156. Lung transplantation: studies in candidates and recipients

P1476

Results of a phase 2b multi-center trial of ALN-RSV01 in respiratory syncytial virus (RSV)-infected lung transplant patients

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ALN-RSV01 is a small interfering RNA targeting RSV replication. A Phase 2a randomized, controlled trial in 24 RSV-infected lung transplant patients administering nebulized ALN-RSV01 or PBO daily for 3 days was previously conducted in which ALN-RSV01 led to a significant decrease in new or progressive bronchiolitis obliterans syndrome (BOS) at Day 90 (p=0.027). We have now performed a Phase 2b multi-center, randomized, double-blind, PBO controlled trial in 87 RSV-infected lung transplant patients to examine the impact of ALN-RSV01 on the incidence of new or progressive BOS at Day 180. RSV positive subjects were randomized (1:1) to receive nebulized ALN-RSV01 or PBO daily for 5 days, alongside the institution's standard-of-care. Patients were prospectively stratified for: 1) days from symptom onset to treatment; and 2) pre-infection BOS grade. Of the 3,985 patients prescreened, 218 were RSV positive, of which 45 were randomized to receive ALN-RSV01 and 42 to receive PBO [intent-to-treat (ITT) population]. Ten patients were without confirmed RSV by central laboratory testing, thus a total of 77 patients (ALN-RSV01, n=44; PBO, n=33) comprised the ITTc (ITT central RSV+) population. Baseline viral load was balanced between both treatments. ALN-RSV01 was generally safe and well tolerated. There was a decrease in new or progressive BOS at Day 180 in ALN-RSV01-treated patients compared to PBO in the ITTc population (13.6% vs 30.3%, p=0.058), which was statistically significant by prospectively defined Last Observation Carried Forward (p=0.028) and Per-Protocol (p=0.025) analyses. ALN-RSV01 had a treatment effect of 54-65% in all of the pre-specified populations.

P1477

The impact of desensitization therapy prior to lung transplantation

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Introduction: Limited data exists on the impact of allosensitization on waitlist time and waitlist mortality in lung transplantation. No published data exists on the impact of desensitzation therapy in lung transplant patients.
Aim: Determine the impact of desensitization therapy on wait time and waitlist mortality.
Methods: We performed a retrospective cohort study based on chart review of all patients listed for lung transplant between 1/1/2007 and 12/31/2010 at The Methodist Hospital. Groups were compared by Chi square test. P-values of 0.05 were considered statistically significant.
Results: Excluding retransplant listings, 299 patients were listed for lung transplant. 30(10%) had panel reactive antibody (PRA) ≥25%, while 269 (90%) had PRA <25%. Median wait time was significantly longer in those with PRA ≥25% (181 days vs. 45 days, p= <0.0001). Waitlist mortality was also significantly higher in those with PRA ≥25% (26.7% vs. 8.6%, p= <0.001). Of the patients with PRA ≥25%, 16 (53.3%) underwent desensitization with intravenous immunoglobulin and plasma exchange +/– rituximab. Of the patients undergoing desensitization therapy, 10 (62.5%) were transplanted, 4 died waiting (25%), and 2 (12.5%) are still waiting. Of the patients with PRA ≥25% that did not receive desensitization therapy, 7 (50%) were transplanted, 4 (28.6%) died waiting, and 3 (21.4%) are still waiting.
Conclusion: PRA ≥25% was associated with a longer wait time and higher waitlist mortality. Desensitization therapy did not improve waitlist time or waitlist mortality when compared to allosensitized patients that did not undergo desensitization. Study is limited by being a retrospective, single center study with low numbers of patients.

