

# Influencing factors of lung transplantation: respiratory tract infections during the first year

Ph.D. Theses

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## **1. INTRODUCTION**

The lung transplantation (LuTX) is indicated in end-stage chronic lung diseases, such as cystic fibrosis (CF), chronic obstructive pulmonary disease (COPD), idiopathic pulmonary fibrosis (IPF), primer pulmonary arterial hypertension (PAH), lymphangiomyomatosis and sarcoidosis. The first Hungarian recipient was transplanted at the Clinic of Surgery of the Medical University Vienna in 1996.

The postoperative care found place initially in the National Korányi Institute of Tuberculosis and Pulmonology. Since October 2008 the Pulmonology Department of the Semmelweis University received the patients from Vienna. Although in the posttransplant period the rebuilding of graft function and improvement of the exercise capacity and the quality of life are the most spectacular and thankful tasks, the real medical challenge is to maintain the immunotolerance and the appropriate protection from infections at the same time. According to these, the basis of the care consists of the combination immunosuppressive therapy, the antimicrobial prophylaxis and the regular patient check-ups, latter completed by symptom indicated visits. There is no detailed international consensus guideline for the postoperative care. The Hungarian practice is based on the

experiences of the LuTX centrum in Vienna, with in comparison frequent surveillance bronchoscopies and strict hygienic rules for proper control of the infections.

The function of lung allografts is limited by acute and chronic graft dysfunctions, various infections and further damages of the lung and other organs.

The significance of infections, apart from the direct damage of the graft is in inducing or enhancing the alloimmune response, which makes early diagnosis and treatment more important than in other immunocompromised patients.

Based on earlier studies from different centres it can be stated that the early postoperative period is dominated by the microorganisms typical for the intensive care unit, while later the opportunistic infections are more characteristic. By the date, no data was present on exact profile of pathogens in Hungarian patients. To our knowledge, no study exists on effect of seasonality and environmental factors on bacterial and fungal infections in LuTX recipients.

## **2. OBJECTIVES**

The infective complications need increased attention during the first year after transplantation. Based on that, the main aims of our studies were:

1. Assessment of the clinical characteristic of the Hungarian LuTX recipients.
2. Determination of the respiratory tract pathogens present in the Hungarian LuTX recipients.
3. Assessment of the variability of respiratory tract pathogens with the postoperative time and between time periods according to intensity of the immunosuppressive treatment intensity.
4. Determination of possible seasonal differences of LRTI after LuTX.
5. Assessment of the effect of the changes in immunosuppression on seasonal differences of LRTI.
6. Assessment of influence of environmental temperature and humidity changes in LuTX patients.
7. Determination of possible differences between patient groups in appearance of the pathogens according to underlying disease (CF and non-CF patients).

### **3. METHODS**

#### *Study subjects*

In the first study, data were analysed from the first 12 LuTX recipients who were transplanted after the 1<sup>st</sup> of October 2008.

In the second study, which assessed effect of seasonality and environmental factors, from the 38 patients transplanted between 1<sup>st</sup> of October 2008 and 31<sup>st</sup> of September 2012 double LuTX recipients with at least one-year survival were involved. All patients underwent LuTX surgery and received postoperative care at the Department of Thoracic Surgery and Intensive Care Units of the Medical University of Vienna. All the analysed data was result from routine patient care.

#### *Detection of the pathogens*

The surveillance bronchoscopies as well as microbiological analysis of the bronchoalveolar lavage (BAL) has been performed after 2,4,8 and 12 weeks and at 6 and 12 months following engraftment in all patients. Samples has been screened for aerobe and if clinically suspected for anaerobe bacteria, *Mycobacteria*, for fungal infections by culture and galactomannan test and for airway viruses (Adenovirus, Respiratory Syncytial Virus, Influenza A, Influenza B and Parainfluenza viruses using polymerase chain reaction). Cytomegalovirus (CMV) infection was screened by polymerase chain

reaction of the BAL fluid and by serum antigen detection. Serology for Epstein-Barr-virus was performed every two months. Grocott staining for *Pneumocystis jirovecii* was a part of the histological examinations. In the case of acute LRTI sputum aerobic and fungal culture were investigated. Virus detection (influenza test) was depending on clinical signs. Serum galactomannan test was performed if the patient had aspergillosis earlier. In absence of sputum if pneumonia was suspected on chest X-ray, BAL samples were analysed for pathogens of earlier examinations and for clinically suspected microbes. In cases without infiltrates and with mild symptoms nasal and throat swabs were taken. Microbiological examination of the BAL was indicated at decline in pulmonary function test or at radiological signs which were suspicious for novel rare pathogens, fungal or mycobacterial infections. Serological test for *Chlamydia pneumoniae* and *Mycoplasma pneumoniae* were indicated only if atypical infection was suspected.

