LECTURES

LIVER AND SMALL INTESTINE I

235 10 YEARS INTESTINAL AND MULTIVISCERAL TRANSPLANTATION: THE BERLIN EXPERIENCE

*A. Pascher, U. Gerlach, O. Guckelberger, P. Neuhaus Charité Campus Virchow-Klinikum, Klinik für Allgemein-, Viszeral und Transplantationschirurgie, Berlin, Deutschland

Background: The intestinal and multivisceral transplant program at the Charité in Berlin, Germany, was established in 2000. We hereby present a single center experience with particular respect to immunosuppressive strategies and post-transplant outcome.
Methods: Thirty patients (21 male, nine female; median age 36.76 ± 9.56 years) out of 80 patients referred to our center with irreversible and complicated intestinal failure due to ultra short bowel syndrome or motility disorder underwent isolated intestine (ITx; n = 18) or multivisceral transplantation (MVTx; n = 12). Data were collected prospectively.
Results: According to immunosuppressive strategies, two time periods were analyzed and compared: 2000–2005 (period I); 2006–2010 (period II). Both, period I (ITx: n = 13; MVTx: n=2) and period II (ITx: n=5; MVTx: n = 10) comprised 15 patients. Immunosuppression in period I and period differed with regard to induction. All patients received tacrolimus (period I: trough 20–25; period II: 10–15 ng/ml), steroids (tapered until mth 3), and delayed onset sirolimus or MMF. Induction in period I consisted of daclizumab (tailored dose) and ATG Fresenius single shot pre-reperfusion (8 mg/kg bw) in n = 10 patients, alemtuzumab (30 mg; POD 0/4) in n = 3 patients, and thymoglobuline in n = 2 patients. In period II, thymoglobuline induction (max. dose 7.5 mg/kg bw), single shot infliximab induction (5 mg/kg bw), tacrolimus (trough 10–15 ng/ml), steroids (tapered until mth 3), and delayed onset sirolimus or MMF have been applied. Both, 1-year and 5-year patient and organ survival rates in period I were 62%, in period II 1-year and actuarial 3-year survival rates 100% for ITx and 90% for MVTx.
Conclusion: Modifications in post-transplant management, particularly modifications in immunosuppressive strategies including infliximab induction have accounted for significant advances in post-transplant patient and graft outcome. Short- and mid-term patient and graft outcomes have reached 90%.

011 SURGERY OF HEPATOCELLULAR CARCINOMA IN CIRRHOSIS: LIVER RESECTION VERSUS LIVER TRANSPLANTATION

*D. Foltys[1], M. Heise[1], M. Hoppe-Lotichius[1], T. Hansen[2], M. Pitton[3], M. Schuchmann[4], G. Otto[1] [1]University Medical Center, Department of Transplantation and Hepatobiliary Surgery, Mainz, Deutschland; [2]University Medical Center, Institute of Pathology, Mainz, Deutschland; [3]University Medical Center, Department of Radiology, Mainz, Deutschland; [4]University Medical Center, Department of Internal Medicine I, Mainz, Deutschland

Introduction: Liver transplantation (LT) appears to be the optimal treatment of hepatocelluar carcinoma (HCC). Replacement of the liver implies the removal of HCC and precancerous cirrhosis. As drop out from the waitlist can only partially be avoided by bridging therapy, initial liver resection (LR) is considered a feasible alternative.
Patients and methods: Two hundred and sixty-four patients suffering from HCC were included in this retrospective analysis. All LT candidates (n = 192) underwent repeatedly performed transarterial chemoembolization (TACE) according to our protocol. Patients with tumor progress during TACE exceeding the RECIST criteria were eliminated from the waitlist. During the same period 72 patients were treated by LR.
Results: Of the 192 LT candidates, 128 patients were finally transplanted. Accordingly, the dropout rate during waiting time was 33%. Five-year survival in the drop out group was 8% and in LT patients 73% compared to 30% in LR patients (P < 0.001). If all transplant candidates are included in the Kaplan Meier analysis (intention-to-treat analysis), 5-year survival dropped to 56%. Even this reduced survival rate is significantly superior to the survival after LR (P = 0.003). Remarkable, patients meeting the MC have a similar prognosis regardless of the surgical approach (P = 0.541).
Conclusion: According to these results LR remains a feasible treatment option in HCC meeting the MC suffering from stable Child A cirrhosis, in particular in view of graft scarcity. In view of this unsolved question a randomised trial comparing LT versus LR for HCC in cirrhosis with defined pretreatment would be necessary.

068 ANALYSIS OF RISK FACTORS FOR EARLY AND LATE LIVER GRAFT FAILURE IN PEDIATRIC LIVER TRANSPLANTATION

*U. Herden[1], R. Ganschow[2], E. Grabhorn[2], A. Briem-Richter[2], B. Nashan[1], L. Fischer[1] [1]University Medical Center Hamburg-Eppendorf, Department of Hepatobiliary Surgery and Transplantation, Hamburg, Deutschland; [2]University Medical Center Hamburg-Eppendorf, Department of Pediatric Hepatology, Hamburg, Deutschland

Purpose: Despite improvements in pediatric liver transplantation (LTX) the rate of pediatric re-LTX remains still high about 10% to 30%. Nowadays, there are few data available regarding risk factors for liver graft failure in children.
Methods: We analyzed all primary pediatric LTX at our institution (2000–2009). Recipients were divided into children with functioning graft and children with early (≤30 days) or late (>30 days) liver graft failure who underwent re-LTX.
Results: Overall 279 primary LTX were performed in the study period, thereof 223 children (median age 1.2 years) are alive with functioning liver graft (follow-up median 45mo) and 44 children underwent re-LTX due to liver graft failure, thereof 22 children early re-LTX (median age 1.9 years, median time to re-LTX 4d) and 22 children late re-LTX (median age 0.9 years, median time to re-LTX 24mo). 12 patients who died with a functioning graft were excluded. We found no significant difference in recipient or donor age, graft-to-recipient-weight ratio, diagnosis, elective versus high-urgent transplantation and in the preoperative recipient prothrombin time, creatinine or bilirubin level between children with functioning graft or early or late liver graft failure. There was no significant difference in the rate of whole organ grafts to technical variant grafts, but the rate of living related LTX was lower in the groups with liver graft failure (early graft failure 13.6% and late graft failure 18.2% living related LTX versus 30.9% living related LTX in patients with functioning graft). Children with early liver graft failure, had a significant longer cold and warm ischemic time (time from beginning of venous anastomosis to portal-venous reperfusion) compared to patients with functioning graft (cold ischemic time 568 versus 504 min, P = 0.036; warm ischemic time 38 versus 31 min, P = 0.004), whereas there was no significant difference in the cold and warm ischemic time between children with late liver graft failure and functioning graft.
Conclusion: Our study revealed an increased warm and cold ischemic time as possible risk factor for early liver graft failure after pediatric LTX. Otherwise, living liver donation could be protective for short- and long-term graft function. No other donor or recipient factors could be identified to influence incidence of graft failure.

072 A DELTA-MELD-SCORE > 12 WITHIN A TIME-WINDOW OF 3 MONTHS PRIOR TO LIVER TRANSPLANTATION RESULTS IN DETERIORATED PATIENT OVERALL SURVIVAL

*A. A. Schnitzbauer[1], M. Sabet-Baktach[1], A. Dönecke[1], G. Kirchner[2], M. Loss[1], A. Proneth[1], S. A. Farkas[1], J. Hartl[2], S. A. Lang[1], S. Fichtner-Feigl[1], B. Sinner[3], T. Bein[1,3], H. J. Schlitt[1], M. N. Scherer[1] [1]Uniklinikum Regensburg, Klinik und Poliklinik für Chirurgie, Regensburg, Deutschland; [2]Uniklinikum Regensburg, Klinik für Innere Medizin I, Regensburg, Deutschland; [3]Uniklinikum Regensburg, Klinik für Anästhesiologie, Regensburg, Deutschland

Background: Organ shortage and MELD-score based organ allocation led to an increase of liver transplantations (LT) in patients with high labMELD-scores. A MELD score of 40 is associated with a mortality of over 90% on the waiting list within 3 months. The hypothesis was that a deltaMELD-score within 3 months prior to LT is a predictive factor for patient survival after LT.
Patients and methods: Between December 27th 2006 and April 11th 2010 a total number of 205 LT were performed at Regensburg University Hospital. All 151 primarily transplanted patients were included in the retrospective analysis. DeltaMELD-scores were calculated as MELD-score at transplantation minus MELD-score 3 months before or minus MELD-score at the time-point of listing in case a patient was on the waiting list for less than 3 months. Patients that were listed high urgency received a deltaMELD-score of 34. Various categories of MELD-intervals, deltaMELD-intervals and the influence of organ quality were evaluated, compared and statistically analyzed with Kaplan–Meier-Survival-Analyses (log rank testing). A P-value < 0.05 was regarded to be statistically significant.
Results: Median patient age was 51 years (range: 23–73 years). The median waiting list time was 6 months (range 0–18 months). The median MELD-score at transplantation was 26 (range: 6–40) and the median deltaMELD-score was 9 (0–34). Overall survival (OS) was 78.5% with a median follow up of 780 days (range: 1–1.513 days), 1-year survival was 82.8% in all primarily transplanted patients. Overall organ survival was 83.9% and 1-year organ survival was 86.6%. Patients with a MELD-score > 35 had a significant reduced OS (62.6% vs. 81.8%) and 1-year-survival (68.7% vs. 86.8%) compared with MELD-scores ≤35 (P = 0.018). A deltaMELD-score >12 was a predictor for mortality ($OS_{deltaMELD>12}$ vs. $OS_{deltaMELD<12}$: 69.6% vs. 84.9%, P = 0.025). Here a subgroup analysis showed, that especially a combination of MELD-score >30 + deltaMELD-score >12 are critical predictive factors for unfavorable outcome (OS 66.9%, n = 29, P = 0.049). Furthermore, utilization of marginal donors led to a lower OS in patients with a MELD-score >30 (54.9%, n = 33, P = 0.045).
Conclusion: MELD-scores >35 result in deterioration of OS. The utilization of marginal donors is possible with excellent outcome if selected for patients with

MELD-scores < 30. A deltaMELD-score > 12 may be a predictive factor for OS in LT. Discrepancy of published data from a multicentric trial in German centers and these single center findings warrant the necessity of a nation wide monitored and quality controlled survey to be able to draw a clear cut picture of Germany-specific patterns in LT that facilitate a precise analysis and amendment of organ allocation and postoperative treatment.

086 POSTINTERVENTIONAL TUMOR NECROSIS PREDICTS RECURRENCE-FREE LONG-TERM SURVIVAL IN LIVER TRANSPLANT PATIENTS WITH HCC

*A. Kornberg[1], U. Witt[1], A. Novotny[1], O. Habrecht[2], B. Krause[2], P. Büchler[1], H. Friess[1] [1]TU München, Klinikum rechts der Isar, Chirurgische Klinik, München, Deutschland; [2]FSU Jena, Chirurgie, Jena, Deutschland

Background: The aim of this trial was to analyze the effect of pretransplant interventional bridging therapy (IBT) on long-term survival after liver transplantation (LT) in patients with hepatocellular carcinoma (HCC).

Patients and methods: A total of 91 liver transplant candidates with HCC were included in this trial. At the time of listing, all patients fulfilled Milan criteria on clinical staging. Patient drop-out was based on significant tumor progression, such as macrovascular permeation and extrahepatic tumor spread, but not on macromorphological progression on radiography. If being eligible, patients underwent pretransplant interventional tumor treatment by transarterial chemoembolisation (TACE) or radiofrequency ablation (RFA). The impact of this treatment on tumor recurrence rate and long-term survival was investigated by uni- and multivariate analysis.

Results: Current overall posttransplant follow-up was ranging between 5 and 165 months (mean: 65.1 months). 57 patients underwent interventional bridging therapy (IBT; 64%; TACE $n = 49$; RFA $n = 8$) pre-LT. Twenty-one patients developed post-LT tumor recurrence (23%), 10 patients in the non-treatment group (29%) and 11 patients in the IBT group (19.3%, $P = 0.27$). Recurrence-free survival rates were 67% in the non-treatment population and 76% in the IBT group after 5 years ($P = 0.3$). Response to IBT resulted in a significantly better 5-year recurrence-free survival rate (93%) than non-response to interventional therapy (50%; $P = 0.001$).

In patients revealing partial or complete tumor necrosis at explant pathology, HCC recurrence rate was significantly lower (2,3%) than in patients without postinterventional tumor necrosis (66.6%; $P < 0.001$). Tumor necrosis was identified as most important independent predictor of recurrence-free survival (odds ratio 52; $P < 0.001$) in patients undergoing IBT. Clinical Milan Out patients with tumor necrosis at explant pathology achieved an excellent recurrence-free survival at 5 years (80% vs. 0%; $P < 0{,}001$). AFP-level < 100 U/ml ($P = 0.01$) and absence of 18F-FDG tumor uptake on PET ($P < 0.001$) were predictable for postinterventional tumor necrosis.

Conclusion: Postinterventional tumor necrosis improves significantly outcome after LT for HCC. Patients with advanced HCC beyond the Milan criteria on clinical staging may, thereby, achieve an excellent long-term survival. Low AFP levels and PET - status identify HCCs that are eligible for IBT-induced tumor necrosis and thereby improved survival.

THORACIC ORGANS

287 PATTERNS OF CHRONIC LUNG ALLOGRAFT DYSFUNCTION

V. Arias Herrera[1], B. Sen[2], P. Huppmann[2], F. Ihle[2], W. von Wulffen[2], C. Baezner[2], S. Carolin[2], R. Hatz[3], U. Peter[4], B. Meiser[5], C. Neurohr[2] [1]Klinikum Großhadern, LMU, Pneumologie/Transplantationszentrum, München, Deutschland; [2]Klinikum Großhadern, LMU, Pneumologie, München, Deutschland; [3]Klinikum Großhadern, LMU, Thoraxchirurgie, München, Deutschland [4]Klinikum Großhadern, LMU, Herzchirurgie, München, Deutschland [5]Klinikum Großhadern, LMU, Transplanstationszentrum, München, Deutschland

Background: Chronic lung allograft dysfunction (CLAD) is the major limitation of survival after lung transplantion (LTx). CLAD is a heterogeneous condition, consisting of bronchiolitis obliterans syndrome (BOS) and the recently described restrictive allograft syndrome (RAS). The goal of this study was to assess prevalence of RAS in our LTx-population, as well as to assess if it was associated with a significant negative impact on lung allograft survival.
Methods: Descriptive, retrospective study over a period of 3.5 years using Kaplan Meier analysis, *t*-test and cross tabulation.
Results: Nineteen of 105 consecutive LTx-recipients were classified as having CLAD (FEV1 < 80% baseline). Of the CLAD patients, 12 had RAS (TLC < 90% predicted), 7 BOS (TLC > 90%). There was no statistical difference with respect to demographics. The first FEV1 after LTx was significantly higher for the RAS-group, 1.75 l ± 0.36 ($P = 0.0031$). FEV1 at last follow-up and best-FEV1 were not statistically different. However overall survival was significantly higher for the BOS-group ($P = 0.038$). The follow-up time for both groups was not statistically different and at the end of follow-up, seven of the 12 RAS-patients had died, whereas none of the 7 BOS-patients had.
Conclusions: Significant difference in long term survival between RAS and BOS-groups, previously described in other studies, suggest that RAS could have an additional negative impact on overall survival after LTx.

233 EXTRACORPOREAL PHOTOPHERESIS - A BENEFICIAL TREATMENT FOR CARDIAC AND LUNG TRANSPLANT REJECTION

S. Rummler, D. Barz Universitätsklinikum Jena, Institut für Transfusionsmedizin, Jena, Deutschland

Introduction: Extracorporeal photopheresis (ECP) is a leukapheresis-based immunomodulatory therapy.

Some studies have suggested that ECP may be beneficial for treating and preventing episodes of acute rejection of lung, liver and renal transplant recipients, although real mechanism of action and optimal treatment schedule are still under investigation.
Methods: ECP received periodical eight patients; two patients after cardiac transplantation and six patients after lung transplantation with acute rejection episodes in their transplant history despite sufficient immunosuppression. All patients treated in the recent past with plasmapheresis. ECP we performed off-line. The collected white cells were transferred into UV illumination bag, mixed with 8-MOP, and after incubation illuminated with UVA 365 nm wavelength, 2.5 J/cm^2. The treated mixture was returned to the patients over 30 min within 4 h.
Results: Until today we performed 97 ECP (2–31 procedures per patient). For each procedure we consumed in average 18.95 μg/U 8-MOP (4.8–42 μg/U) and cumulative 253.4 μg/U (34.4–682.2 μg/U). We transfused in average 5.82 × 10E7/kg body weight (1.26–12.26 × 10E7) illuminated leukocytes to the patient. Approximate 62% (31–82%) of the retransfused cells are lymphocytes. All patients responded to ECP, if reinfused more than 5 × 10E7/kg body weight leukocytes per treatment. In two patients the reinfused WBC were twice below 3 × 10E7/kg and these patients suffered from rejection again. We have observed after reinfusion of WBC >10 × 10E7/kg body weight a superficial venous thrombosis as a side effect.
Conclusion: ECP is a very well-tolerated and powerful procedure. Unfortunately many questions regarding ECP are unanswered, such as when to start, how to monitor and when to stop.

170 CLINICAL EXPERIENCES WITH HEART TRANSPLANTATION OF 11 PATIENTS WITH END STAGE HEART INSUFFICIENCY DUE TO CARDIAC INVOLVEMENT OF A SYSTEMIC MUSCLE DYSTROPHY

U. Fuchs, U. Schulz, B. Schulze, K. Hakim-Meibodi, A. Zitttermann, N. Reiß, J. Gummert HDZ NRW Ruhr Universität, Herzchirurgie Herztransplantation, Bad Oeynhausen, Deutschland

Introduction: Muscle dystrophy (MD) has been described as a contraindication for heart transplantation in patients with progressive heart failure. Nevertheless in the last time many cases have been reported about the feasibility of heart transplantation in patients with inherited myopathies. The aim of our study is to analyze the intra- and postoperative risk and the survival after heart transplantation of patients with muscle dystrophy developing cardiac impairment.
Methods: Between 1.1.1989 and 31.12.2009 1760 patients underwent heart transplantation including 11 patients with muscle dystrophy. For data analysis renal function, adverse events, survival and immunosuppressive regime was evaluated.
Results: Five patients had an Emery-Dreifuss-MD, four patients had a Becker-Kiener-MD, one patient had a Neuromuscular-Typ-MD and one patient had a Multi-Core-MD. Average age was 33.4 years (18–58; standard-deviation; SD: 13.12). Average ischemic time was 205 min(142–266; SD: 40.4) and median Survival was 2443.3 days (17–7168; SD: 2579.5). The 5-year survival – rate was 71.4% ($n = 5/7$). Causes of death: rejection ($n = 1$); graftvasculopathy ($n = 1$); technical complications ($n = 1$). Immunosuppression: Tacrolimus ($n = 6$); Cyclosorine A ($n = 5$) with comparable postoperative course. Currently all eight patient, who survived, have a good function of the transplanted heart. No increased number of adverse events especially such as neurotoxicity or myalgia was observed.
Conclusion: In our opinion heart transplantation in patients with progressive heart failure combined with hereditary muscle dystrophy is a sufficient option realizing acceptable long term results without an increased number of postoperative complications.

211 MYOCARDIAL DEFORMATION ANALYSIS BY ECHOCARDIOGRAPHIC STRAIN IMAGING ALLOWS DIFFERENTIATION BETWEEN PATIENTS WITH AND WITHOUT CORONARY STENOSES AFTER HEART TRANSPLANTATION

C. H. Pang, M. Dandel, E. Wellnhofer, R. Hetzer Deutsches Herzzentrum Berlin, Herz- Thorax und Gefäßchirurgie, Berlin, Deutschland

Purpose: Early non-invasive detection of patients with transplant coronary artery disease (TxCAD) is a major goal. Echocardiographic (ECHO) strain imaging reveals alterations in wall motion and myocardial deformation not detectable by conventional ECHO and allows distinction between active and passive wall motion. We assessed its reliability in distinguishing between patients with and without TxCAD.
Methods: LV radial, circumferential and longitudinal strain and strain-rate (Sr) were measured before coronary angiography in adult heart transplant recipients with normal left ventricular ejection fraction (LVEF ≥ 55%) and without visible wall motion abnormalities. From strain curves also ayssynchrony and dyssynergy indexes were calculated. Strain and Sr parameters and indexes were tested for relationships to angiographic findings. Exclusion criteria: acute rejection, bundle branch block, LVEF < 55%.
Results: Only 56 patients (29.9%) had no angiographic TxCAD (group A), whereas 44 (23.6%) proximal focal stenoses (group B) and 87 (46.5%) diffuse distal coronary type-B lesions group C). Whereas conventional ECHO-parameters showed no predictive value for coronary stenoses, different strain and Sr parameters and indexes appeared predictive for detection of patients with TxCAD. Thus, the time to peak longitudinal strain-rate (TpSrL) and the peak longitudinal strain-rate/TpSrL index showed up to 93.1% and 94.6% sensitivity and specificity, respectively, for differentiation between group A and C. The ayssynchrony and dyssynergy indexes for longitudinal shortening also allowed differentiation between group B and C (sensitivity and specificity: up to 86.3% and 74.4%, respectively).
Conclusions: 2D-strain imaging is reliable for detection of patients with TxCAD and differentiation between patients with and without focal coronary stenoses. Our results recommend 2D-strain as a non-invasive tool with the potential to enable angiographies to be optimally timed.