P1478

Multiple breath washout in bronchiolitis obliterans syndrome following paediatric lung transplantation

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Aim: Bronchiolitis obliterans syndrome (BOS) is a significant cause of morbidity and mortality following lung transplantation. Lung Clearance Index (LCI) measured by multiple breath washout (MBW) detects early structural lung damage in other paediatric obstructive lung disease. The aim was to determine the pattern of LCI values in children with BOS.
Methods: Retrospective analysis of MBW and spirometry data from subjects transplanted between 2002-2010 (date of annual MBW testing introduction). BOS staging was defined using published "all age" reference equations. LCI in BOS 0, 0p and 1 were compared.
Results: 50/56 (89%) subjects had MBW performed (n=162): mean (SD:range) 3.1 (1.85:1-9) times over a mean (SD: range) follow up 1069 (613: 196-2613) days. Abnormal LCI values (>7.5) were common post transplant (63/114 tests, 55%). LCI was increased in subjects with BOS. All those with persistent LCI>10 (n=8) died from severe BOS. Two distinct BOS patterns were seen: gradual vs. very rapid FEV1 decline. Despite infrequent testing, earlier LCI signal was seen in some (3/8) but not all 8 subjects (e.g. not those with rapid FEV1 decline).

	BOS stage post transplant			
	No BOS	Any BOS	BOS 0p	BOS ≥1
Number of MBW tests	70	46	38	8
LCI	7.34 (5.83-11.13)	7.90 (6.32-14.70)*	7.73 (6.32-13.14)*	12.3 (6.51-14.70)*†
LCI > 7.5	33/70 (47.1%)	32/46 (69.5%)	25/38 (65.8%)	7/8 (87.5%)

Data displayed as median (range). p<0.05 vs. *No BOS or †BOS 0p

Conclusion: LCI is frequently abnormal post lung transplantation. LCI is significantly elevated in BOS, and appears to increase with BOS severity. An early signal of subsequent outcome may exist but optimal frequency of testing is yet to be determined.

P1479

Assessment of the restrictive allograft syndrome in patients after lung transplantation

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Background: Chronic lung allograft dysfunction (CLAD) is the leading cause of long term mortality after lung transplantation. The rare but constant finding of fibrotic changes has led to the hypothesis that bronchiolitis obliterans syndrome (BOS) is not the only manifestation of chronic rejection after lung transplantation.
Aims: The purpose of our study was to evaluate the recently reported criteria for the diagnosis of Restrictive Allograft Syndrome (RAS) in our post-lung transplantation population.
Methods: We retrospectively analyzed the lung function tests from 162 patients after lung transplantation from 2000 to 2011 with a conditional survival of 180 days. Established criteria for BOS were used for the definition of CLAD. RAS was defined as a detectable, irreversible decline of more than 10% of the best post-transplant total lung capacity (TLC). CT-scans were evaluated for the detection of possible causes for the TLC-decline.
Results: In our study 68 (42%) patients were diagnosed with CLAD, and 22 (14%) of these patients met the criteria for RAS after exclusion of 13 patients with other causes for a TLC-decline. Mean post-transplant survival was 2873±198 days in patients with no CLAD, 2583±261 days in patients with BOS and 2624±318 days for RAS patients (p=n.s.). In the subgroup of RAS patients CT-scans showed no alterations in 8 patients, honeycombing or reticular pattern in 3 patients and consolidations or infiltrates in 8 patients.
Conclusions: The percentage of restrictive lung function patterns in CLAD patients was comparable to the previously published data. In our study mortality of patients that developed RAS was not different from the BOS group.

P1480

Hyperinflation correlates with poor exercise tolerance in recipients with advanced chronic lung allograft dysfunction

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Background: Advanced chronic lung allograft dysfunction (CLAD) limits the sur-