In the first study, which investigated the appearance of pathogens, nasal and throat samples were also included in the analysis. In the investigation of seasonal effects only the bacterial and fungal infections defined in the consensus document of the International Society of Heart and Lung Transplantation were included. An

analysis of viral infections was not performed because of the low number of cases.

#### *Determination of temperature and relative humidity values*

The monthly mean temperature and relative humidity values were collected from the database of the Hungarian Meteorology Service for every single month of the study period. The calendar year was divided to warm and cold period. The cold period lasted from October to April with monthly average temperature below 12°C and minimum temperature below 0°C. The warm period lasted from May to September with average monthly temperature values above 12°C. During the study period, in the warm periods the minimum temperature was never below 0°C. In contrast to the temperature, relative humidity showed greater over-year variability therefore dry or wet period was not to determine. Correlations were analyzed between number of infections and environmental temperature or humidity for the given month of the given year.

#### *Effect of the immunosuppression*

As stepwise lowering of immunosuppression after LuTX is decreasing the risk of infections and is interfering with seasonal factors, we introduced a correction factor (C<sub>tac</sub>) calculated from the

serum tacrolimus levels. As immunosuppression level is the lowest 7-12 months after LuTX, the risk for infection is less attributable to severe drug induced immunodeficiency and the correction factor was considered with the value of one ( $C_{tac(7-12)}=1$ ).  $C_{tac}$  was calculated as the reciprocal value of the average serum tacrolimus levels for the monthly periods 1 till 3; 4 till 6 and 7 till 12 during the first year. As the highest immunosuppression is used during the first three months, so to reduce the role of drug induced immunodeficiency as a cause of LRTI the  $C_{tac(1-3)}=0.67$  was used, while  $C_{tac(4-6)}=0.81$  for the months 4-6 for intermediate drug induced immunosuppressive state. We compared uncorrected and with  $C_{tac}$  corrected number of LRTI-s (LRTI and cLRTI) per month in the cold and the warm season.

### *Statistical analysis*

The statistical analysis was performed with the GraphPad software (Graph Pad Prism 4.0 by Graph Pad Software Inc., San Diego, USA). The nonparametric values were compared with Wilcoxon signed rank test. The possible correlations with temperature and humidity were analysed with Spearman's rank correlation. The results were considered as statistically significant at  $p<0.05$ .



## 4. RESULTS

The data from the patients of the two studies are presented in Table 1.

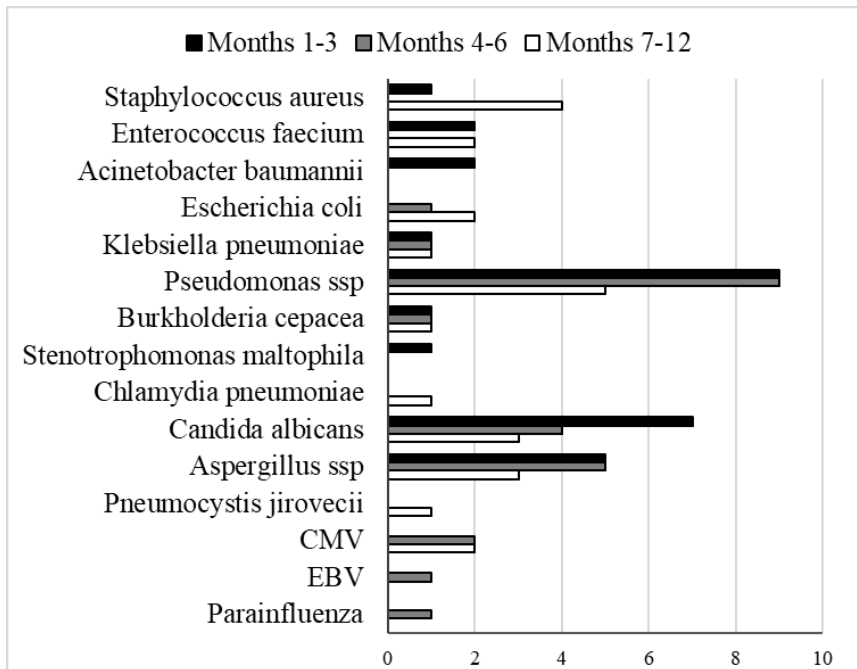
**Table 1.** Clinical data of the investigated patients. Study I: Assessment of the pathogen spectrum, Study II: Assessment of the seasonal effects.