299 EVEROLIMUS, MTORC1 SIGNALING AND EVOLUTION OF BODY WEIGHT AFTER CARDIAC TRANSPLANTATION

G. Junge, S. Witte, A. Zuckermann, H. Lehmkuhl, H. Schwende, Z. Wang, J. Kobashigawa For the B253/A2411/A2403 study groups

Introduction: The mammalian target of rapamycin complex 1 (mTORC1) regulates cellular responses to fuel/energy availability. Recent studies demonstrate that mTORC1 represents an essential intracellular target for the actions of hormones and nutrients on food intake and body weight(BW) regulation. Everolimus(EVR), an mTOR inhibitor effects solely mTORC1 and not mTORC2. Here, the effect of six immunosuppressive regimen on evolution of BW after HTx is assessed.
Results: Seven hundred and forty-three de novo HTx recipients from three randomized, multicenter trials were exposed to: SD-CsA/AZA($n = 167$); SD-CsA/MMF($n = 62$); SD-CsA/h-EVR($n = 161$); SD-CsA/l-EVR($n = 170$); SD-CsA/TDM-EVR($n = 54$); RDCsA/TDM-EVR($n = 129$) [SD/RD=standard/reduced dose; h/l-EVR=3.0/1.5 mg/d; tdm-EVR = C0 3–8ng/ml]. Here, we evaluate evolution of BW 6/12 months(M) post HTx. Overall weight change M1-6/12 are displayed in Table 1.

Analyses for weight change by BL BMI categories for MMF versus RD-CsA/tdm-EVR are shown in Fig. 1 (complete M12 data for all regimes will be presented). The same pattern was consistently observed in gender and age subgroup analyses.
Conclusion: In de novo HTx recipients overall BW increase M1-12 was highest in MPA and AZA. A different pattern was observed when analyzed by BL BMI categories. Here, patients with a low/normal BMI had higher BW increase when exposed to tdm-EVR, whereas patients with a high/very high BMI had greater increase when treated with MMF. These results confirm similar observations in a kidney transplant population. Thus, mTOR treatment seems to not affect weight increase in low/normal BMI categories, but shows less increase in high/very high BMI. A possible explanation may be the effects of EVR on metabolic regulation and adipose tissue differentiation.

BONE MARROW TRANSPLANTATION

038 PD-L1 BLOCKADE EFFECTIVELY RESTORES STRONG GRAFT-VERSUS-LEUKEMIA EFFECTS WITHOUT GRAFT-VERSUS-HOST-DISEASE AFTER DELAYED ADOPTIVE TRANSFER OF T CELL RECEPTOR GENE-ENGINEERED ALLOGENEIC CD8$^+$ T CELLS

*W. Koestner, M. Sauer Medizinische Hochschule Hannover, Pädiatrische Hämatologie und Onkologie, Hannover, Deutschland

Adoptive transfer (AT) of T cells forced to express tumor-reactive T cell receptor (TCR) genes is an attractive strategy to direct autologous T cell immunity against tumor-associated antigens (TAAs). However, clinical effectiveness has been hampered by limited in vivo persistence. We investigated whether the use of MHC-mismatched T cells would prolong the in vivo persistence of tumor-reactive TCR gene expressing T cells by continuous antigen-driven proliferation via the endogenous potentially alloreactive receptor. Donor-derived CD8$^+$ T cells engineered to express a TCR against a leukemia-associated antigen mediated strong graft-versus-leukemia (GVL) effects with reduced graft-versus-host-disease (GVHD) severity when given early post-transplant. AT later post-transplant resulted in a complete loss of GVL. Loss of function was associated with reduced expansion of TCR-transduced T cells as assessed by CDR3 spectratyping analysis and PD-1 upregulation on T cells in leukemia-bearing recipients. PD-L1 blockade in allogeneic transplant recipients largely restored the GVL efficacy without triggering GVHD, whereas no significant anti-leukemia effects of PD-L1 blockade were observed in syngeneic controls. These data suggest a clinical approach in which the AT of gene-modified allogeneic T cells early post-transplant can provide a potent GVL effect without GVHD, while later AT is effective only with concurrent PD-L1 blockade.

169 AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION AS A THERAPEUTIC OPTION FOR PATIENTS WITH SEVERE SYSTEMIC SCLERODERMA

A. Moser[1], S. Straßburg[1], M. Jakob[1], I. Müller[2], G. Leipold[3], S. Corbacioglu[1], *J. Föll[1] [1]Universitätsklinikum, Pädiatrische Hämatologie und Onkologie, Regensburg, Deutschland; [2]Universitätsklinikum, Pädiatrische Hämatologie und Onkologie, Hamburg, Deutschland [3]Praxis, Kinder-und Jugendmedizin, Regensburg, Deutschland

Introduction: Systemic scleroderma is a chronic autoimmune disease which affects primarily the skin and fibrotic tissue. The diffuse form is rapidly progressive and is characterized by the clinical symptoms scalcinosis, Raynaud's phenomenon, esophageal dysfunction, sclerodactylie and telangiectasis. The 10-year-survival is 55% and there is no therapeutic gold standard available. Hematopoietic stem cell transplantation (HSCT) when conducted early leads to a sustain improvement of symptoms.

Case report: Eight years old male diagnosed at age five due to progressive symmetric joint contractions, cutaneous pigmentation and thickened skin. Calcifications present in radiograms and necroses of the fingertips with ANA levels of up to 1:5120 confirmed the diagnosis. At this time pulmonary insufficiency and a decreased mobility of the oesophagus was apparent. Because immune-suppressive drugs failed we decided to carry out an HSCT. He was transplanted with a CD3$^+$ and CD19$^+$ depleted graft to improve recovery and reduce autoimmune T cell clones. With thymoglobulin, fludarabine and cyclophosphamide for conditioning he achieved after an uncomplicated post-transplant recovery a remarkable improvement of his clinical symptoms with an increase of mobility. In the following weeks, we found an improvement of skin thickening, decrease of ANA levels and stabilisation of organ function.

Conclusions: HSCT with depletion of potential autoimmune cells in selected patients with severe progressive systemic scleroderma is a valid therapeutic approach that can contribute to a sustained improvement of quality of life.

208 SUCCESSFUL CORD BLOOD TRANSPLANTATION WITH MULTIPLE MISMATCH FOR DYSKERATOSIS CONGENITA AND TINF2-MUTATION

*M. Jacob, J. Föll, S. Corbacioglu Universitätsklinikum Regensburg, Pädiatrische Hämatologie, Onkologie und Stammzelltransplantation, Regensburg, Deutschland

Introduction: Allogenic hematopoietic stem cell transplantation (HSCT) for Dyskeratosis congenita (DC) is curative. For these patients transplantation-related mortality with conventional conditioning is high due to susceptibility to capillary damage and DNA repair deficiency. To avoid these complications reduced-intensity conditioning regimens were favoured in the past.

Case report: A 2-year old girl with aplastic anemia and molecularly confirmed TINF2-mutation with development delay but no DC related stigmata was transplanted in our unit. Postnatal she presented with refractory neutropenia, anemia and thrombocytosis. Initial bone marrow aspirates showed a hypercellularity without significant dysplasia. A progressive bone marrow failure and frequent pulmonary 'infections' of unknown origin were observed. No matched family or unrelated donor was available. Therefore a mismatch single cord blood (4/6) donor was chosen. The reduced-intensity conditioning regimen included ATG Genzyme, fludarabine and cyclophosphamide. The transplanted cord blood count was 2.8×10^5 CD34$^+$ cells/kg. Bone marrow engraftment was on day +20. She first showed a mixed chimerism on day +36 with 36% overall donor cells, predominantly CD3$^+$. After reduction of immunosuppression chimerism was complete.

Conclusion: DC can occur without stigmata in very young children and must be considered as differential diagnosis for aplastic anemia. Allogeneic HSCT for DC with TINF2 mutation can be successfully with a single multiple mismatch cord blood unit using reduced-intensity conditioning regimen with low toxicities.

178 WISKOTT ALDRICH SYNDROME WITHOUT MICRO THROMBOCYTOPENIA? NOTES FOR NEW MUTATION

E. Votrubec, *S. Weickardt, M. Jakob, J. Föll, S. Corbacioglu Universitätsklinikum Regensburg, Pädiatrische Hämatologie, Onkologie und Stammzelltransplantation, Regensburg, Deutschland

Introduction: The Wiskott Aldrich Syndrome (WAS) is a x-linked disorder that presents with a variety of phenotypes from isolated neutropenia or thrombocytopenia up to the severe form with eczema, combined immunodeficiency and the hallmark of micro-thrombocytopenia with bleedings. Hematological stem cell transplantation (HSCT) is next to yet experimental gene therapy the only cure.

Case report: e report here a 2 months old male who presented with eczema, fever of unknown origin, thrombocytopenia, hepatosplenomegaly and failure to thrive. Micro-thrombocytopenia was absent but the lymphocytopenia, with an almost complete absence of CD8$^+$-T cells and a deficient antigen specific T cell proliferation test was suspicious of a combined immunodeficiency syndrome. The molecular analysis eventually revealed a previously not described homozygous mutation of the WASP gene. Because of the refractory Infections, failure to thrive and progredient lymphocytopenia a HSCT with an HLA-matched unrelated donor was performed. The conditioning regimen with Treosulfan and Fludarabine was tolerated well without serious complications. Currently, the patient is in very good general condition without GVHD or infection, stable haematological reconstitution and developmental catch-up growth.

Conclusions: WAS is a chameleon that needs to be considered in all male patients with a variable hematopoietic and/or immunodeficient phenotype and unexplained eczema even without micro- thrombocytopenia. Prompt HSCT prior to the development of opportunistic infections is currently the only standardized cruative option.

PANCREAS

227 ISLET TRANSPLANTATION IN DAILY DIABETES CARE

*B. Ludwig[1,2], A. Reichel[1], S. Ludwig[3], S. Kersting[3,2], A. Steffen[1,2], J. Schmidt[1], M. Brendel[1], H.-D. Saeger[3,2], S.R. Bornstein[1,2] [1]Universitätsklinikum Carl Gustav Carus Dresden, Medizinische Klinik III, Dresden, Deutschland [2]Paul Langerhans Institut Dresden, Dresden, Deutschland; [3]Universitätsklinikum Carl Gustav Carus Dresden, Klinik für Viszeral-, Thorax- und Gefäßchirurgie, Dresden, Deutschland

Background: For the majority of patients with type 1 diabetes intensive insulin therapy is effective and safe for maintaining glycemia and minimizing diabetes associated complications. However, a rare number of patients show highly labile metabolic control and experience repeated, unpredictable and often life threatening hypoglycaemic episodes despite optimal medical therapy. The rationale for those therapeutic problems is mostly the combination of abolished counter-regulatory mechanisms and severe autonomous neuropathy with impaired gastric emptying. Patients are at high risk for the development of severe acute and chronic diabetes associated complications, often require a safety-hyperglycaemia striving regimen and quality of life (QoL) is considerably impaired. For this small subset of patients, restoration of a basal endogenous and glucose regulated insulin secretion can substantially improve metabolic control and QoL. In our experience, this is irrespective of complete insulin independency.
Methods: Here, we report on our two-year experience with implementing islet transplantation as a potential treatment option for type 1 diabetes. All patients were recruited from the own outpatient clinic, assuring patient's compliance and optimal insulin therapy. All patients were treated by long-term CSII prior to enrolment. The main indication was brittle diabetes and repeated severe hypoglycaemia. The mean HbA1c was 7.9 ± 0.9%. The mean age was 47.3 ± 10.4 years, BMI ranged at 25.7 ± 2.6 kg/m^2. Pre-transplant insulin requirement was 40 ± 15.6 units per day.
Results: Up to date, eight patients have been transplanted in our center with single islet infusion (mean islet mass: 10 200 ± 4.700 IEQ/kg) and followed for up to 24 months. All patients show good graft function, stable glycemic control with a reduction in HbA1c in absence of hypoglycaemia, and a tremendous improvement in quality of life. All patients are kept on minimal insulin via CSII.
Conclusion: In conclusion, islet transplantation can be an excellent therapy for selected patients with type 1 diabetes. Key prerequisite for success and improvement in long-term outcome is a correct and strict indication. The primary goal for islet transplantation should be stabile glycaemia and prevention of hypoglycaemia rather than insulin independence. In fact, maintaining minimal exogenous insulin may protect the islet graft from metabolic stress and exhaustion and even prolong islet graft function.

280 WHERE OFFERED GERMAN PANCREATA GET LOST: PROCESS ANALYSIS OF ORGAN UTILIZATION

*M. Loss[1], K.P. Drewitz[2], K. Kovac[3], T. Breidenbach[4], J. Loss[2] [1]Universitätsklinikum Regensburg, Klinik für Chirurgie, Regensburg, Deutschland; [2]Universitätsklinikum Regensburg, Medizinische Soziologie, Regensburg, Deutschland; [3]Eurotransplant Int. Foundation, Medical Affairs, Leiden, Niederlande [4]Deutsche Stiftung Organtransplantation, Region Bayern, München, Deutschland

Background: The majority of pancreata, offered in allocation, are discarded. This pancreas under-utilization is not well understood, so a thorough analysis of the allocation process seems necessary.
Methods: Allocation protocols of all Eurotransplant (ET)-registered German whole pancreas donors offered to allocation between 2005–2009 (n = 1769) were analysed in detail including turn-down reasons, number of offers required to place (or discard) a pancreas, and time spans of allocation process.
Results: 656/1769 offered pancreata were transplanted (37.1%). 815 were repeatedly rejected and finally withdrawn from the allocation process, after being offered to a median of five centres (eight patients; vs. three centres/three patients for transplanted organs). A further 239 pancreata were discarded following surgical inspection of the organ at the time of intended recovery. 128 pancreata were explanted but not used due to recipient-related reasons (n = 63), or because the organs were damaged or had anatomic abnormalities (n = 65). For the discarded organs, the mean time from registering the organ at ET and the decision to withdraw the organ was 6.8 ± 6.7 h. The reasons for rejecting an offer often differed between centres; a mean of 1.6 ± 1.1 different turndown causes was given for each pancreas (1.4 ± 1.1 for those finally transplanted, 1.8 ± 0.94 for those finally withdrawn). The reasons most frequently named were 'unfavourable lab results' (n = 562), macroscopic damage' (n = 239), 'long ICU stay' (n = 225), and 'resuscitation' (n = 181). Among the 293 organs with favourable P-PASS (<15) and donor age (<30 years), 39% were discarded.
Discussion: The results allow a better understanding of where the organs get 'lost'. Further studies are currently underway to analyse in detail the reasons for rejecting the offered organs, especially those with a favourable P-PASS.

116 "NONSTANDARD" DONORS IN PANCREAS TRANSPLANTATION – A TOO OFTEN UNUTILIZED RESOURCE

*D. Uhlmann, H.-M. Hau, H. Morgül, H.-M. Tautenhan, M. Bartels, S. Jonas Universitätsklinikum Leipzig, 2. Chirurgische Klinik, Leipzig, Deutschland

The current donor organ shortage mandates an ongoing reappraisal of the limits of acceptability when considering pancreas offers from nonstandard or extended-criteria donors.
Patients: Among the 10 pancreas transplantations (PT) in our center in 2010, in five cases (four simultaneous pancreas-kidney transplantations, one pancreas transplantation alone) organs from extended donors were used. These five donor organs were refused by other centers due to cardiac arrest and reanimation, prolonged ICU stay or history of alcohol/drug abuse. Post-operative outcome, organ function and morbidity were analyzed and compared to patients receiving standard-donor organs.
Results: The mean donor age (34 ± 15 years), BMI (23 ± 6 kg/m^2), Lipase (52 ± 36 U/l), P-PASS (16 ± 2), cold ischemia time (12 ± 4 h) showed no differences between the standard and extended donor group. The mean recipient age was 48.6 ± 7.6 years. Recipient median BMI was 25.8 ± 1.6 kg/m^2. Median insulin demand of the patients was 72.8 ± 13.95 units. The PT was performed at a mean of 13.2 ± 3.67 years after the beginning of a medical treatment an a waiting time of 5.2 ± 3.3 months. In the post-operative course, peak CRP (88 ± 24 mg/l), peak lipase (1.8 ± 1.2 μkat/l) and need for insulin substitution was comparable between both groups. In all patients with organs from extended donors, pancreas transplants showed a good primary function without need for insulin after the third day. Two patients of this group needed a re-operation because of a wound infection. Regarding the kidney transplant, two patients revealed a delayed graft function due to acute tubular necrosis without the need for dialysis. The average duration of hospital stay was 29 ± 12 days. In-hospital mortality rate was 0%. Graft-related complications were a mild pancreatitis in one patient, but no fistula or graft thrombosis. There were no cardiac or respiratory complications. At short term follow-up, all recipients receiving grafts from nonstandard donors show a stable function of their grafts and are free of insulin at a mean follow-up of 7.4 months.
Conclusion: These findings suggest a chance to increase the donor pool with good outcomes using nonstandard donors.

255 SIMULTANEOUS PANCREAS AND KIDNEY TRANSPLANTATION IN A SMALL-VOLUME CENTER: TOWARD 100% 1-YEAR GRAFT SURVIVAL

*T. Y. Tsui, E. G. Achilles, J. Pollok, M. Koch, L. Fischer, B. Nashan UKE, Transplantationschirurgie, Hamburg, Deutschland

Simultaneous pancreas and kidney transplantation is currently curative therapy for type I diabetes mellitus with terminal renal dysfunction. The procedure is, however, associated with a higher rate of immunological and surgical complications. The use of new immunosuppressive regimen significantly improves the outcome of SPKTx. The surgical complications vary between centers that might be up to 70%. Here we analyzed the data of SPKTx in our center after implementation of new protocol with alteration of surgical technique (portal venous drainage) and a novel immunosuppressive regimen (induction with ATG and Simulect and a combination of low-dose tacrolimus and everolimus as basic immunosuppression, steriod will be tapered up with 10 days post-operatively) since 2009 (n = 12) and compared the data of SPKTx before the implementation of new protocol (iliac venous drainage, induction with ATG and basic immunosuppression with tacrolimus, MMF and steriod, n = 12, from 2003 to 2008). There is difference in terms of donor profiles after implementation of new protocol, which 42% of organs are center offers and 42% donors with history of ploytrauma in SPKTx with new protocol. Both groups had the same median P-PASS score (median 15). SPKTx with new protocol improved the outcome of PTx with 100% insulin-free pancreas graft survival at one month, 6 and 12 months after Tx., whereas insulin-free graft survival in old protocols are 42%, 42% and 25% respectively. There is no surgical intervention needed in patients with new protocol, whereas surgical interventions are done in 83% of Pat with old protocol. The major causes for the pancreas graft loss in patients with old protocol were venous thrombosis and/or pancreatitis. There is no difference in terms of kidney graft function and survival between two groups.
Conclusion: an excellent result of SPKTx could be achieved in a small-volume transplant center.

250 ACCEPT OR REJECT? FACTORS INFLUENCING THE DECISION-MAKING OF TRANSPLANT SURGEONS WHO ARE OFFERED A PANCREAS: RESULTS OF A QUALITATIVE STUDY

*K. P. Drewitz[1], M. Loss[2], K. Kovac[3], *J. Loss[1] [1]Universitätsklinikum Regensburg, Medizinische Soziologie, Regensburg, Deutschland; [2]Universitätsklinikum Regensburg, Klinik für Chirurgie, Regensburg, Deutschland; [3]Eurotransplant Int. Foundation, Leiden, Niederlande

Background: The majority of offered pancreata are not transplanted. The reasons for this pancreas under-utilization are not well understood. This study aims to analyse the individual organ rejection process.
Methods: Semi-standardised, face-to-face interviews were conducted with 11 consultants from 11 transplant centers in Germany. All interviewees were highly qualified transplant surgeons who are in charge of accepting or

declining organ offers. The interviews focused on the criteria on which the accept/reject decision following a pancreas offer depends. Interviews were recorded, transcribed and underwent content analysis after de-identification.