vival after lung transplantation and reduces health related quality of life (HRQOL).
Aims: The study was performed to find parameters correlating with low exercise capacity of patients with advanced CLAD.
Methods: In this single-center prospective study, all patients with advanced CLAD (FEV1 <50% baseline) in our out-patient clinic were screened between 1.7.2011 and 15.11.2011 by exercise capacity, HRQOL (using SF 36, St. George, HADS), body composition, blood gas analysis, pulmonary function testing, respiratory muscle function and chest x –ray. The patients with low exercise capacity (LEC-CLAD) were defined as 6MWT < 50% predicted or use of oxygen or wheelchair/rollator.
Results: 319 of 785 patients, had CLAD and 53 had the diagnosis of advanced CLAD. A single patient refused consent to this study, 52 patients were included. 19 needed oxygen or had a 6 min walk test (6 MWT) fewer than 50% predicted (LEC-CLAD). Patients with LEC-CLAD demonstrated lower forced vital capacity (1820ml vs 2380ml, p = 0.001), pathologic respiratory muscle function (P0.1/Pimax index: 0.14 vs. 0.06, p < 0.001), decreased inspiratory capacity (IC; 1190ml vs. 1620ml, p = 0.001). We were able to show a positive correlation between 6 MWT and IC (r = 0,367, p < 0.001). Patients with LEC-CLAD demonstrated a decrease in activity and social function.
Conclusion: Advanced CLAD is an inhomogeneity cohort of patients showing different exercise tolerance of reduced lung function. We were able to demonstrated pronounced hyperinflation in patients with worse toleration and pathologic respiratory muscle function.

P1481
Low BMI in emphysema patients: A contraindication for lung transplantation?
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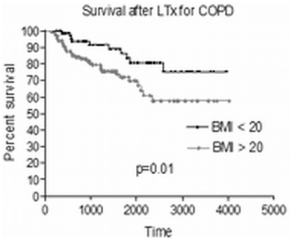
Lung transplantation (LTx) is an accepted therapeutic option for patient with end-stage emphysema. These emphysema patients can be subdivided in blue bloaters and pink puffers,although most patients have characteristics of both groups. BMI is an important tool in distinguishing these subgroups. A low BMI is associated with a poor nutritional status which may predict a poor outcome after LTx. We aimed to investigate the outcome of emphysema patients with a low pre-transplant BMI (<20) in terms of acute rejection (AR), lymphocytic bronchiolitis (LB), infections, BOS and survival. All 193 patients transplanted for emphysema between 1991-2011 and surviving more than 60 days post transplant, were included (53 SLTx(single) and 140 SSLTx(double)). AR, LB and BOS are diagnosed according to the ISHLT criteria. Multivariate analyzes were done using SAS software.
Patients with a lower BMI (<20) had a significant better 10-years survival compared with those with a BMI>20 for the total population (p=0.01) (figure).
Prevalence of BOS was significantly lower within the lower BMI group, independent from other covariates (table). There is a statistical association between the prevalence of BOS, infection and LB (table).



BOS	P-value	Hazard radio(25-75CI)
AR	0.4380	1.25(0.72-2.16)
LB	0.0283	1.86(1.07-3.24)
Age	0.7616	1.01(0.95-1.07)
Gender	0.4690	1.22(0.71-2.11)
Type(SLTX-SSLTX)	0.1538	0.62(0.33-1.19)
Infection	0.0187	1.87(1.11-3.14)
BMI>20	0.0255	1.94(1.09-3.48)

Multivariate analysis for time to BOS with correction for AR, LB, age, gender, type, infection and BMI

We conclude from this single centre observation that emphysema patients with a BMI below 20 have a better outcome in terms of BOS and mortality and this should therefore no longer be regarded as contra- indication for LTx.

P1482
Systemic oxygenation affects post-transplantation edema formation and pulmonary artery hypertension in an ex vivo animal model
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Introduction: By using an ex vivo model of isolated perfused and ventilated rabbit lungs we investigated the influence of systemic oxygenation on pulmonary function during simulated transplantation.
Methods: Lungs of New Zealand White rabbits were flush-perfused with Perfadex® Solution, followed by an ischemic storage for 4h on ice. Thereafter ventilation and reperfusion for 2h were continued to simulate a transplantation situation (oxygenated group, pulmonary artery pO_2=120mmHg). In another series the perfusate inflow was gassed with nitrogen to simulate the typical situation with deoxygenated pulmonary artery blood and not reanastomized private vessels(deoxygenated group, pulmonary artery pO_2=50mmHg). Hemodynamic and ventilatory parameters were continuously detected.
Results: After 2h reperfusion time the oxygenated group showed a significant lower PAP and lung weight compared to the deoxygenated group (p<0.05). PAP and lung weight steadily increased after reestablishment of lung perfusion (PAP 8.78±0.89 to 11.5±1.06cmH_2O; lung weight 22.1±1.32 to 35.4±4.23g). This development was significantly influenced by the intravascular pO_2 (PAP 9.65±0.43 to 8.02±0.63cmH_2O; lung weight 17.9±1.54 to 21.5±2.29g, p<0.05).
Conclusions: Oxygenation of the lung perfusate during simulated transplantation attenuates post transplant edema formation and decreases pulmonal arterial hypertension. Transfering this to the surgical situation, revascularisation of bronchial arteries after lung transplantation might initiate positive effects in the early phase after lung transplantation.