LuTX patients (n)	Study I. (12)	Study II. (28)
Age at LuTX year±SD (range)	36.96±14.7 (18-56)	38.4±11.9 (20-63)
Gender		
male	8 (67%)	16 (57%)
female	4 (33%)	12 (43%)
Underlying disease, n (%)		
CF	7 (58%)	12 (43%)*
COPD	3 (25%)	5 (18%)
IPF	1 (8%)	6 (22%)
PAH	1 (8%)	4 (14%)*
LAM		1 (3%)
Induction therapy, n (%)		
alemtuzumab	0 (0%)	4 (14%)
ATG	0 (0%)	1 (3%)
none	12 (100%)	23 (83%)
Immunosuppressive medication, n (%)		
Tacrolimus	10 (92%)	27 (97%)
Everolimus+Tacrolimus	1 (8%)	0 (0%)
Cyclosporin A	1 (8%)	1 (3%)
Mycophenolate mofetil	9 (75%)	23 (82%)
Azathioprine	3 (25%)	1 (3%)
Steroids	12 (100%)	28 (100%)

CF: cystic fibrosis, COPD: chronic obstructive pulmonary disease, IPF: idiopathic pulmonary fibrosis, PAH: pulmonary arterial hypertension, LAM: lymphangiomyomatosis, ATG: antithymocytoglobulin.

\* 2 retransplantations within the given case number

### Assessment of the pathogen spectrum over the first year



**Figure 1.** Appearance of airway pathogens in the first year after LuTX. The year is divided to three time periods according to the stepwise lowered immunosuppressive medication.

In Hungarian patients the first year pathogens were found to be similar to literature data. Detailed data is shown in Fig.1.

### **The effect of seasonality and the environmental factors**

In the second study, the pathogen spectrum was similar to the first. From the 59 Gram-negative pathogens 28 were *Pseudomonas aeruginosa*, 8 *Stenotrophomonas maltophilia*, 5 *Klebsiella oxytoca*, and 4 *Acinetobacter baumannii*. From the 26 Gram-positive pathogens the most frequent were *Enterococci* with 13 cases, while *Staphylococcus aureus* was present in 6 cultures. The majority of the fungal infections, 25 cases were caused by *Aspergillus* species, both *Penicillium* and *Candida* species were in the background of 2 cases.

Comparison of the cold and the warm period showed significant differences for absolute and corrected number of LRTIs in frequency of all, bacterial and Gram-negative infections. Data is shown in Table 2.

**Table 2.** Seasonal differences in the frequency of LRTI

	Cold period (Oct-Apr)	Warm period (May-Sep)
<b>LRTI</b> (n/month/patient)	(mean±SD)	(mean±SD)
All infections (n=69)	1.68±1.54	0.79±0.92**
Bacterial (n=85)	2.18±2.44	0.82±1.31**
Gram – positive (n=26)	0.64±1.22	0.25±0.80 <sup>ns</sup>
Gram - negative (n=59)	1.54±1.75	0.57±0.96*
Fungal (n=31)	0.68±0.90	0.43±0.88 <sup>ns</sup>
<b>cLRTI</b>		
All infections (n=69)	1.33±1.22	0.66±0.84*
Bacterial (n=85)	1.74±2.00	0.70±1.18**
Gram - positive (n=26)	0.54±1.08	0.21±0.68 <sup>ns</sup>
Gram - negative (n=59)	1.2±1.34	0.49±0.85*
Fungal (n=31)	0,54±0,73	0,38±0,85 <sup>ns</sup>

LRTI: monthly mean values of lower respiratory tract infections, cLRTI: corrected value of LRTI calculated with immunosuppression correction factor, ns: not significant, \*: p<0,05; \*\* p<0,01

Investigating the associations between monthly mean temperature and infections caused by the different pathogen subgroups, significant negative correlation was found for all ( $r = -0.37$ ,  $p < 0.05$ ), the bacterial ( $r = -0.41$ ,  $p < 0.05$ ), the Gram-positive ( $r = -0.59$ ;  $p = 0.001$ ), and the polymicrobial ( $r = -0.64$ ,  $p = 0.001$ ) LRTI frequency. For the Gram-negative LRTI the correlation was nearly significant ( $r = -0.369$ ,  $p = 0.053$ ), while for fungal infections no correlation was found. Only the polymicrobial LRTI correlated significantly with changes in humidity ( $r = 0.492$ ,  $p < 0.05$ ). The Gram-negative LRTI tended to correlate significantly with humidity ( $r = 0.362$ ,  $p = 0.058$ ). For the frequency of the Gram-positive and the fungal LRTI no correlation was detected.

