Posttransplant care of lung transplant recipients using invasive and non-invasive methods

Ph.D. thesis

Dr. Krisztina Czebe

National Koranyi Institute for TB and Pulmonology, Budapest

Graduate Program, Semmelweis Medical School
Clinical Medical Sciences
Budapest

Study supervisor: Dr. Balázs Antus Ph.D.

Committee of Final Examination:

Chairman:	Prof. Dr. György Losonczy D.Sc.
Members:	Dr. Beatrix Bálint Ph.D.
	Dr. Mária Szilasi Ph.D.
Opponents:	Dr. Zoltán Balikó Ph.D.
	Dr. Veronika Müller Ph.D.

Budapest
2008

I. Introduction

In the past few years lung transplantation has become an accepted therapeutic option for the treatment of final stage parenchymal and vascular diseases of the lung. Lung transplant recipients receive lifelong immune suppressive treatment, thus they are susceptible to infections. Infections and rejections are the most important complications affecting the long-term function of lung allografts. Early recognition and accurate treatment of these hazards is of crucial importance in the care of lung transplant recipients.

Non-invasive methods currently in use in the clinical routine are not specific enough to differentiate between these two events. The most reliable differential diagnosis is based on the direct analysis of samples obtained trough bronchoscopy (bronchoalveolar lavage and transbronchial biopsy). Bronhoscopy however is an expensive and invasive method that strains patients and can cause complications.

In the past 15 years several research groups attempted to develop a method that allows non-invasive surveillance of airway inflammations. Among the newly developed methods, the measurement of exhaled nitric oxide (FENO) is gaining popularity in the clinical routine, mostly in the care of asthmatic patients. In contrast, the study of exhaled breath condensate (EBC) is still in research phase. EBC contains a number of biomarkers. pH appears to be the most reliable variable of EBC. In my studies I have attempted to assess the usefulness of the above invasive and non-invasive methods in the diagnosis of pulmonary complications of lung transplant recipients.

II. Study objectives: questions and goals

Study 1: Analysis of bronchoscopy results of Hungarian lung transplant recipients

The objective has been to determine if there was any correlation between clinical symptoms and the data generated by bronchoscopy of lung transplant recipients.

Study 2: Measurement of FENO during pulmonary infections in lung transplant recipients

The objective has been to follow the change, if any, of FENO levels during infections in otherwise stable lung transplant recipients and to establish if this change was an effective marker for diagnosis.

Study 3: Measurement of pH variability in EBC of lung transplant recipients

The main objective has been to determine if there was a change in EBC pH between visits of stable lung transplant recipients.

III. Methods

Study 1

Subjects:

27 Hungarian lung transplant recipients were enrolled in this longitudinal, retrospective study. Mean age: 32 years (17-56); underlying disease: idiopathic pulmonary fibrosis (IPF, n=9), cystic fibrosis (CF, n=8), primer pulmonary hypertension (PPH, n=7), other (n=3); type of surgery: two-sided lung transplantation (TX, n=17), heart-lung TX (n=2), split-TX (n=2), one-sided TX (n=6).

Symptoms (cough, sputum, dyspnoe, fever) were recorded at each visit.

Parameters:

C-reactive protein (CRP) and white blood cell count (WBC) was measured before bronchoscopy.

Lung function test:

Forced vital capacity (FVC), forced expiratory volume in 1 second (FEV_1) and maximal expiratory flow at 50%-FVC (MEF_{50}) was measured at each visit using an electronic spirometer (MEDICOR, MS-11 Piston Ltd, Budapest).

Bronchoscopy:

Surveillance bronchoscopy according to our follow-up protocol was carried out for one group of patients 1., 2., 3., 6. and 12 months following surgery.

For the second group of patients bronchoscopy was carried out as indicated by symptoms (sputum retention, infection or suspected rejection) rather than at fixed intervals following surgery.

In addition to routine bacterial and fungal cultures, Cytomegalovirus (CMV), Mycobacterium tuberculosis, Pneumocystis carinii and Toxoplasma gondii were tested in bronchoalveolar lavage (BAL). 3-5 transbronchial lung biopsies were taken for histology.

Study 2

Subjects:

9 clinically and functionally stable lung transplant recipients were enrolled in this longitudinal cohort. The control cohort included 12 healthy volunteers (*Table 1*).

Table 1. Demographics and lung function data.