P1483
Extracorporeal life support (ECLS) as a bridge to lung transplantation (LTx)
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Purpose: Death on the waiting list remains high in patients awaiting LTx. ECLS as a bridge may increase patient's survival. We present our initial experience.
Materials and methods: Between Feb. 2010 and Dec. 2011 100 patients underwent LTx at our institution. 7 (7%) recipients were supported with ECLS prior to LTx. Outcome, donor and recipient parameters were analysed.
Results: Donor age was 21(20;47) (median (interquartile range)) y.; gender – F/M: 5/2, cause of death: intracranial haemorrhage 3, hypoxic brain injury 2, bacterial meningitis 2; duration of donor mechanical ventilation was 2(2;2.5) days; PaO_2/FiO_2 ratio: 63.4(58.15;69.45) kPa. One patient died during the support.

Table 1. Recipients' demographics and perioperative data

Recipient	1	2	3	4	5	6	7
Age (years)	25	46	21	23	40	29	50
Gender	M	F	F	F	F	F	M
Diagnosis	CF	HP	CF	CF	PH	E-D	PF
Type of ECLS	v-v ECMO	ILA	v-v ECMO	ILA/v-v ECMO	v-a ECMO	v-v ECMO	v-v ECMO
Duration of ECLS (days)	8	1	6	7/8	33	7	9
Duration of postoperative MV (hours)	624	384	72	46	304	398	301
ICU LOS (days)	26	26	10	11	18	19	17
Hospital LOS (days)	70	45	30	41	169#	48	69
FEV1/FVC (%pred.) current	73.6/78.5	79.1/101	85.1/95	59.1/62.2	55.3/70.2	69.6/62	49.3/47
Follow up	739	416	221	182	169	86	67

CF, cystic fibrosis; HP, hypersensivity pneumonitis; PH, pulmonary hypertension; PF, pulmonary fibrosis; E-D, Elerhs-Danlos syndrome related emphysema; ECMO, Extracorporeal Mebrane Oxygenation; ILA, Interventional Lung Assist; LOS, length of stay. #Still in hospital.

Conclusion: ECLS as a bridge to LTx is a feasible option of treatment providing good early results. Longer weaning from mechanical ventilation, hospital and ICU LOS after transplantation should be expected.

P1484
Superior lung preservation with a polyethylene glycol based solution in a porcine lung transplant model
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Objectives: Scot15® is a low-K+ preservation solution including polyethylene glycol (PEG) as a colloid for protection of vascular endothelium during cold ischemia. PEG was previously demonstrated to have "immunocamouflage" properties. The aim of this study was to assess whether these properties would be beneficial in a pig lung transplant model.
Material: Domestic pig donor lungs were flushed either with Scot15®[S;n=6] or Perfadex®[P;n=6] and stored on ice for 22 hours. The left lung was transplanted in a recipient animal observed during 6 hours after reperfusion. Pulmonary vascular resistance (PVR) and partial arterial oxygen tension (PaO_2) were measured hourly. Lung biopsies were taken for HRMAS detection of the respective colloid in the lungs. Bronchoalveolar lavage (BAL) was taken to assess neutrophilic alveolar recruitment. At the end of reperfusion, wet to dry weight ratio (W/D) was measured as a marker of lung edema.
Results: HRMAS showed presence of PEG in the lungs in [S] before and during the reperfusion. Dextran was not detected in [P]. After 6 hours of reperfusion, PaO_2 was significantly better in [S] (310±51mmHg versus 198±71mmHg in [P]; p=0.03). PVR remained lower in [S] but the difference did not reach signifi-

cance. Differential cell count of BAL showed lower neutrophilic cell count in [S] (89±67neutrophils in [S] versus 139±53 neutrophils in [P]; p=0.001). There was no difference in W/D weight ratio (p=0.9).
Conclusion: After 22 hours of cold ischemia, oxygenation capacity was superior with less inflammatory reaction in lung grafts preserved with Scot15®. Further experiments to study the "immunocamouflage" properties of PEG based solutions in lungs are warranted.