Results: The interviewees agreed upon a number of criteria on which they base their accept/reject decision: donor age, BMI, ICU stay, patient history and pancreas macroscopy, although clear cut-offs do not exist. We found completely diverse assessments, however, of the factor 'donor resuscitation' and of P-PASS. Knowing (and trusting) the explant surgeon is also an important factor in organ acceptance. The following criteria play a minor role: previous rejection of another centre, the 'fit' to the intended recipient (although some interviewees stated that the relatively small waiting lists render it more difficult to place the organ). It is rarely only a single factor that accounts for the rejection, but ''the sum of a bad gut feeling''. The majority of interviewed surgeons explained that sometimes, organs are turned down because of staff and capacity shortage. Surgeons admitted they did not always feel certain when turning an offer down, but tended not to doubt their decision afterwards.

Discussion: It transpired that medical accept/reject criteria are used inconsistently and differ between different surgeons. Non-medical aspects play an important role, too, e.g. surgical capacity or trust in the retrieval team. The results are part of a larger study including quantitative analysis of ET-data.

KIDNEY I

124 STEROID-FREE IMMUNOSUPRESSION: EFFECT ON BMI AND GROWTH

*P. Wittenhagen, H. Thiesson, M. Neland Odense University Hospital, Nephrology, Odense, Dänemark

Purpose: Obesity and growth retardation are well known complications to steroid based immunosuppression in transplantation. We evaluated the effects of steroid free immunosuppression on body mass index (BMI) and linear growth in children following renal transplantation.
Method: Retrospective review of 47 children transplanted from 1994–2009 immunosuppressed with calcineurin inhibitor and mycophenolate mofetil. BMI-standard deviation score (SDS) and height-SDS were compared before (pre-Tx) and after (post-Tx) transplantation. Anthoprometric data were calculated using reference data from Nysom (BMI) and Albertsson-Wikland (height). Data from patients treated with steroids due to rejection were excluded.
Results: Pre-Tx 6% (three out of 47) of the patients were obese defined by BMI > 95th percentile (similar to normal Danish children). BMI-SDS remained stable after transplantation, pre-Tx (0.070.15) and 5 years post-Tx (0.040.22) ($P > 0.2$). All groups had a significant catch-up growth, however most pronounced in the youngest age-groups. Average height-SDS in our population are depicted in Table 1. No patient developed diabetes.
Conclusion: With 5 years follow-up we find that steroid-free immunosuppression after renal Tx protects against glucocorticoid induced obesity and to our knowledge this is the first study showing the absence of weight gain. Furthermore we demonstrate catch-up growth especially in the youngest children.

253 UNDIAGNOSED GLUCOSE METABOLISM DISORDERS IN DIALYSIS PATIENTS: AN ANALYSIS USING ORAL GLUCOSE TOLERANCE TESTS IN GERMAN DIALYSIS CENTERS

F. Tokmak[1], M. Banas[2], F. Pallmann[3], M. Baran[1], B. Banas[2], B. Krüger[3], *B. Krämer[3] [1]Universitätsklinikum Bochum, Bochum, Deutschland; [2]Universitätsklinikum Regensburg, Nephrologie, Regensburg, Deutschland; [3]Universitätsklinikum Mannheim, V. Med. Klinik, Mannheim, Deutschland

Background: Post-transplant diabetes mellitus (PTDM) or new-onset diabetes mellitus (NODM) after renal transplantation is considered a major health threat for renal transplant recipients, that goes along with decreased patient and graft survival.
Methods: Screening for undiagnosed diabetes mellitus was done with the use of oral glucose tolerance test (oGTT) in four dialysis centers in Germany according to ADA criteria. Impaired glucose metabolism disorders were defined as a fasting glucose level ≥ 100–125 mg/dl (impaired fasting Glucose IFG) and/or a 2 h glucose level 140–199 mg/dL (impaired glucose tolerance IGT). Overt diabetes mellitus was defined as a fasting glucose level ≥ 126 mg/dl and/or a 2 h glucose level ≥ 200 mg/dl.
Results: Two hundred and thirty-seven adult hemodialysis patients were considered for inclusion in this trial. Nineteen patients (=38.4%), that were known to be diabetic were excluded from the trial leaving 146 non-diabetics. Since oGTT was not performed in 40 of these nondiabetics due to refusal to participate or inability to give informed consent or to participate or nonadherence, 106 patients underwent oGTT. From these 106 patients, 12.3% had an abnormal fasting glucose (≥100 and <126 mg/dl), 18.9% had an impaired glucose tolerance (2 h glucose level ≥ 140 and <200 mg/dl), and 9.4% had overt diabetes mellitus.
Conclusion: There is a considerabe number of undiagnosed glucose metabolism disorders including overt diabetes mellitus in German hemodialysis patients. These patients may (eronneously) be classified as PTDM or NODM after renal transplantation.

081 THE COSTS OF KIDNEY TRANSPLANTATION – WHAT MARGINAL GRAFTS MAY TRIGGER

*K. Lopau, J. Detering, A. L. Herzog, C. Wanner Med. Universitätsklinik und Poliklinik I, Nephrologie, Wuerzburg, Deutschland

Introduction: Transplantation is considered to be the most cost-efficient renal replacement modality. Typically, after a phase of high expenditures in the first three months, costs decrease and fall below dialysis expenses. Knowing this, also the transplantation of worse grafts was supposed to be of benefit for patients and health care systems. However, an distinct revenue account related to graft function in Germany was not undertaken before.
Methods: We evaluated the costs of inpatient- and outpatient-contacts for KTx-recipients surviving more than one year and being constantly in our follow-up care. Patients were classified in four groups in relation to the eGFR at year 1. We used the G-DRG-2010-system to calculate the hospitalisation charges as well as officially fixed prices for medications in Germany.
Results: We evaluated 204 patients, 53 pts with CKD_T I, 64 with II, 69 with III and 18 with IV. Mean age at KTx was 49 years, 63% were male and 17% received an organ from a living donor. eGFR at year1 was negatively correlated to donor age and to recipient BMI. Patients with DGF had a worse graft function, the number of dialysis treatments post transplantation was significantly higher. Patients with worse graft function received significantly less mycophenolate or azathioprin, in most cases due to side effects. Costs for hospitalisation as well as for ambulatory treatment increased significantly with worsening graft function. Hospital charges were two times higher than the costs for ambulatory care. While CKD-I and II-pts consumed approximately 42 200 €/year in total, for III-patients 47 600€ and for IV-patients 57 500 € were spent, this difference was significant. Also expenses for the treatment of adverse events, anemia and antihypertensive therapy and graft failure were higher in respect to worse graft function. The highest expenditures (besides transplantation hospital charges) incurred for the immunosuppressive therapy (26% of total expenses), CMV-treatment (5.9%), treatment of infectious complications (3.7%) and of other adverse events (3.5%). Cardiovascular and metabolic complications were not significantly different during the first year.
Conclusion: Worse graft function after one year is significantly correlated with higher costs pointing towards a more complicated course and more severe complications.

088 ROBOTIC-ASSISTED DONOR NEPHRECTOMY FOR LIVING DONOR KIDNEY TRANSPLANTATION - RESULTS OF THE FIRST SERIES IN GERMANY

*M. Janssen[1], U. Sester[2], B. Kopper[1], M. Gerber[1], C.-H. Ohlmann[1], Z. Akcetin[1], J. Kamradt[1], D. Fliser[2], S. Siemer[1], M. Stöckle[1] [1]Universitätskliniken des Saarlandes, Urologie, Homburg / Saar, Deutschland; [2]Universitätskliniken des Saarlandes, Klinik für Innere Medizin IV, Nephrologie, Homburg / Saar, Deutschland

Introduction: Transplantation after living kidney donation offers longer graft survival. Due to the still existing organ shortage, living donation gets more important. In order to reduce the significant trauma for the donor, caused by the conventional retrieval procedure, the robotic-assisted donor nephrectomy was introduced in our center. The system allows a greater freedom of movement and recreates the hand-eye coordination and 3D-vision that is lost in standard laparoscopy. The results of the first series are presented.
Methods: The daVinci® system was used for a transperitoneal laparoscopic nephrectomy. All patients ($n = 5$) were positioned in an elevated-side-position (about 70° up). The intra-abdominal pressure was 12 mmHg, five trocars were used in total. The camera was positioned supra-umbilically. Vessels were ligated using haemoloc® and titanium clips. The organs were retrieved hand assisted via pfannenstiel incision, in one case via mini laparotomy.
Results: Operational time ranged between 169 and 284 min, blood-loss in all cases < 100 ccm. The warm ischemia time reached from 1 min 30 s to 6 min. No postoperative complications occurred, donors stayed for 4–5 days in hospital.
Conclusion: The application of minimal invasive techniques allow an increased acceptance of the living kidney donation and may decrease the burden the recipients feel for their donors. The introduction of the robotic system holds the potential for a remarkable technical achievement in this matter. In our opinion it may therefore lead to a further increase of the numbers of donors for this life-saving transplant. The initial results are encouraging and clearly prove feasibility.

KIDNEY II

201 A NOVEL URINE-BASED METABONOMIC TEST SYSTEM FOR THE MONITORING OF RENAL ALLOGRAFT FUNCTION

*B. Banas[1], *P. Pagel[2], A. Krumpel[2], F. Huber[2], V. Pfahlert[2], S. Farkas[3], M. Banas[1], B. Jung[1], B. K. Krämer[4], C. A. Böger[1] [1]Universitätsklinikum, Klinik und Poliklinik für Innere Medizin II, Nephrologie, Regensburg, Deutschland; [2]LipoFIT Analytic GmbH, Regensburg, Deutschland; [3]Universitätsklinikum Regensburg, Chirurgie, Regensburg, Deutschland; [4]Universitätsmedizin Mannheim, V. Medizinische Universitätsklinik, Mannheim, Deutschland

Background: Many transplantation centers perform protocol biopsies after kidney transplantation since a non-invasive test for the exclusion of subclinical rejections is currently not available. Our main objective is the development of a NMR-based metabonomic urine test system to detect early renal allograft rejection.
Method: We used a 600 MHz Bruker AVANCE II+ spectrometer for 1D1H NMR spectroscopy. Based on this NMR-analysis of human urine samples, we established a metabonomic fingerprinting method that allowed identifying a network of metabolites that are related to kidney rejection after transplantation.
Results: The prediction method has been successfully established in a retrospective cohort of 78 patients and approximately 400 urine samples. A novel test system was developed showing the risk of allograft rejection. One third of the samples was detected as being at high risk, and developed a biopsy confirmed rejection in 96% during the further course. Another third of the samples was assigned to the low risk group and remained free of rejection in 100%.
Conclusion: We have established a test system for the analysis of urine samples by means of high-resolution NMR in a high-throughput manner on a routine basis and at reasonable costs. We expect further optimization of this approach with the data from an ongoing study (Umbrella Study) including more than 100 consecutive patients at our transplantation center. Our novel test system shows a high potential for a clinically relevant non-invase procedure to exclude allograft rejection, and to further elucidate underlying molecular mechanisms.

005 RIP1-MEDIATED NECROPTOSIS ESSENTIALLY CONTRIBUTES TO RENAL ISCHEMIA/REPERFUSION INJURY

*A. Linkermann[1], J.-H. Bräsen[2], N. Himmerkus[3], U. Kunzendorf[1], S. Krautwald[1] [1]Christian-Albrechts Universität, Klinik für Nieren- und Hochdruckkrankheiten, Kiel, Deutschland; [2]Christian-Albrechts Universität, Institut für Pathologie, Kiel, Deutschland; [3]Christian-Albrechts Universität, Institut für Physiologie, Kiel, Deutschland

Loss of kidney function in renal ischemia/reperfusion injury (IRI) is caused by programmed cell death (PCD) but the contribution of necroptosis, a recently discovered form of programmed necrosis, has not been investigated. Initially, we identify the presence of death receptor-mediated caspase-independent cell death in murine tubular cells and characterize it as necroptosis by addition of necrostatin-1 (Nec-1), a highly specific receptor interacting protein kinase 1 (RIP1)-inhibitor. The detection of the necroptotic key players RIP1 and RIP3 in whole kidney lysates and freshly isolated murine proximal tubules led us to investigate the contribution of necroptosis in a mouse model of renal IRI. Herein, inhibition of RIP1 by Nec-1 reduces organ damage and renal failure, even if administrated after reperfusion, and resulted in a significant survival benefit in a model of lethal renal IRI. We functionally compared these results with the contribution of apoptosis to renal IRI by applying the pan-caspase inhibitor zVAD. Unexpectedly, the specific blockade of apoptosis by zVAD neither prevented organ damage in renal IRI nor the increase of retention parameters *in vivo*. Our results demonstrate the presence and functional relevance of necroptosis in the pathophysiologic course of ischemic kidney injury and a functional predominance of necroptosis over apoptosis in this setting. Above that, we identify the therapeutical potential of Nec-1 as a drug for the prevention and treatment of renal IRI.

103 SERUM ERYTHROPOIETIN-LEVELS OF DECEASED ORGAN DONORS (DEPO) ARE PREDICTIVE OF DELAYED GRAFT FUNCTION (DGF) AFTER RENAL TRANSPLANTATION

T. Kauke[1,2], T. Breidenbach[3], D. Boesebeck[3], U. Schoenermarck[4], B. Banas[5], M. Guba[2], M. Stangl[2], P. Schnuelle[6], M. Vogeser[7], B. K. Kraemer[6], *M. Fischereder[4] [1]Klinikum der LMU, Labor für Immungenetik, Munich, Deutschland; [2]Klinikum der LMU, Chirurgische Klinik, Munich, Deutschland; [3]DSO Bayern, Munich, Deutschland; [4]Klinikum der LMU, Nephrologie, Munich, Deutschland; [5]Uniklinikum Regensburg, Nephrologie, Regensburg, Deutschland; [6]Universitaetsmedizin Mannheim, Klinik für Nephrologie, Mannheim, Deutschland; [7]Klinikum der LMU, Institut für Klinische Chemie, Munich, Deutschland

Background: DGF after renal transplantation affects acute and long-term graft function. As experimental data suggest a benefit from EPO administration prior to organ recovery, we speculated that endogenous dEPO levels might also affect DGF incidence.
Patients and methods: Serum-EPO levels were determined in 55 deceased kidney donors. DGF was defined as requirement for at least one dialysis treatment during the first week after transplantation. Demographic data, DGF incidence and transplant function were extracted from the hospital records.
Results: Mean donor age was 52.9 years (SD ± 16.0), median donor age was 53.0 years (range 15–83), 20 donors were 60 years or older. Mean recipient age was 50.7 years (±12.3). Mean dEPO level was 57.8 mU/ml (±86.4), median dEPO level was 33.7 mU/ml (range 4.6–502). One of 16 recipients with dEPO > 70 mU/ml exhibited DGF whereas significantly more recipients with dEPO < 70 ng/ml exhibited DGF ($P = 0.038$), i.e. 23 of 58 patients. Recipients of dEPO > 70 mU/ml kidneys required on average 0.3 dialysis treatments (±1.0) compared to 2.7 dialysis treatments for patients with dEPO < 70 mU/ml ($P = 0.0447$). dEPO > 70 mU/ml was correlated with lower serum-creatinine of 1.7 mg/dl (±0.4) vs. 2.4 mg/dl (±1.9; $P = 0.14$) on day 28 and shorter hospitalization 22.7 days (±6.4) vs. 29.0 days (±12.8; $P = 0.0809$). Cold ischemia time (CIT), donor and recipient age, PRA and immunosuppressive regimens were not different for dEPO < 70 or >70 mU/ml. dEPO levels < 70 mU/ml have a sensitivity of 95% for prediction of DGF with a specificity of 30%. The negative predictive value of dEPO > 70 mU/ml for DGF is 93.8%.
Conclusion: dEPO levels in deceased kidney donors allow prediction of DGF. Levels > 70 mU/ml appear protective. Modification of dEPO levels to reduce DGF deserves further investigation.

230 THE POTENTIAL ROLE OF MOLECULAR PATTERNS IN 3 MONTH PROTOCOL BIOPSIES FOR THE PREDICTION OF FUTURE ACUTE REJECTION EPISODES

*B. Krüger[1,2], L. Yuan[2], W. Zhang[2], B. Schröppel[3,2], B. Murphy[3,2] [1]Universitätsmedizin Mannheim, V. Medizinische Klinik, Mannheim, Deutschland; [2]Mount Sinai School of Medicine, Division Nephrology, New York, Vereinigte Staaten von Amerika; [3]Mount Sinai School of Medicine, Transplantation Institute, New York, Vereinigte Staaten von Amerika

Routinely performed blood measurements and protocol biopsies offer the potential to identify detrimental subclinical events that predate functional events. However, renal pathology or regular blood work often fail to predict potential graft events. At this, microarrays offer the ability to examine gene activity at a molecular level and herby might overcome limitations of standard pathology and blood work results.

In 66 recipients from the GoCAR study cohort we performed microarray analysis (Affymetrix GeneChip Human Exon 1.0 ST Array) with subsequent hierarchical clustering on 3 month protocol biopsies. 63 of theses biopsies were reported as histologically normal. We performed unsupervised clustering analysis on genes with the most variable expression across patients to identify clusters of patients with similar expression profiles for a set of genes with clinically important biological functions. Gene ranking were based on expression level for each patient and their inter-quartile range of expression ranks was determined for each gene across the 66 patients. We examined the association of gene clusters with clinical outcomes with a minimum follow-up of 12 months.

We revealed three patient clusters by hierarchical clustering stratifying patients into high (H), intermediate (I) and low (L) relative expression of immune response genes. There were no differences on demographic, clinical or histopathological parameters at the time of the biopsy between clusters. However, patient clusters were significantly associated with acute rejection, with the highest risk for acute rejection in patients clustered to group H (H: 66.6% vs. I 21% vs. L: 11%; $P = 0.001$). Furthermore, patient clusters were associated with worse renal function at 6months (H: 40 ± 23, I: 59 ± 20, L: 56 ± 18 ml/min; $P = 0.029$), and potential proactive genes, such as VEGF or IL10, were shown to be elevated during follow-up in the H cluster as compared to L cluster.

Our results show that gene expression profiling of graft tissue can provide a sensitive and reproducible assessment of immune activity. We have identified a molecular pattern that is capable of differentiating immune activation from immune quiescence/suppression, and that this molecular pattern has the potential to stratify patients prior to the development of graft injury, allowing individualized immunosuppressive therapies.

176 KIDNEYS FROM PEDIATRIC DONORS ≤ 5 YEAR OF AGE ARE BEST USED IN PEDIATRIC RECIPIENTS

*A. Gallinat[1], G. Sotiropoulos[1], O. Witzke[2], J. Treckmann[1], A. Paul[1], U. Vester[3] [1]Universitätklinikum Essen, Klinik für Allgemein-, Viszeral und Transplantationschirurgie, Essen, Deutschland; [2]Universitätsklinikum Essen, Klinik für Nephrologie, Essen, Deutschland; [3]Universitätsklinikum Essen, Klinik für Kinderheilkunde II, Essen, Deutschland

Introduction: Kidneys from pediatric donors ≤ 5 year of age are often transplanted en bloc into adult recipients. However, this policy doesn't increase the donor pool and small children are disadvantaged. The purpose of this study was to compare the outcome of pediatric and adult recipients.
Patients and methods: From 3/2003–12/2010 all patients who received single or double kidney transplantation from a pediatric donor ≤ 5 year were included. Donor and recipient data as well as postoperative variables were analysed. Graft and patient survival were calculated using the Kaplan–Meier method.

Results: Eleven pediatric recipients (10 single, one double) and 14 adult recipients (four single, 10 double) received a kidney from a pediatric donor ≤ 5 year. Median donor age was 38 months, median body weight 14 kg. Major postoperative complications were observed in seven recipients (6 = adult, 1 = pediatric).

Primary non function and dilate graft function were observed by adult recipients ($n = 2$ and $n = 1$). All pediatric recipients and 11 adults experienced immediate graft function. After a median follow up of 36 months graft survival was 100% in the pediatrics and 86% in the adults. The calculated 3-year patient survival was 100%. Renal function measured by GFR was significant better in pediatric recipients up to one year post kidney transplantation.