There were no differences in appearance of LRTI between patient groups according to underlying disease (CF and non-CF patients). Detailed data is shown in Table 3.

**Table 3.** Frequency of LRTI according to the underlying diseases

LRTI (n/patient)	non-CF (mean±SD)	CF (mean±SD)
All infections	2.56±2.13	2.33±1.50 <sup>ns</sup>
Bacterial	2.75±3.41	3.33±2.74 <sup>ns</sup>
Gram – positive	0.81±1.76	1.00±1.47 <sup>ns</sup>
Gram – negative	1.94±1.98	2.33±2.31 <sup>ns</sup>
Fungal	1.50±1.67	0.58±0.67 <sup>ns</sup>

LRTI: lower respiratory tract infection, CF: cystic fibrosis,  
ns: not significant

## 5. CONCLUSIONS

1. The mean age of the Hungarian recipients in the study period was similar to the international data. The gender ratio moved from male overweight to equal representation. At the beginning of the study period, CF was the underlying disease in the majority of the recipients. Its dominance diminished to the end of the period, mainly through the increasing number of ILD patients and to a lesser extent by selecting more COPD patients.
2. The pathogen spectrum was similar in Hungarian LuTX recipients to earlier literature, but there was observed a relative high presence of pathogens which are associated with elevated risk of chronic allograft dysfunction, namely *Pseudomonas aeruginosa* and *Aspergillus ssp.* The changes in the ICU flora and its reflection in the changes of pathogens in the early postoperative period could be observed even during the study period, as a consequence of the increasing number of *Enterococci*. With strict surveillance, risk factors leading to graft dysfunction could be detected before the development of the clinical signs. Early adequate therapy changes according to the findings may contribute to the Hungarian survival rates.

3. In the early postoperative phase pathogens are dominating from the ICU, recipient and donor, while later the nosocomial flora was replaced by the opportunistic pathogens.
4. In the cold period, lasting from October to April, more bacterial and Gram-negative LRTI were detected, than in the warm period between May and September. Based on our results, it seems to be appropriate considering seasonal differences in postoperative infections or in the case of deteriorating graft function. Increased attention should be paid during the cold period when starting antimicrobial therapy, in first because of the high number of the polymicrobial infections, while fungal infections have the same presence over the whole calendar year.
5. The finding that no differences were present between LRTI and cLRTI, underlines the significance of seasonality despite of changes in the immunosuppressive medications.
6. The results confirmed the influence of seasonal temperature and in part humidity changes on appearance of bacterial LRTI in the first year after LuTX surgery. The frequency of the bacterial and polymicrobial infections correlated negatively with environmental temperature. Significant positive



correlation was observed between the relative humidity and the occurrence of the polymicrobial LRTI.

7. In the Hungarian LuTX recipients no significant differences according to underlying disease were detected. Presence of *Pseudomonas* should be considered in all recipients, not only in those transplanted because of CF.

## 6. LIST OF PUBLICATIONS

### Publications in English related to the dissertation:

1. **Kovats Z**, Bohacs A, Sutto Z, Vincze K, Lukacsovits J, Losonczy G, Müller V. Seasonal changes of lower respiratory tract infections in lung transplant recipients during the first posttransplant year. The Hungarian experience. *Transpl Inf Dis.* 2017 Apr 19(2) doi: 10.1111/tid.12671. **(IF 1.719)**
2. **Kovats Z**, Süttö Z, Muraközy G, Bohács A, Czebe K, Lang Gy, Rényi-Vámos F, Klepetko W, Müller V. Airway pathogens during the first year after lung transplantation: a single center experience. *Transplant Proc.* 2011 May;43(4):1290-1. **(IF: 1.005)**
3. Erdélyi T, Bohács A, Vincze K, **Kováts Z**, Sárosi V, Sinkovicz A, Balázs G, Müller V. Successful Tacrolimus therapy extended by everolimus in retroperitoneal angiomyolipoma after lung transplantation: A Case Report. *Case Reports in Clinical Medicine*, 2014 3:636-638.
4. Kunos L, **Kováts Z**, Muraközy G, Süttö Z, Bohács A, Czebe K, Lang G, Rényi-Vámos F, Klepetko W, Müller V. Severe mixed sleep apnea after bilateral lung transplantation in a cystic fibrosis patient: a case report. *Transplant Proc.* 2011 May;43(4):1292-3. **(IF: 1.005)**