P1485
Low hypercapnic ventilatory response in long-term lung transplant recipients predicts exercise impairment
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Background: Only few studies reported findings on CO_2 rebreathing in long-term bilateral lung transplant recipients. Bilateral lung transplantation causes denervation of the donor lung. This might have an impact on control of breathing as measured by the hypercapnic ventilatory response (HCVR). The influence of gender on HCVR and the relation to exercise capacity is unknown yet.
Objectives: To study HCVR and the relation to exercise capacity after bilateral lung transplantation.
Methods and patients: Minute ventilation, respiratory rate and HCRV were studied in 24 bilateral lung transplant recipients (12 male). HCVR was measured according to the Read protocol. Exercise testing was performed to evaluate the maximum level of workload. This was expressed as percentage of the corresponding reference value.
Results: Age and body mass index (BMI) of men and women were similar (age: 56±9 years vs. 54±9 years (n.s.), BMI: 26±3 kg/m^2 vs. 25±6 kg/m^2 (n.s.)). Post-transplant period ranged from 6 months to 9 years. HCVR in lung transplant recipients was 1.71±1.25 l/min/mmHg in men and 0.95±0.38 l/min/mmHg in women ($p<0.05$). In healthy controls HCVR at our laboratory were 2.02±0.77 l/min/mmHg for men and 1.51±0.64 l/min/mmHg for women ($p<0.05$). After adjustment for FEV1 and gender in multivariate analysis we found a statistically significant correlation between the exercise capacity and HCVR.
Conclusion: There is a remarkable difference in ventilatory response to carbon dioxide between male and female bilateral lung transplant recipients, suggesting a role for denervation of the lung in the impairment of exercise capacity after bilateral lung transplantation.

P1486
Sleep quality and sleep-disordered breathing in lung transplant recipients
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Background: To date, only a few studies with small sample size analysed sleep-disordered breathing (SDB) in lung transplant recipients (LTX). Therefore, little is known on the prevalence of SDB and the impact of this disorder on morbidity and mortality.
Objective: Our ongoing prospective study aims to determine the prevalence of SDB and the consequences of this disorder in LTX.
Patients and Methods: Between March 2011 and February 2012 we included 28 clinically stable lung transplant recipients (16 men, time range after LTX 6 months to 9 years). Full polysomnography (PSG) was performed to investigate sleep characteristics and SDB. An apnea-hypopnea index (AHI) $\geq$10/h was used as definition for SDB. Daytime sleepiness was measured by the Epworth Sleepiness Scale (ESS) questionnaire.
Results: Indications for LTX were: 15 COPD, 11 idiopathic pulmonary fibrosis, 1 alpha-1 antitrypsin deficiency, 1 pulmonary hypertension. Age was 54.2±8.3 years, body mass index 25.5±4.0 kg/m^2 and FEV1 69±31% predicted. The prevalence of SDB was 57% (50% obstructive sleep apnea, 7% central sleep apnea). Total sleep time (TST) was 372±91 min in the SDB group and 355±93 min in the non-SDB group (n.s). Sleep efficiency was 68.7±17.1% in the SDB group and 67.1±19.9% in the non-SDB group (n.s). ESS was 4.5±4.1 in the SDB group and 3.6±3.2 in the non-SDB group (n.s).
Conclusions: The prevalence of SDB is high in lung transplant recipients, and sleep quality is equally poor for those with and without SDB compared to the general population. Interestingly, neither the occurrence of SDB nor the quality of sleep was associated with symptoms of sleepiness as measured by ESS.