Conclusion: Optimal results can be achieved by single kidney transplantation from pediatric donors in children. Organs from such donors should be preferably allocated to pediatric recipients.

IMMUNOLOGY

163 EARLY REGULATION OF TOLL-LIKE RECEPTOR AND CHEMOKINE EXPRESSION IN ORGANS FROM BRAIN DEAD RATS PRIOR TO EXPLANTATION

M. C. Banas[1], B. Banas[1], A. Steege[1], B. Krüger[2], T. Bergler[1], P. Schnülle[2], B. A. Yard[2], B. Krämer[2], S. Höger[2] [1]Klinikum Regensburg, Klinik und Poliklinik für Innere Medizin II, Regensburg, Deutschland; [2]Universitätsklinikum Mannheim, V. Medizinische Klinik, Mannheim, Deutschland

Introduction: Brain death (BD) is considered an important cause of pre-transplantation allograft injury but the mechanisms causing tissue injury in BD donors are not fully understood. Toll-like receptors (TLR) as receptors of the innate immune system can mediate local danger signals. In the present study we were interested, whether TLR and chemokine expression is different early after inducing brain death.
Methods: BD was induced in Fisher (F344) rats by an epidurally inflation of a fogarty catheter and was maintained for six hours. Ventilated non-BD animals served as controls. In both groups (n = 6 rats per group) kidney, lung, heart, spleen, small bowel and liver were harvested after 6 h and RNA was isolated. Gene expression of TLR1-13, CCL2, CCL3, CCL5, CCL7, CXCL1, CXCL11 and CXCL13 was analysed by real-time PCR.
Results: Whereas the chemokines CCL2, CCL3, CCL7, CXCL1, CXCL11 showed an early and significant upregulation in all investigated BD organs, only TLR2 and TLR3 showed a significant upregulation in the livers of BD animals. In contrast, a significant reduction of TLR5, TLR8, TLR9, TLR11 and TLR12 was seen in organs from BD animals. No differences between BD and non-BD-animals could be detected in the gene expression of TLR1, 4, 7, 9, 10 and CCL5.
Conclusions: In this model system BD organs show a significant and early upregulation of chemokines in thoracal and visceral organs after brain death. Whether the observed simultaneous downregulation of TLRs in several organs is part of a protective local autoregulation and/or the early upregulation of TLR expression in the liver has a specific role for the proinflammatory response needs to be elucidated in further experiments. This will help to better understand the different clinical courses of organs from brain death or living donors used for transplantation.

301 TOLL-LIKE RECEPTOR NINE DOES NOT INFLUENCE THE DEVELOPMENT OF CHRONIC ALLOGRAFT DYSFUNCTION

S. Safi, S. Porubsky, S. Wang, J. Bedke, E. Kiss, C. Schmidt, H.-J. Gröne German Cancer Research Center, Department of Cellular and Molecular Pathology, Heidelberg, Deutschland

Chronic inflammation and progressive fibrosis are the leading causes of chronic allograft dysfunction (CAD). We previously reported that recipients' deficiency of TLR2, TLR4 and their adaptor proteins MyD88 and TRIF can significantly attenuate the development of CAD in murine allogenic kidney transplantation. In contrast, a protective role has been attributed to TLR9-stimulation in inflammatory and fibrotic processes. As nucleic acids are released during graft rejection, we aimed to explore the role of TLR9, which recognizes DNA fragments, in two different models of chronic kidney allograft rejection: In a rat model, the specific TLR9 agonists ODN D19 and ODN 1668 were administered. Furthermore, in a murine model kidney allografts were transplanted into TLR9-deficient mice. TLR9-activation as well as TLR9-deficiency did not lead to a relevant change of the chronic allograft damage, the phenotype of intragraft mononuclear cell infiltrates or alloantigen specific antibodies determined in serum of recipient TLR9-deficient mice. In contrast to prior observations of the effects of other TLRs and despite previous reports on a protective role of TLR9-stimulation in inflammation and fibrosis our study has now shown that TLR9 does not exert morphologic and functional effects on the development of CAD.

Authorship: SS and SP: contributed equally to this study. HJG: designed research, controlled performance of research and data analysis and participated in writing of the manuscript.

182 NON-HLA ANTIBODIES AFTER HEART TRANSPLANTATION ARE LINKED TO CARDIAC ALLOGRAFT VASCULOPATHY

M. Barten[1], D. Dragun[2], S. von Salisch[1], R. Riede[1], S. Klein[1], S. Dhein[1], F. W. Mohr[1], H. Bittner[1] [1]Herzzentrum Leipzig, Chirurgie, Leipzig, Deutschland; [2]Charité- Universitätsmedizin Berlin, Nephrologie und Intensivmedizin, Berlin, Deutschland

Objective: Cardiac allograft vasculopathy (CAV) after heart transplantation (HTx) is a major therapeutic challenge, occuring in over 50% of HTx recipients in the first years after transplantation. Antibodies against human leukocyte antigens (HLA) or non-HLA antigens like major histocompatibility complex class I-related chain A (MICA), angiotensin type 1 receptor (AT1R) or endothelin receptor A (ETAR) gain in importance as modulators of allograft function and survival.
Methods: Sera of 114 HTx recipients were screened post-transplantation by Luminex-technology for HLA and MICA antibodies and by ELISA for AT1R and ETAR antibodies. For statistical analysis gender, age, status of CAV and the number of blood transfusions was documented.
Results: CAV was detected in n = 43 recipients. There was no significant difference in gender and number of blood transfusions between recipients with or without antibodies. HTx recipients developed antibodies against HLA class I or class II to a lower extend than against non-HLA antigens, especially against AT1R (33.3%) and ETAR (45.6%). CAV appeared in 27.1% of recipients with non-HLA antibodies, whereas 5.8% of the recipients with HLA antibodies developed CAV. Interestingly, 30.2% and 37.2% of these CAV-positive HTx recipients were positive for AT1R and ETAR antibodies, respectively, and only 2.3%, 9.3% and 13.9% were positive for HLA class I, HLA class II and MICA antibodies. Furthermore, recipients with non-HLA antibodies developed CAV earlier (69.1 months) than recipients without these antibodies (80.1 months).
Conclusions: Non-HLA antibodies are linked to earlier and higher incidence of CAV after HTx, confirming the necessity for monitoring HLA and non-HLA antibodies after HTx

070 CLINICAL RELEVANCE OF HLA-ANTIBODIES AFTER INTESTINAL TRANSPLANTATION

U. Gerlach[1], C. Schönemann[2], N. Lachmann[2], P. Neuhaus[1], A. Pascher[1] [1]Charité-Universitätsmedizin Berlin CVK, Klinik für Allgemein-, Viszeral- und Transplantationschirurgie, Berlin, Deutschland; [2]Charité-Universitätsmedizin Berlin CVK, Institut für Transfusionsmedizin und Gewebetypisierungslabor, Berlin, Deutschland

Despite a negative crossmatch, intestinal transplant (ITX) recipients are capabale of mounting humoral immune reponses early after transplantation. The development of donorspecific HLA-antibodies (DSA) is associated with severe vascular graft injury and graft failure. We examined the development of HLA-antibodies in association with the clinical course and histopathological findings of 29 ITX recipients.

Between 06/2000 and 05/2011, 29 patients with a median age of 36.76 ± 9.56 years received an isolated intestinal graft (n = 18) or a multivisceral transplantation (n = 11). HLA-antibodies were screened regularly before and after transplantation. Panel reactive antibodies (PRA) of >10% HLA I or II were considered positive. In case of DSA, treatment was initiated with plasmaphereses and ivIG. In the event of DSA-persistence and/or treatment refractory rejection, rituximab and/or bortezomib were added.

15 patients showed HLA-antibodies after transplantation. Five developed non-donor-specific (NDSA) HLA-antibodies, whereas 10 showed strongly positive DSA (20−82%) with significant rejection episodes around the time of positive samples. Interestingly, nine patients showed DSA within 6 months after transplantation, whereas one patient developed DSA 10 years after ITX. All but one patient, who was successfully treated with steroid pulse therapy alone, received plasmapheresis and ivIG. Rituximab was added in eight patients at a dose of 375 mg/m with 1.6 ± 0.9 applications/patient. One patient developed a rituximab-resistant antibody-mediated rejection and was successfully treated with bortezomib. DSA-values decreased with antirejection-therapy in nine of the 10 patients. One patient died of a severe therapy-refractory cellular and humoral rejection.

Development of HLA-antibodies after ITX is often significantly associated with acute rejection episodes. Early diagnosis and therapy is necessary to prevent severe graft injury and graft loss. Combination therapies including rituximab are mainly used to control rejection. However, proteasome inhibitors like bortezomib may serve as a new treatment option in cases of persistent DSA-levels and associated refractory rejection.

028 N-OCTANOYL DOPAMINE TRANSIENTLY INHIBITS T-CELL ACTIVATION: POTENTIAL USE FOR ALLOGRAFT RECIPIENTS

J. Wedel, B. A. Yard Universitätsmedizin Mannheim, Nephrologisches Labor, Mannheim, Deutschland

We have previously prospectively shown that donor dopamine treatment is beneficial on transplantation outcome for renal or heart allograft recipients. Because the protective effect of dopamine is independent of its hemodynamic action, we have developed a non hemodynamic dopamine derivative, i.e. N-octanoyl dopamine (NOD), that is ca 40× more protective than dopamine and has strong anti-inflammatory properties due to NF-kB inhibition. Since T-cell activation partly depends on NF-kB activation, the present study was conducted to test the efficacy of NOD to inhibit T-cell activation.

NOD dose-dependently inhibited T-cell proliferation provoked by anti-CD3/anti-CD28 or PMA/ionomycine stimulation. This occurred transiently and was associated with a G1-arrest. Inhibition of proliferation was reflected by a diminished CD25 expression and retardation of CD45RO expression in naïve T-cells. NOD did not impair early T-cell receptor signaling events, nor did it prevent cytokine production within the first 4 h of stimulation. However, both NF-kB and AP-1 were strongly inhibited by NOD after 24 h of anti-CD3/anti-CD28 or PMA/ionomycine stimulation. NOD inhibited T-cell proliferation in synergy with calcineurin inhibitors (CNI; FK-506, cyclosporine A), thereby reducing the concentration of CNI at least 10-fold for equal inhibition compared to CNI alone.

Based on the strong effects of NOD on both, adaptive and acquired immunity, our results indicate a potential clinical use of NOD in both, donor and recipient. In particular the use of NOD in the recipient might have clinical relevance for safely reducing CNI dosage within the first days after transplantion without affecting overall immunosuppression.

172 AN EXPERIMENTAL APPROACH TOWARD BRONCHIOLITIS OBLITERANS - VERIFICATION OF DIFFERENT IMMUNOSUPPRESSIVE STRATEGIES

K. Lehle[1], M. von Süßkind-Schwendi[1], C. Diez[1], P. Rümmele[2], T. Pühler[1], C. Schmid[1], S. Hirt[1] [1]Uniklinik Regensburg, HTC, Regensburg, Deutschland; [2]Universität, Pathologie, Regenburg, Deutschland

Background: Bronchiolitis obliterans (BO) and vascular sclerosis (VS) hinders successful long-term outcome after lung transplantation (LTX). One risk factor for BO syndrome is frequent early acute rejection (AR) which is often diagnosed too late and inadequate medicated. The Fischer F344-to-Wistar Kyoto WKY rat LTX model was used to study the impact of FTY720, mycophenolate mofetil (MMF) and everolimus on the development of BO.

Methods: Allogeneic (F344-to-WKY) and syngeneic (WKY-to-WKY) left rat LTX without initial immunosuppression was done. During maximum AR (postoperative day (POD) 14) all rats received three methylprednisolon boli (10 mg/kg/day). Study groups included: (i) no additional drug treatment, (ii) FTY720 (3 mg/kg/day), (iii) MMF (30 mg/kg/day, (iv) everolimus (30 mg/kg/day); each POD 14−100. Extent of acute and chronic rejection was classified according to ISHLT. Expression of inflammatory markers was analyzed with specific immunohistochemistry.

Results: AR (POD 15) dominated in untreated animals ending in a fulminant peribronchiolar fibroproliferation and airway obstruction (POD 100). A steroid therapy did not prevent AR and OB. Additional application of FTY720, MMF or everolimus significantly reduced the infiltration of inflammatory cells and activation of endothelial cells in allogenic transplanted lungs. Nevertheless, these lungs developed airway inflammation with destruction of the subepithelial layer of small airways. Finally, none of the drugs prevented the development of chronic vascular and bronchial changes and the destruction of anatomic lung structures.

Conclusion: Compared to successful application after heart transplantation none of the drugs was effective to prevent chronic lung allograft rejection. Alternative strategies might be the application of fibrosis inhibitors.

160 GRAFT TOLERANCE AND IMMUNOLOGICAL CONSEQUENCE FOLLOWING A COMBINED THI / PROGRAF® SUBSTITUTION AFTER LIVER TRANSPLANTATION IN THE RAT: A NOVEL IMMUNSUPPRESANT DRUG

E. Matevossian[1], N. Hüser[1], C. Bald[2], J. Nährig[3] [1]Chirurgische Klinik und Poliklinik, Klinikum rechts der Isar Technische Universität München, Transplantation, München, Deutschland; [2]DSO Bayern, Organisationszentrale München, Deutschland; [3]Klinikum rechts der Isar, Technische Universität München, Institut für Allgemeine Pathologie udn Pathologische Anatomie, München, Deutschland

Background and aim: Acute rejection is still a common complication of liver transplantation (LTx). The mechanism of allograft rejection or tolerance induction is a competitive, complex process that presumably involves interactions between multiple subpopulations of T lymphocytes. CD4+/CD8+ T cells represent a subpopulation of T lymphocytes, and are a marker for allograft rejection. The deposit of CD4+/CD8+ cells provides evidence of activation of humoral immunity. It is known that lymphocyte regress after allograft transplantation depends on sphingosine 1-phosphate (S1P) receptor-1. Treatment with 2-Acetyl-4-tetra-hydroxybutyl imidazole (THI), a potentially immunosuppresant drug, inhibits the S1P-degrading enzyme and plays a fundamental role in the immune response.

The aim of this experimental study is to evaluate the protective effects with mono- or Prograf®-combined THI treatment of the recipients after LTx in a rat model,. An understanding of this preconditioning of the allograft is essential for the design of therapeutic strategies as well as an improvement of survival after LTx for recipients with relevant immunosuppressant toxicity.

Material and methods: Arterialised orthotopic allogeneic liver transplantation was performed in LEWIS/DA . The recipients are divided into four groups: group I (controls without THI or Prograf® pre-treatment/immunosuppression of the recipients, $n = 6$); group II (immunsuppression of the recipients (day 0−28 after LTx) with high-dose Prograf®, $n = 6$); group III (pre-treatment of the recipients (day 0−28 after LTx) with THI, $n = 6$); group IV (pre-treatment and immunosuppression of the recipients (day 0−28 after LTx) with low-dose Prograf® and day 0−14 after LTx with THI, $n = 8$).

Results: Our preliminary data (FACS) with application of a single THI-injection 90 min before liver transplantation show a significant decrease of CD4+/CD8+ populations of the T-lymphocytes in the recipients peripheral blood and liver allograft ($P = 0.002$, group III vs. group I). The histopathologic evaluation of the grafts showed regarding to the number of necrotic areas a significant difference between the group I (mean 11.2) vs. group III (mean 4.2) and group IV (mean 1.6), $P = 0.0013$. The middle maximal size of these areas differs significant between group I (mean 5.2 mm) and group IV (mean 0.22 mm, $P = 0.002$).

Conclusion: These experimental results show that the immunological consequence following a combined THI/Prograf® substitution after liver transplantation has an organ protective effect to the graft. These results gives credence to the theory that the reduction of T-lymphocytes with THI alone or in combination with low-dose Prograf® before reperfusion will prevent or reduce acute rejection episodes in liver allografts. The study is of considerable interest as it draws attention to the modulation of immune function after solid organ transplantation.

EDUCATION IN TRANSPLANTATION MEDICINE

113 TRAINING SKILLS FOR TRANSPLANT SURGERY - EXPERIENCE WITH THE HANDS-ON-TRAINING IN THE TRANSPLANT OPERATIVE PROCEEDINGS (TOP) COURSE

**H. Wolters[1], C. Mönch[2], S. Farkas[2], F. Braun[3], T. Vogel[1], S. Köhler[4], J. G. Brockmann[5] [1]Universitätsklinikum Münster, Klinik und Poliklinik für Allg.-und Viszeralchirurgie, Münster, Deutschland; [2]Universitätsklinikum Regensburg, Klinik und Poliklinik für Chirurgie, Regensburg, Deutschland; [3]Universitätsklinikum Schleswig-Holstein, Campus Kiel, Klinik für Allg. Chirurgie und Thoraxchirurgie, Kiel, Deutschland; [4]Serenis GmbH, Clinical Research and Training, Pullach, Deutschland; [5]UniversitätsSpital Zürich, Klink für Viszeral- und Transplantationschirurgie, Zürich, Schweiz*

Background: Transplant surgery is a highly specialized field of surgery. Most transplant surgeons are trained in a learning-by-doing scenario whilst attending transplant procedures as an assistant. Intensive, structured training of surgical skills still is not common practise. The first experience with standardized skill-evaluation in a well established, international hands-on-course for surgical skills is being presented herein.

Methods: Daily presentations, one-to-one teaching and repetitive hands-on experience of various surgical procedures were carried out in a 5-day hands-on course for abdominal organ transplantation in pigs. Starting in 2011 standardized testing (beginning/end) [A1] evaluating different practical skills of each participant using a score that analyzed use of needle holder, forceps, knot-tying, patency/stenosis of vasc. anastomosis, time for standardized anastomosis aso. Additionally, questionnaires were assessing subjective factors with respect to different surgical skill such as self-confidence.

Results: Since 1999 520 surgeons from 55 international Tx-centers were trained. All of the extensively evaluated participants ($n = 15$) gained self confidence during 5 days regarding more complex procedures. In the heterogeneous experienced group all improved their skills score from a mean of 17.3 ± 3.35 to 24.3 ± 1.57 (max 26). 86,6% of the participants could reduce the time for a standardized end-end- anastomosis up to 53.4% without loss [A1] of quality.

Conclusion: First evaluation indicates that self confidence and technical skills can be improved by a structured hands-on-'TOP'-course. As sufficient clinical training becomes more and more difficult for young surgeons to achieve, hands-on-courses may become an important part of surgical training in near future.

106 EXTERNAL JUGULAR VEIN GRAFT-CAROTID INTERPOSITION IN THE RAT – AN ELABORATE TEACHING VIDEO

**K. Schleimer, A. Greiner, J. Kalder, M. Jacobs, M. Kokozidou Universitätsklinikum Aachen, Klinik für Gefäßchirurgie, Aachen, Deutschland*

Solid organ transplantation in rats and mice is usually performed in cuff technique. The sophisticated transplantations would be easier to learn, if the researcher would practice the cuff technique before. The external jugular vein graft-carotid interposition is an excellent operation for training in cuff anastomoses. Our team has optimized and standardized this model. We have created this comprehensive teaching video.

Female Sprague-Dawley rats weighing 313 ± 20 g were operated under isoflurane anesthesia. An 1 cm long segment of the right external jugular vein was completely prepared. The right common carotid artery was carefully mobilized and dissected in the middle between two ligatures. After positioning of two minibulldog clamps on the distal and the proximal carotid stump, both stumps were cuffed. An 1 cm-long segment of the external jugular vein was excised and interponated between the carotid stumps in cuff technique. Then the venous interposition graft was perfused.

Twenty-one animals were divided in three groups ($n = 7$) and were sacrificed at days 21, 42 and 84 after operation. The survival rate at the time point of sacrifice was 100%, while the graft patency rate was 71% without differences between the different sacrifice time points. The blood flow in the arterialized venous graft was 8 ± 3 ml/min at the time of sacrifice.

Two venous interpositions (one on the right and one on the left side) can be performed in one animal, with two cuff anastomoses each. The authors recommend training in this venous graft-interposition technique, before starting transplantation in small animals.

BASIC SCIENCE I

179 MESENCHYMAL STEM CELLS HAVE BENEFICIAL INFLUENCE ON HEPATIC ISCHEMIA REPERFUSION INJURY

*E. Eggenhofer, V. Benseler, P. Renner, F. Popp, E. Geissler, H.-J. Schlitt, M. Dahlke Universitätsklinikum Regensburg, Klinik und Poliklinik für Chirurgie, Regensburg, Deutschland

Introduction: Mesenchymal stem cells (MSC) are one of the most promising cell populations for cell based immune therapy in solid organ transplantation. These cells are well characterized regarding their bimodal function: immunosuppression as well as tissue regeneration. A transplanted organ is up against immune response of the recipient but also stress and tissue damage caused by ischemia and reperfusion. Here, we investigate the role of MSC in a mouse model of hepatic ischemia reperfusion injury (IRI).