	Patients	**Healthy controls**
n	9	12
Age (year)	28,2 ± 1,9	28,5 ± 4,1
Sex (male/female)	5/4	6/6
FEV_1 (% predicted)	85,1 ± 4,4	99,4 ± 11,6
Time passed since surgery (month)	8,5±3,4	-
Average time between follow ups (month)	27±3,2	-

FEV_1=forced expiratory volume in 1 sec

Underlying diseases: CF (n=5), PPH (n=3) and IPF (n=1). Type of surgery: two-sided TX (n=8) and heart-lung TX (n=1).

Lung function test:

As in Study 1.

Bronchoscopy:

As in Study 1.

FENO measurements:

Chemiluminescent analysis as recommended by ERS/ATS (Model LR2000; Logan Research, Rochester, UK). Values represent the average of 3 successful measurements.

Base FENO level: average of FENO values measured during visits when patients were clinically stable. The increase of FENO is given in percentage of the base FENO level.

Diagnosis, treatment and follow up of airway infections:

Data from 6 upper respiratory tract infection, (URTI) and 10 lower respiratory tract infection, (LRTI) are summarized in *Table 2*.

Table 2. Indications during periods of infection.

	URTI (10 cases)		**LRTI (6 cases)**	
	positive	negative	positive	negative
Bacteria	7	3	2	4
Laboratory indication	8	2	2	4
$FEV_1 \downarrow$	7	3	0	6
X-Ray indication	5	5	0	6

FEV_1=forced expiratory volume in 1 sec

WBC, CRP and blood sedimentation data were considered as laboratory parameters. Sputum or BAL was used for bacterial cultures.

In cases of infection laboratory, X-Ray, lung function tests and FENO measurements were carried out twice: first 1-2 days after the onset of infection and for control, 2-3 weeks later (*Table 3*).

Table 3. Comparison of parameters in clinically stable state vs. during periods of infection.

	Stable	**Infection**
FVC (% predicted)	84,2 ± 5,2	78,7 ± 5,9
FEV_1 (% predicted)	85,0 ± 4,4	76,8 ± 4,2
MEF_{50} (% predicted)	98,7 ± 8,8	89,6 ± 8,4
ESR (mm/h)	14,2 ± 2,7	26,5 ± 6,7*
CRP (mg/L)	5,5 ± 1,4	52,6 ± 23,6*
WBC ($x10^9$/L)	8,6 ± 0,5	8,7 ± 0.9

FVC=forced vital capacity, FEV_1=forced expiratory volume in 1 second, MEF_{50}=maximal expiratory flow at 50% FVC, ESR: erythrocyte sedimentation rate, CRP=C-reactive protein, WBC=white blood cell count; *p<0.05 vs. stable condition

Each case of infection was treated by antibiotics for at least 7 days. Every patient has recovered after treatment therapy, FEV_1 levels have returned to normal base values as measured during stable conditions.

Statistical analysis:

Data are given in mean ± standard error of mean (SEM). ANOVA was used to compare the 2 groups and Bonferroni post hoc test was used for corrections. Correlation analysis was carried out according to the Pearson method. Significance was considered at $p<0.05$.

Study 3

Subjects:

17 clinically stable non-smoker patients were enrolled in this longitudinal study. Underlying diseases: CF (n=11), PPH (n=3), IPF (n=2) and histiocytosis-X (n=1). Type of surgery: two-sided TX (n=16) and heart-lung TX (n=1). Bacterial colonization was evident in 7 CF

patients. The remaining 4 patients and the non-CF patients were not colonized. Bronchiolitis obliterans (BOS) was not diagnosed in any of the patients during the study period.

The control group included 19 non-smoker volunteers who suffered from no chronic disease (*Table 4*). EBC collection was repeated 1, 2 and 3 months later.

Table 4. Demographics and lung function data.

	Lung transplant recipients	**Healthy controls**
n	17	19
Age (year)	31.3 ± 7.1	32.3 ± 4.6
Sex (male/female)	10/7	10/9
FEV_1 (% predicted)	70.9 ± 16.1	96.7 ± 8,6
Average time passed since surgery	6 months	-
Average time passed between follow ups	8.8 ± 5.3 months	-
Average number of EBC collections	4	4

Lung function test:

As described for Study 1.

FENO measurements:

As described for Study 2.

Bronchoscopy:

As described for Study 1.

EBC collection and pH-measurements:

EBC was collected for 10 minutes using an EcoScreen condenser (Jaeger, Hoechberg, Germany) while patients were wearing a nose-clip.