P1487
Comparison of two immunosuppressant triple therapies on airway mucociliary clearance in rats
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The vast majority of lung transplant patients receive a maintenance immunosuppressive therapy consisting of a triple-drug regimen. However, these drugs are related with important side effects and toxicities that limit their clinical use. We hypothesized that different triple therapies could have different effects upon airway mucociliary clearance. Wistar rats were randomly assigned in three groups (n=10 each): Control = saline solution; T1 = tacrolimus + mycophenolate + prednisone; and T2 = cyclosporine + azathioprine + prednisone. After 7 days of treatment by gavage, animals were killed, lungs excised and *in situ* mucociliary transport velocity (MCTV) and ciliary beating frequency (CBF) measured by microscopic direct view of airway ciliated epithelium. Mucus production by goblet cells was quantified in tracheobronchial tissue. All animals from T1 and T2 groups showed a significant decrease in MCTV in comparison with Control group (0.51±0.08, 0.98±0.13, and 1.34±0.23mm/min, respectively; $p<0.001$). The MCTV in T1 was worse than in T2 ($p<0.001$). Indeed, CBF was slower in T1 and T2 versus Control (9.82±0.71, 12.38±1.09, and 13.68±0.60 Hertz, respectively; $p<0.005$). Mucus production was higher in T1 and T2 groups than in Control group (7.30±1.03, 5.92±0.75, and 4.27±1.29%, respectively, $p<0.05$). We conclude that both triple therapies, mainly T1, caused an important impairment in airway mucociliary clearance by reducing MCTV and CBF and increasing mucus production. These data must be considered by clinicians at the best immunosuppressant therapy choice. This study was support by São Paulo Research Foundation.

P1488
Combined CMV prophylaxis reduces short term complications after lung transplantation
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Among solid organ transplant recipients, lung transplant ones are at highest risk of cytomegalovirus (CMV) infection. The advent of CMV prophylaxis and changes in prophylactic protocols have contributed to a steady decline in CMV infection and mortality; however its potential impact on short-term outcome needs further investigation. The aim of the study is to evaluate the effect of combined CMV prophylaxis in reducing short term acute illness after transplant. A consecutive series of 52 CMV high-risk lung transplant recipients who had more than one year follow-up, were studied. The study group (n=26; age 44±2yrs) received ganciclovir or valganciclovir from postoperative day 15 and CMV-Immunoglobulins for six months, while the control group (n=26; 40±2yrs) was treated with pre-emptive therapy. Viral Infection Index (number of BAL with infection/total BAL number), acute rejection index (ARI, number of acute rejections/total transbronchial biopsies number), incidence of CMV pneumonia and early onset BOS were obtained. Viral Infection Index, infection index simply related to CMV as well as ARI were significantly reduced in the study group than in the control group (mean 33%Vs50% p=0.02; 14%Vs27% p=0.05 and 13%Vs26% p=0.04, respectively), while the incidence of CMV-pneumonia and BOS were similar in the two groups. A significant relationship between combined CMV prophylaxis and a reduced prevalence of acute rejection was observed by logistic regression analysis, even when considering grade A3 only (p=0.01). In conclusion our data underline the strong efficacy of combined CMV prophylaxis in reducing infections as well as acute rejections, particularly the most severe ones, in lung transplant recipients.

P1489
Vascularization of heterotopic tracheal transplant in mice
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Heterotopic tracheal transplantation (HTT) is a commonly used model to study obliterative bronchiolitis (OB) in mice. Vascularization of the graft in this model has not been reported. We here describe that vascularization occurs early after HTT.
Methods: HTT (iso- and allograft) was performed (D0-D21). The graft was collected, paraffin-embedded, and cut into sections for anti-CD31 (Santa Cruz) and anti-LYVE1 (Abcam) immunostaining. Groups of 4 grafts were pooled for RNA extraction, and VEGF, angiopoietin-2 and podoplanin mRNAs quantification in grafts versus control non transplanted tracheas. In another series of experiments, biotinylated dextran was injected I.V. 10min before graft harvest.
Results: CD31 immunostaining increased from D0 to D7 in the tracheal tissue of iso- and allograft (72±12 and 43±8 vessels/mm^2), indicating neovascularization of the tissue. VEGF mRNA expression increased with a peak at D1 (8±3-fold) both in iso- and allografts, whereas angiopoietin-2 mRNA increased from D7

