuppressive therapy and management strategies such as pharmacodynamic monitoring of immunosuppressive medications by whole blood flow cytometric analysis of lymphocyte function have the potential to increase the efficacy and safety of individual immunosuppressive therapy after transplantation [24].

References


Appendix A. Conference discussion

Dr A. Patterson (St. Louis, Missouri, USA): I think that the question of alternative immunosuppressive strategies is interesting and I think we should all be thinking about the strategies that we utilize. After all, what everybody receives is a very nonspecific, generalized immunosuppressive regimen, and probably treating everybody the same way does not make a lot of sense. One of the problems that I think you are experiencing here, and I don’t know that you have any monitoring data to show, but the delivery system is questionable when it’s always simply oral. I don’t know what data you have regarding absorption and levels and so on. I think you are in some ways to be congratulated, but in other ways to be criticized. I think you’ve got a very high perioperative morbidity and mortality, and perhaps that represents an early experience, which this is, and I think it also reflects a very complicated patient population. 63% of your patients were fibrotics, significant numbers, and I think that’s a very challenging group of patients to begin a lung transplantation experience with, and it certainly is reflected with respect to your high incidence of early graft dysfunction, and your incidence of bronchial complications is probably in some way a reflection of that.

In terms of the manuscript, I think it would be helpful to grade the early graft dysfunction numerically using the ISHLT grading score. I think that would strengthen the quantification of the data in your manuscript. I must say that I don’t have any specific questions to ask of you. Do you want to make any comments on what I just said?

Dr Bittner: I appreciate having you as a discussant on this paper. We all appreciate the contributions you and your group have made over the last 3 decades to lung transplantation.

I have to state that the in-hospital mortality is fairly high at 20%; however, it still compares well with the ISHLT data reporting. I think the institutional learning curve is steep. However, the high percentage of high-risk patients (more than 60% IPF) and many of these patients presented with pulmonary hypertension and right heart dysfunction, a combination which already leads to a certain degree of failure and adverse outcome.

Five patients had already been on preoperative ECMO support, which is a risk cohort in itself.

In regard to the immunosuppression therapy, I suggested this regimen because it’s very, very simple to use. Comparable to ISHLT reporting, I probably have even a lower rate of acute and chronic rejection over a period of 6 to 7 years observational time, and that is a remarkable point. That’s probably not yet enough to draw serious conclusions. However, characterizing this regimen, it’s so easy to use. You measure your trough levels within the first 24 hours, and you can adjust your next dose of tacrolimus by your level, and you achieve target levels right away. I think that is the advantage. And the adverse effects are much less.

Dr M. Strueber (Hannover, Germany): If you are that aggressive with your maintenance immunosuppression, especially using tac and MMF early at high blood levels, I think that the high incidence of viral infection is a direct consequence of it. We took another route. We stay on cyclosporine or even everolimus to get rid of the viral infections in the first month, and, as you have said, we run into acute rejection in 20% of our patients. Then we identify this subset of 20% of patients rejecting, and we move only this cohort to tacrolimus or a more aggressive maintenance immunosuppressive regimen. I don’t know if our approach is okay, but I think if this immunosuppressive regimen starts up very high in the beginning as in your patients, the viral infection is a real consequence of it.

Dr Bittner: Thank you for those kind remarks and important information. In one particular year, 2005, we had a spike of viral infection, sepsis, and associated death, viral infection-related multi-organ failure. Subsequently we became more aggressive with anti-viral prophylaxis. This was one step. I think the next step is to get back to not only pharmacokinetics, but also to use pharmacodynamic monitoring, which might then allow you to decrease your aggressive immunosuppression. I fully agree. I think the immunosuppression I proposed here is very aggressive and is a factor for not only short-term but also long-term high incidence of deadly infections.

Dr M. Kamler (Essen, Germany): Do you think you can prevent your primary graft failure using cardiopulmonary bypass?

Dr Bittner: I always try to do these operations without cardiopulmonary bypass support or ECMO. However, in a subset of these patients, especially the IPF with the marked pulmonary hypertension, the use of cardiopulmonary bypass is unavoidable from the beginning of the operation. I usually utilize the femoral vessels. The majority of the patients presented, close to 70%, to a certain degree of failure and adverse outcome.

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Dr Bittner: I always try to do these operations without cardiopulmonary bypass support or ECMO. However, in a subset of these patients, especially the IPF with the marked pulmonary hypertension, the use of cardiopulmonary bypass is unavoidable from the beginning of the operation. I usually utilize the femoral vessels. The majority of the patients presented, close to 70%, underwent cardiopulmonary bypass. This graft failure I showed and this plain X-ray depicted is probably not always only related to reperfusion injury, but cardiopulmonary bypass is not the Holy Grail in preventing that.