Methods: MSC were isolated and cultivated from bone marrow of Balb/c mice and 0.5×10^6 cells or PBS as control injections were administred to C57B6 animals. These animals underwent an ischemia reperfusion injury by clamping the median and the right liver lobe for 45 min, followed by reperfusion. Three hours and 24 h post-reperfusion animals were killed and analysed. The readout panel consisted of ALT measurement in the serum, isolation and flow cytometric characterization of liver infiltrating cells (LICs) as well as immunohistochemical analyses and real time PCR of liver tissue.

Results: Therapy with MSC reduced IRI damage significantly. Animals receiving MSC had lower ALT levels compared to PBS treated animals, especially in the early post-reperfusion phase after 3 h. We also observed fewer cellular infiltrates (neutrophiles, $CD4^+$ and $CD8^+$ T cells) in the liver tissue verified by flow cytometry after isolation of LICs as well as histochemical analyses of cryosections of these livers. This might be due to the fact that ischemic livers treated with MSC revealed markedly reduced levels of ICAM-1, an important adhesion molecule in the context of leukocyte transmigration. Along with decreased LICs and ICAM-1 levels, we found significant lower levels of pro-inflammatory cytokines (IFNγ, TNFα) in MSC treated livers.

Discussion: Treatment with MSC in a murine IRI model, had a beneficial influence on early liver damage. Therefore, we conclude that MSC are a promising tool for cell therapy in solid organ transplantation as they can antagonize not only graft-rejection but also transplantation related organ damage.

276 TARGETING THE JAK1/3 PATHWAY TO SUPPRESS ACUTE ALLOGRAFT REJECTION

*X. Hua, T. Gossler, M. Stubbendorff, T. Deuse, V. Taylor, Y. Chen, H. Reichenspurner, R. Robbins, *S. Schrepfer UHZ, Transplant Lab, Hamburg, Deutschland

Background: Selective inhibition of lymphocyte activation represents an optimal strategy for immunosuppression. This is the first report on the novel JAK1/3-inhibitors R507 and R545 for prevention of acute allograft rejection.

Methods: Pharmacokinetic and in vitro enzyme inhibition assays were performed to characterize the drugs. Heterotopic BN-Lew heart transplantation ($n = 48$) were performed to study acute cardiac allograft rejection. Immunosuppressive efficacy and drug toxicity of R507 60 mg/kg, and of R545 20 mg/kg/d was compared to tacrolimus 4 mg/kg/d.

Results: Plasma levels of 30 mg/kg/d R507 and R545 sustained stable *in vivo* (R507: 50 ng/ml and R545: 1800 ng/ml 12 h trough levels), and *in vitro* enzyme assays showed selective inhibition of JAK1/3-dependent pathways. In human primary T-cells, R507 and R545 inhibit IL2-dependent Stat5 phosphorylation (EC50s of 22 and 38 nM, respectively) and the resulting T-cell proliferation (EC50s of 19 and 30 nM, respectively), while showing only modest activity on general cell proliferation (EC50s> 2uM). Both JAK1/3-inhibitors significantly reduced early mononuclear graft infiltration ($P < 0.001$ vs. no medication group). Mononuclear cell infiltration was significantly reduced in the R507, R545, and tacrolimus groups (38 ± 31, 45 ± 36, and 136 ± 43cells/HPF) compared to the no medication group (178 ± 33 cells/HPF; $P \leq 0.038$). Immunohistochemistry revealed significant lower CD68 infiltrating cells in the R507 and R545 groups ($P < 0.001$ vs. no medication). CD3-positive lymphocytes were significantly suppressed in all three treatment groups compared to the no medication group ($P < 0.001$). Donor-specific IgM-antibodies were significantly suppressed in all treatment groups (R507 > R545 > tacrolimus; $P < 0.001$ vs. no medication group).

Conclusions: Both, R507 and R545, are promising novel immunosuppressants with favorable pharmacokinetics and biological activity, effectively diminishing acute cellular rejection.

046 REJECTION OF BRONCHUS TRANSPLANTS IN THIRD-PARTY PORCINIZED MICE IS REGULATED BY ALLOGENEIC $CD4^+CD25^+$ T CELLS

*A.-K. Knöfel[1], N. Madrahimov[1], K. Dreckmann[1], M. Avsar[1], W. Sommer[1], D. Jonigk[2], F. Länger[2], J. Gottlieb[3], A. Haverich[1], M. Strüber[1], G. Warnecke[1] [1]MHH, exp. HTTG, Hannover, Deutschland; [2]MHH, Pathologie, Hannover, Deutschland; [3]MHH, Pneumologie, Hannover, Deutschland

Bronchiolitis obliterans is a limited survival problem in lung transplantation. In our allogenic porcine mice model, we have previously shown that the graft acceptance is better if we injected CD4+CD25+ regulatory T cells.

It is not known whether it is possible to regulate the rejection of the transplanted bronchus in a third-party-model. Therefore, we investigated the role of T cell regulation in an adoptive transfer system using porcine bronchus and allogeneic third-party PBMC transfer into immune deficient mice.

Porcine bronchi were transplanted under the skin of NODrag-/-gammac-/- mice. 5×10^6 porcine PBMC from long-term lung survivor were injected per animal in four groups ($n = 7-12$). Group A received no PBMC, For group B cells and vessels were collected from two MHC mismatched pigs, the cells are from long-term lung-transplantation survivor, so we are able to constructed a third-party model in our mice, group C recipients received allogeneic PBMC depleted of $CD4^+CD25^+$ T cells; group D recipients received allogeneic PBMC enriched of $CD4^+CD25^+$ T cells. Alloinjury of the heterotopic bronchus grafts was assessed by histology of the graft on postoperative day 28.

In the control group A ($n = 11$) epithelial loss ($P < 0.0001$), cell infiltration and luminal obstruction ($P < 0.0001$) were absent and structural damage to the cartilage and the epithelium was low. In group B ($n = 7$) epithelial loss was pronounced and cell infiltration and histological changes were severe. In group C ($n = 12$) these changes were even more severe. In group D ($n = 9$), cell infiltration was reduced and histological damage to the allograft was less severe, as was the epithelial loss.

Thus, heterotopic non-vascularized transplantation of a porcine bronchus graft and reconstitution with allogeneic porcine PBMC in NODrag-/-gammac-/- mice represents a valuable tool for transplant research. In this third-party model, we could show *in vivo* that rejection of bronchus transplants, that is histological reminiscent of bronchiolitis obliterans, is controlled by $CD4^+CD25^+$ T cell regulation.

109 NORMOTHERMIC ACELLULAR *EX VIVO* LIVER PERFUSION (NEVLP) DECREASES HEPATOCYTE AND BILE DUCT INJURY IN LIVERS RETRIEVED AFTER CARDIAC DEATH

*M.U. Boehnert, J.C. Yeung, J.-M. Knaak, F. Bazerbachi, S. Keshavjee, G.A. Levy, D.R. Grant, M. Selzner Toronto General Hospital, Multi Organ Transplant Program, Toronto, Kanada

Problem: Cold static preservation is poorly tolerated by livers retrieved after cardiac death (DCD). We compared cold static liver preservation with normothermic acellular *ex vivo* liver perfusion (NEVLP) for the preservation and assessment of DCD livers.

Methods: One hour cardiac arrest was induced in pig livers as a model of DCD organ retrieval. We compared 12 h cold static preservation with 4 h cold preservation plus 8 h NEVLP. After 12 h preservation whole blood reperfusion was performed as a model of transplantation. In a second set of experiments orthotopic pig liver transplantation was performed.

Results: Using whole blood reperfusion as a model of transplantation AST and ALT were 12-fold reduced after NEVLP when compared to cold static storage. Necrosis was minimal in NEVLP preserved livers (<10%), while cold preserved livers had massive necrosis (>65%, $P < 0.001$). Bile duct necrosis (trichrome) occurred in 100% of the cold preserved vs. 25% of NEVLP treated grafts. The total bilirubin (67 μmol/l vs. 10 μmol/l, $P < 0.0001$), phospholipid (343 μmol/l vs. 62 μmol/l, $P < 0.0001$), and bile acid (8362 μmol/l vs. 1550 μmol/l, $P < 0.0001$) concentration was higher in the bile of NEVLP versus cold preserved grafts. LDH in the bile was five-fold decreased in NEVLP versus cold preserved grafts. Hepatic artery perfusion was decreased in cold preserved grafts while arterial perfusion was normal in NEVLP treated livers. Following DCD pig liver transplantation ALT was five-fold lower in NEVLP versus cold preserved grafts.

Conclusion: NEVLP decreases hepatocellular and biliary injury in DCD livers and improves arterial flow after reperfusion. NEVLP is an attractive new strategy for DCD grafts.

269 GENDERSPECIFIC DIFFERENCES IN INDUCTION OF STEATOSIS BY HIGH FAT DIET

*S. Stöppeler[1], D. Palmes[2], A. Zibert[3], E. Minin[4], R. Bahde[2], M. Fehr[5], H.-U. Spiegel[1] [1]Universitätsklinikum Münster, Chirurgische Forschung, Münster, Deutschland; [2]Universitätsklinikum Münster, Klinik für Allgemein- und Viszeralchirurgie, Münster, Deutschland; [3]Universitätsklinikum Münster, Transplantationsmedizin, Münster, Deutschland; [4]Universitätsklinikum Münster, Pathologie, Münster, Deutschland; [5]Medizinische Hochschule Hannover, Tiermedizin, Hannover, Deutschland

Introduction: NAFLD (nonalcoholic fatty liver disease) as hepatic manifestation of the metabolic syndrome can lead to steatohepatitis and fibrosis followed by end-stage liver disease.The genetic profile plays an important role in disease progression In this study genderspecific differences were investigated in a rat model of high-fat diet induced steatosis.

Methods: Male and female Lewis (Lew-) or Sprague Dawley (Sprague-) rats, respectively, were feed for 3 weeks with standard (SD) or high fat (HF) diet. Body weight was monitored regularly. Liver function and fat metabolism was investigated by laboratory chemistry. Adiponectin levels were analyzed by ELISA. Expression of 23 relevant genes was assessed by qRT-PCR. Liver histology was evaluated by a scoring system with special interest in grade of steatosis and fibrosis, inflammation and cell damage (HE-, Azan-, Oil red staining, electron microscopy).

Results: Both diets, SD and HF, resulted in a higher degree of steatosis in male rats compared to their female counterparts ($P < 0.05$). While Lew rats fed with HF showed a microvesicular steatosis, Sprague developed a macrovesicular steatosis. In female rats liver function (AP, PCHE) and fat metabolism (cholesterine, triglycerides, HDL, LDL) were improved compared to male rats ($P < 0.05$).The level of adiponectin showed a similar gender-specific effect (*Lew male*, HF 2673 ± 174.7 ng/ml, *female* SD 3676 ± 316.5 ng/ml, HF 2780 ± 587.5 ng/ml; *Sprague male* SD 2496 ± 1080.3 ng/ml, HF 2507 ± 853.3 ng/ml, *female* SD 3428 ± 741.1 ng/ml, HF 2461±624.2 ng/ml). qRT-PCR showed lower gene expression in 19 and 17 of 23 investigated genes, respectively, after SH or HF diet in female lew rats and 16 and 14 genes in female SD rats, respectively ($P < 0.05$).

Conclusion: Female gender correlated with improved fat metabolism, less induction of fatty liver and showed an advantageous genetic expression profile. All differences identified in this study may offer therapeutic options for treatment of NAFLD.

023 ANTIGEN-SPECIFIC REGULATORY T CELLS CAN INDUCE TOLERANCE TO IMMUNOGENIC GRAFTS

**N. Seltrecht, N. Mpofu, M. Hardtke-Wolenski, F. Noyan, M.P. Manns, *E. Jaeckel Hannover Medical School, Dept. Gastroenterology, Hepatology & Endocrinology, Hannover, Deutschland*

Regulatory $CD4^+CD25^+Foxp3^+$ T cells (Tregs) play an important role in the induction of allospecific tolerance. However tolerance in solid organ transplantation by mere transfer of Tregs has been difficult. Besides this the stability of the differentiation phenotype of Tregs has recently been questioned.

We therefore aimed in generating large numbers of stable allospecific Tregs from naïve T cells by retroviral transduction with Foxp3. These were tested in an immunogenic skin transplantation model (C57BL/6à BALB/c).

Alloantigen-specific Foxp3 T cells (iTregs) showed high expression for the Treg markers Foxp3, CTLA4 and GITR. They could suppress a MLR in an alloantigen-specific manner. Furthermore, they could be expanded up to 18-fold in vitro while maintaining their Treg phenotype and expression of lymph node homing markers. iTregs prevented skin graft rejection without the need for chronic immunosuppression and recipients showed systemic allospecific allotolerance. Alloantigen-specific Tregs were far more potent than polyspecific Tregs. Mechanisms of tolerance were graft specific homing, expansion and long-term persistence of Tregs within the graft (>100days, 90% of intragraft Tregs were alloantigen-specific). In fact, tolerance could be transferred with re-transplantation of the tolerant graft onto secondary recipients. Third party grafts were readily rejected.

The results prove that large numbers of stable alloantigen-specific Tregs can be generated from a polyclonal repertoire of naïve T cells. This is the first time that allotolerance was achieved in a nonlymphopenic transplant model using skin grafts in an immunogenic strain combination. Therefore, antigen-specific Tregs might have a huge therapeutic potential after solid organ transplantation. (supported by IFB-Tx and SFB738)

BASIC SCIENCE II

096 MOUSE MACROPHAGES DRIVEN TO A NOVEL STATE OF ACTIVATION PROLONG ALLOGRAFT SURVIVAL IN NON-IMMUNOSUPPRESSED RECIPIENTS

*P. Riquelme[1], S. Tomiuk[2], H.J. Schlitt[1], E.K. Geissler[1], J. Hutchinson[1]
[1]Universitätsklinikum Regensburg, Chirurgie, Regensburg, Deutschland; [2]Miltenyi Biotec GmbH, Bergisch-Gladbach, Deutschland

Human regulatory macrophages (M reg) have been extensively investigated as a potential cell-based immunosuppressive treatment. Using culture conditions analogous to those under which human M regs arise, a mouse counterpart of the human cell has been generated. Mouse monocytes are driven to the M reg phenotype over a seven day culture period during which the cells are exposed to M-CSF and IFN-g. The resulting M regs adopt a characteristic morphology and express markers distinguishing them from monocytes, monocyte-derived DCs and M0-, M2a-, and M2c-polarised macrophages. To assess the interrelation of M regs and these other cell types, whole genome expression profiling studies were undertaken: in clustering analyses, M regs were found to constitute a distinct subgroup of macrophages. At a 1:1 ratio, M regs completely suppressed polyclonally-stimulated T cell proliferation through a nitric oxide synthase-dependent mechanism which was blocked by the inhibitor L-NMMA. In a heterotopic heart transplant model, a single intravenous administration of 5×10^6 donor-strain M regs prior to transplantation significantly prolonged allograft survival in unconditioned, nonimmunosuppressed recipients using both the stringent C3H-to-BALB/c (32.6 ± 4.5 vs. 8.7 ± 0.2 days; $P < 0.001$) and B6-to-BALB/c (31.1 ± 12 vs 9.7 ± 0.4 days; $P = 0.002$) strain combinations. The graft-protective effects of M regs were specific to donor cells as recipient (9.6 ± 0.4 days; $P > 001$) and third party-derived M regs (11.0 ± 0.6 days; $P < 0.001$) were not effective. Co-treatment with M regs and 1 mg/kg/day rapamycin for 10 days post-transplant further enhanced the effect of M regs (64.1 ± 8.6 days) compared to M reg treatment alone ($P = 0.006$) or rapamycin alone ($P = 0.022$), and some recipients accepted grafts indefinitely. It is concluded that mouse M regs represent a novel, phenotypically distinct subset of macrophages which bear a resemblence to human M regs in their derivation, marker phenotype and *in vitro* functions.

234 GENETICALLY ENGINEERED HUMAN EMBRYONIC STEM CELLS ESCAPE IMMUNE RESPONSE

*T. Deuse, M. Seifert, A. Fire, H. Volk, X. Hua, H. Reichenspurner, R. Robbins, *S. Schrepfer UHZ, Transplant Lab, Hamburg, Deutschland

Background: Although human embryonic stem cells (hESC) have enormous potential for cell replacement therapy of heart failure, immune rejection of hESC derivatives would inevitably occur after transplantation. We therefore aimed at generating a hypoantigeneic hESC line with improved survival characteristics.

Methods and results: hESC were HLA I positive but negative for HLA II and co-stimulatory molecules. Transplantation of naïve hESC into immunocompetent Balb/c mice induced substantial T_{H1} and T_{H2} responses with rapid cell death. Histology revealed mainly macrophages and T cells, but only scattered NK cells. The human *in vitro* NK response to hESC challenge *in vitro* was negligible.

We successfully generated HLA I knockdown cells ($hESC^{siRNA+IB}$) using both HLA I RNA interference and intrabody technology. HLA I expression was 88−99% reduced after 7 days and remained low for weeks. Cellular immune recognition of these $hESC^{siRNA+IB}$ was strongly reduced in both xenogeneic and allogeneic settings. T cell activation after $hESC^{siRNA+IB}$ transplantation into Balb/c was significantly diminished, antibody production was substantially alleviated, graft-infiltrating immune cells were reduced and even long-term graft survival was achieved in one third of the animals without any immunosuppression.

Conclusions: Because of their very low expression of stimulatory NK ligands, NK-susceptibility of naïve hESC and $hESC^{siRNA+IB}$ was negligible. Thus, HLA I recognition by T cells seems to be the major mechanism of hESC recognition and T cells, macrophages and hESC-specific antibodies participate in hESC killing. HLA I knockdown $hESC^{siRNA+IB}$ provoke T cell ignorance and experience largely mitigated xenogeneic rejection. By generating hypoantigeneic hESC lines, the generation of acceptable hESC derivatives may become a practical concept and push cell replacement strategies forward.

043 INDUCTION OF STABLE PERIPHERAL TOLERANCE IS A RESULT OF ALLOANTIGEN PRIMING AND INDEPENDENT FROM PRETRANSPLANT T CELLS IN A PORCINE LUNG TRANSPLANTATION MODEL

*G. Büchler[1], A.-K. Knöfel[1], N. Madrahimov[1], K. Dreckmann[1], M. Avsar[1], W. Sommer[1], D. Jonigk[2], F. Länger[2], J. Gottlieb[3], A. Haverich[1], M. Strüber[1], G. Warnecke[1] [1]Medizinische Hochschule Hannover, Herz-, Thorax-, Transplantations- & Gefäßchirurgie, Hannover, Deutschland; [2]Medizinische Hochschule Hannover, Institut für Pathologie, Hannover, Deutschland; [3]Medizinische Hochschule Hannover, Klinik für Pneumologie, Hannover, Deutschland

Purpose: We reported successful induction of peripheral tolerance in a porcine lung transplantation model in the presence of high levels of circulating regulatory T cells (Treg). Here, we introduce an adoptive transfer model in immune deficient mice to study function of naïve T cells and naturally occurring porcine Treg *in vivo*.

Methods: Lung transplantation from MHC mismatched donors was performed in 12 adult minipigs. Before transplantation porcine bronchi from the lung donors were transplanted under the skin of NOD*rag*$^{-/-}$gammac$^{-/-}$ mice. Subsequently, $5-10 \times 10^6$ naive or Treg-depleted porcine PBMCs from the lung recipients were injected into these mice. Histological alloinjury of the bronchus graft was assessed after 28 days.

Results: Surviving beyond day 380 five out of 12 lung recipients developed stable tolerance of the graft. In the adoptive transfer model the damage score of the bronchi showed no significant differences between mice injected with PBMCs derived from eventual long term survivors and eventually rejecting recipients. Depletion of $CD4^+CD25^{high}$ T cells from the PBMCs transferred led to more severe damage without differences between groups.

Conclusion: Naive PBMCs from all porcine lung recipients induced rejection of the donor bronchus in mice. This rejection was reliably regulated by naturally occurring Treg in depletion experiments. These results had no impact on eventual development of stable peripheral tolerance in porcine lung recipients following the respective induction protocols. We therefore conclude peripheral tolerance in our model is more likely a result of alloantigen priming, than of pre-existing naturally occurring T cell regulation.