(42±10-fold) to D21 (40±17-fold) in allograft, significantly higher than in isografts. Immunostaining of the lymphatic vessels with LYVE1 occurs at D21 in allografts, and expression of podoplanin mRNA increased at D3 and D7 to return to baseline levels at D21. Functional microvessels labeled with biotinylated dextran were observed in the subepithelium both in iso- and allografts (35±20 and 48±16: D3; 131±42 and 56±11: D7). At D21, labeled microvessels vascularized the trachea (44±03) and the fibroproliferative tissue (OB) (43±17).
Conclusion: The grafts in HTT are vascularized with functional blood and lymphatic vessels. Our data are in strong support of the use of the HTT model for proof of concept studies in OB.

P1490
Study of CD44 expression in fibroblastoid cells isolated from BAL of LTR
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Progressive and irreversible fibroproliferative process leads to BOS. The presence of mesenchymal cells, the primary source of fibrotic cells, has been described in BAL fluid of LTR as predictive of BOS onset (Badri et al, 2011). CD44 cell surface glycoprotein has been found increasingly expressed by graft infiltrating lymphocytes, macrophages and AR fibroblasts with active OB. CD44 has been also associated to an invasive fibroblast phenotype. Inhibition of mTOR, responsible of cell over-proliferation has been found effective in treating fibrotic process.
Aim of this work is to assay CD44 expression in fibroblastoid cells derived from colony-forming units (CFU) of mesenchymal cells isolated from BAL of LTR patients with BOS 0p, 1 and 2 to evaluate its implication in fibrotic process. In addition, in the same cells expression of the active form of mTOR has been assayed to specifically target pharmacological treatment.
Methods: Mesenchymal CFUs from BAL were isolated from two patients with BOS 0p, two with BOS 1 and one with BOS 2, respectively. Proliferation rate was evaluated at 24, 48 and 72 h. CD44 and mTOR expression was assayed by immunocystochemistry.
Results: BOS 1 and 2 patients showed moderate to strong expression of both CD44 and mTOR in 80% cells and weak in 20% cells. BOS 0P subjects displayed moderate to high expression in 5% cells, weak signal in 60% and no detection in 35% cells. We also found that cells isolated from BOS 0p had a significantly ($P<0.01$, ANOVA) lower proliferation rate compared to other cultured fibroblastoid cells.
Conclusion: These results open new perspectives in the identification of a specific fibroblastoid phenotype linked to BOS grades and to target a therapeutic treatment.

P1491
Oxidized-polymers of Z-AT are associated with exaggerated pulmonary inflammation post lung transplantation
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Z antitrypsin (Z-AT) polymerises in the liver and is associated with early onset emphysema. Z-AT polymers are inactive as antiproteinases. We studied patients with and without Z-AT deficiency who had had a lung transplant for emphysema, to examine the relationship between oxidized-polymers (Ox-pZ-AT) and infection/inflammation. BALF was obtained at scheduled surveillance, and as indicated for infection/rejection and airway injury and, assessed by ELISA and immunoblot using a mAb (3F4) to oxidized AT. BALF cell pellets were lysed and HLE activity was used as a measure of PMN count. 16 patients post-transplant were evaluated, 6 Z-AT (15 samples); 9 infective tracheobronchitis, 3 airway stenosis, 1 reflux, 2 normal, and 10 M-AT (20 samples); 7 infective tracheobronchitis, 8 rejection, 5 normal. The Z-AT group with infection had higher Ox-pZ-AT; 386±85ng/ml mean(SEM), $p<0.001$ (compared with non-infected Z-AT group, 100±46ng/ml) and confirmed by immunoblot. The Z-AT group had higher free HLE than M-AT, 139(226-102)ng/ml vs. 74(105-46), (Z vs. M), $p<0.001$. Free HLE in Z-AT was correlated with Ox-pZ-AT levels; $R^2=0.89$. HLE assay of lysed neutrophils showed that the Z-AT group had a higher total PMN count than M-AT; 81±19ng/ml vs. 39±15, $p=0.03$; infected Z-AT (54±9ng/ml) vs. infected M-AT (31±8), $p=0.03$. Ox-pZ-AT present in BALF of Z-AT transplanted individuals are associated with excess PMN and, closely correlated with free HLE. Ox-pZ-AT production results in further reduction of the anti-proteinase and anti-inflammatory protection in the lung and leads to PMN influx. This may predispose Z-AT individuals to exaggerated lung destruction and a worse outcome after lung transplantation.