Condensates were stored frozen at -80ºC. Average length of storage was 4.1±3.2 months.

pH and pCO_2 was measured with a blood gas analyzer (ABL 520, Radiometer, Copenhagen, Denmark) and subsequently, pH was calculated using the CO_2 standardization method.

Statistical analysis:

Data are given as mean ± SD (standard deviation). EBC pH of the 2 groups was compared using the t-test according to Student. Correlation analysis was done using the method of Pearson. The variation coefficient and the Bland-Altman test were used to assess the variability of EBC pH between visits. Significance was considered at $p<0.05$.

IV. Results

Study 1

In the group of patients where bronchoscopy was carried out according to protocol, 34 out of the total 53 visits (64%) necessitated the initiation of treatment. In the group of patients where bronchoscopy was driven by clinical indications, only 72% of cases yielded unambiguous answers about the underlying causes (*Tables 5 and 6*).

Table 5. Parameters followed.

	By protocol n=53 (%)	Clinically indicated n=14 (%)
Complaints	5 (9%)	9 (64%)
Chest X-Ray indication	6 (11%)	1 (7%)
Laboratory (CRP, WBC)	11 (20%)	6 (42%)
Lung function test indication	9 (17%)	11 (78%)
Treatment necessary	34 (64%)	10 (72%)

Table 6. Microorganisms and rejections detected.

	By protocol n=53	Clinically indicated n=14
Bacteria	24	3
CMV	10	7
Mycobacterium	1	0
Parasite	0	0
Fungi	31	7
Rejection	9	7

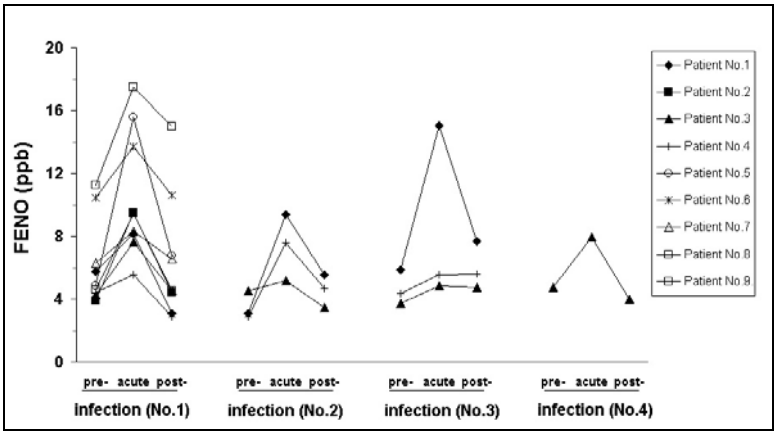
Study 2

FENO values have proven to be reproducible for lung transplant recipients. In clinically stable condition FENO variability between consecutive visits were 8.2% (range: 4.1-15.7). During infections FENO levels increased significantly compared to baseline level (7.6±1.1 vs. 10.8±1.3 ppb; $p<0.05$) in clinically stable condition. In these periods FENO levels increased by 54.2±13.5%, irrespective of

the location of the infection (URTI: 40.4±17.7% and LRTI: 64.9±19.7%). As a result of antibiotic treatment within 2-3 weeks FENO returned to normal levels, and there was no significant difference between FENO values measured before and after infection (6.9±1.1 vs. 7.4±1.3 ppb; $p>0.05$).

During the course of the study 6 patients had 1 infection, 2 patients had 3 and one patient had 4 airway infections (*Figure 1*).

Figure 1. Individual FENO values during infections.



Regarding infections, the sensitivity of FENO measurements was 57%, and their specificity was 96%.

Mean baseline FENO values were similar between lung transplant recipients (7.6±1.1 ppb, range: 4.4-14.4) and healthy volunteers (6.0±0.5 ppb, range: 2.6-8.9).

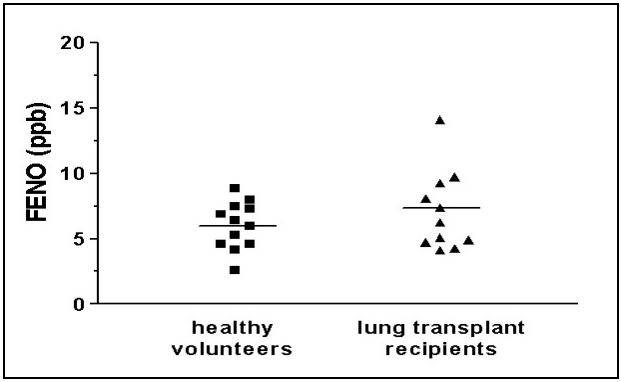
The underlying disease influenced neither the baseline FENO values nor the number of infections.