119 *IN VITRO*-VASCULARISATION OF ENGINEERED HEART TISSUE FROM NEONATAL MICE

*S. Ensminger, *O. Rörick Universitätsklinik, Herzchirurgie, Erlangen, Deutschland

Background: The lack of adequate methods to stimulate the vascularisation of engineered tissue is a bottleneck delaying further developments in the field of Tissue Engineering. The occurrence of tube-like structures within collagen- or fibrin-matrices is a phenomenon exhibited by endothelial cells, smooth muscle cells and pericytes equally. This study was designed to characterise the interactions of all cells involved in EHTs over time.

Methods: Within EHTs from neonatal cardiomyocytes produced with collagen, Matrigel® and Chicken Embryonic Extract, the formation of vessel-like-structures (tube-like-structures) was observed during the first 30 days. Endothelial cells, smooth muscle cells, pericytes and basal membrane compounds were stained immunohistochemically and then examined by immunofluorescence and confocal microscopy over 30 days.

Results: The formation of tube-like structures from ECs occurs within the first days, followed by an expansion of the network during the following 2 weeks. Pericytes are progressively attaching to the EC-grids; attachment of SMCs can be observed to a lesser extent. No markers characteristic for basal membranes and no morphologic correlation to ECs were seen during the observed time frame.

Conclusion: *In vitro*, EHTs form branched networks consisting of endothelial cells that are colonized by pericytes. The observation that smooth muscle cells attach later onto the endothelial cells and in a patchy pattern suggests that arterioles may not mature *in vitro*. The basal membrane showed no adequate reconstitution. Therefore, our current investigations address the question how the ingrowth of recipient-vessels into EHTs can be stimulated.

244 THE ROLE OF RORγT IN T17-MEDIATED ALLOGRAFT REJECTION

*M. Sabet-Baktach, M. Malaise, J. Rovira, H.J. Schlitt, E.K. Geissler, A. Krömer Universitätsklinikum Regensburg, Klinik und Poliklinik für Chirurgie, Regensburg, Deutschland

Introduction: IL-17-producing T cells (T17) may play a major role in allograft rejection, as T17 cells in Th1-deficient mice mediate a pro-inflammatory alloimmune response resulting in accelerated allograft rejection. However, the mechanisms underlying T17-mediated alloimmune responses in transplantation are poorly understood, particularly with respect to regulation by the hallmark T17 transcription factor RORγt.

Methods: To precisely explore the role of RORγt in T17 alloimmunity in the absence of Th1, we created RORγt reporter and knockout mice on a T-bet-deficient (T-bet$^{-/-}$) background by crossbreeding T-bet$^{-/-}$ with Ror$\gamma t^{gfp/wt}$ reporter and Ror$\gamma t^{gfp/gfp}$ knockout mice, respectively.

Results: First, we explored the fate-mapping capacity of our reporter model by polarizing T cells from Ror$\gamma t^{gfp/wt}$T-bet$^{-/-}$ mice towards T17 and measuring GFP expression *in vitro*. We found that GFP was clearly up-regulated in T17-polarized T cells from Ror$\gamma t^{gfp/wt}$T-bet$^{-/-}$ mice and that the expression of GFP paralleled the expression of RORγt (RT-PCR and FACS). Importantly, the T17-polarized T cells from the reporter mice were as capable of producing IL-17 as T cells from controls, as shown by ELISA and FACS, thus demonstrating the efficacy of our reporter model. Next, we tested the hypothesis that RORγt significantly contributes to the differentiation of alloreactive T17 cells by studying T cells from Ror$\gamma t^{gfp/gfp}$T-bet$^{-/-}$ double-knockout mice under T17-polarizing conditions, in terms of expression of T17 effector cytokines *in vitro*. As expected, we found that IL-17 expression was substantially lower in the double-knockout mice when compared to Ror$\gamma t^{gfp/wt}$T-bet$^{-/-}$ and T-bet$^{-/-}$ controls, as indicated by RT-PCR, ELISA, and FACS. Thus, RORγt plays clearly a major, but not exclusive, role in driving IL-17-producing alloreactive T cells in the absence of T-bet.

Conclusion: In conclusion, our novel model is an unprecedented tool in studying RORγt$^+$ T17 cells in alloimmunity. In transplantation, our studies point to an important and as yet understudied role of RORγt.

155 ELEVATED URINARY CYTOKINE CONCENTRATIONS PREDICT ACUTE KIDNEY TRANSPLANT REJECTION IN THE FIRST WEEKS AFTER TRANSPLANTATION

S. Reinhold[1], R. Straub[2], B. Krüger[1,3], B. Kaess[1], T. Bergler[1], C. Weingart[1], M. Banas[1], B. Krämer[1,3], B. Banas[1] [1]University of Regensburg, Klinik und Poliklinik für Innere Medizin II - Nephrologie, Regensburg, Deutschland; [2]University of Regensburg, Laboratory of Neuroendocrino-Immunology, Department of Internal Medicine I, Regensburg, Deutschland; [3]Universitätsklinikum Mannheim, V. Medizinische Klinik, Mannheim, Deutschland

Purpose: We tested the hypothesis that increased urinary cytokine levels may indicate an acute kidney transplant rejection.

Methods: Thirty-five patients after kidney transplantation underwent protocol biopsies about 14 days and 3 months after kidney transplantation. Eight patients with an early rejection in the protocol biopsy about 14 days after transplantation (group A), 9 patients with a biopsy proven rejection 3 months after transplantation (group B) and 18 patients with no rejection at either time point representing the control group (group C) were chosen for this study. On the day of the biopsy, a urine sample was collected and tested for cytokine levels. Concurrent urinary tract infection was excluded by urinary dip stick tests.

Results: The mean urinary levels of interleukin 6 (IL6), soluble IL6 receptor (sIL6R), tumour necrosis factor receptor 1 (TNFR1), and soluble vascular cell adhesion molecule (sVCAM-1) were significantly higher in patients with biopsy proven acute transplant rejection 14 days after transplantation compared to the control group ($P < 0.01$). The urinary sIL6R and sVCAM-1 levels 14 days after transplantation were also significantly higher in group B with a late rejection compared to control ($P < 0.05$). No significant correlation could be shown for IL1ra, TNF, and TNFR2 at any time point.

Conclusion: The individual cytokine profile in the urine of kidney transplant recipients reflects acute transplant rejection and has the potential to replace protocol biopsies in the future.

ORGAN DONATION

153 DONOR SCORING SYSTEM FOR HEART TRANSPLANTATION AND THE IMPACT ON PATIENT SURVIVAL

J. Smits[1], M. De Pauw[2], E. de Vries[1], A. Rahmel[1], B. Meiser[3], G. Laufer[4], A. Zuckermann[4] [1]Eurotransplant, Biostatistics, Leiden, Niederlande; [2]University Hospital, Ghent, Belgien; [3]Hospital Grosshadern Ludwig-Maximilian University, Munich, Deutschland; [4]University Hospital, Vienna, Oesterreich

Background: The aim of this study was to design and validate a heart donor score that reflects experts' perceived risk of allograft failure.
Methods: All heart donors reported to Eurotransplant from January 1, 2005 to December 31, 2008 [$N = 4110$] were used to create a donor score. Based on observed discard rates and using multivariate regression, points were assigned for the following donor factors: age, cause of death, body mass index (BMI), diabetes mellitus (DM), duration of ICU stay, compromised history (drug, abuse, sepsis, meningitis, malignancy, HbsAg+ or Anti-HCV+), hypertension, cardiac arrest, echo cardiography, coronarography, serum sodium and noradrenaline and dopamine/dobutamine dosages. The donor score was obtained by adding all points. All heart donors reported to Eurotransplant in 2009 were used to validate the score [$N = 885$].
Results: All donor factors except BMI, DM and duration of ICU stay, significantly predicted discard. Based on the median value of the score, donors were classified into low risk donors (LRD): 16 points or less and high risk donors (HRD): 17 points or more. In the validation set discard rates were significantly different for HRD: 35% vs. LRD: 7%, ($P < 0.0001$). In addition the heart donor score was significantly associated with 3-year survival: LRD: 81.5% vs. HRD: 70.0% ($P = 0.004$)
Conclusions: The heart donor score accurately reflects the likelihood of organ acceptance and predicts long term patient mortality, its application at time of donor reporting may facilitate donor risk assessment and allow more appropriate matching of extended criteria donor hearts.

050 FAMILY APPROACH - RETROSPECTIVE ANALYSIS OF MORE THAN 5.000 DONATION REQUESTS IN GERMANY

*F. Schaub[1], C.-L. *Fischer-Fröhlich[2], G. Kirste[1] [1]Deutsche Stiftung Organtransplantation, Frankfurt / Main, Deutschland; [2]DSO, Region Baden-Württemberg, Stuttgart, Deutschland*

Introduction: The approach of the family in order to obtain consent for organ donation is a very challenging situation. Since january 2009 the DSO is operating an information system for a detailed documentation of the family approaches for analysis to enhance and improve the family approach.
Methods: The 5132 documented donation requests were spilt into a **s**tudy **g**roup (**sg** $n = 2766$, 01/2009−03/2010, and a **v**alidation **g**roup (**vg** $n = 2366$, 04/2010−04/2011). 54 variables about the donation request were analyzed for their influence on the consent to donation.
Results: Predictive for consent was:
• the participation of a trained DSO-coordinator. The consent rate was 57.7%**sg** / 55.2%**vg** in the case the attending physician only whereas it amounted to 71.7%**sg** / 78.3%**vg** with the participation by a coordinator
• the timepoint when request for organ donation was introduced to donor relatives. The consent rate before brain death certification (BDC) was 55.9%**sg** / 51.5%**vg**; during BDC 74.1%**sg** / 73.2%**vg**; after BDC 61.3%**sg** / 63.1%**vg**.
• in contrast refusal rates increased with missing knowledge about the last wishes of a deceased or dissents within the donor family as well as fears about organ trade, acceptance of death, trust into brain death certification or integrity of the deceased body.
In a logistic regression model the odds ratio [95%CI] for consent to donation vs. refusal ($P < 0.0001$) were for
• participation of a trained coordinator in **sg** 2.92 [2.22−3.87] and **vg** 4.14 [3.03−5.73] compared to no participation,
• request initiated during BDC in **sg** 3.37 [2.18−5.38] and **vg** 2.84 [1.86−4.42] as well as before BDC in **sg** 0.48 [0.33−0.68] and **vg** 0.47 [0.34−0.67] compared to initiated after BDC,
• last whish of deceased unknown, dissents within donor family etc. compared to not having this in **sg** 1278 [407−7719] and **vg** 572 [216−2323].
Conclusion: The low consent rate is one of the mayor factors influencing organ donation rates in Germany. Apparently the participation of a coordinator who has profound knowledge about organ donation and enough time during the decision process can provide comprehensive support to a stable decision. Secondly the adequate point of time introducing donor relatives about organ donation as part in the care at end of life is when decisions and concrete measurement for brain death certification are taken. It seems to be not appropriate to wait until after brain death has been certified. Further raise of public awareness is required in order to ensure that persons make up their mind during life time. Consent to organ donation is unlikely to occur when the wishes of the deceased are unknown or other fears about organ donation exist.
Acknowledgments: *on behalf of the DSO-coordinators who kindly provided the data.

224 PEDIATRIC DONORS AND RECIPIENTS: A COMPARISON OVER THE LAST YEARS

J. Weiss[1], G. Kirste[2], T. Breidenbach[3] [1]Deutsche Stiftung Organtransplantation, Erlangen, Deutschland; [2]Deutsche Stiftung Organtransplantation, Frankfurt am Main, Deutschland; [3]Deutsche Stiftung Organtransplantation, München, Deutschland

Introduction: The situation of pediatric patients, waiting for an organ, is a very special one, as they are not little grown-ups. A dysfunction of an organ has serious effects on the children's further somatically and cognitively development, certainly with different specifications depending on the exact age and the affected organ system. Furthermore, the allocation system reflects this special situation only partially. Little is known about the situation of pediatric donors as well as pediatric recipients in Germany.
Method: We searched within the DSO-data base for all pediatric donors (age 0−15 yrs.) and recipients in Germany from 2007 until 2010. We analyzed the number of donors, the causes of brain death within the different age groups and the number of organs transplanted. The organs, which could be harvested, were opposed to the organs, which were transplanted. In a further step, we will compare the number of transplanted patients with the number of patients on the waiting list.
Results: The overall number of pediatric donors and the numbers within the different age groups remained nearly the same within the last 4 years. But there was a shift concerning the reasons for brain death. In contrast to adult donors, the portion of children who died due to traumatic reasons had significantly increased within the last years. There was also a significant increase in the number of organs retrieved per pediatric donor. An increase of organs transplanted to pediatric recipients in this period could be noticed as well. The number of children dying on the waiting list or transplanted with permanent impairment remains unclear.
Discussion: Besides the risk to die on the waiting list, end stage organ failure in children can lead to severe impairment in development. Therefore everything should be done to transplant these little patients with high priority. Some children can only survive if they receive an organ from a pediatric donor. If brain death is diagnosed in a child, the question for consent to organ donation should be a matter of course. Pediatricians should be informed, that this question does not increase the grieving of the families. Furthermore the allocation rules for pediatric recipients should be reviewed (e.g. liver splitting, if possible, should be mandatory).

294 DONOR DOPAMINE DOES NOT AFFECT GRAFT SURVIVAL AFTER LIVER TRANSPLANTATION: DATA FROM A RANDOMIZED CONTROLLED TRIAL

U. Benck[1], P. Schemmer[2], A. Stöckle[1], S. Hoeger[1], B.A. Yard[1], B. Krämer[1], P. Schnuelle[1] [1]University Medical Centre Mannheim, 5th Department of Medicine, Mannheim, Deutschland; [2]Heidelberg University Hospital, Clinic for Surgery, Liver Transplant Center, Heidelberg, Deutschland

Background: Treatment of the deceased heart-beating donor with low-dose dopamine results in less dialysis requirement after kidney transplantation and appears to improve the outcome after heart transplantation. This study investigates the clinical course of liver allografts from multi-organ donors that were enrolled in the randomized dopamine trial (clinicaltrials.gov Identifier: NCT00115115).
Methods: Between March 2004 and August 2007, 264 brain-dead donors were randomly assigned to receive or to not receive low-dose dopamine. Eligibility criteria included circulatory stability under low-dose norepinephrine. The present study is nested in the randomized controlled trial of donor pre-treatment with dopamine. We assessed the outcomes of 197 liver transplants performed at 32 European centers.
Results: Dopamine was infused at 4 μg/kg/min for a median duration of 362 min (IQR 182 min). Donors and recipients were very similar in baseline characteristics. Thirty-four recipients (16.8%) were transplanted with high urgency and 23 (11.7%) received a repeat transplant. Pretransplant MELD score was not different in recipients of a dopamine treated versus untreated graft (18 ± 8 vs. 19 ± 9, $P = 0.28$). Mean cold ischemic time was 632 ± 172 vs. 600 ± 170 min ($P = 0.20$). Following transplantation, no differences occurred in biopsy-proven rejection episodes (15.3% vs. 17.2%, $P = 0.85$), requirement of hemofiltration (27.6% vs. 27.3%, $P = 0.99$), and in-hospital mortality (13.3% vs. 12.1%, $P = 0.85$). Graft survival was 72.5% vs. 74.8% and 61.2% vs. 63.6% at 1 and 3 years.
Conclusion: Contrasting heart and kidney transplantation, donor pre-treatment with dopamine does not improve the outcome after liver transplantation. Since liver cells specifically express s-COMT with high activity, dopamine is rapidly degraded *in-vivo*, which most likely abrogates its potential to protect the liver graft from oxidative stress under cold storage conditions.

LIVER II

263 HISTOLOGICAL ANALYSIS OF BILE DUCT SPECIMENS RECEIVED DURING LIVER TRANSPLANTATION

*T. Hansen, D. Hollemann, M. Heise, C.J. Kirkpatrick, G. Otto Universitätsmedizin Mainz, Institut für Pathologie, Mainz, Deutschland

Aims: Bile duct lesions belong to the major complications following liver transplantation (LTX). They are generally divided into leakages and strictures, the latter being further subdivided into anastomotic and non-anastomotic strictures. Nonanastomotic strictures are commonly caused by ischemic processes and may even lead to complete bile duct necrosis. Therefore, the term ischemic cholangiopathy or ischemic-type biliary lesion (ITBL) has been designed for this condition. The etiology and pathogenesis of ITBL is not fully understood. In this study, we analyzed the donor bile ducts taken during liver transplantation in order to find novel aspects in the pathogenesis of bile duct necrosis.
Methods: Bile duct tissue specimens of 70 donors (median age: 57.1 years) were isolated during liver transplantation, fixed in PBS-buffered formalin and processed according to standard protocols. The tissue specimens were stained by hematoxylin and eosin.
Results: Almost all specimens showed a loss of epithelium, which affected more than 50% of the epithelium in about 74% of analysed bile ducts. Furthermore, a majority of cases (83%) showed diffuse transmural bleeding of the bile duct. Inflammation was generally only sparsely detected. By contrast, the most remarkable alterations were observed in the arterioles: We found damage of the endothelial lining which was characterized by decrease of the endothelial cells and subendothelial oedema; most importantly, 45% of donors revealed variable numbers of necrotic arterioles. Concomitantly, necrosis of bile duct wall occurred often in these patients.
Conclusions: To our knowledge, this is the first study analyzing the histology of donor bile ducts immediately after transplantation. Thus we were able to examine very early histomorpholigcal changes. Among them, the most interesting finding was a remarkable large number of cases with vascular damage leading to arteriolonecrosis as well as necrosis of the bile duct. Probably, these lesions are associated with bile duct necrosis. Further investigation should thus elucidate whether these lesions might also play a role in the pathogenesis of ITBL.

218 BILIARY STRICTURES FOLLOWING LIVER TRANSPLANTATION: ROLE OF CHEMOKINE RECEPTOR POLYMORPHISMS, DONOR-SPECIFIC ANTI-HLA ANTIBODIES AND SERUM CYTOKINE PROFILE

*S. Beckebaum[1,2], S. Iacob[2,3], A. Dechene[2], M. Lindemann[4], F. Heinemann[4], V. Rebmann[4], S. Ferencik[5], G. Sotiropoulos[1], I. Popescu[3], P. Horn[4], G. Gerken[2], A. Paul[1], *V. Cicinnati[1,2]
[1]Uniklinikum Essen, Allgemein-, Viszeral- und Transplantationschirurgie, Essen, Deutschland;
[2]Uniklinikum Essen, Gastroenterologie und Hepatologie, Essen, Deutschland;
[3]Fundeni Clinical Institute for Digestive Diseases and Liver Transplantation, Bucharest, Rumaenien;
[4]Uniklinikum Essen, Institut für Transfusionsmedizin, Essen, Deutschland;
[5]Uniklinikum Essen, Institut für Immunologie, Essen, Deutschland

Background: Biliary strictures after liver transplantation (LT) are a major cause of morbidity and reduced graft survival.
Aim: The purpose of this study was to investigate genetic, clinical and immunological risk factors for the occurrence of ischemic type biliary lesions (ITBL) and biliary anastomotic strictures (AS) after LT.
Methods: Clinical and laboratory data, chemokine receptor genotypes, serum cytokine profile and inflammatory chemokines were investigated in 162 LT patients.
Results: In the univariate analysis, older donor and recipient age, partial LT, high peak aspartate transaminase levels, and CC chemokine receptor 5 delta32 loss-of-function mutation (CCR5Δ32) were associated with ITBL; whereas LT for acute liver failure (ALF), ABO-compatible non-identical LT, presence of donor-specific anti-HLA class II antibodies, and fractalkine receptor (CX3CR1)-249II allele were associated with AS. In the multivariate analysis, CCR5Δ32 was an independent risk factor for ITBL; whereas LT for ALF, ABO-compatible non-identical LT, and presence of CX3CR1-249II allele remained predictive for AS. Interferon-gamma, interleukin (IL)-6 and IL-10 serum levels were significantly increased in patients with biliary strictures, indicating a chronic inflammatory state and consequently augmented anti-inflammatory cytokine response.
Conclusions: These findings may have relevance for prediction and management of biliary strictures after LT.