P1492
Characterisation and hepatocyte-like differentiation of mesenchymal stem cells derived from adipose tissue of immunodeficient mice
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Background: Mesenchymal stem cells (MSCs) can differentiate into hepatocyte-like cells both in vitro and in vivo. These cells may represent a new resource for the treatment of distinct liver diseases, such as alpha-1 antitrypsin deficiency (AATD), a metabolic disease that has pathological consequences for the liver.
Aims and objectives: To isolate MSCs from mouse adipose tissue and verify their potential for differentiation to explore their use for alleviating the shortage of liver transplantation donors to treat AATD.
Methods: MSCs were isolated from immunodeficient mice and cultured to 90% confluency. After DNA demethylation, a differentiation medium was applied and cellular morphology was assessed by microscopy after 0, 7, 14 and 21 days. Flow cytometry was used to detect mesenchymal (CD13, CD29, CD44, CD105) and haematopoietic (CD34, CD45) cell surface markers to estimate the number of differentiated cells. Gene expression of the hepatocyte-specific markers transferrin, albumin, CK18, CD26 and CYP3A1 was measured by semi-quantitative reverse transcription polymerase chain reaction.
Results: During differentiation, the morphology of MSCs changed from a spindle shape into a more polygonal aspect. Mesenchymal markers were expressed at each time point, whereas haematopoietic markers were hardly detectable after 21 days. The relative gene expression of the hepatic markers was increased at each time point.
Conclusion: MSCs derived from mouse adipose tissue may differentiate into hepatocyte-like cells in vitro. Consequently, the potential therapeutic benefit of MSCs in a syngeneic mouse model of chronic AATD can now be analysed.

P1493
Creatine supplementation attenuates systemic and pulmonary effects of acute lung injury induced by pulmonary ischemia-reperfusion in rats
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Creatine supplementation (CS) presents prophylactic and therapeutic effects for some muscular, cardiovascular and neurological disorders. In case of temporary ischemia, CS improves the capacity to generate ATP reducing cell damage. Ischemia and reperfusion (IR) injury is partially attributed to decreased intracellular ATP turnover, but also to increased oxidative stress and reduced IGF-1. Thus, this study evaluated the effects of 5 days of CS prior 90 minutes ischemia of left pulmonary artery followed by 120 minutes of reperfusion in 40 male Wistar rats divided in 4 groups: Sham-operated, Creatine+Sham, Ischemia/Reperfusion, Creatine+Ischemia/Reperfusion. Lung mechanics, exhaled nitric oxide, cellularity in systemic circulation and in bronchoalveolar lavage (BAL), neutrophils and edema in lung tissue, total proteins in BAL, the levels of IL-1beta, IL-4, IL-6, IL-17, KC, MCP-1 and TNF-alpha in serum and in bronchoalveolar lavage were evaluated by ELISA, as well as the expression of IGF-1, iNOS and caspase-3 in lung tissue. Compared with IR group, CS supplementation (CS+IR group) resulted in a reduction of exhaled nitric oxide ($p<0.05$), tissue damping (GTIS) and tissue elastance (HTIS) ($p<0.05$), total cells and neutrophils number in systemic circulation, in BAL and also in lung tissue ($p<0.01$), BAL levels of total proteins ($p<0.05$) and edema index in lung tissue ($p<0.05$), and systemic and pulmonary IL-1beta levels ($p<0.05$). In addition, CS resulted in increased expression of IGF-1 in lung tissue. CS presents protective effects for the development of pulmonary and systemic manifestations of acute lung injury caused by pulmonary IR.