FENO values did not correlate with any lung function or laboratory parameters regardless of the clinical status of the patient.

Study 3

Mean EBC pH values were similar between clinically stable lung transplant recipients (6.38±0.09, range: 6.26-6.58) and healthy control volunteers (6.44±0.16, range: 6.23-6.69; p>0.05, *Figure 2*).

Figure 2. Individual EBC pH values in the study groups.



■ Mean EBC pH in stable lung transplant recipients (n=17).
▲Mean EBC pH in healthy controls (n=19). The lines represent mean pH values for the respective group. The difference between the groups was not significant (p>0.05).

Sex had no influence on mean EBC pH values either in patients (male: 6.39±0.12 vs. female: 6.38±0.1; p>0.05) or the control group (male: 6.41±0.17 vs. female: 6.46±0.16; p>0.05).

The mean variation coefficient for lung transplant recipients was 2.1±1.1%, while for healthy controls it was 2.3±1.4% (p>0.05).

The range of variability (Bland-Altman test) between visits was -0.29 - 0.46, and -0.53 - 0.44 for lung transplant recipients vs. healthy controls, respectively.

The mean pH was 6.37±0.08 and 6.38±0.13 ($p>0.05$) and the variation coefficient was 2.18±1.03 and 1.9±1.28 ($p>0.05$) for CF and non-CF patients, respectively. The range of variability was between -0.32 - 0.49, and -0.29 - 0.42 for CF and non-CF patients, respectively.

There was no significant difference between the pH values of colonized (n=7) and non-colonized (n=4) CF-patients (6.38±0.08 vs. 6.36±0.08, $p>0.05$). However, although not reaching significance, the variation coefficient for the colonized subgroup was higher than that for the non-colonized subgroup (2.4±1.2 vs. 1.8±0.6%, $p>0.05$). The range of variability was also wider for the colonized vs. the non-colonized subgroup (-0.3 - 0.62 vs. -0.40 - 0.49).

No correlation was found between EBC pH and any lung function parameters (FEV_1: $r=0.09$; $p>0.05$, FVC: $r=0.11$; $p>0.05$ and MEF_{50}: $r=-0.42$; $p>0.05$), or FENO levels ($r=-0.13$; $p>0.05$).

V. Discussion and conclusions

Study 1

The laboratory tests preceding surveillance bronchoscopies indicated abnormality in 20%-of cases yet, the examination detected about three times as many (64%) infections and/or rejections calling for treatment.

More than half of all bronchoscopies indicated by clinical signs confirmed a rejection, but for only 72% of these cases could the underlying cause be determined.

Our results confirm that even stable lung transplant recipients may benefit from surveillance bronchoscopies in regular follow-up protocols. The cause leading to non-protocol indicated bronchoscopies cannot always be identified either due to a sampling error or the presence of microorganisms that are not tested for.

Study 2

The prospective longitudinal study of lung transplant recipients indicates that airway infection is accompanied by a significant increase in FENO levels as compared to the baseline in stable condition. Parallel to clinical recovery the FENO levels are normalized as a result of treatment. Individual FENO levels are stable during periods free of complications. There is no significant difference between the FENO level of stable lung transplant recipients and that of healthy controls.

Despite these results, however, the applicability of FENO measurements in the diagnosis of infections is limited due to low sensitivity.

Study 3

Our results show that the variability of EBC pH is not significantly different between stable lung transplant recipients and the control group. It is important to note that the variability of pH is the lowest among all EBC biomarkers, which is likely due to the method

developed by our research group for EBC pH determination recently. Further studies are needed to assess the potential role of this non-invasive approach in the follow-up of lung transplant recipients.

VI. Acknowledgement

I thank my supervisor, dr. Balazs Antus and the previous head of the Department of Pathophysiology, dr. Ildiko Horvath for help and advice with my research work. I also thank dr. Zoltan Ajkay, as the previous director of the National Koranyi Institute for TB and Pulmonology, who sent me to Vienna University to study the clinical management of lung transplantation. I am grateful to all members of the Lung Transplantation Program in Vienna, especially to dr. Walter Klepetko. My training was supported by the Hungarian Pulmonary Foundation which is gratefully acknowledged. Finally, I thank my current head of clinical department, dr. Eszter Csiszér for support and help in overcoming all my difficulties.