### Hungarian publications related to the dissertation:

1. Müller V, **Kováts Zs**, Horváth G. Szervtranszplantációt kísérő pulmonalis infekciók. [Pulmonary infections following solid organ transplantation]. *Orv Hetil* 2012 Jun 10;153(23):899-903.

### English publications not related to the dissertation:

1. Müller V, Gálffy G, Orosz M, **Kováts Z**, Odler B, Selroos O, Tamási L. Characteristics of reversible and nonreversible COPD and asthma and COPD overlap syndrome patients: an analysis of salbutamol Easyhaler data. *Int J Chron Obstruct Pulmon Dis.* 2016 Jan 12; 11:93-101. **(IF:3.046)**

2. Vincze K, **Kovats Z**, Cseh A, Pasti K, Kiss E, Polgar A, Vasarhelyi B, Szabo AJ, Bohacs A, Tamasi L, Losonczy G, Müller V. Peripheral CD4+ cell prevalence and pleuropulmonary manifestations in systemic lupus erythematosus patients. *Respir Med.* 2014 May;108(5):766-74. **(IF:3.06)**
3. Szondy K, Rusai K, Szabo JA, Nagy A, Gal K, Fekete A, **Kovats Z**, Losonczy G, Lukacsovits J, Müller V. Tumor Cell Expression of Heat Shock Protein (HSP) 72 is Influenced by HSP72 [HSPA1B A(1267)G] Polymorphism and Predicts Survival in Small Cell Lung Cancer (SCLC) Patients. *Cancer Invest* 2012 May;30(4):317-22. **(IF:2.238)**
4. Studinger P, Lénárd Z, **Kováts Z**, Kocsis L, Kollai M. Static and dynamic changes in carotid artery diameter in humans during and after strenuous exercise. *J Physiol.* 2003;550(Pt 2):575-83. **(IF: 1.28)**
5. Lénárd Zs, Studinger P, **Kováts Zs**, Reneman R, Kollai M. Comparisons of aortic arch and carotid sinus distensibility in humans – relation to baroreflex sensitivity. *Auton Neurosci* 2001;92: 92-99. **(IF: 0.93)**

Hungarian publications not related to the dissertation:

1. **Kováts Zs**, Gál K, Tamási L., Müller V. A 72 kDa súlyú indukálható hőszokkfehérje (Hsp72) szerepe és lehetséges felhasználhatósága pulmonológiai kórképekben *Med Thor* 2010. Aug; 63(4):258-263.
2. **Kováts Zs**, Csekeő A, Fillinger J, Csomor J, Magyar P, Losonczy Gy, Tamási L, Müller V. Castleman betegség - benignus angiofollicularis hyperplasia. *Med Thor* 2008 Aug 61(4);177-181.
3. Müller V, Horváth G, Tamási L, Eszes N, Bohács A, Vincze K, **Kováts Zs**, Lázár Zs, Odler B, Kornafeld A, Erdélyi T, Somogyi V, Losonczy Gy. Obstruktív tüdőbetegségekben alkalmazott inhalációs gyógyszerek célpontjai: receptorok a légúti felszín sejtjein. *Med Thor* 2015 Feb; 68(1):6-12.

4. Tamási L; Eszes N, **Kováts Zs**, Velicsek A, Bohács A, Losonczy Gy, Müller V. COPD Assessment Test (CAT) a hazai mindennapi betegellátásban és egészségesekben. *Med Thor* 2012 Aug;65(4):267-271.
5. Gál K, Cseh Á, Szalay B, Rusai K, Vannay Á, Lukácsovits J, Heemann, U, Szabó JA; Losonczy Gy, Tamási L, **Kováts Zs**, Müller V. Dohányfüst és szteroid hatása tüdő-epithelsejtek hősokkfehérje (HSP) 72-rendszerére. *Med Thor* 2011;64(3):152-160.
6. Müller V, **Kováts Zs**, Tamási L. A dohányfüst hatásai az immunrendszerre. *Med Thor* 2007 Feb 60(1):27-33.