

SDC, Materials and Methods

- 1) *Antimicrobial prophylaxis*
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S1) Antimicrobial prophylaxis

All patients initially received a combination of 80 mg of Sulfamethoxazole and 400 mg of Trimethoprim thrice weekly. Patients who tested positive for CMV antibodies at the pretransplant evaluation were given 900 mg Valganciclovir daily for 3 months; the rest of the patients were given the same treatment for 6 months. Adjustments were made based on clinical response.

S2) Description of Bronchoscopy and BAL: Bronchoscopy was performed in a standardized manner. After sedating the patient the bronchoscope was inserted via either a laryngeal or tracheal tube. The lung was inspected and afterwards BAL was performed by infusion of 3 x 50-mL 37° C sterile pyrogen-free phosphate-buffered saline aliquots into a segmental middle lobe or lingula bronchus, with the bronchoscope in a wedged position. Part of the fluid was transferred to a separate container in a sterile manner and frozen at -80° C until analysis.

S3) Primer and probe sequences for quantification of CMV and EBV.

CMV: The primer and probe sequence for quantification of CMV were as follows:

Forward primer: 5'-TGA TCA CTG TTC TCA GCC ACA AT-3', **reverse primer:** 5'-TCC TCT GAT TCT CTG GTG TCA CC-3' and **probe:** 5'FAM-CCC GCS ACT ATC CCT CTG TCC TCA-3'BHQ.

EBV: The primer and probe sequences for quantification of EBV were as follows:

Forward primer: 5'-GGA ACC TGG TCA TCC TTT GC-3', **reverse primer:** 5'-ACG TGC ATG GAC CGG TTA AT-3' and **probe:** 5'FAM-CGC AGG CAC TCG TACT GC TCG CT-3'BHQ.

S4) VRTI distribution amongst EVLP recipients.

EVLP, fisher exact test.

	VRTI pos.	VRTI neg.	P value
EVLP-done	4	4	1

S5) Table describing uni and multivariate cox regression on multiple simultaneous viral infections with CLAD as outcome parameter.

Univariate analysis			Multivariate analysis		
<i>Static covariates</i>	HR(95%CI)	p	<i>Static covariates</i>	HR(95%CI)	p
Female Sex	0.98(0.55-1.73)	0.931			
Age at transplant	1.02(0.99-1.05)	0.114	Age at transplant	1.00(0.97-1.03)	0.99
Retransplant	0.511(0.12-2.12)	0.354			
Single lung transplant	1.64(0.91-2.95)	0.097	Single lung transplant	1.34(0.70-2.80)	0.303
Cyclosporine treatment	3.68(1.44-9.37)	0.006	Cyclosporine treatment	4.36(1.40-11.50)	0.047
COPD	1.48(0.83-2.64)	0.180	COPD	1.23(0.64-2.37)	0.527
Fibrosis	0.97(0.53-1.79)	0.929			
Alpha-1 antitrypsin def.	1.67(0.66-4.62)	0.281			
CF	0.19(0.26-1.38)	0.101	CF	1.03(0.144-13.53)	0.772
PAH	0.42(0.10-1.74)	0.233			
Other	1.07(0.43-2.71)	0.881			
<i>Time-dependent covariates</i>			<i>Time-dependent covariates</i>		
Acute rejection	2.44(1.33-4.50)	0.004	Acute rejection	2.46(1.44-5.61)	0.007
Bacterial infection	1.12(0.63-2.02)	0.697			
Fungal infection	2.16(0.84-5.52)	0.109	Fungal infection	1.50(0.50-3.93)	0.444
Multiple VRTI	3.33(1.29-8.58)	0.031	Multiple VRTI	3.57(1.20-10.58)	0.022
CMV viremia	0.96(0.54-1.70)	0.892			
REED	1.53(0.81-2.90)	0.190	REED	1.40(0.69-2.90)	0.349

The Hazard Ratio in this table is for development of chronic lung allograft dysfunction. The variables in the left column are the individual variables tested and in the right column is the final multivariate cox model.

Abbreviations: **CLAD** = chronic lung allograft rejection, **COPD** = chronic obstructive pulmonary disease, **def.** = deficiency, **CF** = Cystic Fibrosis, **VRTI** = viral respiratory tract viral Infection, **CMV** = cytomegalovirus, **REED** = repeated elevated Epstein-Barr virus DNA levels

S6) Further analysis of respective viral infection type and impact on CLAD development

Data for viral infections with 5 or more events

Univariate Cox proportional hazards with type of viral infection as time dependent covariate

Time dependent Covariate	HR(range)	P value
Coronavirus infection	2,3(1.1-4.78)	0.026
Enterovirus infection	4.36(0,58-32.76)	0.153
HPMV infection	1.11(0.4-3.1)	0.838
Rhinovirus infection	1.7(0.96-3.0)	0.068
RSV infection	1.85(0.83-4.12)	0.136

The Hazard Ratio in this table is for development of chronic lung allograft dysfunction. HPMV: Human metapneumovirus, RSV: Respiratory syncytial virus

S7) Baseline characteristics by choice of CNI treatment

Baseline characteristics for patients treated with Tacrolimus and Cyclosporine, respectively.

Factor	Tac(n=19)	Cya(n=79)	p
Age	41(18-61)	57(31-73)	0.0001
Female Sex	12	48	1.0
Single LTx	5	26	0.78
Retransplantation	4	2	0.012
Diagnose			
CF	6	0	0.0001
PAH	3	3	0.085
COPD	2	34	0.08
Alfa-1 AT-deficiency	0	10	0.2
Fibrosis	5	26	0.78
Other	3	6	0.37

The data shown for the variable 'Age' is the mean and the range is in brackets. Abbreviations: **Tac** = Tacrolimus, **Cya**= Cyclosporine, **LTx** = LungTransplantation, **CF** = CysticFibrosis **COPD** = chronic obstructive pulmonary disease, **AT** = antitrypsin, **PAH** = pulmonary artery hypertension