VII. Publications

The thesis is based on the following publications:

1. **Czebe K,** Csiszér E, Antus B, Lang Gy, Jaksch P, Klepetko W: Tüdőtranszplantált magyar betegek bronchoscopos vizsgálatainak elemzése. Medicina Thoracalis 2005, *58,* 165-170.

2. Antus B, Csiszér E, **Czebe K,** Horváth I: Pulmonary infections increase exhaled nitric oxide in lung transplant recipients: a longitudinal study. Clin Transplant 2005, *19,* 377-382.

3. **Czebe K,** Kullmann T, Csiszer E, Barat E, Horvath I, Antus B: Variability of exhaled breath condensate pH in lung transplant recipients. Respiration 2008, *75,* 339-344.

Further publications related to the thesis:

1. Lang G, Vadász P, Agócs L, Csiszér E, **Czebe K**, Klepetko W: A tüdőtranszplantáció helyzete Magyarországon. Lege Art. Med 2003, *13,* 452-459.
2. Aigner C, Jaksch P, Mazhar S, **Czebe K**, Marta G, S.Taghavi, Lang G, Klepetko W: Treatment of severe acute lung allograft rejection with OKT3 and temporary extracorporeal membrane oxygenation bridging. Eur J Cardiothorac Surg 2004, *25,* 184-187.
3. Csiszér E, Lang G, Füzesi K, **Czebe K**, Jaksch P, Klepetko W: Tüdőátültetés cystás fibrosisban. Gyermekgyógyászat 2004, *55,* 535-540.
4. Lang G, Vadász P, **Czebe K**, Oláh C, Furák J, Papp A, Csekeő A, Klepetko W: Tüdőexplantáció Magyarországon – az első két év eredményei. Magyar Sebészet 2004, *57,* 195-200.
5. Argay K, **Czebe K**, Rényi-Vámos F, Klepetko W, Csiszér E, Herjavecz I: Ritka kórképek. Medicina Thoracalis 2004, *57,* 213-217.
6. Csiszér E, **Czebe K,** Barna E, Csekeő A, Karlócai K, Kovács L, Lang G, Petró J, Zsiray M, Böszörményi NG: Tüdőtranszplantációs bizottsági vizsgálatok elemzése. Medicina Thoracalis 2005, *58,* 41-47.
7. Antus B, Fillinger J, Csiszér E, **Czebe K,** Horváth I: Bronchiolitis obliterans szindróma a tüdőtranszplantáción átesett betegekben. Orvosi Hetilap 2005, *146,* 953-958.
8. Aigner C, Jaksch P, Winkler G, **Czebe K**, Taghavi S, Marta G, Klepetko W: Initial experience with oral valganciclovir for pre-emptive cytomegalovirus therapy after lung transplantation. Wien Klin Wochenschr 2005, *117,* 480-484.
9. Csiszér E, **Czebe K**, Fillinger J, Lang G: Tüdőtranszplantáltak utógondozása. Medicina Thoracalis 2006, *59,* 169-176.
10. **Czebe K,** Antus B, Csiszer E, Horvath I: Pulmonary infections in lung transplant recipients. Eur Respir Dis 2006, *1*, 69-71.
11. **Czebe K,** Antus B, Varga M, Csiszér E: Tüdőtranszplantált betegek pulmonalis infekciói. Orvosi Hetilap 2008, *149*, 99-109.

12. Csiszér E, **Czebe K**, Petró J: Rehabilitáció tüdőtranszplantáció előtt és után. Medicina Thoracalis 2008, *61*, 35-41.
13. Varga M, Remport A, **Czebe K**, Péter A, Toronyi E, Sárváry E, Fehérvári I, Sulyok B, Járay J: A cytomegalovírus-fertőzés rizikófaktorai, hatásai és a megelőzés lehetőségei transzplantációt követően. Orvosi Hetilap 2008*, 149*, 551-558.
14. **Czebe K**, Barta I, Antus B, Valyon M, Horváth I, Kullmann T: Influence of condensing equipment and temperature on exhaled breath condensate pH, total protein and leukotriene concentrations. Respir Med 2008, *102*, 720-725.

Book chapter related to the thesis:

Czebe K, Fillinger J: Tüdőtranszplantált betegek bronchoscopos vizsgálata. In Bronchológia. Szerk.: Strausz J. Medicina Könyvkiadó, Budapest, 2007, 93-103.