284 PEDIATRIC LIVER TRANSPLANTATION: SINGLE CENTRE EXPERIENCE WITH CNI-FREE IMMUNOSUPPRESSION

*E. Sturm[1], R. Handgretinger[2], A. Königsrainer[3], S. Nadalin[3]
[1]Universitätsklinik Tübingen, Kinderklinik, Pädiatrische Gastroenterologie, Tübingen, Deutschland;
[2]Universitätsklinik Tübingen, Kinderklinik, Pädiatrische Hämatologie/ Onkologie, Tübingen, Deutschland;
[3]Universitätsklinik Tübingen, Viszeral- und Transplantationschirurgie, Tübingen, Deutschland

Background: Calcineurin inhibitors (CNI) are the basis for immunosuppression (IS) following pediatric liver transplantation (pLT). However, CNI-attributable side-effects are a major cause for post-transplant morbidity. Approved concepts for CNI-free treatment of pLT do not exist.
Aim: To report about our single centre experience with CNI-free IS after pLT
Methods: Retrospective analysis of patient charts in whom IS conversion to a CNI-free regimen was performed.
Results: CNI lead to non-hepatic organ dysfunction in 9/48 patients. Diagnosis leading to liver failure was biliary atresia in 4, acute liver failure (ALF) due to Wilson's disease in 2 cases. Hepatoportal sclerosis, ALF of unknown etiology and hepatoblastoma were diagnosed in one patient each. CNI toxicity led to renal dysfunction in 5 patients. Pancytopenia, severe eczema, post transplant-lymphoproliferative disease and perioral granulomatous lesions occurred in one patient each. CNI based IS was converted to mTOR inhibitors (mTORi) and mycophenolate mofetil (MMF), mTORi alone, or MMF alone in 3, 4 and 2 cases respectively from 6 months to 9 years after pLT. After 1−3 year follow-up graft function is stable in all patients. Renal toxicity resolved in all cases, non-renal CNI related side effects improved. In one case low level CNI was restarted due to mild acute rejection. 8 patients did not experience side effects of mTORi or MMF, one patient on everolimus developed edema of the lower extremities and was switched to sirolimus.
Conclusion: In pLT, a delayed conversion to CNI-free regimens consisting of mTORi and/ or MMF may be feasible and safe and lead to improvement or resolution of CNI-related side effects. Close monitoring of graft function is required. More studies are needed to establish effective and safe CNI-free regimens containing mTORi and MMF after pLT.

292 LESS THAN 10% OF PATIENTS WITH HEPATOCELLULAR CARCINOMA QUALIFY FOR LIVER TRANSPLANTATION AND SHOW BEST LONG-TERM SURVIVAL RATES

*G. Kirchner[1], A. Hebestreit[1], S. Farkas[2], N. Zorger[3], A. Schnitzbauer[2], J. Hartl[1], M. Loss[2], L. Baier[2], R. Wiest[1], M.N. Scherer[2], H.J. Schlitt[2]
[1]Universitätsklinikum Regensburg, Klinik für Innere Medizin I, Regensburg, Deutschland;
[2]Universitätsklinikum Regensburg, Klinik für Chirurgie, Regensburg, Deutschland;
[3]Krankenhaus der Barmherzigen Brüder, Radiologie, Regensburg, Deutschland

Background and aims: The aim of the study was to determine the survival rates of patients with hepatocellular carcinoma (HCC) in a large German unicenter cohort and to investigate how many patients are qualified for liver transplantation (LT) within the pre-MELD-Score-allocation era.
Patients and methods: In a retrospective study (03/1993 to 11/2006, pre-MELD-score-allocation era) data of 434 patients (364m) with HCC were determined. Clinical parameters, treatment received and survival curves from time of diagnosis were analyzed. Stage-dependent treatment included: LT, partial liver resection, transarterial chemoembolization (TACE), radiofrequence thermoablation (RFTA), systemic chemotherapy or 'best supportive care'.
Results: HCC was diagnosed at a mean-age of 63 ± 11 years. The mean follow-up was 26.7 ± 30.7 months. Median survival of all patients was 14 months. The 1-, 3-, 5-, 7- and 10-year survival rate of all patients was 56%, 29%, 20%, 13%, and 11%, respectively. Liver cirrhosis was diagnosed in 86% of patients (Child A 64%, Child B 24% and Child C 12%). Only 7% ($n = 29$) of all patients were qualified for LT. Patients, who underwent LT, had the best survival rates (median 115 months). The median survival rates without LT were as follows: resection 37 months ($n = 102$), TACE+RFTA 42 months ($n = 22$), RFTA 17 months ($n = 18$), TACE 16 months ($n = 116$), systemically chemotherapy ($n = 30$) and best-supportive care 4 months ($n = 117$), respectively. Independent, negative prognostic parameters for survival were presence of portal vein thrombosis, size of the largest tumor lesion and CLIP stage.
Conclusion: HCC patients, who underwent LT, showed the best long-term survival rates. But only 7% of all HCC patients in this cohort were qualified for LT. Analysis of the following cohort from 12/2006 within the MELD-score-allocation era including HCC exceptional-MELD-scores are currently ongoing.

IMMUNOSUPPRESSION

105 BORTEZOMIB VERSUS RITUXIMAB IN THE TREATMENT OF ANTIBODY MEDIATED RENAL ALLOGRAFT REJECTION

*N. Lachmann[1], C. Schönemann[1], K. Budde[2], M. Schütz[2], L. Liefeldt[2], B. Rudolph[3], H.-H. Neumayer[2], J. Waiser[2] [1]Charité, HLA Laboratory, Berlin, Deutschland;
[2]Charité, Department of Internal Medicine-Nephrology, Berlin, Deutschland;
[3]Charité, Department of Pathology, Berlin, Deutschland*

Treatment with the proteasome inhibitor bortezomib may improve renal allograft survival in patients with antibody-mediated rejection (AMR). Starting in February 2009, we treated ten consecutive patients diagnosed with biopsy-proven AMR using bortezomib (1.3 mg/m^2 i.v., day 1,4,8,11) supplemented by plasma exchange (PEX, 6x) and intravenous immunoglobulins (IVIG, 30 g). This group was compared to a group of patients (n = 9) treated with a fixed single dose of rituximab (500 mg i.v.), PEX (6x) and IVIG (30 g). Patient survival was 100% in both groups. At 18 months after treatment graft survival was 60% (6/10) in the bortezomib group as compared to 11% (1/9) in the rituximab group (P = 0.07). Median serum creatinine was 2.5 mg/dl (2.1−3.4) and 4.3 mg/dl (4.0−4.5), respectively. In the bortezomib group none of the patients with a sustained decrease of the immunodominant donor-specific HLA antibody (iDSA) lost the allograft (0/5). Whereas iDSA levels decreased in 71% of patients, the overall panel reactivity of HLA antibodies decreased in only 18% of patients. Allograft survival was also dependent on the type (acute vs. chronic AMR: 4/13 vs. 4/6) and severity (acute AMR grade I vs. grade II: 2/2 vs. 2/11) of AMR. In conclusion, treatment of AMR with bortezomib was superior to rituximab. Neither of both substances was sufficient to treat severe AMR effectively. Sequential iDSA level measurements help to control the efficacy of bortezomib treatment.

013 INFLUENCE OF DONOR RELATED FACTORS ON OUTCOMES WITH TACROLIMUS-BASED IMMUNOSUPPRESSION AFTER KIDNEY TRANSPLANTATION - THE OSAKA STUDY

*F. Lehner[1], B. Banas[2] [1]Medizinische Hochschule Hannover, Allgemein-, Viszeral- und Transplantationschirurgie, Hannover, Deutschland;
[2]Universitätsklinikum Regensburg, Klinik und Poliklinik für Innere Medizin II, Regensburg, Deutschland*

Background: Extended criteria donors and living donation in kidney transplantation are increasing. The OSAKA study, a large randomised controlled trial, allows analysis of the effect of donor factors on kidney transplant outcomes.
Methods: Patients (n = 1251) were randomised 1:1:1:1 to 24 weeks' treatment with tacrolimus immediate release (BID) 0.2 mg/kg/day (Arm 1; n = 309), prolonged release (QD) 0.2 mg/kg/day (Arm 2; n = 302), tacrolimus QD 0.3 mg/kg/day (Arm 3; n = 304) all with MMF and corticosteroids, or to a corticosteroid avoidance regimen with tacrolimus QD 0.2 mg/kg/day with MMF, basiliximab and a perioperative corticosteroid bolus (Arm 4; n = 283). Primary endpoint was efficacy failure, a composite endpoint defined as incidence and time to first incidence of either graft loss, biopsy confirmed acute rejection (BCAR) or graft dysfunction (eGFR <40 ml/min/1.73m^2) at 24 weeks.
Results: The mean age of donors was ~51.5 years; ~50% were extended criteria donors and ~12% were living donors. Efficacy failure increased markedly with donor age (21.6% in donors <30 years; 63.1% in donors 60-70 years) and recipient age (34.1% in recipients <30 years; 57.2% in recipients 60−70 years). Living organ donation was associated with a better outcome for renal function (GFR was higher with living donors, especially early post-transplant), particularly in the steroid avoidance arm. HLA mismatch increased the incidence of BCAR seen with tacrolimus QD 0.2 mg/kg/day (2.7% with 0−1 and 10.1% with 4−6 mismatches) and tacrolimus BID (7.9% and 17.1%, respectively). Efficacy failure was slightly lower with male than female donors (failure rates of 41.4% and 49.4%) and with living versus deceased donor (34,7% vs 46,4%)
Conclusion: The over-riding factor influencing kidney function after transplantation is donor age. Recipient age, HLA matching and living donations also contribute more than tacrolimus dose or formulation.

057 THREE-YEAR OUTCOMES FROM BENEFIT: A PHASE III STUDY OF BELATACEPT VS CYCLOSPORINE IN KIDNEY TRANSPLANT RECIPIENTS

*F. Vincenti[1], C. Larsen[2], J. Alberu[3], V. Garcia[4], L. Rostaing[5], K. Rice[6], M. Schnitzler[7], J. Xing[8], M. Agarwal[8], B. Charpentier[9] [1]UCSF, Kidney Transplant Service, San Francisco,CA, Vereinigte Staaten von Amerika;
[2]Emory University, Transplant Center, Atlanta,GA, Vereinigte Staaten von Amerika; [3]Instituto Nacional de Ciencias Médicas y Nutrición, "Salvador Zubirán", Mexiko DF, Mexico; [4]Hospital Dom Vicente Scherer, Complexo Hospitalar Santa Casa, Porto Allegre, Brazilien; [5]University Hospital, and INSERM U563, IFR-BMT, Toulouse, Frankreich; [6]Baylor University, Medical Center, Dallas,Texas, Vereinigte Staaten von Amerika; [7]St Louis University, St Louis,MO, Vereinigte Staaten von Amerika; [8]Bristol-Myers Squibb, Princeton, NJ, Vereinigte Staaten von Amerika; [9]Bicêtre Hospital, Kremlin Bicêtre, Frankreich*

Background: Allograft function is associated with patient/graft survival in kidney transplant recipients. The efficacy and safety of belatacept was evaluated to determine if the results at 2 years in the BENEFIT study were sustained at 3 years.
Methods: BENEFIT is a randomized, phase III study in adults receiving a kidney transplant from a living or standard criteria deceased donor. Patients were randomized to a more (MI) or less intensive (LI) regimen of belatacept, or CsA.
Results: 471/666 intent-to-treat patients (n = 158/219 MI; n = 170/226 LI; n = 143/221 CsA) completed at least 3 years of therapy. Patient/graft survival was 92% (MI), 92% (LI), and 89% (CsA) by Year 3. The mean calculated GFR (cGFR) was approximately 21 ml/min higher in the belatacept groups vs CsA by Year 3. The mean cGFR exhibited a positive slope over time in the belatacept groups.Despite an early increase in the rate/grade and impact of acute rejection (AR) in the belatacept groups, no new AR cases occurred in the belatacept groups from Years 2−3, with 1 AR case in the CsA group. There were no new safety signals and no new cases of PTLD after Month 18. A risk prediction model suggested that belatacept would extend the projected graft half-life by 23 months, from 11 to 13 yrs.
Conclusions: Belatacept-treated patients maintained a high rate of patient and graft survival, despite an early increased risk for acute rejection and PTLD. There were no new safety signals. Belatacept was associated with sustained improvements in renal function.

066 EARLY CONVERSION TO A SIROLIMUS-BASED, CALCINEURIN-INHIBITOR-FREE IMMUNOSUPPRESSION IN THE SMART TRIAL: OBSERVATIONAL RESULTS AT 24 AND 36 MONTHS AFTER TRANSPLANTATION

M. Guba[1], J. Pratschke[2], C. Hugo[3], B. Krämer[4], A. Pascher[5], K. Pressmar[6], O. Hakenberg[7], M. Fischereder[8], J. Brockmann[9], J. Andrassy[1], M. Stangl[1], B. Banas[10], K.-W. Jauch[1] [1]Klinikum Großhadern, Chirurgie, München, Deutschland; [2]Universität, Chirurgie, Innsbruck, Oesterreich; [3]Technische Universität, Med III, Dresden, Deutschland; [4]Universität, V. Med. Klinik, Mannheim, Deutschland; [5]Charité - Campus Virchow Klinikum, Chirurgie, Berlin, Deutschland; [6]Universität, Nephrologie, Erlangen, Deutschland; [7]Universität, Urologie, Rostock, Deutschland; [8]Klinikum Großhadern, Med I, München, Deutschland; [9]Universität, Chirurgie, Zürich, Schweiz; [10]Universität, Med II, Regensburg, Deutschland

Early conversion to a calcineurin-inhibitor free maintenance immunosuppression with sirolimus, mycophenolate mofetil and steroids was associated with an improved one-year renal function as compared to a cyclosporine-based regimen (SMART core-study).

This in an observational follow-up of 132 patients for additional 2 years using the same endpoints as the core study.

At 36 months after transplantation (28/64) 43.8% of the patient in sirolimus arm and (41/59) 69.5% in the cyclosporine arm were on treatment. The *per protocol* as well as the *intention-to-treat* analysis at 36 months revealed a significantly better renal function in the sirolimus as compared to the cyclosporine arm (ITT-eGFR$_{@36m}$: 60.80 vs. 53.72 ml/min/1.73 m^2, P = 0.031). Patient- (SRL 96%vs.CsA 94%) and graft (SRL 96% vs. CsA 94%) survival at 36 months was excellent in both arms. Three late (>12 months) biopsy-proven rejections were recorded in the cyclosporine arm, none in the sirolimus arm. De novo malignancy developed in 5 patients in the cyclosporine arm, no malignancy was recorded in the sirolimus arm (P = 0.0259). There were no notable differences between groups in late infections or adverse events during follow-up beyond month 12. Multivariate analysis revealed that substrata of patients may benefit more from a CNI-free sirolimus-based therapy than others, among those were: CMV naive (-) recipients, patients receiving a donor organ <60 years of age and patients with a good initial renal function.

Early conversion to a CNI-free sirolimus based immunosuppression is associated with a sustained improvement of renal function up to 36 months after transplantation. Patient selection will be key to derive long-term benefit and avoid treatment failure using this mTOR-inhibitor-based immunosuppressive regimen.

036 CMV INFECTIONS ARE LESS FREQUENT IN DE NOVO HEART TRANSPLANT RECIPIENTS RECEIVING IMMUNOSUPPRESSION WITH EVEROLIMUS PLUS REDUCED CSA COMPARED TO MMF AND STANDARD CSA

*U. Schulz[1], J. Kobashigawa[2], C. Bara[3], S. Hirt[4], H.B. Lehmkuhl[5], P. Lopez[6], A. Costard-Jaeckle[7] [1]Herzzentrum, Bad Oeynhausen, Deutschland; [2]Mount Sinai School of Medicine, Beverly Hills, Vereinigte Staaten von Amerika; [3]MHH, Hannover, Deutschland; [4]Universitaet, Regensburg, Deutschland; [5]DHZB, Berlin, Deutschland; [6]Novartis Pharma, Basel, Schweiz; [7]Uniklinik, Eppendorf, Deutschland

Purpose: Cytomegalovirus (CMV) is the most clinically relevant infectious agent in heart transplant recipients (HTxR) and may impair long-term graft outcome. Herein we compare CMV outcomes in patients randomized to receive everolimus (EVR) or mycophenolate mofetil (MMF).

Methods: We analyzed 12 months (M) occurrence of CMV infection (defined as laboratory evidence of CMV replication or CMV syndrome/disease) in patients enrolled in A2310. This is a 24M, multicenter, randomized, open-label study designed to compare two EVR arms (target C0 3–8 ng/ml or 6–12 ng/ml) with reduced dose cyclosporine (CsA) to MMF 3 g/day with standard dose CsA for efficacy, renal function and safety. Enrollment in the high EVR dose arm was prematurely interrupted because of excess mortality, and is not included in this analysis. Induction therapy was center-specific (basiliximab/thymoglobulin/no induction). CMV prophylaxis was recommended for patients at high risk for CMV infection (i.e. donor positive/recipient negative, D+/R-), while prophylaxis in low risk patients (i.e. R+) was according to center practice.

Results: Out of the 547 patients comprising the safety population included in this analysis, 279 received EVR and 268 MMF. Significantly lower incidence of CMV infections (8.2% vs. 20.5%, $P < 0.001$) was observed in the EVR vs. MMF arm. CMV infections were less frequent with EVR than MMF in the D+/R- (15% vs. 37%, $P = 0.014$) and D+/R+ subgroups (8% vs 24%, $P = 0.006$). Despite being at higher serological risk, D+/R- patients receiving EVR experienced less CMV infection than D+/R+ patients receiving MMF. Differences remained significant after adjustment for CMV prophylaxis use. Use of induction did not influence the effect of EVR on CMV.

Conclusions: Immunosuppression with EVR and reduced CsA showed a significantly lower incidence of CMV infections compared to MMF with standard CsA in de novo HTxR, regardless of induction, prophylaxis use and CMV serological risk.

034 EFFICACY AND RENAL FUNCTION IN DE NOVO HEART TRANSPLANT RECIPIENTS RECEIVING EVEROLIMUS WITH REDUCED CSA COMPARED TO MMF WITH STANDARD CSA: THE A2310 STUDY

*C. Bara[1], H. Eisen[2], S. Hirt[3], A. Costard-Jaeckle[4], U. Schulz[5], P. Lopez[6], J. Kobashigawa[7], H.B. Lehmkuhl[8] [1]MHH, Hannover, Deutschland; [2]University of Pennsylvania, Philadelphia, Vereinigte Staaten von Amerika; [3]Universitaet, Regensburg, Deutschland; [4]Uniklinik, Eppendorf, Deutschland; [5]Herzzentrum, Bad Oeynhausen, Deutschland; [6]Novartis Pharma, Basel, Schweiz; [7]Mount Sinai School of Medicine, Beverly Hills, Vereinigte Staaten von Amerika; [8]DHZB, Berlin, Deutschland

Aim and methods: A2310, a 24-month (M), multi-center, randomized, open-label study, aimed to compare efficacy and renal function (RF) at Month (M) 12 in de novo heart transplant recipients (HTxR) receiving the mTOR inhibitor everolimus (EVR) dosed at 1.5 or 3 mg/day (target C0 3–8 or 6–12 ng/ml) with reduced dose cyclosporine (CsA) to MMF 3 g/day with standard CsA and steroids. Induction therapy was center-specific (basiliximab (BAS)/thymoglobulin (ATG)/no induction). Key endpoints at M12: composite efficacy failure (acute rejection, BPARs ≥ 3A, death graft loss, lost to follow up) and RF (GFR; MDRD) in EVR arms versus MMF.

Results: 721 HTxR were randomized (EVR 1.5 mg $N = 282$, EVR 3 mg $N = 168$, MMF $N = 271$). Enrollment in the EVR 3 mg arm was prematurely terminated due to higher mortality; only comparison of EVR 1.5 mg vs MMF is presented. At M12, EVR 1.5 mg was non-inferior to MMF for composite efficacy failure, but was inferior for RF (difference in mean GFR: -5.6 ml/min/1.73 m^2; 97.5% CI -10.9, -0.2). GFR (ml/min/1.73 m^2) decrease from baseline (BL) to M12 was -7.1 with EVR 1.5 mg vs -2.9 with MMF (P = NS). GFR decrease from M1 to M12 was significantly lower with EVR 1.5 mg vs MMF (-6.4 vs. -13.7; $P = 0.002$). Post-hoc analyses showed that HTxR at centers which complied with CsA reduction (N=468, 84.6%), had comparable GFR decrease from BL to M12 (EVR: -6.7 vs MMF: -4.4; P = NS) and RF at M12 (difference in mean GFR: -3.6 mL/min/1.73m^2; 97.5% CI -8.9, 1.8) for EVR 1.5 mg and MMF. Imbalance in mortality was seen in EVR 1.5 mg vs MMF (7.8% vs 4.8%), mainly due to infections in the first 3M post-Tx in HTxR receiving ATG induction. Mortality was similar without induction therapy

Conclusion: EVR 1.5 mg was non-inferior to MMF for composite efficacy failure. The higher mortality observed with EVR 1.5 mg seems to be associated with ATG induction. Lack of compliance to CsA reduction in the EVR arm contributed to lower GFR.

041 THE PROTECT STUDY: PRESERVATION OF RENAL FUNCTION IN LIVER TRANSPLANT RECIPIENTS WITH CERTICAN-BASED VS CNI-BASED THERAPY

*H.J. Schlitt[1], F. Lehner[2], P. Neuhaus[3], J. Schmidt[4], T. Becker[5], M.A. Hack[6], S. Ladenburger[6], L. Fischer[7] [1]Uniklinik, Regensburg, Deutschland; [2]MHH, Hannover, Deutschland; [3]Charite, Berlin, Deutschland; [4]Uniklinik, Heidelberg, Deutschland; [5]Uniklinik, Kiel, Deutschland; [6]Novartis Pharma, IDTI, Nürnberg, Deutschland; [7]Uniklinik, Eppendorf, Deutschland

Introduction: With current CNI-based immunosuppressive regimens acute rejection (AR) has become less important as it rarely causes graft loss and death. In fact, excess immunosuppression (IS) is the greater concern which shifted the focus to avoiding long-term complications of IS, especially nephrotoxicity and recurrent liver disease. Even if CNI treatment has become the corner stone in most liver transplant (LTx) programs, its inherent nephrotoxicity still remains a challenge for both, immediate renal complications and long-term outcome. Thus, the introduction of everolimus (EVR) may improve renal function and other safety outcomes by allowing for minimization and later elimination of CNIs early posttransplantation.

Methods: In this 11 month multicenter, prospective, open label study 203 de novo LTx patients with good renal function (calculated glomerular filtration rate ≥50 ml/min) 48 weeks after transplantation were randomized into two groups. Group A continued CNI treatment ($n = 102$; standard CNI dose [tacrolimus or cyclosporine] ±steroids). Group B was switched to everolimus ± steroids ($n = 101$). Everolimus was given.5 mg bid initially, later adjusted to target trough level of 5–12 ng/ml and CNI was withdrawn stepwise until week 16 post randomization.

Results: The primary end point of an inter group-difference of 8 ml/min) in change in GFR calculated by Cockcroft-Gault in the intent-to-treat (ITT) analysis from baseline to Month 11 was not reached. However, the mean difference in GFR calculated by MDRD at Month 11 between the everolimus group and the CNI group was 7.778 ml/min ($P = 0.0209$, ITT population). Further, conversion to an everolimus-based regimen showed comparable efficacy at Month 11 evaluations for incidence of Biopsy Proven Acute Rejection (BPAR), graft loss or death .The adverse effect profile in everolimus group was consistent with earlier transplant studies.

Conclusions: These results demonstrate that in liver transplant recipients with good renal function 4–8 weeks posttransplant, conversion to everolimus (mostly monotherapy) can be achieved without compromising efficacy and may lead to an improvement in renal function during the first postop year.

TISSUE TRANSPLANTATION AND COMPOSITE TISSUE

168 THE INNSBRUCK HAND TRANSPLANT PROGRAM: UPDATE AT 11 YEARS AFTER THE FIRST TRANSPLANT

A. Weißenbacher[1], G. Pierer[2], M. Gabl[3], M. Ninkovic[1], T. Hautz[1], B. Zelger[4], W. Löscher[5], M. Kumnig[6], M. Rieger[7], H. Piza-Katzer[2], R. Margreiter[1], J. Pratschke[1], G. Brandacher[1], S. Schneeberger[1] [1]Medizinische Universität Innsbruck, Universitätsklinik für Visceral-, Transplantations- und Thoraxchirurgie, Innsbruck, Oesterreich; [2]Medizinische Universität Innsbruck, Universitätsklinik für Plastische-, Rekonstruktive- und Ästhetische Chirurgie, Innsbruck, Oesterreich; [3]Medizinische Universität Innsbruck, Universitätsklinik für Unfallchirurgie, Innsbruck, Oesterreich; [4]Medizinische Universität Innsbruck, Institut für Pathologie, Innsbruck, Oesterreich; [5]Medizinische Universität Innsbruck, Universitätsklinik für Neurologie, Innsbruck, Oesterreich; [6]Medizinische Universität Innsbruck, Medizinische Psychologie, Innsbruck, Oesterreich; [7]Medizinische Universität Innsbruck, Department Radiologie, Innsbruck, Oesterreich

Introduction: We describe here the outcome after two bilateral hand, one bilateral forearm and one unilateral hand transplantation at 11/8/5 and 2 years after transplantation.
Methods: Four patients received a bilateral hand ($n = 2$), a bilateral forearm ($n = 1$) or a unilateral hand transplant between March 2000 and July 2009. Induction therapy with ATG ($n = 2$) or alemtuzumab ($n = 2$) was followed by tacrolimus, prednisolon, MMF ($n = 3$) or tacrolimus and MMF ($n = 1$) maintenance IS. Later, sirolimus/everolimus was added under simultaneous withdrawal ($n = 2$) or dose reduction ($n = 1$) of tacrolimus ($n = 1$) or MMF ($n = 1$). Steroids were avoided in one and withdrawn in two patients.
Results: Range of motion reached up to 70% of normal with a grip strength of 2–10kg. Hand function correlated well with time after transplant and amputation level. Intrinsic hand muscle function recovery and discriminative sensation were observed in all patients. Complications included CMV infection, fungal infection, hypertension, hyperglycemia, transient creatinine increase and headache. Three, six, four, and one rejection episode were successfully treated with steroids, anti-CD25, anti-CD52 antibodies and/or intensified maintenance IS. Skin histology at current shows no or mild perivascular lymphocytic infiltrates without signs of progression. Vessels are patent without signs for luminal narrowing or intimal proliferation.
Conclusion: The overall functional outcome and patient satisfaction after bilateral hand, bilateral forearm and unilateral hand transplantation are highly encouraging. All patients are now free of rejection with moderate levels of IS.

246 THE ROLE OF MECHANISMS REGULATING HEPATIC SINUSOIDAL CIRCULATION FOR CELL THERAPY STRATEGIES

R. Bahde[1,2], S. Kapoor[2], K. Bhargava[3], C. Palestro[3], S. Gupta[2] [1]Universitätsklinikum Münster, Klinik und Poliklinik für Allgemein- und Viszeralchirurgie, Münster, Deutschland; [2]Albert Einstein College of Medicine, Marion Bessin Liver Research Center, Departments of Medicine and Pathology, Bronx, NY, Vereinigte Staaten von Amerika; [3]Long Island Jewish Medical Center, Division of Nuclear Medicine and Molecular Imaging, New Hyde Park, NY, Vereinigte Staaten von Amerika

Introduction: Efficient engraftment of transplanted hepatocytes is critical for therapeutic liver repopulation and requires deposition of cells in liver sinusoids. 80–90% of transplanted cells are cleared of the periportal region during the early postoperative period due to acute impairment of hepatic microcirculation and local ischemia/reperfusion injury. Mechanisms regulating hepatic sinusoidal circulation could provide suitable manipulations for enhancing cell engraftment.
Material and methods: Freshly isolated F344 rat hepatocytes were transplanted intraspenically into syngeneic dipeptidyl peptidase IV (DPPIV)-deficient F344 rats. Cell engraftment was analyzed by DPPIV histochemistry and morphometry over 6 h to 2 weeks. Hepatic ischemia (GGT staining), cell activations (neutrophils, PMN, Kupffer cells, KC, hepatic stellate cells, HSC, liver sinusoidal endothelial cells, LSEC), and changes in gene expression (qRT-PCR of 84 relevant genes) were assessed. To define contributions of the renin-angiotensin system (RAS), nitrous oxide (NO), prostacyclin (PGI2) and endothelin (ET) in transplanted cell clearance recipients were treated with ACE inhibitor, AT1R antagonist, prostacyclin, NO donor and ET receptor blocker.
Results: After cell transplantation, hepatic ischemia occurred with PMN, KC, HSC and LSEC activations, and 31 of 84 relevant genes (37%) were upregulated, including in RAS, NO, PGI2, ET and inflammatory chemokine-cytokine systems. ACE inhibitors and AT1R antagonists did not improve cell engraftment, indicating RAS was not a major contributor. Treatment with PGI2, NO donors and ET receptor blocker improved cell engraftment 2–3 fold ($P < 0.05$), indicating that prostanoid and NO systems were more relevant. PGI2 or NO treatment produced fewer changes in gene expression, including inflammatory chemokine-cytokine genes, along with less hepatic ischemia (GGT) and activation of KC. In animals treated with ET receptor blockers some of those parameters were unchanged, others more enhanced such as HSC activation.
Conclusion: Transplantation of hepatocytes led to activation of vascular systems regulating hepatic sinusoidal microcirculation. Hepatic sinusoidal vasodilatation with drugs to alter NO, ET and PGI2 mechanisms improved cell engraftment and will be appropriate for optimizing cell therapy strategies.

063 EFFECT OF PRESERVATION SOLUTIONS HTK (HISTIDINE TRYPTOPHANE KETOGLUTARAT) AND UW (UNIVERSITY OF WISCONSIN SOLUTION) ON ISCHEMIA/REPERFUSION INJURY IN COMPOSITE TISSUE ALLOGRAFTS (CTA)

T. Hickethier[1], S. Schneeberger[1], T. Hautz[1], J. Grahammer[1], C. Krapf[1], M. Kimelman[1], M. Hermann[1], R. Sucher[1], G. Rumberg[1], R. Margreiter[1], J. Pratschke[1], B. Zelger[2], G. Brandacher[3], W.P.A. Lee[3] [1]Medizinische Universität Innsbruck, Univ.-Klinik für Visceral-, Transplantations- und Thoraxchirurgie, Innsbruck, Oesterreich; [2]Medizinische Universität Innsbruck, Institut für Pathologie, Innsbruck, Oesterreich; [3]University of Pittsburgh Medical Center, Division of Plastic Surgery, Pittsburgh, Vereinigte Staaten von Amerika

Background: We investigated the effect of cold ischemia (CI) on damage to skin, muscle, nerve and bone in a composite tissue transplantation model as well as the efficacy of perfusion solutions (HTK and UW) on tissue damage.
Methods: Explanted Lewis rat limbs were flushed and stored for 0, 2, 10, 30 and 40 h in HTK or UW preservation solution. Skin, muscle, bone and nerve biopsies were taken at any time point for H&E-histology.

Furthermore, syngenic hind limb transplantations were performed subsequent to 2, 10 or 30 h of CI. Limbs were flushed and stored either in HTK, UW or NaCl solution. Skin and muscle biopsies were taken after 1 day. Skin, muscle, nerve and bone samples were collected at the endpoint of the study at 10 days posttransplant. Histopathological alterations of tissues were described according to a 4-graded scale (higher scores = higher damage). The muscle biopsies were further analysed applying real time live confocal microscopy.
Results: Appearance and histology of skin, bone and nerve remained unaltered at any time point during preservation. Histomorphologic changes of muscle fibers were observed in some biopsies regardless of preservation solutions and CI-time.

CI and subsequent reperfusion did not cause histomorphologic alterations of skin and muscle at 1 day. At 10 days, skin showed a mild lymphocytic infiltrate in all samples. In muscle a mild lymphocytic infiltrate was found in groups of 10 h CI; after 30 h CI highly affected and necrotic muscle fibers were obtained. Nerve showed alterations, vasculopathy, an infiltrate and was hyper cellular, especially at 10 and 30 h CI. Bone was not affected at all, but secondary infection was observed in some samples, regardless of preservation solution and CI-time. Overall, tissues were more affected of CI when flushed and stored in UW than HTK (overall score: 1.6 vs. 1.1). Tissues flushed with NaCl showed the highest damage of all samples. Results of confocal microscopy revealed increased muscle damage after longer CI-times. At 10 d and 10 h of CI, HTK groups showed 48.06%, UW groups 45.74% and NaCl groups 29.68% vital cells.
Conclusions: Cold ischemia causes mild histomorphologic alterations on muscle, but not on skin, nerve and bone. The longer the time of CI prior to transplantation, the more severe tissue alterations are observed in skin and muscle samples. For preservation of these tissues superiority of HTK over UW is found. Muscle and nerve are identified as tissues being most affected by ischemia/reperfusion injury.

PSYCHOSOMATICS AND ETHICS

115 THE NEED TO CONVEY AND RECEIVE GRATITUDE FOR THE GIFT OF LIFE

A.-B. Blaes-Eise, T. Breidenbach KOSE Öffentlichkeitsarbeit, Deutsche Transplantationsgesellschaft, DTG, Deutschland

Background: Organ transplantation is more than mere therapy of end stage disease. For donor families and organ recipients, organ donation and transplantation have a strong emotional dimension. Organ recipients often search for ways to convey their gratitude for the gift of life they received. Donor families in turn often yearn for positive feedback from recipients, a form of acknowledgement that they took the right decision. Where anonymity between organ donor and recipient is legally mandated, meeting these emotional needs on both sides of organ transplantation is challenging.

Methods: A working group of the German Transplant Society (Deutsche Transplantationsgesellschaft) issued a flyer with information and helpful suggestions for organ recipients on how to write an anonymous thank you letter to donor families. The working group includes representatives of national self-help organizations, medical professionals in the transplantation field, as well as representatives of the German Foundation for Organ Transplantation (DSO). The flyer has been distributed to organ recipients in outpatient follow-up care facilities and by national self-help groups.

Results: Prior to the flyer's release, the DSO central region received 8 thank you letters from organ recipients in a 10 month period (from January/01/2010 to October/01/2010). In the roughly 7 months thereafter, the DSO central region has received 41 thank you letters. The letters were written on average 4.6 years after the respective transplantation surgeries (with a range of 1 month to 20 years). The authors had received a liver (19), kidney (17), lung (2), heart (2), and combined heart and liver (1) transplant.

Conclusions: The flyer with helpful suggestions for organ recipients on how to write an anonymous thank you letter to donor families has struck a nerve with organ recipients eager to convey their appreciation for the gift of life they received. Prior to its release, thank you letters were few and far between. Ever since, the number of thank you letters the DSO has forwarded to donor families has increased considerably. Hence, further attention to the emotional intersection between donor family and organ recipient is warranted. Increasing the number of thank you letters can broaden the currently rather technical image of organ transplantation in Germany by drawing attention to the extraordinary relationship between a grateful recipient and an anonymous organ donor family. Initiatives to encourage one-on-one validation of this invaluable gift of life can help improve the image and acceptance of organ donation in Germany.

134 EXTENDED ADHERENCE OF HEART TRANSPLANT PATIENTS - SINGLE-CENTER STUDY HDZ-NRW

K. Tigges-Limmer[1], G. Schmid-Ott[2], U. Schulz[1], Y. Brocks[1], J. Brakhage[1], A. Benkler[2], J.F. Gummert[1] [1]HDZ-NRW, Klinik für Thorax- und Kardiovaskularchirurgie, Bad Oeynhausen, Deutschland; [2]Berolina-Klinik, Löhne, Deutschland

Introduction: Compliance (adherence) represents a multifactorial concept. Medication non-adherence is a major cause of organ dysfunction following heart transplantation. Beside accurate medicine taking, patients are expected to look out for low-germ food, adhere to nonsmoking and keep to restrictions on domestic animal keeping. The prevalence varies considerably.

Methods: 858 HTX patients of the HDZ-NRW were asked about their adherence in an anonymous cross section investigation. Data was collected on relevant medication adherence (ITAS, ITBS, COMPAD), fears and depression (HADS-D), quality of life (SF-12), prevalence of a PTSD (IES-R), extend of the social desirability (BIDR shortscale) and questions on germ-rich food and domestic animal keeping.

Results: The response rate was 61% ($n = 538$). 79.2% of the patients were male and on average 59.7 years old (range 18−90 years). 72.2% of the patients reported very reliable medicine taking, 14.4% an easily limited, 13.4% a precariously limited adherence. A reduced medicine-referred adherence is significantly associated with an increased degree of a self-deception ($P < 0.025$). 22.6% of the total sample showed remarkably high depression, 16.7% increased fears. A third of the patients indicated to occasionally eating germ-rich food (e.g. soft ice, raw meat, raw egg-products) because 53.3% of the patients negated possible damage and 3.8% admitted to smoking, 14.2% keep at least one animal.

Conclusion: Although the adherence was ascertained only by self-assessment and a positive selection by the response rate has to be assumed, a medicine-referred non adherence of 28% could be demonstrated and a clearly increased non adherence related to germ-poor food and domestic animal keeping is determined. Interventions to increase post HTX adherence should refer to that purposefully.

136 STRESS FACTORS AND PSYCHOSOCIAL PROBLEMS FOR CHILDREN AFTER PEDIATRIC LIVER TRANSPLANTATION

K. Gold[1], M. Melter[1], H. Gruber[2], D. Grothues[1] [1]Universitätsklinikum Regensburg, Klinik und Poliklinik für Kinder- und Jugendmedizin, Regensburg, Deutschland; [2]Universität Regensburg, Institut für Pädagogik, Regensburg, Deutschland

Children undergoing liver transplantations (ltx) have to cope with a huge variety of stressful experiences. The aim of this study was to show the key aspects of psychosocial problems shown after pediatric liver transplantation.

Twenty nine children, older than 4 years (period after ltx >5 years: $n = 20$, period after ltx <5 years: $n = 9$; male $n = 13$, female $n = 16$) were assessed with the *Child Behaviour Checklist (CBCL*/4-1). In general, the *CBCL*/4-1 showed a high significant difference for all tested children in contrast to the regular population ($P < 0.000$), independent of the gender ($P < 0.908$) and the period after liver transplantation (>or < 5 years) ($P < 0.102$). The children scored showed considerable higher rates in somatic complaints, anxiety, depression and social problems. 43.5% of the tested children showed a significantly higher ratio concerning internalizing problems and 20.3% a significantly higher ratio concerning externalizing problems than the normal population. Scores for withdrawn and attention problems showed borderline values.

In summary we could show that there are a lot of psychosocial problems and stress factors liver transplanted children have to cope with. Therefore, there is the urgent need for psychosocial treatment after pediatric liver transplantation.

009 DISAGREEMENT AND INFORMATION DEFICIT - WHICH KNOWLEDGE HAVE TEENAGERS IN A GERMAN CAPITAL CITY REGARDING BRAIN DEATH AND ORGAN DONATION?

J.W. Rey[1], V. Graß[2], A.P. Barreiros[1], N. Haberstroh[2], C. Bahnemann[3], K. Merkator[4], U. Samuel[5], G. Otto[6], P.R. Galle[1], C. Werner[7] [1]University Hospital Mainz, First Department of Internal Medicine, Mainz, Deutschland; [2]Comprehensive School, Mainz-Bretzenheim, Deutschland; [3]University Hospital Mainz, Press Office, Mainz, Deutschland; [4]Member of the City Executive, Mainz, Deutschland; [5]German Organ Procurement Organisation (DSO), Mainz, Deutschland; [6]University Hospital Mainz, Department of Transplant Surgery, Mainz, Deutschland; [7]University Hospital Mainz, Department of Anaesthesiology, Mainz, Deutschland

Background: Organ donation is a major problem in Germany due to the fact that the numbers of donated organs are not sufficient regarding needed organs. Therefore, a significant number of patients on the waiting lists are not transplanted in suitable time. Knowing this situation different strategies have been developed to compensate the organ shortage, a. e. detailed education of German population. So far, data about teenagers' opinion and knowledge about organ donation are of interest and only poor is known.

Methods: Survey papers were distributed in secondary schools in the state capital city of Mainz, Germany. The students were asked to respond to 12 questions concerning brain death and organ donation. The voluntary survey was performed under surveillance and without any resources.

Results: Informations from 1168 surveys were analysed. Overall 11.6% (136/1168) of the teenagers were owners of an organ donor card. 49.6% (579/1168) of the students organ donation and brain death could be identified as a central theme in the pupil's families. 39% (455/1168) of the students decline organ donation. Of these, 72.7% (331/455) named lack of education and informations as the primary reason for this statement. Interestingly, non-German pupils declined organ donation more frequently than Germans (48.2% (68/141) vs. 37.7% (387/1027)).

Conclusion: More than half of reviewed pupils between 14 years and 20 years support the concept of organ transplantation as therapeutic option. Nevertheless the proportion of organ card holders is small among these students. These regional results identify deficiency of informations in young people in Germany as one of the main causes for inadequate acceptance of organ donation. Therefore, information and forceful education should be intensified in German schools as possible tool to increase organ donor card holders in Germany.